Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

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

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

CLH-O-0000001412-86-248/F

Adopted

30 November 2018

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

30 November 2018

CLH-O-0000001412-86-248/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

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

EC Number: **-**

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

The proposal was submitted by **Austria** and received by RAC on **29 August 2017.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Austria has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **14 November 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **12 January 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Stine Husa**

Co-Rapporteur, appointed by RAC: **Marian Rucki**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **30 November 2018** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	potassium (oxido-NNO-azoxy)cyclohexane; cyclohexylhydroxydiazene 1-oxide, potassium salt; [K-HDO]	-	66603-10-9	Flam. Sol. 1 Acute Tox. 3 Skin Irrit. 2 Eye Dam. 1 STOT RE 2 Aquatic Chronic 2	H228 H301 H315 H318 H373 (gastrointestinal tract, liver, kidney) H411	GHS02 GHS05 GHS06 GHS08 GHS09 Dgr	H228 H301 H315 H318 H373 (gastrointestinal tract, liver, kidney) H411			
RAC opinion	TBD	potassium (oxido-NNO-azoxy)cyclohexane; cyclohexylhydroxydiazene 1-oxide, potassium salt; [K-HDO]	-	66603-10-9	Flam. Sol. 1 Acute Tox. 3 STOT RE 2 Skin Irrit. 2 Eye Dam. 1 Aquatic Chronic 2	H228 H301 H373 (liver) H315 H318 H411	GHS02 GHS05 GHS06 GHS08 GHS09 Dgr	H228 H301 H373 (liver) H315 H318 H411		oral: ATE = 136 mg/kg bw	
Resulting Annex VI entry if agreed by COM	TBD	potassium (oxido-NNO-azoxy)cyclohexane; cyclohexylhydroxydiazene 1-oxide, potassium salt; [K-HDO]	-	66603-10-9	Flam. Sol. 1 Acute Tox. 3 STOT RE 2 Skin Irrit. 2 Eye Dam. 1 Aquatic Chronic 2	H228 H301 H373 (liver) H315 H318 H411	GHS02 GHS05 GHS06 GHS08 GHS09 Dgr	H228 H301 H373 (liver) H315 H318 H411		oral: ATE = 136 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The Dossier Submitter (DS) has supplemented the limited toxicological information on K-HDO, especially for repeated dose toxicity, reproduction and carcinogenicity by reading relevant data across from Cu-HDO to K-HDO. The following arguments are relevant for the read-across assessment:

- The HDO^- anion derived by dissociation from Cu-HDO and from K-HDO is structurally identical.
- The toxicological differences in the toxicity profile of Cu-HDO and K-HDO were related to the different effects of the Cu^{2+} and K^+ ions.

