Committee for Risk Assessment

RAC

Annex 1

Background document

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

potassium (oxido-NNO-azoxy)cyclohexane; cyclohexylhydroxydiazene 1-oxide, potassium salt; [K-HDO]

EC Number: -
CAS Number: 66603-10-9

CLH-O-0000001412-86-248/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

30 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: K-HDO (Cyclohexylhydroxydiazene 1-oxide, potassium salt)

EC Number: **not attributed**

CAS Number: **66603-10-9**

Index Number:

Contact details for dossier submitter:

Umweltbundesamt GMbH

on behalf of

AT Competent Authority

Federal Ministry of Agriculture, Forestry, Environment and Water Management

Version number: 5 **Date: 25. August 2017**

CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Cyclohexylhydroxydiazene 1-oxide, potassium salt
EC number:	not attributed
CAS number:	66603-10-9
Annex VI Index number:	***Not available***
Degree of purity:	97,69 % w/w
Impurities:	See 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	Flam. Solid 1; H228 Acute Tox. 3 - H301 Skin Irrit. 2 - H315 Eye Damage 1 -H318: STOT Rep. Exp. 2 - H373: May cause damage to organs (gastrointestinal tract, liver, kidney) through prolonged or repeated exposure Aquatic chronic 2 – H411
Resulting harmonised classification (future entry in Annex VI, CLP	Flam. Solid 1; H228 Acute Tox. 3 - H301 Skin Irrit. 2 - H315

Regulation)	Eye Damage 1 - H318: STOT Rep. Exp. 2 - H373: May cause damage to organs (gastrointestinal tract, liver, kidney) through prolonged or repeated exposure Aquatic chronic 2 – H411

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

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

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives				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	Flam. Solid 1; H228			
2.8.	Self-reactive substances and mixtures				data lacking
2.9.	Pyrophoric liquids				data lacking
2.10.	Pyrophoric solids				data lacking
2.11.	Self-heating substances and mixtures				data lacking
2.12.	Substances and mixtures which in contact with water emit flammable gases				data lacking
2.13.	Oxidising liquids				data lacking
2.14.	Oxidising solids				data lacking
2.15.	Organic peroxides				data lacking
2.16.	Substance and mixtures corrosive to metals				data lacking
3.1.	Acute toxicity - oral	Acute Tox. 3 ; H301: Toxic if swallowed.			
	Acute toxicity - dermal				conclusive but not sufficient for classification
	Acute toxicity - inhalation				conclusive but not sufficient for classification
3.2.	Skin corrosion /	Skin Irrit. 2; H315: Causes skin			

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
	irritation	irritation.			
3.3.	Serious eye damage / eye irritation	Eye Damage 1; H318: Causes serious eye damage.			
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	STOT Rep. Exp. 2; H373: May cause damage to organs (gastrointestinal tract, liver, kidney) through prolonged or repeated exposure			
3.10.	Aspiration hazard				conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Chronic 2 H411: Toxic to aquatic life with long lasting effects			
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: Signal word: Danger

Pictograms: GHS 02/05/06/08/09

Hazard statements:

H228 - Flammable solid

H318 - Causes serious eye damage

H315 - Causes skin irritation

H301 – Toxic if swallowed

H373 – May cause damage to organs (gastrointestinal tract, liver, kidney) through prolonged or repeated exposure

H411 – Toxic to aquatic life with long lasting effects

Precautionary statements:

P 210: Keep away from heat/sparks/open flames/hot surfaces. — No smoking.

P 240: 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

P370 + P378: In case of fire: Use sprayed water, foam, CO_2, extinguishing powder or sand for extinction.

P302 + P352: IF ON SKIN: Wash with plenty of soap and water.

P332 + P313 If skin irritation occurs: Get medical advice/attention.

P362: Take off contaminated clothing and wash before reuse

P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310: Immediately call a POISON CENTER or doctor/physician.

P301 + P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.

P330: Rinse mouth

P391: Collect spillage

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

The active substance "Cyclohexylhydroxydiazene 1-oxide, potassium salt" is not yet included in Table 3.1 of Annex VI of Regulation (EC) No 1272/2008, therefore, no current classification is available.

2.2 Short summary of the scientific justification for the CLH proposal

Flam. Sol 1 H228 Flammable solid	The purified active substance (99.8 %w/w, monohydrate) is highly flammable according Dir 92/69/EEC, Annex V, A.10. In addition read across from Cu-HDO indicates respective classification
Eye Damage 1 H318 - Causes serious eye damage	In vivo eye irritation test
Skin Irrit. 2 H315 - Causes skin irritation	In vivo skin irritation test
Acute Tox. 3 H301 – Toxic if swallowed	In vivo acute gavage test
STOT Rep. Exp. 2 H373 – May cause damage to organs (gastrointestinal tract, liver, kidney) through prolonged or repeated exposure	Read across from Cu-HDO data to K-HDO: WoE analysis of Cu-HDO data 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.
Aquatic chronic 2 H411 – Toxic to aquatic life with long lasting effects	Aquatic acute toxicity: L(E)C_{50} values 10 – 100 mg/L, therefore no acute classification; Aquatic chronic toxicity: chronic NOEC values for all three trophic levels available and lowest chronic NOEC values between 0.1 and 1 mg/L; Fate & behaviour: not rapidly biodegradable;

2.3 Current harmonised classification and labelling

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

No current classification and labelling available.

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

No current classification and labelling available.

2.4 Current self-classification and labelling

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

No current classification and labelling available.

2.4.2 Current self-classification and labelling based on DSD criteria

Please see https://echa.europa.eu/information-on-chemicals/cl-inventory-database/-/discli/details/31132) for self-classification according to CLP.

RAC general comment
The Dossier Submitter (DS) has supplemented the limited toxicological information on K-HDO, especially for repeated dose toxicity, reproduction and carcinogenicity by reading relevant data across from Cu-HDO to K-HDO. The following arguments are relevant for the read-across assessment: • The HDO^- anion derived by dissociation from Cu-HDO and from K-HDO is structurally identical. • The toxicological differences in the toxicity profile of Cu-HDO and K-HDO were related to the different effects of the Cu^{2+} and K^+ ions.

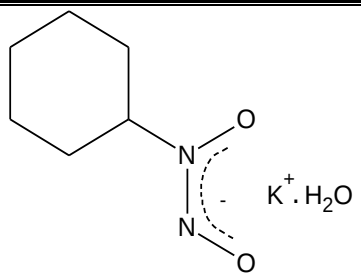


Fig. 1 K-HDO Cyclohexylhydroxydiazene 1-oxide, potassium salt

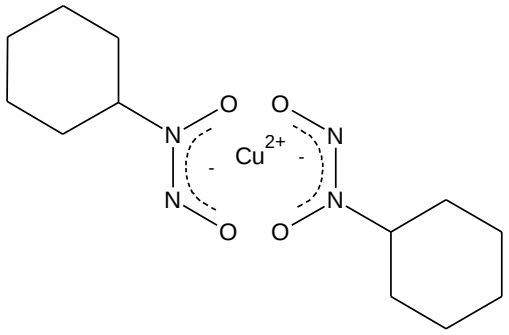


Fig. 2 Cu-HDO Cyclohexylhydroxydiazene 1-oxide, cupper salt

- Cu-HDO contains two HDO^--ions per molecule, while K-HDO only contains one. The atomic weight of K is 39.098 g/mol and the atomic weight of Cu is 63.546 g/mol. The overall molecular weight of K-HDO is 182.3 g/mol and Cu-HDO is 349.9 g/mol. It is assumed that the toxicity is related to the HDO^--ion, therefore it is necessary to take into account that Cu-HDO will release twice as many HDO^-- ions compared to K-HDO. For example, one mole of HDO^- ions will be released by 182.3 g of K-HDO and 174.95 (i.e. 349.9/2) g of Cu-HDO. It can therefore be considered that the difference is very low, and can be ignored in the read-across assessment.
- Cu-HDO and K-HDO showed similar distribution and excretion rates, which are: ready absorption across the GI tract, rapid elimination mainly via urine, no bioaccumulation, plasma levels below 0.1% of the dose and limited absorption via the skin (~8%) (Hoffmann *et al.,* 1993, IIIA.6.2.1, Gamer *et al.*, 2006, IIIA 6.2.4). The kinetics might not have been expected to be comparable since the $\log K_{OW}$ differs (Cu-HDO 2.6 vs. K-HDO -0.2), however, the $\log K_{OW}$ does not necessarily contradict the toxicokinetic findings since biological media are more complex than a simple two-phase-system.
- Comparable kinetics and the identical chemical structure of the HDO^- anion support the assumption of a comparable metabolism.
- Potassium is the quantitatively most important intracellular cation and its concentration gradient towards the extracellular space is responsible for the membrane potential. As such it is important for the functioning of the nervous system, cardiac, skeletal and smooth muscles and epithelia and its homeostasis is usually strictly controlled by renal regulation and influenced by the acid-base state of extracellular liquids. The neurotoxic effects seen only with gavage application of K-HDO (and not with exposure via feed) could be interpreted to result from a K^+ peak in

the plasma disturbing the normally rigidly controlled K^+ homeostasis.

- In contrast, copper is an essential metal, and it is employed in all human cells involved in the reactions and functions of many enzymes, including angiogenesis, neurohormone release, oxygen transport and regulation of genetic expression. Homeostatic maintenance of copper requires the tightly coordinated control of copper uptake, distribution and efflux in cells and the organism as a whole. High dose exposure may lead to local effects in the gastrointestinal-tract, effects in the liver and kidneys.
- Except for the differences that are related to the Cu^{2+} and K^+ cations the toxicity profiles of Cu-HDO and K-HDO do not diverge based on the tests available for both substances.

RAC is of the opinion that a read across of appropriate data from Cu-HDO to K-HDO is fully justified on the above basis in the absence of studies assessing the reproductive toxicity and carcinogenicity of K-HDO and the limited data available for K-HDO for repeated dose toxicity.

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.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON POTASSIUM (OXIDO-NNO-AZOXY)CYCLOHEXANE; CYCLOHEXYLHYDROXYDIAZENE 1-OXIDE, POTASSIUM SALT; [K-HDO]

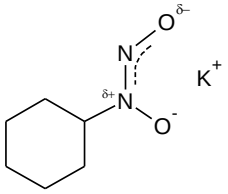
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	not attributed
EC name:	not attributed
CAS number (EC inventory):	not attributed
CAS number:	66603-10-9
CAS name:	Diazene, cyclohexylhydroxy-, 1-oxide, potassium salt
IUPAC name:	Cyclohexylhydroxydiazene 1-oxide, potassium salt potassium (oxido-NNO-azoxy)cyclohexane
CLP Annex VI Index number:	not attributed
Molecular formula:	C6H11KN2O2
Molecular weight range:	182.3

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Cyclohexylhydroxydiazene 1-oxide, potassium salt	97.69 % w/w	95.96 to 99.16 % w/w	

Current Annex VI entry: not yet included

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 yet included

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 as lied down by the 5-batch analysis mentioned above. 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: Summary of physico - chemical properties

PROPERTY	PURITY / SPECIFICATION	RESULT	METHOD[1] / REFERENCE[2]
Melting Point	purified a.s.	163.1°C	OECD Guideline 102; Büldt 2001; **A 3.1.1/01**
Boiling Point	purified a.s.	not detectable due to decomposition at about 210 °C	company's statement; Büldt 2001; **A 3.1.1/01**
Relative Density	purified a.s.	1.431 ± 0.001 at 20°C	OECD Guideline 109; Büldt 2001; **A 3.1.1/01**
Vapour pressure	purified a.s.	< 10^{-6} hPa at 50°C and at 20°C	Dir 92/69/EEC, Annex V, A.4; Büldt 2001; **A 3.1.1/01**
Henry's Law Constant	not applicable	$4.4 \cdot 10^{-11}$ kPa·m^3·mol^{-1}	calculated
Physical state	purified a.s.	solid (crystalline)	visual inspection; Schmidt 2001, **A 3.4/01**; Krack 2004, **A 3.4/03**
	a.s. as 30% aqueous solution	liquid	
Colour	purified a.s.	white	visual inspection; Schmidt 2001, **A 3.4/01**; Krack 2004, **A 3.4/03**
	a.s. as 30% aqueous solution	yellowish	
Odour	purified a.s.	weak	olfactory inspection; Schmidt 2001, **A 3.4/01**; Krack 2004, **A 3.4/03**
	a.s. as 30% aqueous solution	characteristic	
Absorption spectra	purified a.s. and a.s. as 30% aqueous solution	UV/VIS absorption spectra: absorption maximum at 237 nm. The structure of K-HDO is confirmed by all spectra.	OECD Guideline 101; Schmidt 2001, **A 3.4/01**; Euler 2004 **A 3.4/02**; Krack 2004, **A 3.4/03**
Solubility in water	purified a.s.	452 g·L^{-1} at 20°C; pH = 10.4	Dir 92/69/EEC, Annex V, A.6;flask method

			Büldt 2001; **A 3.1.1/01**
Dissociation constant	purified a.s.	pKa = 5.33 ± 0.02	OECD Guideline 112; Büldt 2001; **A 3.1.1/01**
Solubility in organic solvents	purified a.s.	54%(w/w) in ethylene glycol, readily soluble in ethanol, methanol and dimethylformamide	company's statement; Dr. Wolman GmbH 2004, **A 3.7**

Table 9 Summary of physico - chemical properties

contd.

Partition coefficient octanol-water	purified a.s.	log Pow = -0.2 at 25°C and pH 7.2	Dir 92/69/EEC, Annex V, A.8; shake flash method Büldt 2001; **A 3.1.1/01**
Thermal stability	purified a.s.	decomposition at about 210 °C	OECD Guideline 102; Büldt 2001; **A 3.1.1/01**
Flammability	purified a.s.	highly flammable	Dir 92/69/EEC, Annex V, A.10; Löffler 2001a; **A 3.11**
Auto-flammability	purified a.s.	No self ignition at temperatures up to the melting point (163.1°C)	Dir 92/69/EEC, Annex V, A.16; Büldt 2001; **A 3.1.1/01**
Flash Point	purified a.s.	not applicable for solids	Löffler 2001a; **A 3.11**; company's statement
	a.s. as 30% aqueous solution	no flash point due to the high water content	
Surface tension	purified a.s.	71.4 mN/m at 20°C (not surface active; concentration of test solution: 1 g/L)	OECD Guideline 115; Büldt 2001; **A 3.1.1/01**
Viscosity	a.s. as 30% aqueous solution	4.6 mPa s at 20 °C 3.4 MPA S AT 40 °C	OECD GUIDELINE 114; WITTENZELLNER 2004D , **B 3.11**
Explosive properties	purified a.s.	not explosive	Dir 92/69/EEC, Annex V, A.14; Löffler 2001a; **A 3.11**
Oxidising properties	purified a.s.	not oxidising	Dir 92/69/EEC, Annex V, A.17; Löffler 2001a; **A 3.11**
Reactivity towards container material	a.s. as 30% aqueous solution	Xyligen 30 F is stable in the original containers for several years	company's statement; Wittenzellner 2003d; **B 3.7/03**
Granulometry		no data available	

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

[2] bold reference numbers are indicating key studies.

2 MANUFACTURE AND USES

2.1 Manufacture

Detailed information on the manufacturing process(es) is provided in the confidential annex of the DAR.

2.2 Identified uses

K-HDO is a wood preservative (PT 8) fungicide with a broad spectrum of action against wood-destroying Basidiomycetes.

Here K-HDO is evaluated for use as a wood preservative according to product type 8 of the Biocidal Products Directive 98/8/EC.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

PROPERTY	PURITY / SPECIFICATION	RESULT	METHOD[1]
Thermal stability	purified a.s.	decomposition at about 210°C	OECD Guideline 102
Flammability	purified a.s.	highly flammable	Dir 92/69/EEC, Annex V, A.10
Auto-flammability	purified a.s.	relative self- ignition temperature: 250°C	Dir 92/69/EEC, Annex V, A.16
Flash Point	a.s. as 30% aqueous solution	no flash point due to the high water content	company's statement
Explosive properties	purified a.s.	not explosive	Dir 92/69/EEC, Annex V, A.14
Oxidising properties	purified a.s.	not oxidising	Dir 92/69/EEC, Annex V, A.17
Reactivity towards container material	a.s. as 30% aqueous solution	Xyligen 30 F is stable in the original containers for several years	company's statement

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

3.1 Flamability

The purified active substance (99.8 %w/w, monohydrate) has been tested according method A.10 as given in Dir 92/69/EEC, Annex V. The test results showed a burning time of 23 s. The test substance was therefore considered as highly flammable.

For correct classification according EC 1272/2008 a test according UN test N.1 would be necessary, but such data is currently not available. Although the respective test result according Dir 92/69/EEC is not convertible to test conditions as laid down by EC 1272/2008 it can be concluded that K-HDO will also be considered as highly flammable according CLP rules. Nevertheless the data available does not allow to distinguish between Flam. Sol. 1 or Flam. Sol. 2.

