Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

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

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

CLH-O-0000001412-86-248/F

Adopted

30 November 2018

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON POTASSIUM (OXIDO-NNO-AZOXY)CYCLOHEXANE; CYCLOHEXYLHYDROXYDIAZENE 1-OXIDE, POTASSIUM SALT; [K-HDO]

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: potassium (oxido-NNO-azoxy)cyclohexane; cyclohexylhydroxydiazene 1-oxide, potassium salt; [K-HDO]
EC number: -
CAS number: 66603-10-9
Dossier submitter: Austria

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
09.01.2018	France		MemberState	1
Comment received				
According to the CAR of the substance, the typical purity of the TC (technical material) (substance without solvent) is 97.69% and the typical purity of the TK (technical concentrate) (substance with solvent - as manufactured) is 30%w/w. For better clarity, this information should have been reported in the CLH report. According to the CAR of the substance (Appendix 1-List of endpoint), it is stated that the minimum purity of the active substance is 977g/kg. However, the minimum of the concentration range reported in the CLH report is 95.96%. This should be clarified.				
Dossier Submitter's Response				
We agree, the information that the substance as manufactured is an aqueous solution of 30% K-HDO should have been added to the description of the substance in the CLH-report. With regard to the minimum purity of the active substance it has to be noted that the CAR for K-HDO has been finalised in 2008 and at that date no detailed guidance on minimum purity was available. The minimum purity for K-HDO as given in the CAR and finally laid down in Commission Directive 2008/80/EC is in fact based on the mean value of a 5-Batch analysis. The concentration ranges as reported in this 5-Batch analysis are in the range of 95.96 to 99.16 % w/w.				
RAC's response				
Noted.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON POTASSIUM (OXIDO-NNO-AZOXY)CYCLOHEXANE; CYCLOHEXYLHYDROXYDIAZENE 1-OXIDE, POTASSIUM SALT; [K-HDO]

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	2
Comment received				
We welcome this proposal for harmonized classification and labelling. As a general comment, BE CA would stress that studies based on the manufactured product, containing potential co-formulates, should not be regarded as key studies but are only supportive for the evaluation of K-HDO. Comment regarding read-across with Cu-HDO : Although some uncertainties still remain between the two substances (specific toxicity of each metallic ion, consequences of log Pow and size of the molecules in particular), BE CA agrees with the read-across strategy based on Cu-HDO.				
Dossier Submitter's Response				
Thank you for your review and support. With regard to the the purity of the test items related to the manufactured product we would like to clarify that this is 30% (w/w) K-HDO, the rest is water. Impurities between 0.1% and 1% are just chloride and sulphate. Just "K-HDO as manufactured" is available on the market and therefore the use of the respectively available studies for harmonised classification would provide the necessary human health protection in the context of the biocides regulation. This was the reason why we supported their use as key studies for classification.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2018	Germany		MemberState	3
Comment received				
DE-CA supports the CLH proposal. However, the applicability of read across according to Read-Across Assessment Framework (RAAF) on human health between Cu-HDO and K-HDO was not analysed by DE-CA. Nevertheless, we have some general remarks: • SID According to the CA Report DOC IIIA for the active substance "K-HDO", there are two different resonance structures. One resonance structure is a diazeniumdiolate and the other is a nitrosohydroxylamine. Each structure has its own CAS No: Cyclohexylhydroxydiazene 1-oxide, potassium salt CAS No 66603-10-9, EC-No. not attributed N-hydroxy-N-nitroso-Cyclohexanamine, potassium salt CAS No 27697-50-3, EC-No. 248-617-6 With respect to the CLH Report for bis(N-hydroxy-Nitrosocyclohexyl¬aminato-O,O')copper; [CU-HDO] (CAS No 15627-09-5 / 312600-89-8) the situation is similar. The two resonance structures of CU-HDO are also characterized by different CAS numbers. However, for the CLH Report of Cu-HDO both CAS numbers were considered. From our point of view the use of both CAS numbers is correct as the substance Cu-HDO can be described by both CAS numbers, depending on the conditions (e.g. solvent, temperature etc.). Consequently, please add also the CAS No 27697-50-3 and the corresponding EC				

No 248-617-6 to the CLH Report for K-HDO.

• Classification
Since, in addition to M-factors, ATE values should also be the subject of harmonisation the ATE (oral) = 136 mg/kg bw should be considered for discussion.

• Please add for all listed studies the corresponding Reliable Index (RI).

Dossier Submitter's Response

In principle we agree that the oral ATE of 136 mg/kg bw could be indicated. However we note that this is very close to the default c*onverted acute toxicity point estimate* of 100 mg/kg bw. Also the LOAELs in RTD feeding studies are in same range indicating that the acute lethality is likely due to the bolus application, which is not realistic for human exposure. Therefore this ATE refinement may indicate a data-preciseness which is not real.
The reliabilty in terms of Klimisch Score is available in the study summaries (attached document III).

