Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the 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

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON BIS(N-HYDROXY-N-NITROSOCYCLOHEXYLAMINATO-O,O')COPPER; BIS(N-CYCLOHEXYL-DIAZENIUM-DIOXY)-COPPER; [CU-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: bis(N-hydroxy-N-nitrosocyclohexylaminato-O,O')copper; bis(N-cyclohexyl-diazenium-dioxy)-copper; [Cu-HDO]
EC number: 239-703-4
CAS number: 15627-09-5 312600-89-8
Dossier submitter: Austria

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	1
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 Cu-HDO.				
Dossier Submitter's Response				
Basically we agree with the comment by BE. As stated in the CAR the active substance Cu-HDO isn't manufactured as isolated solid, but it is generated in an "all in one approach" during the manufacturing of the product formulation. In order to enable risk assessment which is not biased by co-formulants the manufacturer provided isolated Cu-HDO which has been used for respective studies.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2018	Germany		MemberState	2
Comment received				
DE-CA supports the CLH proposal in general. 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: • Classification Since, in addition to M-factors, ATE values should also be the subject of harmonisation the ATE (oral) = 380 mg/kg bw should be considered for discussion. • Please add for all listed studies the corresponding Reliable Index (RI). • A comment on immunotoxicity is missing.
Dossier Submitter's Response
We agree that the ATE value of 380 mg/kg bw should be considered for harmonised CLP entry. The reliabilty in terms of Klimisch Score is available in the study summaries (attached document III). No specific studies are available for immunotoxicity and no specific findings were oberserved in the standard animal studies.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Germany	BASF Wolman GmbH	Company-Manufacturer	3
Comment received				
Comment on the proposed substance name, EC- and CAS number (CLH report Cu-HDO p. 1, 5, 14-15) For the substance bis(N-cyclohexyl-diazenium-dioxy)-copper; [Cu-HDO] a new CAS number 312600-89-8 had been assigned, as it has been proven by experimental and technical analysis (see attachment number 1 to 3) that the name bis(N-hydroxy-N-nitrosocyclohexylaminato-O,O')copper, which is associated to the old CAS number 15627-09-5, does not represent the real structure of the substance and the name is therefore misleading. The attachments demonstrate that Cu-HDO is accurately referred to as bis(N-cyclohexyl-diazenium-dioxy)-copper, CAS-number 312600-89-8 and that Cu-HDO is not a nitroso compound. This is why the CAS number 15627-09-5, the assigned EC-number 239-703-4 and the name bis(N-hydroxy-N-nitrosocyclohexylaminato-O,O')copper should be removed for the future entry of Annex VI of the CLP regulation. Remark: this item is comparable to the substance K-HDO (CAS 66603-10-9). In the current CLH proposal of this substance only the new CAS number is indicated. ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment CLH Cu-HDO Attachment 1-3.zipx				
Dossier Submitter's Response				
As already described in the CLH-report the x-ray crystallography data which has been submitted for the biocidal active substance approval showed that the diazeniumdiolate form is predominating. During the commenting phase of the CAR some MS experts had concerns that different x-ray crystallography conditions may show another distribution, but in the end they agreed to the results of the study since no contradicting data was available. As a consequence it was decided to assign the respective CAS-No. 312600-89-8				

as the only identifier for the biocidal active substance. The literature provided by the applicant during the public consultation phase for the CLH-report gives additional scientific arguments to substantiate the use of CAS-No. 312600-89-8 as only identifier for Cu-HDO and should therefore considerd by RAC.
RAC's response
Noted.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Sweden		MemberState	4
Comment received				
The Swedish Chemicals Agency agrees with the proposal of the dossier submitter that classification of Cu-HDO for carcinogenicity is not warranted.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	5
Comment received				
The evaluation of the carcinogenic potential of K-HDO is based on one 24 month oral carcinogenicity study 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
11.01.2018	Germany		MemberState	6

Comment received

The only available 2-year rat carcinogenicity feeding study shows evidence of a dose-dependent increase of haemangioma in male and female animals (m: 6-7-12-13, f: 1-1-0-4, from control to high dose). Based on the significant incidence of those tumours in high dose groups, a classification of Cu-HDO as carcinogenic Category 2 should be taken into consideration. When assessing vascular tumours, the incidence of lymphangioma

should be regarded and reported separately from hemangioma and hemangiosacroma due to their different origin.
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. Simple statistics (Cochran-Armitage Tests for trend, Fisher Exact Tests) appeared only positive for the Cochran-Armitage Test for trend for male haemangioma against a cut off p-value of 0.05, for a one-sided test (and negative for a two sided test). Mortality did not appear increased with increasing dose (see attachment to CLP report, Document III A 6.7.1).
RAC's response
RAC considers that the 2-year study in rats does not give limited evidence for carcinogenicity and a classification as Carc. 2 is not justified.