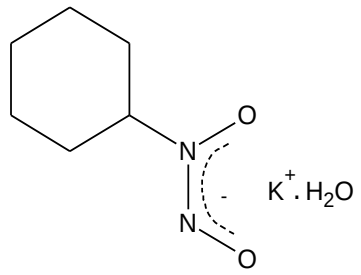

Fig. 1 K-HDO Cyclohexylhydroxydiazene 1-oxide, potassium salt

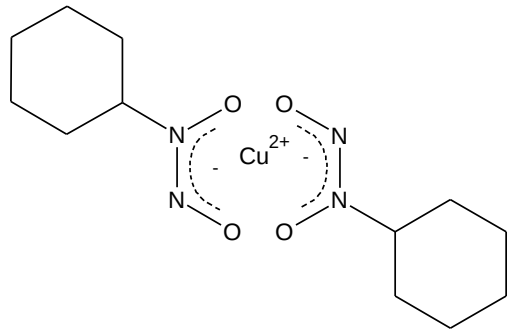

Fig. 2 Cu-HDO Cyclohexylhydroxydiazene 1-oxide, cupper salt

- Cu-HDO contains two HDO^--ions per molecule, while K-HDO only contains one. The atomic weight of K is 39.098 g/mol and the atomic weight of Cu is 63.546 g/mol. The overall molecular weight of K-HDO is 182.3 g/mol and Cu-HDO is 349.9 g/mol. It is assumed that the toxicity is related to the HDO^--ion, therefore it is necessary to take into account that Cu-HDO will release twice as many HDO^-- ions compared to K-HDO. For example, one mole of HDO^- ions will be released by 182.3 g of K-HDO and 174.95 (i.e. 349.9/2) g of Cu-HDO. It can therefore be considered that the difference is very low, and can be ignored in the read-across assessment.
- Cu-HDO and K-HDO showed similar distribution and excretion rates, which are: ready absorption across the GI tract, rapid elimination mainly via urine, no bioaccumulation, plasma levels below 0.1% of the dose and limited absorption via the skin (~8%) (Hoffmann *et al.,* 1993, IIIA.6.2.1, Gamer *et al.*, 2006, IIIA 6.2.4). The kinetics might not have been expected to be comparable since the $logK_{OW}$ differs (Cu-HDO 2.6 vs. K-HDO -0.2), however, the $logK_{OW}$

does not necessarily contradict the toxicokinetic findings since biological media are more complex than a simple two-phase-system.

- Comparable kinetics and the identical chemical structure of the HDO^- anion support the assumption of a comparable metabolism.
- Potassium is the quantitatively most important intracellular cation and its concentration gradient towards the extracellular space is responsible for the membrane potential. As such it is important for the functioning of the nervous system, cardiac, skeletal and smooth muscles and epithelia and its homeostasis is usually strictly controlled by renal regulation and influenced by the acid-base state of extracellular liquids. The neurotoxic effects seen only with gavage application of K-HDO (and not with exposure via feed) could be interpreted to result from a K^+ peak in the plasma disturbing the normally rigidly controlled K^+ homeostasis.
- In contrast, copper is an essential metal, and it is employed in all human cells involved in the reactions and functions of many enzymes, including angiogenesis, neurohormone release, oxygen transport and regulation of genetic expression. Homeostatic maintenance of copper requires the tightly coordinated control of copper uptake, distribution and efflux in cells and the organism as a whole. High dose exposure may lead to local effects in the gastrointestinal-tract, effects in the liver and kidneys.
- Except for the differences that are related to the Cu^{2+} and K^+ cations the toxicity profiles of Cu-HDO and K-HDO do not diverge based on the tests available for both substances.

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

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

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

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

For the structurally very similar substance Cu-HDO, a test according UN test N.1 is available, which showed that the test substance fulfils the criteria for classification as flammable solid, category 1.

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

Oxidising solids

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

Explosive properties

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

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Oral

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

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

Dermal

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

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

Inhalation:

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

Acute oral toxicity

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

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

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

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

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

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

Acute dermal toxicity

Two acute dermal toxicity studies have been evaluated by the DS (both conducted prior to OECD TG and GLP). In the first study rats (Sprague-Dawley, 5 m/f per dose group) were exposed for K-HDO (purified, 99.8%) at a dose of > 1250 mg K-HDO/kg bw corresponding to > 2500 mg (50% w/w K-HDO)/kg bw (A 6.1.2). No mortalities and no signs of toxicity were observed. Further, the animals sacrificed after a 14-day observation period did not show any findings in the internal organs that could be related to the test substance.

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

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

Acute inhalation toxicity

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

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

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

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

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

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

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

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

No comments was received during public consultation.

Assessment and comparison with the classification criteria

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

No narcotic effects were reported and there were no indications of respiratory tract irritation. Hence, classification as STOT SE 3 is not warranted.

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

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

One study (conducted prior to OECD TG and GLP) with male rabbits (White Viennese) exposed to K-HDO (30% w/w aqueous solution) on dorsal skin and the ear were evaluated by the DS. It should be noted that the DS recalculated the scoring for this non–guideline study to scorings according to the current OECD TG 404. The scoring of the non-guideline study ranges from 0-2 while in OECD TG 404 the range is 0-4. Two rabbits were treated for 1, 5 and 15 minutes and two other rabbits were treated for 20 hours under occlusive conditions. No effects were seen on dorsal skin after exposure for 1, 5 and 15 minutes. For the two rabbits treated for 20h, the average score was 4 for erythema/escar formation and 2 for oedema at the 24h scoring according to the revised scoring system. The erythema/escar formation was not completely reversible while the oedema was reversible. For the ear the erythema scores were 2 (reversible) according to the revised scoring system. No results were available to the DS for the 48h or 72h scoring (A 6.1.4). It should be noted that the 20h exposures were performed without washing after exposure and thus the exposure conditions were more severe than those recommended in OECD TG 404.