RAC's response

Noted. Setting of ATE-values has been discussed in the opinion and included for acute oral toxicity.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	4

Comment received

The evaluation of the carcinogenic potential of K-HDO is based on the read-across with Cu-HDO. One 24 month oral carcinogenicity study is available on rat (A6.7, Mellert, 1996). No detailed table is presented regarding the exact type and site of neoplastic findings per dose and per sex.
Table 22b of the CLH proposal dossier (pathology report) indicates that 47/50 males and 46/50 females of the non-exposed control group developed neoplasms. Moreover 24% males and 34% females of this same control-group developed malignant neoplasms. Based on those pooled results, it seems very unlikely to draw any statistically significant observation due to Cu-HDO exposure. These observations raise also some questions about the reliability of the control-group.
When reading the discussion in the CLH proposal dossier, we have been surprised to see in the historical control data's that male rats are 10 folds more at risk than females to develop vascular tumours (22% vs 2%). It seems also inappropriate to pool all vascular tumours together, as well in HCD than in study results, whereas the occurrences and consequences of benign hemangioma and malignant hemangiosarcoma are quite different.
Therefore, BE CA is of the opinion that no conclusion can be drawn on the basis of this study without at least further details about the exact classification, appearance sites and number of all neoplastic findings per sex and group.

Dossier Submitter's Response

Further differentiation of tumor findings are reported in the study summary (attachement document III). The discussion in the CLH report reflects also the discussion of the study author within the original GLP study report.

<u>The applicant provided some further background information as follows</u>:

"When comparing the incidences of vascular tumors of the mesenteric lymph nodes in group 3 and 2 with the control group incidences of 25 comparable in-house studies with the same Wistar rat strain, the observed incidences in groups 3 and 2 are comparable to the upper limits of the historical control data (range from 0% to 25%). In addition, the historical control data of 7 studies derived from the Hannover Tumor Data Base "The Registry Nomenclature Information System"/RENI also offered comparable values (range from 0% to 22%).

Below mentioned are the historical control data for vascular tumours (hemangioma, hemangiosarcoma, and lymphangioma) of the mesenteric lymph nodes from the BASF inhouse evaluation (1) and the Hannover Tumour Data Base (2):

(1)

Males: 1039 animals out of 25 Studies. Mean findings: 10.44% (range: 0-25%); Females: 1040 animals out of 25 Studies. Mean findings: 1.84% (range: 0-6%)

(2)

Males: 320 animals out of 7 Studies. Mean findings: 5.3% (range: 0-22%); Females: 369 animals out of 8 Studies. Mean findings: 0.8% (range: 0-4%)

…

[In addition to the earlier Biocides CAs and ECHA discussion in 2008] the German MAK Commission of the advisory body (AGS) of the Federal Ministry of Labour and Social Affairs (BMAS) on the Ordinance on Hazardous Substances scientifically assessed the above mentioned study as well and they also regarded the study as valid to conclude about a carcinogenic potential of the substance. The full study report was disclosed to the MAK Commission for their assessment. MAK (2013) concluded: "*The tumour incidence was not increased in a carcinogenicity study with N-cyclohexylhydroxydiazene-1-oxide, copper salt administered with the diet in doses of up to 169 mg/kg body weight and day*." [..] "*the overall result of the carcinogenicity study is regarded as negative.*" [..] "*N-Cyclohexylhydroxy-diazene-1-oxide, copper salt yielded negative results in vitro in Salmonella typhimurium and in UDS tests with rat hepatocytes and in vivo in micronucleus tests in the bone marrow cells of mice after oral administration. A carcinogenicity study in rats given oral doses yielded negative results. Therefore, N-cyclohexylhydroxy-diazene-1-oxide, copper salt has not been classified in any of the categories for carcinogens or germ cell mutagens*."

Therefore, at least two official bodies concluded independently that the available study is valid and sufficient to conclude about the carcinogenic potential. Although the study author's discussion and the main results of the study have already been reflected in detail in the CLH report, the only aspect that could increase transparency further is to include additional detailed information in the CLH report if RAC advises accordingly."

RAC's response

Noted. The relevant information on HCD are included in the RAC opinion.

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Sweden		MemberState	5

Comment received

Based on read-across to Cu-HDO, the Swedish Chemicals Agency agrees with the proposal of the dossier submitter that classification of K-HDO for carcinogenicity is not warranted.

Dossier Submitter's Response

Thank you.

