Committee for Risk Assessment

RAC

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

bis(N-hydroxy-N-nitrosocyclohexylaminato-O,O')copper; bis(N-cyclohexyl-diazenium-dioxy)-copper; [Cu-HDO]

EC Number: 239-703-4
CAS Number: 312600-89-8

CLH-O-0000001412-86-249/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

31 November 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: bis(N-hydroxy-N-nitrosocyclohexylaminato-O,O')copper; bis(N-cyclohexyl-diazenium-dioxy)-copper; [Cu-HDO]

EC Number: **239-703-4**

CAS Numbers: **15627-09-5 and *312600-89-8***

Index Number: **not available**

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Umweltbundesamt GMbH

on behalf of

AT Competent Authority

Federal Ministry of Agriculture, Forestry, Environment and Water Management

Version number: 5 **Date: 17 October 2017**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BIS(N-HYDROXY-N-NITROSOCYCLOHEXYLAMINATO-O,O')COPPER; BIS(N-CYCLOHEXYL-DIAZENIUM-DIOXY)-COPPER; [CU-HDO]

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Part A.

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	bis(N-hydroxy-N-nitrosocyclohexylaminato-O,O')copper; bis(N-cyclohexyl-diazenium-dioxy)-copper; [Cu-HDO]
EC number:	***239-703-4***
CAS number:	***15627-09-5*** ***312600-89-8***
Annex VI Index number:	***Not available***
Degree of purity:	***Min. 98.1 % w/w***
Impurities:	See document “Doc IIA confidential” attached to IUCLID section 13

Remarks:

The EC No. 239-703-4 corresponds to CAS No. 15627-09-5

In the context of the biocides regime, Directive 98/8/EC and Regulation (EU) No 528/2012 respectively, this substance has been approved as active biocidal substance with the substance name bis (N-cyclohexyl-diazenium-dioxy)-copper (Cu-HDO) with the CAS No. 312600-89-8.

In the CAR a minimum purity of ≥ 98.1 % w/w has been specified based on the following calculation: (mean -3*SD). For detailed information see document “Doc IIA confidential” attached to IUCLID section 13

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation (including criteria according to 2nd ATP of CLP)
Current entry in Annex VI, CLP Regulation	Not currently in Annex VI, Table 3.1 of the CLP Regulation
Current proposal for consideration by RAC	Flammable Solid 1 - H228 Acute Tox 4 - H302 Eye Damage 1 – H318 STOT RE 2 (GI, liver, kidney)– H373 Aquatic Acute 1 – H 400 (M =1) Aquatic Chronic 1 – H410 (M =1)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Flammable Solid 1 - H228 Acute Tox 4 - H302 Eye Damage 1 – H318 STOT RE 2 (GI, liver, kidney)– H373 Aquatic Acute 1 – H 400 (M =1) Aquatic Chronic 1 – H410 (M =1)

1.3 Proposed harmonised classification and labelling based on CLP Regulation

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

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BIS(N-HYDROXY-N-NITROSOCYCLOHEXYLAMINATO-O,O')COPPER; BIS(N-CYCLOHEXYL-DIAZENIUM-DIOXY)-COPPER; [CU-HDO]

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				conclusive but not sufficient for classification
2.2.	Flammable gases				data lacking
2.3.	Flammable aerosols				data lacking
2.4.	Oxidising gases				data lacking
2.5.	Gases under pressure				data lacking
2.6.	Flammable liquids				data lacking
2.7.	Flammable solids	Flammable Solid 1			
2.8.	Self-reactive substances and mixtures				conclusive but not sufficient for classification
2.9.	Pyrophoric liquids				data lacking
2.10.	Pyrophoric solids				conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures				Data lacking
2.12.	Substances and mixtures which in contact with water emit flammable gases				conclusive but not sufficient for classification
2.13.	Oxidising liquids				data lacking
2.14.	Oxidising solids				conclusive but not sufficient for classification
2.15.	Organic peroxides				conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals				data lacking
3.1.	Acute toxicity - oral	H302: Harmful if swallowed Acute Tox. 4			
	Acute toxicity - dermal				conclusive but not sufficient for classification
	Acute toxicity - inhalation				conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation				conclusive but not sufficient for classification

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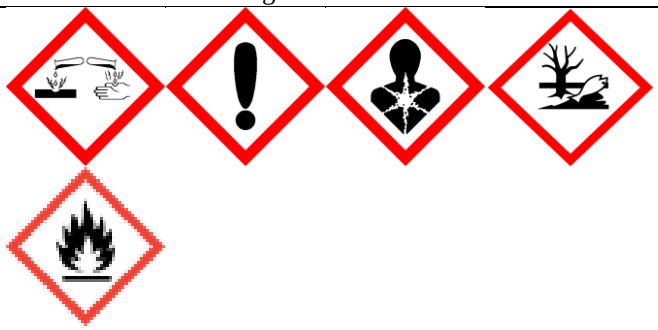
CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.3.	Serious eye damage / eye irritation	H318: Causes serious eye damage. Eye Damage 1			
3.4.	Respiratory sensitisation				data lacking
3.4.	Skin sensitisation				conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity				conclusive but not sufficient for classification
3.6.	Carcinogenicity				conclusive but not sufficient for classification
3.7.	Reproductive toxicity				conclusive but not sufficient for classification
3.8.	Specific target organ toxicity – single exposure				conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	H373: May cause damage to gastrointestinal tract, liver, kidney through prolonged or repeated exposure. STOT Rep. Exp. 2			
3.10.	Aspiration hazard				conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1 H400: Very toxic to aquatic life. Aquatic Chronic 1 H410: Very toxic to aquatic life with long lasting effects.	M-factor =1 M-factor =1		
5.1.	Hazardous to the ozone layer				conclusive but not sufficient for classification

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

Labelling:

Labelling			Justification
GHS Pictograms		 GHS 02/05/07/08/09	
Signal words		Danger	
Classification		Flam Sol 1 Eye Dam 1 Acute Tox. 4 STOT RE 2 Aquatic Acute 1 (M=1) Aquatic Chronic 1 (M=1)	Aquatic Acute 1: L(E)C_{50} values available for all three trophic levels in the range of 0.1 - 10 mg/L; lowest L(E)C_{50} values: LC_{50} (fish) between 0.14 and 0.24 mg/L and E_rC_{50} (algae) =0.194 mg/L. Aquatic Chronic 1: not rapidly degradable and NOEC values available for all three trophic levels. Lowest NOE_rC from algae with 0.056 mg/L.
Hazard statements		H228: Flammable Solid	UN-Test N.1
		H318 - Causes serious eye damage	In vivo eye irritation test
		H302 - Harmful if swallowed	Acute gavage test
		H373 – May cause damage to gastrointestinal tract, liver, kidney through prolonged or repeated exposure	WoE analysis shows toxicological significant effects below guidance value of 100 mg/kg bw/day in sub-chronic studies, which is also supported by results from chronic studies.
		H410 - Very toxic to aquatic life with long lasting effects.	
Precautionary statement	**Prevention**	P210 Keep away from heat/sparks/open flames/hot surfaces. — No smoking: P240 Ground/bond container and receiving equipment. P241 Use explosion-proof electrical/ventilating/lighting/…/equipment. P280 - Wear protective gloves/protective clothing/eye protection/face protection. P264 - Wash thoroughly after handling. P270 - Do not eat, drink or smoke when using this product. P273 – Avoid release to the environment	
	Response	P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P301 + P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P330: Rinse mouth P314: Get medical advice/attention if you feel unwell. P391 – Collect spillage P370 +P378 In case of fire: Use … for extinction.	
	Storage		

	Disposal	P501: Dispose of contents/container in accordance with local/regional/ national/international regulation (to be specified).	

Proposed notes assigned to an entry:

none

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

For the active substance there is no current classification available in Table 3.1 of Annex VI of Regulation (EC) No 1272/2008.

2.2 Short summary of the scientific justification for the CLH proposal

Physico Chemical Porperties:

Flam Sol 1

The results of UN test N.1 showed that the burning time for 100 mm distance was < 45 seconds in five out of six experiments. A moistened zone has stopped the flame front for at least 4 minutes in three of six trials.

Therefore Cu-HDO is considered to fulfil the criteria for classification as flammable solid, category 1 according to EC 1272/2008.

Human Health

STOT RE 2:

Especially the effects in the sub-chronic dog study were toxicologically severe as chronic hepatitis, liver cirrhosis and edema in gall bladder wall. Also the effects in the 28 day and 96 day rat studies are toxicologically significant and appear aggravated in the 12 and 24 months rat studies, mainly as hyperkeratosis and hyperplasia in the GI. In any case the effects observed at the LOAELs were sufficiently significant for the derivation of limit values for risk assessment. It is the dossiers submitters' view that the criterion of representing a relevant point of departure for limit value derivation provides a robust and defensible degree of toxicological significance and should thus also be used for classification purposes and this is in line with the concept for the need of "significant" effects outlined in CLP Annex I, paragraph 3.9.2.1.7.3. and 3.9.2.9.2. Significant effects were observed at LOAELs that meet the STOT RE 2 guidance value for 90 day rat studies, if scaled for allometric species differences and exposure time differences and if it is considered that the "real" LOAEL may be located between the NOAEL and the LOAEL, or in other words with repeating the study with a different dose spacing the LOAEL may be considerably lower.

No exposure route is specified, since there is no evidence that the liver and kidney effects would not appear with respiratory or dermal exposure.

Eye damage cat 1

Non reversible effects with high scores in a rabbit test supports classification for severe eye damage.

Acute tox oral cat 4

An acute oral toxicity study in rats is available indicating an LD50 of 380 mg/kg bw, which is within the oral toxicity range for category 4, 300 to 2000 mg/kg bw. The other available studies indicating higher LD50 values are of lower reliability, but still support category 4 classification. Available dermal and respiratory studies do not support classification for acute dermal or respiratory toxicity.

Environment:

According to the Guidance on the Application of the CLP Criteria v.4.1, Annex IV Metals and inorganic metal compounds, "*Organometals that do not release metal ions are thereby excluded from the guidance of this section and should be classified according to the general guidance provided in part 4 Environmental hazards, of the Guidance on the Application of the CLP Criteria. Metal compounds that contain an organic component but that dissociate easily in water or dissolve as the metal ion should be treated in the same way as metal compounds and be classified according to this annex."*

Cu-HDO is stable to hydrolysis under environmental relevant conditions, it is not rapidly degradable in the aquatic and terrestrial environment and high rates of parent compound were found in the water/sediment degradation study (water phase: 75.4% TAR at day 0, decreasing to 2.8% TAR at day 30; sediment phase (extractable): 16.6% TAR at day 0, increasing to 45.2% at day 10 and again decreasing to 21.5% at day 30). These data show that Cu-HDO, being an organometal compound, cannot dissociate easily in water or dissolve as a metal ion and should therefore be classified according to the general guidance provided in part 4 Environmental hazards, of the Guidance on the Application of the CLP Criteria.

Therefore measured toxicity data for Cu-HDO were taken as basis for C&L of Cu-HDO:

Acute aquatic toxicity: L(E)C_{50} values: 0.1 – 10 mg/L; lowest E(L)C_{50} values: LC_{50} (fish) 0.14 – 0.24 mg/L and E_rC_{50} (algae) =0.194 mg/L.

Chronic aquatic toxicity: NOEC values available for all three trophic levels. Lowest NOE_rC (algae) =0.056 mg/L;

Fate & behaviour: not rapidly degradable;

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

- Classification with Aquatic Acute 1, M factor =1, since the lowest EC_{50} values are LC_{50} (fish) 0.14 – 0.24 mg/L and E_rC_{50} (algae) =0.194 mg/L.
- Classification with Aquatic Chronic 1, M factor =1, since the substance is not rapidly biodegradable and the lowest chronic NOE_rC value (algae) =0.056 mg/L.

2.3 Current harmonised classification and labelling

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

Not available

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

Not available

2.4 Current self-classification and labelling

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

Classification and labelling according to ECHA C&L Inventory:

Acute Tox 4 – H302

Eye Dam 1 – H318

Aquatic Acute 1 – H400

Aquatic Chronic – H410, P273, 391, 501, GHS09

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

Preliminary Note: where references are made to Doc. III-A (=Document III-A) these references refer to the key study summary for the respective endpoint of the biocidal draft Competent Authority Report, which can be found attached to section 13 of the IUCLID dossier.

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	239-703-4
EC name:	Bis(N-hydroxy-N-nitrosocyclohexylaminato-O,O')copper
CAS number (EC inventory):	15627-09-5
CAS number:	15627-09-5 312600-89-8
CAS name:	bis(N-hydroxy-N-nitrosocyclohexylaminato-O,O')copper; bis(N-cyclohexyl-diazenium-dioxy)-copper; [Cu-HDO]
IUPAC name:	bis[1-cyclohexyl-1,2-di(hydroxy-kappa*O*)diazeniumato(2-)]copper
CLP Annex VI Index number:	Not available
Molecular formula:	$C_{12}H_{22}CuN_4O_4$
Molecular weight range:	349.9

Note:

In the context of the biocides regime, Directive 98/8/EC and Regulation (EU) No 528/2012 respectively, this substance has been approved as active biocidal substance with the substance name bis (N-cyclohexyl-diazenium-dioxy)-copper (Cu-HDO) and the CAS No. 312600-89-8.

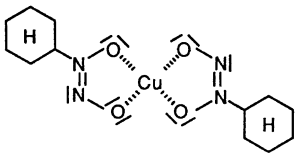
This decision was based on the following considerations. Cu-HDO has two different resonance structures namely a diazeniumdiolate form and a nitrosohydroxylamine form. Each form has its own CAS-No. and EC No.:

• diazeniumdiolate form:	CAS-No. 312600-89-8	EC-No. not attributed
• nitrosohydroxylamine form:	CAS-No. 15627-09-5	EC-No. 248-617-6

The x-ray crystallography data, for details see Doc IIA and Doc III A2, which has been submitted with the dossier for biocidal active substance approval showed that the diazeniumdiolate form is predominating; therefore it was decided to keep only CAS-No. 312600-89-8 as identifier for the biocidal active substance.

Nevertheless it should be kept in mind that different x-ray crystallography conditions may show another distribution. Therefore it is justified to use both CAS numbers and the respective EC number as identifier for this substance within the CLH process.

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Copper, bis[1-cyclohexyl-1,2-di(hydroxy-.kappa.O)diazeniumato(2-)]	99.2 % w/w	98.7 to 99.6 % w/w	In the CAR a minimum purity of ≥ 98.1 % w/w has been specified based on the following calculation: (mean - 3*SD). The mean concentration, derived from a 5-batch analysis amounts to: 99.2 % w/w

Current Annex VI entry: not available

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
See Doc IIA confidential attached to IUCLID section 13			

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

Current Annex VI entry: not available

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
none				

The substance does not contain any additives.

Current Annex VI entry: not applicable

1.2.1 Composition of test material

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

1.3 Physico-chemical properties

Table 9 Physico-chemical properties

PROPERTY	PURITY / SPECIFICATION	RESULT	METHOD[1] / REFERENCE
Melting Point	purified a.s. 99%w/w	**149°C**	OECD Guideline 102; **study A 3.1.1/01, document III A 3**
Boiling Point	purified a.s. 99%w/w	**Not detectable due to decomposition at about 182°C**	company's statement; **study A 3.1.1/01, document III A 3**
Relative Density	purified a.s. 99%w/w	**1.514±0.005 at 20°C**	OECD Guideline 109; **study A 3.1.1/01, document III A 3**
Vapour pressure	purified a.s. 99%w/w	**$<10^{-6}$ hPa at 50°C and at 20°C**	Dir 92/69/EEC, Annex V, A.4; **study A 3.1.1/01, document III A 3**
Henry's Law Constant	n.a. (calculated)	$<5.7 \cdot 10^{-6}$ kPa·m^3·mol^{-1}	calculated **document III A 3**
Physical state	purified a.s. 99%w/w	**Solid (crystalline powder, homogenous at inspection)**	visual inspection; **study A 3.4/02, document III A 3**
Colour	purified a.s. 99%w/w	**blue (blue violet)**	visual inspection; **study A 3.4/02, document III A 3**
Odour	purified a.s. 99%w/w	**Odourless**	olfactory inspection **document III A 3**
Absorption spectra	purified a.s. 99%w/w	**UV/VIS absorption maxima: E[1 cm/1%] = 293 at 238 nm E[1 cm/1%] = 1.2 at 629 nm The structure of Cu-HDO is confirmed by all spectra.**	**study A 3.4/01 study A 3.4/02 document III A 3**
Solubility in water	purified a.s. 99% w/w	**34.6 mg/L (pH = 4) 6.1 mg/L (pH = 7) 8.6 mg/L (pH = 9) (flask method)**	Dir 92/69/EEC, Annex V, A.6; **study A 3.5, document III A 3.5**
Dissociation constant	purified a.s. 99%w/w	**Not determinable by neither conductometric method nor spectrophotometric method nor titration method due to the low water solubility**	OECD Guideline 112; **study A 3.6, document III A 3**

Table 9 Physico-chemical properties

contd.

PROPERTY	PURITY / SPECIFICATION	RESULT	METHOD[1] / REFERENCE
Solubility in organic solvents	purified a.s. >98%w/w	n-octanol: 6100mg/L at 25°C	**study A 3.7/01, document III A 3**
		General: soluble in non-polar organic solvents within a range of 1000–10 000mg/L	Dir 79/831/EEC, Annex V, A.7 (deleted 1992); **study A 3.7/02, document III A 3**
Partition coefficient octanol-water	purified a.s. 99%w/w	2.6 at pH 6.1 and 25°C 1.6 at pH 4 and 25°C	Dir 92/69/EEC, Annex V, A.8 **study A 3.1.1/01**
Thermal stability	purified a.s.	Decomposition at 182°C; expected disintegration products: NO_x, CO_2, H_2O	OECD Guideline 102; **study A3.1.1/01, document III A 3**
Flammability	purified a.s. 99%w/w	>164°C not "highly flammable"	Dir 92/69/EEC, Annex V, A.10 **study A 3.11, document III A 3**
	purified a.s. 99%w/w	The test determines the burning time for a measuring section of 100 mm. The test was performed six times and the determined burning rate was between 23.8 and 51.2 s. A moistened zone has stopped the flame front for at least 4 minutes in three of six trials.	EC 1272/2008 UN test N.1 **study A 3.15, document III A 3**
Auto-flammability	purified a.s. 99%w/w	Self-ignition temperature ca. 170°C	Dir 92/69/EEC, Annex V, A16 **study A 3.11, document III A 3**
Surface tension	solution of purified a.s. 99%w/w in water (90% of the saturation solubility)	70.1 mN/m (not surface active)	OECD Guideline 115; **study A3.1.1/01, document III A 3**
Explosive properties	purified a.s. 99%w/w	Result according Directive 67/548/EEC (Dangerous Substances Directive; DSD): Danger of explosion in the sense of the directive (thermal sensitivity; no sensitivity to impact or friction) This study is not compliant to CLP Regulation and has been replaced by Study A 3.15/1 which is summarised below	Dir 92/69/EEC, Annex V, A14 **study A 3.11, document III A 3**

	purified a.s. 99%w/w	Onset-temperature: 178 °C Decomposition heat: 1908 J/g and 1831 J/g	EC 1272/2008 Differential scanning calorimetry **study A 3.15/1, document III A 3**
	purified a.s. 99%w/w	The test result is negative, because the diameter of the steel core is < 2mm.	EC 1272/2008 Koenentest -UN test 2(b) **study A 3.15, document III A 3**
	purified a.s. 99%w/w	In three tests the pressure arises from 670 kPA to 2070 kPa in 198 ms, 304 and 105 ms. According to UN 1(c) (i) positive, because pressure is> 2070 kPa. According to UN 2(c) (i) negative, because time for pressure increase is > 30 ms.	EC 1272/2008 Pressure/time test-UN test 1(c)(i)/2(c)(i) **study A 3.15, document III A 3**
	purified a.s. 99%w/w	For 10 g substance the expansion was 3 mL in the lead bock test. The test result is clearly negative and no further testing is required.	EC 1272/2008 Trauzl test, UN test F.3 **study A 3.15, document III A 3**
Oxidising properties	purified a.s. 99%w/w	Result according Directive 67/548/EEC (Dangerous Substances Directive; DSD): Oxidising This study is not compliant to CLP Regulation and has been replaced by Study A 3.15/1 which is summarised below	Dir 92/69/EEC, Annex V, A17 **study A 3.11, document III A 3**
	purified a.s. 99%w/w	The test substance Cu-HDO was tested in a mixture with Cellulose in a ratio of 1:1 and 4:1 [mass-%]. The averaged burning rate was 81.9 s [1:1] and 51.8 s [4:1]. Based on these test results, the burning rate of Cu-HDO was tested with an inert substance (Kieselguhr) in a ratio of 4:1 [mass-%]. The averaged burning rate was 35.7 s.	EC 1272/2008 UN test O. **study A 3.15, document III A 3**
Reactivity towards container material	purified a.s. 99%w/w	Please see document II-A – Effects assessment for the active substance – Appendix – Confidential data and information	company's statement **document III A 3**

[1] "OECD Guideline" is short for "OECD Guideline for the testing of chemicals"

2 MANUFACTURE AND USES

2.1 Manufacture

See document "Doc IIA confidential" attached to IUCLID section 13

2.2 Identified uses

Biocide for use as: Wood Preservative (PT 8)
Film preservatives (PT 7)
Fibre, leather, rubber and polymerised materials preservatives (PT 9)
Masonry preservatives (PT 10)

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

PROPERTY	PURITY / SPECIFICATION	RESULT	METHOD[1] / REFERENCE
Thermal stability	purified a.s.	**Decomposition at 182 °C; expected disintegration products: NO_x, CO_2, H_2O**	OECD Guideline 102; **study A3.1.1/01, document III A 3**
Flammability	purified a.s. 99%w/w	The test determines the burning time for a measuring section of 100 mm. The test was performed six times and the determined burning rate was between 23.8 and 51.2 s. A moistened zone has stopped the flame front for at least 4 minutes in three of six trials.	EG 1272/2008 UN test N.1 **study A 3.15, document III A 3**
Auto-flammability	purified a.s. 99%w/w	**Self-ignition temperature ca. 170°C**	Dir 92/69/EEC, Annex V, A16 **study A 3.11, document III A 3**
Flash Point	purified a.s. 99%w/w	n.a.	–
Explosive properties	purified a.s. 99%w/w	Onset-temperature: 178 °C Decomposition heat: 1908 J/g and 1831 J/g	company's statement; EC 1272/2008 Differential scanning calorimetry **study A 3.15/1, document III A 3**
	purified a.s. 99%w/w	The test result is negative, because the diameter of the steel core is < 2mm.	company's statement; EC 1272/2008

			Koenentest -UN test 2(b) **study A 3.15, document III A 3**
	purified a.s. 99%w/w	In three tests the pressure arises from 670 kPA to 2070 kPa in 198 ms, 304 and 105 ms. According to UN 1(c) (i) positive, because pressure is> 2070 kPa. According to UN 2(c) (i) negative, because time for pressure increase is > 30 ms.	company's statement; EC 1272/2008 Pressure/time test-UN test 1(c)(i)/2(c)(i) **study A 3.15, document III A 3**
	purified a.s. 99%w/w	For 10 g substance the expansion was 3 mL in thelead bock test. The test result is clearly negative and no further testing is required.	company's statement; EG 1272/2008 Trauzl test, UN test F.3 **study A 3.15, document III A 3**
Oxidising properties	purified a.s. 99%w/w	The test substance Cu-HDO was tested in a mixture with Cellulose in a ratio of 1:1 and 4:1 [mass-%]. The averaged burning rate was 81.9 s [1:1] and 51.8 s [4:1]. Based on these test results, the burning rate of Cu-HDO was tested with an inert substance (Kieselguhr) in a ratio of 4:1 [mass-%]. The averaged burning rate was 35.7 s. The average burning rate of the reference mixture were 60.0 s [2:3] and 17.9 s [3:2].	company's statement; EG 1272/2008 UN test O.1 **study A 3.15, document III A 3**
Reactivity towards container material	purified a.s. 99%w/w	**Please see document II-A – Effects assessment for the active substance – Appendix – Confidential data and information**	company's statement **document III A 3**

3.1 Flammability, oxidising and explosive properties

3.1.1 Summary and discussion

On 25 March 2004, Austrian competent authorities received a dossier for the biocidal active substance Cu-HDO in the context of the work programme for the review of existing active substances with the view to the possible inclusion of this substance into Annex I or IA of Directive 98/8/EC. On of 31 January 2014 Cu-HDO has been approved as biocidal active substance according Regulation (EU) No 528/2012, which replaced Directive 98/8/EC on 1 September 2013.

With regard to flammability, oxidising and explosive properties of Cu-HDO the studies submitted in 2004 were compliant with Directive 67/548/EEC (Dangerous Substances Directive; DSD) and resulted in classification as E: R2 and O: R8.

Since DSD has been replaced by CLP a revision of the flammability, oxidising and explosive properties of Cu-HDO was necessary. Therefore a CLP compliant study has been submitted on 21 October 2013. The results of this study are summarised below.

Flammability:

The results of UN test N.1 showed that the burning time for 100 mm distance was < 45 seconds in five out of six experiments. A moistened zone has stopped the flame front for at least 4 minutes in three of six trials.

Therefore Cu-HDO is considered to fulfil the criteria for classification as flammable solid, category 1 according to EC 1272/2008.

Oxidizing properties:

The test result of the UN-test O.1 showed that the tested Cu-HDO/cellulose mixture (ratio 4:1) exhibited a mean burning time of 51.8 s, which is clearly below the burning time of 64.0 s for the reference mixture (ratio 2:3). This would have meant classification as oxidising solid, category 2.

The test substance has been tested again mixed with an inert substance (diatomaceous earth) at a ratio of 4:1, exhibiting an average burning time of 35.7 s. This test showed that Cu-HDO does not increase the burning rate of Cellulose but burns itself. Therefore the results of the UN-test 0.1 are considered as false positive and consequently Cu-HDO should not be classified as oxidising.

Explosive properties:

Due to the structure and the high decomposition energy (approx.. 1900 J/g) it could not be excluded that the test substance Cu-HDO may be considered as explosive substance according to EC 1272/2008. Therefore the acceptance procedure according to No. 10.3 of the UN testing manual was performed. According to this test procedure, Cu-HDO is to insensitive for classification in class 1 “explosive substances”. Therefore no classification as explosive is required.

3.1.2 Comparison with criteria

See above

3.1.3 Conclusions on classification and labelling

Not oxidising

No classification as explosive is required.

Cu-HDO should be classified as Flam Sol 1. The labelling is therefore GHS02, danger, H 228, flammable solid.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Flammability

The UN test N.1 was carried out with Cu-HDO with a purity of 98%.

The test determines the burning time for a measuring section of 100 mm of the tested substance. The test was performed six times and the determined burning rate was between 23.8 and 51.2s. A moistened zone stopped the flame front for at least 4 minutes in three of the six trials. Classification is based upon the shortest burning time obtained in six test runs.

Based on the test results of UN test N.1, the Cu-HDO fulfils the criteria for classification as flammable solid Category 1 (Flam Sol. 1; H228).

Oxidising solids

The test result of the UN-test O.1 showed that the tested Cu-HDO/cellulose mixture (ratio 4:1 w/w) exhibited a mean burning time of 51.8s, which is clearly below the burning time of 64.0s for the reference mixture potassium bromate/cellulose (ratio 2:3). This would have met the classification criteria for oxidising solid, Category 2.

The test substance was tested again, mixed with an inert substance (diatomaceous earth) at a ratio of 4:1, exhibiting an average burning time of 35.7s. This test showed that Cu-HDO does not increase the burning rate of cellulose but burns itself. Therefore, the results of the UN-test O.1 are considered to be false positives and consequently Cu-HDO should not be classified as an oxidising solid.

Explosive properties

Due to the structure and the high decomposition energy (approx. 1900 J/g) it could not be excluded that the test substance "Cu-HDO" may be considered as an explosive substance according to CLP. Therefore, the following tests were performed to assess the explosive properties of Cu-HDO.

Trauzl test (UN test F.3, test substance Cu-HDO, purity 98%)

This test is used to measure the explosive power (strength) of a substance by determining of the volume increase, which is produced by the detonation of a tested explosive charge in the cavity of a lead block of a defined quality and size. For 10 g substance the expansion was 3 mL in the lead bock test (at least 10 mL expansion is required). The test result is clearly negative and no further testing is required.

Pressure/time test (UN test 1(c)(i)/2(c)(i), test substance Cu-HDO, purity 98%)

In three tests the pressure rises from 670 to 2070 kPa in 198 ms, 304 ms and 105 ms. According to UN 1(c)(i) one test is positive, because the pressure is > 2070 kPa. According to UN 2(c)(i) the result is negative, because time for pressure increase is > 30 ms.

The Koenen test (UN test 2(b), test substance Cu-HDO, purity 98%): The test result is negative, because the diameter of the steel core is < 2 mm.

Based on the above mentioned tests, no classification of Cu-HDO as an explosive solid was proposed by the dossier submitter (DS).

Based on the results of the available studies, the classification of Cu-HDO proposed by the DS is flammable solid, Category 1. According to the DS, Cu-HDO should not be classified as oxidising or as explosive.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

Overall, RAC agrees with the DS proposal to classify **Cu-HDO** only as **Flam Sol. 1; H228** for physical hazards.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Four toxicokinetic studies were submitted for the evaluation of Cu-HDO (see document A 6.2.1. to 6.2.4.)

Within the first study (A 6.2.1.) the dermal uptake rate for Cu-HDO was found to be 3%. However the reliability of this value is limited since for 5 from the 8 tests with Cu-HDO carried out within this study the recovery rates were below 90%. Moreover the dermal uptake data for Cu-HDO from the technical preparation (Wolmanit CX50) generated within the same study were not valid since the recovery rate was only 77%. The dermal uptake was not investigated in the subsequent study A 6.2.2.

