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

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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.**

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Biocides: No need for justification.

Also conclusion for non-classification for the various endpoints is of utmost importance for European harmonisation. RMS proposals for classification and non-classification were not discussed in detail within the European Biocides Technical Meetings.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

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

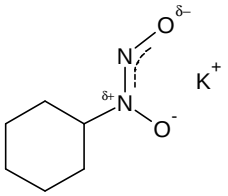
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	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.

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.

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.

[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

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.

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.

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.

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 GLP	0, 10, 25, 50 % (v/v) of Xyligen LP 15670 (with a K-HDO content of 30.4%) in water and 25% hexyl cinnamic aldehyde in acetone:olive oil, (4:1)	not sensitising (stimulation index < 3): 1, 1.5; 2.4; 1.9 and with positive control 53.3. With 50% (v/v)	**A 6.1.5** Weber, 2004

		as positive control	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.

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.

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.

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)

BASF Department of Toxicology 106
PATHOLOGY REPORT 70C0679/89113
BIS-(N-CYCLOHEXYL-DIAZENIUMDIOXY)-COPPER Jan/30/1996 CBGE
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	65
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.

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.

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

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**
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 · 10^{-12} cm³ · $molecule^{-1}$ · s^{-1}

OH radical concentration: [OH·] = 1.5 • 10^{6} OH / cm³.

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 · 10^{-12} cm³ · $molecule^{-1}$ · s^{-1}

OH radical concentration: [OH·] = 0.5 • 10^{6} OH / cm³.

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 para-meter	Inoculum			Additional substrate	Test substance concentr.	Degradation		Reference
			Type	Concen-tration	Adap-tation			Incubation period	Degree [%]	
DIN 38409, part 51; / BOD "Ready Biode-gradability"	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 bio-degradability: 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;

- **è 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:

- **è No classification**

Aquatic Chronic 2:

- **è 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).

6 OTHER INFORMATION

Not available

7 REFERENCES

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
A 2.6	2004	"Product identity and Composition of (N-cyclohexyldiazeniumdioxy)-potassium"	Y	Dr. Wolman GmbH
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
A 3.1.1/01	2001	"Physico-chemical properties of (N-Cyclohexyl-diazeniumdioxy)-potassium"; BASF AG, Germany; BASF Report 01L00057; GLP; unpublished	Y	BASF
A 3.4/01	2001	"Characterisation of N-Cyclohexyl-diazeniumdioxy-potassium"; BASF AG, Germany; BASF Report 01L00234; GLP; unpublished	Y	BASF
A 3.4/02*	2004	"Spectroscopic characterisation of Xyligen Betriebsprobe"; BASF AG, Germany; Study No. 03L00371; GLP; unpublished	Y	BASF
A 3.4/03	2004	"Determination of the identity of Xyligen Betriebsprobe"; BASF AG, Germany; Study No. 04L00210; GLP; unpublished	Y	BASF
A 3.7	2004	"Solubility of K-HDO in organic solvents"	Y	Dr. Wolman GmbH
A 3.11	2001a	"Evaluation of safety characteristics according to 92/69/EEC, annex A9 – A17"; BASF AG, Germany; BASF Report SIK 01/0222; GLP; unpublished	Y	BASF

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
A 4.1/01	1992	Colorimetric determination of Xyligen potassium in Xyligen 30-F and in reaction preparations, BASF AG, Germany, BASF method M 92/9, unpublished, no GLP	Y	BASF
A 4.1/02	2003	"Determination of Chloride and Bromide by potentiometric Titration", BASF AG, GKA Analytik, test method 0021/05, unpublished, no GLP	Y	BASF
A 4.1/04	2005	"Determination of Sulphate in Xyligen 30F by ion-chromatography", BASF AG, GKA Kompetenzzentrum Analytik, method M05/0043/01, unpublished, no GLP	Y	BASF AG
A 4.1/05	2005	Validation of a Photometer method for the determination of K-HDO in water	Y	Dr. Wolman GmbH
A 4.1/06	2005	Concentration control analysis of N-Cyclohexyl-diazeniumdioxy-potassium in non Chlorinated Charcoal filtered tap water (Frankenthal, Germany, mixed with deionized water	Y	Dr. Wolman GmbH
A 4.2/01	2004a	Validation of a HPLC method for the determination of K-HDO in soil, Dr. Wolman GmbH, Germany, no GLP, unpublished	Y	Dr. Wolman GmbH
A 5.3	2004	Bio-testing report - Effectiveness of K-HDO against wood destroying fungi, Biological Testing Laboratory, Dr. Wolman GmbH, Lab. Ref. B 1335-1983, no GLP, unpublished	Y	Dr. Wolman GmbH
A 6.1.1	1977	Report on the test of the acute oral toxicity of K-HDO in the rat, BASF AG AG Ludwigshafen, Germany, no GLP, unpublished	Y	BASF AG
A 6.1.2	1979	Report on the test of the acute dermal toxicity of K-HDO in the rat, BASF AG AG Ludwigshafen, Germany, no GLP, unpublished	Y	BASF AG
A 6.1.3.1	2001	Xyligen 30-F, acute inhalation toxicity study in Wistar rats, Report 13/0069/017001, BASF AG AG, GLP, unpublished	Y	BASF AG
A 6.1.3.2	1971a	Report on the test of the acute inhalation toxicity (inhalation risk) of Xyligen 30F in the rat, BASF AG AG, Ludwigshafen, Germany, no GLP, unpublished	Y	BASF AG
A 6.1.4	1971a	Report on the test of the primary irritant effect of Xyligen 30F on the skin and mucosa of rabbits, BASF AG Aktiengesellschaft, Ludwigshafen, Germany, no GLP, unpublished	Y	BASF AG
A 6.1.5	2004	Xyligen LP 15670: Testing of the skin sensitizating potential with the Local Lymph Node Assay, Laboratory Project ID Wol91, ARC Seibersdorf research GmbH, Austria, unpublished	Y	BASF AG
A 6.2.1	1993	Study on the Comparison of the adsorption and excretion of the potassium, copper and aluminium salt of 14-C-N Cyclohexyl-hydroxi-diazeniumoxide after oral, dermal and intravenous administration to Wistar rats, Report: 22B0638/896001, BASF AG AG, unpublished	Y	BASF AG
A 6.2.2	2001	14C-Cu-HDO Study of the Biokinetics in Rats, Report: 02B0881/006037, BASF AG AG, unpublished	Y	BASF AG