Currently the C&L inventory holds only one notification for K-HDO, stating the substance as Flam. Sol. 1.

For the structural very similar substance Cu-HDO (bis[1-cyclohexyl-1,2-di(hydroxyl-κO)diazeniumato(2-)]copper; CAS No. 312600-89-8) a test according UN test N.1 is available, which shows that the test substance fulfils the criteria for classification as flammable solid, category 1. For details see the CLH-report for Cu-HDO.

Considering the arguments listed above it is suggested to classify K-HDO as Flam. Sol. 1.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

An experimental study on K-HDO was included by the DS in the CLH report. In addition, based on the structural very similar substance Cu-HDO (bis[1-cyclohexyl-1,2-di(hydroxyl)diazeniumato(2-)]copper; CAS No. 312600-89-8) the DS suggested to classify K-HDO as Flam. Sol. 1 and no classification as explosive or oxidising substance.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

Purified K-HDO (99.8% w/w, monohydrate) was tested according to method A.10 as given in Dir 92/69/EEC, Annex V. The test results showed a burning time of 23 s. The test substance was therefore considered as highly flammable.

For correct classification according EC 1272/2008 a test according UN test N.1 would be necessary, but such data is currently not available. Although the respective test result according Dir 92/69/EEC is not convertible to test conditions as laid down by EC 1272/2008 it can be concluded that K-HDO will also be considered as highly flammable according CLP . Nevertheless the data available does not allow any distinction between Flam. Sol. 1 or Flam. Sol. 2.

For the structurally very similar substance Cu-HDO, a test according UN test N.1 is

available, which showed that the test substance fulfils the criteria for classification as flammable solid, category 1.

Considering the arguments listed above RAC agrees with the DS proposal to classify K-HDO as **Flam. Sol. 1**.

Oxidising solids

In the train test, the maximum burning rate of test mixtures is 3.4 mm/s (80% w/w of test substance, 20% w/w of cellulose) compared to 5 mm/s of the test reference (bariumnitrate/cellulose mixture). RAC concludes K-HDO should **not be classified as oxidising solid**.

Explosive properties

Based on the data provided by Löffler (2001), according to 92/69/EEC, annex A9-A17), the test substance is not considered to present a danger of explosion and therefore RAC concludes K-HDO should **not be classified as explosive**.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The available rat study with purified K-HDO (Hoffmann 1993, docIIIA6.2.1.) shows that, after oral administration either by gavage or in the diet, the organic moiety is readily absorbed across the GI tract and rapidly eliminated with virtually no bioaccumulation. This contention is supported by the fact that 48 hours after application no radioactivity was found in urine and faeces, the level in carcass after 72 hours was below 1% and plasma levels remained below 0.1% of the applied radioactivity during the whole study. Excretion occurred mainly via the urine (>95%). A significant amount of radioactivity was detected in bile samples (31%) which suggest that HDO is subject to enterohepatic circulation.

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 IIIA6.2.4, discussion see below) 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.

Within the study of Fabian 2002 (docIIIA6.2.3) it was shown that after administration by oral gavage the major part (58-72%) of Cu-HDO is metabolised to the glucuronide of the free ligand, N-cyclohexyldiazeniumdioxyglucuronide. 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). This indicates a deglucuronidation process in the gut, and since the total faeces excretion is considerably lower than the amount recovered from bile, it is concluded that re-absorption occurs in the gut as part of an enterohepatic circulation. 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.

The **dermal absorption tests** carried out within the study of Hoffmann 1993 indicate that K-HDO uptake via the skin is limited to 4 % of the applied dose. However since the recovery rate for several of the tests were below 90% and only 2 instead of 4 animals were used for the toxicokinetic tests a new in vitro absorption study with human skin samples was carried out by Gamer et al. 2006a (doc IIIA6.2.4). The study was carried out with K-HDO as manufactured that is the 30% aqueous solution and an exposure time of 24 hours. The total decrease in the donor fluid was about 20% 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. The discussion of the dermal absorption rate to be deduced from this study for the risk assessment has lead to two positions: 18.7 % and 8%. The discussion is reflected in the table 11.

Table 11: Discussion of dermal absorption rate

% of applied dose		Pro 18.7 % absorption rate	Pro 8% absorption rate
tape strips after 24h	2.2	Should be considered as absorbed, since 1. No single tape strips analysis is available, the 6 tape strips were pooled for analysis, and therefore the tape strip analysis is not reliable enough. 2. It is a 'precautious' common practise to include that in the calculation of the absorbed percentage. However, in this case it even seems likely that the amount in the stratum corneum will be absorbed, because, at the 24 hours time-point, much more substance had passed through the skin (14%) than was present in the skin (2.2% in SC, and in total 5% if including the epidermis). This indicates a quite fast transport through the skin, and that the substance in the skin is indeed available to absorption, even the amount in the stratum corneum.	Should be considered as unabsorbed, since 1. one difference between the study with K-HDO and the one with Dichlofluanid is that with the latter it is not clear how many tape strips were used to detach the stratum corneum. The OECD Guidance document for the conduct of skin absorption studies (No 28) recommends 15-25 tape strips to take off the total stratum corneum. In the case with K-HDO only 6 tape strips were used, consequently the detached amount should not represent the total but only the superficial stratum corneum. A more detailed balance of the amount detached with each individual strip is a question of analytical sensitivity. For this reason the strips have been pooled to arrive at an accurate determination of this fraction of the test substance. 2. The argument on the relative amount passed and retained in the skin is not sufficiently convincing to conclude a complete incorporation of the stratum corneum bound amount. It has to be considered that exposure continued for 24 hours and it could be that the high external concentration is important for the driving force through the stratum corneum. It should be taken into consideration that K-HDO has a very high water solubility of 452 g/l and very low log Pow of -0,2 which is (e.g. according to the TGD p 263) rate limiting. It could well be that low amounts of the substance are sufficient for the saturation of the retention capacity in the stratum corneum and uptake happens only for the excess substance continuously supplied from the external side
membrane washing (24h)	80.4	Not absorbed	Not absorbed
Skin preparation after 24 h	2.9	Should be included in the amount absorbed after 24 hours.	Should be used as estimate for amount remaining in the skin at the 10 hours-time point since the amount in the skin is not likely to decrease with continuous exposure between 10 and 24 hours. Furthermore the increase of the cumulative absorbed dose over time was linear between 4 and 24 hours.
Receptor fluid after 10h	5	The 10 hour value should not be used (for arguments see below)	The cumulative absorption in the receptor fluid after 10 hours should be used since it is assumed that the worker will wash his hands latest after 10 hours of work.

Table 11: Discussion of dermal absorption rate

contd.

Receptor fluid after 24h	13.6	The 24 hour value should be used for the risk assessment, since 1. the proper method for working place exposure would have been to apply the product for 10h and analyse the absorption into the receptor fluid till 24 hours. Estimates for the 10 hour time point are not reliable enough. 2. a short-coming with this study is that the applied amount (3 mg K-HDO/cm^2) is ca. 10-fold higher than the highest exposure estimate in the exposure assessment (260 µg/cm2). It is clear that the percentage absorption is highly dependent on the amount applied (the higher amount applied, the lower the percentage), and the obtained absorption percentage may thus not completely correspond with the exposure conditions in the risk assessment. 3.. the nominal amount of test substance preparation applied (10mg or µl/cm^2) is useful for finite dose experiments (preferred for occupational scenarios) whereas for infinite dose doses >100 µl/cm2 are required to obtain steady-state conditions from which the steady-state flux or absorption rate and the permeability coefficient Kp can be calculated. Estimates of steady state flux and permeability coefficients should include data only from times greater than the time to reach steady state. Including data for times before the steady state is established will lead to a false estimate of the permeability coefficient (Environmental Health Criteria 235, WHO, 2006). The way the Kp was calculated by the applicant is not in accordance with the guidance above.	The 24 hour value should not be used for the risk assessment, since 1. the amount in the receptor fluid was measured after 10 hours and the amount in the skin is not likely to decrease with continuous exposure between 10 and 24 hours. The amount in the skin after 24 hours is a reliable estimate for the 10 hours time point. 2. the dose results from the necessary volume of the product to cover the skin sample. Furthermore the difference between the experimental dose and exposure dose is a general problem with all kinetic studies and difficult to meet since several diverse exposure doses can be relevant. As long as the difference of dose/cm2 is not higher than a factor of about 10, the results should be sufficiently reliable considering that usually total safety factors of at least 100 are applied. Furthermore in the specific case of the K-HDO study an increase of the cumulative uptake is evident after 4 hours indicating a better functional barrier with short term exposure situations, eventually also because of the irritant properties of K-HDO. It is expected that in many cases exposure will be shorter than 4 hours; nevertheless the upake rate from 10 hours exposure was used for the risk assessment. Therefore overall some conservative assumption is included in the derivation of the dermal uptake rate which could partly compensate the difference between experiment and real-life exposure concentrations. 3. the total decrease in the donor fluid till 24 hours is below 20% and since after a lag phase of 4 hours the cumulative absorbed dose is linear with time up to 24 hours the situation could be considered at least semi-static and the calculation of the permeability constant (based on the steepest part of the cumulative dose – time curve) at least approximately correct. However the permeability coefficient is not used in the risk assessment.

Overall there seems to be sufficient evidence to support both interpretations. In order to understand the impact of the two interpretations the risk characterisation is calculated with both values (18.7% and with 8%) resulting in 2 sub-tiers. For product authorisation the 18.7% value should be used as tier 1 assessment whereas the 8% value could be used for higher tier refinements.

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 12 acute toxicity tests, oral route

Test substance	Method Guideline	Species Strain Sex no/group	dose levels duration of exposure	Value LD50/LC50	Remarks	Reference[1] (Doc IV)
purified K-HDO (99.8%)	BASF test – 1977 no GLP	Sprague-Dawley rats m/f	56,2, 68,1, 82,5, 100, 121, 147, 178, 215, 261 mg/kg single gavage administration	136 mg K-HDO/kg bw corresponds to 452 mg (30%w/w K-HDO) /kg bw	acute neurological effects also at low dose level	**A 6.1.1** Munk, Gelbke, 1977
K-HDO as manufactured (30%w/w)	The study was performed before the instillation of the respective OECD guideline; no GLP	Sprague-Dawley rats; 10 male and 10 female/group	200/250/320/400/800/1600 mm^3/kg bw ~ 226/282.5/361.6/452/904/1808 mg/kg bw	400 mm^3 (30%w/w K-HDO) / kg ~ 452 mg (30%w/w K-HDO) /kg bw corresponds to 136 mg K-HDO/kg bw	Acute neurotoxic effects, necropsy animals which died: flaccid intestinal tract containing much fluid	**B 6.1.1.** Hofmann 1971b

[1] bold references are indicating key studies.

4.2.1.2 Acute toxicity: inhalation

Table 13 acute toxicity tests, inhalative route

Test substance	Method Guideline	Species Strain Sex no/group	dose levels duration of exposure	Value LD50/LC50	Remarks	Reference[1] (Doc IV)
manufactured (30%w/w)	OECD Guideline method 403 GLP	Wistar rat ClrGlxBrl Han:WI 10 (5 males / 5 females) per group	aerosol; 1.2 mg/l and 7.8 mg/l; 4 hours exposure	LC_{50} > 7.8 mg K-HDO/L	No mortality; observed effects: accelerated or slower respiration, squatting posture, smeared fur; generally until including day 2; apathy only day 0, attempts to escape ≤ 1h	**A 6.1.3.1** Gamer, Leibold, Hofmann, 2001
K-HDO as manu-factured (30%w/w)	Acute inhalation hazard test, BASF AG, 1971t No GLP	rats 12 animals (m+f)	Atmosphere saturated with vapour at 20°C 8 h exposure	LC_{50} > 1.33 mg K-HDO/L	No effects observed, but study not reliable, since no exposure measurements	A 6.1.3.2 Hofmann, 1971a

[1] bold references are indicating key studies.

4.2.1.3 Acute toxicity: dermal

Table 14 acute toxicity tests, dermal route

Test substance	Method Guideline	Species Strain Sex no/group	dose levels duration of exposure	Value LD50/LC50	Remarks	Reference[1] (Doc IV)
purified K-HDO (99.8%)	BASF test-1979 no GLP	Sprague-Dawley rats	2500 mg (50%w/w K-HDO) /kg bw for 24 h	> 2500 mg (50%w/w K-HDO) /kg bw corresponds to > 1250 mg K-HDO/kg bw	No effects observed; reliability 2	**A 6.1.2** Zeller 1979
K-HDO as manufactured (30%w/w)	method of D.N. Noakes and D.M. Sanderson: "A Method for Determining the Dermal Toxicity of Pesticides"; Brit. J. Industr. Med. 26, 59 (1969) no GLP	Sprague-Dawley rats, 10 males and 10 females	5000 mm³/kg bw for 24 h ~ 5650 mg/kg bw for 24 h	> 5650 mg (30%w/w K-HDO)/ kg bw corresponds to > ~1700 mg K-HDO /kg bw	No signs of toxicity were observed. No local effects. Internal organs showed no gross pathological abnormalities	**B 6.1.2** Zeller 1971b

[1] bold references are indicating key studies.

4.2.1.4 Acute toxicity: other routes

No data available.

4.2.2 Human information

Not available

4.2.3 Summary and discussion of acute toxicity

The acute toxicity of K-HDO was tested by the oral and dermal route as well as by the inhalative route. All tests were conducted using male and female rats. Most studies were performed prior to requirement of GLP and of the corresponding OECD guidelines. Since the studies are well reported and consistent they are acceptable.

The LD50, rat, oral of purified K-HDO amounts to 136 mg/kg bw and should lead to the assignment of acute toxicity category 3, H301, Toxic if swallowed.

By contrast, the active substance as manufactured (K-HDO as 30%w/w aqueous solution) is not toxic due to the high water content: K-HDO as 30%w/w aqueous solution has an LD50, rat, oral given by 452 mg/kg bw and should be classified as acute toxicity category 4, H302 – Harmful if swallowed.

The active substance K-HDO does not display any acute systemic toxicity by the dermal route: The acute dermal LD50 performed in rats is > 1700 mg/kg bw, and no mortality, no clinical signs of toxicity and no gross pathological effects were observed. The substance was tested as a 30% and a 50% aqueous solution. With higher concentrations the substance could possibly be corrosive with consequent systemic effects. However, considering the substance as available on the market (i.e. a 30% aqueous solution) and in the absence of other data, no classification for acute dermal toxicity is proposed.

The inhalative toxicity was tested in rats in a not guideline conform inhalation hazard test and an acute toxicity study according to GLP and OECD guideline 403. Within the latter study some clinical effects were observed at high doses applied (see table above), but the acute inhalation LC50 is > 7.8 mg/l/4h, which is above the concentration range which leads to classification. The substance was tested as manufactured, i.e. a 30% aqueous solution.. With higher concentrations the active substance could possibly be corrosive with consequent effects in the respiration tract leading to lethality. However, considering the substance as available on the market and in the absence of other data, no classification is proposed.

4.2.4 Comparison with criteria

Classification for acute oral toxicity category 3 is proposed on the basis of the available animal study providing an LD50 estimate in the category 3 range, i.e. between 50 and 300 mg/kg bw.

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

4.2.5 Conclusions on classification and labelling

Classification as acute oral toxicity category 3, H301- Toxic if swallowed is proposed.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral

Two acute oral toxicity studies with K-HDO (both conducted prior to OECD guideline and GLP) were evaluated by the DS.

The DS proposed to classify K-HDO (purified) in category 3 for acute oral toxicity based on the LD_{50} value of 136 mg/kg bw. Furthermore, the DS suggested to classify K-HDO (30% aqueous solution) as category 4 for acute oral toxicity based on an LD_{50} value of 452 mg/kg bw.

Dermal

Two acute dermal toxicity studies were evaluated by the DS (both conducted prior to OECD TG and GLP). In the first study no effects were observed.

From the second study, the DS evaluated the LD_{50} to be > 5650 mg (30% w/w K-HDO)/kg bw, corresponding to > 1700 mg K-HDO/kg bw. No classification was proposed.

Inhalation:

Two acute toxicity studies by the inhalation route for K-HDO were evaluated, on the basis of which the DS proposed not to classify K-HDO for acute toxicity by inhalation.

Comments received during public consultation

One Member State Competent Authorities (MSCA) supported the proposed classification for acute oral toxicity in Category 3 for K-HDO. Another MSCA pointed out that information regarding details for the B 6.1.1 acute oral toxicity study and the B 6.1.2 acute dermal toxicity study were lacking. This MSCA also pointed out that an ATE-value for acute oral toxicity should be considered.

Assessment and comparison with the classification criteria

Acute oral toxicity

Two acute oral toxicity studies were included in the CLH report. In the first study (conducted prior to OECD TG and GLP) Sprague-Dawley rats (10 m/f per dose group) were exposed by single gavage to K-HDO (purity 99.8%) at doses of 56.2, 68.1, 82.5, 100, 121, 147, 178, 215 and 261 mg/kg bw.

