Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

2-bromo-2-(bromomethyl)pentanedinitrile; [DBDCB]

EC Number: 252-681-0
CAS Number: 35691-65-7

CLH-O-0000007329-67-01/F

Adopted

8 June 2023

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2-BROMO-2-(BROMOMETHYL)PENTANEDINITRILE; [DBDCB]

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during 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 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 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. Journal articles are not confidential; however they are not published on the website due to Intellectual Property Rights.

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Substance name: 2-bromo-2-(bromomethyl)pentanedinitrile; [DBDCB]
EC number: 252-681-0
CAS number: 35691-65-7
Dossier submitter: Czech Republic

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
05.10.2022	Germany		MemberState	1
Comment received				
The DE CA supports the proposed classification and labelling of the Dossier Submitter. CLH Dossier, p. 18 Table 9 and p. 21 section 4.2.4: Please note that the LD50 from the oral LD50 test [non key A6.1.1, 1978] differs in the table (541 mg/kg BW) and in the text (514 mg/kg BW). Classification as Acute Tox. 4, H302 is not influenced by this. CLH Dossier, p. 18 Table 9, p. 20 section 4.2.1.2, p. 21 section 4.2.3 and p.21 section 4.2.4: Please note that the LC50 from the inhalation LC50 test [key study 6.1.3, 2003] differs: Table 9 and section 4.2.1.2 (0.265 mg/L) vs. section 4.2.3 and 4.2.4 (0.264 mg/L). Classification as Acute Tox 2, H330 is not influenced by this.				
Dossier Submitter's Response				
Thank you for your comment. The correct values are 541 mg/kg bw and 0.265 mg/L for LD50 and LC50,respectively.				
RAC's response				
Thank you for your comments.				

Date	Country	Organisation	Type of Organisation	Comment number
29.09.2022	Netherlands		MemberState	2
Comment received				
It is noted that the study summaries (for example regarding reproduction toxicity and STOT RE) as included in the CLH-report contain very limited details, which may hamper a				

proper evaluation based on the CLH report alone. There is no link/direct reference to the background documents (CAR) available via the relevant CLH-pages (ECHA-site or CIRCABC). As a result the NL-CA can only request a reflection on some issues rather than agree or disagree with the proposed classifications.
Dossier Submitter's Response
Thank you for your comment. The documents you mention should be available at CIRCABC. We wonder if ECHA could make them available to you?
RAC's response
RAC agrees with opinion of the MSCA that the CLH report contains limited details regarding reproduction toxicity and STOT RE.

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
06.10.2022	France		MemberState	3
Comment received				
FR supports the proposal to not classify DBDCB for carcinogenicity.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your comment.				

MUTAGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
06.10.2022	France		MemberState	4
Comment received				
FR supports the proposal to not classify DBDCB for mutagenicity.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for comment.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
29.09.2022	Netherlands		MemberState	5
Comment received				
In the key rat developmental toxicity study, there was significant difference in weight change in the dams between treated and control groups. It is also noted that an increased number of resorptions was observed already at the mid dose group. However, no quantitative information is presented. These findings might be related. The reduction in weight change in the dams may not be maternal toxicity as the DS suggests, but rather attributable to reduced growth of the developing fetuses and resorptions. If there is any information on body weight gain with/without the uterus weight this should be taken into account. To the perspective of the NL-CA, the information might be indicative of a developmental effect and should be considered for classification. The Dossier Submitter is kindly requested to reflect on this.				