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

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS presented one study (conducted prior to OECD TG and GLP) where eye irritation was investigated in two rabbits (white Vienna (Gaukler), 1 male/1 female) exposed to a single instillation of 50 µL K-HDO (30% w/w aqueous solution) (A 6.1.4). Corneal opacity, redness of the conjunctiva and chemosis were observed, these effects being reversible by day 8. On the basis of the corneal opacity with a score of 3 at 24 hours after instillation of K-HDO, the DS proposed to classify K-HDO for severe eye damage in category 1.

Comments received during public consultation

One MSCA pointed out that it should be clarified if the tested substance contains co-formulants. However, considering that K-HDO were tested in a pure-water-based solution with appropriate negative control, the proposed classification is supported.

Assessment and comparison with the classification criteria

Eye irritation was investigated in a study (conducted prior to OECD TG and GLP) in two rabbits (white Vienna (Gaukler), 1 male/1 female) exposed to a single application of 50 µL K-HDO (30% w/w aqueous solution) (A 6.1.4). The following scores were reported:

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

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

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

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS summarised in their evaluation one Local Lymph Node Assay (LLNA) (OECD TG 429 and GLP) in mice where K-HDO (30% w/w in aqueous solution) was diluted in water and administered to three groups of 6 female CBA/Ca mice. The test substance was applied at concentrations of 10, 25 and 50% (25 µL per ear) epicutaneously to the dorsal surface of both ears, once a day for three consecutive days. Hexyl cinnamic aldehyde (25% in acetone: olive oil (4:1)) was used as the positive control. In the high dose group, signs of toxicity were observed as reduced motor

activity, hunched posture and white crusts between days 3-5. There were no effects of irritation at any dose level. The stimulation index were 1.0 (negative control), 1.5 (low dose (10%)), 2.4 (mid dose (25%)), 1.9 (high dose (50%)) and 53.3 (positive control).

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

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

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

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

Summary of the Dossier Submitter's proposal

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

K-HDO oral studies

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

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

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

K-HDO inhalation study

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

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

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

Studies with Cu-HDO

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

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

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

The DS argued that in addition to the LOAEL values, the NOAEL to LOAEL ranges should also be considered in the assessment for a classification for STOT RE, since the "real" LOAEL may be located between the NOAEL and the LOAEL. This is because by repeating the study with a different dose spacing considerable differences in the LOAEL values may be obtained, including values below the STOT guidance value (GV). The LOAEL of the 90-day dog study (68 mg/kg bw/d) is below the STOT RE 2 GV of 100 mg/kg bw and justify classification as STOT RE 2. Futhermore, the LOAEL of the 28-day rat study at 139 mg/kg bw/d was below the extrapolated STOT RE 2 GV for a 28-day study (300 mg/kg bw/d based on the scaling to take into accound the different study durations (i.e. factor 3, CLP Annex I, paragraph 3.9.2.9.6).

Moreover, the DS considered that the NOAEL to LOAEL range of the 90-day rat study (38 to 153 mg/kg bw/d) included the STOT RE 2 GV of 100 mg/kg bw/d. The NOAEL to LOAEL ranges of the 12- and 24-months rat studies (18 to 61 and 6 to 33 mg/kg bw/d respectively) should be compared with the extrapolated GV of 5 to 50 (factor 2 for a 12- to 24-month study, REACH guidance chapter R.8.4.3.1) leading to a NOAEL to LOAEL range including or being below the STOT RE 2 GV, which is considered to provide further support for classification.

Based on the read across assessment from Cu-HDO to K-HDO and the effects reported in the liver, kidney and GI tract in the repeated dose toxicity studies with Cu-HDO, the DS proposed classification as STOT RE 2; H373 (liver, kidney and GI tract). No exposure route was specified, since there was no evidence that the liver and kidney would not be affected after inhalation or dermal exposure.