Therefore a new in vitro dermal absorption study with human skin samples was carried out (A 6.2.4.c). The study was carried out with a 2% solution of Wolmanit CX and an exposure time of 24 hours. The total decrease in the donor fluid was 22% over 24 hours; this means that steady-state conditions were approximately achieved. The amount penetrating to the receptor fluid till 0.5, 1, 2, 4, 6, 10 and 24 hours, the amount remaining in the skin preparation after 24 hours and the amount remaining in the superficial stratum corneum after 24 hours (tape stripping) were analysed. For the risk assessment it was assumed that the worker will wash his hands latest after 10 hours of work. Therefore the mean value of the cumulative absorption in the receptor fluid after 10 hours was taken into consideration (3.8%). Since with continuous exposure the amount in the skin should not decrease and since the increase of the cumulative absorbed dose over time was linear between 4 and 24 hours, the recovery from skin preparation at the end of the experiment (24 hours) was used as an estimate for the amount remaining in the skin at the 10 hours time point (3.9%). The amount remaining in the superficial stratum corneum (6 tape strips, ca. 6%) was considered as unabsorbed. Consequently the uptake rate for worker exposure situations was estimated with ca. 8%.

This value of 8% dermal absorption is supported by considering that an indicative value of 3% appears from the in vivo rat dermal absorption study (A6.2.1, see above). This in vivo study is not fully valid because of low

recovery rate of 77% with Cu-HDO in Wolmanit CX. However, published data (van Ravenzwaay and Leibold 2004) support that under in vitro conditions rat skin is more permeable than human skin. Furthermore the in vitro rat data overestimate the absorption through in vivo rat skin. Taking into account the uncertainties in these studies, and incomplete data for a more precise calculation as suggested in van Ravenzwaay and Leibold 2004 [in vivo human absorption = in vivo rat x (in vitro human/in vitro rat)] an overall dermal absorption rate of 8% is considered to give sufficient confidence for risk assessment including the aspects of remaining uncertainties.

The other results from the available in vivo rat studies with purified Cu-HDO described in document A 6.2.1. were basically confirmed by the subsequent study described in document III-A 6.2.2. It was shown that after gavage administration the organic moiety is completely absorbed across the GI tract: 48 hours after the application of the low dose (15 mg/kg bw) the applied radioactivity was already excreted via urine at least to 78% and recovered from bile to 34%. With the higher dose of 150 mg/kg bw the biliary excretion seemed saturated, since 93% of the applied radioactivity was excreted via urine and only 12% recovered from bile. Accordingly excretion via faeces was 14% of the applied radioactivity for low dose and 2% for high dose. Since the total faeces excretion is considerably lower than the amount recovered from bile, it was concluded that re-absorption occurs in the gut as part of an enterohepatic circulation. With repeated high dosing (150 mg/kg bw day) the excretion pattern and time course of excretion did not change in comparison to the single dosing. However since the terminal plasma half time was about 24 hours some potential for bioaccumulation is evident. Throughout the time course of the experiments, highest radioactivity concentrations were found in the GI tract, liver and kidney whereas radioactivity levels were lowest in bone, brain and muscles.

Within the following study, described in A 6.2.3, it was shown that after administration by oral gavage the major part (58%, 65%, 72% for 15, 150 and 15x150 mg/kg bw, respectively) of Cu-HDO is metabolised to the glucuronide of the free ligand, N-cyclohexyl-diazenium-dioxy-glucuronide. Besides this major metabolite and the parent compound, several minor metabolites with less than 2.5% of dose were found in the chromatograms. No further structural identification was performed in these cases. The parent compound was found in urine (15-24% of dose), bile (0-1.5% of dose) and faeces (0.8-13% of dose), whereas the glucuronide metabolite was detected only in urine (58-72% of dose) and bile (9-33% of dose). Considering the evidence for complete absorption and enterohepatic circulation this indicates a deglucuronidation process in the gut. There are no substantial differences of the metabolic patterns observable between the single high dose group and the 15 times repeated high dose group (both 150 mg/kg bw) which demonstrates that an induction of metabolic enzymes by the test substance is unlikely.

In summary it is concluded that Cu-HDO is orally absorbed by about 100% and dermally absorbed by 8%. In the absence of other data 100% inhalative absorption is assumed. Highest concentration throughout the toxicokinetic time course is found in GI, liver and kidney. The terminal plasma half live is about 24 hours, indicating some limited potential for bioaccumulation. The main route of excretion is in urine and to a lesser extent in feces, there is evidence for enterohepatic circulation. As metabolite only the glucuronide of the free HDO was identified. Other minor metabolites (< 2.5% of dose) were not identified.

Within the study of Hoffmann in 1993 (docIIIA6.2.1.), the toxicokinetics of K-HDO, Cu-HDO and of Al-HDO were investigated in parallel. Since the log $P_{o/w}$ differs between Cu-HDO (2.6) and K-HDO (-0.2) it could be expected that differences might be found for the rate and extent of the absorption and excretion or the general bioavailability of the various compounds. However, within this study virtually no difference in the amount of radioactivity in body fluids or excreta was found. Also the in vitro dermal absorption studies carried out in parallel with K-HDO (Gamer et al. 2006a, doc IIIA6.2.4k) and with Cu-HDO (Gamer et al. 2006b) resulted in similar dermal absorption rates. This indicates that the bioavailability of the organic anion HDO is not – or to a minor extent – influenced by the type of cation bound to it. The latter might be explained by the fact that biological media are more complex than a simple two-phase-system: The behaviour of Cu-HDO and K-HDO is not only influenced by differences in polarity of the surrounding medium, but also e.g. by various ions (e.g. Ca^{2+}, Mg^{2+}), proteins and lipoproteins. However with the comparable kinetics, a read-across of the metabolism data from Cu-HDO to K-HDO appears justified.

4.1.2 Human information

Not available

4.1.3 Summary and discussion on toxicokinetics

See discussion above

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Table 11 Acute toxicity tests, oral route

Test substance	Method Guideline	Species Strain Sex no/group	Dose levels duration of exposure	Value LD_{50}	Remarks	Reference
Cu-HDO suspended in aqueous 0.5% carboxy methyl cellulose	BASF test (prior to OECD Guideline 401 and GLP)	Sprague-Dawley rats Male/female 10 m and 10 f per dose group	215, 261, 316, 383, 464, 562, 681 mg/kg bw single administration	**380 mg/kg bw**	no GLP; reliability 1	**Study A6.1.1/01;** **Document IIIA6.1.1/01**
Cu-HDO suspended in aqueous 0.5% carboxy-methyl-cellulose	Not indicated in the study report; test prior to OECD Guideline 401 and GLP	Not indicated in the study report;	0.15–15% Cu-HDO suspension in 0.5% aqueous carboxy-methyl-cellulose	500 mg/kg bw	no GLP; reliability 3 (not sufficient experimental details)	Study A6.1.1/02; Document IIIA6.1.1/02
Cu-HDO (no more detailed specification in the study report)	Test prior to OECD Guideline 401 and GLP; LD_{50} Calculation according to the method of Weil C.S. (1952), Biometrics 8, 249	Rats, CFY strain Male/female 5 m and 5 f per dose group	0, 400, 640, 1000, 1600, 2500 mg/kg bw oral intubation	860 mg/kg bw	no GLP; reliability 2	Study A6.1.1/03; Document IIIA6.1.1/03

4.2.1.2 Acute toxicity: inhalation

Table 12 Acute toxicity tests, inhalative route

Test substance	Method Guideline	Species Strain Sex no/group	Dose levels duration of exposure	Value LC_{50}	Remarks	Reference
solid Cu-HDO	Acute inhalation hazard test; test prior to OECD Guideline 403 and GLP	Rats 12 animals per dose group	Atmosphere saturated with vapour or enriched with dust at 20°C (concentration not measured); exposure: 3min, 10min, 30min, 1h, 3h, 8h	**No mortality after 8h exposure**	no GLP; reliability 3	**Study A 6.1.3;** **Document IIIA 6.1.3**

4.2.1.3 Acute toxicity: dermal

Table 13 Acute toxicity tests, dermal route

Test substance	Method Guideline	Species Strain Sex no/group	Dose levels duration of exposure	Value LD_{50}	Remarks	Reference
Cu-HDO	In accordance with D.N. Noakes and D.M. Sanderson (A method for determining the dermal toxicity of pesticides; Brit. Journ. Ind. Med. 26, 1969) test prior to OECD Guideline 402 and GLP	Sprague-Dawley rats/SPF Male/female 5 m and 5 f per dose group	2500 mg/kg bw (duration of exposure is not nearer specified)	**> 2500 mg/kg bw**	no GLP; reliability 2	**Study A 6.1.2;** **Document IIIA 6.1.2**

4.2.1.4 Acute toxicity: other routes

Not available

4.2.2 Human information

Not available

4.2.3 Summary and discussion of acute toxicity

The acute toxicity of Cu-HDO was tested by the oral and dermal route as well as by the inhalative route. All tests were conducted using rats.The studies were performed prior to the requirement of GLP and of the adequate OECD guidelines, but since the studies are consistent and since they support each other, they are acceptable.

The $LD_{50,oral,rat}$ of Cu-HDO was determined in a study using 146 animals (**study** A 6.1.1/01); it amounts to 380 mg/kg bw and should lead to the assignment of "H332 – Harmful if swallowed"(according to Regulation 1272/2008/EC)..

The active substance Cu-HDO does not display any acute sytemic toxicity by the dermal route: The $LD_{50,\ dermal,\ rat}$ is >2500mg/kg bw (**study** A 6.1.2). No mortality occurred, no clinical signs of toxicity were observed, and the animals sacrificed after the 14-day observation period exhibited no finding of the internal organs attributable to the applied test substance.

Regarding the inhalative route, an acute inhalation hazard test was carried out with rats (**study** A 6.1.3). After 8 hours of exposure, no mortality was observed. Only slight irriation of the eyes and not other clinical signs of toxicity were reported.

As concentration and particle size distribution of the active substance as well as the rate of air flow were not measured, it is not possible to set the lower limit of the LC_{50} value. Anyway, as heavy dust development was stated and as the duration of exposure was 8 hours instead of 4 hours as recommended in the OECD guideline

403, it can be assumed that the LC_{50} is above the concentration range which leads to classification. The available data do not meet the EU criteria for classification as acute toxic via inhalation.

However the human inhalation exposure is limited compared to the dermal exposure which justifies providing reliable results primarily for the oral and dermal route.

4.2.4 Comparison with criteria

See discussion above

4.2.5 Conclusions on classification and labelling

Classification for acute oral toxicity category 4 is proposed on the basis of the available animal studies providing LD50 estimates in the category 4 range, i.e. between 300 and 2000 mg/kg bw.

Available dermal and respiratory studies do not support classification for acute toxicity.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

Three acute oral toxicity studies with Cu-HDO (all conducted prior to OECD test guideline (TG) and GLP) were evaluated by the DS.

In a study rats (Sprague-Dawley, 10 m/f) were exposed to a single dose of Cu-HDO at dosed up to 681 mg/kg bw (A 6.1.1/01). Signs of toxicity included poor general state, apathy, dyspnoea, diarrhoea. All animals of the 681 mg/kg bw dose group, 13 animals (7m/8f) of the 563 mg/kg bw, 13 animals (8m/5f) of the 464 mg/kg bw, 13 animals (6m/7f) of the 383 mg/kg bw dose group, 5 animals (3m/2f) of the 316 mg/kg bw dose group and 5 animals (4m/1f) of the 261 mg/kg dose group died within 4 days after application. The LD_{50} value was found to be 360 mg/kg bw for males, 399 mg/kg bw for females and 380 mg/kg bw for males and females combined.

A study of low quality rats (strain and number of animals not specified) were exposed to 0.15-15% Cu-HDO suspension in 0.5% aqueous carboxy methyl cellulose. Signs of toxicity included apathy, diarrheic faeces, exsiccosis, dyspnoea, and anaemia. The LD_{50} was evaluated to be 500 mg/kg bw (A 6.1.1/02).

In the third study rats (strain: CFY, 5 males/5 females per dose group) were exposed to doses of 0, 400, 640, 1000, 1600 and 2500 mg/kg bw by oral intubation. Signs of toxicity included lethargy, piloerection and moderate diarrhoea. At doses above 400 mg/kg bw an increase in salivation was observed. At the two highest doses (1600 and 2500 mg/kg bw) female rats showed moderate diuresis and at the high dose of 2500 mg/kg bw moderate ataxia was observed. All animals of the 2500 mg/kg bw dose group, 9 animals (5m/4f) of the 1600 mg/kg bw dose group, 9 animals (5m/4f) of the 1000 mg/kg bw dose group, 0 animals of the 640 mg/kg bw dose group and 1 female of the 400 mg/kg bw dose group died within 5 days after application (A 6.1.1/03). The LD_{50} was evaluated to be 860 mg/kg bw.

The DS proposed to classify Cu-HDO in Category 4 for acute oral toxicity based on the LD_{50} value of 380 mg/kg bw.

Acute dermal toxicty

One acute dermal toxicity study (prior to OECD TG and GLP) was evaluated by the DS. In this study rats (Sprague-Dawley, 5 males/5 females) were exposed for Cu-HDO at a dose of 2500 mg/kg bw for 24h. The animals were sacrificed after a 14 days observation period (A 6.1.2). No mortality or clinical signs of systemic toxicity were seen and the DS concluded that no classification was warranted for acute dermal toxicity of Cu-HDO.

Inhalation

One acute toxicity study (prior to OECD TG and GLP) for Cu-HDO by the inhalation route has been evaluated by the DS. Rats (12 animals per dose group, no information on strain/ sex) were exposed to atmospheres saturated with vapour or enriched with dust at 20ºC. No mortality was reported after 8 hours exposure, and signs of toxicity were limited to slightly irritation of eyes (A 6.1.3). The concentration and particle size distribution of Cu-HDO as well as the air flow were not measured in this study. The DS noted that it is not possible to set a lower limit for the LC_{50} value, and concluded that it can be assumed that the LC_{50} value is above the concentration range relevant for classification. Therefore, no classification was proposed.

Comments received during public consultation

One Member State Competent Authority (MSCA) supported the proposed classification as acute oral toxicity Category 4, however they requested some clarifications regarding the purity of the test substance. Another commenting MSCA was of the opinion that the study presented for acute toxicity by inhalation is of low reliability and that the basis for no classification is rather weak.

Assessment and comparison with the classification criteria

Acute oral toxicity

Based on the data presented, the oral LD_{50} are evaluated to be 360 mg/kg bw for males, 399 mg/kg bw for females and 380 mg/kg bw for males and females combined. According to CLP, LD_{50} values for acute oral toxicity ranging from 300 to 2000 mg/kg bw warrants classification in Category 4. RAC is in agreement with the DS, that Cu-HDO meets the criteria for classification in Category 4 for acute oral toxicity.

The ATE-value for classifying mixtures should be equal to the lowest oral LD_{50} which was observed for male rats, that is 360 mg/kg bw.

Acute dermal toxicity

According to CLP, LD_{50} values for acute dermal toxicity > 2000 mg/kg bw do not warrant classification. RAC is in agreement with the DS that no classification for acute dermal toxicity is warranted for Cu-HDO.

Acute inhalation toxicity

RAC considers that it is not possible to evaluate the result of the acute inhalation study presented and no classification for acute toxicity via inhalation is proposed for Cu-HDO due to insufficient data.

Overall, RAC agrees with the DS to classify **Cu-HDO as Acute Tox. 4; H302** with an **ATE value of 360 mg/kg bw**.

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

The acute respiratory study did not indicate local respiratory effects though these may be expected considering the very severe local eye effects in the rabbit eye study. No other specific target organs were identified in the acute studies.

No classification is proposed for STOT SE.

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

Summary of the Dossier Submitter's proposal

Several clinical signs were observed after acute oral administration of doses up to 2500 mg/kg bw of Cu-HDO to rats, including a poor general state, apathy, dyspnoea, diarrhoea, lethargy, piloerection, increased salivation, diuresis, ataxia, exsiccosis and anaemia. Dermal administration to rats elicited no clinical signs. During inhalation exposure signs of eye irritation were seen. The DS evaluated that the acute inhalation toxicity study did not show any local respiratory effects, and pointed out that no other specific target organ effects were identified in the acute toxicity studies, and on this basis did not propose any classification for STOT SE.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

A STOT SE classification is assigned on the basis of findings of 'significant' and/or 'severe' toxicity at generally low doses (Category 1) or with significant toxicity at more moderate doses (Category 2). There is insufficient evidence of specific target organ toxicity at low or moderate doses via oral, dermal or inhalation routes which is not already covered by the proposed classifications for acute oral toxicity and serious eye damage.

As regards STOT SE Category 3 there was no evidence of respiratory tract irritation or narcotic effects in any studies.

RAC agrees with the DS that **no classification for STOT SE warranted**.

4.4 Irritation

4.4.1 Skin irritation

Table 14: Summary table of relevant skin irritation studies

Test subst.	Species Strain Sex no/group	Method	Duration of exposure	Average score 24, 48, 72h		Reversibility	Result	Reference
				Erythema and eschar	Edema			
Cu-HDO (50% paste in aqua dest.)	Rabbit White Vienna (Gaukler) 4m, 2f	BASF test (prior to OECD Guideline 404 and GLP)	20 hours (occlusive)	animal 1= 0	animal 1 = 0	yes	**Not irritating**	**Study A 6.1.4/01; Document IIIA 6.1.4/01**
				animal 2 = 0	animal 2 = 0			
				animal 3 = 1	animal 3 = 0			
				animal 4 = 0,3	animal 4 = 0			
				animal 5 = 0	animal 5 = 0			
				animal 6 = 0,3	animal 6 = 0			

4.4.1.1 Non-human information

Skin irritation was examined in rabbits in a non GLP and non OECD or EC guideline conform test. The test item was moistened to produce a 50% paste in distilled water. This may be moistened more than recommended by TG404: "When testing solids (which may be pulverised, if considered necessary), the test chemical should be moistened with the smallest amount of water (or, where necessary, of another suitable vehicle) sufficient to ensure good skin contact" and "5 g of solid or paste is applied to the test site". However the testing conditions were harder compared to the OECD 404 test in respect of duration (20 versus 4 hours) and exposure (occlusive versus semi-occlusive).

Slight spotty erythema on the dorsal skin and ear was observed in 3 of 6 animals after the 20 hours exposure. All effects resolved by day 8, the last day of observation. The results of this study indicating that the skin irritation potential of Cu-HDO is low are supported by the respective observations from the acute dermal toxicity test.

4.4.1.2 Human information

Not available

4.4.1.3 Summary and discussion of skin irritation

See discussion above

4.4.1.4 Comparison with criteria

Skin irritation scores in the rabbit test clearly below 2 for all endpoints indicate that no classification for skin irritation is necessary.

4.4.1.5 Conclusions on classification and labelling

No classification necessary.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The skin irritation potential of Cu-HDO was investigated in a study (prior to OECD TG and GLP) in rabbits (white Vienna (Gaukler), 4 males/2 females). Male and female rabbits (6 animals/group) were exposed to Cu-HDO (50% paste in distilled water) for 1, 5 and 15 minutes and 20 hours under occlusive conditions. There are no further information on the composition of the Cu-HDO paste used in this study. No erythema or oedema were reported after exposure for 1, 5 or 15 minutes followed by a 8 days post exposure period. The animals were examined after 24 hours and 8 days. Exposure for 20 hours revealed slight spotty erythema in 3 out of 6 animals (score after 24h: 1, 0.3 and 0.3), and no oedema. The effects were reversible within 8 days after exposure (A 6.1.4/02). Based on this study the DS concluded that no classification is warranted for skin corrosion/irritation of Cu-HDO.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

The scores for erythema and oedema was clearly below the limits for classification as skin irritation category 2 in this study. On this basis, RAC agrees with the DS that **no classification for skin corrosion/irritation is warranted**.

4.4.2 Eye irritation

Table 15: Summary table of relevant eye irritation studies

Test subst.	Species Strain Sex no/group	Method	Average score 24, 48, 72h				Reversibility	Result	Reference
			Cornea Opacity[#]	Iris	Redness Conjunc-tiva[#]	Chemosis[#]			
50 µl Cu-HDO (solid)	Rabbit	BASF test:	3* 8d: 3	not reported	2* 8d: 2	3* 8d: 3	no	**Severe damage**	**Study A 6.1.4/02;**

	White Vienna (Gaukler) 2f	Single application of 50 ml (prior to OECD Guideline 405 and GLP)						**to the eye**	**Document IIIA 6.1.4/02**

*two animals were tested, they yielded identical scores

The scores presented in this table by the RMS represent a translation of the scores from the study report to the OECD 405 scoring system: The scoring in the study ranges from 0 to 3, but in the OECD guideline only for the endpoint redness from 0-3 and for the other endpoints from 0 to 4. This means that the score 2 given in the study for cornea opacity and chemosis corresponds to a score between 2 and 3 according to the OECD guideline.

4.4.2.1 Non-human information

Eye irritation was examined in two female rabbits. The two animals were exposed with 50µl of test-substance (solid). The eyes were not washed out after 24 hours as specified in OECD guideline 405, which could be critical for solids, which thus remained on the cornea for several days, causing mechanical damage, which would probably be less severe if it had been washed out after 1 day.

Within the study report the effect to the iris was not evaluated separately as mandatory in the OECD guideline. The scores from the study report were translated to the OECD 405 scoring system by the RMS: The scoring in the study ranges from 0 to 3, but in the OECD guideline only for the endpoint redness from 0-3 and for the other endpoints from 0 to 4. This means that the score 2 given in the study for cornea opacity and chemosis corresponds to a score between 2 and 3 according to the OECD guideline.

24 to 72 hours after application distinct corneal opacity, Erythema and edema as well as corrosion, suppuration and scar formation was observed.

8 days after application when the study was terminated distinct erythema, edema and corneal opacity, were observed in the exposed animals. One animal showed corrosion the other animal showed white nictitating membranes, partly white conjunctivae, suppuration scar formation and staphyloma.

The other eye was treated with talcum as control. In this control eye only slight erythema was seen 24 hours after application. No signs of irritation were seen 48 and 72 hours after application as well as 8 days after application.

4.4.2.2 Human information

Not available

4.4.2.3 Summary and discussion of eye irritation

See discussion above

4.4.2.4 Comparison with criteria

Since cornea opacity was equal to 3 for both animals tested and since the ocular lesions were still present at the end of the observation time, although only 50 µl Cu-HDO were instilled instead of the recommended 100 µl (OECD 405), there is sufficient evidence for assigning the classification “Eye damage1, H318 - Causes serious eye damage.”

4.4.2.5 Conclusions on classification and labelling

Classification for severe eye damage category 1, H318 is proposed.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS presented one study (prior to OECD TG and GLP) where eye irritation were investigated in two female rabbits (white Vienna (Gaukler)) exposed to a single application of 50 µL Cu-HDO (solid) (A 6.1.4/02).

The following scores were reported:

	Average score 24, 48, 72 hours	Score, 8 days
Corneal opacity	3	3
Iris	Not reported	Not reported
Redness conjunctivae	2	2
Chemosis	3	3

The DS noted that the scores reported were translated by the DS (RMS) from the system used in the study report to the OECD TG 405 scoring system. The scoring in the study ranges from 0-3, but in the OECD guideline the scores for the endpoint redness ranged from 0-3 and for the other endpoints from 0-4. This means that the score 2 given in the study for corneal opacity and chemosis corresponds to a score between 2 and 3 according to the OECD guideline.

At termination of the study distinct erythema, oedema, and corneal opacity were observed in the exposed animals. One animal showed corrosion while the other showed white nictitating membranes, partly white conjunctivae, suppuration, scar formation and staphyloma. At this time point the same scores for these effects were reported. The iris was not evaluated separately in this study. It is unknown why the study was terminated after 8 days, i.e. if it was due to the corrosion observed after 8 days.

Based on a corneal opacity of 3 for both animals and that the effects persisted until the end of the observation period, DS proposed to classify Cu-HDO for severe eye damage Category 1.

Comments received during public consultation

One MSCA supported the proposed classification, however requested some clarifications on the test substance.

Assessment and comparison with the classification criteria

Eye irritation was investigated in a study (prior to OECD TG and GLP) in two female rabbits (white Vienna (Gaukler)) exposed to a single application of 50 µL Cu-HDO (solid) (A 6.1.4/02).

The DS noted that the eyes were not rinsed after 24 hours as required in the current OECD TG 405. This could have influenced the result since solids could have caused mechanical damage which potentially could have been less severe if rinsing had been performed after 24 hours. On the other hand it should also be noted that the tested dose was 50 µL, while the OECD TG 405 indicates that when testing solids, pastes, and particulate substances, the amount used should have a volume of 0.1 mL or a weight of not more than 100 mg.

According to Table 3.3.1 of the CLP Regulation classification criteria for irreversible eye effects are as follows:

A substance is considered to cause irreversible effects on the eye (Category 1) if, when applied to the eye of an animal, it produces:

- at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or

- at least in 2 of 3 tested animals, a positive response of: corneal opacity ≥ 3 and/or iritis > 1.5 (calculated as the mean score following grading at 24, 48, 72 hours after installation of the test material).

On the basis of corneal opacity with a mean score of 3 which persisted until the termination of the study at day 8 after application, RAC agrees with the DS that a classification for **Eye Dam. 1; H318** is warranted.

4.4.3 Respiratory tract irritation

Not specific information available.

4.5 Corrosivity

See chapter 4.4.

4.6 Sensitisation

4.6.1 Skin sensititsation

4.6.1.1 Non-human information

Table 16: Summary table of relevant skin sensitisation studies

Species	Method	Number of animals sensitised / total number of animals	Result	Reference
Guinea pig Strain: Pirbright White, Dunkin Hartley HOE DHPK [SPF-LAC] BÖ	Guinea pig maximisation test OECD guideline 406 or B.6 GLP	0/10	**Cu-HDO does not have a sensitising effect on the skin of the guinea pig**	**Study A 6.1.5;** **Document IIIA 6.1.5**

In a Guinea Pig Maximisation test where relatively high dermal induction/challenge concentrations (50%/25%) were used, Cu-HDO did not show sensitising properties: With intradermal induction well-defined erythema and slight oedema were observed at the site of injection in control animals treated with Freund´s adjuvant in 0.9% aqueous solution – whereas necrotic skin changes were observed in animals treated with 1% Cu-HDO with and without Freud´s adjuvant. At topical induction well -defined erythema and slight oedema were observed in the control animals treated with vehicle only and necrotic skin lesions and slight oedema were observed in animals treated with 50%. From the relatively strong effect at the negative control sites with topical induction it may be assumed that residual skin damage from intradermal application was still present (which also limits the interpretation of these data with regard to the skin irritation endpoint). However no skin reactions were observed following challenge with a 25% solution of Cu-HDO in 0.5% aqueous Tylose at 24 and 48 hours after patch removal. The positive control was clearly positive (1-chlor-2.4-dinitro-benzol by 1% in ethanol).

4.6.1.2 Human information

Not available

4.6.1.3 Summary and discussion of skin sensitisation

See discussion above

4.6.1.4 Comparison with criteria

No skin reactions were observed in any of the 10 animals after epidermal challenge, therefore the classification criteria (at least 30% positive animals with GPMT) are not met.

4.6.1.5 Conclusions on classification and labelling

No classification necessary.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The skin sensitisation potential of Cu-HDO was investigated in a standard GLP and guideline compliant (OECD TG 406) guinea pig maximisation test using 10 animals in the test group (strain; Pirbright White, Dunkin Hartley HOE DHPK [SPF-LAC] BÖ). Intradermal induction showed well defined erythema and slight oedema at the site of injection in control animals treated with Freund's adjuvant in 0.9% aqueous NaCl solution. In animals treated with 1% Cu-HDO with and without Freund's adjuvant, necrotic skin changes were observed. At topical induction, erythema and slight oedema were observed in the control animals treated with vehicle only and necrotic skin changes and slight oedema were seen in animals treated with 50% Cu-HDO in 0.5% Tylose CB 30000 in 0.9% aqueous NaCl solution. No skin reactions were observed following challenge with 25% Cu-HDO in 0.5% Tylose CB 30000 in 0.9% aqueous NaCl solution at 24 and 48 hours after patch removal (A 6.1.5).

The DS did not propose classification for skin sensitisation.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Cu-HDO showed no potential for skin sensitisation in any animal in the guinea pig maximisation test (OECD TG 406, GLP). There were no human data available to evaluate for the potential for skin sensitisation. RAC agrees with the DS's conclusion that **no classification is warranted for skin sensitisation**.

4.6.2 Respiratory sensitisation

Not information available.

4.7 Repeated dose toxicity

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

All repeated dose toxicity studies submitted were considered to be key studies: A 28 day feeding study with rats, a 3 months feeding study with rats, a 3 months feeding study with dogs and a 12 months feeding study with rats.