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Sweden		MemberState	7
Comment received				
The Swedish Chemicals Agency agrees with no classification of Cu-HDO for germ cell mutagenicity.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	8
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.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	9
Comment received				
No assessment of Cu-HDO potential toxicity on fertility can be made because no 2-generation study is available for Cu-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 Cu-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. We would not exclude that the massive reduction of food intake in the top dose group (see first bullet point answer to this rabbit study) relates to a stress that could finally also affect spontaneous malformation rates.
- 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*

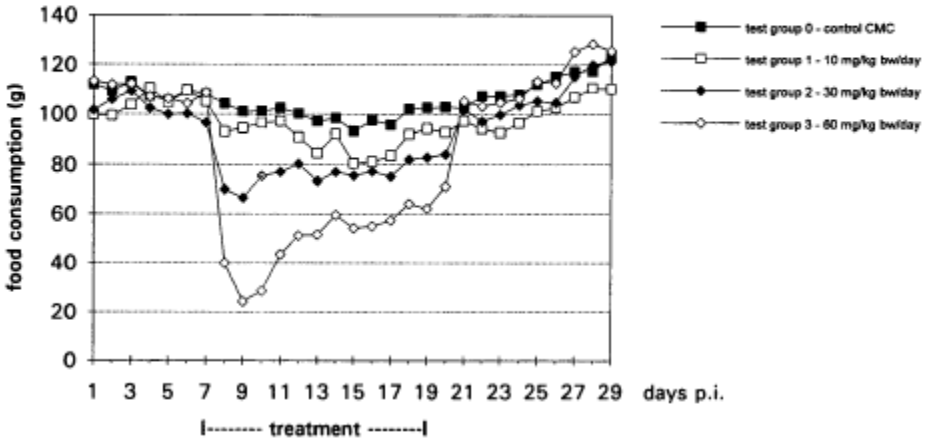
that did not show defecation for several treatment days can be explained by the drastically reduced food consumption, as well as the one female with blood in bedding due to litter loss." As mentioned we would not exclude that the massive reduction of food intake in the top dose group (see first bullet point answer to this rabbit study) represents a stress that could finally also affect spontaneous malformation rates. 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. Effects oberserved in the rabbit developmental study may be due to drastically reduced food consumption and related stress.
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

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

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON BIS(N-HYDROXY-N-NITROSOCYCLOHEXYLAMINATO-O,O')COPPER; BIS(N-CYCLOHEXYL-DIAZENIUM-DIOXY)-COPPER; [CU-HDO]

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2018	Germany		MemberState	10

Comment received

Two prenatal development studies with Cu-HDO, one in rats and one in rabbits are available in the CLH report. While the study in rats does not exert relevant findings for classification, the rabbit study shows an increased rate of resorption in the high dose group and therefore an increase in post-implantation loss that exceeded the concurrent and the historical control values. In parallel, maternal effects, primarily reduced food consumption during the treatment phase, are described. The dossier submitter´s proposal is no classification based on inadequate evidence for reproductive toxicity.
However, in view of the relationship between developmental effects and general maternal toxicity the CLP regulation states in Annex I, paragraph 3.7.2.4.2:
"Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies."
Regarding the relationship between developmental effects and general maternal toxicity please consider a recent publication: D. Nitzsche (2017) Effect of maternal feed restriction on prenatal development in rats in rabbits – A review of published data Reg. Tox. Pharm., Vol. 90, pp.95-103.
The author evaluated studies with feed restriction down to 10% of control (in rabbits) and states that
"[…]effects like embryo/fetal lethality and malformations in rats and in rabbits are not impaired by feed restriction. This indicates that, when they appear in developmental toxicity safety studies of specific compounds, these effects are likely to be regarded as more specific indicators for developmental toxicity."
and
"Malformation indicating substance-specific developmental toxicity does not seem to be attributed to maternal toxicity in rats and rabbits. This also applies to embryo/fetal lethality due to no increase in resorption or numbers of dead fetuses observed in feed restriction studies."
Please consider these findings when deciding on the classification for developmental toxicity.