Further, the NL-CA notices the following:
- The section on toxicokinetics (page 16, section 4.1.3) states that "DBDCB is completely debrominated prior to systemic distribution,…. ".
- Further, page 49 (section 4.10.1.1) of the CLH-report states: "The assessment of reproductive toxicity of DBDCB should take into account the effects of bromide ion released from the DBDCB molecule and cumulating in tissues at higher daily intakes. Exposure to DBDCB at LOAE levels of 60 – 250 mg/kg bw corresponds to daily bromide intake of 36 - 150 mg/kg bw. LOAEL/ NOAEL values of 1200/300 mg bromide/kg of diet determined in a 3-generation test in rats (cited in JMPR, 1988) correspond approximately to 72/18 mg bromide/kg bw per day. Fertility and the viability of the offspring were significantly reduced at 4800 mg bromide/kg of diet (approx. 300 mg bromide/kg bw per day). Exposure to DBDCB at NOAEL level corresponds to daily bromide intake of 18 mg/kg bw." This is understood by the NL-CA that it is considered there is not enough bromide ion systemically available in the developmental toxicity studies to cause effects based on previous findings by JMPR, 1988. However this does not mean bromide could not induce developmental toxicity at somewhat higher dose levels. Moreover, developmental toxicity already occurs as noted in our first discussion point.
- Previously, RAC agreed on the classification of ammonium bromide for the endpoint reproductive toxicity as Repr. 1B (H360FD) and Lact. (H362). (https://echa.europa.eu/documents/10162/61e8d5d7-2ebd-fd02-a9c5-89671c2aef3b). Here, it was considered that "…the bromide ion is the relevant ion for determination of the toxicological profile…".
It seems that when considering the bromide ion in relation to reproductive toxicity, the level of exposure is taking into account by the Dossier Submitter. However, the classification and labelling criteria are based upon the presence of a hazard rather than a risk, and the above cited DS' statement should therefore not be driven by the exposure level, but rather consider the hazard of the bromide ion. The Dossier Submitter is kindly requested to reflect on this.

Dossier Submitter's Response

Thank you for your comment. Regarding the early resorptions in the developmental toxicity study it is noted that these were primarily clustered in two animals. One animal in the high dose (175 mg/kg bw) accounted for 13 out of 26 early resorptions and one animal in 100 mg/kg bw dose accounted for the other 14 out 28 early resorption in this dose group. Unfortunatelly, no historical control data are available on early resorptions in controls. However, in the dose ranging study early resorptions accounted for 20.2 % in the control group (15 of 18 clustered in 2 animals) which is two fold higher than 10% in the highest dose group in the main study. No significant difference in the live fetus body weights were indetified among the control and any of the treastment groups. Furthermore, reduced growth of foetuses is, in our opinion, usually linked to late organogenesis which can hardly be linked to early resorptions. Though, we can agree that early resorptions and reduced growth can be due to to the same pathway such effects would be probably accompanied by other adverse effects (e.g. malformations)
In the main study, the values of the actual body weight gain (mg/kg bw) from day 6-20 are 86.0±15.10, 88.7±14.47, 75.0±28.64 and 70.1±22.8* for the doses (mg/kg.bw) of 0, 25,100 and 175, respectively. The values of the body weight gain corrected for the gravid uterus (mg/kg bw) from day 6-20 are 25.8±7.32, 28.3±10.26, 16.8±21.34 ,and 13.7±19.58 * for the doses (mg/kg.bw) of 0, 25,100 and 175, respectively. Thus, the body weight change corrected for the uterus decreased with the increased dose (no statistical significance detected) which indicates maternal toxicity could be the causal factor. In the 90 day study following in utero exposure the parent animals were dietarily exposed to ♂: 5.6, 33.0, 195.9 mg/kg bw/day ♀: 6.7, 41.5, 247.8 mg/kg bw/day starting 7 days prior to mating. Yet, no litter effects were observed. The body weights of the pups in the treated

groups did not show any singnificant difference from the control group on the day 1 following the parturition. Histopathology of the uterus did not reveal any adversity. This again indicates that the substance does not show any developmental toxicity in doses applied in the studies. Rather, the early resorptions observed in the developmental studies were due to systemic toxicity in the dams which could have been at least partly due to the high blood peaks of the a.s. resulting from the administration via gavage.

We are sceptical regarding "read across" from ammonium bromide as ammonium can make a difference.
Regarding the "low dose" we note that the dose was determined by the dose range finding study and the highest dose in the developmental study is based on the adversity (including mortality) beyond MTD observed in the dams treated with 250 mg/kg bw/d. We therefore agree with the highest dose of 175 mg/kg bw for DBDCB in the main developmental study.

RAC's response

Thank you for your comments. Data on the potential mechanism of action is secondary to clear presentation of numerical and qualitative description results of studies that make the comparison with the classification criteria more convincing.

Date	Country	Organisation	Type of Organisation	Comment number
06.10.2022	France		MemberState	6

Comment received

FR supports the justifications proposed for not classifying DBDCB for reproductive/developmental toxicity.