Table 17.1: Summary table of relevant repeated dose toxicity studies

Route	**duration study**	**Species Strain Sex no/group**	**Dose [mg/ day]**	**Results**	**NOAEL** [mg/kg bw day]	**Reference**
oral, feeding	about 28 days	Wistar rats. 5 males and 5 females per group	0, 13, 44, 131 (m); 0, 5, 49, 146 (f)	~139 mg/kg bw day: iron pigment deposition (m+f) and goblet cell hyperplasia within intestine (m+f) interpreted as irritation of the mucosa of the intestine No other adverse effects in any other dose group, but histopathology was restricted to stomach, duodenum, jejenum, ileum, cecum, colon, and rectum; organ weights did not include adrenals, testes, epididymis, thymus, spleen, heart. Therefore conclusions as to the overall toxicological profile of Cu-HDO cannot be drawn.	47	**Study A6.3.1** **Doc IIIA 6.3.1.** **GLP**
oral, feeding	about 96 days	Wistar rats; 10 m +10 f per group	35, 139, 275 (m); 41, 167, 322 (f)	~299 mg/kg bw day: ↑alanine-aminotransferase & aspartate-aminotransferase & cholesterol in the serum (m); ↓ triglycerides in the serum (m); ↑ granulated casts in the urine sediment (m); ↓alkaline phosphatase & globulins in the serum (f); minimal to slight hepatic single cell necrosis (10m); swelling and pigmentation of Kupffer's cells (8f, 10m); slight ↓in hepatocellular lipid content (m); minimal and slight bile duct hyperplasia (2m); hyaline droplets in the proximal tubular epithelial cells and protein precipitates in the renal tubular lumina (10m, 8f); minimal to slight diffuse hyperkeratosis in the forestomach; iron-positive pigment in the tunica propria of the small intestine ~153 mg/kg bw day: minimal hepatic single cell necrosis (3m) and swelling and pigmentation of Kupffer's cells (6m, 3f); hyaline droplets in the proximal tubular epithelial cells (5m) and protein precipitates in the renal tubular lumina (10m); minimal diffuse hyperkeratosis in the forestomach; iron-positive pigment in the tunica propria of the small intestine ~38 mg/kg bw day: no substance-induced changes	38	**Study A 6.4.1/01; Doc IIIA 6.4.1./01; GLP**

Route	**duration study**	**Species Strain Sex no/group**	**Dose [mg/ day]**	**Results**	**NOAEL** [mg/kg bw day]	**Reference**
oral, feeding	about 96 days	Beagle dogs 5 m + 5 f per test group	8.3; 25.2; 64.6 (m); 9.3; 27.4; 71.9 (f)	~68 mg/kg bw day Vomiting mainly in the first week of administration; reduced food consumption (m~22%, f~26%); marked impairment of food efficiency (especially m); ↓ body weight (m~12%, f~5 %); ↑alanine aminotransferase,↑a spartate aminotrans-ferase, ↑potassium; ↑prothrombin time (m); ↓calcium, ↓total protein, ↓albumin, ↓globulins; ↓cholesterol in both sexes; ↓glucose (f); ↓mean absolute and relative liver weights (m); gross lesions in the liver (4 m+3f) indicative for liver cell damage represented by foci, necrosis and/or capsular retractions; chronic hepatitis (all dogs); liver cirrhosis in (5 m+3f); copper pigment storage in hepatocytes and Kupffer cells (all dogs); edema in the gall bladder wall (2 m+4f); edema in the pancreas and in the mesentery (2 m); minimal hyperplasia in the mucosa of the esophagus (3 m+1f); lymphoid depletion in the thymus (3 m) 8-27 mg/kg bw day No substance-induced changes	26	**Study A 6.4.1/02; Doc IIIA 6.4.1/02; GLP**
Oral, feeding	about 12 months	Wistar rats. 20 males and 20 females per group.	0, 6, 18, 61, 183	6 and 18 mg/kg bw day: no effects 61 mg/kg day: Thickening of the forestomach wall (m+f); Hyperkeratosis of the forestomach mucosa (f); Hyperplasia of glandular stomach mucosa (f); Swollen and pigmented Kupffer's cells in the liver (11/20m, 4/20f) 183 mg/kg bw day: ↑total bilirubin; ↑white blood cells, lymphocytes, alanine aminotransferase, aspartate aminotransferase and cholesterol (m); ↑squamous epithelial cells in the urine sediment (f); ↑relative and absolute kidney weights (m); ↑relative liver weight(f); thickening of the forestomach wall; hyperkeratosis and hyperplasia of the forestomach mucosa and edema in the submucosa; hyperplasia of the glandular stomach mucosa; hyperplasia of the duodenal mucosa; swollen and pigmented Kupffer's cells in the liver (19/20m + 14/20f) and single cell necrosis (m); hyaline (fluorescent) droplets in the renal proximal tubules (m) and proteinaceous casts in the tubular lumina (m)	18	**STUDY A6.5; DOC IIIA 6.5. GLP**

The aim of the 28 day feeding study with rats was the clarification of the mechanistic action of Cu-HDO on the digestive tract and the detection of possible neurotoxic effects using a functional observational battery

which included various parameters of sensory and motor functions. This investigation indicates that Cu-HDO is irritating to the mucosa of the intestine, which is in line with the observation of its severe eye damaging property. Also within the subchronic studies and the chronic study the GI tract was identified as the main target organ besides the liver. The neurofunctional observations were without adverse findings as was the histological analysis of brain and nerves in the subchronic and chronic studies.

As described in the table above the subchronic toxicity studies with Cu-HDO carried out in the rat and in the dog indicate the same target organs for both species, that is the GI tract and the liver, though in the dogs the liver effects were stronger including gross lesions, hepatitis and cirrhosis and as sequelae additionally edema in the gall bladder (2 m, 4 f) and in the pancreas and mesentery lymph nodes (2 m). Vomiting was found only in dogs (m+f) mainly in the first week of administration, but this of course cannot be found in rats for physiological reasons. Thus no additional target organs were found in the dog. The NOAELs of the dog and rat subchronic study are similar with 26 and 38 mg/kg bw day respectively. Thus from the data submitted no concern is evident about interspecies differences between rat and dog.

The chronic toxicity study in rats carried out with Cu-HDO resulted in a NOAEL of 18 mg/kg bw day based on histological effects in the forestomach, stomach and Kupffer`s-cells in the liver at 61 mg/kg bw day. In the higher doses besides GI tract and liver also the kidneys were identified as target organs.

Waiving of the chronic toxicity study with a second species was accepted based on the arguments that 1) the NOAELs from the rat and dog 3 months studies are similar and no additional toxicological targets are identified in the dog, supporting that a priori interspecies differences with 24 months studies are not expected, 2) the NOAELs from the rat 12 months studies is just slightly lower compared to the NOAEL from the 3 months study, that is 18 compared to 38 mg/kg bw/day, also the target organs liver, GI and kidney are similar, supporting that quantitative or qualitative differences between sub-chronic and chronic NOAELs are not expected, and 3) because Cu-HDO is applied only in industrial fully automatic processes which limits the potential for exposure.

4.7.1.2 Repeated dose toxicity: inhalation

Not available

4.7.1.3 Repeated dose toxicity: dermal

Not available

4.7.1.4 Repeated dose toxicity: other routes

Not available

4.7.1.5 Human information

Not available

4.7.1.6 Other relevant information

Not available

4.7.1.7 Summary and discussion of repeated dose toxicity

See discussion above

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

See below in chapter 4.8.2.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Table 17.2

Studies relevant for STOT RE classification	**STOT Guidance values**	**NOAEL to LOAELs range [**mg/kg bw day]	**Effects at LOAEL**
96 day, oral feeding in dog	STOT RE 2: 90 day oral rat: 10 -100 mg/kg bw day	26 to 68 May be allometrically scaled from dog to rat* and considered to correspond to sub-chronic rat **NOAEL to LOAEL range of 75 to 197 mg/kg bw day** i.e. corresponding "real" sub-chronic rat LOAEL may be below 100 mg/kg bw day	**esophagus, liver, kidney**: Vomiting mainly in the first week of administration; reduced food consumption (m~22%, f~26%); marked impairment of food efficiency (especially m); ↓ body weight (m~12%, f~5 %); ↑alanine aminotransferase,↑a spartate aminotrans- ferase, ↑potassium; ↑prothrombin time (m); ↓calcium, ↓total protein, ↓albumin, ↓globulins; ↓cholesterol in both sexes; ↓glucose (f); ↓mean absolute and relative liver weights (m); gross lesions in the liver (4 m+3f) indicative for liver cell damage represented by foci, necrosis and/or capsular retractions; chronic hepatitis (all dogs); liver cirrhosis in (5 m+3f); copper pigment storage in hepatocytes and Kupffer cells (all dogs); edema in the gall bladder wall (2 m+4f); edema in the pancreas and in the mesentery (2 m); minimal hyperplasia in the mucosa of the esophagus (3 m+1f); lymphoid depletion in the thymus (3 m)
28 day, oral feeding in rat	STOT RE 2: 90 day oral rat: 10-100 mg/kg bw day	46 to 139 May be considered to correspond to sub-chronic **NOAEL to LOAEL range[+] of 15 to 46 mg/kg bw day** i.e. corresponding sub-chronic LOAEL is below 100 mg/kg bw day	**Intestine**: iron pigment deposition (m+f) and goblet cell hyperplasia within intestine (m+f) interpreted as irritation of the mucosa of the intestine
96 day, oral feeding in rat	STOT RE 2: 90 day oral rat: 10-100 mg/kg bw day	38 to 153 i.e. "real" LOAEL may be below 100 mg/kg bw day	**liver, kidney, forestomach, small intestine**: minimal hepatic single cell necrosis (3m) and swelling and pigmentation of Kupffer's cells (6m, 3f); hyaline droplets in the proximal tubular epithelial cells (5m) and protein precipitates in the renal tubular lumina (10m); minimal diffuse hyperkeratosis in the forestomach; iron-positive pigment in the tunica propria of the small intestine
12 months, oral feeding in rat	STOT RE 2:	18 to 61	**forestomach, glandular stomach, liver**: Thickening of the forestomach wall (m+f); Hyperkeratosis of the

	90 day oral rat: 10-100 mg/kg bw day	May be considered to correspond to sub-chronic **NOAEL to LOAEL range of 36 to 120 mg/kg bw day**[#] i.e. corresponding "real" sub-chronic LOAEL may be below 100 mg/kg bw day	forestomach mucosa (f); Hyperplasia of glandular stomach mucosa (f); Swollen and pigmented Kupffer's cells in the liver (11/20m, 4/20f)
24 months, oral feeding in rat	STOT RE 2: 90 day oral rat: 10-100 mg/kg bw day	6 to 33 May be considered to correspond to sub-chronic **NOAEL to LOAEL range of 12 to 66**[#]; i.e. corresponding sub-chronic LOAEL is below 100 mg/kg bw day	**Forestomach:** slight ↑ of graded severity of cellular hyperplasia of the forestomach's epithelium (11/50m vs. control 2/50); ↑ number of males with hyperkeratosis of the forestomach's wall (40/50m vs. control 20/50)

*see REACH guidance chapter R.8.4.3.1: Interspecies kinetic factor = (bw dog/bw rat) /(bw dog/bw rat) $^{0.75}$= (18/0.25)/(18/0.25) $^{0.75}$= 2.9

+: factor 3, see CLP Annex I, paragraph 3.9.2.9.6

factor 2, REACH guidance chapter R.8.4.3.1, table R 8-5, factor 2 from sub-chronic to chronic; CLP Annex I, paragraph refers to Haber´s rule (which would indicate a factor of 8), however the geometric mean values of data based exposure time extrapolation factors are closer to the REACH recommendation of factor 2 than the Haber´s rule (for a summary see e.g. Paparella et al. 2013 ALTEX 30, p 131f, table 1). CLP Regulation recommends to take a total weight of evidence approach (Annex I, paragraph 1.1.1.).

The observed effects at the LOAELs are indicated in the table 17.2 above and effects at dose levels above the LOAELs are listed in the tables in chapters 4.7.1 and 4.10.1.Especially the effects in the sub-chronic dog study were toxicologically severe as chronic hepatitis, liver cirrhosis and edema in gall bladder wall. Also the effects in the 28 day and 96 day rat studies are toxicologically significant and appear aggravated in the 12 and 24 months rat studies, mainly as hyperkeratosis and hyperplasia in the GI. In any case the effects observed at the LOAELs were sufficiently significant for the derivation of limit values for risk assessment. It is the dossiers submitters' view that the criterion of representing a relevant point of departure for limit value derivation provides a robust and defensible degree of toxicological significance and should thus also be used for classification purposes and this is in line with the concept for the need of "significant" effects outlined in CLP Annex I, paragraph 3.9.2.1.7.3. and 3.9.2.9.2.

The following discussion includes not just the LOAEL values but the NOAEL to LOAEL ranges, since the "real" LOAEL may be located between the NOAEL and the LOAEL, or in other words with repeating the study with a different dose spacing the LOAEL may vary considerably and by this be located below the STOT guidance value. The LOAEL of the 96 day dog study (68 mg/kg bw/day) is below the STOT RE 2 guidance value of 100 mg/kg bw and also after allometric scaling of the dog doses to the corresponding rat doses the NOAEL to LOAEL range of the 90 day dog study (factor 2.9 leading to a range of 75 to 197 mg/kg bw/day, see footnote* to table above) still includes the STOT RE guidance value of 100 mg/kg bw/day (recommended in CLP Annex I, table 3.9.2. for rats). Furthermore scaling the LOAEL of the 28 day rat study to 90 day duration (factor 3, CLP Annex I, paragraph 3.9.2.9.6) leads to a LOAEL below 100 mg/kg bw/day. Moreover the NOAEL to LOAEL range of the 96 day rat study (38 to 153 mg/kg bw day) includes the STOT RE 2 guidance value of 100 mg/kg bw/day. The NOAEL to LOAEL ranges of the 12 and 24 months rat may be corrected to a sub-chronic estimate (factor 2, see footnote[#] to table above; 36 to 120 mg/kg bw day for 12 months study, 12 to 66 mg/kg bw/day for 24 months study) leading to a NOAEL to LOAEL range including or being below the STOT RE guidance value, which is considered as further supportive for classification.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Classification for STOT RE 2 H373 (gastrointestinal tract, liver, kidney) is proposed.

No exposure route is specified, since there is no evidence that the liver and kidney effects would not appear with respiratory or dermal exposure.

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

Summary of the Dossier Submitter's proposal

For the assessment of STOT RE the DS included five experimental animal studies, four in rats and one in dogs. No human data was available. The rat studies were a 28-day, a 90-day, a 1-year and a 2-year study in Wistar rats performed in accordance with OECD test guidelines and GLP. The dog study was a 90-day study performed according to Directive 87/302/EEC, part B and GLP.

The DS considered the effects reported in the 90-day dog study to be toxicologically severe. In this study chronic hepatitis, liver cirrhosis and oedema in the gall bladder wall were reported at 68 mg/kg bw/d (A 6.4.1/02). Also the effects reported in the 28 day rat study at 139 mg/kg bw/d (A 6.3.1) and in the 90-day rat studies at 153 mg/kg bw/d (A 6.4.1/01) were considered toxicologically significant. The effects (mainly hyperkeratosis and hyperplasia in the GI) appeared to have been of greather severity in the rat 12 and 24 month studies from 61 mg/kg bw/d and 33 mg/kg bw/d, respectively (A 6.5.1/A 6.7.1) (see the table below).

Further, the DS argued that in addition to the LOAEL values, the NOAEL to LOAEL ranges should also be considered, since the "real" LOAEL may be located between the NOAEL and the LOAEL. This is because by repeating the study with a different dose spacing considerable differences in the LOAEL values may be obtained, including values below the STOT RE guidance values (GV). The LOAEL of the 90 day dog study (68 mg/kg bw/d) is below the STOT RE 2 GV of 100 mg/kg bw/d and justify classification as STOT RE 2. Furthermore, the LOAEL of the 28 day rat study at 139 mg/kg bw/d was below the STOT RE 2 GV for a 28-day study (300 mg/kg bw/d).

Moreover, the DS considered that the NOAEL to LOAEL range of the 90-day rat study (38 to 153 mg/kg bw/d) included the STOT RE 2 GV of 100 mg/kg bw/d due to the considerations described above. The NOAEL to LOAEL ranges of the 12 and 24 month rat studies may be corrected to a sub-chronic (90-day study) estimate (factor 2, see footnote* to table the table below; 36 to 120 mg/kg bw/d for 12 months study, 12 to 66 mg/kg bw/d for 24 months study) leading to a NOAEL to LOAEL range including or being below the STOT RE GV for a 90-day study, which is considered to provide further support for classification.

***Table**: Studies included by the DS for the assessment of STOT RE classification*

Study	NOAEL/LOAEL	STOT RE 2 GV	Effects
Rat: 28 day	46/139 mg/kg bw/d	30-300 mg/kg bw/d	Intestine: iron pigmentation, goblet cell hyperplasia.
Rat: 90 day	38/153 mg/kg bw/d i.e. "real" LOAEL may be below 100 mg/kg bw day.	10-100 mg/kg bw/d	Liver: necrosis. Kidney: hyaline droplets in tubular epithelial cells. Protein precipitates in the renal tubular lumina. Forestomach: minimal diffuse hyperkeratosis. Small intestine: iron-positive pigment in tunica propria.
Dog: 90 day	26/68 mg/kg bw/d	10-100 mg/kg bw/d	Liver: Chronic hepatitis and cirrhosis. Gall bladder: oedema in wall. GI tract: minimal hyperplasia in the mucosa of the oesophagus.
Rat: 12 month	18/61 mg/kg bw/d May be considered to correspond to sub-chronic (90-day) NOAEL to LOAEL range of 36 to 120 mg/kg bw/d*; i.e. corresponding sub chronic LOAEL is below 100 mg/kg bw day.	2.5-25 mg/kg bw/d	Forestomach: Thickening of wall, hyperkeratosis of mucosa. Stomach: Hyperplasia of mucosa. Liver: Swollen and pigmented Küpffer's cells.
Rat: 24 month	6/33 mg/kg bw/d May be considered to correspond to sub-chronic NOAEL to LOAEL range of 12 to 66 mg/kg bw/d* i.e. corresponding sub chronic LOAEL is below 100 mg/kg bw day	1.25-12.5 mg/kg bw/d	Forestomach: hyperplasia in epithelium and hyperkeratosis of wall.

*IR-CSA Chapter R 8.4.3.1, Table R8-5: Default assessment factor of 2 for sub-chronic 90-day to chronic.

Based on the effects reported in the liver, kidney and GI tract in the repeated dose toxicity studies described above, the DS proposed classification as STOT RE 2; H373 (liver, kidney and GI tract). No exposure route was specified, since there was no evidence that the liver and kidney would not be affected after inhalation or dermal exposure.

Comments received during public consultation

Comments were received from two MSCAs. One MSCA supported the classification proposed by the DS based on the liver effects seen in the dog study and considering the gap between the NOAEL and LOAEL in the rat studies, the MSCA was also in favour of a STOT RE 2 classification for effects in the GI tract and kidney. The other MSCA supported

the DS proposal but considered that the oedema seen in the pancreas in the 90 day dog study should also be taken into account leading to a classification as STOT RE 2 (liver, kidney, GI tract and pancreas).

Assessment and comparison with the classification criteria

For the assessment of STOT RE the DS included five experimental animal studies, four in rats and one in dogs. No human data were available. The rat studies were a 28-day, a 90-day, and a 1-year and 2-year study in Wistar rats with oral exposure to Cu-HDO performed in accordance with OECD TGs and GLP. The dog study was a 90-day oral study performed according to Directive 87/302/EEC, part B and GLP. In all these studies clinical findings included a discoloration of the faeces which was considered to represent a chemical reaction of the test substance in the digestive tract rather than being related to toxicity.

In the rat oral 28-day study, Wistar rats (5/sex/group), no mortality was reported. No effects were reported on body weight gain, food consumption, organ weights as well as blood and urine analysis. In the gross- and histopathological examination discoloration of the digestive tract, iron pigment deposition and goblet cell hyperplasia within the intestine was reported in both males and females. The effects reported in the intestine were interpreted in the study report as being related to a weakly irritating effect of Cu-HDO of the mucosa of the intestine. No effects were reported in the low and mid dose group. However, it was evident from the Competent Authority Report that a very limited number of parameters were investigated in the 28-day study (e.g. the liver and kidney was not submitted to gross- and histopathological examination), therefore no clear conclusion regarding the toxicity of Cu-HDO can be drawn from this study.

In the rat oral 90-day study, Wistar rats (10/sex/group), no mortality and no effects were reported on body weight gain and food consumption and organ weights. In the gross- and histopathological examination effects were reported in the liver, kidney, forestomach and small intestine in the mid and high dose group. These were evident in the high dose group as minimal to slight hepatic single cell necrosis (10 males) and swelling and pigmentation of Küpffer's cells (6 males and 3 females). In the kidney, hyaline droplets in the proximal tubular epithelial cells (10 males and 7 females) and protein precipitates in the renal tubular lumina (10 males and 8 females) were reported. In the forestomach, minimal diffuse hyperkeratosis was seen (9 males and 7 females), and in the small intestine there was iron positive pigment in the tunica propria (7 males and 5 females). In the mid dose group minimal hepatic single cell necrosis (3 males) and swelling and pigmentation of Küpffer's cells was reported in both sexes (incidences (6) only given for males). In the kidney, hyaline droplets in the proximal tubular epithelial cells and protein precipitates in the renal tubular lumina were seen in males only. In the forestomach, minimal diffuse hyperkeratosis as well as iron-positive pigment in the tunica propria of the small intestine were reported in both sexes, without any incidences given. In the low does group no substance induced changes were observed. The group exposed to $CuSO_4$ showed substance-induced changes in the forestomach, liver and kidneys which were similar to those observed in the high dose Cu-HDO group with the exception of iron positive pigment in the tunica propria of the small intestine that was not seen in the $CuSO_4$ exposed group. These results indicated that the effects observed in forestomach, liver and kidneys might be caused by the Cu^{2+} ion.

RAC considers that the effects reported in the liver, kidney and forestomach at 140/167 and 275/322 mg/kg bw/d in the 90-day study were above the GV for a STOT RE 2 classification (10-100 mg/kg bw/d).

In the rat oral 1-year study, Wistar rats (20/sex/group), two male rats in the control group and two female rats in the mid dose group died intercurrently, and it was not considered treatment related. No effects on body weight gain and food consumption were reported that were considered related to treatment. Organ weight changes were only reported in the high dose group. These were evident as a statistically significant increase in kidney weight in males and liver weight in females. Gross examination revealed in the high dose group thickening of forestomach wall in 20/20 males and in 16/20 females. Histopathological examination showed in the high dose group effects in the GI tract, liver and kidney in males and females. The effects were evident as hyperkeratosis, hyperplasia of forestomach mucosa and oedema in the submucosa as well as hyperplasia of the glandular stomach mucosa in males and females. Further, hyperplasia of the duodenal mucosa and swollen and pigmented Küpffer's cells in the liver were seen in males (11/20) and females (4/20), as well as single cell necrosis in males. In the kidney in males, hyaline (fluorescent) droplets in the renal proximal tubules and proteinaceous casts in the tubular lumina were reported. In the mid dose group, hyperkeratosis of forestomach mucosa and hyperplasia of glandular stomach mucosa were reported in females as well as swollen and pigmented Küpffer's cells in the liver of males and females. No effects were reported in the low-dose group. When comparing the group exposed to $CuSO_4$ with the high dose group exposed to Cu-HDO some effects on the digestive tract, the liver and the kidneys were reported in both groups and therefore could be related to the Cu^{2+} ion. However, some effects were only reported in the high-dose Cu-HDO group. These included: hyaline droplets in renal proximal tubules and proteinaceous casts in tubular lumina (males), increase in relative liver weight (females), forestomach mucosa and oedema in the submucosa (male and female), storage of iron-containing pigment in the macrophages of the duodenum (male and females), increased incidences of cysts in the liver (female), as well as increase in white blood cells and leucocytes.

RAC considers that the effects reported in the liver, kidney and GI tract at 54/67 and 161/205 mg/kg bw/d (mid and high doses, respectively) in the 1-year study were above the GV for a STOT RE 2 classification (2.5-25.0 mg/kg bw/d).

In the rat oral 2-year study, Wistar rats (50/sex/group), non-neoplastic lesions were reported in the GI tract and liver in males and females. These were evident in the high dose group in the forestomach as, e.g. hyperplasia of epithelium, hyperkeratosis and submucosal oedema (39/50 in males and 33/50 females), in the duodenum as storage of an iron-containing pigment in the macrophages (16/50 males and 19/50 females), and in the liver as, e.g. centrolubular liver cell vacuolation (26/50 males), single liver cell necrosis (11/50 females), copper storage in Küpffer's cells and in hepatocytes and increased incidence of hepatic cysts (23/50 females). In the mid dose group, some of the above mentioned findings in the forestomach were seen. These findings were comparable to the findings in the group exposed to $CuSO_4$ with the exception of hepatic cysts in the liver that was not seen in female rats exposed to $CuSO_4$, and storage of iron-containing pigment in the macrophages of the duodenum that was not seen in male and female rats exposed to $CuSO_4$. These effects were also reported in the 1-year study in Wistar rats exposed to 3000 ppm Cu-HDO (high dose). These two effects were considered attributed to exposure to Cu-HDO and not to $CuSO_4$.

RAC considers that the effects reported in the liver and GI tract at 29/37 and 148/189 mg/kg bw/d in the 2-year study were above the GV for a STOT RE 2 classification (1.25-12.5 mg/kg bw/d).

In the dog oral 90-day study, Beagle dogs (5/sex/group) were exposed to 0, 300, 900, 2700 ppm Cu-HDO in the diet corresponding to approximately 0, 9, 26 and 69 mg/kg bw/d. This study did not include a group exposed to $CuSO_4$ corresponding to the same amount of Cu^{2+} ions as in the high dose group exposed to Cu-HDO. Therefore, it is not possible to assess whether the effects reported in the dogs were related to the exposure to Cu^{2+} or the HDO^- anion. Results: No mortality was reported. In the high-dose group clinical signs were evident as vomiting in both sexes mainly in the first week of dosing. Lower body weight relative to control were reported (males approx. 12% and females approx. 5%). Food consumption was reduced (males approx. 22% and females approx. 26%). These effects are considered to be substance related. Blood analysis revealed in the high dose group an increase in alanine aminotransferase, aspartate aminotransferase and potassium in both sexes. Further, prolonged prothrombin time in the males, decrease in calcium, total protein, albumin, globulins and cholesterol in both sexes as well as a decrease in glucose were seen in the females. No changes in clinical chemistry were reported in the low and mid dose groups. Urinalysis revealed in the high dose group at the end of the study a statistically significantly increased bilirubin level. No further treatment-related changes were seen in the other urinary parameters. Organ weight changes were reported in the liver in the high dose males (absolute and relative weight) and females (relative weight). Macroscopic and histopathological changes were only reported in the high-dose group. These included gross lesions in the liver of 4 male and 3 female dogs, which were indicative of liver cell damage represented by foci, necrosis and/or capsular retractions. Further, chronic hepatitis was seen in all male and female dogs and liver cirrhosis in 5 male and in 3 female dogs. Copper pigment storage was reported in the hepatocytes and Küpffer's cells in all dogs. In the gall bladder wall, oedema in 2 male and 5 female dogs were reported. Oedema was seen in the pancreas and in the mesentery in 2 male dogs. Minimal hyperplasia in the mucosa of the oesophagus was seen in 3 males and 1 female, and lymphoid depletion in the thymus of 3 males.

RAC considers that the effects reported especially in the liver at approximately 69 mg/kg bw/d in the 90-day study in dogs are within the GV for a STOT RE 2 classification (10-100 mg/kg bw/d). RAC considers, in agreement with the DS, that the oedema reported in the pancreas in 2 male dogs is not considered sufficiently severe for a STOT RE classification, and was only reported in 2/5 males and in no females.

In summary: From the four repeated dose toxicity studies in rats and the 90-day study in dogs RAC considers that the dog is a more sensitive species than rats following exposure to Cu-HDO. In the dogs macroscopic and histopathological examinations revealed severe effects in the liver observed as chronic hepatitis, liver cirrhosis and necrosis at approx. 69 mg/kg bw/d that are relevant for a STOT RE classification and are within the GV for a classification in Category 2 (10-100 mg/kg bw/d). Effects in the liver were also supported from the repeated dose toxicity studies in rats, however, these were reported as adverse outside the GV for a STOT RE 2 classification. The DS proposed to include a STOT RE 2 classification for both liver, GI tract and kidney. RAC is however of the opinion that the effects on kidney and GI tract reported in the rat repeated dose toxicity studies were outside the GV for a STOT RE 2 classification.

In conclusion, RAC considers that a **classification as STOT RE 2 (liver)** is justified for Cu-HDO.

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

4.9.1.1 In vitro data

Table 18 Compilation of in vitro genotoxicity studies

Test system Method Guideline	Organism/ strain(s)	Concentrations tested	Result	Reference
Ames test OECD 471; no GLP, 4 instead of 5 strains, positive control was not guideline conform for S9 mix	Salmonella typhimurium TA 1535, TA 100, TA 1537, TA 98	0–5000 µg per plate. Triplicate plating in presence and in absence of S9	No dose-related increases in revertant counts in any of the four strains in presence or in absence of metabolic activation. Bacterial toxicity at ≥ 5 µg per plate without S9 and ≥ 50 with S9.	**Study A6.6.1** **Doc IIIA 6.6.1**
Unscheduled DNA synthesis OECD 482; GLP	Primary rat hepatocytes	0.0003–0.1 µg/ml^{-1} in 5% DMSO. Incubation: 18h.	Cell viability: > 60% Cytotoxicity: ≥ 1 µg/ml^{-1} No increases in the mean number of net nuclear grain counts compared with negative controls.	**Study A6.6.3/01** **Doc IIIA 6.6.3/01**
In vitro gene mutation in mammalian cells OECD 476; GLP **carried out with K-HDO**	L5178Y (TK+/-) mouse lymphoma cells	K-HDO: 312–5000 µg/ml Incubation: 3 and 24h.	K-HDO: no gene mutation; no change of colony size indicating no cytogenetic effects	Study A 6.6.3/02

4.9.1.2 In vivo data

Table 19 Compilation of in vivo genotoxicity studies

Type of test Method/ Guideline	Species Strain Sex no/group	Frequency of application	Sampling times	Dose levels	Results	Reference
Micro-nucleus assay OECD 474; GLP	Mouse NMRI Male/female 6 animals per group	1 gavage application	24, 48 and 72h post-treatment	50, 170 and 500 mg/kg	PCE/NCE ratios at 24 and 72 h sampling time comparable with the negative controls. At 48 h sampling time, decrease in PCE/NCE ratio indicative of cytotoxicity. No significant increase in the number of micronucleated PCEs in treated animals or negative controls at any sampling time No genotoxic activity towards bone marrow erythroblasts in the mouse. Signs of toxicity: reduction in spontaneous activity, eyelid closure and apathy at 500 mg/kg bw; no other signs reported	**Study A6.6.4;** **Document IIIA6.6.4**

4.9.2 Human information

Not available

4.9.3 Other relevant information

Not available

4.9.4 Summary and discussion of mutagenicity

Cu-HDO did not show genotoxic effects in the Ames-test, in the in vitro UDS test and in the in vivo micronucleus test.