Comments received during public consultation

Comments were received from two MSCAs. One MSCA agreed with the DS proposal to classify K-HDO for STOT RE 2 (liver and kidney) mainly based on read-across from Cu-HDO. The MSCA did not agree that the GI tract should be included as a target organ in the STOT RE 2 classification. This was based on the fact that in the 2-year study in rats with exposure to Cu-HDO or $CuSO_4$, it was evident that the effects in the GI tract was mainly cause by copper. The MSCA also asked for a careful discussion in RAC of the neurotoxic effects observed after K-HDO gavage administration. The second MSCA asked for a more quantitative comparison of the organ toxicity

of Cu-HDO vs. relevant Cu-salts to increase the robustness of the read-across approach. The DS commented that Cu-ions may penetrate deeper into the GI-mucosa mediated by the organic HDO-residue than Cu-salts. This could increase the cytotoxic effects of the copper-ion as a toxophore. A 2-year study in rats showed for example storage of an iron-containing pigment in macrophages in the submucosa of the duodenum of male and female animals after oral exposure with 169 mg/kg bw/d of Cu-HDO. This was not observed after comparable exposure with $CuSO_4$. Consequenty, the DS considered that a STOT RE 2 classification for GI tract, liver and kidney are supported by experimental evidence.

Assessment and comparison with the classification criteria

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

Assessment of the studies with K-HDO

Four repeated dose toxicity studies with K-HDO were included in the CLH-report, three by oral exposure (one gavage and two in feed) and one by inhalation exposure. In the 90-day gavage study in Sprague-Dawley rats acute clinical neurotoxic effects were reported from 50 mg/kg bw/d K-HDO. The acute neurotoxic effects were reported only with the bolus application of K-HDO, which are probably disturbing the tightly controlled K^+ homeostasis, an effect that was not observed in oral feeding studies where the K^+ uptake is expected to be slower. RAC considers that the neurotoxic effect was reported in the same dose-range that resulted in similar effects in the acute toxicity gavage studies (see the acute toxicity section) and is not considered relevant for a STOT RE classification. It should be mentioned that liver and/or stomach damage were also reported in this study from 50 mg/kg bw/d. Microscopic examinations showed degenerative liver damage, which was seen in some cases as severe dystrophy. Gastric ulcers were reported occasionally. However, the bolus application of K-HDO may have disturbed the K^+ homeostasis and seriously compromising the health status of the rats. Therefore, the effects reported in liver and stomach may be considered as secondary to the health status of the animals.

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

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

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

Liver: In the 90-day gavage study with K-HDO in Sprague-Dawley rats microscopic examinations showed degenerative liver damage seen in some cases as severe dystrophy from 50 mg/kg bw/d. However, bolus application of K-HDO may have disturbed the K^+ homeostasis, seriously compromising the health status of the rats. Therefore, the effects reported in liver and stomach may be considered as secondary to the health status of the animals. In the 28-day inhalation study with K-HDO (one dose, uncertain exposure) in Sprague-Dawley rats effects were reported in females as focal-like liver necrosis and slight fatty metamorphosis. No repeated dose toxicity were performed in Beagle dogs with K-HDO, which were considered to the most sensitive species for effects in the liver following exposure to Cu-HDO. In the 90-day study with Cu-HDO in dogs, chronic hepatitis and cirrhosis as well as oedema in the gall bladder wall was reported at 68 mg/kg bw/d, adverse effects that were within the GV for a STOT RE 2 (between 10 - 100 mg/kg bw/d). However, in this dog-study, a group exposed to $CuSO_4$ corresponding to the same amount of Cu^{2+} ions as in the high dose group exposed to Cu-HDO was not included. Therefore, it is not possible to assess whether the effects reported in the dogs were related to the exposure to Cu^{2+} or to the HDO^- anion. Liver as a target organ following exposure to Cu-HDO was also supported from the repeated dose toxicity studies in rats, however, RAC considers that the effects reported as adverse in the rat studies with Cu-HDO were outside the GV for a STOT RE 2 classification.

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

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

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

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

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

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

For the evaluation of germ cell mutagenicity, the DS included three *in vitro* studies; one Ames test (OECD TG 471, GLP) and one TK mouse lymphoma assay (OECD TG 476, GLP). In addition,

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

In vitro studies

The Ames test (OECD TG 471, GLP) was performed with *S. typhimurium* (TA1535, TA100, TA1537, TA98) at concentrations of 15-5000 µg with and without metabolic activation. K-HDO did not show any dose-related increase in revertant counts in any of the four strains either with or without metabolic activation. However, there are some limitations to this study since one test strain is missing and 2-aminoanthracene was used as the only positive control with S9 activation (A 6.6.1).