RAC's response
Noted.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	6
Comment received				
BE CA agrees with the Dossier Submitter that no classification is warranted based on the available studies in the CLH proposal dossier.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Sweden		MemberState	7
Comment received				
Based on the information given by the dossier submitter, the Swedish CA agrees with no classification of K-HDO for germ cell mutagenicity. However, we have a few comments: 1) The negative result of the in vivo micronucleus test is difficult to interpret, as bone marrow exposure was not confirmed. However, the neurotoxicity seen at the maximum tolerated dose may be considered as evidence of systemic bioavailability. 2) We consider the negative carcinogenicity test with Cu-HDO as not relevant for the conclusion of the genotoxic potential of K-HDO. Cancer is a separate endpoint.				
Dossier Submitter's Response				
We agree with these observations and note that also the in vitro genotoxicity studies were negative and for the (non-)classification proposal for carcinogenicity it is relevant to integrate all relevant available data.				
RAC's response				
Noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	8
Comment received				
The evaluation of the reproductive toxicity of K-HDO is based on the read-across with Cu-HDO. No assessment of K-HDO potential toxicity on fertility can be made because no 2-generation study is available neither for Cu-HDO or K-HDO. Two OECD TG 414 are available for Cu-HDO on rat and rabbit (respectively Hellwig, 1991 and Hellwig, 1994) to evaluate the developmental toxicity of K-HDO. First, we would like to stress some uncertainties regarding the reliability of the rat OECD 414 study : - The historical control data's indicate 0 dead foetuses in 418 litters (5528 foetuses). Although they do not have any consequence on the study results, these observations seem very unlikely, especially considering the reporting of 3,6% of skeletal malformations				

and 0,2% of soft tissue malformations in HCD, among other observations (cf. Table 12.4 of the CLH proposal dossier).
- The historical control data's indicate that respectively 40,5% and 39,4% of rat foetuses expressed skeletal retardations or variations, but also 33,6% with soft tissue variations. These results raise questions about the chosen rat strain (Wistar rat).
- Non-exposed control group pregnancy rate is also 9% lower than historical control data's 83% vs 92%). Again, these observations question the validity of the control-group.
- Finally, the same control group expressed lung edema in 20% and marginal emphysema in 3,3% of the dams reported as "dead before the end of test" (Table 23.2 of the CLH proposal dossier). These observations are unconsistent with the mortality percentage of dams, reported as 0% in the same table.

Regarding the specific observations in the rat OECD TG 414 study, Table 12.4 of the CLH proposal dossier reports that all three tested Cu-HDO doses induced an increase in soft tissue malformations (0% for control group vs. 2,2 – 1,8 and 1,9% for low, medium and high doses, respectively), which are 10-fold over the HCD range (0,2%). We are surprised to find that those results are not considered to be statistically significant, especially noting that the total number of foetuses varies between 320 and 368 per group.

Moreover, no detail is given in the dossier about these specific malformations, and they do not seem to have been considered in the evaluation of the developmental toxicity of Cu-HDO. BE CA would appreciate further details about these specific findings. On the basis of those partial informations, soft tissue malformations might be sufficient to warrant a developmental toxicity classification.

Secondly, in the rabbit OECD TG 414 study, the CLH proposal dossier states that a conception rate of 100% was reached in all groups. However, in the high dose group (60 mg/Kg bw), 4/15 dams had no viable foetuses at all due to early resorption. Considering that no acceptable justification has been given to explain these results and that resorptions and post-implantation losses are over the range of HCD in this group, BE CA is of the opinion that this observation should be considered as substance-related.

The examination of rabbit foetuses (Table 12.7) reported statistically significant external malformations on medium and high doses groups (respectively 1,2% and 2,8% vs. 0% in control-group). No detailed informations about these malformations are provided, but BE CA is of the opinion that skeletal malformations cannot be related to a non-specific stress, and have therefore to be taken into consideration.

We also express our surprise to read that 65% of the non-exposed control group showed skeletal retardations. 27% of the same group demonstrated soft tissue variations and even 2,4% had soft tissue malformations. Again, these observations raise questions about the chosen rabbit strain, but also about the experimental conditions if the skeletal retardations would be explained by a non-specific stress. We would appreciate to have some informations about the historical control data's regarding all retardations, variations and malformations.

No major maternal toxicity has been specifically highlighted. Although there is a decrease in body weight gain, the terminal body weight without uterus weight is not statistically different between all groups. No maternal mortality has been reported. However, a decrease of food consumption has been reported in medium and high group. Although this observation might explain foetal retardation, BE CA is of the opinion that this is not linked to developmental malformations. Moreover, the food reduction starting from day 7, we do not believe that this should be considered as the cause of the observed early resorption in the 4 dams in the high dose group.