The reliability of the Ames-test is considered to be somewhat restricted since 2-aminoanthracene was used as the sole positive control with S9 activation, which is not guideline conform, and one test strain (e.coli WP2 uvrA or WP2 uvrA (pKM101) or *S.typhimurium* TA102) is missing. Approximately 7.5% of the bacterial mutagens identified are detected by *E.coli* WPuvrA but not by the standard set of 4 Salmonella strains (CPMP/IHC/1141/95). However, the test was carried out before the respective revision of the guideline 471.

Yet a fully valid in vitro UDS test with primary rat hepatocytes was carried out with Cu-HDO. The advantage of the in vitro UDS test with primary hepatocytes is that no external metabolising system is necessary, means that metabolism occurs inside the cells which enhances the chance to detect potential genotoxic metabolites that are short living or that do not enter the cell easily. The endpoint of the UDS test (genetic repair) is considered to correlate with mutagenic events. We agree that the negative in vitro UDS test with Cu-HDO further supports the negative genotoxicity test battery.

Furthermore also the in vivo micronucleus test was considered fully valid. A slight cytotoxicity indicated by a slight decrease of the ratio of immature polychromatic to mature normochromatic erythrocytes was observed in the high dose group with the 48 hours sampling time point. This provides some evidence that the Cu-HDO dose reached the bone marrow and thus the absence of micronucleated polychromatic erythrocytes can be considered to be a reliable indicator for the absence of genotoxicity within this test system.

Further evidence for the absence of genotoxicity of the HDO^- anion can be derived from the TK-mouse-lymphoma assay carried out with K-HDO, a substance that dissociates in water into the HDO^- anion and the potassium cation (for read across justification see chapter 4.1.1., especially last paragraph). This assay is considered to be sensitive for mutagenic and clastogenic events (CPMP/IHC/1141/95).

Taking all genotoxicity test results together and considering insufficient evidence for carcinogenicity in the 2 year study with Cu-HDO (see below, chapter 3.7.) there is no indication for a genotoxic potential of Cu-HDO.

This might appear contradicting with the earlier description of the HDO anion as a nitrosamine. Nitrosamines are metabolised to alpha-hydroxynitrosamines which are instable and break down to the alkyldiazohydroxides and further to carbenium compounds. However a nitrosamine-like activation of the HDO^- ion is not likely since the material is a primary (and not secondary) amine and has no α-oxidisable alkyl group linked to the nitrogen, which seem to be essential features of genotoxic nitrosamines (see e.g. Marquardt and Schäfer, 2004[1]). Moreover, mutagenic nitrosamines show positive results in the in vitro mutagenicity and UDS assays, which is not the case for Cu-HDO.

4.9.5 Comparison with criteria

No positive genotoxicity results were observed, so the substance does not meet the criteria for classification.

4.9.6 Conclusions on classification and labelling

No classification necessary.

[1] Marquardt H., Schäfer S. (2004): Lehrbuch der Toxikologie Stuttgart, Wissenschaftliche Verlags-Gesellschaft, ISBN 3-8047-1777-2.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

For the evaluation of germ cell mutagenicity the DS included three *in vitro* studies; one Ames test and one USD test both performed with Cu-HDO. In addition, the DS included one TK–mouse lymphoma assay performed with K-HDO. Further, the DS included one *in vivo* study: a micronucleus assay performed with Cu-HDO.

In vitro studies

The Ames test (OECD TG 471, no GLP) performed with *S. typhimurium* (TA1535, TA100, TA1537, TA98) at concentrations of 1.25-5000 µg (without S9 mix) and 3-5000 µg (with S9 mix) Cu-HDO did not show any dose-related increase in revertant counts in any of the four strains, either with or without metabolic activation. However, there are some limitations to this study since one test strain was missing and 2-aminoanthracene was used as the only positive control with S9 activation (A 6.6.1).

The study of unscheduled DNA synthesis (OECD TG 482, GLP) performed with Cu-HDO (0.0003-0.1 µg/mL in 5% DMSO) on primary rat hepatocytes did not show any increase in the mean number of net nuclear grain counts compared with negative controls (A 6.6.3/01).

In addition, the DS included one study of *in vitro* gene mutation in mammalian cells (OECD TG 476, GLP) performed with K-HDO (312-5000 µg/mL) on mouse lymphoma cells. This study did not show any gene mutations and no change in colony size indicating no cytogenetic effects (A 6.6.3/02).

In vivo studies

One micronucleus assay (OECD TG 474, GLP) was performed with male and female NMRI mice at dose levels of 50, 170 and 500 mg/kg bw. This study did not show any significant increase in the number of micronucleated PCEs in treated animals or negative controls at any sampling time. Slight cytotoxicity was indicated by a slight decrease in the PCE/NCE ratio observed at the 48h sampling point which provided some evidence that the Cu-HDO reached the bone marrow (A 6.6.4).

Cu-HDO did not show genotoxic effects in either the Ames test, USD test or the *in vivo* micronucleus test. Further, no effects were seen in the TK–mouse lymphoma assay performed with K-HDO. Based on these results, the DS is of the opinion that no classification for germ cell mutagenicity is warranted for Cu-HDO.

Comments received during public consultation

Two commenting MSCA supported the DS' proposal for no classification of Cu-HDO for germ cell mutagenicity.

Assessment and comparison with the classification criteria

There were no human data available for Cu-HDO, therefore a classification as Muta. 1A is not justified.

Further, a classification as Muta. 1B or Muta. 2 is not justified since there are no positive results from the *in vivo* micronucleus assay in mice and no positive results from the *in vitro* studies.

Altogether, RAC agrees with the DS that **classification for germ cell mutagenicity is not warranted.**

4.10 Carcinogenicity

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Table 20a Carcinogenicity of purified Cu-HDO

Route	**Species Strain Sex no/group**	average equivalent dose levels [mg/kg bw/day] frequency of application	**Effects observed**	**NOAEL**	**Reference**

Oral, feeding	Wistar rats. 50 males and 50 females per group	ca. 6, 33, 169 of Cu-HDO and 31 of Cu-SO_4 (Cu^{2+} ~equivalent to highest Cu-HDO dose) in diet for 24 months	6 mg/kg bw day: no effects 33 mg/kg bw day: slight ↑ of graded severity of cellular hyperplasia of the forestomach's epithelium (11/50m vs. control 2/50); ↑ number of males with hyperkeratosis of the forestomach's wall (40/50m vs. control 20/50) 169 mg/kg bw day: impairment of body weight (m), resulting in reduced values of about 10% after 24 months. No such effects were seen after administration of $CuSO_4$; impairment of body weight change in males, resulting in reduced values of about 12% after 24 months. No such effects were seen after administration of CuSO4; thickening of the forestomach's mucosa at necropsy in 25/50 males and in 23/50 females, either focal (in the region of the limiting ridge/margo plicatus) or diffusely. Similar effects were seen after administration of $CuSO_4$; ↑ numbers of cysts in the liver in female animals (18/50) at necropsy. This effect was not observed after treatment with $CuSO_4$; slight ↑ of graded severity of cellular hyperplasia of the forestomach's epithelium (m+f). Similar effects were seen after administration of $CuSO_4$; ↑ number of animals affected with hyperkeratosis of the forestomach's wall as well as ↑ graded severity of it (m+f). Similar effects were seen after administration of $CuSO_4$; ↑ incidences of submucosal edema in the forestomach's wall (m 39/50, f 33/50). Similar effects were seen after administration of $CuSO_4$ in males (36/50) but not in females (23/50); storage of an iron-containing pigment in macrophages in the submucosa of the duodenum (m 16/50, f 19/50). This effect was not observed after treatment with $CuSO_4$; centrilobular liver cell vacuolization in males (26/50). Similar effects were seen in principle after administration of $CuSO_4$; single liver cell necrosis in 11/50 female rats. Similar effects were seen in principle after administration of $CuSO_4$; copper storage in Kupffer cells and in hepatocytes (13 f affected with one or the other location of storage or both). Similar effects were seen in principle after administration of $CuSO_4$; Insufficient evidence for carcinogenicity.	Local NOAEL = 600 mg/kg food (~0.06%) Systemic NOAEL = 33 mg/kg bw day	**Study A6.7; Doc IIIA 6.7; GLP**

Table 20b Overview on observed tumours:

Group 0 = control, Group 1= low dose (6 mg/kg bw day Cu-HDO), Group 2 = mid dose (33 mg/kg bw day Cu-HDO), Group 3 = high dose (169 mg/kg bw day Cu-HDO), Group 4 = 31 mg/kg bw day Cu-SO4 (Cu 2+ ~equivalent to highest Cu-HDO dose)

BASF Department of Toxicology
PATHOLOGY REPORT 106
BIS-(N-CYCLOHEXYL-DIAZENIUMDIOXY)-COPPER 70C0679/89113
24-Month Feeding Study in Rats Jan/30/1996 CEGE
acopat system

LIST OF TUMOR BEARING ANIMALS AND SUMMARY OF TUMORS
GROUPS 0-3 - ALL ANIMALS

Sacrifice	F1							
Sex	M				F			
Group	0	1	2	3	0	1	2	3
Animals in selected Group	50	50	50	50	50	50	50	50
Number of Animals with:								
Neoplasms	47	38	44	41	46	44	49	44
1 Primary Neoplasm	17	20	20	18	21	19	23	14
2 and > Primary Neoplasms	30	18	24	23	25	25	26	30
Number of Animals with:								
Benign Neoplasms	43	35	42	38	43	42	45	40
Benign Neoplasms only	35	28	37	28	29	31	35	25
Malignant Neoplasms	12	10	7	13	17	13	14	19
Malignant Neoplasms only	4	3	2	3	3	2	4	4
Systemic Neoplasms	2	2	1	2	.	1	1	3
Metastasized Neoplasms	1	2	2	1	1	2	2	1
Total Number of:								
Primary Neoplasms	96	62	84	79	86	82	88	92
Benign Neoplasms	82	52	77	66	67	69	70	69
Malignant Neoplasms	14	10	7	13	19	13	18	23
Systemic Neoplasms	2	2	1	2	.	1	1	3
Metastasized Neoplasms	1	2	2	1	1	2	3	1

LIST OF TUMOR BEARING ANIMALS AND SUMMARY OF TUMORS
GOUPS 3 AND 4 - ALL ANIMALS

Sacrifice	F1			
Sex	M		F	
Group	3	4	3	4
Animals in selected Group	50	50	50	50
Number of Animals with:				
Neoplasms	41	46	44	44
1 Primary Neoplasm	18	15	14	19
2 and > Primary Neoplasms	23	31	30	25
Number of Animals with:				
Benign Neoplasms	38	42	40	38
Benign Neoplasms only	28	32	25	26
Malignant Neoplasms	13	14	19	18
Malignant Neoplasms only	3	4	4	6
Systemic Neoplasms	2	2	3	.
Metastasized Neoplasms	1	3	1	1
Total Number of:				
Primary Neoplasms	79	96	92	84
Benign Neoplasms	66	79	69	63
Malignant Neoplasms	13	17	23	21
Systemic Neoplasms	2	2	3	.
Metastasized Neoplasms	1	3	1	1

4.10.1.2 Carcinogenicity: inhalation

Not available

4.10.1.3 Carcinogenicity: dermal

Not available

4.10.2 Human information

Not available

4.10.3 Other relevant information

Not available

4.10.4 Summary and discussion of carcinogenicity

One 2 year rat carcinogenicity feeding study is available including control, low, mid and high dose groups with Cu-HDO and a parallel CuSO4 dose group with a Cu dose corresponding to the high dose Cu-HDO. The study report is not explicit on the statistics used for tumour analysis. However in this study a higher incidence of mesenteric lymph nodes hemangioma was observed for the groups 2 and 3 when compared to the control (from control to high dose: male 6-7-12-13, female 1-1-0-4). Mesenteric lymph node hemangiosarcoma was observed only in one female control animal. Mesenteric lymph node lymphangioma was also not increased in males (control to high dose: 4-1-1-1) or females (control to high dose: 0-1-1-1). The combined incidence of all vascular tumours (hemangioma, hemangiosarcoma and lymphangioma) in mesenteric lymph nodes shows a comparable incidence in all male groups (10-8-13-14) as well as in female groups (2-2-1-5). The historical control range for vascular tumours in mesenteric lymph nodes is reported in the study report for males from 0 to 11 animals (22%) and for females from 0 to 2 animals (2%) indicating that in this study controls were at the upper edge of the historical control and mid (males) and top doses (males+females) slightly above. In other organs vascular tumours (hemangioma, hemangiosarcoma and lymphangioma) were not increased with dose at all. The total number of animals with vascular tumours and the total number of vascular tumours (hemangioma, hemangiosarcoma and lymphangioma) in all organs was also comparable between groups (number of animals with vascular tumours, males: 13-9-16-15, females: 4-3-3-6; total number of vascular tumours males: 13-11-18-18, females 4-4-3-6). The same was reported for comparison of group 3 (Cu-HDO) and group 4 (CuSO4): In the mesenteric lymph node hemangioma was comparable (group 3-group 4: males 13-13, females 4-3) as was lymphangioma (males 1-2, females 1-1) as well as total number of animals with vascular tumours (males 15-20, females 6-6) and total number of vascular tumours (males 18-21, females 6-6). For all other organs no increase of animals with specific tumour types is reported in this study.

As outlined in the table above the study report further supports that there is inadequate evidence for a carcinogenic potential: The number of animals with neoplasms, the number of animals with one or more than one primary neoplasm, as well as the number of animals with benign, malignant systemic or metastasized neoplasms, respectively, and the total number of primary neoplasms, comprising benign, malignant, systemic or metastasised primary tumours did not differ biologically from controls. All tumor types noted are commonly found in Wistar rats and no rare tumors gew in particular tissues with an abnormal higher incidence. The total number of rats with tumors and the total number of tumors – benign and malignant- were comparable between the control group and dose groups 3 (top dose Cu-HDO) and 4 (CuSO4) on the one hand and between groups 3 and 4 on the other hand. Within this study also the rest of the toxicity profile appeared similar for the high dose Cu-HDO group and the corresponding CuSO4 group with regard to all observations except that body weight impairment and increased numbers of cysts in the liver in female animals and the storage of

iron-containing pigment in the macrophages of the duodenum were attributable to Cu-HDO only, but not to CuSO4.

The mortality rate was smaller than 34% in all dose groups and the body weight was reduced in high dose female group by 12% and male group by 10% which supports that the maximum dose was adequate. The local NOAEL of 6 mg/kg bw/day and 0.06% (w/w) in food is based on histological effects in the forestomach at 33 mg/kg bw day. With 169 mg/kg bw/day additionally an effect on weight and weight gain in males, further histological forestomach, liver and duodenum effects were observed. Thus the results are in agreement with the results from the chronic study with Cu-HDO indicating the GI tract as primary target organ. The systemic NOAEL is 33 mg/kg bw day.

Waiving of the carcinogenic study with a second species was accepted based on the arguments that the 1) NOAELs from the rat and dog 3 months studies were similar and no additional toxicological targets were identified in the dog, supporting that a priori interspecies differences with 24 months studies are not expected, 2) the systemic NOAELs from the rat 3, 12 and 24 months studies were within the same magnitude, that is 38 compared to 18 and 33 mg/kg bw/day and also the target organs liver, GI and kidney were similar, supporting that quantitative or qualitative differences between sub-chronic and chronic NOAELs are not expected. 3) Furthermore the genotoxicity tests (in vitro bacterial mutation test, in vitro UDS, in vivo micronucleus test) were negative and 4) Cu-HDO is applied only in industrial fully automatic processes which limits the potential for exposure.

4.10.5 Comparison with criteria

No positive genotoxicity was observed in the related specific genotoxicity studies and the vascular tumours observed in the mesenteric lymph node were limited to a benign nature, at a single organ site, in one species, i.e. rat, in a single study. In terms of total mesenteric lymph node vascular tumours, the actual controls were at the upper edge of the historical control range with a mid-dose group (males) and top-dose groups (males + females) slightly exceeding this range. On this basis it is concluded that there is inadequate evidence for carcinogenicity and the substance does not meet the criteria for classification.

4.10.6 Conclusions on classification and labelling

No classification is necessary.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

For the assessment of carcinogenicity, the DS included one 2-year oral carcinogenicity study in Wistar rats (A 6.7.1, 1996). In this study rats (50/sex/group) were exposed to Cu-HDO in the diet at concentrations of 0, 100, 600 and 3000 ppm corresponding, respectively, to 0, 5, 29 and 148 mg/kg bw/d Cu-HDO in males and 0, 6, 33 and 189 mg/kg bw/d Cu-HDO in females. One group was exposed to 67 mg/kg bw/d of $CuSO_4$ corresponding to the same amount of Cu^{2+} as in the highest dose group exposed to Cu-HDO. The mortality rate in the study was less than 34% in all dose groups. Body weight was reduced in the high dose

females by 12% and in high dose males by 10%. For other systemic effects see the STOT RE section. The main concern related to carcinogenicity was an increase in vascular tumours in the mesenteric lymph node and the incidences are shown in the table below. When comparing the incidences in the high dose group exposed to Cu-HDO with the group exposed to $CuSO_4$ (with equal levels of Cu^{2+}) no difference in the incidences of vascular tumours were reported.

***Table**: Incidences of vascular tumours in the mesenteric lymph nodes*

Parameter	HCD	Control 0 mg/kg bw/d	5/6 mg/kg bw/d (m/f)	29/33 mg/kg bw/d (m/f)	148/189 mg/kg bw/d (m/f)	$CuSO_4$: 67 mg/kg bw/d
Lymph node haemangioma		6M/1F (12/2 %)	7M/1F (14/2 %)	12M/0F (24/0%)	13M/4F (26/8 %)	13M/3F (26/6 %)
Lymph node haemangiosarcoma		0M/1F (0/2 %)	0M/0F (0/0 %)	0M/0F (0/0 %)	0M/0F (0/0 %)	
Lymph node lymphangioma		4M/0F (8/0 %)	1M/1F (2/2 %)	1M/1F (2/2 %)	1M/1F (2/2 %)	2M/1F (4/2 %)
Combined incidences	M: 0-11, 20%* F: 0-2, 2%*	10M/2F (20/4 %)	8M/2F (16/4 %)	13M/1F (26/2 %)	14M/5F (28/10 %)	

*Additional HCD for combined vascular tumours provided by DS during public consultation:

- BASF (1983-1993): male 10.44% (range 0-25%) from 1039 rats/25 studies and females 1.84% (range 0-6%) from 1040 rats/25 studies.
- Hannover tumour data base (1985-1990): male 5.3% (range 0-22%) from 320 rats/7 studies and females 0.8% (range 0-4%) from 369 rats/8 studies

It was observed from the data that the combined incidences of all vascular tumours (haemangioma, haemangiosarcoma and lymphangioma) in mesenteric lymph nodes in the control animals was at the upper edge of the HCD range and in the top dose in females above the HCD, however, this was related to an increase in benign haemangioma.

In other organs there were no increase in vascular tumours with increasing dose, see table below:

***Table**: Incidences of vascular tumours in all organs assessed*

Parameter	Control 0 mg/kg bw/d (m/f)	5/6 mg/kg bw/d (m/f)	29/33 mg/kg bw/d (m/f)	148/189 mg/kg bw/d (m/f)	$CuSO_4$: 67 mg/kg bw/d (m/f)
# animals with vascular tumours	13M/4F	9M/3F	16M/3F	15M/6F	20M/6F
# vascular tumours	13M/4F	11M/4F	18M/3F	18M/6F	21M/6F

The DS considered that the incidences of vascular tumours were comparable in all groups including the controls and exposed animals.

The DS also included an overview of the number of all observed tumours in the animals, see table below. When comparing the incidences in the high dose group exposed to Cu-HDO with the group exposed to $CuSO_4$ (with equal levels of Cu^{2+}) no difference in the incidences of neoplasms were reported.

***Table**: An overview of all tumours*

Parameter	**Control 0 mg/kg bw/d (m/f)**	**5/6 mg/kg bw/d (m/f)**	**29/33 mg/kg bw/d (m/f)**	**148/189 mg/kg bw/d (m/f)**	**$CuSO_4$: 67 mg/kg bw/d (m/f)**
# animals	50	50	50	50	50
# rats with: - neoplasms	47M/46F	38M/44F	44M/49F	41M/44F	46M/44F
- 1 primary neoplasm	17M/21F	20M/19F	20M/23F	18M/14F	15M/19F
- 2 and > primary neoplasms	30M/25F	28M/25F	24M/26F	23M/30F	31M/25F
# rats with: - Benign neoplasms	43M/43F	35M/42F	42M/45F	38M/40F	42M/38F
- Benign neoplasms only	35M/29F	28M/31F	37M/35F	28M/25F	32M/26F
- Malignant neoplasms	12M/17F	10M/13F	7M/14F	13M/19F	14M/18F
- Malignant neoplasm only	4M/3F	3M/2F	2M/4F	3M/4F	4M/6F
- Systemic neoplasms	2M/0F	2M/1F	1M/1F	2M/3F	2M/0F
- Metastasized neoplasms	1M/1F	2M/2F	2M/2F	1M/1F	3M/1F
# of: - Primary neoplasms	96M/86F	62M/82F	84M/88F	79M/92F	96M/84F
- Benign neoplasms	82M/67F	52M/69F	77M/70F	66M/69F	79M/63F
- Malignant neoplasms	96M/86F	62M/82F	84M/88F	79M/92F	17M/21F
- Systemic neoplasms	14M/19F	10M/13F	7M/18F	13M/23F	2M/0F
- Metastasized neoplasms	1M/1F	2M/2F	2M/3F	1M/1F	3M/1F

The DS considered that the results support that there is inadequate evidence for carcinogenic potential following exposure to Cu-HDO or $CuSO_4$ in rats. This was based on the argument, that the findings do not differ biologically from the control animals in terms of the following:

1. the number of animals with neoplasms
2. the number of animals with one or more primary neoplasm
3. the number of animals with benign, malignant systemic or metastasized neoplasms
4. the total number of primary neoplasms, comprising benign, malignant, systemic or metastasized primary tumours

The DS also argued that all tumour types reported were commonly seen in Wistar rats and no rare tumours were reported in particular tissues with an abnormal higher incidence. The total number of rats with tumours and the total number of tumours, benign and malignant, were comparable between the control group, the high dose group and the control group and the group exposed to $CuSO_4$, as well as between the high dose group and the group exposed to $CuSO_4$.

The DS concluded that there is inadequate evidence for carcinogenicity following 2-year exposure to Cu-HDO to rats, therefore, the results from the study do not meet the criteria for classification for carcinogenicity.

Comments received during public consultation

Comments were received from three MSCA. One MSCA agreed with the DS proposal for no classification for carcinogenicity. The other MSCA questioned the reliability of the control group since 47/50 males and 46/50 females in the control group developed neoplasms including 24% males and 34% females with malignant neoplasms. The MSCA also found it strange that the HCD for combined vascular tumours in males was 20% and in females 2% and also considered it inappropriate to pool all vascular tumours both in the study and in the HCD together, since the consequences of benign haemangioma and malignant haemangiosarcoma are quite different. Therefore, the MSCA asked for further details regarding the tumour appearance site and number per sex per group before being able to conclude on a classification for carcinogenicity. In response the DS included in the RCOM more data on the HCD for vascular tumours, which were included in the RAC Opinion.

The third MSCA considered that on the basis of the increased incidences of haemangioma in males and females in the high dose group, a classification as Carc. 2 should be considered.

Assessment and comparison with the classification criteria

For the assessment of carcinogenicity the DS included one 2-year oral carcinogenicity study in Wistar rats. In this study 50 rats/sex/group were exposed to Cu-HDO in the diet at concentrations of 0, 100, 600 and 3000 ppm corresponding, respectively, to 0, 5, 29 and 148 mg/kg bw/d Cu-HDO in males and 0, 6, 33 and 189 mg/kg bw/d Cu-HDO in females. One group was exposed to 67 mg/kg bw/d of $CuSO_4$, corresponding to the same amount of Cu^{2+} as in the highest dose group exposed to Cu-HDO. In the study there was some concern for carcinogenicity arising from vascular tumours in the mesenteric lymph nodes. However, RAC supports the DS in their assessment that the combined incidences of all vascular tumours (haemangioma, haemangiosarcoma and lymphangioma) in the mesenteric lymph nodes in the control animals was at the upper edge of the HCD range, and in the top dose in females above the HCD, however, this was related to an increase in benign haemangioma with no progression to malignancy. The incidences of vascular tumours in all organs assessed were comparable in all groups including the controls and exposed animals. RAC therefore considers that the vascular tumours reported in the 2-year rat study do not justify classification for carcinogenicity. However, as the combined incidence of vascular neoplasms in the control group was at the upper edge of the HCD range, there is concern regarding the reliability of the study and the findings should be interpreted with caution.

The DS also assessed all the neoplasms reported in the study including benign and malignant neoplasms as well as systemic and metastasized neoplasms. RAC agrees with the DS that the tumour types reported were commonly seen in Wistar rats. The total number of rats with tumours and the total number of tumours, benign and malignant, were comparable between the control group and the high dose group, the control group and the group exposed to $CuSO_4$, as well as between the high dose group and the group exposed to $CuSO_4$.

RAC is of the opinion that a classification as Carc. 1A is not justified since no human data was available for the assessment of carcinogenicity following exposure to Cu-HDO.

For a classification as Carc. 1B according to the CLP criteria the animal data should demonstrated sufficient evidence of animal carcinogenicity. RAC considers that the 2-year

study in rats did not provide sufficient evidence for carcinogenicity and classification as Carc. 1B is not justified.

For a classification as Carc. 2 according to the CLP criteria the animal data should demonstrated limited evidence of animal carcinogenicity. RAC considers that the 2-year study in rats did not provide limited evidence for carcinogenicity and thereforeclassification as Carc. 2 is not justified.

In conclusion, RAC is of the opinion that **no classification for carcinogenicity** is justified.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

4.11.1.1 Non-human information

So far, no 2-generation study has been undertaken for Cu-HDO.

The applicant provided waiving arguments which were essentially based on the absence of gross- and histopathological effects within the reproductive organs within the repeated dose studies and the absence of developmental effects and the requirement of neglegible exposure. The approach is supported by a probabilistic evaluation of NOAEL subchr./NOAEL2-gen ratios for about 120 substances as well as a probabilistic evaluation of classification triggers for fertility effects in repeated doses studies for more than 70 substances and consideration of product composition as skin corrosive and only industrial intended use.

In specific with regard to regard C&L it was recognized that within the review of Janer et al 2007 (Reproductive Toxicology 24, 103-113), 67% of 30 reproductive toxic substances can be identified as such on the basis of a rat sub-chronic toxicity study. Dent 2007 (Reg.Tox.Pharm 48, 241-258) found that even 93% of 73 reproductive toxic substances showed detectable pathology in the male and in some cases in the female tract within well performed sub-chronic toxicity studies. Furthermore Dent 2007 describes that by taking into consideration also the developmental toxicity studies 96% of the 73 reproductive substances can be identified as such without a 2-gen study. Mangelsdorf et al. 2003 (Reg Toxicol Pharmacol 37: 356-369) quotes an analysis of 32 substances that show adverse effects with regard to male reproduction and for which a complete data set with regard to male reproductive toxicity endpoints was available (reproductive organ histopathology and weights, sperm analysis, mating trial). 30 from these 32 substances showed effects in histopathology and/or organ weight. This is consistent with another analysis cited that indicates that 89% of the considered reproductive toxicants produced histopathological effects in the gonads. These parameters measured after 4 and 9 weeks of exposure were shown to be on average more sensitive than the pregnancy index. (see also BAuA Forschungsbericht Fb 984, 2003).

4.11.1.2 Human information

Not available

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Table 21.1 Summary of developmental toxicity studies with Cu-HDO

Route of exposure	Guideline	Species Strain Sex no/group	Exposure Period	Doses [mg/kg bw day]	Critical effects maternal developmental	NO(A)EL maternal toxicity	NO(A)EL Teratogenicity embryotoxicity	Reference
gavage	OECD guideline 414	Wistar rats Females 20 pregnant animals	day 6 to 15 of gestation	0, 10, 30, 100	No mortality at any dose group. **maternal NOEL: 30 mg/kg bw day** based on slightly and transiently impaired food consumption and marginally impaired body weight gain in top dose dams. **developmental NOAEL>100 mg/kg bw day**, since no treatment related developmental effects following administration of up to 100 mg/kg The maximum applied dose is only slightly below any toxicologically meaningful dose, since the acute LD_{50} is about 380 mg/kg bw.			**Study A6.8.1.1** **Doc IIIA 6.8.1.1; GLP**
gavage	OECD guideline 414	Himalayan rabbits 15 pregnant females	day 7 to 19 of gestation	0, 10, 30, 60	10 mg/kg bw day: no effects on does and fetuses. 30 mg/kg bw day: ↓ food consumption on days 7–20 p.i.[1] (with statistical significance on most of these days); statistically significant ↓ body weight gain (if the weight gain over the total treatment period is calculated; net weight gain not reduced); statistically significantly ↑ numbers of litters with skeletal variations 60 mg/kg bw day: statistically significant ↓ food consumption (day 7–20 p.i.[1]) [only about half of the food-intake of the controls]; body weight loss and/or statistically significantly impaired weight gains during the treatment period (days 7–19 p.i.[1], but net weight gain not reduced); reduced mean gravid uterus weight (only about 76% of the control value); one doe with blood in bedding and another female with no defecation during several treatment days; slightly ↑resorption rate (predominantly early ones) and consequently increased post-implantation loss (31.6%) predominantly due to the fact that 4 females had no viable foetuses at all but only dead implants in uterus; ↓ mean placental and foetal body weights; ↑ occurrence of skeletal variations and 2 skeletal retardations (incomplete ossification of sacral vertebral arch(es) and /or talus **maternal NOAEL: 10 mg/kg bw day** **developmental NOAEL: 10 mg/kg bw day**			**Study A6.8.1.2** **Doc IIIA 6.8.1.2; GLP**

[1]p.i. = post insemination

The developmental toxicity of Cu-HDO has been evaluated in the **rat** and in the **rabbit**.