Symptoms observed in the low dose group included clonic spasms, twitching, dyspnoea and poor general condition. With increasing doses also tremor, tonic spasms, salatory spasms, salivation, staggering, spastic gate, lateral position, apathy and agitation (A 6.1.1). The LD_{50} value was calculated to be 136 (117-161) mg/kg bw.

The second study was performed with K-HDO as manufactured (30% w/w). Sprague-Dawley rats (10 m/f per dose group) were exposed to doses of ~226, 282.5, 361.6, 452, 904 and 1808 mg/kg bw (Hofmann, 1971b). Clinical signs seen were acute neurotoxic effects and necropsy showed flaccid intestinal tract with much fluid (B 6.1.1). The LD_{50} value was found to be 452 mg/kg bw of K-HDO as 30% w/w aqueous solution, which corresponds to 136 mg/kg bw for K-HDO (purified). This is consistent with the finding in the first study.

Based on the data presented, the oral LD_{50} is evaluated to be 136 mg/kg bw in rats. According to CLP, oral LD_{50} values ranging from 50 to 300 mg/kg bw warrant classification in category 3. RAC agrees with the DS, that K-HDO meets the criteria for classification in category 3 for acute oral toxicity.

The ATE-value for classifying mixtures should be equal to the lowest oral LD_{50} for rats, which was 136 mg/kg bw.

In addition the DS suggested to classify K-HDO (30% w/w aqueous solution) in category 4 for acute oral toxicity based on the LD_{50} value of 452 mg/kg bw, since this value is within the range 300 to 2000 mg/kg bw. RAC is however of the opinion that K-HDO (30% w/w aqueous solution) should not be classified separately, as it is covered by the classification of purified K-HDO.

Acute dermal toxicity

Two acute dermal toxicity studies have been evaluated by the DS (both conducted prior to OECD TG and GLP). In the first study rats (Sprague-Dawley, 5 m/f per dose group) were exposed for K-HDO (purified, 99.8%) at a dose of > 1250 mg K-HDO/kg bw corresponding to > 2500 mg (50% w/w K-HDO)/kg bw (A 6.1.2). No mortalities and no

signs of toxicity were observed. Further, the animals sacrificed after a 14-day observation period did not show any findings in the internal organs that could be related to the test substance.

In the second study, rats (Sprague-Dawley, 10 m/f) were exposed for K-HDO (as manufactured, 30% w/w) at a dose corresponding to ~5650 mg (30% w/w K-HDO)/kg bw for 24 hours. No signs of toxicity were observed (B 6.1.2). The DS evaluated the LD_{50} to be > 5650 mg (30% w/w K-HDO)/kg bw, corresponding to > 1700 mg K-HDO/kg bw. No classification was proposed.

Classification via the dermal route is required where the LD_{50} is ≤ 2000 mg/kg bw. The LD_{50} was found to be > 1700 mg/kg bw. RAC agrees with the DS that based on the available data **no classification is warranted for acute dermal toxicity**.

Acute inhalation toxicity

The DS included two acute toxicity studies by the inhalation route for the evaluation of acute inhalation toxicity.

In the first study (OECD TG 403, GLP) rats (Wistar, 5 m/f per group) were exposed to 1.2 or 7.8 mg/L K-HDO (as manufactured, 30% w/w) for 4 hours in a head-nose inhalation system. No mortalities were observed. Signs of toxicity included accelerated or slower respiration, squatting posture, apathy, smeared fur and attempts to escape (A 6.1.3.1). The LC_{50} was evaluated to be > 7.8 mg/L for K-HDO (as manufactured, 30% w/w). This corresponds to an LC_{50} > 2.3 mg/L for K-HDO.

In the second study (conducted prior to OECD TG and GLP) rats (12 per group, m/f) were exposed to approximately 1.3 mg/L K-HDO (as manufactured, 30% w/w) for 8 hours as an atmosphere saturated with vapour at 20ºC (A 6.1.2.3). No effects were observed, however the DS regarded the study as not reliable due to lack of exposure measurements.

On the basis of these studies, the DS proposed **not to classify K-HDO for acute toxicity by inhalation**.

Classification via the inhalation route is required where the LC_{50} value is ≤ 5 mg/L (dusts and mists). The rat 4h LC_{50} for K-HDO is > 2.3 mg/L. RAC agrees with the DS that based on the available data no classification is warranted for acute inhalation toxicity.

Overall, RAC agrees with the DS, to classify **K-HDO as Acute Tox. 3; H301** – Toxic if swallowed with an **ATE-value** of **136 mg/kg bw**.

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

The acute clinical neurotoxic oral effects in the acute gavage study at doses between 50 and 60 mg/kg bw and in the 96 day gavage study between 25 and 50 mg/kg bw day were not observed in the 28 day and 42 day feeding studies up to and including 724 mg/kg bw (see chapter 4.7.). The 28 day feeding study carried out with a single dose of 90 mg/kg bw day included also a functional observation test battery (see chapter 4.12).

It is concluded that the neurotoxic effects observed only in the gavage studies could be due to the bolus application of the K+ ion that overwhelmed the naturally tightly controlled K+ homeostasis. The slower uptake in the feeding studies did not induce neurotoxic effects, though applied with much higher doses. It is noted that e.g. potassium chloride and potassium carbonate are not classified for acute toxicity in the EU and the REACH registration dossiers indicate LD50 values above concentrations relevant for acute toxicity classification[1]. However a bolus effect is to be expected from a physiological and kinetic perspective[2] and this bolus effect is clearly demonstrated with the available (gavage versus feeding study) data for K-HDO.

Thus under realistic human exposure scenarios (which do not include a high dose bolus application) no specific concern for neurotoxicological effects can be deduced from the data submitted. Consequently no STOT SE classification is proposed for this effect.

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

Summary of the Dossier Submitter's proposal

The DS highlighted that neurotoxic effects were observed in the acute oral toxicity studies at doses of 50 and 60 mg/kg bw and in the 90-day study at doses of 25 and 50 mg/kg bw/d. These were all gavage studies. Similar effects were not observed in the 28 and 42 day feeding studies with doses up to 724 mg/kg bw/d. The DS suggested that the neurotoxic effects observed in the gavage studies could be related to the bolus dose of K^+-ions overwhelming the K^+ homeostasis. The feeding studies on the other hand result in a slower uptake and in these studies higher doses of K-HDO did not show the same neurotoxic effects as in the gavage studies. No classification for neurotoxic effects was suggested by the DS since under realistic human exposure the bolus effect is not relevant.

Comments received during public consultation

No comments was received during public consultation.

Assessment and comparison with the classification criteria

RAC notes that the acute toxicity studies by oral exposure showed acute neurological effects starting from the lowest tested dose of 56.2 mg/kg bw. Also, repeated dose toxicity studies showed neurotoxic effects after gavage administration of K-HDO but not following exposure to K-HDO in feed. This effect could be relevant for a classification for STOT SE category 1. However, the LD_{50} value of 136 mg/kg bw used for a classification for acute oral toxicity is within the guidance value (≤ 300 mg/kg bw) for STOT SE 1. According to CLP, acute toxicity takes precedence over STOT SE when lethality occurs at relevant doses. A classification as STOT SE 1 or 2 is thus not warranted.

No narcotic effects were reported and there were no indications of respiratory tract

[1] https://echa.europa.eu/registration-dossier/-/registered-dossier/15221; https://echa.europa.eu/registration-dossier/-/registered-dossier/14341

[2] see e.g. http://www.inchem.org/documents/pims/pharm/potasscl.htm

irritation. Hence, classification as STOT SE 3 is not warranted.

In conclusion, RAC supports the DS's proposal for **no classification of K-HDO for STOT SE**.

4.4 Irritation

4.4.1 Skin irritation

4.4.1.1 Non-human information

Table 15: Summary table of relevant skin irritation studies (test item: K-HDO as 30%w/w aqueous solution)

Species	Method	Average score/animal on day 1 and day 8 applied to the back and the ear of the animals		Result	Remark	Reference[1] (Doc IV)
Rabbit White Viennese	BASF test 1971 application for 20h on the back (2 males) and on the ear (2males) no GLP	Erythema and Eschar[2] <u>day 1</u> back: 4 erythema ear: 2 erythema <u>day 8</u> back: 4 erythema & eschar ear: 0	Edema[2] <u>day 1</u> back: 2 ear: 0 <u>day 8</u> back: 0 ear: 0	Skin irrit. 2, H315 since average score is ≥ 2.3 for erythema or edema and additionally persistent till day 8	The conditions of 20h exposure without washing after exposure were more severe compared to the conditions of 4h with washing recommended by OECD guideline 404; the given OECD scores are a translation of the non-OECD conform scores of the study report produced in 1971	**A 6.1.4** Zeller, 1971a

[1] bold references are indicating key studies.
[2] two animals were tested, identical scores were obtained for both of them. Effects were only measured after 20 hours exposure and at day 8 post exposure, but not at 48 hours and not at 72 hours post exposure. Therefore only the 20 hours (day 1) values and the day 8 values can be reported here. More details of the study are available in the attached study summary.

4.4.1.2 Human information

Not available

4.4.1.3 Summary and discussion of skin irritation

Skin irritation was examined in rabbits in a test representing a worst case compared to the OECD 404 test in respect of duration (20 versus 4 hours) and exposure (occlusive versus semi-occlusive).. However, without a more adequate test the reported results have to be used for hazard assessment as worst case situation. The average score for 24h, 48h and 72h cannot be calculated, since no results are documented for 48h and 72h. Therefore the 24h score has to be used instead.

In the tests K-HDO (active substance, 30% aqueous solution) displayed acute dermal irritation and has to be classified for skin irritant category 2, H315: Causes skin irritation, because average score is ≥ 2.3 for erythema or edema and additionally erythema is persistent till day 8 and at that time accompanied by severe eschar formation. Solubility in water is about 450 g/L and results in a pH of 10.4, which also indicates skin irritating, but not skin corrosive properties at that concentration.

With concentrations above 31%w/w active substance could possibly be corrosive; however, since the active substance as manufactured is generated only as a maximal 31% aqueous solution, the study cited above is sufficient, as long as methods of manufacturing do not lead to higher concentrations. If concentrations of K-HDO above 31% w/w are achieved by any production process, tests on dermal corrosion should be performed (e.g. OECD guideline 430 or 431). However in the moment on the basis of available data no other data based conclusion than "skin irritation" is possible.

Therefore the proposed classification for the active substance is skin irritant category 2, H315. It should be reconsidered when the active substance is available at higher concentrations and other data are available.

4.4.1.4 Comparison with criteria

See discussion above

4.4.1.5 Conclusions on classification and labelling

Classification for skin irritant category 2, H315: causes skin irritation, is proposed.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS presented one study (conducted prior to OECD TG and GLP) where male rabbits exposed to a 30% w/w aqueous solution of K-HDO for up to 20 hours showed an average score exceeding 2.3 for erythema and oedema at 24h scoring. No results are available for the 48h or 72h scoring. The erythema persisted for 8 days and was also accompanied by severe escar formation (A 6.1.4). There was no information on pure K-HDO, and the classification as Skin. Irrit. 2 as proposed by the DS is based on results from the testing of a 30% w/w aqueous solution of K-HDO. The DS cannot rule out that higher concentrations of the active substance could be corrosive.

Comments received during public consultation

One MSCA pointed out that it should be clarified if the tested substance contains co-formulants. Further, they questioned the testing K-HDO in a water based solution since the results indicate that undiluted K-HDO might be corrosive.

Assessment and comparison with the classification criteria

There was no information on the skin corrosion/irritation potential of pure K-HDO.

One study (conducted prior to OECD TG and GLP) with male rabbits (White Viennese) exposed to K-HDO (30% w/w aqueous solution) on dorsal skin and the ear were evaluated by the DS. It should be noted that the DS recalculated the scoring for this non–guideline study to scorings according to the current OECD TG 404. The scoring of the non-guideline study ranges from 0-2 while in OECD TG 404 the range is 0-4. Two rabbits were treated for 1, 5 and 15 minutes and two other rabbits were treated for 20 hours under occlusive conditions. No effects were seen on dorsal skin after exposure for

1, 5 and 15 minutes. For the two rabbits treated for 20h, the average score was 4 for erythema/escar formation and 2 for oedema at the 24h scoring according to the revised scoring system. The erythema/escar formation was not completely reversible while the oedema was reversible. For the ear the erythema scores were 2 (reversible) according to the revised scoring system. No results were available to the DS for the 48h or 72h scoring (A 6.1.4). It should be noted that the 20h exposures were performed without washing after exposure and thus the exposure conditions were more severe than those recommended in OECD TG 404.

According to the CLP criteria, a substance should be classified in category 2 for skin irritation if the mean score of ≥ 2.3 - ≤ 4.0 for erythema/eschar or for oedema is observed in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal. K-HDO (30% w/w aqueous solution) showed a score for erythema/escar formation of 4 at the 24h scoring for both of the tested rabbits. Scores from 48h and 72h were not available to the DS, however the average score (24, 48 and 72h) was reported to be 3 (re-calculated) according to the applicant. The erythema/escar formation was not completely reversed after 8 days with a score for erythema/escar formation of 2. On the basis of this of this observation RAC agrees with the DS that classification of **K-HDO for skin irritation in category 2** is warranted.

4.4.2 Eye irritation

4.4.2.1 Non-human information

Table 16: Summary table of relevant eye irritation studies (test item: K-HDO as 30%w/w aqueous solution)

Species	Method	Average Score				Result	Reversible yes/no	Ref.[1]
		Cornea Opacity[2]	Iris	Conjunctiva Redness[2]	Conjunctiva Chemosis[2]			
Rabbit White Viennese	BASF test 1971 Application: Instillation of about 50 µL of the product into the conjuctival sac; no GLP	1h: 2-3, clouding of cornea day 1: 3, clouding of cornea day 8: 0	not indicated	1h: 2 day 1: 3 day 8: 0	1h: 4 edema & bleeding day 1: 4, edema & bleeding day 8: 0	Risk for serious damage to eye; The study report's scores were translated into OECD conform scores	Yes, reversible over the course of 8 days	**A 6.1.4** Zeller, 1971a

[1] bold references are indicating key studies.

[2] Two animals were tested and effects were nearly identically. Effects were only measured after 1 hour and after 20 hours exposure and at day 8, but not at 48 hours and not at 72 hours. Therefore no 24/48/72 hour average can be presented here. More details on the study are available in the attached study summary.

4.4.2.2 Human information

Not available

4.4.2.3 Summary and discussion of eye irritation

Regarding irritation to eyes, K-HDO (as manufactured, 30%w/w aqueous solution) displays severe eye damaging effects when applied undiluted to the conjunctival sac (rabbit). Clouding of the cornea, conjunctival redness, swelling and bleeding were observed within 1 h post treatment and persisted at least till day 1, but not till day 8. Eye damage category 1, H318: causes serious eye damage, should be applied, as the value of cornea opacity equals 3.

Since this result already represents the worst possible damage to the eyes, it can be directly transferred to any higher concentration of K-HDO.

4.4.2.4 Comparison with criteria

See discussion above

4.4.2.5 Conclusions on classification and labelling

Classification for eye damage category 1; H318: causes serious eye damage, is proposed.

RAC evaluation of serious eye damage/irritation
Summary of the Dossier Submitter's proposal The DS presented one study (conducted prior to OECD TG and GLP) where eye irritation was investigated in two rabbits (white Vienna (Gaukler), 1 male/1 female) exposed to a single instillation of 50 µL K-HDO (30% w/w aqueous solution) (A 6.1.4). Corneal opacity, redness of the conjunctiva and chemosis were observed, these effects being reversible by day 8. On the basis of the corneal opacity with a score of 3 at 24 hours after instillation of K-HDO, the DS proposed to classify K-HDO for severe eye damage in category 1. **Comments received during public consultation** One MSCA pointed out that it should be clarified if the tested substance contains co-formulants. However, considering that K-HDO were tested in a pure-water-based solution with appropriate negative control, the proposed classification is supported. **Assessment and comparison with the classification criteria** Eye irritation was investigated in a study (conducted prior to OECD TG and GLP) in two rabbits (white Vienna (Gaukler), 1 male/1 female) exposed to a single application of 50

µL K-HDO (30% w/w aqueous solution) (A 6.1.4). The following scores were reported:

	Score 1 hour	Score 24 hours	Score, 8 days
Corneal opacity	2-3 (clouding of the cornea)	3 (clouding of the cornea)	0
Iris	Not reported	Not reported	Not reported
Redness conjunctiva	2	3	0
Chemosis	4 (oedema, bleeding)	4 (oedema, bleeding)	0

It should be noted that the reported scores were translated by the DS (RMS) from the system used in the study report to the OECD TG 405 scoring system.

RAC considered the reasons provided by the DS to propose classification, i.e. corneal opacity with a score of 3 at 24 hours after instillation of K-HDO, reversible within the 8 day observation period and took into account that the tested substance was a 30% w/w aqueous solution, noting that it can be argued that pure K-HDO if tested would show a more persistent effect on the eyes. In addition it should be noted that only 50 µL of the 30% w/w aqueous solution of K-HDO were applied to the rabbit eyes, while according to OECD TG 405, 100 µL of the test substance should be applied. In conclusion, RAC is of the opinion that based on an overall weight of evidence a classification of **K-HDO as Eye Dam. 1; H318** is justified.