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
A 6.2.3	2002	The Metabolism of 14C-Cu-HDO in Rats, Report: 2002/1004467, BASF AG AG, unpublished	Y	BASF AG
A 6.2.4	2006	Study of penetration through human skin in vitro; BASF AG laboratory report number 52H0892/052243, unpublished	Y	BASF AG
A 6.3.1	1976	Study of the toxicity of Xyligen K powder in the 42-days feeding study, BASF AG Aktiengesellschaft, Ludwigshafen, Germany report, No. XXIII/280, no GLP, unpublished	Y	BASF AG
A 6.3.3	1978	Bericht über die Prüfung der subakuten Inhalationstoxizität von Reu-E 3403 (Xyligen-K) in Sprague-Dawley Ratten, BASF AG Aktiengesellschaft, Ludwigshafen, Germany, kein GLP, unpublished	Y	BASF AG
A 6.4.	1995	Subchronic oral toxicity study with Bis-(N-Cyclohexyldiazeniumdioxy)-copper in beagle dogs, Report: 31D0141/92060, BASF AG, unpublished	Y	BASF AG
A 6.4.	1991	Report on the study of the oral toxicity of Bis-(N-cyclohexyl-diazeniumdioxy)-copper in rats Administration via the diet for 3 months; Report: 30C0679/89041, BASF AG, unpublished	Y	BASF AG
A 6.4.1	1978	3-Monate-Toxizität von Xyligen K, Charge 77/267 - kurz "Xyligen K" genannt - an Sprague-Dawley Ratten bei Verabreichung per Magensonde, Laboratorium für Pharmakologie und Toxikologie Prof. Dr. F. Leuschner, Hamburg, Germany, no GLP, unpublished	Y	BASF AG
A 6.5	1993	Report on the study of the chronic toxicity of Bis-(N-cyclohexyldiazeniumdioxy)-copper in rats, Report: 50C0679/89080, BASF AG AG, GLP, unpublished	Y	BASF AG
A 6.6.1	1989	Report on the study of N-Cyclohexyl-diazenium-dioxy-potassium-hydrat in the Ames Test (Standart plate test and preincubation test with Salmonella typhimurium), BASF AG Aktiengesellschaft, Ludwigshafen, Germany, GLP, unpublished	Y	BASF AG
A 6.6.3.1	1992	Rat hepatocyte DNA repair assay [UDS] in vitro: 81MO679/894495, BASF AG AG, GLP, unpublished	Y	BASF AG
A 6.6.3.2	2005	Mutagenicity study of Xyligen LP 15671 in the mouse lymphoma forward mutation assay –in vitro- Laboratory of Pharmacology and Toxicology KG, Hamburg, Germany LPT No. 18342/04, unpublished	Y	Dr. Wolman GmbH
A 6.6.4	1982	Cytogenetic investigations in NMRI mice after single oral administration of Xyligen-K powder, Micronucleus test, BASF AG Aktiengesellschaft, Ludwigshafen, Project No. 26MO172/8215, Germany, no GLP, unpublished	Y	BASF AG