In the **rat developmental toxicity study** (Study A6.8.1.1, Doc IIIA 6.8.1.1) no developmental and no maternal effects were observed up to the highest applied dose of 100 mg/kg bw day, except for slight and transiently impaired food consumption and marginally impaired weight gain in the top dose dams. This slight maternal effect should not be considered to represent an adverse effect. However 100 mg/kg bw/day is only slightly below any meaningful toxicological dose, since the acute toxic LD50 is 380 mg/kg bw. Therefore the assay is considered to be fully valid. Considering also the results of the dose finding study which showed significantly reduced food intake and significantly reduced maternal weight gain with 50 mg/kg bw the maternal NOAEL could be set to 30 mg/kg bw though this maternal NOAEL cannot be related to the developmental NOAEL generated independently in the final study.

Table 12.2. Maternal effects in the rat developmental toxicity study

Parameter	**control data**		**low dose**	**medium dose**	**high dose**	**dose-response**
	historical	study	**10 mg/kg bw Cu-HDO**	**30 mg/kg bw Cu-HDO**	**100 mg/kg bw Cu-HDO**	**+ / -**
Number of dams examined		30	30	30	30	
Clinical findings during application of test substance						
Mortality of dams *%*		0	3.3*	6.6*	10*	–
Abortions		0	0	0	0	
Body weight gain					↓ days 6-8 p.c (corrected bw gain = 92% of control) ↑ days 8-10 p.c.	+
Food consumption					↓days 6-8 (by 18%)	+
Pregnancies *pregnancy rate or %*	92%	83%	90%	90%	90%	–
Necropsy findings in dams dead before end of test						
Lungs: edema		20%	6.7%	6.7%	6.7%	–
Lungs marginal emphysema		3.3%	0%	0%	0%	–
Particular find. on implants in dams sacr. morib./died interc.		0%	3.3%	6.7%	10%	

*The rats died accidentally on day 7 p.c. (after the second gavaging) due to the unintentional use of a faulty stomach tube

The conception rate varied between 83% (control group) and 90% (all substance treated groups). No substance-related and/or statistically significant differences between the groups in conception rate, in the mean number of corpora lutea and implantation sites or in the values calculated for the pre- and the post-implantation losses, the number of resorptions and viable foetuses. The differences evident are considered to be incidental and within the normal range of deviations for animals of this strain and age

Table 12.3. Litter response (Caesarean section data) in the rat developmental toxicity study

Parameter	**control data**		**low dose**	**medium dose**	**high dose**	**dose-response + / -**
	historical	study	**10mg/kg bw Cu-HDO**	**30mg/kg bw Cu-HDO**	**100mg/kg bw Cu-HDO**	
Corpora lutea *total/number of dams*	6599/420	403/25	442/27	403/27	391/27	–
Implantations *total/number of dams*	5999/420	344/25	393/27	367/27	345/27	–
Resorptions *total/number of dams*	420/248	18/25	25/26	23/25	25/24	
total number of foetuses	5528	326	368	344	320	
pre-implantation loss *[%]*	9.1	14.8	11.8	9.0	13.2	
post-implantation loss *[%]*	7.9	5	6.1	6.0	7.2	
total number of litters	418	25	26	25	24	
foetuses / litter	13.2	13.0	14.2	13.8	13.3	
live foetuses / litter	5528/418	326/25	368/26	344/25	320/24	
dead foetuses / litter	0	0	0	0	0	
foetus weight (mean) *[g]*	3.9	3.8	3.9	3.9	4.0	
placenta weight (mean) *[g]*	0.43	0.45	0.46	0.45	0.45	
crown-rump length (mean) *[mm]*						
Foetal sex ratio *[m/f]*	2759/2769 (1 : 1.003)	164/162 (1 : 0.99)	173/195 (1 : 1.13)	187/157 (1 : 0.84)	174/146 (1 : 0.84)	–

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With the exception of two specific skeletal variations in group 1 (13th rib shortened, sternebrae of irregular shape) there are no statistically significant differences between the control and the substance-treated groups concerning fetal external, soft tissue, skeletal and overall observations. The lower number of group 1 fetuses with shortened 13th rib(s) and the increased number of group 1 fetuses with sternebra (e) of irregular shape (both findings are skeletal variations), are assessed as being of spontaneous nature and not related to the test substance administration. All other findings appeared without a clear dose-response relationship and most of them appeared either in the actual or in the historical control group at a comparable frequency.

Table 12.4 Examination of the foetuses in the rat developmental toxicity study

Parameter	**control data**		**low dose**	**medium dose**	**high dose**
	historical	study			
External malformations *[%]*	0.05	0	0	0.6	0.3
External variations *[%]*	0	0	0	0	0
External unclassified *[%]*		0.3	0	0.3	0
Skeletal malformations *[%]*	3.6	6.5	3.2	5.1	4.3
Skeletal retardations *[%]*	40.5	41	38	48	42
Skeletal variations *[%]*	39.4	36	41	42	33
Soft tissue malformations *[%]*	0.2	0	2.2	1.8	1.9
Soft tissue variations *[%]*	33.6	22	20	17	27

Within the **rabbit developmental toxicity study** (Study A6.8.1.2 Doc IIIA 6.8.1.2) the primary maternal effect seems to be reduced food consumption during the treatment phase. There was a sharp decrease of food consumption at day 7, i.e. the first day of exposure, that increased sharply again at day 21, the first post-exposure period. During the exposure period the daily food consumption decreased to levels between 26% to 69% of control in the high dose and 66% to 82% of control in the mid dose. During the post-treatment period (day 20 to 29), food consumption of the 30 and 60mg/kg groups reached or even exceeded control values. This resulted in a reduced body weight gain in the medium dose group (30 mg/kg bw day), which seems to produce a (not statistically significant) maternal net weight reduction without effects on uterus weight and fetal weight. In contrast in the high dose group (60 mg/kg bw) the drastically reduced food consumption resulted in a body weight loss in terms of (not statistically significant) maternal net-weight reduction. Also a (not statistically significant mean) uterus weight reduction was observed, due to complete resorption in 4 dams (No 47, 53, 56, 54). Individual correlation of complete resorption with drastically reduced food consumption appears for dams 47, 53, 56: Dams No 47 and 53 reduced their daily food consumption to less than 10% of their pre-exposure consumption for period of 6 consecutive days (showed also drastically reduced food consumption over the complete exposure period) and were among the three animals with most severely total day 7 to day 19 reduced food consumption. Dam 56 reduced its daily food consumption to less than 10% of its pre-exposure consumption for 2 consecutive day and also showed drastically reduced food consumption over the compete exposure period. Also the two clinical observations can be related to this: Dam 47 did not show defecation for several treatment days, which can be explained by the drastically reduced food consumption. With dam 53 blood was found in bedding (due to litter loss). Other animals in group 3 showed severely reduced food consumption without litter loss, which indicates individual variability. Dam 54 reduced its food consumption to 35% and 68% of pre-exposure consumption for 2 consecutive days, but it was the

animal of dose group 4 with highest food consumption in the treatment period, thus the complete resorption may also have other reasons. There was also one dam (No 12) in the control group with complete litter resorption.

Parameter	**Group 0** **0 mg/kg bw**	**Group 1** **10 mg/kg bw**	**Group 2** **30 mg/kg bw**	**Group 3** **60 mg/kg bw**
Number of dams examined	15	15	15	15
Clinical findings during application of test substance				1 dam: No defecation on days 10 –13 p.i. (1 animal) 1 dam: Blood in bedding during days 14 – 19 p.i.
Mortality of dams *%*	0	0	0	0
Abortions	0	0	0	0
Body weight gain Mean (SD) d 0-7	45.3 (29.63)	24.6 (53.99)	19.9 (58.17)	36.1 (62.86)
Body weight gain Mean (SD) d 7-19	87.7 (45.35)	44.3 (45.07)	25.9* (52.49)	-82.5** (101.25)
Body weight gain Mean (SD) d 19-29	173.3 (73.41)	147.8 (67.88)	188.7 (73.45)	181.5 (59.71)
Body weight gain Mean (SD) d 0-29	306.3 (112.56)	216.7 (69.80)	234.5 (103.48)	135.1** (147.87)
Gravid uterus Mean (SD)	313.1 (141.32)	298.6 (88.61)	317.0 (93.53)	236.7[1] (158.97)
Carcass (terminal bw – uterus weight) Mean (SD)	2504.09 (191.76)	2444.4 (174.78)	2435.0 (173.57)	2463.3 (196.61)
Net weight change from day 7 (carcass weight – d7 bw) Mean (SD)	-52.1 (91.10)	-106.5 (82.03)	-102.3 (64.7)	-137.7 (142.07)
Food consumption			Significantly reduced on days 7 to 13 and 15 to 20 (between 67% and 84% of control)	Significantly reduced on days 7 to 20 (between 24% and 71% of control)
Pregnancies *pregnancy rate or %*	100%	100%	100%	100%
Necropsy findings in dams dead before end of test	—	—	—	—

[1] due to high standard deviation not significantly reduced;
p.i. = post insemination

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON BIS(N-HYDROXY-N-NITROSOCYCLOHEXYLAMINATO-O,O')COPPER; BIS(N-CYCLOHEXYL-DIAZENIUM-DIOXY)-COPPER; [CU-HDO]

A conception rate of 100% was reached in all groups.

Concerning test groups 1 and 2, there were no substance-related and/or statistically significant differences in conception rate, in the mean number of corpora lutea and implantation sites or in the values calculated for the pre- and the post-implementation losses, the number of resorptions and viable foetuses. The differences evinced are considered to be incidental and within the normal range of deviations for animals of this strain and age. One low dose foetus was already dead when the uterus and the foetal membranes were opened.

As discussed above, in test group 3, the mean resorption rate was increased, due to the fact, that 4 out of 15 pregnant does of this group had no viable foetuses at all but only (predominantly early) resorptions. (As a consequence, the post-implantation loss of the 60mg/kg group was increased (31.6%) to a level outside the historical control range, i.e. 3.0% - 23.1%). However the mean number of live foetuses/dam, was not reduced in the remaining 11 high dose females.

Table 12.6. Litter response (Caesarean section data) in the rabbit developmental toxicity study

Parameter	**Group 0 0 mg/kg bw**		**Group 1 10 mg/kg bw**	**Group 2 30 mg/kg bw**	**Group 3 60 mg/kg bw**
	historical	study			
Corpora lutea *total/number of dams*	mean 8.0 range 7.2 – 8.8	111/15 (7.4)	112/15 (7.5)	116/15 (7.7)	112/15 (7.5)
Implantations *total/number of dams*	mean 6.8 Range 5.4-8.1	91/15 (6.1)	97/15 (6.5)	93/15 (6.2)	94/15 (6.3)
Resorptions *total/number of dams*	mean 0.7 range 0.2-1.3	7/15 (=0.47)	11/15 (=0.73)	8/15 (=0.53)	23/15 (=1.5)
total number of foetuses	2425	84	85	85	71
pre-implantation loss *% (SD)*	mean 14.0 range 6.1 - 28.5	19.2 (SD:25.46)	14.2 (SD:14.43)	19.8 (SD:18.80)	14.0 (SD:17.17)
post-implantation loss *% (SD)*	mean 11.2 range 3.0 - 23.1	12.4 (SD:29.91)	11.2 (SD:16.11)	8.2 (SD:18.55)	31.6 (SD:44.08)
total number of litters	394	14	15	15	11
foetuses / litter	6.08	84/14 (=6)	86/15 (=5.7)	85/15 (=5.7)	71/11 (=6.5)
live foetuses / litter *ratio*	mean 6.1 range 4.5-7.2	84/14 (6:1)	85/15 (5.7:1)	85/15 (5.7:1)	71/11 (6.5:1)
dead foetuses / litter *ratio*	0.005	0	1/15 (0.07:1)	0	0
foetus weight (mean) *[g]*	mean 41.1 2.5 - 97.5 percentile: 33.5 - 48.7	41.8	38.6	41.8	36.5
placenta weight (mean) *[g]*	4.62	4.9	4.4	4.7	4.2

crown-rump length (mean) *[mm]*	n.d.	n.d.	n.d.	n.d.	n.d.
Foetal sex ratio *[m/f]*	1109:1314 (1 : 1.2)	42:42 (1 : 1)	48:37 (1 : 0.77)	45:40 (1 : 0.89)	35:36 (1 : 0.97)

The morphological examinations failed to reveal significant evidence of foetal external, soft tissue, skeletal or total malformations. The total malformation rate was low, substantially similar in all groups and did not show a clear relation to dosing. Moreover, the isolated and disparate nature of the observed malformations does not suggest any treatment-related aetiology.

The statistically significantly increased number of group 2 and group 3 litters and the higher percentage of high dose foetuses/litter with total skeletal variations however are assessed as embryotoxic effects representing manifestations of a non-specific stress on the does; these findings are not interpreted as the indication of a teratogenic effect of the test substance at these dose levels.

The increased occurrence of single skeletal retardations (delayed ossification of sacral vertebral arch (es) and (or talus) at 60mg/kg are in-line with the reductions in foetal body weights in this group.

There were no further statistically significant and/or biologically relevant differences between the substance-treated groups and the control in respect to external, soft tissue or skeletal findings. As already discussed with the exception of the increased rate of skeletal variations (at group 2 and 3) and the increased occurrence of two skeletal retardations (at group 3) – all foetal findings are considered to be of spontaneous nature, because no dose-response relationship is given and/or the respective values are within the historical control range.

Table 12.7 Examination of the foetuses in the rabbit developmental toxicity study

Parameter	**Group 0 0 mg/kg bw**	**Group 1 10 mg/kg bw**	**Group 2 30 mg/kg bw**	**Group 3 60 mg/kg bw**
External malformations *[%]*	**0**	**0**	**1.2**	**2.8**
External variations *[%]*	**0**	**5.8**	**1.2**	**0**
Skeletal malformations *[%]*	**2.4**	**1.2**	**1.2**	**2.8**
Skeletal variations *[%]*	**13**	**17**	**20**	**30**
Skeletal retardations *[%]*	**65**	**58**	**47**	**69**
Soft tissue malformations *[%]*	**2.4**	**2.3**	**0**	**2.8**
Soft tissue variations *[%]*	**27**	**21**	**25**	**23**

4.11.2.2 Human information

Not available

4.11.3 Other relevant information

Not available

4.11.4 Summary and discussion of reproductive toxicity

See discussion above

4.11.5 Comparison with criteria

Two developmental toxicity studies are available, in rat and in rabbits. Classification for category 1B would require "clear evidence of an adverse effect on reproduction in the absence of other toxic effects or if occurring together with other toxic effects the adverse effect on reproduction should not considered to be a secondary non-specific consequence of other toxic effects". Classification in category 2 should be based on "some evidence from humans or experimental animals, possibly supplemented with other information … and not considered to be secondary, non-specific consequence of the other toxic effects".

In the rat study no developmental effects were observed. In the rabbit study strongly reduced daily food consumption was observed in the high dose group: sharply between day 7, i.e. the first day of exposure, and day 20, between 26% to 69% of control. During the post-treatment period (day 19 to 29), food consumption reached or even exceeded control values. Food consumption is recognised as critical according to CLP Annex I, paragraph 3.7.2.4. and considered to be related to several non-specific consequences, as the observed net weight reduction, gravid uterus weight reduction, the complete litter resorption in 3 dams, the clinical findings of no defecation (day 10-13) in one dam and observed blood in bedding in another dam (due to litter loss), increase in skeletal variations and skeletal retardations. There is no other supplementing information that may support a concern for developmental toxicity. Consequently it is considered that there is inadequate evidence for reproductive toxicity.

4.11.6 Conclusions on classification and labelling

No classification is necessary.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

No human data were available for the assessment of effects on fertility and sexual function or for effects on development following exposure to Cu-HDO.

Effects on sexual function and fertility

No 2-generation study for the assessment of effects on reproduction following exposure to Cu-HDO was included in the CLH-dossier. The waiving argument provided by the Applicant were based on the absence of gross- and histopathological effects in the male and female reproductive organs in the repeated dose toxicity studies following exposure to Cu-HDO. The support for the waiving was based on several studies analysing the link between effects in male reproductive organs and effects on functional fertility. In these studies a clear link between effects in male reproductive organs and effects on reproduction was found (Dent,

2007, Janer *et al.*, 2007 and Mangelsdorf *et al.*, 2003). Based on the absence of effects in the reproductive organs in males and females evident from repeated dose toxicity studies following exposure to Cu-HDO and the waiving arguments for a 2-generation study no classification for effects on fertility and sexual function was proposed by the DS.

Developmental toxicity

Two developmental toxicity studies performed according to OECD TG 414 and which were GLP compliant were included in the CLH-dossier, one in rats and one in rabbits.

In the **rat** developmental toxicity study no developmental effects were reported following exposure to 0, 10, 30 and 100 mg/kg bw/d Cu-HDO from gestation day (GD) 6-15 (A 6.8.1/01).

In the **rabbit** developmental toxicity study the animals were exposed from GD 7-19 to 0, 10, 30 and 60 mg/kg bw/d Cu-HDO (A 6.8.1/02).

Maternal toxicity included a statistically significant reduction in the daily food consumption in the mid and high dose groups starting on the first day of exposure (GD 7) and persisting to the end of exposure (GD 19). The reduction in food consumption from GD 7-19 was accompanied by a statistically significant reduction in body weight gain during the exposure period. During the post-treatment period (GD 20 to 29) food consumption reached or even exceeded control values, and the maternal body weight gain was comparable to the control group. Reduction in gravid uterus weight was also reported in the high dose group, however, this was not statistically significant due to the high variability in the results. Clinical findings in the high dose group included no defecation in one dam (day 10-13) and blood in the bedding of another dam (due to litter loss).

Embryo/foetal toxicity included an increase in resorptions (early) in the high dose group. In this dose group 4 out of 15 pregnant dams had no viable foetuses. As a consequence, an increase in post-implantation losses was also reported in the high dose group. However, the standard deviation was very high in the high dose group since the mean number of live foetuses was not reduced in the remaining 11 high dose does.

The morphological examinations did not show significant evidence of foetal external, soft tissue, skeletal or total malformations. The total malformation rate was low, similar in all groups and did not show a clear dose-relationship. Moreover, the isolated and disparate nature of the observed malformations did not suggest any treatment-related aetiology. The statistically significantly increased number of litters in the mid and high dose group and the higher percentage of high dose foetuses/litter with total skeletal variations were assessed as embryotoxic effects related to non-specific stress in the dams. Therefore, these findings were not interpreted by the DS as an indication of a teratogenic effect of Cu-HDO at these dose levels. The increased occurrence of single skeletal retardations (delayed ossification of sacral vertebral arch(es) and/or talus) in the high dose group were in-line with the reductions in foetal body weights in this group.

There were no further statistically significant and/or biologically relevant differences between the exposed groups and the control group for external, soft tissue or skeletal findings. In summary, all foetal findings, including those described above, were considered by the DS to be of spontaneous nature, since no dose-response relationship was seen and/or the respective values were within the historical control range.

In their conclusion for no classification for effects on development the DS pointed out that the food consumption is recognised as critical according to CLP Annex I, paragraph 3.7.2.4. and

is considered to be related to several non-specific consequences. These were reported as reduction in body weight gain, gravid uterus weight reduction, complete litter resorption in 4 dams, the clinical findings of no defecation in one dam (day 10-13) and observed blood in bedding in another dam (due to litter loss), as well as an increase in skeletal variations and skeletal retardations. The DS also recognised that there was no other information that may support a concern for developmental toxicity. Consequently, the DS considered that there is inadequate evidence for developmental toxicity and no classification was proposed.

Comments received during public consultation

Comments were received from two MSCA. Both MSCA supported no classification for effects on fertility and sexual function, but one strongly regretted the absence of fertility study.

One MSCA had some questions regarding the use of Wistar rats in the OECD TG 414 study due to the high incidences of skeletal retardations or variations in the HCD. They also considered that due to the deficiencies in the reporting of the effects in the offspring from the rat and rabbit developmental toxicity studies, it was difficult to perform a proper assessment of the developmental toxicity.

However, the MSCA believed that despite the major deficiencies in the reporting of the two developmental toxicity studies, the findings were sufficient to warrant a developmental toxicity classification. The MSCA considered that at least a Repr. 2 classification for developmental toxicity was warranted. This was based on the fact that malformations were observed in two different studies. With further clarifications of the details about the observed variations and malformations in the two studies it might even lead to a Repr. 1B classification for developmental toxicity.

The second MSCA considered that the study in rats did not show relevant findings for classification. However, the rabbit study included an increased rate of resorption in the high dose group and therefore an increase in post-implantation loss that exceeded the concurrent controls and the HCD range. In parallel, maternal effects were evident as reduced food consumption during the treatment period. The MSCA asked for more clarification relating to the maternal effects reported in the rabbit study and if the developmental toxicity was considered as a primary effect of the substance or if it was secondary to maternal toxicity before deciding on a classification for developmental toxicity.

Further information regarding the effects reported in the rat and rabbit developmental toxicity studies was provided by the DS in the RCOM and included in the assessment and comparison with the classification criteria section of the opinion.

Assessment and comparison with the classification criteria

Effects on sexual function and fertility

Information on the potential effects of Cu-HDO on sexual function and fertility was only available from repeated dose toxicity studies. In these studies no gross- and histopathological effects in the male and female reproductive organs were reported. For further information see the section of this opinion on STOT RE. No 2-generation reproductive toxicity study was available due to waiving arguments provided by the applicant and agreed upon in the technical meeting for biocides focusing on risk assessment. Based on the absence of effects in the reproductive organs in males and females evident from repeated dose toxicity studies following exposure to Cu-HDO RAC agrees with the DS that no classification of Cu-HDO for effects on sexual function and fertility is justified based on the data available. However, RAC recognises

the absence of a 2-generation reproductive toxicity study. Data from a 1- or 2-generation study is considered by RAC to be needed to fully assess effects on sexual function and fertility.

Developmental toxicity

The DS included two developmental toxicity studies performed according to OECD TG 414 in the CLH dossier, one in rats and one in rabbits.

In the ***rat*** developmental toxicity study performed in accordance with OECD TG 414 and GLP pregnant Wistar rats were exposed to 0, 10, 30 and 100 mg/kg bw/d Cu-HDO from GD 6-15. Maternal toxicity included a slight and transient reduced food consumption and marginally reduced body weight gain at 100 mg/kg bw/d, see table below.

Table: *Maternal effects in rat developmental toxicity study*

Parameter	HCD	Control	10 mg/kg bw/d	30 mg/kg bw/d	100 mg/kg bw/d
# dams		30	30	30	30
Mortality of dams %		0	3.3*	6.6*	10*
BW gain				↓ GD 6-8 (corrected bw gain = 92% of control) ↑ GD 8-10	
Food consumption				↓ GD 6-8 (18%)	
Pregnancies %	92%	83%	90%	90%	90%
Necropsy findings of dams dead before end of test					
Lungs: oedema		20%	6.7%	6.7%	6.7%
Lungs: marginal emphysema		3.3%	0%	0%	0%
Particular findings on implants in dams sacr. Morbid/died interc.		0%	3.3%	6.7%	10%

*the rats died accidentally on GD 7 (after the second gavage) due to unintentional use of a faulty stomach tube

No effects following exposure to Cu-HDO were reported on the conception rate, number of corpora lutea and implantation sites as well as post-implantation losses, resorption, and viable foetuses. The difference between the control and exposed groups was considered to be within the normal range of this rat stain (see table below).

Table: *Litter response in the rat developmental toxicity study*

Parameter	HCD	Control	10 mg/kg bw/d	30 mg/kg bw/d	100 mg/kg bw/d
Corpora lutea Total/# dams	6599/420 (15.7)	403/25 (16.1)	442/27 (16.4)	403/27 (14.9)	391/27 (14.5)
Implantations Total/# dams	5999/420 (14.3)	344/25 (13.8)	393/27 (14.6)	367/27 (13.6)	345/27 (12.8)
Resorptions Total/# dams	420/248 (1.7)	18/25 (0.7)	25/26 (1.0)	23/25 (0.9)	25/24 (1.0)
Total # foetuses	5528	326	368	344	320
Pre-implantation loss %	9.1	14.8	11.8	9.0	13.2
Post-implantation –loss %	7.9	5.0	6.1	6.0	7.2

Total # litters	418	25	26	25	24
Live foetuses/litter	13.2	13.0	14.2	13.8	13.3
Dead foetuses/litter	0	0	0	0	0
Foetus weight (g)	3.9	3.8	3.9	3.9	4.0

No association with exposure to Cu-HDO was reported for external variations and malformations. As regards skeletal variations, retardation and malformations, questions were raised during the public consultation on the selection of the rat strain used since there was a high incidence of skeletal retardation and variations in the HCD as well as in the control and exposed groups, however, without a dose-response relationship. In response, the DS provided ranges of HCD (included in the table below) and replied that the ranges were quite usual. An increase in soft tissue malformation was also reported in all exposed groups, without a dose-response relationship, but at the upper range of the HCD. The incidence of external, skeletal and soft tissue variations and malformations is included in the table below. A table with more detailed information regarding the incidences of soft tissue malformations is also included since this was in the upper range of the HCD.

***Table**: Incidences of variations and malformations*

Parameters	HCD	Control	10 mg/kg bw/d	30 mg/kg bw/d	100 mg/kg bw/d
External malformations %	0.09 (0-1.2)	0	0	0.6	0.3
External variations %	0	0	0	0	0
Skeletal malformations %	3.2 (0-10.1)	6.5	3.2	5.1	4.3
Skeletal retardations %	46.5 (0.0–72.0)	41	38	48	42
Skeletal variations %	47.8 (31.8–88.4)	36	41	42	33
Soft tissue variations %	15.5 (4.9-33.1)	22	20	17	27
Soft tissue malformations %	0.3 (0–2.2)	0	2.2	1.8	1.9

***Table**: Incidences of soft tissue malformations*

Parameters	Control	10 mg/kg bw/d	30 mg/kg bw/d	100 mg/kg bw/d
Soft tissue malformations, foetuses affected/foetuses	0/157	4/178	3/166	3/157
Soft tissue malformations, litters affected/litters	0/25	4/26	3/25	3/24
- sinus inverses	0	0.6	0.6	0
- hydrocephaly	0	0.6	0	0.6
- microcephalia	0	0	0.6	0
- malformations of great vessels	0	0	0	0.6
- hearth dilatation of right ventricle	0	0	1.2	0
- hearth dilatation of both ventricles	0	1.1	0	0

- septal defect	0	0	0	0.6

RAC agrees with the DS that based on the reported observations in the rat developmental toxicity study, no effects were reported which could justify classification for developmental toxicity. However it could be noted that higher doses could have been considered since limited maternal toxicity was seen in the high dose group.

In the ***rabbit*** developmental toxicity study performed in accordance with OECD TG 414 and GLP, pregnant rabbits were exposed from GD 7-19 to 0, 10, 30 and 60 mg/kg bw/d Cu-HDO. Maternal toxicity: No mortality or abortions were reported. The pregnancy rate was 100% in all dose groups. A statistically significant reduction in the daily food consumption in the mid and high dose groups starting from the first day of exposure (GD 7) to the end of exposure (GD 19) was reported (see table below). The reduction in food consumption from GD 7-19 was accompanied by a statistically significant reduction in body weight gain during the exposure period. During the post-treatment period (GD 20 to 29) food consumption reached or even exceeded control values, and the maternal body weight gain was comparable to the control group. Reduction in gravid uterus weight was also reported in the high dose group, however, this was not statistically significant due to high standard deviations. Clinical findings in the high dose group included no defecation in one dam (day 10-13) and blood in bedding in another dam (due to litter loss). For further details see the table below:

***Table**: Maternal toxicity in the rabbit developmental toxicity study*

Parameter	Control	10 mg/kg bw/d	30 mg/kg bw/d	60 mg/kg bw/d
# dams	15	15	15	15
Bw gain GD 0-7 mean (SD)	45.3 (29.63)	24.6 (53.99)	19.9 (58.17)	36.1 (62.86)
Bw gain GD 7-19 mean (SD)	87.7 (45.35)	44.3 (45.07)	**25.9*** (52.49)	-**82.5**** (101.25)
Bw gain GD 19-29 mean (SD)	173.3 (73.41)	147.8 (67.88)	188.7 (73.45)	181.5 (59.71)
Bw gain GD 0-29 mean (SD)	306.3 (112.56)	216.7 (69.80)	234.5 (103.48)	**135.1**** (147.87)
Gravid uterus mean (SD)	313.1 (141.32)	298.6 (88.61)	317.0 (93.53)	236.7[a] (158.97)
Food consumption			Significantly reduced GD 7-13 and GD 15-20 (between 67% and 84% of controls)	Significantly reduced GD 7-20 (between 24% and 71% of controls)

*p ≤ 0.05/** p ≤ 0.01, SD: standard deviation

[a]Due to high SD not statistical significant reduced

Litter data included an increase in resorptions (early) in the high dose group. In this dose group 4 out of 15 pregnant dams had no viable foetuses and the number was outside the HCD range so the increase in resorptions could be considered as substance related. However, in these four dams a marked reduction in food consumption was reported, down to 10% of their pre-exposure consumption, as well as no defecation in one dam (day 10-13) and blood in bedding in another dam (due to litter loss). As a consequence, an increase in post-implantation losses was also reported in the high dose group (12.4%, 11.2%, 8.2% and 31.6% in the control, low, mid and high dose groups, respectively) that were outside the HCD range in the high dose group. However, the standard deviation was very high in the high dose group since the mean number of live foetuses was not reduced in the remaining 11 high dose does. As can be seen from the table below, there were no effects on the number of corpora lutea,

implantations, pre-implantation losses, foetuses/litter, live foetuses/litter, dead foetuses/litter and the bw of the foetuses.

