Committee for Risk Assessment

RAC

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

8 June 2023

CLH-O-0000007329-67-01/F

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

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

Chemical name: **2-bromo-2-(bromomethyl)pentanedinitrile; [DBDCB]**

EC Number: **252-681-0**

CAS Number: **35691-65-7**

The proposal was submitted by the **Czech Republic** and received by RAC on **25 February 2022.**

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

PROCESS FOR ADOPTION OF THE OPINION

The **Czech Republic** has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **8 August 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **7 October 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bogusław Barański**

Co-Rapporteur, appointed by RAC: **Dania Esposito**

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

The RAC opinion on the proposed harmonised classification and labelling was adopted on **8 June 2023** by **consensus**.

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

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	2-bromo-2-(bromomethyl)pentane dinitrile; [DBDCB]	252-681-0	35691-65-7	Acute Tox. 4 Acute Tox. 2 Eye Dam. 1 Skin Sens. 1 Aquatic Chronic 2	H302 H330 H318 H317 H411	GHS06 GHS05 GHS09	H302 H330 H318 H317 H411			
RAC opinion	TBD	2-bromo-2-(bromomethyl)pentane dinitrile; [DBDCB]	252-681-0	35691-65-7	Acute Tox. 4 Acute Tox. 2 STOT RE 2 Eye Dam. 1 Skin Sens. 1A Aquatic Chronic 2	H302 H330 H318 H317 H411	GHS06 GHS05 GHS08 GHS09	H302 H330 H318 H317 H411		Inhalation: ATE = 0,27 mg/L (dusts or mists) Oral: ATE = 500 mg/kg bw Skin sens 1A: SCL = 0,001 %	
Resulting Annex VI entry if agreed by COM	TBD	2-bromo-2-(bromomethyl)pentane dinitrile; [DBDCB]	252-681-0	35691-65-7	Acute Tox. 4 Acute Tox. 2 STOT RE 2 Eye Dam. 1 Skin Sens. 1A Aquatic Chronic 2	H302 H330 H373 (thyroid, central nervous system) H318 H317 H411	GHS06 GHS05 GHS08 GHS09	H302 H330 H373 (thyroid, central nervous system) H318 H317 H411		Inhalation: ATE = 0,27 mg/L (dusts or mists) Oral: ATE = 500 mg/kg bw Skin sens 1A: SCL = 0,001 %	

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GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

2-bromo-2-(bromomethyl)pentanedinitrile (DBDCB), with molecular formula $C_6H_6Br_2N_2$, is an organic substance used as a preservative in a wide array of detergent products for household and car cleaning, wax and other polishing preparations for floors, adhesives, paints, and metal working fluids. In addition, it is used in veterinary (e. g. in dog shampoos) and cosmetic products at a maximum authorised concentration of 0.1 %.

DBDCB is preregistered under the REACH Regulation (EC) No 1907/2006 and is manufactured in and/or imported to the European Economic Area at ≥ 1 to < 10 tonnes per year. It is included in the inventory of substances likely to meet the criteria of Annex III to the REACH Regulation and has been assessed as an active substance for biocidal products according to the Regulation (EU) 528/2012. The present opinion and the CLH report prepared by the Dossier Submitter (DS) – Czech Republic – are thus mainly based on data reported in the Assessment Report developed in accordance with the Regulation (EU) 528/2012.

Although the hazard class "Aspiration hazard" was inadvertently open for comments during the consultation of the CLH report, no data or CLH proposal were included in the CLH report and no comments on this hazard class were received during the consultation, therefore this hazard class has not been assessed in this opinion.

Currently, DBDCB is not listed in Annex VI to the CLP Regulation (EC) No 1272/2008 (the CLP Regulation). Throughout the opinion, reliability categories are according to Klimisch *et al.* (1997) and ECHA "Guidance on the Application of the CLP Criteria Version 5.0 – July 2017" is referred to as "CLP Guidance".

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

DBDCB is a granular solid with a water solubility of 0.63 to 2.62 g/L, depending on pH and temperature (OECD TG 105), and a measured Log K_{ow} of 2.0 (EC method A.8).

A summary of the physicochemical properties as provided by DS is presented below.

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20 °C and 101,3 kPa	Granular solid	J. (1992., 3.3/01, 3.6/01, 3.10/01, 3.17/01)	Visual inspection according to Pesticide Assessment Guideline, Subdivision D, Series 63-2.
Melting/freezing point	50.3 °C	3.1/01	OECD TG 102 (DTA)
Boiling point	Up to the exothermic decomposition no boiling point could be observed	3.1/02	OECD TG 103 (DTA)
Relative density	1.918 at 20 °C	3.1/03	OECD TG 109 (pycnometer method)
Vapour pressure	3.81 × 10-3 Pa at 20 °C 7.77 × 10-3 Pa at 25 °C	3.2/01	OECD TG 104 (gas saturation method)

Property	Value	Reference	Comment (e.g. measured or estimated)
Surface tension	72.99 mN/m at 20 °C	3.13/01	OECD TG 115 (ring tensiometer) concentration 1g/L
Water solubility	Results at pH 5: 1.03 g/L at 10 °C 1.68 g/L at 20 °C 2.62 g/L at 30 °C Results at pH 7: 1.05 g/L at 10 °C 1.70 g/L at 20 °C 2.62 g/L at 30 °C Results at pH 9: 0.42 g/L at 10 °C 0.79 g/L at 20 °C 2.09 g/L at 30 °C No pH-influence between pH 5 and pH 7 was detected. Only at pH 9 a slightly lower water solubility was observed. Temperature dependence was detected. The water solubility increased between 10 °C and 30 °C.	3.5/01	OECD TG 105
Partition coefficient n-octanol/water	1) Log Kow prediction: Log Kow = 1.63 2) Result at pH 5, 7 and 9 and 25 °C: Log Kow = 2.0 3) Results of temperature dependence: Log Kow = 0.95 at 10 °C Log Kow = 0.96 at 20 °C Log Kow = 1.02 at 30 °C The partition coefficient is not influenced by the pH in the range of pH 5 and 9. Correspondence with the log Kow prediction is sufficient. No temperature dependence could be observed between 10 and 30 °C.	3.9/01	EC method A.8 (The log Kow was calculated with the software KOWWIN v1.66, US EPA. The temperature dependence was calculated based on its solubilities in 1-octanol and water).
Flash point	Not relevant since DBDCB is solid.	-	-
Flammability	DBDCB is not highly flammable.	3.11	EC method A.10
Explosive properties	DBDCB contains none of the functional groups which may indicate explosive properties. It can therefore be concluded that the active substance is not explosive.	3.15	EC method A.14
Self-ignition temperature	DBDCB does not undergo spontaneous combustion.	3.11	EC method A.16
Oxidising properties	DBDCB contains none of the functional groups which may indicate oxidising properties. It can therefore be concluded that the active substance has no oxidising properties.	3.16	EC method A.17

Property	Value	Reference	Comment (e.g. measured or estimated)
Granulometry	-	-	-
Stability in organic solvents and identity of relevant degradation products	DBDCB as manufactured does not include an organic solvent. Therefore no study regarding its stability in organic solvents was performed.	-	-
Dissociation constant	DBDCB has no dissociation constant.	3.6/01	Pesticide Assessment Guideline, Subdivision D, Series 63-10 (titration).
Viscosity	Not relevant since DBDCB is solid.	-	-

Based on physico-chemical data reported above, the DS concluded that DBDCB warrants no harmonised classification for the hazard classes 'Explosives', 'Flammable solids', 'Self-reactive substances and mixtures', 'Pyrophoric solids', 'Self-heating substances', 'Substances or mixtures which in contact with water emit flammable gases', 'Oxidising solids', 'Organic peroxides', and 'Corrosive to metals'.