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

In addition, the DS included one study of unscheduled DNA synthesis (OECD TG 482, GLP) performed with Cu-HDO (0.0003-0.1 µg/mL in 5% DMSO) on primary rat hepatocytes. This study did not show any increase in the mean number of net nuclear grain counts compared with negative controls (A 6.6.3.1).

In vivo studies

One micronucleus assay (OECD TG 474, no GLP) was performed with 5 male and 5 female NMRI mice per group at dose levels of 0, 6.8, 21.5 and 68 mg/kg bw. This study did not show any significant increase in the number of micronucleated PCEs in treated animals or negative controls at any sampling time. The study did not provide evidence that K-HDO reaches the bone marrow since the ratio of PCE to NCE was not affected at the highest dose tested. However, higher doses could not be tested due to the toxicity observed at the highest tested dose (A 6.6.4).

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

Comments received during public consultation

Two commenting MSCAs supported the proposed no classification for mutagenicity.

Assessment and comparison with the classification criteria

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

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

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

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No carcinogenicity study following exposure to K-HDO was available, therefore the DS used a read across data from Cu-HDO. The arguments for read across from Cu-HDO to K-HDO are

provided in the section "RAC general comment". The results from the 2-year carcinogenicity study with exposure to Cu-HDO are included below.

For the assessment of carcinogenicity the DS included one 2-year oral carcinogenicity study in Wistar rats (A 6.7.1, 1996). In this study rats (50/sex/group) were exposed to Cu-HDO in the diet at concentrations of 0, 100, 600 and 3000 ppm corresponding, respectively, to 0, 5, 29 and 148 mg/kg bw/d in males and 0, 6, 33 and 189 mg/kg bw/d in females. One group was exposed to 67 mg/kg bw/d of $CuSO_4$ corresponding to the same amount of Cu^{2+} as in the highest dose group exposed to Cu-HDO. The mortality rate in the study was less than 34% in all dose groups. Body weight was reduced in the high dose females by 12% and in high dose males by 10%. For other systemic effects see the STOT RE section. The main concern related to carcinogenicity was an increase in vascular tumours in the mesenteric lymph node and the incidences are shown in the table below. When comparing the incidences in the high dose group exposed to Cu-HDO with the group exposed to $CuSO_4$ (with equal levels of Cu^{2+}) no difference in the incidences of vascular tumours were reported.

Incidences of vascular tumours in the mesenteric lymph nodes:

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

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

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

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

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

Incidences of vascular tumours in all organs assessed:

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

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

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

An overview of all tumours:

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

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

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

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

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

Comments received during public consultation

Comments were received from two MSCAs; one supported the proposed read across o data from Cu-HDO, and that no classification for K-HDO was warranted. The other questioned the reliability of the control group since 47/50 males and 46/50 females in the control group developed neoplasms including 24% males and 34% females with malignant neoplasms. The MSCA also found it unusual that the historical control data (HCD) for combined vascular tumours in males was 20% and in females 2% and also considered it inappropriate to pool all vascular tumours both in the study and in the HCD together, since the consequences of benign haemangioma and malignant haemangiosarcoma are quite different. Therefore, they asked for further details regarding the tumour appearance site and number per sex per group before being able to conclude on a classification for carcinogenicity. In response, the DS included in the RCOM more data on the HCD for vascular tumours, which were also included in the RAC Opinion.

Assessment and comparison with the classification criteria

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

In the only carcinogenicity study (2-year, oral), there was some concern for carcinogenicity arising from vascular tumours in the mesenteric lymph nodes. However, RAC supports the DS in their assessment that the combined incidences of all vascular tumours (haemangioma, haemangiosarcoma and lymphangioma) in the mesenteric lymph nodes in the control animals were at the upper edge of the HCD range and in the top dose in females above the HCD, however, this was related to an increase in benign haemagioma with no progression to malignancy. The incidences of vascular tumours in all organs assessed were comparable in all groups including the controls and Cu-HDO and $CuSO_4$ exposed animals. RAC therefore considers that the vascular tumours reported in the 2-year rat study do not justify classification for carcinogenicity. However, as the combined incidence of vascular neoplasms in the control group was at the upper edge of the HCD range, there is concern regarding the reliability of the study and the findings should be interpreted with caution.