Table: *Litter data in the rabbit developmental toxicity study*

Parameter	HCD	Control	10 mg/kg bw/d	30 mg/kg bw/d	60 mg/kg bw/d
Corpora lutea (total/#dams)	mean 8.0 range 7.2-8.8	111/15 (7.4)	112/15 (7.5)	116/15 (7.7)	112/15 (7.5)
Implantations (total/#dams)	mean 6.8 range 5.4-8.1	91/15 (6.1)	97/15 (6.5)	93/15 (6.2)	94/15 (6.3)
Resorptions (total/#dams)	mean 0.7 range 0.2-1.3	7/15 (0.47)	11/15 (0.73)	8/15 (0.53)	**23/15 (1.5)**
Pre-implantation loss % (SD)	mean 14.0 range 6.1–28.5	19.2 (SD:25.46)	14.2 (SD:14.43)	19.8 (SD:18.80)	14.0 (SD:17.17)
Post-implantation loss % (SD)	mean 11.2 range 3.0-23.1	12.4 (SD:29.91)	11.2 (SD:16.11)	8.2 (SD:18.55)	**31.6 (SD:44.08)**
Foetuses/litters (total #)	2425/394 (6.08)	84/14 (6)	85/15 (5.7)	85/15 (5.7)	71/11 (6.5)
Live foetuses/litter (ratio)	mean 6.1 range 4.5-7.2	84/14 (6:1)	85/15 (5.7:1)	85/15 (5.7:1)	71/11 (6.5:1)
dead foetuses/litter (ratio)	0.005	0	1/15 (o.007:1)	0	0
Foetal weight (g)	mean 41.1 range 2.5-97.5	41.8	38.6	41.8	36.5

The external, skeletal and soft tissue variations and malformations is shown in the tables below including further information from the DS due to a request from public consultation

Parameter	HCD	Control	10 mg/kg bw/d	30 mg/kg bw/d	60 mg/kg bw/d
Number of foetus examined	2425	84	86	85	71
% External malformations	8/2425 (0.3%)	0	0	**1.2**	**2.8**
% External variations		0	05.8	1.2	0
% Skeletal malformations	31/2425 (1.3)	2.4	1.2	1.2	**2.8**
% Skeletal variations	314/2425 (12.9%)	13	17	20	30
% Skeletal retardations	1365/2425 (56.3%)	65	58	47	69
% Soft tissue malformations	48/2425 (2.0%)	2.4	2.3	0	2.8
% Soft tissue variations	741/2425 (30.6%)	27	21	25	23

Table: *Further data on the external malformations*

Parameter (% foetal incidence)	Control	10 mg/kg bw/d	30 mg/kg bw/d	60 mg/kg bw/d
Gastroschisis	0	0	0	1.4
Toes shortened	0	0	1.2	0
Polydactyly	0	0	0	1.4
Shortened and thickened hind limbs	0	0	0	1.4*

*the thickened and shortened hind limb in one of the high dose foetuses was also the one that had polydactyly

An increased incidence above the HCD was reported for skeletal malformations, however, in the control animals the incidence of skeletal malformations was also above the HCD range and no clear dose-response was seen. Further, the DS informed that during the skeletal examination, the shortened and bent tibia and fibula observed was identified as the cause for the thickened and shortened hind limb. The same picture was also observed for the soft tissue malformations with incidences above the HCD in the control group without a clear dose response relationship. RAC considers that this information lowers the concern arising from these malformations.

Regarding the external malformations, incidences were reported in the mid and high dose groups that were outside the HCD range and a dose-response relationship was reported. However, the increase was not statistically significantly increased. Further, the DS informed that gastroschisis and different malformations of the extremities sporadically occur in control foetuses of the strain used, however, no further data was provided. It could also be considered whether the maternal toxicity reported in the mid and high dose group evident as a statistically significant reduced food consumption during GD 7-19 leading to a statistically significant reduced bw gain during the same time period could affect the malformation rate reported in the mid and high dose group. This aspect was raised during the public consultation and in the review by Nitzsche (2017) in which an analysis of effects of maternal feed restriction on prenatal development in rats and rabbits was included. This review concluded that effects on embryo lethality and malformations in rabbits and rats were not impaired by feed restriction up to 10% of the control group. Only in one of the six studies included in the review, the study by Clark *et al.* (1986), was an increased incidence of foetuses with malformations such as omphalocele (2%), clubbed forefoot (3%) and sternebrae malformations (4%) reported at a maternal feed intake of 10% of the control group. HCD from the study by Ema *et al.* (2012) was also included in the review for comparison with incidences of 0.07% foetuses with omphalocele (range 0-2.22% performed from 1994-2000) and 0.08% foetuses with clubbed forefoot (range 0-1.43% performed from 2001 to 2010, Ema *et al.*, 2012). RAC therefore considers that the external malformations observed in one or two foetuses from one litter with no dose-response relationship are not considered associated with treatment to Cu-HDO but instead are considered to be spontaneous.

Comparison with the CLP classification criteria

No human data were available for the assessment of developmental toxicity, so a classification according to the CLP criteria as Repr. 1A is not justified.

A classification as Repr. 1B according to the CLP criteria is based on "*clear evidence of an adverse effect on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on development is considered not to be a secondary non-specific consequence of other toxic effects*".

A classification as Repr. 2 is be based on "*some evidence from humans or experimental animals, possibly supplemented with other information of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1*"

Two developmental toxicity studies were included in the CLH report, one in rats and one in rabbits. In the rat developmental toxicity study no effects considered related to *in utero* exposure to Cu-HDO were observed.

In the rabbit developmental toxicity study a statistically significantly reduced daily food consumption was observed in the mid and high dose group, especially during the exposure period starting GD 7, i.e. the first day of exposure, persisting to GD 19. During the post-

treatment period (GD 20 to 29), food consumption reached or even exceeded control values. The reported developmental toxicity effect that could be of concern was the full resorption of all foetuses in 4/15 dams. However, in these four dams a marked reduction in food consumption was observed, down to 10% of their pre-exposure consumption, as well as no defecation in one of them (day 10-13) and blood in bedding in another of the dams (due to litter loss). The mean number of live foetuses in the remaining 11 dams in the high dose group was not reduced. Further, an increase in external malformations that that was outside the HCD range in the mid and high dose groups could be of concern. However, when analysing these malformations, it was noted that two of the malformations were reported in the same foetus. This information lowers the concern arising from these malformations. There were no other supplementing information that might support a concern for developmental toxicity.

Overall, RAC agrees with the DS that the available data **does not warrant classification of Cu-HDO for sexual function and fertility or for developmental toxicity**.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Cu-HDO was investigated within subacute, subchronic and chronic oral administration regimens and in prenatal toxicity studies. In no case, the results indicated a clinical neurotoxic effect of this material or the brain as target organ (see studies and discussion in section 4.7.1., 4.8.2., 4.11.2)

Furthermore, within the frame of a subacute toxicity study in rats (Study A6.3.1, Doc IIIA 6.3.1.) neurotoxicity investigations along a functional observation battery (FOB) were carried out: General appearance (general state of health), tremors, convulsions, piloerection, lacrimation/secretion of pigmented tears, salivation, pupil size, diarrhoea, vocalization while handling, paresis, paralysis, ataxia, body tone, posture, animal body (appearance), locomotor activity, respiration, urination, skin colour, righting reflex, behaviour, grip strength, papillary reflex, winking reflex, vision, audition, olfaction, sensitivity of the body surface, pain perception, tail pinch, toe pinch, visual placing response, miscellaneous (all other visible clinical signs). The observation of the neurofunction was made on all animals once prior to the start of the test substance administration, 24 hours after the first administration and on days 7, 14 and on day 27. No functional effects were observed.

Hence, there are no indications for concerns of neurotoxicity of Cu-HDO and therefore no additional neurotoxicity study (according to Annex IIIA of the BPD) was considered necessary.

4.12.1.2 Immunotoxicity

4.12.1.3 Specific investigations: other studies

4.12.1.4 Human information

The applicant states that Cu-HDO and Cu-HDO containing wood preservatives were used in practice over more than 10 years. During this time, Cu-HDO based products would have been processed in more than 150 treatment plants and more than 5 million m^3 of wood would have been treated. In addition the applicant reports no cases of poisoning from manufacture or professional use

4.12.2 Summary and discussion

See discussion above

4.12.3 Comparison with criteria

See discussion above

4.12.4 Conclusions on classification and labelling

No classification necessary.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

5.1.1 Stability

Hydrolysis

Table 22 Hydrolysis

Guideline / Test method	pH	Temperature [°C]	Initial TS concentration, C_0 [mg/L]	Reaction rate constant, K /d^{-1}	Half-life, DT_{50} [h]	Coefficient of correlation, r^2	Reference
OPPTS 835.2130 / Hydrolysis as a function of pH and temperature	3	Pre-test: 50°C Main-test: 25, 40, 55 and 70°C	1.79-2.43 mg Cu-HDO/L	-	Pre-test: 50°C: 95h **Main-test**: **25°C: stable** **40°C: 1087h** **55°C: 305h** **70°C: 60h**	Pre-test: 0.99 Main-test: 40°C: 0.68 55°C: 0.99 70°C: 0.99	**Study A 7.1.1.1.1/02, document III A 7.1.1.1.1/02**
	7	Pre-test: 50°C Main-test: 25, 40 and 55°C	2.18-2.69 mg Cu-HDO/L	-	Pre-test: 50°C: 415h **Main-test:** **25°C: stable** **40°C: stable** **55°C: 1449h**	Pre-test: 0.65 Main test: 55°C: 0.96	
	11	Pre-test: 50°C Main-test: 40 and 55°C	1.97-2.62 mg Cu-HDO/L	-	Pre-test: 50°C: 302h **Main-test:** **40°C: stable** **50°C: stable**	Pre-test: 0.72	
EC C.7 / Hydrolysis as a function of pH	Pre- and Main-Test: 4	Pre-test: 50°C; Main-test: 35 and 50°C	Pre-test: 38, 6.3 and 8.5 mg Cu-HDO/L Main-test: 34 and 47 mg Cu-HDO/L	0.153 at 25°C, (calculated)	108h (4.5 d) at 25°C (calculated)	-	Study A 7.1.1.1.1

Guideline / Test method	pH	Temperature [°C]	Initial TS concentration, C_0 [mg/L]	Reaction rate constant, K /d^{-1}	Half-life, DT_{50} [h]	Coefficient of correlation, r^2	Reference
	Pre-test: 7	Pre-test: 50°C	Pre-test: 38, 6.3 and 8.5 mg Cu-HDO/L		stable		
	Pre-test: 9	Pre-test: 50°C	Pre-test: 38, 6.3 and 8.5 mg Cu-HDO/L		stable		

The hydrolytic behaviour of Cu-HDO has been investigated in two studies.

In the study according to OPPTS guideline 835.2130 **(study A 7.1.1.1.1/02, document III-A 7.1.1.1.1/02)** the hydrolytic behaviour has been experimentally determined at environmentally relevant temperature (25°C) at pH 3 and 7. Under these conditions no hydrolysis occurred. In addition the transformation products have been determined from a sample which was run at pH 3 and 70°C. It could be shown that Cu-HDO hydrolyzes in a parallel reaction to compounds identified as Cyclohexanone (68.8% of HDO) and as Cyclohexanol (6.35% of HDO). Dissolved copper, was not measured in the study, but it is clear that it will additionally contribute to the transformation products.

The second study (study A 7.1.1.1.1) confirms the general tendency of the key study. Measurable hydrolysis occurs under acidic conditions (pH 3-4) and temperatures ≥ 35°C. At neutral pH hydrolysis is only observed at even higher temperatures (55°C). In alkaline pH Cu-HDO is stable for all tested temperatures.

Conclusion:
Cu-HDO has been shown to be hydrolytically stable at 25°C and at pH3 and 7. Hydrolysis for all tested pHs (3, 7, 9 and 11) only occurs at temperatures ≥ 35°C. It is therefore assumed that under relevant environmental conditions (5 -25°C) no hydrolysis will take place in the pH range 4 – 9. The identified transformation products, including dissolved copper are therefore not considered relevant.
According to the Guidance on the Application of the CLP Criteria v. 4.1, Annex II, chapter 4 Decision scheme, it is therefore concluded that the available data on hydrolysis give no indication for the fulfilment of the criteria for rapid degradation (half-life < 16 days) of Cu-HDO.

Photolysis in water

As the draft OECD Guideline for the testing of chemicals "Phototransformation of Chemicals in Water – Direct and Indirect Photolysis" points out that direct photolysis can be an important dissipation pathway for some chemical pollutants which exhibit significant light absorption above the 295 nm cut-off of solar irradiation at the earth's surface. Indirect photolysis can also be an important dissipation pathway for some chemical pollutants that come in contact with photo-sensitisers in electronically excited triplet states or with short-lived photo-chemically generated oxidants such as hydroxyl radicals and singlet oxygen. In some cases both direct and indirect photolysis can contribute significantly to the dissipation of a chemical in natural waters.

The draft guideline suggests using a filtered xenon arc lamp capable of simulating natural sunlight in the 295 to 800 nm region or sunlight for direct photolysis studies, and sunlight for indirect photolysis studies, whereas the already existing guideline OPPTS 835.2210 (US-EPA, 1998) gives the instruction to use natural sunlight in any case.

In the submitted test report (study A 7.1.1.1.2/03), photolysis of Cu-HDO in water showed rapid degradation (Lamp: Xenon lamp; intensity: 3 mW/cm² simulating a clear summer day; filter: UV filter to cut off wavelengths < 290 nm) of the test item [U-^{14}C] Cu-HDO and the formation of cyclohexanone (45% total applied radioactivity TAR after 48 hours) and cyclohexanone oxime (51% TAR after 48 hours), which further degraded to volatile degradation products of low molecular weight, e.g. carbon dioxide. No other metabolite above 5% TAR occurred. Again dissolved copper, was not measured in the study, but it is clear that it will additionally contribute to the transformation products.

Cu-HDO is readily degraded by aqueous photolysis; the experimental half-life (DT_{50}) of Cu-HDO was 6 hours under irradiation. The DT_{90} of Cu-HDO is calculated to be 19.4 hours. In the dark control, no degradation of Cu-HDO was observed. The calculated half-life for the top-layer of aqueous systems under Central European conditions considering the quantum yield of Cu-HDO was estimated to be less than 1 hour during the months April-August.

Estimated photolysis rate constant k_p(1/d) for the test substance (pH 7) =0.1185
Quantum yield Φ for the test substance =0.0276

A literature method also was submitted (study A 7.1.1.1.2/01) where a filtered xenon arc lamp capable of simulating natural sunlight in the 295 to 800 nm region was used (800 W/m², 25°C). It is stated that Cu-HDO in aqueous solution undergoes rapid fragmentation upon irradiation with light (λ > 290 nm) (concentration and degradation time not nearer specified). The main degradation products of Cu-HDO are derivates of cyclohexane (cyclohexanone, methoxy-cyclohexane and 1,1-dimethyl-cyclohexane).

Conclusion:
Cu-HDO degrades rapidly by photolysis in water under formation of several degradation products, including dissolved copper. However, due to the adsorption coefficient of 30 277.4 L/kg (section 5.1.2) this process won't represent a major degradation pathway in the environment, since Cu-HDO will adsorb very quickly and almost irreversible onto organic matter.
Therefore rapid photolysis should not be taken as an indication for rapid degradation of Cu-HDO in the environment according to the Guidance on the Application of the CLP Criteria v. 4.1, Annex II, chapter 4.

Phototransformation in air

The specific degradation rate constant of Cu-HDO with OH-radicals (k_{OH} [cm^3 x $molec.^{-1}$ x s^{-1}]) was estimated with the Atmospheric Oxidation Programme AOP 1.91, Epi Suite, Syracuse Research Corporation (See document III-A7.3.1):

$$k_{OH} \text{ (Cu-HDO)} = 68.72 \times 10^{-12} \text{ cm}^3 \times \text{molecule}^{-1} \times \text{s}^{-1}$$

By relating k_{OH} to the average OH-radical concentration in the atmosphere ($c(OH)_{air}$ [molec. x cm^{-3}]), the pseudo-first order rate constant for degradation in air ($k_{deg, air}$, [d^{-1}]) can be derived:

$$k_{deg,\,air} = k_{OH} \times c(OH)_{air} \times 24 \times 3600$$

According to the TGD on Risk Assessment, $c(OH)_{air} = 5 \times 10^5$ molecules/cm^{-3}, and according to the Atmospheric Oxidation Programme AOP 1.91, $c(OH)_{air} = 1.5 \times 10^6$ molecules/cm^{-3}, which leads to

$k_{deg,\,air}$ (Cu-HDO) = 2.97 d^{-1}, $T_{1/2}$ = 5.6 h (TGD)

$k_{deg,\,air}$ (Cu-HDO) = 8.91 d^{-1}, $T_{1/2}$ = 1.87 h (AOP)

Conclusion:
Due to adsorption processes the amount of Cu-HDO which is present in the atmosphere is considered marginal. The half-life of Cu-HDO was estimated to be 1.87 hours and 5.6 hours, respectively. Because of the short lifetime in the atmosphere due to the very low vapor pressure, and due to the fact that Cu-HDO does not contain any atoms of chlorine, bromine or fluorine, an effect of Cu-HDO on stratospheric ozone is not expected.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No data available

5.1.2.2 Screening tests

Table 23 Biodegradation, screening tests

Guideline / Test method	Test type[1]	Test parameter	Inoculum			Additional substrate	Test substance concentration	Degradation		Reference
			Type	Concen-tration	Adaptation			Incubation period	Degree [%]	
OECD Guideline 301 D / Ready Biodegradability: Closed Bottle Test	Ready	BOD / ThOD	Effluent from a laboratory waste water plant treating municipal sewage	-	Not pre-adapted	-	2 mg Cu-HDO/L	56 d	**<10%**	**Study A 7.1.1.2.1, Document III A 7.1.1.2.1**
OECD Guideline 302 B / Inherent biodegrade-ability: Modified Zahn-Wellens Test	Inherent	DOC	Activated sludge from laboratory plants with municipal waste water	-	No pre-adaptation	-	6 mg Cu-HDO/L	28 d	**100% elimination (50% elimination due to adsorption)**	**Study A 7.1.1.2.2, Document III A 7.1.1.2.2**

[1] Test on *inherent* or *ready* biodegradability according to OECD criteria

The biodegradability of Cu-HDO has been investigated in a ready test **(study A 7.1.1.2.1, document III-A 7.1.1.2.1)** and in an inherent test **(study A 7.1.1.2.2, document III-A 7.1.1.2.2)**. In both studies the concentration of copper (II) ion was not measured.

In the Closed Bottle Test **(study A 7.1.1.2.1, document III-A 7.1.1.2.1)** < 10% biodegradation was measured, even after prolongation of the study up to 56 days. The substance is therefore considered as being “not readily biodegradable”.

In the Zahn-Wellens Test **(1993; study A 7.1.1.2.2, document III-A 7.1.1.2.2)** a total elimination rate of 100% was already reached after 17 days. 50% of that elimination took place within the first two hours, which indicates elimination due to adsorption. In that study Cu-HDO was tested at a concentration of 6 mg/L, which in the Activated Sludge, Respiration Inhibition Test **(2001; study A 7.4.1.4, document III-A 7.4.1.4)** was later shown to be an inhibitory concentration**.** These inhibitory effects were not taken into account.

Conclusion:

Cu-HDO is not readily biodegradable and therefore also not rapidly degradable according to the criteria (70% DOC removal or 60% theoretical oxygen demand) given in the Guidance on the Application of the CLP Criteria v. 4.1, Annex II, chapter 4.

5.1.2.3 Simulation tests

Biodegradability in water/sediment system:

Table 24 Biodegradation, water/sediment

Guideline / Test method	Test type	Test parameter	Inoculum			Additional substrate	Test substance concentration	Degradation		Reference
			Type	Concen-tration	Adaptation			Incubation period	Degree [%]	
US-EPA Subdivision N, Section 162-4 (835.4300); Study performed before revision of guideline in October 2008	Aerobic water /sediment simulation test with ^{14}C Cu-HDO	Determination, identification and quantification of %TAR through LSC, GC-MS and HPLC.	Water and associated sediment from a pond located in Wabasha County, Minnesota.			-	2.2 mg ^{14}C Cu-HDO/L	30 d, dark conditions at 25°C	**DT_{50} dissipation, water phase 2.4 days (25°C);** biphasic kinetic (FOMC) DT_{50} dissipation sediment phase 20.3 days (25°C); first order kinetic (SFO) DT_{50} degradation, total system 14.5 days (25°C); first order kinetic (SFO) Mineralisation rate 13.2% after 30 days (25°C) Converted to standard conditions: **DT_{50} water phase 6.8 days (12°C)** DT_{50} sediment phase 57 days (12°C) DT_{50} total system 41 days (12°C) Mineralisation rate 13.2% after 84.9 days (12°C)	**Study A 7.1.2.2.2 and addendum, Document III A 7.1.2.2.2**

The degradation of ^{14}C Cu-HDO in a water/sediment system was investigated in a study according to US-EPA test guideline section 162-4 (835.4300) before revision of the guideline in 2008. Therefore only one water/sediment system was tested (pond), the test duration was limited to 30 days and the temperature was maintained at 25°C. The applied test substance concentration was 2.2 mg ^{14}C Cu-HDO/L.

A DT_{50} dissipation value was calculated for the water phase with 2.4 days (biphasic kinetics, $r^2 = 0.988$). In the sediment phase the DT_{50} for dissipation was calculated with 20.3 days (first order kinetics, $r^2 = 0.910$). The DT_{50} value for degradation in the total system was calculated with 14.5 days (first order kinetics, $r^2 = 0.966$).

Mineralisation was determined with 13.2% after 30 days of incubation. The calculated DT_{50} for mineralisation was 89.1 days (logistic kinetics, $r^2 = 0.981$). This value exceeds the limit of observed data and is therefore considered beyond the range of reliable extrapolation.

Immediately after application 78.2% of the totally applied radioactivity (TAR) was found in the water phase. The radioactivity in water decreased to 5.5% TAR at day 30. The major component in the water phase was parent (75.4% TAR at day 0 and 2.8% TAR at day 30).

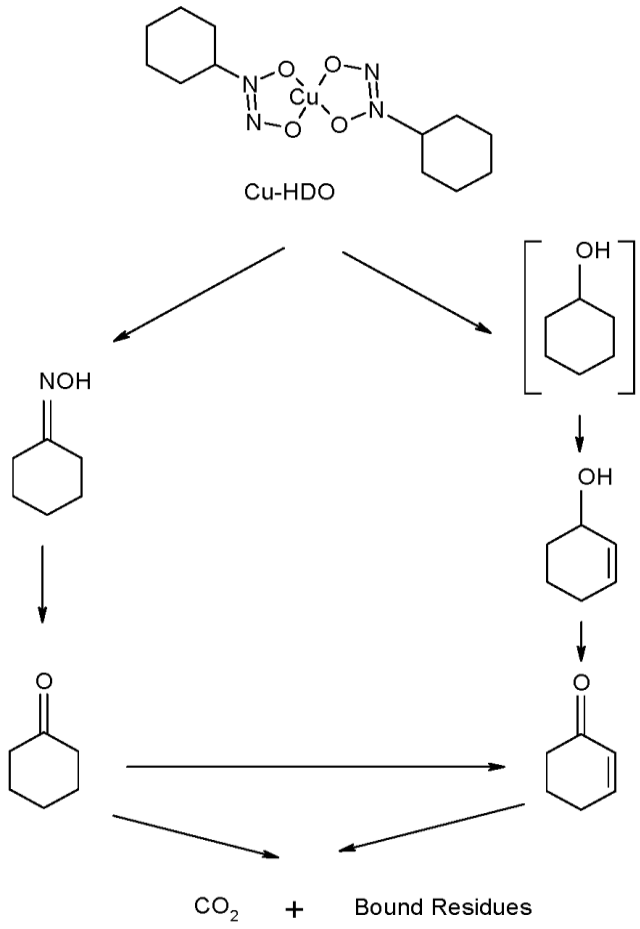
In the sediment phase 25.9% TAR was found at day 0 (16.6% TAR as extractable and 9.3% TAR as non-extractable residues).The extractable radioactivity content in the sediment increased to 45.2% at day 10 and then decreased to 21.5% at day 30. Most of the extractable radioactivity was parent. The non-extractable residues continually increased up to 44% at day 30.

In the water phase as well as in the sediment phase a number of minor metabolites were observed. The only identifiable metabolite was Cyclohexanone which never exceeded 4.3% TAR (day 10) and declined over time. The only major metabolite (13.2% TAR) found was CO_2. Though not detected and measured in this study it is clear that copper will also add to the transformation products. Copper, being a chemical element is not biodegradable. The most important parameters determining the distribution of copper in the aquatic compartment is adsorption onto solid materials and therefore the copper partitioning coefficients. As all metals copper becomes complexes to organic and inorganic matter in waters and sediments and this affects copper speciation, bioavailability and toxicity (AR, France, 2011).

<u>Two degradation pathways are proposed for Cu-HDO in water/sediment:</u>

- Cu-HDO will either degrade to Cyclohexanone Oxime and further to Cyclohexanone which can be further transformed to 2-Cyclohexene-1-one or degraded to CO_2.
- Cu-HDO is degraded to 2-Cyclohexanol, then further to 2-Cyclohexen-1-ol and 2-Cyclohexen-1-one, which is then mineralized to CO_2.

Figure 1: Proposed degradation pathway of Cu-HDO in water/sediment:



Conclusion:

A DT_{50} dissipation value was calculated for the water phase with 2.4 days, in the sediment phase the DT_{50} for dissipation was calculated with 20.3 days.

Cu-HDO undergoes degradation in the total system (water and sediment) with a DT_{50} of 14.5 days at 25°C, corresponding to 41 days (12°C).

But Cu-HDO mineralizes only up to 13.2% after 30 days at 25°C in the total system (water- and sediment phase), which corresponds to a calculated DT_{50} for mineralization of 89.1 days).

The major component in the water phase (75.4% TAR at day 0 and 2.8% TAR at day 30) was parent. In the sediment phase the major component of the extractable TAR was parent as well (extractable: 16.6% TAR at day 0, 45.2% at day 10 and 21.5% at day 30). The non-extractable residues increased from 9.3% (day 0) up to 44% at day 30.

In the water phase as well as in the sediment phase a number of minor metabolites were observed. CO_2 is the only major metabolite (13.2% TAR). Though not detected and measured in this study it is clear that copper adds to the degradation products. Copper will not undergo rapid transformation in the aquatic environment, but it will strongly adsorb onto solid matter and it will get complexed to organic and inorganic matter in waters and sediments (AR, France, 2011).

Therefore, according to the Guidance on the Application of the CLP Criteria v.4.1, Annex II, chapter 4, the substance is considered to be not rapidly degradable, since:

- The criterion for ultimate degradation in a surface water test or a sediment simulation test, with a half-life < 16 days is neither met for the water phase nor for the sediment phase of the water/sediment simulation test.

- The transformation product copper fulfils the criteria for classification as hazardous to the aquatic environment.

Furthermore according to the Guidance on the Application of the CLP Criteria v.4.1, Annex IV, chapter IV, metal compounds that contain an organic component but that dissociate easily in water or dissolve as the metal ion should be treated in the same way as metal compounds and classified according to this annex. However, organometals that do not release metal ions are thereby excluded from the guidance of this section and should be classified according to the general guidance provided in section 4 (Environmental hazards).

- The high rates of parent compound found (water phase: 75.4% TAR at day 0 and 2.8% TAR at day 30; sediment phase (extractable): 16.6% TAR at day 0, 45.2% at day 10 and 21.5% at day 30) show that Cu-HDO, being an organometal compound, cannot dissociate easily in water or dissolve as a metal ion. Therefore Cu-HDO should not be treated in the same way as metal compounds but it should be classified according to part 4 Environmental hazards, of the Guidance on the Application of the CLP Criteria.

Degradability in soil

Table 25 Biodegradation, soil

Guideline / Test method	Test type	Test parameter	Inoculum: Type	Inoculum: Concen-tration	Inoculum: Adap-tation	Test substance concentration	Degradation: Incubation period	Degradation: Degree [%]	Reference
BBA 4.1 / Destination of pesticides in the ground - degradation, transformation and metabolism (BBA leaflet No. 36 and 56)	Degradation in soil / HDO content in soil		Slightly loamy sand Sand: 87.4% Silk: 9.1% Clay: 3.5% Organic Carbon: 0.7% pH: 6.2			5 mg HDO/kg soil (Wolmanit CX-S a formulation containing Cu-HDO was used as test-substance)	DT_{50} ca. 16 days DT_{90} ca. 88 days (graphically determined) converted to standard conditions (12°C): DT_{50} = 35.6 days		Study A 7.2.1, Document III A 7.2.1
OECD 307, Commission Regulation (EC) No 440/2008 C. 23	Transformation in soil of C^{14} Cu-HDO under aerobic conditions at 21.9°C Duration:120 days 40% max. WHC 9 sampling times (without day 0)		Loamy sand 1: pH 5.5, organic carbon 1.9%, microbial biomass (start): 376 mg C/kg dw Silty sand 2: pH 6.7, organic carbon 1.0%, microbial biomass: 302 mg C/kg dw Clay loam 3: pH 7.1, organic carbon 2.5%, microbial biomass: 608 mg C/kg dw Loamy sand 4: pH 7.2, organic carbon 1.3%, microbial biomass: 351 mg C/kg dw			Application of the test substance ^{14}C-CuHDO by aliquots of an acetonic stock solution on quartz sand. Resulting concentration 3980 µ g/kg (dry weight)	1^{st} order kinetic, SAS-Procedure NLIN: Soil 1: DT_{50}: 2.3; DT_{90} : 7.7 Soil 2: DT_{50}: 2.2; DT_{90}: 7.4 Soil 3: DT_{50}: 9.5; DT_{90}: 31 Soil 4: DT_{50}: 11; DT_{90}: 35 FOMC, Model Maker 4.0, r^2>0.96: Soil 1: DT_{50}: 2.0; DT_{90}: 88.3 $T_{1/2}$: 79.1 Soil 2: DT_{50}: 2.3; DT_{90}: 20.9 $T_{1/2}$: 66.4 Soil 3: DT_{50}: 1.5; DT_{90}: 104.3 $T_{1/2}$: 107.2 Soil 4: DT_{50}: 4.5; DT_{90}: 76 $T_{1/2}$: 64		**Study A 7.2.2.1_02, Document III A 7.2.2.1_02**

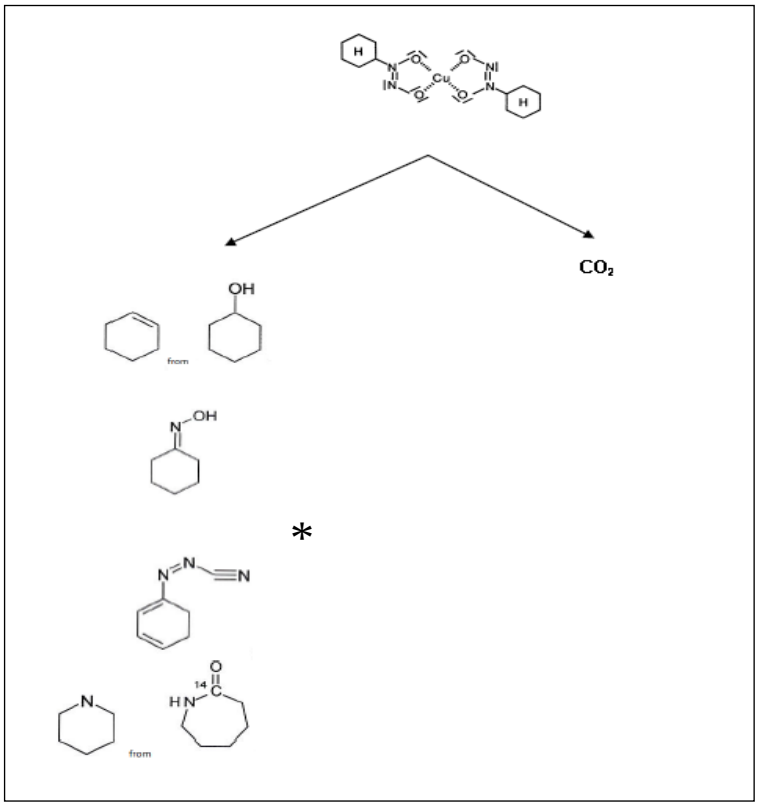
The degradability of Cu-HDO in soil has been investigated in a laboratory test according to BBA guideline 4.1 (study A 7.2.1, document III-A 7.2.1). A DT_{50} value of 16 days and a DT_{90} value of 88 days were graphically determined. Since the DT_{90} value was < 100 days no further testing was required according to the cited guideline. This study was not accepted as key study since important endpoints (primary and ultimate degradation, identification and quantification of metabolites, etc.) were not provided in the test report.