As a general conclusion, BE CA believes that, although the major deficiencies in the reporting of the two developmental toxicity studies, the findings are sufficient to warrant a developmental toxicity classification. In an OECD TG 414 developmental toxicity study in rat, soft tissues malformations have been observed out of HDC range for the three

tested doses (respectively 2,2% - 1,8% and 1,9% after 10 mg – 30 mg or 100 mg/kg bw Cu-HDO). The OECD TG 414 developmental toxicity study in rabbit also reported an increase in early resorption in high dose group for 4 dams out of 15. Moreover, external malformations have been observed in the medium and high dose groups (30 mg and 60 mg/kg bw Cu-HDO). To our opinion, at least a Repr. 2 classification for developmental toxicity is warranted. Considering the fact that malformations have been observed in two different studies and the lack of details about the observed variations and malformations in the two studies, further clarifications might even lead to a Repr. 1B classification for developmental toxicity. We strongly regret the absence of fertility study.

Dossier Submitter's Response

Please note that accepting the absence of a fertily study was based on scientific considerations focussing on risk-assessment and was agreed in the technical meeting.
With regard to the rat TG414 study:

- The heading within table 23.2. needs correction as follows: Necropsy findings in dams ~~dead before end of test~~
- Please also note that for soft tissue malformations no dose-response relationship is apparent from low to high dose. The incidence for foetuses affected/foetuses analysed is (from control to high dose): 0/157, 4/178, 3/166 and 3/157 or 0, 2.2%, 1.8%, 1.9%. The litter incidence was 0/25, 4/26, 3/25, 3/24 or 0%, 15%, 12%, 13%.

- The soft tissue malformations were (from control to high dose, % fetal incidence) sinus inversus: 0, 0.6, 0.6, 0; hydrocephaly: 0, 0.6, 0, 0.6; microcepahlia: 0, 0, 0.6, 0; malformation of great vessels: 0, 0, 0, 0.6; heart-dilatation of right ventricle: 0, 0, 1.2, 0; septal defect: 0, 0, 0, 0.6; dilatation of both ventricles (globular shaped heart): 0, 1.1, 0, 0.

<u>The applicant provided some further remarks and background information as follows:</u>
With regard to: "- *The historical control data's indicate 0 dead foetuses in 418 litters (5528 foetuses)*.[..]" The table should be read in that way that no dead foetuses have been evaluated. Usually in historical control data the number of live and dead foetuses evaluated are given. We propose to rephrase the table for clarification.

With regard to: "*The historical control data's indicate that respectively 40,5% and 39,4% of rat foetuses expressed skeletal retardations or variations, but also 33,6% with soft tissue variations. These results raise questions about the chosen rat strain (Wistar rat).*"
We disagree. Please see additional BASF-in-house historical control data incl. ranges between years 1990 – 1998 (Wistar Rats; supplier: Thomae) (%fetuses and %range per study):

Total fetal external malformations: 0.09% (0-1.2%)
Total fetal external variations: 0% (0%)
Total fetal external unclassified: 0.2% (0-0.7%)
Total fetal skeletal malformations: 3.2% (0-10.1%)
Total fetal skeletal variations: 47.8% (31.0-88.4%)
Total fetal skeletal retardations: 46.5 (0.0-72.0%)
Total fetal soft tissue malformations: 0.3 (0-2.2%)
Total fetal soft tissue variations: 15.5% (4.9-33.1%)

Those values and ranges are quite usual and do not pose a risk of invalidity. Besides the percentage of affected foetuses the ratio of affected foetuses per litter is an important number in order to conclude about developmental effects. Unfortunately the range of the historical control data was not given e.g. in table 12.4 of the CLH report, however, the range is important to assess whether the study data are out of historical control range or not. Focusing on the above

mentioned historical control ranges all study mean values of table 12.4 (CLH report) are within the historical control range. This strengthens the conclusion that no developmental effects were observed in the rat study. Also the German MAK Commission (2013) concluded: "*A developmental toxicity study with N-cyclohexylhydroxy-diazene-1-oxide, copper salt in rats did not reveal any substance-induced findings in the offspring up to the high dose of 100 mg/kg body weight and day, the dose that coincided with the onset of maternal toxicity.*"

With regard to the rabbit TG414 study:

- In section 4.11.5 it is explained that "*In the rabbit study strongly reduced daily food consumption was observed in the high dose group: sharply between day 7, i.e. the first day of exposure, and day 20, between 26% to 69% of control. During the post-treatment period (day 19 to 29), food consumption reached or even exceeded control values. Food consumption is recognised as critical according to CLP Annex I, paragraph 3.7.2.4. and considered to be related to several non-specific consequences…*"
- The external malformations in medium and high dose group were (from control to high dose, % fetal incidence in 84, 86, 85, 71 fetuses evaluated): Gastroschisis: 0, 0, 0, 1.4; toes shortened: 0, 0, 1.2, 0; polydactyly: 0, 0, 0, 1.4; shortened and thickend hindlimbs: 0, 0, 0, 1.4. For further explanation: The thickened and shortened hindlimb in the one high dose fetus was also the one that had two supernumerary toes (polydactyly). After the skeletal examination shortened and bent tibia and fibula were identified as the cause for the thickening and shortening. Gastroschisis and different malformations of the extremities occur also sporadically in control foetuses of the rabbit strain used. Therefore the occurrence of the above described malformations in just one or two foetuses from one litter was not considered as associated with the treatment, but as being of spontaneous nature.
- Historical control data as referenced in the study report: total fetal external malformations 8/2425 = 0.3%; total fetal skeletal malformations 31/2425=1.3%;total fetal skeletal variations 314/2425=12.9%; total fetal skeletal retardations 1365/2425=56.3%; total fetal soft tissue malformations 48/2425=2%; total fetal soft tissue variations 741/2425=30.6%
- Within the study summary (document III6.8.2, section "evaluation by Competent Authority) it is explained: "*A primary maternal effect seems to be reduced food consumption during the treatment phase. This reduced body weight gain already in the medium dose group (30 mg/kg bw day), which seems to produce a (not statistically significant) maternal net weight reduction without effects on uterus weight and fetal weight. In contrast in the high dose group (60 mg/kg bw) the drastically reduced food consumption resulted in a body weight loss due to resorptions, subsequent litter loss and reduced uterus weight. Also the one dam that did not show defecation for several treatment days can be explained by the drastically reduced food consumption, as well as the one female with blood in bedding due to litter loss.*" Potential influence of the massive reduction of food intake in the top dose group on maternal stress and spontaneous malformation rates should be considered. Please also note that exposure started at day 7 and was continued to day 19 post insemination.

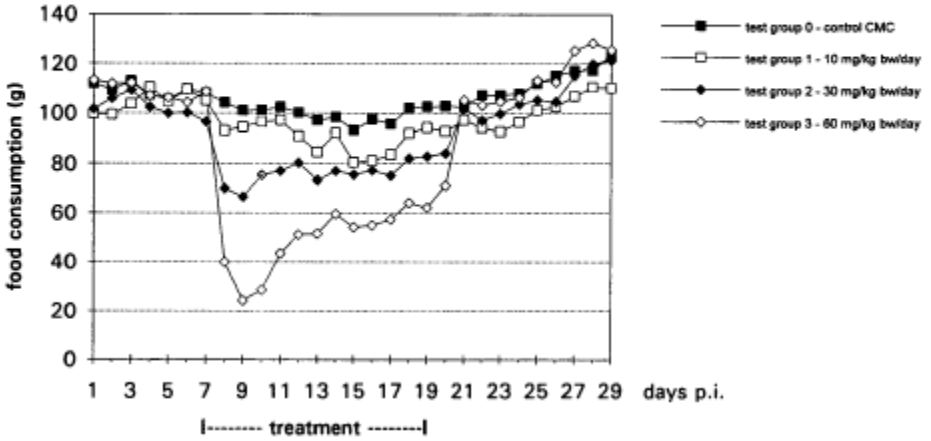
With regard to the BE CA general conclusion:

We have provided some further background information in this RCOM that would in our perspective not support classification. As indicated no dose-response relation for soft tissue malformations is apparent from low to high dose. Potential influence of the massive reduction of food intake in the top dose group on maternal stress and spontaneous malformation rates should be considered. We recommend to RAC to consider the new publication indicated by the German comment (D. Nitzsche 2017, Reg. Tox. Pharm., Vol.

90, pp.95-103); the database analysed was not completely devoid of findings of increased malformation rates in correlation with maternal food reduction.
Please note that accepting the absence of a fertily study was based on scientific considerations focussing on limit values and risk-assessment and agreed in the technical meeting.

<u>The applicant provided some further considerations and background informaton as follows:</u>
"As shown in below mentioned figure, drastic reduction of mean food consumption in the high (and mid) dose was observed during the treatment. Some animals of the high dose group reduced their food intake up to 90% for several treatment days which affected body weight and body weight gain. These maternal toxicity effects correlated with developmental findings on a single-animal level and are already discussed in the CLH report.

Fig. 4.2.1.1.1.: Mean food consumption (g/animal/day)



It should, in addition, be noted that rabbits have a more delicate gut microflora than other laboratory animals (e.g. rats) and it is well known that bacteriostatic substances such as biocidal substances disturb the balance of the rabbit intestinal/caecal microflora which in turn may lead to malnutrition and subsequent maternal toxicity, while humans might be exposed to higher doses without similar concern (ECHA, 2016; ADI and ARfD derivation for biocidal active substances). In addition, unlike rats, laboratory rabbits have a different eating behaviour including coprophagy, which is required in rabbits to receive sufficient nutritional intake (*Note coprophagy: rabbits (herbivores) do not have a complex ruminant digestive system. They extract extra nutrition from grass by giving their food a second pass through the gut. Rabbits produce cecotropes which are called "soft feces' or 'night feces'. The cecotropes are the material resulting from the fermentation of food in a part of the digestive system, the cecum. Rabbits also excrete another kind of feces which is their typical hard fecal pellet, but they do not normally consume that. Cecotropes arenutrient-rich and are passed out of the body, like feces, but are re-ingested by the rabbit so that more nutrients can be absorbed. Cecotropes have twice the protein and half the fiber of their typical hard fecal pellets. They also contain high levels of vitamin K and B vitamins (Vitamin B 12 in particular). After ingestion, on the second pass through, the extra nutrients are absorbed by the small intestine.).* Without this process, many of the nutrients in the food would be lost and passed through the colon, and out as typical feces.