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

RAC is of the opinion that no classification for carcinogenicity is justified for K-HDO. This is based on the absence of a carcinogenicity study for K-HDO and supported by a read across from Cu-HDO data.

In the carcinogenicity study for Cu-HDO it was assessed if Cu^{2+} could be responsible for carcinogenicity, however no increased incidence of tumours was reported in the groups exposed to Cu-HDO or $CuSO_4$. On this basis and bearing in mind that Cu-HDO and K-HDO both form the same HDO^- ion, a read across of the carcinogenicity study data from Cu-HDO to K-HDO is fully

justified. RAC is therefore of the opinion that **no classification for K-HDO for carcinogenicity is justified**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

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

Effects on fertility and sexual function

No 2-generation study with exposure to K-HDO or Cu-HDO was available and the waiver provided by the Applicant was based on the absence of gross- and histopathological effects in the male and female reproductive organs in the repeated dose toxicity studies following exposure to K-HDO and Cu-HDO, i.e. by analysing the clear link observed between effects in male reproductive organs and effects on functional fertility in several studies (Dent, 2007, Janer *et al.*, 2007 and Mangelsdorf *et al.*, 2003). Based on the absence of effects in the reproductive organs in males and females evident from repeated dose toxicity studies following exposure to K-HDO and Cu-HDO and the waiving arguments for a 2-generation study, no classification for effects on fertility and sexual function was proposed by the DS.

Developmental toxicity

No developmental toxicity study with exposure to K-HDO was available, therefore the DS used a read across approach from Cu-HDO data (see "RAC general comments" above. For Cu-HDO two developmental toxicity studies performed according to OECD TG 414 and which were GLP compliant were included in the CLH dossier, one in rats and one in rabbits.

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

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

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

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

The morphological examinations did not show significant evidence of foetal external, soft tissue, skeletal or total malformations. The total malformation rate was low, similar in all groups and did not show a clear dose-relationship. Moreover, the isolated and disparate nature of the observed

malformations did not suggest any treatment-related aetiology. The statistically significantly increased number of litters in the mid and high dose groups and the higher percentage of high dose foetuses/litter with total skeletal variations were assessed as embryotoxic effects related to non-specific stress in the dams. Therefore, these findings were not interpreted by the DS as an indication of a teratogenic effect of Cu-HDO at these dose levels. The increased occurrence of single skeletal retardations (delayed ossification of sacral vertebral arch(es) and/or talus) in the high-dose group were in line with the reductions in foetal body weights in this group.

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

In addition, for effects on development, the DS pointed out that the food consumption is recognised as critical according to CLP Annex I, paragraph 3.7.2.4. and is considered to be related to several non-specific consequences. These were reported as reduction in body weight gain, gravid uterus weight reduction, complete litter resorption in 4 dams, the clinical findings of no defecation in one dam (GD 10-13) and observed blood in bedding in another dam (due to litter loss), as well as an increase in skeletal variations and skeletal retardations. The DS also recognised that there was no other information that may support a concern for developmental toxicity. Consequently, the DS considered that there is inadequate evidence for developmental toxicity and no classification was proposed.

Comments received during public consultation

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

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

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

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

Assessment and comparison with the classification criteria

Effects on sexual function and fertility

Information on potential effects of K-HDO and Cu-HDO on sexual function and fertility was only available from repeated dose toxicity studies, as no studies on sexual development and fertility were available. In these studies, no gross- and histopathological effects in the male and female reproductive organs were reported. For further information see the section of this opinion on

STOT RE. Based on the absence of effects in the reproductive organs in males and females evident from repeated dose toxicity studies following exposure to Cu-HDO, RAC agrees with the DS that no classification of K-HDO for effects on sexual function and fertility is justified based on the data available.