4.4.3 Respiratory tract irritation

No data available.

4.5 Corrosivity

See discussion above, chapter 4.4.1 and 4.4.2.

4.6 Sensitisation

4.6.1 Skin sensititsation

4.6.1.1 Non-human information

Table 17: Summary table of relevant skin sensitisation studies
(test item: K-HDO as 30%w/w aqueous solution)

Species	Method	Dose levels	Result	Reference[1] (Doc IV)
mouse	Local Lymph Node Assay, OECD guideline 429	0, 10, 25, 50 % (v/v) of Xyligen LP 15670 (with a K-HDO content of 30.4%) in water and 25% hexyl	not sensitising (stimulation index < 3): 1, 1.5; 2.4; 1.9 and with positive control	**A 6.1.5** Weber, 2004

	GLP	cinnamic aldehyde in acetone:olive oil, (4:1) as positive control	53.3. With 50% (v/v) reduced motor activities (3 animals) and hushed posture and white crusts between days 3 and 5.	

[1] bold references are indicating key studies.

4.6.1.2 Human information

Not available

4.6.1.3 Summary and discussion of skin sensitisation

In the local lymph node assay, K-HDO did not show sensitising properties according to the guideline, as the stimulation index was below 3.

As the concentrations of the active substance applied in the test should maximise exposure whilst avoiding systemic toxicity and excessive local skin irritation, and as the active substance as manufactured (30%w/w aqueous solution) is already irritant, the results apply to any concentration of the active substance in water that is higher than 30%w/w.

In conclusion, no classification under the provisions of Commission Directive 93/21/EEC, Annex VI (1993) is required.

4.6.1.4 Comparison with criteria

See discussion above

4.6.1.5 Conclusions on classification and labelling

No classification is necessary.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS summarised in their evaluation one Local Lymph Node Assay (LLNA) (OECD TG 429 and GLP) in mice where K-HDO (30% w/w in aqueous solution) was diluted in water and administered to three groups of 6 female CBA/Ca mice. The test substance was applied at concentrations of 10, 25 and 50% (25 µL per ear) epicutaneously to the dorsal surface of both ears, once a day for three consecutive days. Hexyl cinnamic aldehyde (25% in acetone: olive oil (4:1)) was used as the positive control. In the high dose group, signs of toxicity were observed as reduced motor activity, hunched posture and white crusts between days 3-5. There were no effects of irritation at any dose level. The stimulation index were 1.0 (negative control), 1.5 (low dose (10%)), 2.4 (mid dose (25%)), 1.9 (high dose (50%)) and 53.3 (positive control).

According to the DS, K-HDO does not meet the criteria for classification as a skin sensitiser based on the results of the LLNA in mice.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

Based on the results of the LLNA included in the CLH report, RAC is of the opinion that **K-HDO does not warrant any classification for skin sensitisation**.

4.6.2 Respiratory sensitisation

No data available

4.7 Repeated dose toxicity

4.7.1 Non-human information

The following table summarises the repeated dose toxicity studies submitted for the evaluation of the active substance K-HDO. For key studies the references are indicated in bold.

Table 18: Summary table of relevant repeated dose toxicity studies

Tested substance	**Route**	**duration of study**	**Species Strain Sex no/group**	**dose**	**Results**	**NOAEL** [mg/kg bw day]	**Reference**
Purified K-HDO	Oral, feeding	28 days	Wistar Rat 5 males and 5 females per group	0 (control); only one dose: 82(m) and 90 (f) mg/kg bw day	No clinical signs and no functional effects in the functional observation test battery No pathological effects, no damage or irritation of intestinal mucosa (histopathological analysis restricted to GI) Clinical chemistry effects: magnesium↑ (m+f); inorganic phosphatase↑ (f); calcium↑ (f); glucose and triglycerides ↓ (f)!	> 90 (~ 300 of 30% K-HDO)	**A6.9.** **Mellert 1992; GLP**
Purified K-HDO	Oral, feeding	about 42 days;	Sprague-Dawley rat. 10 males and10 females per group	ca. 0, 10, 30, 100 and 1000 mg/kg bw day	No mortality during study. No significant body weight changes, slight effects on food consumption. No clinical signs No substance induced gross-pathological organ findings or organ weight changes (no histopathology carried out)	724 (~ 2413 of 30% K-HDO)	**A6.3.1** **Hofmann H. Th., Freisberg K. O.; 1976; no GLP**
Purified K-HDO	inhalation	Exposure about 28 days	Sprague-Dawley rat 10 males and 10 females per group.	0.6 mg /l	Exposure concentration and aerosol size not measured, but since effects at the single dose level, evidence that significant proportion was taken up. No NOAEL can be derived from this study due to effects observed at the single dose level of 0,6 mg/l: total lipids ↓, alkaline phosphatase↑, urine sediment round epithelia ↑ (m) and leucocytes↑ (f), liver weight ↓ (m), liver necrosis (3f), foam cells number ↑ (m)	< 0.6 mg/l (~ < 2 mg/l of 30% K-HDO)	**A6.3.3 Klimisch H.-J., Deckardt K., Mirea D., Schulz V. ;1978; no GLP**

Table 18: Summary table of relevant repeated dose toxicity studies

contd.

Purified K-HDO	Oral, gavage	about 96 days	Sprague-Dawley rat, 20 m + 20 f per group	12, 25, 50, 100 mg/kg bw day	12.5 and 25 mg/kg bw day: no effects 50 mg/kg by just below the LD_{50}. : aggressiveness↑; salivation↑, incidents of mild tonoclonic spasms with atatic intervals↑ ; second week onwards, 8 (m) + 9 (f) died or moribund; prelethal spasms and dispnoe; apathy↑; food intake↓; scrubby fur; haemoglobin↓, erythrocytes↓, haematocrit ↓; GOT↑, GPT↑, AP↑, liver weight ↓, brain weight ↓, liver-damage 100 mg/kg bw day: above LD50	25 (~ 83 of 30% K-HDO)	**A6.4.1 Leuschner, F. Hübscher, F. Dontenwill, W.; 1978; no GLP**
Purified Cu-HDO	oral, feeding	about 96 days	Wistar rats; 10 m +10 f per group	35, 139, 275 (m) and 41, 167, 322 (f) mg/kg bw day	~ 298 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 (m); swelling and pigmentation of Kupffer's cells (f weaker than m); 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; 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 and swelling and pigmentation of Kupffer's cells; hyaline droplets in the proximal tubular epithelial cells and protein precipitates in the renal tubular lumina (m); 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	35 Cu-HDO corresp. to 36.4 K-HDO (~121.5 30% K-HDO)	A 6.4 Mellert 1991; GLP
Purified Cu-HDO	oral, feeding	about 96 days	Beagle dogs 5 m + 5 f per test group	8.3; 25.2; 64.6 (m) and 9.3; 27.4; 71.9 (f) mg/kg bw day (Cu-HDO)	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, ↑aspartate-aminotransferase, ↑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 (4m+3f) indicative for liver cell damage represented by foci, necrosis and/or capsular retractions; chronic hepatitis (all dogs); liver cirrhosis in (5m+3f); copper pigment storage in hepatocytes and Kupffer cells (all dogs); edema in the gall bladder wall (2m+4f); edema in the pancreas and in the mesentery (2m); minimal hyperplasia in the mucosa of the esophagus (3m+1f); lymphoid depletion in the thymus (3m) 8 - 27 mg/kg bw day No substance-induced changes	26 Cu-HDO; corresp. to 27 K-HDO (~90,3 of 30% K-HDO)	A 6.4 Hellwig 1995; GLP

Table 18: Summary table of relevant repeated dose toxicity studies

contd.

Purified Cu-HDO	Oral, feeding	about 12 months	Wistar rats. 20 males and 20 females per group.	0, 6, 18, 61 and 183 mg/kg bw day (Cu-HDO!)	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 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 (m+f) and single cell necrosis (m); hyaline (fluorescent) droplets in the renal proximal tubules (m) and proteinaceous casts in the tubular lumina (m)	18 Cu-HDO; corresp. to 18.7 of K-HDO or 62.5 of 30% K-HDO	**A6.5 MELLERT; 1993; GLP**

4.7.1.1 Repeated dose toxicity: oral

See chapter 4.7.1

4.7.1.2 Repeated dose toxicity: inhalation

See chapter 4.7.1

4.7.1.3 Repeated dose toxicity: dermal

No information available.

4.7.1.4 Repeated dose toxicity: other routes

No information available.

4.7.1.5 Human information

No information available.

4.7.1.6 Other relevant information

Not available

4.7.1.7 Summary and discussion of repeated dose toxicity

The toxicity-tests conducted with the purified K-HDO are relevant for the hazard evaluation of the K-HDO as manufactured, since the latter does not contain toxicologically relevant impurities in concentrations above 0.1%.

As summarised in the table above for K-HDO no adverse effects were observed in the 28 day (one dose, 90 mg/kg bw) and in the 42 day studies with food administration (see III A 6.3.1, Hofmann, Freisberg, 1976; III A 6.9, Mellert 1992) up to and including 724 mg/kg bw day. Within the 28 day one dose study also a functional observation test battery was carried out, which did not show any adverse effect. However

histopathology was restricted to the gastro-intestinal tract in the 28 day study and was not performed in the 42 day study.

Nevertheless the results of the 96 day gavage study, which included also histopathology, basically confirmed the results of the two shorter studies. The only effects observed in the 96 day gavage study but not in the 28 day and 42 day feeding studies were the acute clinical neurotoxic effects. These effects may be to be due to the bolus application of K-HDO disturbing the normally strictly controlled K+ homeostasis, an effect that could not be mediated with feeding studies where the K+ uptake is expected to be slower. (It is already noted in section 4.3. that e.g. potassium chloride and potassium carbonate are not classified for acute toxicity in the EU and the REACH registration dossiers indicate LD50 values above concentrations relevant for acute toxicity classification. However the bolus effect is clearly demonstrated with the available (gavage versus feeding study) data for K-HDO).

In summary the acute clinical neurotoxic effects are considered to be of low concern, since they were observed only with the bolus application, which is an unlikely situation for human exposure, and because within the 96 day study the LOAEL for these acute neurotoxic effects was between 25 and 50 mg/kg bw day which is in the same range that results in acute neurotoxic effects in the acute gavage study, (means the adverse effect level did not significantly decrease from the acute to the sub-chronic study) and because within the 28 day study with 90 mg/kg bw day no effects were observed within the behavioural test battery.

The results from the inhalation toxicity study are difficult to interpret since exposure concentration and aerosol size were not measured. Furthermore the results do not show toxicologically consistent effects but intersexes differences. However, assuming a nominal dose of 0.6 mg/l, an aspiration rate of 0.2 l/min, 6 hours exposure, 100% uptake and a body weight between 90g and 150g from start to the end of the study this would result in a dose of approximately 500 to 300 mg/kg bw and day, respectively. This dose is relatively high so that the study result should not be considered as contradicting the other sub-acute and (sub)chronic studies.

Thus in summary under realistic exposure situations that do not include a high dose bolus application, the repeated dose toxicological studies available for K-HDO result in a NOAEL of at least 90 mg/kg bw day.

However no metabolism, no sub-chronic second species, no chronic, no carcinogenic and no developmental toxicity studies were carried out with K-HDO. These studies were carried out only with Cu-HDO and the results were read across to evaluate the respective toxicological hazards of K-HDO. Cu-HDO LOAELs [mg/kg bw day] may be multiplied by a factor of 1.04 to estimate equivalent K-HDO doses [mg/kg bw day], since both substances contain the same HDO anion and in terms of molecular weight these are compensating the difference of molecular weight of Copper and Potassium. Or in other words one microgram Cu-HDO contains practically the same amount of HDO compared to one microgram K-HDO. The essential arguments for the read across strategy are the following:

- Cu-HDO and K-HDO showed similar distribution and excretion rates, which is ready absorption across the GI tract, rapid elimination mainly via urine, no bioaccumulation, plasma levels below 0.1% of the dose and limited absorption via the skin (~8%) (Hoffmann et al. 1993, IIIA.6.2.1, Gamer et al. 2006, IIIA6.2.4). The kinetics might not have been expected to be comparable since the log P_{ow} differs (Cu-HDO 2.6 vs. K-HDO -0.2). However, the log P_{ow} does not necessarily contradict the toxicokinetic findings since 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. Furthermore the comparable kinetics and the identical chemical structure of the HDO^- anion support the assumption of a comparable metabolism.
- The HDO^- anion derived from Cu-HDO and from K-HDO is structurally identical.
- The toxicological differences in the toxicity profile of Cu-HDO and K-HDO were related to the different effects of the Cu^{2+} and K^+ ions.
    - Potassium is the quantitatively most important intracellular cation and its concentration gradient towards extracellular space is responsible for the membrane potential. As such it is important for the functioning of the nervous system, cardiac, skeletal and smooth muscles

and epithelia and its homeostasis is usually strictly controlled by renal regulation and influenced by the acid-base state of extracellular liquids. The neurotoxic effects seen only with gavage application of K-HDO (and not with exposure via feed) could be interpreted to result from a K+ peak in the plasma disturbing the normally rigidly controlled K+ homeostasis.

- In contrast copper is an essential metal. It is employed in all human cells involved in the reactions and functions of many enzymes, including angiogenesis, neurohormone release, oxygen transport and regulation of genetic expression. Homeostatic maintenance of copper requires the tightly coordinated control of copper uptake, distribution and efflux in cells and the organism as a whole. High dose exposure may lead to local effects in the gastro-intestinal-tract, effects in the liver and kidney (see e.g. Biocides assessment report for basic copper carbonate, 2011). However the toxicity of Cu appears to be lower compared to Cu-HDO, since the AEL for Cu (0.04 mg/kg bw day for Cu corresponding to 0.22 mg/kg bw day Cu-HDO; molar ratio 5.5) is more than 6 fold above the AEL for Cu-HDO (0.033 mg/kg bw).

- Except for the differences that are related to the Cu^{2+} and K^{+} cations the toxicity profile of Cu-HDO and K-HDO does not diverge with the tests available for both. Both showed severe eye damaging effects, neither of them induced sensitisation and neither of them showed genotoxic effects in the in vitro and vivo assays.
- The fact that the available Ames test, TK mouse lymphoma assay and the micronucleus test do not indicate genotoxicity reduces concern for carcinogenic effects.
- With K-HDO no adverse effects were seen in the 28 day (one dose) study at 90 mg/kg bw day (including behavioural test battery, but histopathology analysis restricted to GI) and in the 42 day feeding studies at 724 mg/kg bw day (but no histopathology analysis) and only clinical neurotoxic effects were seen in the 96 day gavage study with a LOAEL between 25 and 50 mg/kg bw day that is in the same range of the dose inducing similar neurotoxic effects in the acute gavage study. This available repeated dose toxicological profile of K-HDO supports the assumption that reading across the critical NOAEL of Cu-HDO from the 2-year study of 6 mg/kg bw day (corresponding to the equivalent K-HDO dose of 6.25 mg/kg bw day) is a sufficiently conservative estimate of the overall NOAEL of K-HDO.

Irritating and histological effects in the GI tract and kidney effects were observed within the repeated dose studies only with Cu-HDO and not with K-HDO. There are 2 potential explanations for this: (1) It was a Cu^{2+} specific effect that resulted from increased intracellular cytotoxic Cu^{2+} levels that were the consequence of the slow dissociation of Cu-HDO or (2) the effects could have been observed also with K-HDO if the same doses would have been analysed histologically: A histopathological analysis is available for K-HDO only with maximal 50 mg/kg bw for 96 days or with 90 mg/kg bw for 28 days, whereas the histopathological effects with Cu-HDO were observed only with 132 mg/kg bw for 28 days or 153 mg/kg bw for 96 days or 61 mg/kg bw for 12 months or 33 mg/kg bw for 24 months. However in any case these results do not raise specific concerns for K-HDO or contradict the read across arguments.

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 (2m, 4f) and in the pancreas and mesentery lymph nodes (2m). 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 35 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 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. The equivalent

NOAEL for K-HDO is 18.7 mg/kg bw day. As discussed above according to the available repeated dose toxicity profile of K-HDO this is considered to be a sufficiently conservative estimate.

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

See chapter 4.8.2

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

See chapter 4.8.2

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

As discussed in chapter 4.3 the acute neurotoxic effects were observed only with gavage studies, but not with feeding studies. They are a result of the bolus application and may be related to the K+ ion overwhelming naturally tightly controlled K+ homeostasis. Consequently these effects were not considered as relevant for STOT SE classification.

Within the 96 day gavage study similar neurotoxic effects were observed at similar doses compared to the acute gavage study, but no neurotoxic effects were observed at 724 mg/kg bw day in the 42 day feeding study and at the single dose of 90 mg/kg bw day in the 28 day study that included also a functional observation test battery. Consequently these neurotoxic effects in the 96 day gavage study were also not considered as relevant for STOT RE classification.