Dossier Submitter's Response

OECD Guidance Document ENV/JM/Mono(2008)16 references Chappon et al 2005 indicating that abortions in rabbits may be a consequence of severe feed restriction. Please also note that the feed uptake in top dose animals rapidly dropped from start of dosing, i.e. d7 to d9 when mean was about 20% of control. Thereafter it slowly increased till day 19 to about 60% of control and increased quickly to 100% and above with cessation of dosing after day 19. Therefore reduced feed intake is unlikely a consequence of systemic toxicity. We would not exclude that the massive reduction of food intake in the top dose group represents a stress that could finally also affect spontaneous malformation rates. The available data are described in the CLP report also dam-specific, which will support the independend RAC review.

RAC's response

Noted. The information from Nitzsche (2017) has been included in the RAC opinion, and 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	11
Comment received				
The evaluation of Cu-HDO acute toxicity is mainly based on two studies, using as test-item Cu-HDO suspended in aqueous 0.5% carboxymethylcellulose. 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. BE CA is of the opinion that a study based on the manufactured product should only be considered with the greatest caution, unless appropriate negative control have been applied. Considering that Cu-HDO would have been tested with appropriate negative control, BE CA agrees with the proposed Acute Tox. 4 classification (H301) for Cu-HDO. The resulting oral LD50 as 380 mg Cu-HDO/kg bw warrants this classification (Study A6.1.1/01). The other available studies of lower reliability also support a category 4 classification.				
Dossier Submitter's Response				
The test item purity is of p.a. quality, please see the study summary (Doc IIIA 6.1.1/01), section "Evaluation by Competent Authorities".				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2018	Germany		MemberState	12
Comment received				
Inhalation RAC may indicate that the data base for the proposal "no classification" is rather weak, as the concentration applied in the only test is not known and the reliability is 3.				
Dossier Submitter's Response				
We agree. However please also note that "heavy dust development was stated and as the duration of exposure was 8 hours instead of 4 hours as recommended in the OECD guideline 403, it can be assumed that the LC50 is above the concentration range which leads to classification."				
RAC's response				
RAC considers that it is not possible to evauate the result of the study presented and no classification for acute toxicity via inhalation is proposed for Cu-HDO due to insufficient data.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	13
Comment received				
BE CA agrees with the proposed Eye dam. 1 classification for Cu-HDO. However, we would appreciate further clarifications about the dilution protocol of Cu-HDO, as Table 15 indicates the application of 50 ml of solid Cu-HDO. Although the BASF protocol was prior				

to GLP guidelines, the observations of non-reversible effects with high score after a single application of 50 ml warrant this classification.
Dossier Submitter's Response
The substance was not diluted, but applied as a solid. The amount of substance was indicated as 50µl.
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	14
Comment received				
BE CA agrees with the STOT RE 2 (GI, liver, kidney) classification proposal for Cu-HDO. In a 96 days oral study in rat, toxic effects in liver (hepatic single cell necrosis, and swelling and pigmentation of Kupffer's cells), gastro-intestinal tract (diffuse hyperkeratosis in the forestomach and iron-positive pigment in the small intestine) and kidney (hyaline droplets in the proximal tubular epithelial cells and protein precipitates in the renal tubular lumina) from 153 mg/kg bw/day (NOAEL 38 mg/kg bw/day). Dose-related increase of the toxic effects. Some similar observations in the liver and GI of rats have been made in a 24 month study from 61 mg/kg bw/day (NOAEL 18 mg/kg bw/day) (Study A6.5). Moreover, a 96 days oral study in Beagle dogs indicated severe toxic effects from 68 mg/kg bw/day (Study A6.4.1/02). Liver observations include necrosis, chronic hepatitis, liver cirrhosis associated with copper pigment storage in hepatocytes and Kupffer cells. GI tract findings showed minimal hyperplasia in the mucosa of the esophagus and edema in the gallbladder wall. The observations in the dog warrant a STOT RE 2 classification for the liver. Considering the gap between the NOAEL and LOAEL in the rat studies, BE CA is also in favour of a STOT RE 2 classification for GI tract and kidney.				
Dossier Submitter's Response				
Thank you.				
RAC's response				
From the four repeated dose toxicity studies in rats and the 96-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 evident 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 are 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. No exposure route should be specified, since there was no evidence that the liver would not be affected by other exposure routes.				