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

Developmental toxicity

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

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

Maternal effects in rat developmental toxicity study:

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

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

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

Litter response in the rat developmental toxicity study:

Parameter	**HCD**	**0 mg/kg bw/d**	**10 mg/kg bw/d**	**30 mg/kg bw/d**	**100 mg/kg bw/d**
Corpora lutea Total/# dams	6599/420 (15.7)	403/25 (16.1)	442/27 (16.4)	403/27 (14.9)	391/27 (14.5)
Implantations Total/# dams	5999/420 (14.3)	344/25 (13.8)	393/27 (14.6)	367/27 (13.6)	345/27 (12.8)
Resorptions Total/# dams	420/248 (1.7)	18/25 (0.7)	25/26 (1.0)	23/25 (0.9)	25/24 (1.0)

Total # foetuses	5528	326	368	344	320
Pre-implantation loss %	9.1	14.8	11.8	9.0	13.2
Post-implantation loss %	7.9	5.0	6.1	6.0	7.2
Total # litters	418	25	26	25	24
Live foetuses/litters	13.2	13.0	14.2	13.8	13.3
Dead foetuses/litters	0	0	0	0	0
Foetus weight (g)	3.9	3.8	3.9	3.9	4.0

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

Incidences of variations and malformations

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

Incidences of soft tissue malformations:

Parameters	**Control**	**10 mg/kg bw/d**	**30 mg/kg bw/d**	**100 mg/kg bw/d**
Soft tissue malformations, foetuses affected/foetuses	0/157	4/178	3/166	3/157
Soft tissue malformations, litters affected/litters	0/25	4/26	3/25	3/24
- sinus inversus	0	0.6	0.6	0
- hydrocephaly	0	0.6	0	0.6
- microcephalia	0	0	0.6	0

- malformations of great vessels	0	0	0	0.6
- hearth dilatation of right ventricle	0	0	1.2	0
- hearth dilatation of both ventricles	0	1.1	0	0
- septal defect	0	0	0	0.6

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

In the rabbit developmental toxicity study performed in accordance with OECD TG 414 and GLP, pregnant rabbits were exposed from GD 7-19 to 0, 10, 30 and 60 mg/kg bw/d Cu-HDO.

Maternal toxicity: No mortality or abortions were reported. The pregnancy rate was 100% in all dose groups. A statistically significant reduction in the daily food consumption in the mid and high dose groups starting from the first day of exposure (GD 7) to the end of exposure (GD 19) was reported (see table below). The reduction in food consumption from GD 7-19 was accompanied by a statistically significant reduction in body weight gain during the exposure period. During the post-treatment period (GD 20 to 29) food consumption reached or even exceeded control values, and the maternal body weight gain was comparable to the control group. Reduction in gravid uterus weight was also reported in the high dose group, however, this was not statistically significant due to high standard deviations. Clinical findings in the high dose group included no defecation in one dam (GD 10-13) and blood in bedding of another dam (due to litter loss). For further details see the table below:

Maternal toxicity in the rabbit developmental toxicity study:

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

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

[a]Due to high SD not statistically significantly reduced

Litter data: included an increase in resorptions (early) in the high dose group. In this dose group, 4 out of 15 pregnant dams had no viable foetuses and the number was outside the HCD range so the increase in resorptions could be considered as substance related. However, in these four dams a marked reduction in food consumption was reported, down to 10% of their pre-exposure consumption, as well as no defecation in one dam (day 10-13) and blood in bedding in another dam (due to litter loss). As a consequence, an increase in post-implantation losses was reported

in the high dose group (12.4%, 11.2%, 8.2% and 31.6% in the control, low, mid and high dose groups, respectively) that were outside the HCD range in the high dose group. However, the standard deviation was very high in the high-dose group since the mean number of live foetuses was not reduced in the remaining 11 high dose dams. As can be seen from the table below there were no effects on the number of corpora lutea, implantations, pre-implantation losses, foetuses/litter, live foetuses/litter, dead foetuses/litter and the bw of the foetuses.

Litter data in the rabbit developmental toxicity study:

Parameter	**HCD**	**Control**	**10 mg/kg bw/d**	**30 mg/kg bw/d**	**60 mg/kg bw/d**
Corpora lutea (total/#dams)	mean 8.0 range 7.2-8.8	111/15 (7.4)	112/15 (7.5)	116/15 (7.7)	112/15 (7.5)
Implantations (total/#dams)	mean 6.8 range 5.4-8.1	91/15 (6.1)	97/15 (6.5)	93/15 (6.2)	94/15 (6.3)
Resorptions (total/#dams)	mean 0.7 range 0.2-1.3	7/15 (0.47)	11/15 (0.73)	8/15 (0.53)	**23/15 (1.5)**
Pre-implantation loss % (SD)	mean 14.0 range 6.1–28.5	19.2 (SD: 25.46)	14.2 (SD: 14.43)	19.8 (SD: 18.80)	14.0 (SD: 17.17)
Post-implantation loss % (SD)	mean 11.2 range 3.0-23.1	12.4 (SD: 29.91)	11.2 (SD: 16.11)	8.2 (SD: 18.55)	**31.6 (SD: 44.08)**
Foetuses/litters (total #)	2425/394 (6.08)	84/14 (6)	85/15 (5.7)	85/15 (5.7)	71/11 (6.5)
Live foetuses/litters (ratio)	mean 6.1 range 4.5-7.2	84/14 (6:1)	85/15 (5.7:1)	85/15 (5.7:1)	71/11 (6.5:1)
dead foetuses/litters (ratio)	0.005	0	1/15 (0.07:1)	0	0
Foetal weight (g)	mean 41.1 range 2.5-97.5	41.8	38.6	41.8	36.5

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

Parameter	**HCD**	**Control**	**10 mg/kg bw/d**	**30 mg/kg bw/d**	**60 mg/kg bw/d**
Number of foetus examined	2425	84	86	85	71
% External malformations	8/2425 (0.3%)	0	0	**1.2**	**2.8**
% External variations		0	5.8	1.2	0
% Skeletal malformations	31/2425 (1.3)	2.4	1.2	1.2	**2.8**
% Skeletal variations	314/2425 (12.9%)	13	17	20	30
% Skeletal retardations	1365/2425 (56.3%)	65	58	47	69
% Soft tissue malformations	48/2425 (2.0%)	2.4	2.3	0	2.8
% Soft tissue variations	741/2425 (30.6%)	27	21	25	23

Further data on the external malformations:

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

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

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

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

Comparison with the CLP classification criteria

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

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

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Degradation

a) Ready biodegradability:

K-HDO was tested in an enhanced ready test; study A 7.1.1.2.1, document III-A 7.1.1.2.1 (pre-adaptation of the inoculum for 69 days, test duration 30 days, BOD/ThOD measurement lead to ca. 60% biodegradation). Therefore based on this study results, K-HDO was not rapidly

degradable according to the criteria (70% DOC removal or 60% theoretical oxygen demand, within 28 days).

b) Ultimate degradation in a surface water simulation test:

There are no data available.

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

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

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

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

Bioaccumulation

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

Aquatic toxicity

Aquatic acute toxicity

The DS included the results from a 96h fish study, two 48h *D. magna* studies and a 72h algae study. All studies were conducted using K-HDO 30% and the final $L(E)C_{50}$ recalculated to 100% K-HDO.

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

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

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

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

Aquatic chronic toxicity

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

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

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

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

Biodegradation

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

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

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

In the Zahn–Wellens test (Haid, 1995, Document A7.1.1.2.2) almost no adaptation (< 1 day) of the inoculum took place. An elimination rate of 98% was reached after 28 days. 57% of this elimination took place within the first three hours, which indicates elimination due to adsorption. DOC measurement was performed, but no abiotic control was run in parallel. Therefore, there is no proof for biodegradation in the test system. K-HDO adsorbs strongly onto organic matter with

a mean KFoc of 6006 L/kg. Therefore, it is concluded that K-HDO is well eliminated from water, mainly through adsorption. K-HDO may possibly be regarded as primary inherently biodegradable, but in no case as ultimately inherently biodegradable.

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

Bioaccumulation

Measured BCF data are not available for K-HDO. According to the Guidance on the Application of the CLP Criteria v.5.0, Annex III, chapter II.5, Decision scheme, the measured $\log K_{OW}$ = -0.2 was used. Because the $\log K_{OW}$ < 4, the substance does not meet the criterion and does not have a potential for bioconcentration in aquatic organisms.

Aquatic toxicity

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

Aquatic Acute

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

Aquatic Chronic

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

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

Additional references

Ema *et al.*, 2012. Historical control data on prenatal developmental toxicity studies in rabbits. Congenit. Anom. (Kyoto) 52; 155-161.

Nitzsche, 2017. Effect of maternal feed restriction on prenatal development in rats and rabbits - A review of published data. Reg. Tox. Pharmacol. 90; 95-103.

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).