However as summarized in the bullet points in chapter 4.7.1.7 above read across from the Cu-HDO data to the K-HDO is supported. The following studies carried out with Cu-HDO support STOT RE 2 classification of K-HDO. Cu-HDO LOAELs [mg/kg bw day] may be multiplied by a factor of 1.04 to estimate equivalent K-HDO doses [mg/kg bw day], but since this leads to virtually identically values no transformation is given here.

Table 19:

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: 90 day oral rat: 10-100 mg/kg bw day	18 to 61 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, glandular stomach, liver**: 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)
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 19 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 in STOT RE Cat2 and attribution of H373 – may cause damage to organs (gastrointestinal tract, liver kidney) is required.

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

The DS included four repeated dose toxicity studies with K-HDO in the CLH-report, three with oral exposure (one gavage and two in feed) and one with inhalation exposure. They also included five studies with Cu-HDO, one 28-day study, one 90-day study, one 12-month study and one 2-year study in Wistar rats as well as one 90-day study in Beagle dogs based on a read-across from Cu-HDO to K-HDO.

K-HDO oral studies

In a 28-day oral feeding study Wistar rats (5/sex/group) were exposed to one dose of K-HDO, 82 mg/kg bw/d (males) and 90 mg/kg bw/d (females). Results: No clinical signs were reported as well as no effects in a functional observation battery test. Histopathological examinations were only performed in the gastrointestinal tract (GI),

and no damage or irritation of intestinal mucosa was observed.

In a 42-day oral feeding study Sprague-Dawley rats (10/sex/group) were exposed to approximately 0, 10, 30, 100 and 1000 mg/kg bw/day K-HDO. Results: No clinical signs were seen, and no effects were reported on gross-pathology, however, no histopathological examination was performed.

In a 90-day oral gavage study Sprague-Dawley rats (20/sex/group) were exposed to 0, 12, 25, 50 and 100 mg/kg bw/d. Results: At 12.5 and 25 mg/kg bw/d no effects were reported. At 50 mg/kg bw/d aggressiveness, salivation and incidents of mild tonoclonic spasms with atatic intervals was increased. Further, from the second week and onwards, 8 male + 9 females died or were moribund with pre-lethal spasms and dyspnoea. Apathy was increased, food intake decreased, haemoglobin, erythrocytes and haematocrit were decreased as well as liver and brain weight. Liver and/or stomach damage was also reported. Microscopic examinations showed degenerative liver damage which was severe in some cases and seen as dystrophy. Gastric ulcers were also reported occasionally. At 100 mg/kg bw/d the rats developed increasing aggressiveness, and 11 male and 12 female animals died within the first 9 weeks or were sacrificed prematurely. Pre-lethal symptoms and morphological changes were consistent with those reported at 50 mg/kg bw/d.

K-HDO inhalation study

In a 28-day inhalation study Sprague-Dawley rats (10/sex/group) were exposed to 0.6 mg/L 6h per day. The exposure concentration and aerosol size was not measured, however, as effects were seen in the rats at this single dose level, there is evidence that a significant amount of K-HDO was taken up by the animals. Results: One female died and there was a slight reduction in body weight. A decrease in total lipids in 2/10 males, an increase in alkaline phosphatase, an increase in urine sediment, round epithelia in males and leucocytes in females were reported. Further, the liver weight was decreased in males. In females slight fatty metamorphosis of liver was reported as well as focal-like liver necrosis in three females. The foam cell number was increased in males.

In summary, in the K-HDO studies no adverse effects were reported in the 28-day (one dose, 90 mg/kg bw/d) and in the 42-day studies with oral exposure to K-HDO via feed up to approximately 1000 mg/kg bw/d. However, histopathology was restricted to the GI-tract in the 28-day study, and was not performed in the 42-day study. In the 90-day oral gavage study liver and/or stomach damage was reported from 50 mg/kg bw/d. Microscopic examinations showed degenerative liver damage which was severe in some cases and seen as dystrophy. Gastric ulcers was also reported occasionally. Further, in the 90-day study clinical neurotoxic effects as also seen in the acute toxicity studies were reported. The DS considered that these effects may be due to the bolus application of K-HDO that disturbed the normally strictly controlled K^+ homeostasis, an effect that could not be mediated with feeding studies where the K^+ uptake is expected to be slower. The DS concluded that the acute clinical neurotoxic effects were considered to be of low concern, since they were observed only with the bolus application, which is an unlikely human exposure situation, and because within the 90-day study the LOAEL for these acute neurotoxic effects was between 25 and 50 mg/kg bw/d which is the same range that results in acute neurotoxic effects in the acute toxicity gavage study. This may indicate that the adverse effect level did not significantly decrease from the acute to the sub-chronic study since in the 28-day study with exposure to 90 mg/kg bw/d no effects were observed in the functional behavioural test battery. Consequently, the neurotoxic

effects in the 90-day gavage study were not considered relevant for a STOT RE classification.

The results from the inhalation toxicity study were difficult to interpret since exposure concentration and aerosol size were not measured. However, in females slight fatty metamorphosis of the liver was reported as well as focal-like liver necrosis in three females. On the other hand, the results do not show toxicologically consistent effects but intersex differences.

Studies with Cu-HDO

Study	**NOAEL/LOAEL (mg/kg bw/d)**	**STOT RE 2 GV (mg/kg bw/d)**	**Effects**
Rat 28-day oral in feed	46/139	30-300	Intestine: iron pigmentation, goblet cell hyperplasia.
Rat: 90-day oral in feed	38/153	10-100	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 oral in feed	26/68	10-100	Liver: chronic hepatitis and cirrhosis. Gall bladder: oedema in wall. GI tract: minimal hyperplasia in the mucosa of the oesophagus.
Rat: 12-month oral in feed	18/61	2.5-25	Forestomach: thickening of wall, hyperkeratosis of mucosa. Stomach: hyperplasia of mucosa. Liver: swollen and pigmented Küpffer's cells.
Rat: 24-month oral in feed	6/33	1.25-12.5	Forestomach: hyperplasia in epithelium and hyperkeratosis of wall.

In the repeated dose toxicity studies with Cu-HDO used for the read-across assessment to K-HDO, irritation and histopathological effects were reported in the GI tract, but not following exposure to K-HDO. The DS included two explanations for this: (1) the GI tract effect was a Cu^{2+} specific effect that resulted from increased intracellular cytotoxic Cu^{2+} levels due to the slow dissociation of Cu-HDO or (2) the effects could have been observed also with K-HDO if the same doses would have been analysed histologically.

As described in the table above, the subchronic toxicity studies with Cu-HDO in the rat and in the dog indicate the same target organs for both species, the GI tract and the liver, with the dogs having a more pronounced effect in the liver including gross lesions, hepatitis and cirrhosis and as sequelae additionally oedema in the gall bladder (2 male and 4 female). Thus from the data submitted, the DS considered that there were no concerns regarding the interspecies differences between rats and dogs. The chronic toxicity study carried out with Cu-HDO resulted in a NOAEL of 18 mg/kg bw/d with a LOAEL of 61 mg/kg bw/d based on histological effects in the forestomach, stomach and Küpffer's cells in the liver. In the higher doses besides GI tract and liver also the kidneys were identified as a target organ. The equivalent NOAEL for K-HDO was estimated to be 18.7 mg/kg bw/d based on the assessment that one microgram of Cu-HDO contains practically the same amount of HDO^- compared to one microgram K-HDO (see the section "RAC general comment").

The DS argued that in addition to the LOAEL values, the NOAEL to LOAEL ranges should also be considered in the assessment for a classification for STOT RE, 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 guidance value (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 and justify classification as STOT RE 2. Futhermore, the LOAEL of the 28-day rat study at 139 mg/kg bw/d was below the extrapolated STOT RE 2 GV for a 28-day study (300 mg/kg bw/d based on the scaling to take into accound the different study durations (i.e. factor 3, CLP Annex I, paragraph 3.9.2.9.6).

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. The NOAEL to LOAEL ranges of the 12- and 24-months rat studies (18 to 61 and 6 to 33 mg/kg bw/d respectively) should be compared with the extrapolated GV of 5 to 50 (factor 2 for a 12- to 24-month study, REACH guidance chapter R.8.4.3.1) leading to a NOAEL to LOAEL range including or being below the STOT RE 2 GV, which is considered to provide further support for classification.

Based on the read across assessment from Cu-HDO to K-HDO and the effects reported in the liver, kidney and GI tract in the repeated dose toxicity studies with Cu-HDO, 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 agreed with the DS proposal to classify K-HDO for STOT RE 2 (liver and kidney) mainly based on read-across from Cu-HDO. The MSCA did not agree that the GI tract should be included as a target organ in the STOT RE 2 classification. This was based on the fact that in the 2-year study in rats with exposure to Cu-HDO or $CuSO_4$, it was evident that the effects in the GI tract was mainly cause by copper. The MSCA also asked for a careful discussion in RAC of the neurotoxic effects observed after K-HDO gavage administration. The second MSCA asked for a more quantitative comparison of the organ toxicity of Cu-HDO vs. relevant Cu-salts to increase the robustness of the read-across approach. The DS commented that Cu-ions may penetrate deeper into the GI-mucosa mediated by the organic HDO-residue than Cu-salts. This could increase the cytotoxic effects of the copper-ion as a toxophore. A 2-year study in rats showed for example storage of an iron-containing pigment in macrophages in the submucosa of the duodenum of male and female animals after oral exposure with 169 mg/kg bw/d of Cu-HDO. This was not observed after comparable exposure with $CuSO_4$. Consequenty, the DS considered that a STOT RE 2 classification for GI tract, liver and kidney are supported by experimental evidence.

Assessment and comparison with the classification criteria

The DS proposal was in favour of classification as STOT RE 2 (liver, kidney and GI tract) based on data from repeated dose toxicity studies with K-HDO and in addition a read across from Cu-HDO. A justification for read across is included in the section "RAC general comments".

Assessment of the studies with K-HDO

Four repeated dose toxicity studies with K-HDO were included in the CLH-report, three by oral exposure (one gavage and two in feed) and one by inhalation exposure. In the 90-

day gavage study in Sprague-Dawley rats acute clinical neurotoxic effects were reported from 50 mg/kg bw/d K-HDO. The acute neurotoxic effects were reported only with the bolus application of K-HDO, which are probably disturbing the tightly controlled K^+ homeostasis, an effect that was not observed in oral feeding studies where the K^+ uptake is expected to be slower. RAC considers that the neurotoxic effect was reported in the same dose-range that resulted in similar effects in the acute toxicity gavage studies (see the acute toxicity section) and is not considered relevant for a STOT RE classification. It should be mentioned that liver and/or stomach damage were also reported in this study from 50 mg/kg bw/d. Microscopic examinations showed degenerative liver damage, which was seen in some cases as severe dystrophy. Gastric ulcers were reported occasionally. However, the bolus application of K-HDO may have disturbed the K^+ homeostasis and seriously compromising the health status of the rats. Therefore, the effects reported in liver and stomach may be considered as secondary to the health status of the animals.

RAC considers that the other repeated dose toxicity studies had limitations; in the 28-day study with oral exposure in feed to 82/90 (m/f) mg/kg bw/d K-HDO no effects were observed within the functional behavioural test battery. No other effects were reported, however, histopathology was only performed in the GI tract. In the 42-day study with doses from approximately 10 to 1000 mg/kg bw/d of K-HDO in feed no effects were reported, however, no histopathological examinations were performed. The results from the 28-day inhalation toxicity study were difficult to interpret since the exposure concentration was described to be 0.6 mg/L K-HDO, however the aerosol size was not measured. In three females focal-like liver necrosis was reported and slight fatty metamorphosis of the liver was also seen.

Assessment of the studies with Cu-HDO and read across to K-HDO:

GI tract: RAC acknowledges the differences in the doses used in the K-HDO and Cu-HDO studies. Histopathology of the GI tract were assessed from 12.5 mg/kg bw/d in the 90-day gavage study with K-HDO where gastric ulcers were reported occasionally from 50 mg/kg bw/d, however with no further information. For Cu-HDO effects in the GI tract were seen in the histopathological assessment following exposure to higher doses; 132 mg/kg bw/d in a 28-day study and at 153 mg/kg bw/d in a 90-day study or 61 mg/kg bw/d in a 12-month study and 33 mg/kg bw/d in a 2-year study. However, effects following exposure to Cu-HDO in the GI tract considered as adverse were only seen at doses outside the GV for STOT RE 2.

Liver: In the 90-day gavage study with K-HDO in Sprague-Dawley rats microscopic examinations showed degenerative liver damage seen in some cases as severe dystrophy from 50 mg/kg bw/d. However, bolus application of K-HDO may have disturbed the K^+ homeostasis, seriously compromising the health status of the rats. Therefore, the effects reported in liver and stomach may be considered as secondary to the health status of the animals. In the 28-day inhalation study with K-HDO (one dose, uncertain exposure) in Sprague-Dawley rats effects were reported in females as focal-like liver necrosis and slight fatty metamorphosis. No repeated dose toxicity were performed in Beagle dogs with K-HDO, which were considered to the most sensitive species for effects in the liver following exposure to Cu-HDO. In the 90-day study with Cu-HDO in dogs, chronic hepatitis and cirrhosis as well as oedema in the gall bladder wall was reported at 68 mg/kg bw/d, adverse effects that were within the GV for a STOT RE 2 (between 10 - 100 mg/kg bw/d). However, in this dog-study, 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 was not included. Therefore, it is not possible to assess whether the effects reported in the dogs were related to the exposure to Cu^{2+} or to the HDO^- anion. Liver as a target organ following exposure to Cu-HDO was also supported from the repeated dose toxicity studies in rats, however, RAC considers that the effects reported as adverse in the rat studies with Cu-HDO were outside the GV for a STOT RE 2 classification.

Kidney: No effects on kidney were reported in the studies with K-HDO. As regards the repeated dose toxicity studies with Cu-HDO, RAC is of the opinion that the effects of Cu-HDO on kidney reported in the rats were outside the GV for a STOT RE 2 classification.

The DS proposed to classify K-HDO as STOT RE 2 (liver, GI tract and kidney) based on a read across from Cu-HDO. In the read across assessment for a STOT RE classification it has to be considered if the effects reported in the liver were related to effects of the Cu^{2+} or the HDO^- ion. Therefore, in the 90-day, 1-year and 2-year repeated dose toxicity studies in rats with Cu-HDO, an additional group receiving $CuSO_4$ corresponding to the same amount of Cu^{2+} ions as in the high dose group exposed to Cu-HDO of each study was included. The $CuSO_4$ groups were included to assess if the effects reported for the Cu-HDO exposed groups were related to an effect caused by Cu^{2+} ions or the HDO^- ion. In the 1- and 2-year studies an increased incidence of cyst in the liver of female rats were observed only for the high dose Cu-HDO group, and not in rats exposed to $CuSO_4$.

In summary, RAC considers that a classification as STOT RE 2 (liver) is justified based on the observation that the liver was seen as a target organ following exposure to K-HDO in rats and supported by the read across from Cu-HDO. However, RAC acknowledges the absence of a repeated dose toxicity study with K-HDO in dogs, considering that dog was the most sensitive species following exposure to Cu-HDO.

The classification is further supported by the increased incidence of cysts in the liver that was only reported in the group receiving Cu-HDO and not $CuSO_4$ in the 1- and 2-year studies indicating that it was not the Cu-ion alone, but rather the HDO^- ion that was responsible for the increased incidence of hepatic cysts.

In conclusion, RAC considers that classification as **STOT RE 2 (liver)** is justified based on the liver effects observed following exposure to K-HDO and supported by a read-across of data from Cu-HDO.

