Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

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

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

CLH-O-0000001412-86-249/F

Adopted

30 November 2018

30 November 2018

CLH-O-0000001412-86-249/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: **bis(N-hydroxy-N-nitrosocyclohexylaminato-O,O')copper; bis(N-cyclohexyl-diazenium-dioxy)-copper; [Cu-HDO]**

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

CAS Number: **312600-89-8**

The proposal was submitted by **Austria** and received by RAC on **23 October 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: **Christine Bjørge**

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	bis(N-hydroxy-N-nitrosocyclohexylaminato-O,O')copper; bis(N-cyclohexyl-diazenium-dioxy)-copper; [Cu-HDO]	239-703-4	312600-89-8 15627-09-5	Flam. Sol. 1 Acute Tox. 4 Eye Dam. 1 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H228 H302 H318 H373 (gastrointestinal tract, liver, kidney) H400 H410	GHS02 GHS05 GHS07 GHS08 GHS09 Dng	H228 H302 H318 H373 (gastrointestinal tract, liver, kidney) H410		M=1 M=1	
RAC opinion	TBD	bis(N-hydroxy-N-nitrosocyclohexylaminato-O,O')copper; bis(N-cyclohexyl-diazenium-dioxy)-copper; [Cu-HDO]	239-703-4	312600-89-8 15627-09-5	Flam. Sol. 1 Acute Tox. 4 Eye Dam. 1 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H228 H302 H318 H373 (liver) H400 H410	GHS02 GHS05 GHS07 GHS08 GHS09 Dng	H228 H302 H318 H373 (liver) H410		Oral: ATE=360 mg/kg bw M=1 M=1	
Resulting Annex VI entry if agreed by COM	TBD	bis(N-hydroxy-N-nitrosocyclohexylaminato-O,O')copper; bis(N-cyclohexyl-diazenium-dioxy)-copper; [Cu-HDO]	239-703-4	312600-89-8 15627-09-5	Flam. Sol. 1 Acute Tox. 4 Eye Dam. 1 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H228 H302 H318 H373 (liver) H400 H410	GHS02 GHS05 GHS07 GHS08 GHS09 Dng	H228 H302 H318 H373 (liver) H410		Oral: ATE=360 mg/kg bw M=1 M=1	

GROUNDS FOR ADOPTION OF THE OPINION

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Flammability

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

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

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

Oxidising solids

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

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

Explosive properties

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

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

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

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

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

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

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

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

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

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

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

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

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

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

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

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

Acute dermal toxicty

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

Inhalation

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

Acute oral toxicity

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

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

Acute dermal toxicity

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

Acute inhalation toxicity

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

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

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

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

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

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

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

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

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

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

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

The following scores were reported:

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

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

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

The DS noted that the eyes were not rinsed after 24 hours as required in the current OECD TG 405. This could have influenced the result since solids could have caused mechanical damage which potentially could have been less severe if rinsing had been performed after 24 hours. On

the other hand it should also be noted that the tested dose was 50 µL, while the OECD TG 405 indicates that when testing solids, pastes, and particulate substances, the amount used should have a volume of 0.1 mL or a weight of not more than 100 mg.

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

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

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

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

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

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

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

The DS did not propose classification for skin sensitisation.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

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

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

Summary of the Dossier Submitter's proposal

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

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

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

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

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

Study	NOAEL/LOAEL	STOT RE 2 GV	Effects
Rat: 28 day	46/139 mg/kg bw/d	30-300 mg/kg bw/d	Intestine: iron pigmentation, goblet cell hyperplasia.
Rat: 90 day	38/153 mg/kg bw/d i.e. "real" LOAEL may be below 100 mg/kg bw day.	10-100 mg/kg bw/d	Liver: necrosis. Kidney: hyaline droplets in tubular epithelial cells. Protein precipitates in the renal tubular lumina. Forestomach: minimal diffuse hyperkeratosis. Small intestine: iron-positive pigment in tunica propria.
Dog: 90 day	26/68 mg/kg bw/d	10-100 mg/kg bw/d	Liver: Chronic hepatitis and cirrhosis. Gall bladder: oedema in wall. GI tract: minimal hyperplasia in the mucosa of the oesophagus.