Explosives

The active substance contains a feature (contiguous nitrogen atoms) associated with explosive properties and no information on the exothermic decomposition energy is available; DBDCB did not fulfil the criteria of the screening procedure and therefore the acceptance procedure should have been performed. A substance is considered for classification as explosive where a positive result is obtained in the UN test series 2-8 (see figure 2.1.2 of Annex I of the CLP regulation). The substance was tested only according with EU method A.14, hence despite the negative results, it cannot be conclusively argued that DBDCB is not explosive.

Flammable solids

DBDCB was tested according to EU A.10 method and found to be not highly flammable. It does not liberate flammable gases in hazardous amounts, does not deliver indications of pyrophoric properties and does not undergo spontaneous combustion. Negative (not highly flammable) results of an EC A.10 method are considered acceptable to replace the UN N.1 for classification purposes (see ECHA Guidance on Information Requirements and Chemical Safety Assessment Chapter R.7a: Endpoint specific guidance, 7.1.10.3).

Self-reactive substances

DBDCB contains a feature (contiguous nitrogen atoms) associated with explosive properties. In the absence of other data, there is not sufficient information to conclude on DBDCB self-reactive properties.

Pyrophoric solid

No studies are available. DBDCB has, however, been handled in air in other studies conducted and referred to in this dossier, where no incidences of self-ignition when exposed to air have been reported.

Self-heating substances

DBDCB has a low melting point, *i.e.* < 160 °C, and it should not be considered for classification in this class since the melting process is endothermic and the substance-air surface is drastically

reduced. In addition, it gave negative results in an EC A.16 method, which is considered supportive information.

Substances which in contact with water emit flammable gases

DBDCB does not fulfil any of the criteria for classification in this hazard class: the chemical structure does not contain metals or metalloids, experience in production or handling shows that the substance does not react with water and the substance is known to be soluble in water to form a stable solution.

Oxidising solids

DBDCB does not contain oxygen, fluorine or chlorine atoms, thus fulfilling criteria 2.14.4.1(a) for no classification. In addition, DBDCB tested negative in an EC A.17 test. However, as these results are not directly comparable with the CLP criteria, they are considered supportive information.

Organic peroxides

Hazard class not applicable, the substance is not an organic peroxide.

Corrosive to metals

DBDCB was not tested for corrosive to metals according to method UN C.1, therefore it is not possible to conclude on this hazard class.

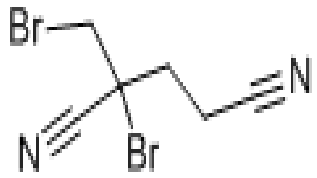
Comments received during consultation

No comments on physical hazards were received during public consultation.

Assessment and comparison with the classification criteria

Explosives

The substance has the following formula:



According to CLP Guidance (CLP Annex I, 2.1.4.3a) a substance or mixture is not classified as explosive when there are no chemical groups associated with explosive properties present in the molecule and further testing is not required. Since the DBDCD molecule does not contain any of the chemical groups listed on the Guidance, including contiguous nitrogen atoms, RAC is of the opinion that DBDCD does not require classification as explosive.

Flammable solid

RAC agrees with the DS that DBDCB does not warrant classification as a flammable solid based on data from EU A.10 method.

Self-reactive substances

According to CLP Guidance (CLP Annex I, 2.8.4.2a), the classification of a self-reactive substance or mixture in one of the seven categories 'types A to G' is dependent on its detonation, deflagration and thermal explosion properties, its response to heating under confinement, its explosive power and the concentration and the type of diluent added to desensitize the substance or mixture. The Substances and mixtures must be considered for classification in this hazard class unless there are no chemical groups present in the molecule associated with explosive or self-reactive properties; examples of such groups are given in Tables A6.1 and A6.2 in Appendix 6 of the UN RTDG, Manual of Tests and Criteria. Since such chemical groups are not present DBDCB it does not warrant classification for self-reacting substances.

Pyrophoric solid

Pyrophoricity, i.e. the ability to spontaneously ignite in air, is the result of a reaction of a substance or mixture with the oxygen in the air. The reaction is exothermic and has the particularity that it starts spontaneously, i.e. without the aid of a supplied spark, flame, heat or other energy source.

According to point 2.10.2.1. of the CLP Regulation, a pyrophoric solid shall be classified in a single category for this class using test N.2 in Part III, subsection 33.3.1.4 of the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria in accordance with Table 2.10.1: The solid ignites within 5 minutes of coming into contact with air.

However, according to the additional classification considerations in CLP Annex I, 2.10.4, the classification procedure for pyrophoric solids need not be applied when experience in manufacture or handling shows that the substance or mixture does not ignite spontaneously on coming into contact with air at normal temperatures (i.e. the substance or mixture is known to be stable at room temperature for prolonged periods of time (days). The DS has reported that although no test is available DBDCB has been handled in air in other studies conducted and referred to in this dossier, where no incidences of self-ignition when exposed to air have been reported. Based on this observation, RAC is of the opinion that DBDCB does not warrant classification as a pyrophoric solid.

Self-heating substances

RAC agrees that given the DBDCB melting point < 160 °C, and the negative results gathered through EC A.16 method, no classification is warranted as a self-heating substance.

Substances which in contact with water emit flammable gases

RAC agrees that since DBDCB does not contain metals/metalloids in its chemicals structure and is soluble and stable in water it does not warrant classification as a substance which in contact with water emits flammable gases.

Oxidising solid

In line with the DS conclusion, RAC notes that DBDCB does not contain oxygen, fluorine or chlorine atoms; thus, **no classification is warranted as an oxidizing solid**.

Organic Peroxides

RAC agrees that since the substance is not a peroxide, this hazard class is not applicable.

Corrosive to metals

A substance or a mixture that is corrosive to metals means a substance or a mixture which by chemical action will materially damage, or even destroy, metals.

According to the classification criteria only substances and mixtures for which the application of the UN Test C.1 (described in part III, Section 37.4.1.1 of the UN-MTC) is relevant need to be considered. However, it is known that solids are currently difficult to test according to the current CLP requirements, as the UN Test C.1 was designed for liquids. Solids having a melting point lower than 55 °C (which is the test temperature required in UN Test C.1) must then be taken into consideration. This condition is met by DBDCB as its melting point is 50.3 °C.

According to CLP Guidance the following substances and mixtures should be considered for classification in this class:

- substances and mixtures having acidic or basic functional groups;
- substances or mixtures containing halogen;
- substances able to form complexes with metals and mixtures containing such substances.

DBDCB does not have acidic or basic functional groups, however no information is provided on whether it may form complexes with metals and it contains halogen. Thus, in the absence of a test according to method UN C.1 as required based on the CLP regulation, RAC proposes no classification for corrosive to metals due to lack of data.

Desensitised explosives

According to the CLP Regulation, certain physical hazards (due to explosive properties) are altered by dilution, as is the case for desensitized explosives, by inclusion in a mixture or article, packaging or other factors. Since DBDCB is not an explosive, this hazard class is not applicable for this substance.