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

4.9.1.1 In vitro data

Table 20: Summary table of relevant in vitro and in vivo mutagenicity studies in vitro genotoxicity of K-HDO and Cu-HDO

Tested substance	Test system Method Guideline	organism/ strain(s)	concentra-tions tested *(give range)*	Result	Remark *give information on cytotoxicity and other*	Reference
Purified K-HDO	Ames test OECD 471; GLP	Salmonella typhimurium TA 1535, TA 100, TA 1537, TA 98	15 – 5000 µg per plate With and without metabolic activation	No dose-related increases in revertant counts in any of the four strains in presence or in absence of metabolic activation.	No mutagenic potential, but insufficient positive control for S9 mix and only 4 instead of 5 strains tested	**A6.6.1** **Hoffmann H. D., Engelhardt G. 1989;**
30% K-HDO	In vitro gene mutation in mammalian cells OECD 476; GLP	L5178Y (TK+/-) mouse lymphoma cells	312 – 5000 µg/ml Incubation: 3 and 24h.	no gene mutation; no change of colony size indicating no cytogenetic effects	-	**A6.6.3.2** **Uhde, H, Leuschner, J. ; 2005;**
purified Cu-HDO	Unscheduled DNA synthesis OECD 482 GLP	Primary rat hepatocytes	0.0003 – 0.1 $\mu g/ml^{-1}$ in 5% DMSO. Incubation: 18h.	Viability of cell preparation: > 60%. Cytotoxicity: ≥ 1 $\mu g/ml^{-1}$. No increases in the mean number of net nuclear grain counts compared with negative controls.	Results for Cu-HDO read across to K-HDO No induction of unscheduled DNA repair.	A6.6.3.1 Jäckh 1992

4.9.1.2 In vivo data

Table 21: Summary table of relevant in vitro and in vivo mutagenicity studies in vivo genotoxicity of K-HDO and Cu-HDO

Tested substance	Type of test meth od	Species Strain Sex no/group	frequency of application	sampling times	dose levels	Results *give dose, sampling time and result +/-/+*	Remarks	Reference
Purified K-HDO	Micronucleus assay OECD 474; no GLP	Mouse NMRI 5 m + 5 f animals per group	Administered once orally to male and female	16, 24 and 48 h after treatment	0; 68; 21.5; 6.8 mg/kg bw	Number of polychromatic erythrocytes containing micronuclei in the same range as that of control for all dose groups and all sacrifice intervals Erythropoiesis (polychromatic to normochromatic erythrocytes) not influenced, but higher doses could not be tested because of acute neurotoxic effects: Acute neurological effects at max. tolerated dose of 68.1 mg/ kg bw from 15 min to next day : most mice: irregular respiration, excitation; some mice: tremors, twitchings, tonic and clonic convulsions, ruffled fur, apathy At 21.5 and 6.8 mg/kg bw 15-30 min after admin., not on next day : irregular respiration, slight excitation, ruffled fur	No chromosome-damaging (clastogenic) effect no indications of any impairment of distribution in the course of mitosis. But: Study does not demonstrate that K-HDO reaches the bone marrow. Also the kinetic study shows that plasma levels remain very low at all time points.	**A6.6.4 Gelbke H.-P., Engelhardt G. 1982;**

4.9.2 Human information

Not available

4.9.3 Other relevant information

Not available

4.9.4 Summary and discussion of mutagenicity

The toxicity-tests conducted with the purified K-HDO are relevant for the hazard evaluation of the K-HDO as manufactured, since the latter does not contain toxicologically relevant impurities in concentrations above 0.1%.

K-HDO did not show genotoxic effects in the Ames-test, the TK-mouse-lymphoma 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.

The reliability of the micronucleus test is also somewhat restricted since the relation of polychromatic to normochromatic erythrocytes is not affected in the highest dose, means that there is no evidence that K-HDO reached the bone marrow. Furthermore also the kinetic studies show that plasma levels remain below 0.1% of the applied dose. However higher doses could not be applied because of the acute neurotoxic effects of K^+.

In contrast the TK-mouse-lymphoma assay is fully valid. This assay is considered to be sensitive for mutagenic and clastogenic events (CPMP/IHC/1141/95).

Furthermore a fully valid in vitro UDS test with primary rat hepatocytes was carried out with Cu-HDO to further support the negative genotoxicity test battery of K-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 provides some further support for the negative genotoxicity test battery with K-HDO, since it could well be that after solution in the complex culture medium and after cellular uptake the stability and the metabolism of the HDO stemming from Cu-HDO or K-HDO are comparable.

Taking all genotoxicity test results together and considering the negative carcinogenicity test with Cu-HDO (see below, chapter 3.7.) there is no indication for a genotoxic potential of K-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-oxidizable alkyl group linked to the nitrogen, which seem to be essential features of genotoxic nitrosamines. (see e.g. Marquardt & Schäfer 2004, ISBN 3-80-47-1777-2). Moreover, mutagenic nitrosamines show positive results in the in vitro mutagenicity and UDS assays, which is not the case for K-HDO.

4.9.5 Comparison with criteria

See discussion above

4.9.6 Conclusions on classification and labelling

No classification is necessary.

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 (OECD TG 471, GLP) and one TK mouse lymphoma assay (OECD TG 476, GLP). In addition, one USD test (OECD TG 482) using Cu-HDO was included. Furthermore, the DS included one *in vivo* study: a micronucleus assay performed with purified K-HDO (OECD TG 474, non GLP).

In vitro studies

The Ames test (OECD TG 471, GLP) was performed with *S. typhimurium* (TA1535, TA100, TA1537, TA98) at concentrations of 15-5000 µg with and without metabolic activation. K-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 is missing and 2-aminoanthracene was used as the only positive control with S9 activation (A 6.6.1).

The DS included one 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 addition, the DS included one 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. This study did not show any increase in the mean number of net nuclear grain counts compared with negative controls (A 6.6.3.1).

In vivo studies

One micronucleus assay (OECD TG 474, no GLP) was performed with 5 male and 5 female NMRI mice per group at dose levels of 0, 6.8, 21.5 and 68 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. The study did not provide evidence that K-HDO reaches the bone marrow since the ratio of PCE to NCE was not affected at the highest dose tested. However, higher doses could not be tested due to the toxicity observed at the highest tested dose (A 6.6.4).

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

Comments received during public consultation

Two commenting MSCAs supported the proposed no classification for mutagenicity.

Assessment and comparison with the classification criteria

There were no human data available for K-HDO, therefore classification with Muta. 1A is not justified.

Further, a classification with 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

Data for Cu-HDO and arguments to read across these data to K-HDO were submitted. The essential read-across arguments are already listed in chapter 4.7.1.7, see bullet points. The carcinogenicity study is considered to be a key study; the critical long term NOAEL is derived from this study.

Table 22a: Summary table of relevant carcinogenicity studies of purified Cu-HDO

Substance tested	Route	Species Strain Sex no/group	dose levels frequency of application	Effects observed	NOAEL [mg/kg bw day]	Reference
Purified Cu-HDO	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 (m); ↑ number of males with hyperkeratosis of the forestomach's wall 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 $CuSO_4$; No indication of a carcinogenic potential.	6 Cu-HDO corr. to 6,25 K-HDO (~20.8 of 30% K-HDO)	**A6.7 Mellert; 1996; GLP**

Table 22b 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)

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PATHOLOGY REPORT — 70C0679/89113
BIS-(N-CYCLOHEXYL-DIAZENIUMDIOXY)-COPPER — Jan/30/1996 CEGE
24-Month Feeding Study in Rats — 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.

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. Why these histological findings in the GI tract were observed only with Cu-HDO and not with K-HDO might be explained – as discussed with the read across arguments – by the slow dissociation of Cu-HDO leading to intracellular cytotoxic Cu^{2+} levels and the other observations given under 4.7.1.7. The NOAEL of 6 mg/kg bw day corresponds to an equivalent NOAEL for K-HDO of 6.25 mg/kg bw day. As mentioned in chapter 4.7.1.7. (see bullet points) according to the toxicological repeated dose profile available for K-HDO this is considered to be a sufficiently conservative estimate.

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 as well as K-HDO are 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 of Cu-HDO and the substance does not meet the criteria for classification. This conclusion is read across to K-HDO based on the arguments listed in chapter 4.7.1.7.

4.10.6 Conclusions on classification and labelling

No classification necessary.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No carcinogenicity study following exposure to K-HDO was available, therefore the DS used a read across data from Cu-HDO. The arguments for read across from Cu-HDO to K-HDO are provided in the section "RAC general comment". The results from the 2-year carcinogenicity study with exposure to Cu-HDO are included below.

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 in males and 0, 6, 33 and 189 mg/kg bw/d 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.

Incidences of vascular tumours in the mesenteric lymph nodes:

Parameter	HCD	0 mg/kg bw/d	5/6 mg/kg bw/d	29/33 mg/kg bw/d	148/189 g/kg bw/d	$CuSO_4$: 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 the table below):

Incidences of vascular tumours in all organs assessed:

Parameter	0 mg/kg bw/d	5/6 mg/kg bw/d	29/33 mg/kg bw/d	148/189 g/kg bw/d	$CuSO_4$: 67 mg/kg bw/d
# animals with vascular tumours	13M/4F	9M/3G	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 numbers 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.

An overview of all tumours:

Parameter	**0 mg/kg bw/d**	**5/6 mg/kg bw/d**	**29/33 mg/kg bw/d**	**148/189 mg/kg bw/d**	**$CuSO_4$: 67 mg/kg bw/d**
# 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 the conclusion that there is inadequate evidence for a carcinogenic potential following exposure to Cu-HDO or $CuSO_4$ in rats. This was based on the arguments, that the findings do not differ biologically from the control animals interms 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. The DS concluded that the results from the study do not meet the criteria for classification for carcinogenicity and the read across from Cu-HDO to K-HDO is supported.

Comments received during public consultation

Comments were received from two MSCAs; one supported the proposed read across o data from Cu-HDO, and that no classification for K-HDO was warranted. The other 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 unusual that the historical control data (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, they 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 also included in the RAC Opinion.

Assessment and comparison with the classification criteria

No carcinogenicity study following exposure to K-HDO was available, therefore the DS used a read across approach from Cu-HDO; this is supported by RAC (see "RAC general comments" above).

In the only carcinogenicity study (2-year, oral), 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 were 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 haemagioma with no progression to malignancy. The incidences of vascular tumours in all organs assessed were comparable in all groups including the controls and Cu-HDO and $CuSO_4$ 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 no classification for carcinogenicity is justified for K-HDO. This is based on the absence of a carcinogenicity study for K-HDO and supported by a read across

from Cu-HDO data.

In the carcinogenicity study for Cu-HDO it was assessed if Cu^{2+} could be responsible for carcinogenicity, however no increased incidence of tumours was reported in the groups exposed to Cu-HDO or $CuSO_4$. On this basis and bearing in mind that Cu-HDO and K-HDO both form the same HDO^- ion, a read across of the carcinogenicity study data from Cu-HDO to K-HDO is fully justified. RAC is therefore of the opinion that **no classification for K-HDO 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 or K-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 NOAELsubchr./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 23.1: Summary of developmental toxicity studies with Cu-HDO

Substance tested	**Route of exposure**	**Test type Method Guideline**	**Species Strain Sex no/group**	**Exposure Period**	**Doses** [mg/kg bw day]	**Critical effects dams fetuses**	**NO(A)EL maternal toxicity**	**NO(A)EL Teratogenicity embryotoxicity**	**Reference**
Purified Cu-HDO	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** (~ 31.2 mg/kg bw day for K-HDO or 104.2 mg/kg bw day for 30% K-HDO) 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** (~104,2 mg/kg bw day for K-HDO or 347.3 mg/kg bw day 30% K-HDO), 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.			**A6.8.1.1 Hellwig; 1991; GLP**
Purified Cu-HDO	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. (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 (days 7 – 20 p.i.) [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., 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** (~10.4 mg/kg bw day for K-HDO or 34.7 mg/kg bw day for 30% K-HDO) **developmental NOAEL: 10 mg/kg bw day** (~10.4 mg/kg bw day for K-HDO or 34.7 mg/kg bw day for 30% K-HDO)			**A6.8.1.2 Hellwig; 1994; GLP**

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 23.2. 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 23.3. 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)	–

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

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 detailed discussions 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 for Cu-HDO. This conclusion is read across to K-HDO based on the arguments listed in chapter 4.7.1.7.

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 was available for the assessment of effects on fertility and sexual function or for effects on development following exposure to K-HDO.

Effects on fertility and sexual function

No 2-generation study with exposure to K-HDO or Cu-HDO was available and the waiver provided by the Applicant was 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 K-HDO and Cu-HDO, i.e. by analysing the clear link observed between effects in male reproductive organs and effects on functional fertility in several studies (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 K-HDO and 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

No developmental toxicity study with exposure to K-HDO was available, therefore the DS used a read across approach from Cu-HDO data (see "RAC general comments" above. For Cu-HDO 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 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 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 29 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 (GD 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 dams.

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 groups 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 addition, 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 (GD 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 one MSCA, which supported no classification for effects on fertility and sexual function, and strongly regretted the absence of fertility study.

The 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 range. 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, 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.

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 potential effects of K-HDO and Cu-HDO on sexual function and fertility was only available from repeated dose toxicity studies, as no studies on sexual development and fertility were available. 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. 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 K-HDO for effects on sexual function and fertility is justified based on the data available.

However, RAC notes the absence of a 2-generation reproductive toxicity study, data from which is considered by RAC to be needed to fully assess effects on sexual function and fertility under CLP.

Developmental toxicity

The DS included two developmental toxicity studies performed according to OECD TG 414 with Cu-HDO 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 to 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).

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.

Litter response in the rat developmental toxicity study:

Parameter	**HCD**	**0 mg/kg bw/d**	**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/litters	13.2	13.0	14.2	13.8	13.3
Dead foetuses/litters	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 relatioship. 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 malformations were also reported in all exposed groups, without a dose-response relatioship, 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 at the upper range of the HCD.

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

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 inversus	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, there were no effects that 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 (GD 10-13) and blood in bedding of another dam (due to litter loss). For further details see the table below:

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 statistically significantly 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 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 dams. 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.

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/litters (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/litters (ratio)	0.005	0	1/15 (0.07:1)	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 are 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	5.8	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

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*

*both the thickened and shortened hind limb and the polydactyly were observed in the same the high dose foetuses.

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 relationship was seen. Furthermore, the DS informed that during the skeletal examination, the shortened and bent tibia and fibula observed were 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 range 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 group 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 be considered whether the maternal toxicity reported in the mid and high dose groups evident as statistically significantly reduced food consumption during GD 7-19 leading to a statistically significantly 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 public consultation and in the review by Nitzsche (2017) in which an analysis of the effects of maternal feed restriction on prenatal development in rats and rabbits was included. This review concluded that effects on embryolethality 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 sternebral malformations (4%) reported at a maternal feed intake of 10% of the control group. HCD from the study by Ema *et al.* (2012) were 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 is not considered associated with treatment to Cu-HDO but instead are considered to be spontaneous.

Comparison with the CLP classification criteria

RAC is of the opinion that no classification for developmental toxicity is justified for K-HDO based onread across of relevant data from Cu-HDO.

Overall, RAC is of the opinion that **no classification for effects on fertility and sexual function, and development is warranted for K-HDO**.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Table 24. Neurotoxicity of K-HDO

Substance applied	**Route**	**duration of study**	**Species Strain Sex no/group**	**dose levels frequency of application**	**Results**	**NO(A)EL for neurological effects** [mg/kg bw day]	**Reference**
Purified K-HDO	Oral, **feeding**	28 days	Wistar Rat 5 males and 5 females per group	0 (control); 82 (m) and 90 (f) mg/kg bw day	**No clinical signs** and **no functional effects in the functional observation test battery** …	**> 90**	**A6.9.** **Mellert 1992; GLP**
Purified K-HDO	Oral, **feeding**	about 42 days	Sprague-Dawley rat. 10 males and10 females per group	ca. 0, 10, 30, 100 and 1000 mg/kg bw	No mortality during study. No significant body weight changes, slight effects on food consumption. **No clinical signs** No substance induced gross-pathological organ findings or organ weight changes.	**> 724**	**A6.3.1** **Hofmann H. Th., Freisberg K. O.; 1976; no GLP**
Purified K-HDO	Oral **gavage**	acute, single administration	Sprague-Dawley rats 5 males + 5 females per group	56,2, 68,1, 82,5, 100, 121, 147, 178, 215, 261 mg/kg	acute neurological effects also at low dose level	**< 56**	**A6.1.1** **Munk, Gelbke, H.-P. 1977; no GLP**

Substance applied	Route	duration of study	Species Strain Sex no/group	dose levels frequency of application	Results	NO(A)EL for neurological effects [mg/kg bw day]	Reference
Purified K-HDO	Oral, **gavage** Micronucleus assay	acute, single administration ; analysis 16, 24 and 48 h after treatment	NMRI mouse Male/female 10 animals per group	0; 6,8; 21,5; 68,1 mg/kg bw	… **Acute neurological effects** at max. tolerated dose of 68,1 mg/ kg bw from 15 min to next day : most mice: irregular respiration, excitation; some mice: tremors, twitchings, tonic and clonic convulsions, ruffled fur, apathy At 21,5 and 6,8 mg/kg bw 15-30 min after admin., not on next day: irregular respiration, slight excitation, ruffled fur	**< 6,8**	**A6.6.4 Gelbke H.-P., Engelhardt G. 1982; no GLP**

4.12.1.2 Immunotoxicity

Not available

4.12.1.3 Specific investigations: other studies

Not available

4.12.1.4 Human information

Not available

4.12.2 Summary and discussion

Clinical signs of neurotoxicity have been analysed in acute gavage, subacute feeding and subchronic gavage studies (see table below). Within the gavage studies the clinical neurological effect of the K^+ ion is evident at doses between 50 and 60 mg/kg bw, whereas within the feeding studies no clinical neurological effects are seen up to and including 724 mg/kg bw which is presumably due to the slower uptake of the K^+ ion.

Within the 96 day study the LOAEL for the acute neurotoxic effects was between 25 and 50 mg/kg bw day which is in the same range where the same acute neurotoxic effects were seen in the acute gavage study, this means that the adverse effect level did not significantly decrease from the acute to the subchronic study.