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2018	Germany		MemberState	15
Comment received				
The sub-chronic and the chronic toxicity studies in rats compared the effects of Cu-HDO and CuSO4. Some effects were observed in both test groups and were attributed to the copper ion while some effects were specific for Cu-HDO. A more elaborative assessment of these findings may help to decide if the read-across from Cu-HDO to K-HDO is applicable. It is suggested to include oedema observed in the pancreas and in the mesentery into the classification for STOT RE2 (GI, kidney, liver), as the incidence of those effects was observed in 2 male dogs at LOAEL (96-d. oral dog feeding study).				
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. See response to comment no 14.				

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2018	Netherlands		MemberState	16
Comment received				
Aquatic toxicity and degradability. Proposed comments - In the annex VI report it is argued that the classification should be performed according to the general guidance, as Cu-HDO is an organo metal that does not dissociate easily in water or dissolves as a metal ion. Although NL agrees that the substance should be considered as not rapidly degradable based on the criteria in the guidance, this conclusion is based on data determining complete mineralization. Slow mineralization could be due to slow degradation of the metabolites, which does not indicate that an earlier copper releasing step will also be a slow process. In the water-sediment study, 21.5% and 2.8% of the parent is detected in the sediment and water respectively after 30 days. These data				

show that primary degradation is not completed within 30 days, but it also shows that more than 70% of the copper could have been released. In the annex VI report a comparison is made between the toxicity of free copper and that of Cu-HDO to support the classification based on Cu-HDO, but data on the parent is very limited to make such a comparison. Furthermore, the data available for both Cu(II) and Cu-HDO does not concern the same species, e.g. for Cu-HDO only an acute fish toxicity endpoint is available for Onchorhychus mykiss while the lowest acute value for Cu(II) is for Pimephales promelas, and the lowest chronic value for Cu(II) is for Ceriodaphnia dubia while for Cu-HDO only an endpoint for Daphnia magna is available. The difference between the calculated ERV values and the toxicity values for Cu-HDO is less than a factor 10, which could be explained by differences in species sensitivity. On the basis of a potential high release of Cu(II) ion, NL is in the opinion that RAC should discuss if the classification should not be based on or Cu(II) ion instead of Cu-HDO. As shown in the annex VI report, classification based on Cu(II) ion would lead to a stricter classification with M-factors of 10 instead of 1.
- NL would further like to note that it is inconsistent to in one place calculate a chronic toxicity value for fish based on the copper content while in another instance is stated that the toxicity from Cu-HDO differs from that of free copper (II) and that therefore the classification should not be based on Cu(II). Although the calculated value is proposed as a worst case, the dossier or report does not contain sufficient information to support that the calculated value is actually worst case as comparable data on individual animal species is not presented or available for both Cu-HDO and Cu(II). Also, this can be considered a read-across and a justification is not presented. Therefore, NL is of the opinion that the surrogate approach should be applied for fish when the classification is to be based on the toxicity data for Cu-HDO. Although it should be noted that the classification based on Cu(II) ion is preferred, see our other comment on this.

Dossier Submitter's Response

Replying to the comments it was discovered that in the CLH-report a mistake was made concerning the derived chronic classification based on Cu(II) ion. The calculated chronic $ERV_{CU\text{-}HDO}$ is 0.041 mg/L, which in combination with no rapid biodegradability results in a chronic classification with Aquatic Chronic 1, **M=1** instead of M=10, as mentioned in the report. We appologize for that mistake!