Page 36; in the subchronic dietary exposure of dogs to DBDCB, the effects observed in thyroid are considered relevant as they are associated to developmental effects observed in rat on skeletal ossification and to effects on CNS in dog. Similar effects in thyroid (hypertrophy or hyperplasia), on CNS (degeneration in brain), effects on testis) are observed in oral toxicity dog studies with methyldibromo glutaronitrile (MDBGN).
For DBDCB, in summary section 4.7.1, you stated that the guidance value for classification with STOT RE 2 is very close to 100 mg/kg bw/d for both rat and dog. However, we do not suggest to use the threshold in dog for the rat as nothing in CLP criteria is applicable for other species than the rat. The use of the allometric function approach you propose is not mentioned in CLP criteria to fix limit of classification, moreover it could be a source of uncertainties. The dog is more sensitive than rat in absence of specific value according to CLP criteria for a classification STOT RE 2. If dog is considered relevant model for human, a classification STOT RE 2 could be proposed and discussed by RAC members.

Dossier Submitter's Response

Thank you for your support and your comment. We insist on non classification. It is generally known that physiological rates are higher in smaller animals when normalised per body weight. This in this case predicts faster elimination of Br- from rats as opposed to dogs. This difference is reflected in the allometric scaling factor which is a common part of the inter species assessment factor. Using the same arguments the dog is predicted to be nearer to humans than the rat in terms of Br- elimination. Thus, the difference between rats and dogs is predictable based on this general knowledge. The higher "distance" between the rat and the human is reflected in the CLP guidance value for rat of 100 mg/kg bw. This implies lower value for the dog (not explicitelly mentioned in the CLP but implied by the general knowledge). Applying the same value to the dog would mean being uduly conservative.

While we agree that the more sensitive species could be used, in our opinion such sensitivity should be specific to this species and due to other factors rather than the predictable differnces in physiological rates. This is not the case here where the higher sensitivity of the dog is predictable as explained above and therefore we do not agree with the classification.
RAC's response
Thank you for your comments. Data on the potential mechanism of action is secondary to clear presentation of numerical and qualitative description results of studies that make the comparison with the classification criteria more convincing.

RESPIRATORY SENSITISATION

Date	Country	Organisation	Type of Organisation	Comment number
06.10.2022	France		MemberState	7
Comment received				
FR supports the conclusions for no classification for respiratory sensitization.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your comment.				

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
05.10.2022	Germany		MemberState	8
Comment received				

Oral
Two oral LD50 studies [key study 6.1.1, 1991a and non-key A6.1.1, 1978] in rats are available.

In the study similar to OECD TG 401 [key study A6.1.1, 1991a], decedents showed signs of lethargy, ataxia, ptosis, dyspnoea, tremors, coma, flaccid muscle tone, prostration, diarrhoea and hyperactivity prior to death. Survivors also showed signs such as lethargy, ataxia, chromodacryorrhoea, chromorhinorrhoea, diarrhoea, emaciation, hyperactivity, wetness of the anogenital area and brown staining of the nose/mouth area.

The LD50 values observed were 640 mg/kg BW [key study 6.1.1, 1991a] and 514 mg/kg BW [non key A6.1.1, 1978].
Both LD50 values justify a classification as Acute Tox. 4, H302.

Inhalation
For acute inhalation toxicity, two LC50 tests, one according to OECD TG 403 [key study 6.1.3, 2003] and the other one similar to OECD TG 403 [non key A6.1.3, 1992] are available. While in the key study a high mortality (LC50 = 0.264 mg/L) was observed due to the inhalation of dust, no significant toxic effects from inhalation exposure were observed in the non key study. Observed mortality mentioned above, occurred at ≥ 0.217 mg/L and was caused by an acute alveolar oedema. The non-key study provides inconsistent results, whereas 3/5 males and 2/5 females died at a concentration of 4.76 mg/L. However, no deaths occurred at 8.31 mg/L and 13.09 mg/L after DBDCB exposure.

Besides, clinical signs were reported in the key study but returned up to mid-term of the

second post-exposure week. Necropsy findings in all surviving rats were unremarkable, on the contrary for those who succumbed during the study the following effects were included: lungs less collapsed and with discolorations/white foci, firm consistency; watery to yellowish/red discharge from nose or content in the nasal cavities, nose/nostrils with red encrustations; trachea with foamy content; corneal opacity; gastrointestinal tract bloated and yellowish content in lumen, mucosa reddened; discolorations of parenchymatous organs.