Within the functional observation test battery of the subacute feeding study no functional effects were observed at the single dose level analysed (90 mg/kg bw).

Thus under realistic human exposure scenarios (which do not include a high dose bolus application) no specific concern for neurotoxicological effects can be deduced from the data submitted.

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 25: Summary of relevant information on 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
OECD 111; EC C.7; / Hydrolysis as a function of pH;	4 7 9	30, 42 and 50°C 50°C 50°C	1 g K-HDO monohydrate / L	0,5485 d^{-1} (25°C)	**DT_{50} = 1.26 d at 25°C** **stable** **stable**		**Wittenzellner J. (2004b)** A 7.1.1.1.1

K-HDO is hydrolytically stable at pH 7 and 9. It hydrolyses at pH 4 with an estimated half-life time of 1.26 days at 25°C. The reaction does not follow first order kinetic. The only degradation product identified but not quantified at pH 4 was Cyclohexanone oxime.

Conclusion:
K-HDO is stable to hydrolysis at pH 7 and 9. At pH 4 hydrolysis occurs with a DT_{50} value of 1.26 d under formation of Cyclohexanone oxime (not quantified). There is no harmonised classification available for that metabolite.
According to the Guidance on the Application of the CLP Criteria v.4.1, Annex II, chapter 2 the longest half-life value determined within the pH range 4-9 has to be shorter than 16 days in order that hydrolysis data may be used for classification purposes. Since K-HDO is stable at pH 7 and 9 this is not the case.
Therefore, according to the Guidance on the Application of the CLP Criteria v.4.1, Annex II, chapter 2 and 4, it is concluded that the results of the hydrolysis study indicate that K-HDO is not rapidly biodegradable.

Photolysis in water

There are no data available on potolysis in water.
Due to the adsorption coefficient of 6006 L/kg photolysis in water is not expected to represent a major degradation pathway in the environment, since K-HDO will adsorb very quickly onto organic matter.

Photo-oxidation in air

The degradation rate constant of K-HDO with OH-radicals (k_{OH} in $cm^3 \cdot molecule^{-1} \cdot s^{-1}$) was estimated with an Atmospheric Oxidation Program (AOP 1.91, Epi Suite, Syracuse Research corporation):

Specific first-order degradation rate

constant of K-HDO with OH-radicals: k_{OH} (K-HDO) = $34.36 \cdot 10^{-12}$ $cm^3 \cdot molecule^{-1} \cdot s^{-1}$

OH radical concentration: $[OH\cdot] = 1.5 \bullet 10^6$ OH / cm^3.

half-life of K-HDO: $T_{1/2}$ = 3.7 hours

Estimation according to EU TGD, Part II, Chapter 2.3.6.3:

Specific first-order degradation rate

constant of K-HDO with OH-radicals: k_{OH} (K-HDO) = $34.36 \cdot 10^{-12}$ $cm^3 \cdot molecule^{-1} \cdot s^{-1}$

OH radical concentration: $[OH\cdot] = 0.5 \bullet 10^6$ OH / cm^3.

half-life of K-HDO: $T_{1/2}$ = 11.2 hours

Conclusion:

Because of the low vapour pressure and the short lifetime in the atmosphere, and due to the fact that K-HDO does not contain any atoms of chlorine, bromine or fluorine, an effect of K-HDO on stratospheric ozone is not expected.

In addition, the very low vapour pressure (< 10^{-6} hPa at 20 °C) suggests that the amounts of K-HDO which are present in the atmosphere are marginal.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

No data available.

5.1.2.2 Screening tests

Table 26: Summary of relevant information on Biodegradation, Screening tests

Guideline / Test method	Test type[1]	Test parameter	Inoculum			Additional substrate	Test substance concentr.	Degradation		Reference
			Type	Concentration	Adaptation			Incubation period	Degree [%]	
DIN 38409, part 51; / BOD "Ready Biodegradability"	enhanced ready	BOD / ThOD	Activated sludge	1g/l dry matter	**Yes 69 days**	-	6.1 mg K-HDO / L (corresponds to 20.4 mg 30% w/w K-HDO)	30 d	**Ca. 60%**	**Haid M. (1996)** A 7.1.1.2.1
OECD 302 B / "Inherent biodegradability: Modified Zahn-Wellens Test	Inherent	DOC - removal	Activated sludge from laboratory plants with municipal waste water	1g/l dry matter	**< 1 day**	-	2 mg K-HDO / L	28 d	**98% elimination (57% elimination due to adsorption)**	**Haid M. (1995)** A7.1.1.2.2

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

The biodegradability of K-HDO 30% in water has been investigated in an enhanced ready test (Haid M., 1996, Document A7.1.1.2.1, key study) and in an inherent test (Haid M., 1995, Document A7.1.1.2.2, key study).

In the BOD-test (Haid M., 1996, Document A7.1.1.2.1) a biodegradation degree of 60% for K-HDO has been reached after 30 days. In this test the inoculum has been pre-adapted to the test substance for 69 days. In addition K-HDO has been tested at inhibitory concentrations relative to the results of the Activated Sludge, Respiration Inhibition Test (Taeger K., 1995, Document A III 7.4.1.4). The test substance concentration of 6.1 mg/L was chosen, because evaluation was only possible around this concentration range. With concentrations >7 mg/L the oxygen consumption was too high in order to calculate a BOD value and with concentrations <3 mg/L oxygen consumption was too low to be measured. Biodegradation of K-HDO was therefore not inhibited at the used concentration, despite the results of the Activated Sludge Inhibition Test. The result of the BOD test is not regarded as a proof for a ready bio-degradability of K-HDO and the substance is therefore considered as being “not readily biodegradable”.

Since in the BOD test after 69 days of pre-adaption the pass-level for ready biodegradability was just reached after 30 days, and in addition a negative inherent biodegradation test has been submitted (Haid M., 1995, Document A7.1.1.2.2), no new study on ready biodegradability has been asked for.

In the Zahn–Wellens test (Haid M., 1995, Document A7.1.1.2.2) almost no adaptation (<1 day) of the inoculum took place. An elimination rate of 98% was reached after 28 days. 57% of this elimination took place within the first three hours, which indicates elimination due to adsorption. DOC measurement was performed, but no abiotic control was run in parallel. Therefore there is no proof for biodegradation in the test system. As can further be seen in section 5.2.1 Adsorption/Desorption K-HDO adsorbs strongly onto organic matter with a mean K_{Foc} of 6006 L/kg. Therefore it is concluded that K-HDO is well eliminated from water, mainly through adsorption. K-HDO may possibly be regarded as primary inherently biodegradable, but in no case as ultimately inherently biodegradable.

Conclusion:

Based on the results of the screening tests it is concluded that K-HDO is not rapidly 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. 4.1, Annex II, chapter 4.

5.1.2.3 Simulation tests

Biodegradation, STP

Table 27: Summary of relevant information on Biodegradation, STP

Guideline / Test method	Test type[1]	Test para-meter	Inoculum			Additional substrate	Test substance concentr.	Degradation		Reference
			Type	Concen-tration	Adap-tation			Incubation period	Degree [%]	
OECD 303 A / Aerobic Sewage Treatment; Activated Sludge Units	Simulation test		Activated sludge			-	100 mg K-HDO / L	41 d	> 90%	Reuther (1980)

Conclusion:

The simulation test (Reuther, 1980) is very badly documented which makes it impossible to evaluate the test report properly. Therefore the results of this test report are considered as being not valid.

According to the Guidance on the Application of the CLP Criteria v.4.1, Annex II, chapter 2 results from sewage treatment plant simulation tests (e.g. OECD 303) cannot be used for the assessment of rapid degradation in the aquatic environment. Therefore and also due to the very poor quality, the data were not used further for classification purposes.

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:

K-HDO was tested in an enhanced ready test; **study A 7.1.1.2.1, document III-A 7.1.1.2.1** (pre-adaptation of the inoculum for 69 days, test duration30 days, BOD/ThOD measurement lead to ca. 60% biodegradation).
Therefore **K-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 are no data available.

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 of K-HDO** occurs only at pH 4, with a DT_{50} of 1.26 days at 25°C. K-HDO has been shown to be **hydrolytically stable at 50°C and at pH 7 and 9 (study A 7.1.1.1.1, document III-A 7.1.1.1.1)**. According to the Guidance on the Application of the CLP Criteria v.4.1, Annex II, chapter 2, data on hydrolysis might only be considered for the determination of rapid degradation, if the longest half-life within the pH range 4-9 is <16 days. Since K-HDO is stable at pH 7 and 9 this is not the case. Therefore the results of the hydrolysis study indicate that **K-HDO is not rapidly degradable** through hydrolysis, according to the same criteria, chapter 4, Decision scheme.

- Due to the **adsorption coefficient of 6006 L/kg** photolysis in water is not expected to **represent a major degradation pathway** in the environment, since K-**HDO will adsorb very quickly onto organic matter.**

5.2 Environmental distribution

No data available

5.2.1 Adsorption/Desorption

Table 29: Summary of relevant information on Adsorption onto / desorption from soils (substance: purified K-HDO)

Guideline	**Soil**	**A_{eq} [%] at $R_{S/T}$ = 2**	**K_F^{ads} [-]**	**$K_{F,OC}^{ads}$ [-]**	**K_F^{des} [-]**	**$K_{F,OC}^{des}$ [-]**	**K_F^{ads} / K_F^{des} [-]**	**Reference**
OECD 106	Bruch West (loamy sand)	32.8	20.5	805	27	1064	0.76	**Groß G. (2006)**
	LUFA 2.1 (loamy sand)	65.6	66.3	10518	103.3	15472	0.64	A 7.1.3/03
	LUFA 2.2 (loamy sand)	88.3	233.8	10606	343.8	16293	0.68	
	LUFA 2.3 (loamy sand)	47.2	38.1	3739	53.7	5261	0.71	
	LUFA 6S (clay loam/clay)	62.7	79.8	4360	93.5	5112	0.85	
	mean			**6006**		**8640**		

The adsorption/desorption behaviour of purified K-HDO has been investigated (Groß G. 2006, A 7.1.3/03, key study; see Table 29) according to OECD 106. Freundlich adsorption and desorption coefficients for five different soils were determined; they give indication of irreversible adsorption which was >25% at equilibration time and a ratio soil / test item solution = 2 for all five soil types tested. The mean Freundlich adsorption coefficient was determined with 6006 L/kg.

Table 30a: Summary of relevant information on Adsorption onto / desorption from soils; **supportive studies**

Guideline	**Soil**	**Substance**	**K_{oc}**(K-HDO)	**K_{oc}**(Cu)	**K_{OC}**(HDO)	**Reference**

OECD 121 / Estimation of the Adsorption Coefficient using HPLC	Cyanopropyl stationary phase pH = 2.5	purified K-HDO	log K_{oc} = 1.25			Büldt (2001)
calculation according to EPIWIN	Soil	K-HDO	log K_{oc} = 2.17			

Other supportive studies and statements concerning this endpoint were a HPLC screening test (Büldt, 2001) and a calculation of the K_{oc} value for K-HDO (EPIWIN model).

None of these supportive reports were considered valid for the following reasons: In the HPLC screening test (Büldt, 2001) an acceptable chromatogram could only be obtained at pH 2.5 and not between 5.5 and 7.5, which is normal for agricultural soils or tanks of sewage treatment plants. Therefore it was concluded that the HPLC screening method is not applicable for K-HDO.

The calculation with the EPIWIN model has not been accepted since there is no common agreement within the EU on the use of (Q)SAR calculations for the determination of intrinsic properties of substances.

5.2.2 Volatilisation

Table 30b: vapour pressure

PROPERTY	PURITY / SPECIFICATION	RESULT	METHOD / REFERENCE
Vapour pressure	purified a.s.	< 10^{-6} hPa at 50°C and at 20°C	Dir 92/69/EEC, Annex V, A.4; Büldt 2001; **A 3.1.1/01**

5.2.3 Distribution modelling

See discussion above

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Table 30c: Estimations on aquatic bioconcentration

Basis for estimation	log K_{OW} (measured)	Estimated BCF for K-HDO	Reference
Calculation	-0.2	The log BCF-value can be calculated using the log K_{ow} values log BCF =0.85 x log Pow - 0.7 Therefore the calculated value is -0.87 and the **BCF** 0.134.	**Büldt A. (2001**) A 3.1.1/01

The BCF of K-HDO is 0.134.

5.3.1.2 Measured bioaccumulation data

No data available

5.3.2 Summary and discussion of aquatic bioaccumulation

Measured BCF data are not available for K-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 -0.2 has to be used.

5.4 Aquatic toxicity

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

In a standard laboratory test K-HDO shows low acute toxicity to fish, as indicated by the LC_{50}-value of 51.3 mg/L for the golden orfe.

Laboratory studies conducted with 30% w/w K-HDO to assess the toxicity to aquatic organisms are summarised in Tables 31to 36.

Table 31: Acute toxicity to fish

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results (mg/L) nominal			Remarks	Reference
			design	duration	LC_0	LC_{50}	LC_{100}		

DIN 38412	Golden orfe	mortality	static	96 h	30 (corresponds to 100 mg 30% w/w K-HDO/L)	**51.3** (corresponds to 171 mg 30% w/w K-HDO/L)	94.81 (corresponds to 316 mg 30% w/w K-HDO/L)	Test with Xyligen 30 F	**Gelbke H.-P., Munk R. (1980)** A 7.4.1.1

5.4.1.2 Long-term toxicity to fish

A fish juvenile growth test according to the OECD 215 guideline was carried out with K-HDO for a period of 28 days following the OECD guideline for Testing of Chemicals No. 215, adopted January 2000 "Fish, Juvenile Growth Test" (study A 7.4.3.2).

Juvenile zebra fish (Danio rerio) were exposed to 0.033 / 0.11 / 0.33 / 1.1 and 3.3 mg K-HDO/L. The test concentrations were selected on the basis of preliminary tests, which indicated mortality at 10.0 mg/L within test duration of 4 days. The study was performed under flow-through conditions with 5 concentrations of the test substance and a dilution water control. The temperature was maintained generally at 24°C, the dilution water was none-chlorinated drinking water obtained from the municipal water works mixed with deionised water to achieve a hardness of 1.4 mmol/L.

In the control and the concentration groups up to 0.33mg/L all fish survived until sacrifice. In the highest concentration group (3.3 mg/L), all fish died during the first day of exposure. In the concentration group 1.1 mg/L the survival rate was 30%. Mortalities were observed from days 1 – 14 after start of exposure.

In comparison to the control group the growth rate was statistically significantly reduced in the surviving animals of the concentration group 1.1 mg/L after 14 days. No effects on the growth rate were detected in the lower concentration groups (nominal 0.033, 0.11 and 0.33 mg/L).

Over the exposure period, no toxic signs and no abnormalities in the control and in the surviving fish of the concentration groups were observed.

In conclusion, the overall NOEC was 0.33 mg/L (nominal concentration) and 0.29 mg/L (based on the mean analytically determined concentrations) and the LOEC was 1.1 mg/L (nominal concentration) and 0.74 mg/L (based on the mean analytically determined concentrations).

The two highest concentrations showed deviations of >20% of nominal. So the toxicity endpoints are given in mean analytically determined concentrations. Please see Table 32.

Table 32: Chronic toxicity to fish

Guideline /Test method	**Species**	**Endpoint /Type of test**	**Exposure**		**Results in mg/L mean measured**		**Reference**
			Design	**Duration**	**NOEC**	**LOEC**	
OECD guideline 215	*Danio rerio*	Growth rate	Flow through	28 days	**0.29**	0.74	**Study A 7.4.3.2 (2005)**

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

K-HDO is also of low toxicity to *Daphnia magna* with an EC_{50} of > 30 mg/L.

Table 33: Acute toxicity to invertebrates

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results (mg/L) nominal			Remarks	Reference
			design	duration	EC_0	EC_{50}	EC_{100}		
OECD 202	*Daphnia magna*	immobility	static	48 h	30* (corresponds to 100 mg 30% w/w K-HDO/L)	**> 30*** (corresponds to >100 mg 30% w/w K-HDO/L)	> 30* (corresponds to >100 mg 30% w/w K-HDO/L)	Test with Xyligen 30 F	**Jatzek , H.J. (2002).** A 7.4.1.2
DIN 38412	*Daphnia magna*	immobility	static	48 h	23.4** (corresponds to 78 mg 30% w/w K-HDO/L)	39** (corresponds to 130 mg 30% w/w K-HDO/L)	57.9** (corresponds to 193 mg 30% w/w K-HDO/L)	Test with 30 % K-HDO	Buchen, G. (1993a)

***** nominal confirmed

****** no measurement of test concentration

5.4.2.2 Long-term toxicity to aquatic invertebrates

The chronic toxicity to Daphnia magna was determined in a 21-day reproduction study and the NOEC, based on numbers of offspring per adult, results in 0.47 mg a.i./L.