Rat: 12 month	18/61 mg/kg bw/d May be considered to correspond to sub-chronic (90-day) NOAEL to LOAEL range of 36 to 120 mg/kg bw/d*; i.e. corresponding sub chronic LOAEL is below 100 mg/kg bw day.	2.5-25 mg/kg bw/d	Forestomach: Thickening of wall, hyperkeratosis of mucosa. Stomach: Hyperplasia of mucosa. Liver: Swollen and pigmented Küpffer's cells.
Rat: 24 month	6/33 mg/kg bw/d May be considered to correspond to sub-chronic NOAEL to LOAEL range of 12 to 66 mg/kg bw/d* i.e. corresponding sub chronic LOAEL is below 100 mg/kg bw day	1.25-12.5 mg/kg bw/d	Forestomach: hyperplasia in epithelium and hyperkeratosis of wall.

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

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

Comments received during public consultation

Comments were received from two MSCAs. One MSCA supported the classification proposed by the DS based on the liver effects seen in the dog study and considering the gap between the NOAEL and LOAEL in the rat studies, the MSCA was also in favour of a STOT RE 2 classification for effects in the GI tract and kidney. The other MSCA supported the DS proposal but considered that the oedema seen in the pancreas in the 90 day dog study should also be taken into account leading to a classification as STOT RE 2 (liver, kidney, GI tract and pancreas).

Assessment and comparison with the classification criteria

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

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

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

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

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

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

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

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

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

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

In summary: From the four repeated dose toxicity studies in rats and the 90-day study in dogs RAC considers that the dog is a more sensitive species than rats following exposure to Cu-HDO. In the dogs macroscopic and histopathological examinations revealed severe effects in the liver

observed as chronic hepatitis, liver cirrhosis and necrosis at approx. 69 mg/kg bw/d that are relevant for a STOT RE classification and are within the GV for a classification in Category 2 (10-100 mg/kg bw/d). Effects in the liver were also supported from the repeated dose toxicity studies in rats, however, these were reported as adverse outside the GV for a STOT RE 2 classification. The DS proposed to include a STOT RE 2 classification for both liver, GI tract and kidney. RAC is however of the opinion that the effects on kidney and GI tract reported in the rat repeated dose toxicity studies were outside the GV for a STOT RE 2 classification.

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

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

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

In vitro studies

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

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

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

In vivo studies

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

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

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

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

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

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

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

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

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

It was observed from the data that the combined incidences of all vascular tumours (haemangioma, haemangiosarcoma and lymphangioma) in mesenteric lymph nodes in the

control animals was at the upper edge of the HCD range and in the top dose in females above the HCD, however, this was related to an increase in benign haemangioma.

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

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

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

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

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

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

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

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

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

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

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

Comments received during public consultation

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

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

Assessment and comparison with the classification criteria

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

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

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

For a classification as Carc. 1B according to the CLP criteria the animal data should demonstrated sufficient evidence of animal carcinogenicity. RAC considers that the 2-year study in rats did not provide sufficient evidence for carcinogenicity and classification as Carc. 1B is not justified.

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

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

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

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

Effects on sexual function and fertility

No 2-generation study for the assessment of effects on reproduction following exposure to Cu-HDO was included in the CLH-dossier. The waiving argument provided by the Applicant were based on the absence of gross- and histopathological effects in the male and female reproductive organs in the repeated dose toxicity studies following exposure to Cu-HDO. The support for the waiving was based on several studies analysing the link between effects in male reproductive organs and effects on functional fertility. In these studies a clear link between effects in male reproductive organs and effects on reproduction was found (Dent, 2007, Janer *et al.*, 2007 and Mangelsdorf *et al.*, 2003). Based on the absence of effects in the reproductive organs in males and females evident from repeated dose toxicity studies following exposure to Cu-HDO and the waiving arguments for a 2-generation study no classification for effects on fertility and sexual function was proposed by the DS.