In summary, the observed LC50 of 0.264 mg/L justifies a classification as Acute Tox 2, H330.

Dermal
After treatment with the dermal limit dose some effects on the skin including moderate erythema (day 1), moderate to severe eschar (day 7), moderate to severe eschar in 3/10 animals and slight erythema in 4/10 animals (both noted on day 14), as well as oedema ranging from slight to severe (day 1) and slight to absent (days 7 and 14) were observed in an acute dermal limit test (similar to OECD TG 402) [key study 6.1.2]. The main toxic sign observed was diarrhoea. Necropsy revealed eight animals show no effects and two had crusted skin at the treated area.

During the observation period no deaths occurred from the treatment with the dermal limit dose. Therefore, the proposed non-classification is justified.

Dossier Submitter's Response
Thank you for your evaluation. As we arrived at the same conclusions no further response is needed.
RAC's response
Thank you for your comments.

Date	Country	Organisation	Type of Organisation	Comment number
06.10.2022	France		MemberState	9
Comment received				
Acute toxicity oral: FR supports the proposed classification based on the only supported study which is not robust. In page 18 table 9, should it be read 541 or 514 mg/kg bw/d as it is written in section 4.2.4 for LD50 oral?. Acute toxicity inhalation: FR agrees with the proposed classification. Should it be read LC50 (inhalation, rat) of 264 mg/L (section 4.2.4) or 265 mg/l (table 9 page 18)? Respiratory tract irritation: section 4.4.3.3 for summary. It should be written in the text …acute toxicity via inhalation Did you consider adding ATE for acute toxicity oral and inhalation as stipulated in CLP guidance (2017)?				
Dossier Submitter's Response				
Thank you for your comment. The correct values are 541 mg/kg bw and 0.265 mg/L for LD50 and LC50,respectively. We agree with correcting the word inhaltion to inhalation. We did not consider adding ATE for the two routes.				
RAC's response				
Thank you for your comments and corrections.				

OTHER HAZARDS AND ENDPOINTS – Eye Hazard

Date	Country	Organisation	Type of Organisation	Comment number
05.10.2022	Germany		MemberState	10
Comment received				
DBDCB caused strong eye irritation in an in vivo rabbit eye test according to OECD TG 405 [key study 6.14 / 02, 1982a]. Effects included damage of the cornea, iris, conjunctival redness as well as chemosis. Eye irritation reactions occurred in all rabbits and did not ease by the end of the study period. Thus, classification as Eye Dam. 1, H318 is warranted.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for comments.				

Date	Country	Organisation	Type of Organisation	Comment number
06.10.2022	France		MemberState	11
Comment received				
FR supports the proposed classification.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Thank you for your comment				

OTHER HAZARDS AND ENDPOINTS – Skin Sensitisation Hazard

Date	Country	Organisation	Type of Organisation	Comment number
05.10.2022	Germany		MemberState	12
Comment received				
In general, the classification as a skin sensitiser can be supported. However, the result of the key study (6.1.5/01) is questionable because no dose-range finding test was performed for this study. Thus, there is a possibility that the negative result of the study is not based on the lack of a respective inherent property of the substance but on the test concentration being too low. The second key study (6.1.5/02 19939) also has major uncertainties, because neither the test substance nor the test system are adequately characterised. The results of the human studies ultimately provide an indication of the inherent skin sensitising properties of the test substance. Here, the CLP Guidance also provides detailed information on how to assess these studies. The results of the positive patch tests suggest that a "Relatively high frequency of occurrence of skin sensitisation" (c.f. Table 3.2, Guidance on the Application of CLP Criteria, Reference: ECHA-17-G-21-EN) is occurring. With regard to Annex I: 3.4.2.2.4. (Regulation (EC) No 1272/2008) a weight-of-evidence				

approach can be performed using the human data alone. Because a classification as Skin Sens. 1A cannot be excluded, the classification Skin Sens. 1 is appropriate.