Conclusion on physical hazard classification

RAC agrees with the DS that, based on the available information on DBDCB physical properties, **the substance does not warrant classification for any of the assessed physical hazard classes**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

The DS provided results of two acute oral toxicity studies aimed at determining the oral median lethal dose (LD_{50}) in rats.

The first study, chosen by the DS as a key study, was carried out under GLP conditions according to an internationally recognised test guideline (TG) US EPA 81-1, which is similar to OECD TG 401. In this study, five male and five female Wistar rats per dose received DBDCB at 492, 553, 622 or 700 mg/kg bw, respectively, as a 50 % suspension in corn oil by single-dose oral gavage. Two, five, five, and six animals died out of the 10 treated in the 492, 553, 622 and 700 mg/kg bw groups, respectively. However the sex of the animals that died was not provided in the study report. Lethargy, ataxia, ptosis, dyspnoea, tremors, coma, flaccid muscle tone, prostration, diarrhoea and hyperactivity were noted in decedents prior to death. Survivors showed lethargy,

ataxia, chromodacryorrhoea, chromorhinorrhoea, diarrhoea, emaciation, hyperactivity, wetness of the anogenital area and brown staining of the nose/mouth area. The calculated LD_{50} for rats of both sexes was 640 mg/kg bw; however the DS noted that the „higher sensitivity of female animals was remarkable and does not permit to estimate one LD_{50} value, mortality data were probably distorted by aspiration of the DBDCB suspension". The DS scored the reliability of this study as 3 (based on Klimisch).

In the second acute toxicity study (no GLP, no guideline), 10 female and 10 male rats per group were given DBDCB by gavage, but details of the doses given were not provided. The oral LD_{50} reported in this study was 541 mg/kg bw.

Based on these study results, the DS concluded that, according to the criteria of the CLP Regulation, the oral LD_{50} values in rats of 640 mg/kg bw and 514 mg/kg bw trigger classification as Acute tox 4, H302, as they fall within the range of values (300 mg/kg bw – 2 000 mg/kg bw) for this category. No acute toxicity estimate (ATE) value was proposed.

Acute dermal toxicity

The DS provided results of one acute dermal toxicity study aimed at determining the dermal LD_{50} in rabbits.

In this limit test (reliability 1), performed under GLP and according to the internationally recognised TG US EPA 81-2, which is similar to OECD TG 402, 2 000 mg/kg bw DBDCB was applied dermally to 5 male and 5 female New Zealand White (NZW) rabbits. The test article was applied on the abraded abdomen and the site was occluded for 24 h. No deaths resulted from the treatment at the dermal limit dose.

Diarrhoea was the principal toxic sign noted during the observation period. Moderate erythema of the skin was present on day 1 and moderate to severe eschar on day 7. On day 14, moderate to severe eschar was noted in 3/10 animals and slight erythema in 4/10 animals. Oedema ranged from slight to severe on day 1 and slight to absent on days 7 and 14. Body weights were within expected limits. At necropsy, 8 animals appeared normal; two animals had crusted skin at the treated area.

No classification for acute dermal toxicity of DBDCB was proposed by the DS.

Acute inhalation toxicity

The DS presented the results of two acute inhalation toxicity studies aimed at determining the inhalation median lethal concentration (LC_{50}) in rats.

The first study, chosen by the DS as a key study (with reliability 1), was carried out in GLP conditions and according to OECD TG 403. In this study, four groups of rats (5/sex/group) were exposed nose-only to mean aerosol concentrations of 0.217*, 0.226, 0.272*, and 0.239 mg/L in air (the concentrations denoted with an asterisk were generated using the non-micronized test article, whilst in all other cases the micronized test article was used). The mass median aerodynamic diameter (MMAD) at the LC_{50} of the micronized and non-micronized DBDCB were 3.5 µm (with geometric standard deviation (GSD) of 2 µm) and MMAD 6.8 µm (GSD 2.4 µm), respectively. Mortality occurred at 0.217 mg/L and above. Based on the gross necropsy findings, mortality was causally related to acute alveolar oedema. The 4-h LC_{50} for rats of both sexes was 0.265 mg/L.

The following clinical signs were observed in the study: bradypnoea, tachypnoea, laboured breathing pattern, irregular breathing pattern, dyspnoea, breathing sounds, nasal discharge (serous), reddened nose, red encrustations (nose, muzzle, nostrils), stridor (muzzle), motility reduced, limp, tremor, high- legged gait, piloerection, ungroomed hair-coat, pallor, cyanosis,

prostration (lying on belly), corneal opacity, mydriasis, emaciation, decreased reflexes, hypothermia, and decreased body weights. The clinical signs resolved by the middle of the second post-exposure week.

The second acute toxicity study (no GLP, no guideline) produced inconsistent results. While 3 out of 5 males and 2 out of 5 females died during exposure to DBDCB at a concentration of 4.76 mg/L, no mortality was observed at concentrations of 8.31 and 13.09 mg/L. Therefore, this study does not enable evaluation of the acute inhalation toxicity of DBDCB.

Based on the calculated 4-h LC_{50} in rats of 0.265 mg/L air in the key study, the DS concluded that DBDCB warrants classification as Acute Tox 2, H330.

Comments received during consultation

Two MSCAs supported the classification conclusions for acute oral, dermal and inhalation toxicity of DBDCB as proposed by the DS.

Assessment and comparison with the classification criteria

Oral

Noting that in two acute oral toxicity studies in rats the medial lethal doses of DBDCB of 640 mg/kg bw and 541 mg/kg bw were found for animals of both sexes, thus within the range of 300 mg/kg bw to 2 000 mg/kg bw, RAC is of the opinion that the substance **warrants classification as Acute Tox. 4, H302 (Harmful if swallowed)**. However, taking into account that the reported data suggest that a) there might be considerable variation in sensitivity between males and females towards acute oral toxicity, b) a clear dose-response relationship was not presented in either study, and c) both studies were of limited reliability, RAC considers that the **default ATE of 500 mg/kg bw should be used** for DBDCB for classification of mixtures.

Dermal

Since the LD_{50} of DBDCB in the acceptable acute dermal toxicity study in rabbits was above 2 000 mg/kg bw, RAC concurs with the DS proposal that the substance **does not require classification for acute dermal toxicity**.

Inhalation

The 4-h LC_{50} of DBDCB aerosol for rats of both sexes was 0.265 mg/L in the acceptable acute inhalation toxicity study. Since this value is within the range 0.05 mg/L – 0.5 mg/L, RAC considers that the substance **warrants classification as Acute Tox. 2, H330 (Fatal if inhaled)**, as proposed by the DS, with an **ATE of 0.27 mg/L (dusts or mists).**

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

Summary of the Dossier Submitter's proposal

The information on the potential of DBDCB to cause irritation of the respiratory tract comes from a study on acute toxicity via the inhalation route. Necropsy observations such as dark red discoloration, foamy content, yellowish/red discharge from nose and in-life clinical signs such as

bradypnoea are indicative of marked irritation of the respiratory tract. Since DBDCB is a strong eye irritant, a strong irritant reaction towards inhaled DBDCB is also expected.