In consequence of the strong reduction in feed intake in the rabbit study, one animal of the high dose group did not show defaecation for several treatment days, which - in line

of the above mentioned - increased its nutritional shortage further. It is very likely that also other animals which did not consume sufficient food also reduced the defaecation rate and, thus, received a comparable nutritional shortage. It is very likely that animals from the mid and high dose group were deficient in essential nutrients required for the development of their developing offspring. Altogether, it is highly plausible that the clear maternal toxicity (e.g. up to 90% reduced food consumption) is linked to the observed effects, particularly in the high dosed rabbits. At doses of Cu-HDO, where no nutritional shortage persisted, no developmental effects were observed in the rabbit study. With regard to the above mentioned, it is highly plausible that animals suffering from critical nutritional shortage during the organogenesis-phase, that is highly critical for development, are not able to provide sufficient nutritional supply for their developing offspring.

This is furthermore supported by published feed restriction studies. They are summarized by Nitsche (2017), however, the cited original studies should also be taken into consideration. Consequences of reduced feed intake are body weight loss and reduced body weight gain as maternal toxicity parameters. These are accompanied by reduced fetal body weights or associated with embryo-fetal deaths and abortions or premature birth. In rabbits the resorption rate was 3-18% in dams with restricted feeding (~10% of the control group) during organogenesis (HCD 3-8%). Post-implantational losses up to 19% are also observed in a study from Clark et al (1986). Clark et al observed also malformations after feed restriction, including omphalocele, clubbed feet and sternebral malformations. Another well documented consequence of feed restriction is the retarded development of the foetuses, indicated by unossified sternebrae, metatarsals, metacarpals, or caudal vertebrae (e.g. Cappon et al, 2005). In line with the review of the German authority BAUA (Nitzsche, 2017) those effects can be interpreted as non-specific and would not indicate a specific developmental toxicity in the context of hazard classification of chemicals.

With regard to the risk assessment and the already established AEL/AEC for Cu-HDO it is not expected that applicants are at risk. Also the MAK Commission (2013) concluded that if the workplace levels comply with the safe exposure levels for Cu-HDO there is no reason to fear damage to the embryo or foetus. Furthermore, due to the current classification and harmonized proposal for STOT RE2 classification sufficient risk mitigation measures are in place at the respective workplaces to protect from hazardous properties."

RAC's response

After considering the information provided by the DS, which has been included in the RAC opinion, RAC agrees with the DS that the effects reported in the rat and rabbit developmental toxicity study do not justify a classification for developmental toxicity.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	9

Comment received

BE CA agrees with the proposed Acute Tox. 3 classification (H301) for K-HDO on the basis of an acute toxicity test realised on purified K-HDO in rat (Munk & Gelbke, 1971). The resulting oral LD50 as 136 mg K-HDO/kg bw warrants this classification.

Dossier Submitter's Response

Thank you.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2018	Germany		MemberState	10
Comment received				
Oral Page 21, table 12: The study Hofmann 1971b is not included in annex2 (study summaries). Please attach. Dermal Page 23, table 14: The study Zeller 1971b is not included in annex2 (study summaries). Please attach.				
Dossier Submitter's Response				
The CLH report cannot be updated at this time point, but we can provide these documents to RAC if needed.				
RAC's response				
Noted. Relevant information is available to RAC.				