The results of the second report (**study A 7.2.2.1_02, document III A 7.2.2.1_02**) show that the behaviour of ^{14}C Cu-HDO in soils was characterized by significant degradation (mineralization) and adsorption onto soil. The degradation rate of ^{14}C Cu-HDO reached 10% TAR in all soils in the first 20 days of exposure. Then the degradation rate increased constantly to about 50-60% TAR (measured as $^{14}CO_2$) at the end of exposure. The geometric mean DT_{50}value for mineralization (FOMC) is 77.6 days (171.3 days at 12°C) for all four soil types. The amount of formed carbon dioxide showed that the ring system was broken down.
In the representative soil (loamy sand 1) there were four potential metabolites detected after 1 day. Because of matrix contamination no additional metabolites could be identified via HPLC-MS. The applicant stated that no further clean-up and analytical methods for identification and quantification for the transformation products were available. From day 85 of exposure no relevant peaks with a content ≥ 10% TAR could be detected by HPLC. Four metabolites were identified in the samples of day 1: Cyclohexene (from Cyclohexanol), Cyclohexanonoxime and Piperidine (from Caprolactam). The occurrence of metabolite $C_7H_7N_3$ (isomers possible) is nebulous, a reaction product (workup artefact) of ^{14}C Cu-HDO with the solvent acetonitrile was suggested. The study failed to gain full information on the amounts, nature and rates of formation and decline of transformation products. Therefore the description of the degradation pathway cannot be considered as complete. The suggested pathway based on the available data is shown in Figure 4.1.1.1-2. Though not detected and measured in this study it is clear that copper will also add to the transformation products. The not extractable radioactivity (NER) in soils reached about 20 to 25% TAR after the first 2-3 days and remained in this concentration range until study termination with a max. of 35% TAR in loamy sand 1 after 10 days, with a max. of 23% TAR after 10 and 23 days in silty sand and with a max. of 28% TAR in loamy sand 4. The clay loam showed the highest percentage of NER formation starting with 40% TAR after day 1 that gradually declined after day 10 to around 22% TAR after 120 day. No characterisation of the NER was performed.

The analytical measurements of ^{14}C Cu-HDO had some shortcomings (matrix effects, shifted retention times). Additionally the mass balance for the sampling times was outside the recommended range of 90-110% TAR. According to the study report this was due to the complex and difficult soil matrix and the behaviour of the test compound in soil (formation of NER). In addition the extraction solution (phosphate buffer) increased the matrix effects according to the applicant.
The rates of degradation and dissipation were analysed under the considerations of the FOCUS kinetics workgroup in an amendment to the original study. According to this study amendment the SFO (single first order) model did not match the measured degradation pattern of the parent compound. Using a first order multi compartment model (FOMC) the following geometric mean values were calculated for ^{14}C Cu-HDO: DT_{50} 2.4 days and DT_{90} 62 days (at 12°C: DT_{50} 5.7 days and DT_{90} 136 days). The original report presented the following geometric mean values (using a single first order model): DT_{50} 4.8 and DT_{90} 15.8 days for the four soils (at 12°C DT_{50} 11 and DT_{90} 34.9 days). However, only a small subset of data points was included in the calculations.
Therefore the DT_{50} of 5.7 days at 12°C (FOMC) was used for the risk characterisation. In addition PECs were also calculated based on the degradation DT_{50} (mineralization) of 171.3 days at 12°C because of the analytical shortcomings. For the groundwater FOCUS exposure modelling the DT_{50} was conservatively derived from the $DT_{90}/3.32$ = 41 day (136 days/3.32) because of the FOMC fit according to the FOCUS guidance (2006)[2].

[2] http://focus.jrc.ec.europa.eu/dk/docs/finalreportFOCDegKin04June06linked.pdf

Figure 2: Proposed pathway of degradation of ^{14}C Cu-HDO at day 1



*Molecule might be an artefact, please see text above

Conclusion:
In the study (OECD 307) a DT_{50} value for mineralization of 77.6 days at 21.9°C was determined for soil, corresponding to a DT_{50} of 171.3 days at 12°C (geometric mean value, n=4).
Therefore, according to the Guidance on the Application of the CLP Criteria v.4.1, Annex II, chapter 4, the substance is considered to be not rapidly degradable, since the pass level of an ultimate degradation of < 16 days wasn't reached.

5.1.3 Summary and discussion of degradation

5.1.3.1 According to the decision scheme concerning rapid degradation in the Guidance on the Application of the CLP Criteria v. 4.1, Annex II, chapter II.4

a) Ready biodegradability:

Cu-HDO is not readily biodegradable (< 10% biodegradation, even after prolongation of the study up to 56 days, in a Closed Bottle Test; **study A 7.1.1.2.1, document III-A 7.1.1.2.1).**
Therefore **Cu-HDO is not rapidly degradable** according to the criteria (70% DOC removal or 60% theoretical oxygen demand, within 28 days).

b) Ultimate degradation in a surface water simulation test:

There is no surface water simulation test available for Cu-HDO. In the submitted **water/sediment degradation study (Study A 7.1.2.2.2 and addendum, Document III A 7.1.2.2.2)** the mineralization rate was determined with 13.2% after 30 days of incubation. The corresponding **DT_{50} value for mineralisation was calculated with 89.1 days**, which exceeds the limit of the observed data and is therefore considered beyond the range of reliable extrapolation.
Therefore **Cu-HDO is not rapidly degradable**, since the criterion for ultimate degradation in a surface water simulation test or in a sediment simulation test, with a half-life < 16 days is neither met for the water phase nor for the sediment phase of the water/sediment simulation test.

c) Primary degradation, biotically or abiotically e.g. via hydrolysis, and demonstration that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment:

- **Hydrolysis** only occurs at temperatures ≥ 35°C for all tested pHes (3, 7, 9 and 11). Cu-HDO has been shown to be **hydrolytically stable at 25°C and at pH3 and 7 (study A 7.1.1.1.1/02, document III-A 7.1.1.1.1/02)**. It is therefore assumed that under relevant environmental conditions (5 - 25°C; pH 4 - 9) no hydrolysis will take place.
  Therefore it is concluded that the available data on hydrolysis give **no indication for rapid degradation of Cu-HDO.**

- Cu-HDO undergoes rapid primary degradation through **photolysis in water** with an experimental half-life **(DT_{50}) of 6 hours (study A 7.1.1.1.2/03).** During degradation several major and minor degradation products are formed. Though not detected and measured in this study it is clear that **copper, which fulfils the criteria for classification as hazardous to the aquatic environment**, adds to the degradation products.
  Due to the **adsorption coefficient of 30 277.4 L/kg** photolysis in water **won't represent a major degradation pathway** in the environment, since **Cu-HDO will adsorb very quickly and almost irreversible onto organic matter.**
  Therefore the DT_{50} < 16 days should **not be taken as an indication for rapid degradation of Cu-HDO** in the environment.

- In the submitted **water/sediment degradation study (Study A 7.1.2.2.2 and addendum, Document III A 7.1.2.2.2),** already mentioned under b) it could be shown that the substance undergoes **primary degradation in the total system (water and sediment) with a DT_{50} of 14.5 days at 25°C (corresponding to 41 days at 12°C).** The only major metabolite found was CO_2 (13.2% TAR). Though not detected and measured in this study it is clear that **copper, adds to the degradation products.**
  Therefore, **Cu-HDO is considered to be not rapidly degradable**, although the DT_{50} for degradation of 14.5 days (25°C) meets the criterion (DT_{50} < 16 days), according to the Guidance since the

transformation product copper, fulfils the criteria for classification as hazardous to the aquatic environment.

Additionally available data:

a) Ultimate degradation in a soil simulation test:

In the laboratory **soil degradation study (Study A 7.2.2.1/02, Document III A 7.2.2.1/02)** a DT_{50} (mineralization) of 77.6 days, at 21.9°C was determined, corresponding to 171.3 days at 12°C (geometric mean value, n=4).

Therefore, **Cu-HDO doesn't meet the criterion of ultimately degradation with a half-life < 16 days.**

5.1.3.2 Comparison with the Guidance on the Application of the CLP Criteria v. 4.1, Annex IV

"Metal compounds that contain an organic component but that dissociate easily in water or dissolve as the metal ion should be treated in the same way as metal compounds and be classified according to this annex. Organometals that do not release metal ions are thereby excluded from the guidance of this section and should be classified according to the general guidance provided in part 4 Environmental hazards, of the Guidance on the Application of the CLP Criteria."

The fact that Cu-HDO is stable to hydrolysis under environmental relevant conditions, that it is not rapidly degradable in the aquatic and terrestrial environment, and the high rates of parent compound found in the **water/sediment degradation study** (water phase: 75.4% TAR at day 0, decreasing to 2.8% TAR at day 30; sediment phase (extractable): 16.6% TAR at day 0, increasing to 45.2% at day 10 and again decreasing to 21.5% at day 30) show that Cu-HDO, being an organometal compound, cannot dissociate easily in water or dissolve as a metal ion. Cu-HDO should therefore not be treated in the same way as metal compounds but should be classified according to part 4 Environmental hazards, of the Guidance on the Application of the CLP Criteria v.4.1.

Overall conclusion:

The provided data fail to demonstrate the rapid degradability of Cu-HDO.

Cu-HDO should be classified according to part 4 Environmental hazards, of the Guidance on the Application of the CLP Criteria v.4.1.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Table 27 Adsorption onto / desorption from soils, key study

Guideline / Test method	Soil	Substance	Freundlich Koc_{ads}	Freundlich Koc_{des}	Reference
OECD 106 / Adsorption – Desorption Using a Batch Equilibrium Method	Loamy sand Sand Loamy sand Sandy silt loam Clayey loam **Mean (geometric)**	Cu-HDO	32167 8739 24884 31655 114910 **30 277.4**	893081 33339 133902 133479 - **151 883.6**	**Study A 7.1.3, document III A 7.1.3**

Adsorption onto / desorption from soil

The adsorption/desorption behaviour of Cu-HDO has been investigated in a study according to OECD 106 **(study A 7.1.3, document A 7.1.3).** Freundlich adsorption and desorption coefficients for five different soils were determined in this study. Cu-HDO showed practically irreversible adsorption, which was > 85% at equilibration time.

Conclusion: Cu-HDO strongly adsorbs to soil with a geometric mean K_{oc} value of 30 277.4 L/kg.

The most important parameters determining the distribution of copper in the soil compartment is adsorption onto solid materials and therefore the copper partitioning coefficients. As all metals, copper becomes complexed to organic and inorganic matter in waters, soil and sediments and this affects copper speciation, bioavailability and toxicity (AR, France, 2011).

Table 28a Adsorption onto / desorption from soils (additional information)

Guideline / Test method	Soil	Substance	K_{oc}(Cu-HDO)	K_{oc}(Cu)	K_{OC}(HDO)	Reference
OECD 121/ Estimation of the Adsoption Coefficient using HPLC	Cyanopropyl stationary phase	Cu-HDO	Log Koc = 1.25 Koc = 17.78	-	-	Study A 3.1.1/01
OECD 106 / Adsorption – Desorption Using a Batch Equilibrium Method	Slightly loamy sand Humous sand Loamy sand	Wolmanit CX-S and Wolmanit CX-50 (containing Cu-HDO as active substance) and their leaching samples from treated wood	-	911-5663 967-17726 1289-7269	436-5497 632-5715 238-768	Study A 7.2.3.2/01

Guideline / Test method	Soil	Substance	K_{oc}(Cu-HDO)	K_{oc}(Cu)	K_{OC}(HDO)	Reference
Lysimeter test, according to UBA concept	Slightly loamy sand	Wolmanit CX-S and its leaching samples	-	Copper concen-trations dropped from 0.28 mg/L to ≤ 0.02 mg/L after 9 months in the seepage water;	HDO could not be detected at any time in the seepage water (< 0.05 mg/L)	Study A 7.2.3.2/02

Adsorption onto / desorption from soil (additional information)

Additionally submitted studies concerning the adsorption/desorption behaviour of Cu-HDO were a HPLC screening test (study A 3.1.1/01) and a test according to OECD 106 reporting the adsorption/desorption behaviour of Cu-HDO as an active substance in complex formulations and of the corresponding leaching samples from treated wood (study A 7.2.3.2/01).

None of these two reports were considered valid or relevant for the following reasons:

In the HPLC screening test (study A 3.1.1/01) an acceptable chromatogram could only be obtained at pH 2.5 and not between pH 5.5 and 7.5, which would be normal for agricultural soils or tanks of sewage treatment plants. Therefore it was concluded that the HPLC screening method is not applicable for Cu-HDO.

In the study according to OECD 106 (study A 7.2.3.2/01) which was performed with Wolmanit CX-S and Wolmanit CX-50 (both complex formulations containing Cu-HDO as an active substance) separate K_{oc} values for Cu and for HDO were determined. No K_{oc} value for Cu-HDO was determined.

The additionally submitted lysimeter study (study A 7.2.3.2/02) was performed with Wolmanit CX-S and its leaching samples as test substances. The study was not performed according to an internationally agreed guideline. Cu and HDO were measured separately in the seepage waters. No negative control was performed.

Therefore it is not clear whether or not Cu from Wolmanit CX-S was measured in the seepage water.

Conclusion: The results of the submitted non-key studies are therefore only considered as further information.

5.2.2 Volatilisation

Table 28b

PROPERTY	PURITY / SPECIFICATION	RESULT	METHOD / REFERENCE
Vapour pressure	purified a.s. 99%w/w	**$<10^{-6}$ hPa at 50°C and at 20°C**	Dir 92/69/EEC, Annex V, A.4; **study A 3.1.1/01, document III A 3**

5.2.3 Distribution modelling

No data available

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Table 29 Estimations on aquatic bio-concentration

Basis for estimation	log KOW (measured)	Estimated BCF for Cu-HDO	Reference
Calculation	2.6	The log BCF-value can be calculated using the log K_{ow} value log BCF =0.85 x log K_{ow} -0.7 Therefore the calculated value is 1.51 and the BCF_{fish} 32.36.	TGD on Risk Assessment

The calculated log BCF of Cu-HDO in fish is 1.51, the resulting BCF_{fish} is 32.36. According to the BCF there is no risk of accumulation.

Because of the homeostasis of metals (i.e. copper), BCF values are not indicative of the potential bioaccumulation. There is therefore limited evidence of accumulation and secondary poisoning of inorganic forms of metals, and bio-magnification in food webs (AR, France, 2011). For the accumulation potential of copper and the risk for secondary poisoning please see section 4.2.4.

5.3.1.2 Measured bioaccumulation data

Not available

5.3.2 Summary and discussion of aquatic bioaccumulation

Measured BCF data are not available for Cu-HDO. According to the Guidance on the Application of the CLP Criteria v.4.1, Annex III, chapter II.5, Decision scheme, a calculated BCF value should not be used for C&L purposes. Instead the measured log K_{ow} of 2.6 has to be used.

5.4 Aquatic toxicity

Laboratory studies conducted with Cu-HDO as a test substance to assess its toxicity to aquatic organisms are summarised in Tables 30 to 35. In none of these studies the amount of dissolved copper has been measured.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

In standard laboratory tests Cu-HDO is toxic to fish, as indicated by the acute LC_{50}-values of 0.14–0.24

mg/L for rainbow trout (*Oncorhynchus mykiss*).

Table 30 Acute toxicity to fish

Guideline/Test method	Species	Endpoint/ Type of test	Exposure		Results (mg/L) measured			Remarks	Reference
			Design	Duration	LC_0	LC_{50}	LC_{100}		
OECD 203	Rainbow trout	Mortality	Static	96 h	0.066	**0.14-0.24***	0.24	—	**Study A 7.4.1.1, Document III A 7.4.1.1**

* 10% mortality at 0.14 mg/L, 100% mortality at 0.24 mg/L, no calculation of a LC_{50}

5.4.1.2 Long-term toxicity to fish

No long term test in fish was carried out with Cu-HDO, so the long-term toxicity is derived from the copper in the Cu-VRAR 2008. In this report "species mean" NOEC values for freshwater fish range from 11.6 µg/L to 120 µg/L Cu. The worst case value is used for deriving the long term toxicity of fish for Cu-HDO (Cu-HDO contains 18.16% copper).

Table 31 Comparison of the ecotoxicity data available for Cu-HDO and Cu-HDO predicted from the copper content based on the copper toxicity estimated in the Cu-VRAV 08

Substance	Fish	Daphnia	Algae
	NOEC mg/L	NOEC mg/L	NOE_rC mg/L
Cu-HDO (Test)	?	0.75	0.0562
Cu-HDO (calculated from Cu), worst case	**0.064**	Not available for daphnids (0.033)	0.236
Cu-HDO (calculated from Cu HC5 of Cu-VRAV 08)	0.043		

In the Cu-VRAV 08, the "species mean" NOEC values for freshwater algae range from 43 µg/L Cu to 138 µg/L. Using the worst case value, the equimolare toxicity of Cu-HDO for algae is 0.236 mg/L. This value is approximately 4 times higher than the toxicity value in the test with Cu-HDO which is in the range of the biological variation. So the $NOEC_{algae}$ for Cu-HDO predicted from the copper content and the measured NOE_rC value for Cu-HDO, can be seen in the range of the biological variation for algae tests.

The "species mean" NOEC values as reported in the Cu-VRAV 08 for freshwater invertebrates range from 6.0 µg Cu/L to 50.3 µg/ Cu/L. As these data cover the toxicity data of all invertebrates, the comparison with the data of daphnids only is not advisable. Additionally it should be mentioned that the lowest NOEC for freshwater invertebrates is 6.0 µg Cu/L which is lower than the lowest HC_5-50 value for Cu, calculated for an ecoregion in the VRAR which is 7.8 µg/L. This value results in a predicted HC_5-50 of 43.0 µg/L Cu-HDO, calculated on an equimolar basis.

The $NOEC_{fish}$ for Cu-HDO predicted from the copper content is reasonable when compared to the measured EC_{50}-fish (0.14-0.24 mg/L) and equal to the NOEC for Cu-HDO in the acute test (0.066 mg/L). So the toxicity of Cu-HDO can be derived on the basis of Cu in Cu-HDO

Conclusion: The long term $NOEC_{fish}$ based on the toxicity of Cu in Cu-HDO is **0.064 mg/L** (on equimolar basis).

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Cu-HDO is toxic to *Daphnia magna* with an acute EC_{50} of 1.1 mg/L.

Table 32 Acute toxicity to invertebrates

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results in mg/L (nominal confirmed)			Remarks	Reference
			Design	Duration	LC_0	LC_{50}	LC_{100}		
Directive 79/831/ EEC, Annex V, Part C.2	*Daphnia magna*	Mobility	Static	48h	0.75	**1.1**	1.5	—	**Study A 7.4.1.2, Document III A 7.4.1.2**

5.4.2.2 Long-term toxicity to aquatic invertebrates

The chronic toxicity to *Daphnia magna* was determined in a 21-day reproduction study (**study A 7.4.3.4**). The chronic NOEC, based on numbers of offspring per adult, was determined to be 0.75 mg a.i./L.

Table 33 Chronic toxicity to aquatic invertebrates

Guideline	Species	Endpoint / Type of test	Exposure		Results mg a.i./L (nominal confirmed)		Remarks	Reference
			Design	Duration	Effect	NOEC		
EEC guideline XI/681/86	*Daphnia magna*	Reproduction and mortality / chronic	Semi-static	21 days	Reproduction	**0.75**	10 concentra-tions tested, effects obser-ved in the 2 highest con-centrations (all animals dying)	**Study A 7.4.3.4 document IIIA 7.4.3.4**

5.4.3 Algae and aquatic plants

The EC_{50}-value of green algae (*Scenedesmus subspicatus*) was determined in a static test. The inhibition of the growth was determined to be 0.194 mg a.s./L, the EC_{50} of the biomass inhibition is 0.079 mg a.s./L. The NOEC of the growth rate is 0.056 mg a.s./L.

Table 34 Growth inhibition on algae

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results in mg/L (nominal confirmed)			Re-marks	Reference
			Design	Duration	NOE_rC	E_bC_{50}	E_rC_{50}		
Directive 79/831/ EEC, Annex V, Part C.3	*Scenedesmus subspicatus*	Growth and biomass inhibition	static	72h	**0.056**	0.079	0.194	—	**Study A 7.4.1.3 document III A 7.4.1.3**

5.4.4 Other aquatic organisms (including sediment)

Aquatic micro-organisms

The inhibitory effect of Cu-HDO against aquatic microbial activity was investigated in a study according to OECD 209 **(study A 7.4.1.4, document III-A 7.4.1.4)**. The nominal EC-values were graphically determined. The lowest concentration tested was 2 mg/L, which caused already about 17% inhibition of the test system. Neither a NOEC nor an EC_{10} value was determined, but instead an EC_{20} with ca. 2.5 mg/L, an EC_{50} with ca. 9 mg/L and an EC_{80} with ca. 50 mg/L of Cu-HDO.

Conclusion: Inhibitory effects at nominal concentrations ≥ 2.5 mg/L may be expected.

Table 35 Inhibition of microbial activity (aquatic)

Guideline / Test method	Species / Inoculum	Endpoint / Type of test	Exposure		Results			Re-marks	Reference
			Design	Duration	EC_{20}	EC_{50}	EC_{80}		
OECD 209 / Activated Sludge, Respiration Inhibition Test	Activated sludge	Inhibition of oxygen consumption / Respiration inhibition test	—	180 min	ca. 2.5 mg/L nominal	**ca. 9 mg/L nominal**	ca. 50 mg/L nominal	—	**Study A 7.4.1.4, document III A 7.4.1.4**

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

5.5.1 Cu-HDO

Aquatic Acute Category:

The submitted acute aquatic L(E)C_{50} values for Cu-HDO for all three trophic levels are in the range of 0.1 - 10 mg/L. The lowest reliable L(E)C_{50} value is the E_rC_{50} of 0.194 mg/L for algae (*Scenedesmus subspicatus).*

Aquatic Acute 1:

Aquatic acute toxicity: L(E)C_{50} values available for all three trophic levels in the range of 0.1 - 10 mg/L;
Lowest L(E)C_{50} values:
LC_{50} (fish) not calculated, between 0.14 and 0.24 mg/L, corresponding to 10% and 100% mortality, respectively;
E_rC_{50} (algae) =0.194 mg/L

- ➔ **Classification with Aquatic Acute 1**
- ➔ **M factor = 1**

Studies used:

- Doc. III-A 7.4.1.1: Munk R. (1993), OECD 203, Study report Acute toxicity study on the rainbow trout *(Oncorhynchus mykiss Walbaum 1792)* of Bis-(N-Cyclohexyldiazeniumdioxy)-kupfer in a static system (96 hours); -> **LC_{50} (fish) =0.14 – 0.24 mg/L**
- Doc. III-A 7.4.1.2: Elendt-Schneider (1992), Directive 79/831/ EEC, Annex V, Part C.2, Determination of the acute toxicity of Bis-(N-Cyclohexyldiazeniumdioxy)-kupfer (Cu-HDO) to the water flea *Daphnia magna Strauss* -> **EC_{50} (crustacea) =1.1 mg/L**
- Doc. III-A 7.4.1.3: Siebel-Sauer (1993), Directive 79/831/ EEC, Annex V, Part C.3, Determination of the inhibitory effect of Bis-(N-Cyclohexyldiazeniumdioxy)-kupfer, (Cu-HDO) on cell division of the green alga *Scenedesmus subspicatus* -> **E_rC_{50} (algae) =0.194 mg/L**

Aquatic Chronic Categories:

Cu-HDO isn't rapidly degradable [ready test: <10% degradation in 28 days; water/sediment simulation test: $t_{1/2}$ (mineralization; 25°C) = 89.1 days for the whole system (water and sediment); $t_{1/2}$ (degradation; 25°C) = 14.5 days for the whole system, no major metabolites found besides copper(II) ions; major component in the water phase was parent (75.4% TAR at day 0 and 2.8% TAR at day 30); in the sediment phase the major component of extractable TAR was parent as well (16.6% TAR at day 0, 45.2% at day 10 and 21.5% at day 30); non-extractable residues increased from 9.3% (day 0) up to 44% at day 30. Cu-HDO is hydrolytically stable under environmental relevant conditions (pH 3 and 7 at 25°C). Photolysis in air and water were not considered, since Cu-HDO shows a low volatility and fast and strong adsorption onto organic matter. Therefore it is assumed that only a very limited quantity of Cu-HDO will be subjected to photolysis.]

Adequate chronic toxicity data are available for all three trophic levels. The lowest chronic value is the NOE_rC from algae with 0.056 mg/L.

Aquatic Chronic 1:

Cu-HDO is not rapidly degradable. In combination with the lowest NOE_rC from algae with 0.056 mg/L this leads to a classification with Aquatic Chronic 1.

- ➔ **Classification with Aquatic Chronic 1**
- ➔ **M factor = 1**

Studies used:

- Doc. III-A 7.1.1.2.1**:** Schwarz (2001), OECD 301 D, Bis-(N-Cyclohexyldiazeniumdioxy)-copper, Determination of the biodegradability in the closed bottle test -> **<10% degradation in 28 days**
- Doc. III-A 7.1.2.2.2**:** Singh M. (2008), US-EPA subdivision N, Section 162-4 (835.4300 - study performed before revision of 835.4300 guideline in October 2008) Aerobic aquatic metabolism of ^{14}C Cu-HDO -> **$t_{1/2}$ (mineralization; 25°C) = 89.1 days; $t_{1/2}$ (degradation; 25°C) = 14.5 days**
- Doc. III-A 7.1.1.1.1/02**:** Dolich Th. (2005), EPA guideline OPPTS 835.2130, Hydrolysis as a Function of pH and Temperature of Bis-(N-Cyclohexyldiazeniumdioxy)-copper -> **Hydrolytically stable under environmental relevant conditions**
- Doc. III-A 7.4.3.2: Effects on reproduction and growth rate of fish, Justification for non-submission of data -> **NOEC (fish) =0.064 mg/L**
- Doc. III-A 7.4.3.4: Elendt-Schneider (1992), EEC XI/681/86, draft 4, Determination of the chronic toxicity of Bis-(N-Cyclohexyldiazeniumdioxy)-Kupfer, Cu-HDO to the water flea *Daphnia manga Straus* -> **NOEC (crustacea) =0.75 mg/L**
- Doc. III-A 7.4.1.3: Siebel-Sauer (1993), Directive 79/831/ EEC, Annex V, Part C.3, Determination of the inhibitory effect of Bis-(N-Cyclohexyldiazeniumdioxy)-kupfer, (Cu-HDO) on cell division of the green alga *Scenedesmus subspicatus* -> **NOE_rC (algae) =0.056 mg/L**

5.5.2 Metabolite copper (II) ion

In the dossier on Cu-HDO no data were submitted for the metabolite copper (II) ion. Meanwhile RAC opinions for several copper compounds were adopted in December 2014 (available online at http://echa.europa.eu/opinions-of-the-committee-for-risk-assessment-on-proposals-for-harmonised-classification-and-labelling), based on CLH reports prepared by France (July and December 2013).

Decisions taken in the RAC opinions:
RAC came to the final conclusion that copper (II) ions are not subject to rapid environmental transformation for the purposes of classification and labelling.
The geometric mean LC_{50} of 8.1 µg/L for *Pimephales promelas* was considered to be the relevant acute toxicity value for hazard classification.
The lowest chronic value chosen for hazard classification was the NOEC of 7.4 µg/L for *Cerodaphnia dubia.*
In the mentioned RAC opinions $ERV_{compound}$ values for the different inorganic copper compounds were calculated on the basis of the ERV values of the dissolved copper (II) ion.