Dossier Submitter's Response

Thank you for your support of Skin Sens. 1 . Regarding the human data we note that many human subjects may have already been sensitised (induced) prior to the conduct of the tests due to the use of this substance in cosmetics. This may lead to lower values causing an effect than in the intact (by this substance) people. This should be taken into account when deciding on classification as adversity should be shown in intact organisms.

RAC's response

Thank you for your comments. Human data appeared to be crucial in determining the classification.

Date	Country	Organisation	Type of Organisation	Comment number
06.10.2022	France		MemberState	13

Comment received

FR supports the proposed classification based on the use of DBDBCB in cosmetic products.

Dossier Submitter's Response

Thank you for your comment. Regarding the human data we note that many human subjects may have already been sensitised (induced) prior to the conduct of the tests due to the use of this substance in cosmetics. This may lead to lower values needed to cause an effect than in the intact (by this substance) people. This should be taken into account whan deciding on classification as adversity should be measured in intact organisms.

RAC's response

Thank you for your comments. Human data appeared to be crucial in determining the classification.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
05.10.2022	Germany		MemberState	14

Comment received

The data leading to classification as STOT RE are well described and indicate that there are large interspecies differences regarding thyroidal effects. The dog is apparently more sensitive than the rat species required in Table 3.9.2 and Table 3.9.3 of the Regulation (EC) No 1272/2008. Nevertheless, the dog species could be used for classification. Based on the principle of using the results of the most sensitive species and that „Evaluation […] on all existing data "(Annex I: 3.9.2.4, Regulation (EC) No 1272/2008) are mandatory for assessment.

One of the major difference between dogs and rats is the half-life of bromide (as shown in the CLH proposal). Here, the dog species is more comparable to humans than the rat (dog: 15 – 46 days, rat: 3 – 8 days, human: 12 days). Therefore, it would be plausible to use the results of the dog studies to conduct a classification. This means that the limit value determined from the dog study (102 mg/kg BW/d) can be regarded as a borderline case to the limit value for classification according to Annex I: 3.9.2.9.7 (< 100 mg/kg

BW/d).

In summary, based on these data non-classification of thyroid effects as STOT RE can be justified; however, an in-depth analysis is required.

Dossier Submitter's Response

Thank you for your comment. We insist on non classification.
As you mentioned the dog is near to the human than the rat. For the rat the distance from the human is predictable as it is generally known that physiological rates are higher in smaller animals when normalised per body weight.
This in this case predicts faster elimination of Br- from rats as opposed to dogs. This difference is reflected in the allometric scaling factor which is a common part of the inter species assessment factor. Using the same arguments the dog is predicted to be near to humans than the rat in terms of Br- elimination. Thus, the difference between rats and dogs is predictable based on this general knowledge. The generally higher "distance" between the rat and the human is reflected in the CLP guidance value for rat of 100 mg/kg bw. This implies lower value in for the dog (not explicitelly mentioned in the CLP but implicitly from the logic and general knowledge). Applying the same value to the dog as for the rat makes littile sense here.
While we agree that the more sensitive species could be used, in our opinion such sensitivity should be specific to this species and due to other factors than predictable differences in physiological rates. This is not the case here where the higher sensitivity of the dog is predictable as explained above and therefore we do not agree with the classification.

RAC's response

Thank you for your comments. Data on the potential mechanism of action is secondary to clear presentation of numerical and qualitative description results of studies, which make comparison with classification criteria more convincing.

Date	Country	Organisation	Type of Organisation	Comment number
29.09.2022	Netherlands		MemberState	15

Comment received

Similarly as for the endpoint reproductive toxicity, the NL-CA notices the following for the endpoint STOT RE:
- The section on toxicokinetics (page 16, section 4.1.3) states that "DBDCB is completely debrominated prior to systemic distribution,…. ".
- Previously, RAC agreed on the classification of ammonium bromide for the endpoint STOT RE as STOT RE 1 (H372) with nervous system as the primary target organ. (https://echa.europa.eu/documents/10162/61e8d5d7-2ebd-fd02-a9c5-89671c2aef3b).

Here, it was considered that "…the bromide ion is the relevant ion for determination of the toxicological profile…".
With respect to thyroid effects, RAC considered that the severity of these effects were not sufficient to include thyroid as a target organ.

The Dossier Submitter is kindly requested to reflect on these points.

Dossier Submitter's Response

Thank you for your your comments.
We insist on non classification.