The DS concluded, however, that classification of DBDCB as STOT SE 1; H370 (Causes damage to respiratory system on inhalation) is not justified, because according to section 3.8.1.1. of the CLP Regulation, the specific target organ toxicity on single exposure is defined as a specific, non lethal target organ toxicity. Since mortality was induced after short-term, low exposure by inhalation, a classification as Acute Tox. 2, H330 (Fatal if inhaled) is warranted, covering this endpoint. The DS did not identify any effects justifying classification as STOT SE category 2 or 3 in the evaluated studies, therefore classification for specific target organ toxicity – single exposure (STOT SE) was not proposed.

Comments received during consultation

No comments were provided regarding STOT SE.

Assessment and comparison with the classification criteria

In the presented acute oral, dermal and inhalation toxicity studies no adverse, systemic effects meeting classification criteria were described in animals after single exposure to DBDCB at non-lethal doses or concentrations. Thus, RAC is of the opinion that this substance **does not require classification for STOT SE**. The systemic and local effects induced by DBDCB at lethal doses or concentrations are covered by the classifications in the respective categories for acute oral and inhalation toxicity.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS provided results from one acute dermal irritation/corrosion study in rabbits (with reliability 2). This study was carried out under GLP conditions and according to an internationally recognised method US EPA 81-5, which is similar to OECD TG 404.

The DBDCB (0.5 g, moistened with distilled water) was applied for 4 h to the intact skin on the back of six female NZW rabbits under semi-occlusive conditions. Skin reactions were scored at 30-60 min after removal of the test substance (washing) and again at 24, 48, and 72 h. Reversibility was verified by an examination after 7 days.

The relevant average scores at 24, 48 and 72 h after patch removal for erythema and oedema were 1.56 and 0.78, respectively. The skin changes were fully reversible within 7 d.

Based on these results the DS concluded that DBDCB does not warrant classification as a skin irritant.

Comments received during consultation

No comments were provided regarding evaluation of DBDCB for skin corrosion/irritation.

Assessment and comparison with the classification criteria

Noting that in the acceptable skin irritation study in rabbits, the mean 24-48-72 h scores for erythema and oedema in three rabbits were well below the classification criteria (≥ 2.3 - ≤ 4.0

for erythema/eschar or for oedema in at least 2 of 3 tested animals) and that the observed skin inflammation was fully reversible within 7 days, RAC concurs with the DS proposal that DBDCB **does not require classification for skin irritation**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS provided results of one acute eye irritation/corrosion study in rabbits(with reliability 1)which was carried out under GLP conditions and according to OECD TG 405.

DBDCB (0.1 g) was instilled into the conjunctival sac of six young adult NZW rabbits. The test substance was not removed. Treated eyes were examined at 1, 2, 3, 4, 7, 14, and 21 days after instillation.

DBDCB produced ocular irritation characterised by corneal and iridial effects and conjunctival irritation. Strong irritation reactions were observed in all animals, which did not resolve by the end of study on day 21. The results of the study are summarized in the following table:

Score (average of animals investigated)	**Corneal opacity**	**Iritis**	**Conjunctiva**	
			Redness	**Chemosis**
	0 to 4	0 to 2	0 to 3	0 to 4
24 h	4.00	2.00	2.00	4.00
48 h	4.00	2.00	2.00	4.00
72 h	4.00	2.00	2.00	4.00
Average 24 h, 48 h, 72 h	**4.00**	**2.00**	**2.00**	**4.00**
Reversibility in 21 days after instillation	not reversible	not reversible	not reversible	not reversible

The DS concluded that DBDCB should be classified with Eye Dam. 1; H318 (Causes serious eye damage).

Comments received during consultation

Two MSCAs supported classification of DBDCB as Eye Dam. 1; H318.

Assessment and comparison with the classification criteria

In the acceptable acute eye irritation/corrosion study in rabbits, DBDCB induced considerably more severe eye effects than required for classification in category 1 under the criteria in the CLP Regulation, which are:

- at least in one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or
- at least in 2 of 3 tested animals, a positive response of corneal opacity ≥ 3 and/or iritis > 1.5 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.

Therefore, RAC concludes that DBDCB requires classification as **Eye Dam. 1; H318 (Causes serious eye damage)** as proposed by the DS.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS has informed that no animal and/or human data are available for evaluation of the respiratory sensitisation of DBDCB. The DS noted that according to the CLP Regulation (table 3.4.1.) no recognized and validated animal models for testing of respiratory hypersensitivity are available. It was further noted that neither in the available acute nor in the repeated dose toxicity studies in animals were there any findings which would have indicated that DBDCB had respiratory sensitisation potential.

Based on this information, the DS concluded that the substance does not cause respiratory sensitisation in humans. This conclusion is further supported by the medical surveillance examinations performed on a regular basis in the DBDCB manufacturing site in the USA, where no cases of respiratory sensitisation were reported. (Medical Statement Lanxess Corporation US, 2015)

Comments received during consultation

No comments were provided regarding evaluation of DBDCB respiratory sensitisation.

Assessment and comparison with the classification criteria

Taking into account that there are no data suggesting a respiratory sensitisation of DBDCB in humans and animals, RAC concludes that the substance **does not warrant classification as a respiratory sensitizer due to lack of data**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS provided results from several clinical studies on skin sensitisation of DBDCB in humans.
In a Repeat Insult Patch Test (RIPT) (M., 1982, A6.1.5), 100 human subjects were repeatedly exposed to 0.2 mL of the test solution (0.5 % DBDCB in petrolatum or in ethanol) by means of an occlusive bandage. In the 3-week induction phase, patches were applied 3 times per week and the subjects were instructed to leave the patches on for 48-72 h following the application. The elicitation of potential skin sensitisation induction with a solution of the same DBDCB concentration took place approximately two weeks after the induction phase. A positive response was observed in 8 % of the subjects treated with DBDCB in petrolatum and in 14 % of persons treated with DBDCB in petrolatum in ethanol. When individuals showing a positive response were re-challenged in a provocative use test with a non-ionic cream containing 500 ppm DBDCB, 4 out of 12 individuals displayed adverse skin reactions. No further details on exposure or effects observed were provided.

In a second the Repeat Insult Patch Test (RIPT) (M., 1984, A6.1.5), induction and challenge of *ca.* 100 human subjects was done with solution of DBDCB in water at a concentration of 0.25 %.

Only one positive response was noted at the challenge. No further details on exposure or effects observed were provided.

In the third Repeat Insult Patch Test (RIPT) with exposure to natural sunlight (photoallergy test), a solution of DBDCB in water at 0.20 % was used for induction of sensitisation and for challenge of 24 persons (W., 1983; A6.1.5). A positive skin response was noted after challenge in 6 persons. No further details on exposure or effects observed were provided.

In the fourth Repeat Insult Patch Test (RIPT) with exposure to UVA/UVB (photoallergy test), a solution of DBDCB in water at a concentration of 0.20 % was used for induction of sensitisation and for challenge of 26 persons (B., 1988, A6.1.5). A positive skin response was noted after challenge in 11 out of 26 treated persons. Presence or absence of irradiation with UV light had no effect. No further details on exposure or effects observed were provided.

In the fifth Repeat Insult Patch Test (RIPT) with exposure to UVA/UVB (photoallergy test), a solution of DBDCB in water at concentration of 0.08 % was used for induction of sensitisation and for challenge of 50 persons (K., 1993, A6.1.5). A positive skin response was noted after challenge in 2 out of 50 treated persons. The presence or absence of irradiation with UV light had no effect. No further details on exposure or effects observed were provided.