Derivation of $ERV_{Cu\text{-}HDO}$ on basis of the copper content of the compound:
Acute $ERV_{Cu\text{-}HDO}$: 0.044 mg/L [{acute ERV of metal ion x molecular weight of the metal compound / (atomic weight of the metal x number of metal ions)}, so 0.0081 x 349.9 / (63.55 x 1)].
Chronic $ERV_{Cu\text{-}HDO}$: 0.041 mg/L [{chronic ERV of metal ion x molecular weight of the metal compound / (atomic weight of the metal x number of metal ions)}, so 0.0074 x 349.9 / (63.55 x 1)].

These calculated $ERV_{Cu\text{-}HDO}$ result in the following classification:
Aquatic acute category:

Lowest available $ERV_{Cu\text{-}HDO}$ is 0.044 mg/L.

Aquatic Acute 1:

- ➔ Classification with Aquatic Acute 1
- ➔ M factor = 10

Aquatic choronic categories:

Lowest available chronic $ERV_{Cu\text{-}HDO}$ is 0.041 mg/L.

Aquatic chronic 1:

- ➔ Classification with Aquatic Chronic 1
- ➔ M factor = 10

Guidance on the Application of the CLP Criteria v.4.1, Annex IV: Metals and inorganic metal compounds:

According to the guidance given, "*Organometals that do not release metal ions are thereby excluded from the guidance of this section and should be classified according to the general guidance provided in part 4 Environmental hazards, of the Guidance on the Application of the CLP Criteria. Metal compounds that contain an organic component but that dissociate easily in water or dissolve as the metal ion should be treated in the same way as metal compounds and be classified according to this annex."*

Cu-HDO is stable to hydrolysis under environmental relevant conditions, it is not rapidly degradable in the aquatic and terrestrial environment and high rates of parent compound were found in the water/sediment degradation study (water phase: 75.4% TAR at day 0, decreasing to 2.8% TAR at day 30; sediment phase (extractable): 16.6% TAR at day 0, increasing to 45.2% at day 10 and again decreasing to 21.5% at day 30). These data show that Cu-HDO, being an organometal compound, cannot dissociate easily in water or dissolve as a metal ion.

5.5.3 Overall conclusion

Degradation data show, that Cu-HDO does not dissociate easily in water or dissolve as the metal ion and it should therefore be classified according to the general guidance provided in part 4 Environmental hazards. Classification of Cu-HDO on basis of the hazards identified for the metabolite copper (II) ion, by calculating $ERV_{Cu\text{-}HDO}$ values and thereby assuming 100% release of the ion, would present an overestimation of the hazards posed for the environment by Cu-HDO. This is confirmed by the measured toxicity values for Cu-HDO, which show less toxicity than those for dissolved copper (II) ions.

It is therefore finally concluded that the proposal for classification and labelling of Cu-HDO should be based on the measured toxicity values for Cu-HDO.

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

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		 GHS 05/07/08/09	
Signal words		Danger	
Classification		Eye Dam 1 Acute Tox. 4 STOT RE 2 Aquatic Acute 1 (M=1) Aquatic Chronic 1 (M=1)	Aquatic acute 1: L(E)C_{50} values available for all three trophic levels in the range of 0.1 - 10 mg/L; lowest L(E)C_{50} values: LC_{50} (fish) between 0.14 and 0.24 mg/L; LC_{50} (fish) not calculated and E_rC_{50} (algae) =0.194 mg/L. Aquatic Chronic 1: not rapidly degradable; NOEC values available for all three trophic levels; lowest NOE_rC from algae with 0.056 mg/L.
Hazard statements		H318 - Causes serious eye damage	In vivo eye irritation test
		H302 - Harmful if swallowed	Acute gavage test
		H373 – Causes damage to organs (gastrointestinal tract) through prolonged or repeated exposure	Carcinogenicity study: local effects in GI at ~ 34 mg/kg bw
		H400 - Very toxic to aquatic life H410 - Very toxic to aquatic life with long lasting effects	
Precautionary statement	**Prevention**	P280 - Wear protective gloves/protective clothing/eye protection/face protection. P264 - Wash thoroughly after handling. P270 - Do not eat, drink or smoke when using this product. P273 – Avoid release to the environment	
	Response	P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P301 + P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P330: Rinse mouth P314: Get medical advice/attention if you feel unwell. P391: Collect spillage	
	Storage		

	Disposal	P501: Dispose of contents/container in accordance with local/regional/ national/international regulation (to be specified).	

[1]The studies submitted for the endpoints "explosives" and "oxidising properties" do not allow for a classification according to Reg. 1272/2008/EC, therefore there is no C&L with regard to these endpoints due to lacking data.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Cu-HDO is stable to hydrolysis under environmental relevant conditions, it is not rapidly degradable in the aquatic and terrestrial environment and high concentrations of parent compound were found in the water/sediment degradation study (water phase: 75.4% TAR at day 0, decreasing to 2.8% TAR at day 30; sediment phase (extractable): 16.6% TAR at day 0, increasing to 45.2% at day 10 and again decreasing to 21.5% at day 30).

Overall, the DS concluded that Cu-HDO is not rapidly degradable.

The acute aquatic toxicity L(E)C_{50} values were in the range 0.1 – 10 mg/L for all three trophic levels. The lowest E(L)C_{50} values both in the same range between 0.1 and 1 mg/L, were observed in fish, LC_{50} 0.14 – 0.24 mg/L, and in algae, E_rC_{50} = 0.194 mg/L.

The chronic aquatic toxicity, NOEC values, were available for two trophic levels only, crustaceans and algae, the lowest is the NOE_rC (algae) = 0.056 mg/L. No chronic fish study is available for Cu-HDO. In its initial proposal the DS considered using the Cu(II) ion chronic toxicity value from the Cu-VRAV 08 to fill the fish data gap. An LC_{50} = 0.064 mg/L was proposed by the DS based on the Cu content of Cu-HDO.

Initiilly, the DS proposed to classify Cu-HDO as Aquatic Acute 1, M factor = 1, since the lowest EC_{50} values are LC_{50} (fish) 0.14 – 0.24 mg/L and E_rC_{50} (algae) = 0.194 mg/L, and as Aquatic Chronic 1, M factor = 10, since the substance is not rapidly biodegradable and the lowest chronic NOE_rC value (algae) = 0.056 mg/L.

Comments received during public consultation

Comments were received by MSCAs, one comment was mainly editorial, while the other 5 concerned the use of Cu(II) data from Cu-VRAV 08 to fill the fish data gap and M-factor for the aquatic chronic classification. The DS concurred with the commenting MSCAs that there are sufficient data on the Cu-HDO to base the classification on the substance data itself and the use of the surrogate approach due to the absence of chronic fish data. They also agreed that the correct M-factor should be 1 instead of 10 as indicated in the CLH report.

Assessment and comparison with the classification criteria

Hydrolysis

The hydrolytic behaviour of Cu-HDO has been investigated in two studies.

In the study according to OPPTS guideline 835.2130 (study A 7.1.1.1.1/02, document III-A 7.1.1.1.1/02) the hydrolytic behaviour has been experimentally determined at environmentally relevant temperature (25°C) at pH 3 and 7. Under these conditions no hydrolysis occurred, the compound is stable.

In addition, the transformation products have been determined from a sample which was run at pH 3 and 70°C (Half-life, DT_{50} = 60h). The analytical determination focused on analysis for Cu-HDO and cyclohexanol, cyclohexanone, and cyclohexanonoxime as transformation products of HDO. These transformation products are the expected transformation products. Cu-HDO and cyclohexanonoxime were determined by HPLC with a UV/VIS detector. Cyclohexanol and cyclohexanone were determined by GC-FID. It could be shown that Cu-HDO hydrolyses in a parallel reaction to compounds identified as cyclohexanone (68.8% of HDO) and as cyclohexane (6.35% of HDO). Cyclohexanoneoxime was not detected (limit of detection = 0.5 mg/L). Remaining Cu-HDO was determined to be 0.45 mg/L (19.48% of HDO). Dissolved copper, was not measured in the study.

The second study (study A 7.1.1.1.1) confirms the general tendency of the key study. Measurable hydrolysis occurs under acidic conditions (pH 3-4) and temperatures ≥ 35°C. At neutral pH, hydrolysis is only observed at even higher temperatures (55°C). In alkaline pH, Cu-HDO is stable for all tested temperatures.

Conclusion:

Cu-HDO is hydrolytically stable at 25°C and at pHs 3 and 7. Hydrolysis for all tested pHs (3, 7, 9 and 11) only occurs at temperatures ≥ 35°C. It is therefore assumed that under relevant environmental conditions (5 - 25°C) no hydrolysis will take place in the pH range 4 – 9. The identified transformation products, including dissolved copper are therefore not considered relevant.

Photolysis in water

In the submitted test report (study A 7.1.1.1.2/03), photolysis of Cu-HDO in water showed rapid degradation (Lamp: Xenon lamp; intensity: 3 mW/cm² simulating a clear summer day; filter: UV filter to cut off wavelengths < 290 nm) of the test item [U-14C] Cu-HDO and the formation of cyclohexanone (45% total applied radioactivity (TAR) after 48 hours) and cyclohexanone oxime (51% TAR after 48 hours), which further degraded to volatile degradation products of low molecular weight, e.g. carbon dioxide. No other metabolite above 5% TAR occurred. Again dissolved copper, was not measured in the study, but it is clear that it will additionally contribute to the transformation products.

Cu-HDO is readily degraded by aqueous photolysis; the experimental half-life (DT_{50}) of Cu-HDO was 6 hours under irradiation. The DT_{90} of Cu-HDO is calculated to be 19.4 hours. In the dark control, no degradation of Cu-HDO was observed.

A literature method also was submitted (study A 7.1.1.1.2/01) where a filtered xenon arc lamp capable of simulating natural sunlight in the 295 to 800 nm region was used (800 W/m², 25°C). It is stated that Cu-HDO in aqueous solution undergoes rapid fragmentation upon irradiation with light (l > 290 nm) (concentration and degradation time not specified). The main degradation products of Cu-HDO are derivates of cyclohexane (cyclohexanone, methoxy-cyclohexane and 1,1-dimethyl-cyclohexane).

Biodegradation

The biodegradability of Cu-HDO has been investigated in a ready test (study A 7.1.1.2.1, document III-A 7.1.1.2.1) and in an inherent test (study A7.1.1.2.2, document III-A 7.1.1.2.2). In both studies the concentration of copper (II) ion was not measured.

In the closed bottle test (study A 7.1.1.2.1, document III-A 7.1.1.2.1), the biodegradation measured was below 10%, even after prolongation of the study up to 56 days. The substance is therefore considered as being "not readily biodegradable".

In the Zahn-Wellens test (1993; study A 7.1.1.2.2, document III-A 7.1.1.2.2) a total elimination rate of 100% was already reached after 17 days. However, 50% of that elimination took place within the first two hours, which indicates elimination due to adsorption. In that study, Cu-HDO was tested at a concentration of 6 mg/L, which in the activated sludge, respiration inhibition test (2001; study A 7.4.1.4, document III-A 7.4.1.4) was later shown to be an inhibitory concentration. These inhibitory effects were not taken into account.

In conclusion, Cu-HDO is not considered to be readily biodegradable according to the criteria (70% DOC removal or 60% theoretical oxygen demand) given in the guidance on the application of the CLP criteria v. 5.0 (CLP guidance), Annex II, chapter 4.

The degradation of ^{14}C Cu-HDO in a water/sediment system was investigated in a study according to US-EPA test guideline section 162-4 (835.4300) before revision of the guideline in 2008. Therefore, only one water/sediment system was tested (pond), the test duration was limited to 30 days and the temperature was maintained at 25°C. The applied test substance concentration was 2.2 mg ^{14}C Cu-HDO/L. A DT_{50} dissipation value of 2.4 days was calculated for the water phase (biphasic kinetics, $r^2 = 0.988$). In the sediment phase the DT_{50} for dissipation of 20.3 days was calculated (first order kinetics, $r^2 = 0.910$). The DT_{50} value for degradation in the total system was calculated to be 14.5 days (first order kinetics, $r^2 = 0.966$).

Mineralisation was determined with 13.2% after 30 days of incubation. The calculated DT_{50} for mineralisation was 89.1 days (logistic kinetics, $r^2 = 0.981$). This value exceeds the limit of observed data and is therefore considered beyond the range of reliable extrapolation.

Immediately after application, 78.2% of the TAR was found in the water phase. The radioactivity in water decreased to 5.5% TAR at day 30. The major component in the water phase was parent (75.4% TAR at day 0 and 2.8% TAR at day 30).

In the sediment phase 25.9% TAR was found at day 0 (16.6% TAR as extractable and 9.3% TAR as non-extractable residues). The extractable radioactivity content in the sediment increased to 45.2% at day 10 and then decreased to 21.5% at day 30. Most of the extractable radioactivity was the parent. The non-extractable residues continually increased up to 44% at day 30.

In the water phase as well as in the sediment phase, a number of minor metabolites were observed. The only identifiable metabolite was cyclohexanone which never exceeded 4.3% TAR (day 10) and declined over time. The only major metabolite (13.2% TAR) found was CO_2. Though not detected and measured in this study it is clear that copper will also add to the transformation products. Copper, being a chemical element is not biodegradable.

In conclusion, Cu-HDO mineralizes only up to 13.2% after 30 days at 25°C in the total system (water and sediment phase), which corresponds to a calculated DT_{50} for mineralization of 89.1 days.

The major component in the water phase (75.4% TAR at day 0 and 2.8% TAR at day 30) was the parent substance. In the sediment phase, the major component of the extractable TAR was Cu-HDO as well (extractable: 16.6% TAR at day 0, 45.2% at day 10 and 21.5% at day 30). The non-extractable residues increased from 9.3% (day 0) up to 44% at day 30.

In the water phase as well as in the sediment phase a number of minor metabolites were observed. CO_2 is the only major metabolite (13.2% TAR). Although not detected and measured in this study it is clear that copper adds to the degradation products.

According to the CLP guidance, Annex II, chapter 4, the substance is considered to be not rapidly degradable, since the criterion for ultimate degradation in a surface water test or a sediment simulation test, with a half-life < 16 days is neither met for the water phase nor for the sediment phase of the water/sediment simulation test.

Overall conclusion

Based on the above mentioned studies and information, the Cu-HDO is considered to be not rapidly degradable.

Bioaccumulation

Measured BCF data are not available for Cu-HDO. According to the CLP guidance, Annex III, chapter II.5, decision scheme, the measured log K_{OW} of 2.6 was used. Because the log K_{OW} < 4, the substance does not meet the criterion and does not have a potential for bioconcentration in aquatic organisms.

Aquatic toxicity

RAC notes that the data on degradation show that Cu-HDO, being an organometallic compound, cannot dissociate easily in water or dissolve as a metal ion and should therefore be classified according to the general guidance provided in part 4 Environmental hazards, of the Guidance on the Application of the CLP Criteria. Therefore, the only measured toxicity data for Cu-HDO were taken as basis for the classification of Cu-HDO. As a consequence, the Cu(II) fish chronic value provided by the DS was not used by RAC for the classification.

Short-term toxicity to fish

In standard laboratory tests Cu-HDO (OECD TG 203) is toxic to fish, as indicated by the acute LC_{50} values of 0.14 – 0.24 mg/L for rainbow trout (*Oncorhynchus mykiss*), study A 7.4.1.1.

Long-term toxicity to fish

No long term test in fish was carried out with Cu-HDO. So, the surrogate approach was used to cover the fish chronic study data gap (LC_{50} 0.14-0.24 mg/L, *Oncorhynchus mykiss)*.

Short-term toxicity to aquatic invertebrates

Cu-HDO is toxic to *Daphnia magna* with an acute EC_{50} of 1.1 mg/L, see study A 7.4.1.2.

Long-term toxicity to aquatic invertebrates

The chronic toxicity to *Daphnia magna* was determined in a 21-day semi-static reproduction study (study A 7.4.3.4). The chronic NOEC, based on numbers of offspring per adult, was determined to be 0.75 mg/L.

Algae and aquatic plants

The EC_{50} value of green algae (*Scenedesmus subspicatus*) was determined in a static test (72h). The inhibition of the growth (E_rC_{50}) was determined to be 0.194 mg/L, the E_bC_{50} of the biomass inhibition is 0.079 mg/L. The NOE_rC of the growth rate is 0.056 mg/L.

Comparison with the criteria

Aquatic Acute Category

The submitted acute aquatic $L(E)C_{50}$ values for Cu-HDO for all three trophic levels are in the range of 0.1 - 10 mg/L. The lowest reliable $L(E)C_{50}$ value is the E_rC_{50} of 0.194 mg/L for algae (*Scenedesmus subspicatus).*

Based on the information above, Cu-HDO fulfils the criteria to be classified as Aquatic Acute 1 with an M factor = 1.

Aquatic Chronic Category

Cu-HDO is not rapidly degradable, the most sensitive species in the long-term studies was the alage with a NOE_rC of 0.056 mg/L. Therefore, Cu-HDO fulfils the criteria to be classified as Aquatic Chronic 1 with an M factor = 1.

The same classification results after the application of the surrogate approach to cover the absence of chronic fish study. The acute fish LC_{50} 0.14-0.24 mg/L, *Oncorhynchus mykiss,* was used against the criteria in Table 4.1.0(b)(iii).

Overall, RAC agrees with the DS' proposal to classify Cu-HDO as **Aquatic Acute 1** and **Aquatic Chronic 1** with an **M factor = 1 for both acute and chronic**.

6 OTHER INFORMATION

Not available

7 REFERENCES

List of studies for the active substance:

Section No / Reference No	Year	Title	Data Protection Claimed (Y/N)	Owner
A 2.6	2004	Product identity and Composition of Bis- (N-Cyclohexyl-diazeniumdioxy)-copper, Dr. Wolman GmbH, BAS/04/1503, Germany, no GLP, unpublished	Y	Dr. Wolman GmbH
A 2.10.2	2005	Doc II-B 3.3.2 Predicted Environmental concentrations and environmental risk characterisation of Cu-HDO, Dr. Wolman GmbH, Wol 3101/2005, Germany, no GLP, unpublished	Y	Dr. Wolman GmbH
A 3.1.1/01	2001	Physico-chemical properties of Bis-(N-Cyclohexyl-diazeniumdioxy)-copper, BASF AG, Germany, BASF Report 01L00056, GLP, unpublished	Y	BASF AG
A 3.1.1/02	1999	Kalorimetrische Bestimmung der Schmelztemperatur, BASF AG, Germany, BASF Report 99 M 01618, no GLP, unpublished	Y	BASF AG
A 3.2/01	1987	Vapor pressure of cyclohexyldiazenium oxide, BASF AG, Germany, BASF Report Bru 87.129, no GLP, unpublished	Y	BASF AG
A 3.4/01	2002	Characterization of "Bis-(N-Cyclo hexyldiazeniumdioxy)-copper, BASF AG, Germany, BASF Report 02L00244, GLP, unpublished	Y	BASF AG
A 3.4/02	2001	Characterization of "Bis-(N-Cyclo hexyldiazeniumdioxy)-copper before start of ecological studies, BASF AG, Germany, BASF Report 01L00055, GLP, unpublished	Y	BASF AG
A 3.5	1992	Wasserlöslichkeit bei pH 4, pH 7 und 9 von Bis-(N-cyclohexyldiazeniumdioxy) -Kupfer, BASF AG, Germany, BASF Report 92.15.1, GLP, unpublished	Y	BASF AG
A 3.6	1992	Dissoziationskonstante von Bis-(N-cyclohexyldiazenium-dioxy)-Kupfer, BASF AG, Germany, BASF Report 92.15.2, GLP, unpublished	Y	BASF AG

A 3.7/01	1989	Solubility of bis-(N-Cyclohexyldiazenium-dioxy)-copper at 25°C in water and octanol, BASF AG, Germany, BASF Report BRU 88.277, no GLP, unpublished	Y	BASF AG
A 3.7/02	1992	Fettlöslichkeit von Bis(N-cyclohexyl diazeniumdioxy)-Kupfer bei 37°C, BASF AG, Germany, BASF Report 92.12.2, no GLP, unpublished	Y	BASF AG
A 3.9	1989	Octanol-Wasser-Verteilungskoeffizient POW von Bis-(N-cyclohexyldiazenium dioxy)-Kupfer bei 25 °C, BASF AG, Germany, BASF Report 88.276, no GLP, unpublished	Y	BASF AG
A 3.11	2001	Evaluation of safety characteristics according to 92/69/EEC, annex A9-A17, BASF AG, Germany, BASF Report SIK 01/0223, GLP, unpublished	Y	BASF AG
A 4.1	2002	Validation of a Photometer method for the determination of Bis-(N-cyclohexyl -diazeniumdioxy)-copper ($Cu(HDO)_2$ in wood preservatives, Dr. Wolman GmbH, Germany, no GLP, unpublished	Y	Dr. Wolman GmbH
A 4.2/01	2002	DIN 38414 and DIN 38406, Normausschuss Wasserwesen (NAW) im DIN Deutsches Institut für Normung e.V., 2002	N	Publication
A 4.2/03	2004	Validation of an HPLC method for the determination of Bis-(N-Cylohexyl-diazeniumdioxy-copper) in surface water; BASF AG, GKA Analytik, Study No. 03L00272, March 18, 2004, GLP, unpublished,	Y	BASF AG
A 5.3/01	1988	Bestimmung der Grenze der Wirksamkeit von LP 10458 gegenüber holzzerstörenden Basidiomyceten gemäß DIN EN 113 nach Auswaschbeanspruchung gemäß DIN EN 84 – BAM Berlin – 1988, no GLP, unpublished	Y	Dr. Wolman GmbH
A 5.3/02	1988	Bestimmung der Grenze der Wirksamkeit von LP 10458 gegenüber Coriolus versicolor gemäß DIN EN 113 nach Auswaschbeanspruchung gemäß DIN EN 84 – BAM Berlin – 1988, no GLP, unpublished	Y	Dr. Wolman GmbH

A 5.3/03	1988	Prüfung der moderfäulewidrigen Wirksamkeit von LP 10458. Bestimmung von Gewichtsverlusten nach Auswaschung der getränkten Kiefernsplintholz-Proben mit dem Vermiculit- und dem Erd-Eingrabe-Verfahren – BAM Berlin – 1988, no GLP, unpublished	Y	Dr. Wolman GmbH
A 5.3/04	1987	Giftwertbestimmung von Wolmanit CX gegenüber Eilarven des Hausbockkäfers gemäß DIN EN 47 nach Auswaschbeanspruchung des behandelten Holzes gemäß DIN EN 84 – BAM Berlin – 1987, no GLP, unpublished	Y	Dr. Wolman GmbH
A 5.3/05	2004	Composition of the formulation LP 10458 and Wolmanit CX used in the efficacy tests, no GLP, unpublished	Y	Dr. Wolman GmbH
A 5.3/06	1950	Wissenschaftliche Abhandlungen der deutschen Materialprüfungsanstalten, II. Folge, 1950, Heft 7, Ergebnisse einer vergleichenden Prüfung der insektentötenden Wirkung von Holzschutzmitteln. II. teil	N	Public
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A 6.1.1/02	1975	Report on the study of the acute oral toxicity of Cu-NCH in the rat, BASF AG, Germany, Report gl2206-1 BASF AG, no GLP, unpublished	Y	BASF AG
A 6.1.1/03	1975	Acute toxicity to rats of copper salt of NCH, Huntingdon Research Centre, division of Toxicology, BASF AG, 75/0075, no GLP; unpublished	Y	BASF AG
A 6.1.2	1975	Report on the study of the acute dermal toxicity of Cu-NCH in the rat, BASF AG, ck 180682, no GLP, unpublished	Y	BASF AG
A 6.1.3	1975	Report on the study of the acute inhalation of Kupfer-NCH in rats (inhalation hazard test), Report 2206-5 BASF AG, Department of toxicology, no GLP, unpublished	Y	BASF AG
A 6.1.4/01	1975	On the study of the acute dermal irritation/corrosion of Kupfer-NCH in the rabbit, Report ck220682 BASF AG, no GLP, unpublished	Y	BASF AG

A 6.1.4/02	1975	Report on the Study of the acute eye irritation of Cu-NCH in the rabbit Report ck220681 BASF AG, no GLP; unpublished	Y	BASF AG
A 6.1.5	1992	Report on the maximization test for the sensitising potential of Bis-(N-Cyclo-hexyldiazeniumdioxy)-Kupfer (Cu-HDO) in guinea pigs Report rr-gl:2566, BASF AG, GLP, unpublished	Y	BASF AG
A 6.2/01	1993	Study on the Comparison of the adsorption and excretion of the potassium, copper and aluminium salt of 14-C-N Cyclohexyl-hydroxi-diazeniumoxide after oral, dermal and intravenous administration to Wistar rats, Report: 22B0638/896001, BASF AG, GLP, unpublished	Y	BASF AG
A 6.2/02	2001	^{14}C-Cu-HDO Study of the Biokinetics in Rats, Report: 02B0881/006037, BASF AG, GLP, unpublished	Y	BASF AG
A 6.2/03	2002	The Metabolism of ^{14}C-Cu-HDO in Rats, Report: 2002/1004467, BASF AG, GLP, unpublished	Y	BASF AG
A 6.2/04	2006	^{14}C-Reg. No. 4041387 (Bis-(N-Cyclo-hexyldiazenium-dioxy)-copper) in Wolmanit CX (2% solution) Study of penetration through human skin in vitro, Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany, laboratory report number 52H0893 /052242, unpublished	Y	BASF AG
A 6.2/04-1	2004	A comparision between in vitro rat and human and in vivo rat skin absorption studies Human and Experimental Toxicology 23, 421-430	N	published
A 6.3.1	1991a	Report on the limited study of the oral toxicity of Bis-(N-cyclohexyldia-zenium-dioxy)-copper in rats after administration via the diet for 4 weeks, Report: 20C0124/88078, BASF AG, GLP, unpublished	Y	BASF AG
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A 6.5	1993	Report on the study of the chronic toxicity of Bis-(N-cyclohexyldiazen-ium-dioxy)-copper in rats, Report: 50C0679/89080, BASF AG, GLP, unpublished	Y	BASF AG
A 6.6.1	1987	Report on the study of Cu-HDO in the AMES TEST: 40MO254/874050, BASF AG, no GLP, unpublished	Y	BASF AG
A 6.6.3/01	1992	Rat hepatocyte DNA repair assay [UDS] in vitro: 81MO679/894495, BASF AG, GLP, unpublished	Y	BASF AG
A6.6.3/02	2005	Mutagenicity study of Xyligen LP 15671 in the mouse lymphoma forward mutation assay –in vitro-; Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany; LPT No. 18342/04, unpublished	Y	Dr.Wolman GmbH
A 6.6.4	1990	Micronucleus assay in bone marrow cells of the mouse with Bis-(N-Cyclohexyldiazeniumdioxy)-Kupfer: 26M0679/899010, Cytotest Cell Research GmbH, GLP, unpublished	Y	BASF AG
A 6.7	1996	Carcinogenicity study with Bis-N-cyclohexyl-diazeniumdioxy)-copper in Wistar rats Administration in the diet for 24 months: 70C0679/89113, BASF AG, GLP, unpublished	Y	BASF AG
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A 6.8.2/01	2007	A retrospective analysis of the added value of the rat two-generation repro ducetive toxicity study versus the rat subchronic toxicity study. Reproductive Toxicol 24: 103-113	N	published

A 6.8.2/02	2007	Strengths and limitations of using repeat-dose toxicity studies to predict effects on fertility. Reg.Tox.Pharm 48, 241-258	N	published
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A 6.8.2/04	2003	Extrapolation from results of animal studies to humans for the endpoint male fertility. Forschungsbericht Fb 984.	N	published
A 7.1.1.1.1	1993	Hydrolyse von Bis-(N-cyclohexyl-diazeniumdioxy)-Kupfer bei pH = 4, 7 und 9: Report: 92.15.3, BASF AG, GLP, unpublished	Y	BASF AG
A 7.1.1.1.1/02	2005	Hydrolysis as a Function of pH and Temperature of Bis-(N-Cyclohexyl-diazeniumdioxy)-copper, Study No: 05L00057	Y	BASF AG
A 7.1.1.1.2/01	1993	Thermal and photochemical degradation of wood preservatives, published in Fres. Envir. Bull., 2, (1993), 576-581	N	Publication
A 7.1.1.1.2/02	not indicated	Degradation of HDO in aqueous solutions exposed to UV radiation, Internal report, no GLP, unpublished	Y	Dr.Wolman GmbH
A 7.1.1.1.2/03	2006	Aqueous photolysis of Cu-HDO [-U-14C]; BASF AG., Ludwigshafen, Germany; unpublished	Y	BASF AG
A 7.1.1.2.1	2001	BIS-(N-CYCLOHEXYL-DIAZENIUMDIOXY)-COPPER, Determination of the Biodegradability in the Closed Bottle Test, Project No: 00/0801/23/1, BASF AG, GLP, unpublished	Y	BASF AG
A 7.1.1.2.2	1993	Determination of the biodegradability or the Elimination of BIS-(N-CYCLOHEXYL-DIAZENIUM-DIOXY)-COPPER, Cu-HDO in the Zahn-Wellens-Test: Report 92/1699 /10/1, BASF AG, no GLP, unpublished	Y	BASF AG
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A 7.2.3.2/02	1992	Mobility of Active Ingredients from Wolmanit CX Pressure Treated Wood in soil – Lysimeter Test, Report No: 314, no GLP, unpublished	Y	Dr.Wolman GmbH
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A 7.4.1.4	2001	Determination of the Inhibition of Oxygen Consumption by Activated Sludge in the Activated Sludge Respiration Inhibition test: Report 00/0801/08/2, BASF AG, GLP, unpublished	Y	BASF AG
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A 7.4.3.2/08	1979	Chronic effect of copper on the bluntnose minnow, *Pimephales notatus* (Rafinesque). Arch Environ Contam Toxicol, 8: 545-552.	N	Public
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1 ANNEXES

Throughout the CLH-Report references are made to the Competent Authority Report (CAR) on bis (N-cyclohexyl-diazenium-dioxy)-copper (Cu-HDO), which has been finalised by the Standing Committee on Biocidal Products during its meeting held on 13 December 2013.

Attached to IUCLID section 13 you will find the following parts of the CAR

DOC IIA

DOC IIA confidential

DOC IIIA (confidential version)

DOC IIIA (non-confidential version)