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
A 6.7	1996	Carcinogenicity study with Bis-N-cyclohexyl-diazeniumdioxy)-copper in Wistar rats Administration in the diet for 24 months: 70C0679/89113, BASF AG AG, GLP, unpublished	Y	BASF AG
A 6.8.1.1	1991	Study of the Prenatal Toxicity of BIS-(N-CYCLOHEXYL-DIAZENIUMDIOXY)-COPPER in rats after oral administration (gavage): 30R0679/89059, BASF AG AG, GLP, unpublished	Y	BASF AG
A 6.8.1.2	1994	Study of the Prenatal Toxicity of BIS-(N-CYCLOHEXYL-DIAZENIUMDIOXY)-COPPER in rabbits after oral administration (gavage)administration (gavage): 40R0141/92031, BASF AG AG, GLP, unpublished	Y	BASF AG
A 6.9	1992	Report to the study of the oral toxicity of N-Cyclohexyldiazeniumdioxy-potassium-hydrate in Wistar rats, administration via the diet for 4 weeks, BASF AG Aktiengesellschaft, Ludwigshafen, Germany unpublished	Y	BASF AG
A 7.1.1.1.1	2004b	**Hydrolysis** of K-HDO as function of pH, Report: 01/2002, Dr. Wolman GmbH, no GLP, unpublished	Y	Dr Wolman GmbH
A 7.1.1.2.1	1996	**Ready Biodegradability** Prüfung der biologischen Abbaubarkeit von K-HDO, techn. 30 %ig im Verdünnungs BSB-test nach 30 Tagen, Report No: 95/0179/04/2; BASF Aktiengesellschaft, Emissionsüberwachung und Ökologie, Ludwigshafen, Germany, no GLP, unpublished	Y	BASF
A 7.1.1.2.2	1995	**Inherent Biodegradability** Determination of the Biodegradability and the Elimination of K-HDO, techn. 30 %, respectively from water in the modified static Zahn-Wellens-Test, Project Number 95/0179/10/2, Emission monitoring and Ecology, Laboratory for Microbiology, BASF Aktiengesellschaft, Ludwigshafen, Germany, no GLP, unpublished	Y	BASF
A 7.1.2.1.1	1980	**STP Simulation study** Hydroxydiazeniumoxide (HDO) potassium salt – determination of the biological degradability in a long-term test, J-No. 63529, Analytical Laboratory, BASF Aktiengesellschaft, Ludwigshafen, Germany, no GLP, unpublished	Y	BASF
A 7.1.3/03	2006	**Adsorption/desorption study** with K-HDO according to OECD 106, Biochem. agrar, Report no 05 10 35 2029, BioChem agrar, BASF Aktiengesellschaft, Ludwigshafen, Germany, GLP, unpublished	Y	BASF
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**
A 7.4.1.1	1980	Report on the test of the **acute toxicity** of Xyligen 30F **in fish** (golden orfe - Leuciscus idus L.) BASF AG, Ludwigshafen, Germany, no GLP, unpublished	Y	BASF
A 7.4.1.2/01	2002	Xyligen K 30 F – Determination of the **acute effect on the swimming ability of the water flea Daphnia magna** Straus, Report 01/0069/50/2, Experimental Toxicology and Ecology, BASF AG, Ludwigshafen, Germany, GLP, unpublished	Y	BASF

A 7.4.1.2/02	1993a	**Daphnia acute toxicity study** Test Protokoll für den akuten 24 h/48 h Daphnientest nach DIN 38412, Teil 11, Umweltbundesamt (UBA), Institut für Wasser-, Boden- und Lufthygiene (BGA), Berlin, Germany, no GLP, unpublished	Y	BASF
A 7.4.1.3	2002	N-cyclohexyl-diazenium-dioxy-potassium - **Determination of the inhibitory effect on the cell multiplication of unicellular green algae**, Report 01/0069/60/1, BASF AG, GLP, unpublished	Y	BASF
A 7.4.1.4/01	1995	**Activated Sludge Respiration Inhibition** Prüfung der Atmungshemmung von Belebtschlamm durch K-HDO, techn. 30%ig im Kurzzeitatmungstest, Report 95/0179/08/1, BASF Aktiengesellschaft, Ludwigshafen, Germany, no GLP, unpublished	Y	BASF
A 7.4.1.4/02	1993b	**Growth Inhibition test** Testprotokoll für den Zellvermehrungshemmtest mit **Pseudomonas putida** nach DIN 38412, Teil 8, (1993) Umweltbundesamt (UBA), Institut für Wasser-, Boden- und Lufthygiene (BGA), Berlin, Germany, unpublished	Y	BASF
A 7.4.1.4/03	1993 c	**Luminescence Inhibition study** Testprotokoll für den Leuchtbakterientest nach DIN 38412, Teil 34, Umweltbundesamt (UBA), Institut für Wasser-, Boden- und Lufthygiene (BGA), Berlin, Germany, no GLP, unpublished	Y	BASF
A 7.4.3.2	2005	**Long-term toxicity to fish** N-Cyclohexyldiazeniumdioxy-potassium – juvenile growth test in the zebra fish (Danio rerio) in a flow through system (28 days), Laboratory for Wildlife and Fish Toxicology of Experimental Toxicology and Ecology, BASF AG, Germany, Report No. 44F0069/015137, 25 July 2005, unpublished	Y	Dr. Wolman GmbH
A 7.4.3.4	2002	**Long-term toxicity to daphnia** Influence of Xyligen K 30 F on Survival and Reproduction of Daphnia magna in a semi static test over 21 days. Report 13601221, IBACON, GLP, unpublished	Y	BASF

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

DOC IIA confidential

DOC IIIA (confidential version)

DOC IIIA (non-confidential version)