In a study performed in the Netherlands, 0.5 % of subjects with 0.05 % DBDCB in petrolatum in patients suspected of contact dermatitis showed a positive reaction (De Groot *et al*. 1993, as cited in the NICNAS report). In a follow-up study, positive reactions to at least one of the three DBDCB concentrations (i.e. 0.05 %, 0.1 % and 0.3 % w/w) were observed in 4 % of 119 patients (De Groot *et al.* 1996a, as cited in the NICNAS report). In a study by Okkerse (1996, as cited in the NICNAS report), 2.4 % of the subjects suspected of contact dermatitis showed a positive reaction to 0.1 % DBDCB in petrol ether. In a study by Zachariae *et al* (2003, as cited in the NICNAS report), 2.9 % of 1 019 patients suspected of contact dermatitis showed an allergic reaction to 0.3 % DBDCB in petrol-ether. The following year the same authors reported 4.9 % positive cases in 776 patients suspected of contact dermatitis in reaction to patch test with the same DBDCB concentration.

All the above studies provided evidence that at relatively low concentration (0.5 % or lower) DBDCB may induce skin sensitisation in humans.

Animal studies

A Guinea Pig Maximization Test (GMPT) (G., 1982b, 6.1.5/01) was performed under GLP conditions and in compliance with OECD TG 406, but with the following deviations: the test substance was not characterised; a pre-test to identify the lowest irritating/highest non-irritating concentration was not performed; the topical induction with 5 % solution of DBDCB in 80/20 ethanol/water was not preceded by creating a local irritation; the vehicle control group was not challenged with the test material; 1-chloro-2,4-dinitro benzene (DNCB) was used as a positive control (moderate sensitiser required); duration of challenge exposure was 21 h, scoring was performed 24 and 48 h after the beginning of challenge exposure. In this test none of 20 guinea pigs induced with DBDCB showed a skin reaction 24 or 48 hours after challenge. The vehicle control group did not show any skin reaction. Nine out of 10 guinea pigs of the positive control group showed skin reactions 24 h after beginning (3 h after the end) of challenge with CDNB. No skin reactions were seen in positive controls at 27 h after the end of challenge exposure. The study is considered by the DS as reliable with restrictions (Klimisch 2).

In a published, non- guideline study (H.,1993, 6.1.5/02), with reliability considered by the DS to be Klimisch 2/3, the animals received three intradermal induction treatments with Freunds Complete Adjuvant (FCA, 6 injections of 0.1-0.15 mL). Induction was done with DBDCB at a concentration of 0.2 %. Challenge was performed with two concentrations of DBDCB: 0.1 and 0.3 %. A concentration-dependent response to the challenge exposure was observed. A first DBDCB elicitation concentration of 0.3 % (0.05 mL) caused moderate reactions (distinct erythema restricted to the application area) in 1, 2 and 1 animals at 24, 48 and 72 h, respectively. The remaining animals showed either a weak reaction (slight spotted erythema) in 6, 5 and 6 animals after 24, 48 and 72 h, respectively, or no reaction at all. A second DBDCB elicitation concentration of 0.1 % (0.05 mL) caused moderate reactions in 1, 1 and 2 animals at 24, 48 and 72 h, respectively. The remaining animals showed either a weak reaction (slight spotted erythema) in 1, 2 and 3 animals in 24, 48 and 72 h, respectively, or no reaction at all. Two out of 20 animals were sensitised 24 h after challenge, and 3 out of 20 at 48 h and 72 h after challenge. The test substance was not sufficiently described and the test system was not checked for reliability. No further details on exposure or effects observed were provided.

In a non-guideline study using the Ritz-Buehler-Method (G.,1982 c, 6.1.5) (Current Concepts in Cutaneous Toxicity, Academic Press, 1980), 10 guinea pigs were given 0.4 mL of 5 % test substance solution in ethanol/water 80:20 on intact skin for 6 h per day, three times weekly for 3 consecutive weeks (10 applications in total). The challenge application, using 0.4 mL of 5 % test substance solution in ethanol/water 80:20, took place at a skin site different from the original application site, two weeks following the termination of the induction phase. No positive skin response was noted in the challenge test in any of the guinea pigs. No further details on exposure or effects observed were provided.

In a second, non-guideline study using the Ritz-Buehler-Method (6.1.5), 20 guinea pigs were treated for induction and for challenge with the test substance at a concentration of 75 % w/v in acetone. The doses used were not specified in the report. The study gave negative results, however, it is unclear whether the induction concentrations were sufficiently high to elicit mild skin irritation. No further details on exposure or effects observed were provided.

The DS also noted that some studies are summarised in the Existing Chemical Hazard Assessment Report on DBDCB compiled by the Department of Health and Ageing of Australian Government in 2009 (www.nicnas.gov.au). In this report, summaries of 7 non adjuvant and 6 adjuvant tests are provided. It is concluded that no or only minimal evidence of skin sensitising potential is shown in non-adjuvant tests. Regarding the adjuvant test, it is concluded that in these tests no or only minimal skin sensitisation potential is shown except for two tests (cumulative contact enhancement test (CCET) and modified FCA procedure (H.,1993, 6.1.5/02), also provided by the applicant). In addition, summaries of 3 local lymph node assay (LLNA) test provided in the report showed positive reactions.

Based on the data presented above, the DS proposed to classify DBDCB as Skin Sens. 1; H317 (May cause an allergic skin reaction).

Comments received during consultation

One MSCA supported classification of DBDCB as a Skin Sensitiser. They noted that the result of the key study (6.1.5/01) is questionable because no dose-range finding test was performed. Thus, it is possible 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 had 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. The results of the positive patch tests, taking into account recommendations of CLP Guidance, suggest that a "Relatively high frequency of occurrence of skin sensitisation" is occurring (c.f. Table 3.2, CLP Guidance). With regard to Annex I: 3.4.2.2.4 of the CLP Regulation, a weight-of-evidence approach can be performed using the human data alone. Because a classification as Skin Sens. 1A cannot be excluded, classification as Skin Sens. 1 was considered appropriate.

A second MSCA supported proposed classification noting the use of DBDCB in cosmetic products.

In their response the DS noted 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 cosmetic products.

Assessment and comparison with the classification criteria

RAC notes that all provided animal studies on skin sensitisation with DBDCB were of very low quality with serious deviations from the recommended test methods, were poorly described and there was uncertainty concerning the identity of the substance, thus they do not provide reliable evidence of lack of skin sensitising properties of DBDCB. In fact, one animal study (H.,1993, 6.1.5/02) indicated weak skin sensitising potential of DBDCB. With such a database, the evaluation of this hazard needed to be based solely on results of studies in humans.

CLP Guidance further outlines how frequency of occurrence of skin sensitisation or level of human exposure shall be assessed and how they should be considered in decision on need for subcategorization (Section 3.4.2.2.3.1, Tables 3.2, 3.3 and 3.4, as reproduced below):

Table 3.2 CLP Guidance: *Frequency of occurrence of skin sensitisation*

Human diagnostic patch test data	**High frequency**	**Low/moderate frequency**
General population studies	≥ 0.2 %	< 0.2 %
Dermatitis patients (unselected, consecutive)	≥ 1.0 %	< 1.0 %
Selected dermatitis patients (aimed testing, usually special test series)	≥ 2.0 %	< 2.0 %
Workplace studies: 1: all or randomly selected workers 2: selected workers with known exposure or dermatitis	 ≥ 0.4 % ≥ 1.0 %	 < 0.4 % < 1.0 %
Number of published cases	≥ 100 cases	< 100 cases

Table 3.3 CLP Guidance*: Relatively high or low exposure*

Exposure data)	Relatively low exposure (weighting)	Relatively high exposure (weighting)
Concentration / dose	< 1.0 % < 500 µg/cm^2 (score 0)	≥ 1.0 % ≥ 500 µg/cm^2 (score 2)
Repeated exposure	< once/daily (score 1)	≥ once/daily (score 2)
Number of exposures (irrespective of concentration of sensitizer)	< 100 exposures (score 0)	≥ 100 exposures (score 2)

The scores in Table 3.3 represent weightings whose purpose is to enable an exposure index to be derived which best reflects our understanding of the relative importance of dose versus frequency of exposure. An additive exposure score of 1-4 equates to low exposure, whereas 5-6 reflects high exposure.