Developmental toxicity

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

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

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

Maternal toxicity included a statistically significant reduction in the daily food consumption in the mid and high dose groups starting on the first day of exposure (GD 7) and persisting to the end of exposure (GD 19). The reduction in food consumption from GD 7-19 was accompanied by a statistically significant reduction in body weight gain during the exposure period. During the post-

treatment period (GD 20 to 29) food consumption reached or even exceeded control values, and the maternal body weight gain was comparable to the control group. Reduction in gravid uterus weight was also reported in the high dose group, however, this was not statistically significant due to the high variability in the results. Clinical findings in the high dose group included no defecation in one dam (day 10-13) and blood in the bedding of another dam (due to litter loss).

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

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

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

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

Comments received during public consultation

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

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

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

malformations in the two studies it might even lead to a Repr. 1B classification for developmental toxicity.

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

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

Assessment and comparison with the classification criteria

Effects on sexual function and fertility

Information on the potential effects of Cu-HDO on sexual function and fertility was only available from repeated dose toxicity studies. In these studies no gross- and histopathological effects in the male and female reproductive organs were reported. For further information see the section of this opinion on STOT RE. No 2-generation reproductive toxicity study was available due to waiving arguments provided by the applicant and agreed upon in the technical meeting for biocides focusing on risk assessment. Based on the absence of effects in the reproductive organs in males and females evident from repeated dose toxicity studies following exposure to Cu-HDO RAC agrees with the DS that no classification of Cu-HDO for effects on sexual function and fertility is justified based on the data available. However, RAC recognises the absence of a 2-generation reproductive toxicity study. Data from a 1- or 2-generation study is considered by RAC to be needed to fully assess effects on sexual function and fertility.

Developmental toxicity

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

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

Table: *Maternal effects in rat developmental toxicity study*

Parameter	HCD	Control	10 mg/kg bw/d	30 mg/kg bw/d	100 mg/kg bw/d
# dams		30	30	30	30
Mortality of dams %		0	3.3*	6.6*	10*
BW gain				↓ GD 6-8 (corrected bw gain = 92% of control) ↑ GD 8-10	
Food consumption				↓ GD 6-8 (18%)	
Pregnancies %	92%	83%	90%	90%	90%

Necropsy findings of dams dead before end of test					
Lungs: oedema		20%	6.7%	6.7%	6.7%
Lungs: marginal emphysema		3.3%	0%	0%	0%
Particular findings on implants in dams sacr. Morbid/died interc.		0%	3.3%	6.7%	10%

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

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

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

Parameter	HCD	Control	10 mg/kg bw/d	30 mg/kg bw/d	100 mg/kg bw/d
Corpora lutea Total/# dams	6599/420 (15.7)	403/25 (16.1)	442/27 (16.4)	403/27 (14.9)	391/27 (14.5)
Implantations Total/# dams	5999/420 (14.3)	344/25 (13.8)	393/27 (14.6)	367/27 (13.6)	345/27 (12.8)
Resorptions Total/# dams	420/248 (1.7)	18/25 (0.7)	25/26 (1.0)	23/25 (0.9)	25/24 (1.0)
Total # foetuses	5528	326	368	344	320
Pre-implantation loss %	9.1	14.8	11.8	9.0	13.2
Post-implantation – loss %	7.9	5.0	6.1	6.0	7.2
Total # litters	418	25	26	25	24
Live foetuses/litter	13.2	13.0	14.2	13.8	13.3
Dead foetuses/litter	0	0	0	0	0
Foetus weight (g)	3.9	3.8	3.9	3.9	4.0

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

Table*: Incidences of variations and malformations*

Parameters	HCD	Control	10 mg/kg bw/d	30 mg/kg bw/d	100 mg/kg bw/d
External malformations %	0.09 (0-1.2)	0	0	0.6	0.3
External variations %	0	0	0	0	0