OTHER HAZARDS AND ENDPOINTS – Skin Hazard

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	11
Comment received				
The evaluation of K-HDO skin irritating proposal is based on a single study, using as test-item 30% (w/w) K-HDO in aqueous solution (Zeller, 1971a). First, the purity of the test-item remains unclear. We would appreciate some clarifications whether the study is based on the manufactured product, with other co-formulates, or on a pure water-based solution. BE CA is of the opinion that a study based on the manufactured product does not met the minimal conditions for a suitable evaluation of K-HDO skin irritation potential, unless appropriate negative control have been applied. The appropriate conclusion would therefore be "no classification due to data lacking". Secondly, considering that K-HDO would have been tested in a pure water-based solution, BE CA believes that the study results should be considered with caution. Although the testing conditions were more severe compared to the OECD 404 guideline, the resulting observations after a low concentration K-HDO exposure, with eschar and erythema persisting after 8 days, suggest that undiluted K-HDO might possibly be a corrosive agent. In general, considering that the CLP criteria's evaluate the hazard of the substance, and not its potential risk in a mixture, BE CA is also of the opinion that the maximal concentration of K-HDO generated in the manufactured product should not be used as an argument justifying any CLH classification.				
Dossier Submitter's Response				
The purity of the test item relates to the manufactured product and it is 30% (w/w) K-HDO, the rest is water. Impurities between 0.1% and 1% are just chloride and sulphate. Just "K-HDO as manufactured" is available on the market and therefore the use of the respectively available study for harmonised classification would provide the necessary human health protection in the context of the biocides regulation. This was the reason why we supported classification.				
RAC's response				
Noted. RAC agrees with the DS and on the basis of the data available is of the opinion that a classification of K-HDO for skin irritation in category 2 is warranted.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	12
Comment received				
The evaluation of K-HDO eye irritating proposal is based on a single study, using as test-item 30% (w/w) K-HDO in aqueous solution (Zeller, 1971a). The same comment regarding the purity of the test-item applies as for the skin irritation endpoint. BE CA is of the opinion that a study based on the manufactured product does not met the minimal conditions for a suitable evaluation of K-HDO eye irritation potential. The appropriate conclusion would therefore be "no classification due to data lacking". Considering that K-HDO would have been tested in a pure water-based solution, or compared to the appropriate negative controls, BE CA would agree with the proposed eye damage category 1 (H318).				
Dossier Submitter's Response				
Please see our response to comment 11.				
RAC's response				
Noted.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	13
Comment received				
BE CA agrees with the STOT RE 2 (liver, kidney) classification proposal for K-HDO, mainly based on the read-across with Cu-HDO. However, the neurotoxic effects observed after K-HDO gavage should be carefully discussed in Committee. We also disagree with the gastro-intestinal route for classification. Major deficiencies have been observed in the K-HDO feeding studies in rat. First, in the Mellert study (A6.9, 1992), only one dose has been investigated and the histopathological analysis has been restricted to the gastrointestinal tract. The second study (A6.3.1, Hofmann & Freisberg, 1976) did not carried out any histopathology after K-HDO exposure. However, the observed increase in magnesium, calcium and inorganic phosphate in the Mellert study show an electrolytic disequilibrium that should be carefully assessed, especially considering the observations of neurological effects in the K-HDO oral gavage (A.6.4.1, Leuschne et al, 1978). If the normal homeostasis is already disturbed after K-HDO feeding exposure, the gavage bolus as the potential cause of the observed neurotoxicity might be reconsidered. A 24 months Cu-HDO oral carcinogenicity study in rat (study A6.7) showed that the same adverse effects on gastro-intestinal tract are observed after eiher administration of Cu-HDO or CuSO4. These findings strongly suggest that the toxic effects on gastro-intestinal tract are mainly caused by copper. Therefore, BE CA is of the opinion that a STOT RE classification for gastro-intestinal tract is not warranted based on observations after Cu-HDO exposure. This justification does not apply for the liver, considering that in the same study, liver cysts were only observed after Cu-HDO exposure and not CuSO4.				

Dossier Submitter's Response
To make sure that there is no misunderstanding, we would like to underline that the suggestion was to mention gastrointestinal tract as a target organ, not restrict the STOT classification to the gastrointestinal route. We recommend RAC to consider the observations of the BE CA. Please note the explanation in the CLH dossier (page 34), supporting the read across also for the GI: "*Irritating and histological effects in the GI tract and kidney effects were observed within the repeated dose studies only with Cu-HDO and not with K-HDO. There are 2 potential explanations for this: (1) It was a Cu2+ specific effect that resulted from increased intracellular cytotoxic Cu2+ levels that were the consequence of the slow dissociation of Cu-HDO or (2) the effects could have been observed also with K-HDO if the same doses would have been analysed histologically: A histopathological analysis is available for K-HDO only with maximal 50 mg/kg bw for 96 days or with 90 mg/kg bw for 28 days, whereas the histopathological effects with Cu-HDO were observed only with 132 mg/kg bw for 28 days or 153 mg/kg bw for 96 days or 61 mg/kg bw for 12 months or 33 mg/kg bw for 24 months. However in any case these results do not raise specific concerns for K-HDO or contradict the read across arguments*"
RAC's response
Noted. RAC considers that a classification as STOT RE 2 (liver) is justified based on a read across from Cu-HDO to K-HDO and is supported by liver effects observed following exposure to K-HDO. The read across is further supported by the increased incidence of cysts in the liver that was only reported in the group receiving Cu-HDO and not CuSO4 in the 1- and 2-year studies indicating that it was not the Cu-ion alone, buth rather the HDO^--ion that was responsible for the increased incidence of hepatic cysts.