Table 34: Chronic toxicity to aquatic invertebrates

Guideline	Species	Endpoint / Type of test	Exposure		Results mg /L (nominal confirmed)			Remarks	Reference
			Design	Duration	NOEC	LOEC	EC_{50}		
OECD 211	*Daphnia magna*	reproduction and mortality	semi-static	21 days	**0.47** (corresponds to 1.56 mg 30% w/w K-HDO/L)	0.94 (corresponds to 3.13 mg 30% w/w K-HDO/L)	2.91 (corresponds to 9.7 mg 30% w/w K-HDO/L)	Test with Xyligen 30 F	**Hertl , J. (2002)** A 7.4.3.4

5.4.3 Algae and aquatic plants

K-HDO is only slightly toxic to algae, as shown by E_rC_{50} and E_bC_{50} values >30 and 15.6 mg a.i./L, respectively.

Table 35: Growth inhibition on algae

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results (mg/L) (nominal confirmed)			Remarks	Reference
			design	duration	NOE_rC	E_bC_{50}	E_rC_{50}		
OECD 201	*Desmodesmus subspicatus* (algae)	growth rate/biomass	static	72 h	**3.75** (corresponds to 12.5 mg 30% w/w K-HDO/L)	**15.6** (corresponds to 52 mg 30% w/w K-HDO/L)	**> 30** (corresponds to >100 mg 30% w/w K-HDO/L)	Test with Xyligen 30 F	**Werner , D.I.** (2002) A 7.4.1.3

5.4.4 Other aquatic organisms (including sediment)

The inhibitory effects of K-HDO to microbial activity have been investigated in 3 tests. In the only valid test (Taeger K., 1995, Document A7.4.1.4/01) the EC50 of K-HDO was graphically determined with ca. 9 mg/L (nominal), the EC20 was ca. 1.44 mg/L (nominal) and the EC10 was ca.1.1 mg/L (nominal; corresponds to ca. 3.6 mg 30% K-HDO/L).

The other tests (Buchen G., 1993b and Buchen G., 1993c) were not considered valid due to a very poor documentation of the data in the test report, which made a proper evaluation of the reports impossible.

Table 36: Inhibition of microbial activity (aquatic)

Guideline / Test method	Species / Inoculum	Endpoint / Type of test	Exposure		Results (nominal)			Remarks	Reference
			design	duration	EC_{20}	EC_{50}	EC_{80}		
OECD 209 / Activated Sludge, Respiration Inhibition Test	Activated sludge	Oxygen consumption/ Respiration inhibition	-	30 min	**Ca.1.44 mg/L** (corresponds to ca. 4.8 mg 30% w/w K-HDO/L; graphically determined)	**Ca. 9 mg/L** (corresponds to ca. 30 mg 30% w/w K-HDO/L; graphically determined)	Not reached	Test with K-HDO 30% w/w in water	**Taeger K. (1995)** A 7.4.1.4 / 01
DIN 38412, Part 8 / Pseudomonas putida growth inhibition test	Pseudomonas Putida	Growth inhibition	DIN 38412	16 h		Ca. 6.9 mg/L (corresponds to ca. 23 mg 30% w/w K-HDO/L; graphically determined)	Ca. 11.4 mg/L (corresponds to ca. 38 mg 30% w/w K-HDO/L; graphically determined)	Test with Xyligen K (= K-HDO 30% w/w in water)	Buchen G. (1993b)
DIN 38412, Part 34 / Luminescence inhibition test	Vibrio fischeri	Luminescence inhibition	DIN 38412	30 min	7.9 mg/L (corresponds to 26.3 mg 30% w/w K-HDO/L)	40.5 mg/L (corresponds to 135 mg 30% w/w K-HDO/L)	-	Test with Xyligen K (= K-HDO 30% w/w in water)	Buchen G. (1993c)

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4, according to Reg. (EU) No 1272/2008, Table 3.1 and Reg. (EU) No 286/2011)

All data discussed in this section and used for classification purposes always refer to 100% K-HDO.

Aquatic Acute 1:

Available aquatic acute toxicity values (L(E)C_{50}) for all three trophic levels are between 10 – 100 mg/L;

➔ **No classification**

Studies used:

- Doc. III A 7.4.1.1: Gelbke H.-P., Munk R. (1980), DIN 38412 -> **LC_{50} (fish) = 51.3 mg/L**
- Doc. III A 7.4.1.2: Jatzek H.J. (2002), OECD 202 -> **EC_{50} (crustacea) > 30 mg/L**
- Doc. III A 7.4.1.3: Werner D.I. (2002), OECD 201 -> **E_rC_{50} (algae) > 30 mg/L**

Aquatic Chronic Categories:

There are chronic data available for all three trophic levels and K-HDO is not rapidly degradable (after 69 days of pre-adaptation 60% biodegradation in an enhanced ready test; hydrolytically stable at pH 7 and 9, at pH 4 DT_{50} of 1.26 days at 25°C).
Chronic NOEC values for all three trophic levels are between 0.1 and 10 mg/L; the lowest chronic NOEC values are the NOEC for fish with 0.29 mg/L and the NOEC for daphnia with 0.47 mg/L.

Aquatic Chronic 1:

➔ **No classification**

Aquatic Chronic 2:

➔ **classification with Aquatic Chronic 2**

Studies used:

- Doc. III A7.1.1.2.1: Haid M. (1996), DIN 38409, part 51 –> **ca. 60% degradation in 28 days, after 69 days of pre-adaptation**
- Doc. III A7.1.1.1.1: Wittenzellner J. (2004b), EEC C.7, OECD 111 -> **hydrolytically stable at pH 7 and 9 at 50°C, at pH 4 DT_{50} = 1.26 days at 25°C**
- Doc. III A7.4.3.2: Zok S. (2005), OECD 215 -> **NOEC (fish) = 0.29 mg/L**
- Doc. III A7.4.3.4: Hertl J. (2002), OECD 211 -> **NOEC (crustacea) = 0.47 mg/L**
- Doc. III A 7.4.1.3: Werner D.I. (2002), OECD 201 -> **NOE_rC (algae) = 3.75 mg/L**

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

Proposed classification according to Reg. (EU) No 1272/2008, Table 3.1 and Reg. (EU) No 286/2011(proposed by RMS)

Classification		**Justification**
Classification	Aquatic chronic 2	($L(E)C_{50}$ values for all three trophic levels are > 1 mg/L, therefore no acute classification. Not rapidly degradable and chronic NOEC values available for all trophic levels. Lowest available chronic NOEC value (fish) = 0.29 mg/L.
Hazard statements	H411: Toxic to aquatic life with long lasting effects	See above

Proposed labelling according to Reg. (EU) No 1272/2008, Table 3.1 and Reg. (EU) No 286/2011(proposed by RMS)

Labelling		
GHS Pictograms		 GHS09
Signal words		-
Hazard statements		H411: Toxic to aquatic life with long lasting effects
Precautionary statement	Prevention	P273 – Avoid release to the environment
	Response	P391 – Collect spillage
	Storage	-
	Disposal	P501 - Dispose of contents/container in accordance with local/regional/ national/international regulation (to be specified).

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Degradation

a) Ready biodegradability:

K-HDO was tested in an enhanced ready test; study A 7.1.1.2.1, document III-A 7.1.1.2.1 (pre-adaptation of the inoculum for 69 days, test duration 30 days, BOD/ThOD measurement lead to ca. 60% biodegradation). Therefore based on this study results, K-HDO was 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 are no data available.

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 of K-HDO was investigated in a study according to OECD TG 111 (study A 7.1.1.1.1). K-HDO hydrolysis occurred only at pH 4, with a DT_{50} of 1.26 days at 25°C. K-HDO has been shown to be hydrolytically stable at 50°C and at pH 7 and 9. According to the Guidance on the Application of the CLP Criteria v.5, Annex II, chapter 2.3.8, data on hydrolysis might only be considered for the determination of rapid degradation, if the longest half-life within the pH range 4 - 9 is < 16 days. Since K-HDO is stable at pH 7 and 9 this is not the case. Therefore, the results of the hydrolysis study indicate that K-HDO is not rapidly degradable through hydrolysis, according to the same criteria, chapter 4, and decision scheme.

There are no data available on photolysis in water. Due to the adsorption coefficient of 6006 L/kg photolysis in water is not expected to represent a major degradation pathway in the environment, since K-HDO will adsorb very quickly onto organic matter.

Overall, the DS concluded that K-HDO is not rapidly degradable in the sense of CLP Regulation.

Bioaccumulation

The bioconcentration factor was not measured for K-HDO; in its absence, the bioaccumulation potential of K-HDO was evaluated using the measured $\log K_{OW}$ which is -0.2, namely below the cut-off criteria of 4, as indicated in the CLP Regulation. Therefore the DS concluded K-HDO has a low potential to bioaccumulate.

Aquatic toxicity

<u>Aquatic acute toxicity</u>

The DS included the results from a 96h fish study, two 48h *D. magna* studies and a 72h algae study. All studies were conducted using K-HDO 30% and the final $L(E)C_{50}$

recalculated to 100% K-HDO.

In a standard laboratory test (DIN 38412) K-HDO shows low acute toxicity to fish, as indicated by the LC_{50} value of 51.3 mg/L for the golden orfe (*Leuciscus idus*).

K-HDO is also of low toxicity to *Daphnia magna* with an EC_{50} of > 30 mg/L, (OECD TG 202, study A 7.4.1.2, and Buchen, 1993a according to DIN 38412).

K-HDO is only slightly toxic to algae (*Desmodesmus subspicatus)*, as shown by E_rC_{50} and E_bC_{50} values > 30 and 15.6 mg/L, respectively (OECD TG 201, study A 7.4.1.3).

Overall, the aquatic acute toxicity values ($L(E)C_{50}$) for all three trophic levels are between 10 – 100 mg/L, therefore no classification was proposed by the DS.

Aquatic chronic toxicity

The DS included a chronic study for each trophic level, all studies were considered reliable by the DS.

A fish juvenile growth test (zebra fish, *Danio rerio*) according to the OECD TG 215 guideline was carried out with K-HDO for a period of 28 days (study A 7.4.3.2). In conclusion, the overall NOEC was 0.33 mg/L (nominal concentration) and 0.29 mg/L (based on the mean analytically determined concentrations) and the LOEC was 1.1 mg/L (nominal concentration) and 0.74 mg/L (based on the mean analytically determined concentrations).

The chronic toxicity to *Daphnia magna* was determined in a 21-day reproduction study and the NOEC, based on numbers of offspring per adult, resulted in 0.47 mg/L (OECD TG 211, study A 7.4.3.4).

K-HDO is only slightly toxic to algae (*Desmodesmus subspicatus)*, as shown by NOE_rC value of 3.75 mg/L (OECD TG 201, study A 7.4.1.3).

The chronic NOEC values for all three trophic levels are between 0.1 and 10 mg/L, and the lowest chronic NOEC values are the NOEC for fish (0.29 mg/L) and for daphnia (0.47 mg/L). The DS considered K-HDO not rapidly degradable, and based on these NOECs, they proposed a classification as Aquatic Chronic 2.

Comments received during public consultation

Four MSCAs commented, one agreed with the proposed classification. The other MSCAs commented on the studies about their uncertainties, being conducted using the 30% K-HDO solution and the absence of biodegradation in soil study from the CLH dossier. The DS replied confirming that the study were indeed conducted with K-HDO at 30%, but the results were recalculated to consider 100% pure substance and that the biodegradation in soil study was not included because it was not considered valid.

Assessment and comparison with the classification criteria

Biodegradation

The biodegradability of K-HDO 30% in water has been investigated in an enhanced ready test (Haid, 1996, Document A7.1.1.2.1, key study) and in an inherent test (Haid, 1995, Document A7.1.1.2.2, key study).

In the BOD-test (Haid, 1996) a biodegradation degree of 60% for K-HDO has been reached after 30 days. In this test the inoculum has been pre-adapted to the test substance for 69 days. In addition K-HDO has been tested at inhibitory concentrations relative to the results of the Activated Sludge, Respiration Inhibition Test (Taeger, 1995, Document A III 7.4.1.4). The EC_{50} of K-HDO was graphically determined with ca. 9 mg/L (nominal), the EC_{20} was ca. 1.44 mg/L (nominal) and the EC_{10} was ca. 1.1 mg/L (nominal; corresponds to ca. 3.6 mg 30% K-HDO/L).

The test substance concentration of 6.1 mg/L was chosen, because evaluation was only possible around this concentration range. With concentrations above 7 mg/L, the oxygen consumption was too high in order to calculate a BOD value and with concentrations below 3 mg/L, oxygen consumption was too low to be measured. Biodegradation of K-HDO was therefore not inhibited at the used concentration, despite the results of the Activated Sludge Inhibition Test. The result of the BOD test is not regarded as a proof for a ready bio-degradability of K-HDO and the substance is therefore considered as being "not readily biodegradable".

In the Zahn–Wellens test (Haid, 1995, Document A7.1.1.2.2) almost no adaptation (< 1 day) of the inoculum took place. An elimination rate of 98% was reached after 28 days. 57% of this elimination took place within the first three hours, which indicates elimination due to adsorption. DOC measurement was performed, but no abiotic control was run in parallel. Therefore, there is no proof for biodegradation in the test system. K-HDO adsorbs strongly onto organic matter with a mean KFoc of 6006 L/kg. Therefore, it is concluded that K-HDO is well eliminated from water, mainly through adsorption. K-HDO may possibly be regarded as primary inherently biodegradable, but in no case as ultimately inherently biodegradable.

Conclusion: based on the results of the screening ready biodegradation tests RAC agrees with the DS and concludes that K-HDO is not rapidly biodegradable according to the CLP criteria.

Bioaccumulation

Measured BCF data are not available for K-HDO. According to the Guidance on the Application of the CLP Criteria v.5.0, Annex III, chapter II.5, Decision scheme, the measured $\log K_{OW}$ = -0.2 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

It should be mentioned that the studies were performed with 30% formulation in water with the results adjusted accordingly to 100% compound. Despite this limitation, RAC considers the study are reliable and suitable for classification purposes.

<u>Aquatic Acute</u>

For category Aquatic Acute 1, the aquatic acute toxicity $L(E)C_{50}$ values available for all three trophic levels should be in the range of 0.1 - 1 mg/L. The submitted acute aquatic $L(E)C_{50}$ values for K-HDO for all three trophic levels are in the range of 10 - 100 mg/L. Therefore, K-HDO does not fulfil the criteria for classification as aquatic acute, hence RAC agrees with the DS' proposal **not to classify K-HDO as aquatic acute toxicity**.

Aquatic Chronic

RAC considers K-HDO as not rapidly degradable. For non-rapidly degradable substances, classification as Aquatic Chronic 2 applies when the NOEC or ECx is in the range < 0.1 and ≤ 1 mg/L for the most sensitive trophic level (fish, crustacea and/or algae or aquatic plants). For K-HDO, the lowest NOEC has been observed in the fish chronic study, NOEC equal to 0.29 mg/L, which leads to a classification as Aquatic Chronic 2.

Overall, RAC agrees with the DS proposal to classify **K-HDO as Aquatic Chronic 2; H411**.

6 OTHER INFORMATION

Not available

7 REFERENCES

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A 2.8	2003	"Chemical 5 batch analysis of Xyligen 30 F"	Y	BASF
A 2.10.2.2	2005b	"Surface coatings of wood based panels"	Y	Dr. Wolman GmbH
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CAR Section No	**Year**	**Title** **Source (where different from company) Company, Report No.** **GLP or GEP status (where relevant)** **Published or not**	**Data Protection Claimed** **Y/ N**	**Owner**
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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON POTASSIUM (OXIDO-NNO-AZOXY)CYCLOHEXANE; CYCLOHEXYLHYDROXYDIAZENE 1-OXIDE, POTASSIUM SALT; [K-HDO]

CAR Section No	**Year**	**Title** **Source (where different from company) Company, Report No.** **GLP or GEP status (where relevant)** **Published or not**	**Data Protection Claimed** **Y/ N**	**Owner**
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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON POTASSIUM (OXIDO-NNO-AZOXY)CYCLOHEXANE; CYCLOHEXYLHYDROXYDIAZENE 1-OXIDE, POTASSIUM SALT; [K-HDO]

CAR Section No	**Year**	**Title** **Source (where different from company) Company, Report No.** **GLP or GEP status (where relevant)** **Published or not**	**Data Protection Claimed** **Y/ N**	**Owner**
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Nitzsche, 2017. Effect of maternal feed restriction on prenatal development in rats and rabbits - A review of published data. Reg. Tox. Pharmacol. 90; 95-103.

8 ANNEXES

Throughout the CLH-Report references are made to the Competent Authority Report (CAR) on Cyclohexylhydroxydiazene 1-oxide, potassium salt (K-HDO), which has been finalised by the Standing Committee on Biocidal Products during its meeting held on 22 February 2008.

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

DOC IIA

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON POTASSIUM (OXIDO-NNO-AZOXY)CYCLOHEXANE; CYCLOHEXYLHYDROXYDIAZENE 1-OXIDE, POTASSIUM SALT; [K-HDO]

DOC IIA confidential

DOC IIIA (confidential version)

DOC IIIA (non-confidential version)