Skeletal malformations %	3.2 (0-10.1)	6.5	3.2	5.1	4.3
Skeletal retardations %	46.5 (0.0–72.0)	41	38	48	42
Skeletal variations %	47.8 (31.8–88.4)	36	41	42	33
Soft tissue variations %	15.5 (4.9-33.1)	22	20	17	27
Soft tissue malformations %	0.3 (0–2.2)	0	2.2	1.8	1.9

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

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

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

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

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

Parameter	**Control**	**10 mg/kg bw/d**	**30 mg/kg bw/d**	**60 mg/kg bw/d**
# dams	15	15	15	15
Bw gain GD 0-7 mean (SD)	45.3 (29.63)	24.6 (53.99)	19.9 (58.17)	36.1 (62.86)

Bw gain GD 7-19 mean (SD)	87.7 (45.35)	44.3 (45.07)	**25.9*** (52.49)	-**82.5**** (101.25)
Bw gain GD 19-29 mean (SD)	173.3 (73.41)	147.8 (67.88)	188.7 (73.45)	181.5 (59.71)
Bw gain GD 0-29 mean (SD)	306.3 (112.56)	216.7 (69.80)	234.5 (103.48)	**135.1**** (147.87)
Gravid uterus mean (SD)	313.1 (141.32)	298.6 (88.61)	317.0 (93.53)	236.7[a] (158.97)
Food consumption			Significantly reduced GD 7-13 and GD 15-20 (between 67% and 84% of controls)	Significantly reduced GD 7-20 (between 24% and 71% of controls)

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

[a]Due to high SD not statistical significant reduced

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

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

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

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

Parameter	**HCD**	**Control**	**10 mg/kg bw/d**	**30 mg/kg bw/d**	**60 mg/kg bw/d**
Number of foetus examined	2425	84	86	85	71
% External malformations	8/2425 (0.3%)	0	0	**1.2**	**2.8**

% External variations		0	05.8	1.2	0
% Skeletal malformations	31/2425 (1.3)	2.4	1.2	1.2	**2.8**
% Skeletal variations	314/2425 (12.9%)	13	17	20	30
% Skeletal retardations	1365/2425 (56.3%)	65	58	47	69
% Soft tissue malformations	48/2425 (2.0%)	2.4	2.3	0	2.8
% Soft tissue variations	741/2425 (30.6%)	27	21	25	23

Table: *Further data on the external malformations*

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

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

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

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

Comparison with the CLP classification criteria

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

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

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

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

In the rabbit developmental toxicity study a statistically significantly reduced daily food consumption was observed in the mid and high dose group, especially during the exposure period starting GD 7, i.e. the first day of exposure, persisting to GD 19. During the post-treatment period (GD 20 to 29), food consumption reached or even exceeded control values. The reported developmental toxicity effect that could be of concern was the full resorption of all foetuses in 4/15 dams. However, in these four dams a marked reduction in food consumption was observed, down to 10% of their pre-exposure consumption, as well as no defecation in one of them (day 10-13) and blood in bedding in another of the dams (due to litter loss). The mean number of live foetuses in the remaining 11 dams in the high dose group was not reduced. Further, an increase in external malformations that that was outside the HCD range in the mid and high dose groups could be of concern. However, when analysing these malformations, it was noted that two of the malformations were reported in the same foetus. This information lowers the concern arising from these malformations. There were no other supplementing information that might support a concern for developmental toxicity.

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

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

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

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

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

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

Hydrolysis

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

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

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

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

Conclusion:

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

Photolysis in water

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

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

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

Biodegradation

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

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

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

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

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

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

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

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

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

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

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

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

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

Overall conclusion

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

Bioaccumulation

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

Aquatic toxicity

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

Short-term toxicity to fish

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

Long-term toxicity to fish

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

Short-term toxicity to aquatic invertebrates

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

Long-term toxicity to aquatic invertebrates

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

Algae and aquatic plants

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

Comparison with the criteria

Aquatic Acute Category

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

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

Aquatic Chronic Category

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

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

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

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).