AT CA is of the opinion that since there are enough data available on Cu-HDO, classification should be based on these data, only.
The new proposal therefore replaces the long-term NOEC value for fish (0.064 mg/L; recalculated on equimolar basis from Cu(II) in the Cu-VRAR 2008) by the acute fish value for Cu-HDO of 10 - 100% mortality at 0.14 – 0.24 mg/l, respectively. In combination with "not rapidly biodegradable" and applying the criteria given in Table 4.1.0(b)(iii) of Part IV, Annex I to CLP Regulation this leads to the same C&L proposal as in the CLH report: Aquatic Acute 1, H400; M=1 and Aquatic chronic 1, H410; M=1;

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
12.01.2018	Belgium		MemberState	17

Comment received

BE CA agrees with the proposal to classify the substance Cu_HDO as Aquatic Acute 1, H400; Aquatic Chronic 1, H410 and assignment of an acute M-factor =1 and chronic M-

factor = 1.

From the aquatic/sediment degradation study it can be concluded that Cu-HDO is an organometal compound that does not dissociate easily in water, nor dissolves as metal ion. BE CA is of the opinion that classification should not be based on the calculation of the NOEC from the Cu in Cu-VRAR 2008, neither from Cu HC5in Cu-VRAR 2008.

In other words BE CA doesn't share the opinion that chronic toxicity studies are available for all the 3 trophic levels. This means that the surrogate approach should be considered. However this will not change the final conclusion as both outcomes are giving the same chronic classification and M-factor.
- Based on lowest available NOEC :
Algae (Scenedesmus subspicatus) with 72h NOErC=0.056 mg/L and substance not rapidly degradable
☐ Aquatic Chonic 1, H410; M=1
- Based on acute toxicity fish: only range of mortality (10 at 0.14 mg/L -100% at 0.24mg/L) available
☐ range between 0.1 and 1 mg/l : Aquatic Chronic 1, H400; M=1

Dossier Submitter's Response

Thank you for your comment, AT CA completely agrees with your proposal.

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
08.01.2018	France		MemberState	18

Comment received

Section 5.4.1.2 – long term toxicity to fish: Chronic toxicity of Cu-HDO was calculated from the lowest NOEC value of 0.064 mg/L, for freshwater fish issued from the Cu-VRAR 2008. It should be noted that the ecotoxicity database on copper has been updated for the classification dossier of granulated copper which was under public consultation in the Echa website in 2017 (https://echa.europa.eu/fr/harmonised-classification-and-labelling-previous-consultations/-/substance-rev/16213/term?_viewsubstances_WAR_echarevsubstanceportlet_SEARCH_CRITERIA_EC_NUMBER=-&_viewsubstances_WAR_echarevsubstanceportlet_DISS=true).
This dossier will be discussed at the RAC level at the beginning of 2018. Nevertheless, a lowest chronic data for fish of 5.9 µg/L is reported for P. promelas. We are of the opinion that this value should be taken into account for the classification of Cu-HDO. This leads to a NOECfish of 0.032 mg/l (on equimolar basis) which indicates that fish is the most sensitive species.

Section 5.5.1 – Comparison with criteria for environment hazards: Regarding aquatic chronic toxicity data, as no 'real' chronic data is available for Cu-HDO, the proposed approach should be compared with the criteria given in Table 4.1.0(b)(iii) as recommended in the figure 4.1.1 of the Guidance on the application of the CLP criteria (v5). Both approaches lead to the same classification.

Section 5.5.3 – overall conclusion: it is mentioned that "It is therefore finally concluded that the proposal for classification and labelling of Cu-HDO should be based on the measured toxicity values for Cu-HDO". In contrast, based on the new available data, the

chronic classification should be based on the lowest NOEC for fish which is not a measured toxicity value for Cu-HDO. Taking into account the above remarks, we agree to the proposed classification aquatic acute 1 with M-factor = 1 and aquatic chronic 1 with M-factor = 1. Additional minor comments: page 73 the following sentence, referred to the CAR of Copper, is not relevant for the CLH dossier: "For the accumulation potential of copper and the risk for secondary poisoning please see section 4.2.4."
Dossier Submitter's Response
Thank you for your comment. AT CA is of the opinion that since there are enough data available on Cu-HDO, classification should be based on these data, only. The new proposal therefore replaces the long-term NOEC value for fish (0.064 mg/L; recalculated on equimolar basis from Cu(II) in the Cu-VRAR 2008) by the acute fish value for Cu-HDO of 10 - 100% mortality at 0.14 – 0.24 mg/l, respectively. In combination with "not rapidly biodegradable" and applying the criteria given in Table 4.1.0(b)(iii) of Part IV, Annex I to CLP Regulation this leads to the same C&L proposal as in the CLH report: Aquatic Acute 1, H400; M=1 and Aquatic chronic 1, H410; M=1; We completely agree with your minor comment, but at this stage it is not forseen anymore to change the CLH report.
RAC's response
Noted.