Regarding "read across" from ammonium bromide, CNS is a known target organ for ammonium and it is likely that this part of the ammonium bromide is responsible for its classification.
As no other effects relevant for classification as STOT RE were observed we consider only thyroid in the following text.
We have doubts regarding the relevancy of the guidance value of 100 mg/kg bw (oral, rats) for dogs. It is generally known that physiological rates are higher in smaller animals when normalised per body weight. This in this case predicts faster elimination of Br- from rats as opposed to dogs.
This difference is incarnated in the allometric scaling factor which is usually a part of the inter species assessment factor. By the same arguments, the dog is predicted to be nearer to humans than the rat in terms of Br- elimination. Thus, the difference between rats and dogs is predictable based on this general knowledge. The higher "distance" between the rat and the human is reflected in the CLP guidance value for rat of 100 mg/kg bw. This implies lower value for the dog.
Thus using the same guidance value for the dog is not justified.

While we agree that the more sensitive species could be used, in our opinion such sensitivity should be specific to this species and due to other factors than predictable differences in physiological rates. This is not the case here where the higher sensitivity of the dog is predictable as explained above and therefore, we do not agree with the classification.

RAC's response

Thank you for your comments. Data on the potential mechanism of action is secondary to clear presentation of numerical and qualitative description results of studies that make comparison with classification criteria more convincing.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
06.10.2022	United Kingdom	Health and Safety Executive	National Authority	16

Comment received

DBDCB (CAS: 35691-65-7)
We note that the chronic fish NOEC based on survival is in the same concentration range from 0.1-1 mg/L as the key algal chronic endpoint and therefore supports the proposed chronic classification. Given the importance of these two endpoints, please could the CLH DS confirm whether the OECD TG 210 and the OECD TG 201 validity criteria were met in these fish and algal studies, respectively? Additionally, please could the CLH DS provide EC10 values for the chronic fish study if these are available and reliable, noting that these are preferred over NOEC values for the purpose of hazard classification.

Dossier Submitter's Response

The requested information was searched for in the available study reports and, if available, is presented below:
For the OECD TG 201 study with algae, the validity criteria for the factor of the biomass parameter and the coefficients of variation for replicates were met. The EC10 values were provided in the study report and amounted to 0.0318 mg/L (for yield at 72h) and 0.1987 mg/L (for growth rate at 72h).
For fish, the long-term toxicity of DCDCB was determined in a fish early life-stage toxicity test according to US-EPA guideline 72-4, equivalent to OECD 210, in rainbow trout. The test was performed under flow-through conditions with the measurement of concentrations.

In order to improve the quality of the data and to allow additional 3-8 weeks to the test duration, several changes were proposed for the study:

1. Embryos used to initiate the test will be 2-24 hours post ferlizzaiton or at the eyed stage at least 7 days before hatch.
2. The animals will be exposed for 32 days after swim up rather than 32 days after hatching.
3. The photoperiod will be adjusted to 24 hours of darkness or dim light until swim up.
4. Live fish will be counted and released into the test vessels at swim up rather than at hatching.
5. The number of live fish will be thinned to 30-40 perc test vessel between hatching and release.
6. Fish will be fed daily after swim up rather than after hatching.

The changes were suggested and implemented in order to improve the quality of data collected during the study via initiating the test with eggs at an earlier development stage. Regarding the results and validity criteria, it was observed that the mean measured concentrations were in good agreement with the nominal concentrations. The control and solvent control survival rates were 96.7-100%. The time to hatch averaged 34.9 days for the control and 33.6 days for the solvent control, and the time to swim up was 49 days for both controls. The relative standard deviation of the weights for surviving fish in the control test chambers was less than 40%. Water quality parameters were within acceptable limits throughout the test.

As for the results, the overall NOEC was determined to be 0.75 mg/L and the overall LOEC to be 1.0 mg/L, based on mean measured concentrations. EC10 values were not provided.

RAC's response

RAC agrees with the need to clarify the aspects reported by UKNA and took the DS response into account for the opinion development.

Date	Country	Organisation	Type of Organisation	Comment number
06.10.2022	France		MemberState	17

Comment received

FR agrees with the aquatic chronic toxicity classification proposed.

Dossier Submitter's Response

Thank you for your comment.

RAC's response

Noted.