***Table 3.4 CLP Guidance**: Sub-categorisation decision table*

	Relatively low frequency of occurrence of skin sensitisation	Relatively high frequency of occurrence of skin sensitisation
Relatively high exposure (score 5-6)	Sub-category 1B	Category 1 or case by case evaluation
Relatively low exposure (score 1-4)	Category 1 or case by case evaluation	Sub-category 1A

CLP Article 7 (3) states that human induction studies such as HRIPT or HMT must not be performed, although historical data may be used as weight of evidence for the sub-categorisation (see below).

In the first Human Repeated Insult Patch Test (HRIPT) (M., 1982, A6.1.5), a frequency of occurrence of skin sensitisation among 100 human subjects amounted to 8-14 %, depending upon the vehicle used for dissolution of DBDCB (petrolatum or ethanol), thus it was extremely high, well above the frequency of 0.2 % foreseen in Table 3.2 for the general population. The level of exposure in this study was low (0.5 % DBDCB in petrolatum or in ethanol) thus below the 1 % foreseen in Table 3.3 for relatively low exposure and the frequency of *ca*. 21 days for three weeks was also below 100 exposures. Thus, noting a relatively high frequency of occurrence of skin sensitisation and relatively low exposure in this study, the effects observed in this study meet criteria for skin sensitisation subcategory 1A.

In the third and fourth Human Repeat Insult Patch Test (HRIPT) with exposure to natural sunlight or UVA/UVB (photoallergy test), a solution of DBDCB in water at a concentration of 0.20 % was used for induction of sensitisation and for challenge of 24 persons (W., 1983; A6.1.5) or 26 persons ((B., 1988, A6.1.5). The frequency of occurrence of skin sensitisation in the third HRIPT was 6/24 (25 %) and in the fourth HRIPT it was 11/26 (42.3 %), thus extremely high. The exposure level in these studies were low (0.2 % DBDCB in water), thus below the 1 % foreseen in Table 3.3 for relatively low exposure. The number of exposures in these two HRIPT was not given, but it is highly probable that they were below 100 exposures, thus taking into account relatively low exposure in these studies the effects observed meet criteria for skin sensitisation in subcategory 1A.

In **the fifth Human Repeat Insult Patch Test (HRIPT)** the frequency of occurrence of skin sensitisation in 2/50 persons (4 %) after repeated dermal exposure to a solution of DBDCB in water at a concentration of 0.08 % also provided evidence of high skin sensitising potency of DBDCB in humans meeting criteria for subcategory 1A for the incidence of sensitisation and level of exposure, but the number of exposures was not provided.

In several patch test studies of selected dermatitis patients the frequency of skin sensitisation to DBDCB was above 2 %, thus it was high according to Table 3.2:

- 4 % of 119 patients (De Groot *et al.* 1996a, as cited in the NICNAS report);
- 2.4 % of the subjects suspected of contact dermatitis showed a positive reaction to 0.1 % DBDCB in petrol ether (Okkerse, 1996, as cited in the NICNAS report);
- 2.9 % of 1019 patients suspected of contact dermatitis showed an allergic reaction to 0.3 % DBDCB in petrol-ether (Zachariae *et al*, 2003, as cited in the NICNAS report).
- 4.9 % positive cases in 776 patients suspected of contact dermatitis in reaction to patch test with the same DBDCB concentration (Zachariae *et al*, 2003, as cited in the NICNAS report).

The main criteria for classification for a HRIPT (based on CLP Guidance) include the frequency of occurrence of skin sensitisation, the level of exposure and the number of exposures. In one HRIPT, DBDCB meets all the criteria for classification as Skin Sens. 1A, and in three other HRIPTs DBDCB meets the criteria for subcategory 1A regarding high frequency of occurrence of skin sensitisation in exposed persons and of low level of exposure, although numbers of exposures, most probably below 100, are not known. In addition, a high frequency of skin sensitisation to DBDCB was seen in patch testing of selected patients. Therefore RAC is of the opinion that DBDCB **warrants classification as Skin Sens. 1A, H317 (May cause an allergic skin reaction)**. A **specific concentration limit of ≥ 0,001 %** should apply for classification of mixtures, since it was demonstrated that DBDCB at a concentration of 0.08 % is able to sensitise the skin of humans.

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

Summary of the Dossier Submitter's proposal

The DS provided several repeated dose toxicity studies by the oral and dermal routes in rats and dogs.

Oral route

In a **repeated dose and *in utero* exposure study in rats,** the parental animals (10/sex/dose) were fed diet containing DBDCB at concentrations of 83.5, 500, 3 000 ppm, leading to dose-levels of 6.3, 37, and 275 mg/kg/day for males and 7.5, 43 and 360 mg/kg/day for females, respectively. The dams during mating, pregnancy (21 d) and during lactation (21 d) and the offspring (20/sex/dose) were provided the same concentrations of DBDCB in the diet as the parental generation during 13 weeks post weaning. The study was conducted in accordance with GLP and according to US-EPA 82-1 /OECD TG 408, with reliability = 1 (6.4.1/01, key study). Thus, the weanlings were first potentially exposed to DBDCB or its metabolites *in utero* (21 d) and via nursing (21 d). At the highest dose, the offspring showed lower birth weights and impaired body weight development throughout the duration of the study. Histopathological examination revealed a slight increase in extramedullary haematopoiesis in spleen sections of high-dose animals. Changes in absolute and/or relative weights of some organs did not show a conclusive dose-related pattern. The toxicological significance of these findings is thus doubtful. It is concluded that the subchronic NOAEL for this study is 37/43 mg/kg bw per day (males/females).

In a **repeated dose toxicity study, Beagle dogs** (4/sex/dose) were fed for 13 weeks a diet containing DBDCB at concentrations of 167, 1 000, 4 000 ppm, equivalent to daily doses of 4.7, 28.9 and 101.5 mg/kg/day for males and 5.3, 37.7, 109.8 mg/kg/day for females, respectively. The study was conducted under GLP conditions and according to OECD TG 409, with reliability = 1 (1980b, 6.4.1/02, key study). DBDCB caused clinical signs of toxicity (diarrhoea and/or soft stool, feed emesis and ataxia) at the highest dose level of 102 / 110 mg/kg bw per day (males/females) (1980b, 6.4.1/02). Feed consumption and body weight development were also depressed in this dose group. Increased thyroid weights (glandular hyperplasia) and follicular cell height were noted at the top dose. It was concluded that the subchronic LOAEL was 102/110 mg/ kg bw/day (males/females) and NOAEL for this dog study was 30 / 38 mg/kg bw per day (males/females).