Date	Country	Organisation	Type of Organisation	Comment number
11.01.2018	Germany		MemberState	19
Comment received				
Chapter 5.1.2.3 Simulation tests page 62 – biodegradability in water/sediment system: Table 24 Biodegradation, water/sediment The relevant value is DegT50 total system, so it might be better to mark in bold this one instead of DissT50 water phase.				
Dossier Submitter's Response				
Thank you for your comment. We completely agree, but at this stage it is not forseen anymore to change the CLH report.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
29.12.2017	Finland		MemberState	20
Comment received				
FI CA supports the conclusion that Cu-HDO should be classified based on the measured toxicity values rather than hazards identified for the metabolite copper (II) ion. Toxicity test for algae (Scenedesmus subspicatus) used for classification of Cu-HDO is				

considered valid. The lowest acute toxicity was EC50 value of 0.194 mg/l and the lowest chronic toxicity was NOEC value of 0.056 mg/l. FI CA supports the conclusions that Cu-HDO is neither rapidly degradable or potentially bioaccumulative.

Based on classification criteria FI CA supports the proposed environmental classification Aquatic Acute 1, H400 with M-factor of 1 and Aquatic Chronic 1, H410 with M-factor of 1 for Cu-HDO.

Dossier Submitter's Response

Thank you for your comment.
AT CA is of the opinion that since there are enough data available on Cu-HDO, classification should be based on these data, only.
The new proposal therefore replaces the long-term NOEC value for fish (0.064 mg/L; recalculated on equimolar basis from Cu(II) in the Cu-VRAR 2008) by the acute fish value for Cu-HDO of 10 - 100% mortality at 0.14 – 0.24 mg/l, respectively. In combination with "not rapidly biodegradable" and applying the criteria given in Table 4.1.0(b)(iii) of Part IV, Annex I to CLP Regulation this leads to the same C&L proposal as in the CLH report: Aquatic Acute 1, H400; M=1 and Aquatic chronic 1, H410; M=1;

RAC's response

Noted.

Date	Country	Organisation	Type of Organisation	Comment number
10.01.2018	United Kingdom		MemberState	21

Comment received

Cu-HDO is an organo-metallic compound. Data on dissolved copper concentrations in fate studies is not available and that dissolution/transformation data is not available. Therefore, it is unclear if/what rate copper ions were released and if the observed ecotoxicity is attributable (potentially in part) to the metal ion. On that basis there is some uncertainty whether the classification should be based on Cu-HDO or the metal copper ion. The CLH proposal is based on Cu-HDO although the CLH report includes the classification based on the copper ion for comparison – the later results in a more stringent classification due to an additional factor of 10 to the M-factor.
Based on the available data and uncertainty, we wonder if the proposal should be based on the more stringent classification.
We note that if further fate data becomes available, the environmental classification should be re-considered.

Dossier Submitter's Response

Replying to the comments it was discovered that in the CLH-report a mistake was made concerning the derived chronic classification based on Cu(II) ion. The calculated chronic $ERV_{CU\text{-}HDO}$ is 0.041 mg/L, which in combination with no rapid biodegradability results in a chronic classification with Aquatic Chronic 1, **M=1** instead of M=10, as mentioned in the report. We appologize for that mistake!

AT CA is of the opinion that since there are enough data available on Cu-HDO, classification should be based on these data, only.
The new proposal therefore replaces the long-term NOEC value for fish (0.064 mg/L; recalculated on equimolar basis from Cu(II) in the Cu-VRAR 2008) by the acute fish value for Cu-HDO of 10 - 100% mortality at 0.14 – 0.24 mg/l, respectively. In combination with

"not rapidly biodegradable" and applying the criteria given in Table 4.1.0(b)(iii) of Part IV, Annex I to CLP Regulation this leads to the same C&L proposal as in the CLH report: Aquatic Acute 1, H400; M=1 and Aquatic chronic 1, H410; M=1;
RAC's response
Noted.

CONFIDENTIAL ATTACHMENTS
1. CLH Cu-HDO Attachment 1-3.zipx [Please refer to comment No. 3]