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2018	Germany		MemberState	14
Comment received				
The proposal for STOT RE2 is largely based on read-across from data on Copper-HDO and the argument that the toxicity of this compound following repeated exposure cannot be attributed to the copper content alone. A specific target organ toxicity of HDO was concluded and formed the basis for proposing classification also of Potassium-HDO. A more detailed, quantitative comparison of the organ toxicity of Cu-HDO vs. relevant Cu-salts could improve the robustness of this read-across approach. It is noted that for the purpose of the assessment under the biocides legislation, the read-across has recently been accepted.				
Dossier Submitter's Response				
We would like to refer to the study summaries (document III attachment) of the chronic (Doc III A6.5.) and the carcinogenicity (Doc III A.7) studies with Cu-HDO, which contain equimolar CuSO4 dose groups. We would think that the results of this comparision, carried out within the same studies is useful for the purpose of this assessment. <u>The applicant provided some further comments and background information as follows:</u> "We also conclude that classification as STOT RE2 for gastrointestinal tract is warranted, because in difference to copper sulfate, copper-ions may penetrate deeper into the gastrointestinal mucosa mediated by the organic HDO-residue. This could increase cytotoxic effects of the copper-ions as toxophore. Available studies show for instance 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 $CuSO_4$. In consequence STOT RE2				

classification for GI tract, liver and kidney are supported by experimental evidence. Also the German MAK Commission concluded (2013) for Cu-HDO that "the gastrointestinal tract, liver and kidneys are the target organs of the toxicity of N-cyclohexylhydroxydiazene- 1-oxide, copper salt."
RAC's response
Noted. RAC considers that a classification as STOT RE 2 (liver) is justified based on a read across from Cu-HDO to K-HDO and is supported by liver effects observed following exposure to K-HDO. The read across is further supported by the increased incidence of cysts in the liver that was only reported in the group receiving Cu-HDO and not CuSO4 in the 1- and 2-year studies indicating that it was not the Cu-ion alone, buth rather the HDO^--ion that was responsible for the increased incidence of hepatic cysts.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
29.12.2017	Finland		MemberState	15
Comment received				
Toxicity tests with fish and crustacea are recommended for classification purposes of aquatic hazards. The key studies for this proposal are Danio rerio juvenile growth test (OECD 215) and Daphnia magna reproduction test (OECD 211). The lowest chronic toxicity NOEC values for K-HDO were reported as 0,29 mg/l and 0,47 mg/l, respectively. FI CA supports the conclusions that the substance is neither rapidly degradable nor bioaccumulative. Based on the available information and the classification criteria FI CA supports the proposed classification of Aquatic Chronic 2, H411 for K-HDO.				
Dossier Submitter's Response				
Thank you for your kind support!				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	16
Comment received				
BE CA is of the opinion that studies based on the manufactured product, containing potential co-formulates, should not be regarded as key studies but are only supportive for the evaluation of K-HDO. Moreover, in general CLP criteria evaluate the hazard of the substance as such and derivation of the hazard of a substance from his mixture, based on his maximal concentration in the manufactured product should not be used as an argument justifying any CLH classification. Therefore we conclude that, at present, data are lacking to decide on the environmental classification of K-HDO.				
Dossier Submitter's Response				
All aquatic toxicity studies were performed with a product which is a 30%(w/w) solution of the active substance K-HDO in water. All test results were recalculated to pure K-HDO content in the test solutions. This is clearly explained and stated under chapter 5.4 Aquatic toxicity in the CLH report.				

Therefore it is concluded, that the CLH proposal is based on reliable and scientifically sound key studies.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2018	Germany		MemberState	17
Comment received				
5.1.2 Biodegradation, page 58 DOC II A contains two studies for biodegradation in soil (Dr. Wolmann GmbH-year unknown, Anonymous-1976). Could you please include these studies in the CLH report as a matter of completeness?				
Dossier Submitter's Response				
During active substance evaluation these studies were not considered valid due to a very poor documentation of the data in the test reports, which made a proper evaluation impossible. Therefore, no Doc IIIAs were requested from the applicant and are therefore not available.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2018	United Kingdom		MemberState	18
Comment received				
We note there is uncertainty regarding the toxicity to fish endpoint given: - the acute study uses a non-standard test species and analytical verification is not available, and - it is unclear if growth is the most sensitive chronic endpoint for fish meaning. Should additional fish toxicity data become available, the classification should be reconsidered.				
Dossier Submitter's Response				
The used test species in the acute fish study is mentioned as standard species for the testing of acute aquatic toxicity in fish in Reg. (EC) No. 440/2008. In the key studies with Algae and Daphnia, analytical results were in the range of 80% of the nominal values comfirming the stability of the test substance during these tests. Thus, it is very likely that the test substance was also stable during the acute fish test as well. The chronic fish study was conducted according OECD 215 and without deficiencies. Growth rate as endpoint is required according to guideline. Therefore no further testing is necessary.				
RAC's response				
Noted.				