The thyroid effects seen in the study by W.(1980b, 6.4.1/02) were re-investigated in **a special 13-week feeding study in which Beagle dogs** (4/sex/dose) were fed diet containing DBDCB at concentration of 167 ppm, equivalent to 5.9 / 5.7 mg/ kg/day (males/females) (W., 1982, 6.10). An increase in thyroid weight was seen in females, although this might be an incidental finding because one of the control females had an unusually small thyroid. The small group size in dog studies (n = 4) leads to an overly high influence on such outliers on group means. No effects were noted on basal or TSH-stimulated levels of serum T3/T4 concentrations. The histomorphological appearance of thyroids was not affected at the top dose of 5.9 / 5.7 mg/ kg/day (males/females).

In **a repeated dose study Beagle dogs** (4/sex/dose) were fed for 13 weeks a diet containing DBDCB at concentrations of 10, 100 and 4 000 ppm, leading to a daily dose of 0.29, 3.1 and 102 mg/kg/day for males and 0.33, 3.1 and 119 mg/kg/day for females, respectively, followed by a 3 month recovery period. The study was conducted according to OECD TG 409, reliability = 1 (R. *et al*. 1994, A 6.4.1). Only in the highest dose group were adverse effects observed. The clinical signs included food-like emesis, thin or weak appearance, diarrhoea, prostration, trembling and reduced food consumption and body weight. Haematological changes included increased white blood cell counts and decreases in red blood cell count, haemoglobin concentration and haematocrit and increases in mean corpuscular volume and decrease in mean corpuscular haemoglobin concentration. Reticulocyte counts, platelet counts and segmented neutrophil counts were increased. In both male and female dogs, effects on bone marrow were observed, including hypercellularity in the erythropoietic cells and decrease in total myelogenous cells. Biochemical changes in the serum included decreases in calcium, phosphorus, alkaline phosphatase, albumin, glucose, alanine aminotransferase and total protein (females only) and a slight increase of globulin in males. Urology revealed decreased urinary pH. All the differences seen in biochemical, haematological and urological parameters were reversed within the 3 month recovery period. The thyroid enlargement is attributed to the decreases in the thyroid hormones, T3 and T4. On necropsy, effects on the central nervous system (CNS), consisting of trace to moderate axonal degeneration within all sections of the spinal cord and brain were observed. Further effects included a degeneration of the seminiferous tubules, hypospermia (2/4 males) and prostatic atrophy. All of the test article related effects partially or totally receded within the 3 month recovery period.

No effects were reported at lower dose levels so the NOAEL of the study of by R. (1994, A6.4.1) was the second highest dose of 3.1 mg/kg bw/day.

Dermal route

In a **repeated dose toxicity study, the rats** (5/sex/dose) were given on skin DBDCB once a day for 6 h/day at doses of 1 000, 2 000, 4 000 mg/kg/day, 7 days per week for 3 weeks. The method of dermal exposure (open or closed) was not provided (6.3.2; Key Study). The study was done under GLP conditions and according to US-EPA 82-2/OECD 410, reliability-1.

Only severe local irritation was noted at ≥ 1 000 mg/kg bw/day. No systemic effects were observed up to and including the highest dose level tested (4 000 mg/kg bw/day). Therefore, the systemic NOAEL after dermal application is greater than 4 000 mg/kg bw/day.

The DS noted that effects on the thyroid were observed at 102 mg/kg bw/day in dogs, slightly above 100 mg/kg bw/day, the guidance value boundary for STOT RE 2.

According to the DS, these effects are likely to be due to the effect of bromide on the thyroid. To determine whether this effect on dogs is relevant for a classification, the elimination rate of bromide and T_3/T_4 hormones in rats and dogs obtained from publicly available sources were compared. The T_3/T_4 half-lives were 6 hours/ 12-24 hours in rats and 6 hours/ 10-16 hours in

dogs. The half-life of bromide in dogs ranges between 15-46 days whereas that in rats ranged from 3 to 8 days. Thus, in terms of T_3/T_4 half-lives, rats and dogs were comparable, whereas in terms of bromide accumulation dogs were more prone to adverse effects of bromide. The latter argument corresponds to allometric scaling (table R.8-3 in Guidance on information requirements and chemical safety assessment, ECHA) where the factor for an extrapolation from rats to dogs is about 3 (4 ÷ 1.4 = 2.9). using allometric scaling to adjust the guidance value, the classification limit for STOT RE 2 based on an oral dog study is equivalent to 100 mg/kg bw/day/2.9 = 34.5 mg/kg bw/day which is virtually equivalent to the NOAEL in the dog study. No effects on the thyroid were observed in repeated dose study in rats at the top dose of 240 mg/kg bw/day which is greater than the guidance value of 100 mg/kg bw/day for subchronic rat studies.

Based on this analysis DS concluded that classification of DBDCB as STOT RE 2 is not justified.

Comments received during consultation

One MSCA noted that the study summaries (for example regarding reproductive toxicity and STOT RE) included in the CLH-report contain very limited detail, 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, this MSCA in their view, could only request a reflection on some issues rather than agree or disagree with the proposed classifications.

This MSCA also noted that the section on toxicokinetics (page 16 of the CLH report, section 4.1.3) states that "DBDCB is completely debrominated prior to systemic distribution …". They also pointed to the previously agreed RAC opinion on the classification of ammonium bromide[1] for the endpoint STOT RE as STOT RE 1 (H372) with nervous system as the primary target organ, where 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 for the classification of ammonium bromide.

In their response the DS argued that no classification for STOT RE should apply.

The DS expressed doubts regarding the relevance of the guidance value of 100 mg/kg bw (oral, rats) for dogs, pointing to the higher rates of physiological processes in smaller animals when normalised to body weight. In this case, a faster elimination of the bromide ion (Br^-) in rats as opposed to dogs is predicted. This difference is taken into account in the allometric scaling factors which are used in the assessment of exposure to chemicals between species. By the same arguments, the dog is predicted to be nearer to humans than the rat in terms of Br- elimination. The DS argued that since the difference between rats and dogs is predictable based on this general knowledge, the greater "distance" between rats and the humans is reflected in the CLP guidance value for rats of 100 mg/kg bw. This implies that a lower guidance value is needed for the dog and therefore using the same guidance value for the dog is not justified.

The DS agreed that the more sensitive species could be used, however such sensitivity should be specific to the species and due to factors other 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 the DS did not agree with the proposal to classify.

[1] https://echa.europa.eu/documents/10162/61e8d5d7-2ebd-fd02-a9c5-89671c2aef3b

A second MSCA noted that data leading to classification as STOT RE are well described and indicate that there are large interspecies differences regarding thyroid effects. The dog is apparently more sensitive than the rat, which is indicated in Table 3.9.2 and Table 3.9.3 of the CLP Regulation as the recommended species for setting guidance values to assist in justifying classification for STOT RE. Nevertheless, the results in dogs could be used for classification, based on the principle of using the most sensitive species and that "Evaluation shall be based on all existing data" (Annex I: 3.9.2.4, CLP Regulation).

One of the major differences between dogs and rats is the half-life of elimination of bromide (as shown in the CLH report). 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 from 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). The MSCA concluded that based on these data, non-classification of thyroid effects as STOT RE can be justified, but also noted that an in-depth analysis is required.

The points made by the DS in response to this comment were similar to those stated in response to the comment from the first MS, above.

Assessment and comparison with the classification criteria

There is no information on the repeated dose toxicity of DBDCB in humans. However, there is one repeated dose toxicity diet study in rats, one 21-day repeated dose dermal toxicity study in rats, one carcinogenicity study in rats and mice treated with DBDCB via the dermal route and three 90-day diet studies.

According to the summary of the repeated dose toxicity study in rats in the CLH report (6.4.1/01, key study) the toxic effects, such as reduced weight at birth and decreased body weight development were seen only in animals exposed in the diet to DBDCB at the top doses of 239.8 mg/kg/day (males) and 317.3 mg/kg/day (females) during pregnancy *in utero* (21 d), during lactation (21 d) and during 13 weeks post weaning. Histopathological examination of these animals revealed only a slight increase in extramedullary haematopoiesis in the spleen. Changes in absolute and/or relative weights of some organs did not show a conclusive dose-related pattern. No toxic adverse effects were seen, according to the DS, in rats exposed via the diet to DBDCB at the lower doses of 5.7 and 33.8 mg/kg/day (males) and 7.0 and 39.3mg/kg/day (females).

No systemic toxic effects were observed in rats in the repeated dose dermal toxicity study (6.3.2; key study), in which animals were given DBDCB on the skin once a day for 6 h/day at doses of 1 000, 2 000, 4 000 mg/kg/day, 7 days per week for 3 weeks and in rats treated via the dermal route with DBDCB in a 2-year carcinogenicity study at doses of 2, 6, or 18 mg/kg bw/day, and in mice receiving 0.6, 2, or 6 mg DBDCB/kg bw/day (NTP technical report 555, June 2010).

The data gathered in these repeated dose toxicity studies in rats and mice did not provide evidence based on which it could be presumed that DBDCB have the potential to be harmful to human health following repeated exposure.

However, adverse effects of repeated exposure to DBDCB were found in dogs.

In a 13-week oral repeated dose toxicity study (1980b, 6.4.1/02, key study) DBDCB induced in male dogs at 101.5 mg/kg/day and in female dogs at 109.8 mg/kg/day clinical signs of toxicity (diarrhoea and/or soft stool, feed emesis and ataxia), reduced feed consumption and body weight development, increased thyroid weights (glandular hyperplasia) and follicular cell height. No

adverse effect was reported in this study in dogs exposed to DBDCB at doses of 4.7 and 28.9 mg/kg/day (males) and 5.3 and 37.7 mg/kg/day (females).

In the second 13-week repeated dose toxicity study (W., 1982, 6.10), DBDCB did not induce significant alterations in basal or TSH-stimulated levels of serum T3/T4 concentrations or pathomorphological changes in the thyroid. However the applied daily dose of 5.9 / 5.7 mg/ kg/day (males/females) was almost 20 times lower that the DBDCB dose causing effects in the thyroid in the first study (1980b, 6.4.1/02).

In the third 13-week repeated dose toxicity study (R. *et al*. 1994, A 6.4.1), DBDCB at a dose of 102 mg/kg/day for males and 119 mg/kg/day for females induced general signs of toxicity such as emesis, thin or weak appearance, diarrhoea, prostration, trembling, reduced food consumption and body weight. There were also some changes in haematological, biochemical and urological parameters in these animals, but their adversity could not be assessed as only qualitative data were reported. In the provided dossier it was reported that observed thyroid enlargement could be attributed to the decreases in T3 and T4. It is also stated that on necropsy, effects on the CNS consisting of trace to moderate axonal degeneration within all sections of the spinal cord and brain were observed. Further effects included a degeneration of the seminiferous tubules, hypospermia (2/4 males) and prostatic atrophy. All of the test article related effects partially or totally reversed within the 3-month recovery period. According to the CLH report, the authors of the study ascribed most of these findings to the thyroid gland hypofunction. At lower doses used in this study (0.29 and 3.1 mg/kg/day for males and 0.33 and 3.1 and 119 mg/kg/day for females) no toxic effects were observed.
Limited detail on thyroid weight, levels of T3 and T4 or histopathology of the thyroid were provided to assess the effects produced in the thyroid of dogs.
The changes in the thyroid and CNS which were observed following exposure to DBDCB in dogs were considered adverse, and it was not possible to exclude that these changes are not relevant for humans. Therefore, the evidence from studies in dogs showed that this substance has the potential to be harmful to human health following repeated exposure.

It is noted that these adverse changes were only seen at doses very close to the upper limit of the guidance values (100 mg/kg bw/d) provided in the CLP Regulation to assist in Category 2 classification. It is stated in Annex I, 3.9.2.9.8. of the CLP Regulation that "*the guidance values and ranges […] are intended only for guidance purposes, i.e. to be used as part of the weight of evidence approach, and to assist with decisions about classification. They are not intended as strict demarcation values*." It is also noted that in the past RAC has not used different guidance values for different species based on allometric scaling.

Therefore, taking into account the adverse effects described above induced by DBDCB in the thyroid and CNS in repeated toxicity studies in dogs at doses close to the guidance values, RAC is of the opinion that DBDCB **warrants classification as STOT RE 2; H373 (May cause damage to organs (thyroid, central nervous system) through prolonged or repeated exposure).**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS provided for evaluation of germ cell mutagenicity several *in vitro* and *in vivo* genotoxicity studies which are summarised below:

Test system, Method, Guideline GLP Reliability	**Organism/ strain(s)**	**Concentrations tested**	**Result**		**Remark**	**Reference**
			– S9	**+ S9**		
Salmonella/ Microsome test No guideline, but ≅ OECD 471 Non-GLP Reliability-2	*S. typhimurium*: TA 98, TA 1537, TA 1538, TA 100, TA 1535 *E. coli:* WP2uvrA	0.001-1 000 µg/ plate (prelim. test) 0.2-100 µg/ plate (main test)	neg.	neg.	Cytotoxicity: –S9: ≥ 50 µg/plate +S9: 1 000 µg/plate	O, 1985, 6.6.1 **KEY STUDY**
Salmonella/ Microsome test No guideline, but ≅ OECD 471 Non-GLP	*S. typhimurium* TA 98, TA 1538, TA 100, TA 1535	1st test: 0.25-25 µg/plate 2nd test: 0.66-2 500 µg/plate	neg.	Neg.	Cytotoxicity: –S9: ≥ 100 µg/plate +S9: ≥ 500 µg/plate	T., Z., 1978, 6.6.1
Salmonella/ Microsome test No guideline, but ≅ OECD 471	*S. typhimurium* TA 98, TA 1537, TA 1538, TA 100, TA 1535	1st test: 100-10 000 µg/plate 2nd test: 1-100 µg/ plate 3rd test: 5-25 µg/ plate	neg.	Neg.	Cytotoxicity: –S9: ≥ 25 µg/plate +S9: ≥ 100 µg/plate	R, 1983a, 6.6.1
Chromosome aberration No guideline, but ≅ OECD 473 GLP – yes Reliability- 2 (The number of evaluated metaphases is only 50 per concentration. This can impair the system's ability to detect a weak clastogen)	CHO cells	Cytotoxicity test 0.58-2000 µg/mL Cytogenicity test: –S9: 2.6-19.6 µg/ mL +S9: 60-600 µg/ mL	pos.	pos.	Cytotoxicity: –S9: at ≥ 11.03 µg/mL +S9: at ≥ 189.84 µg/mL	T., 1982, 6.6.2/01 **KEY STUDY**
UDS-test US EPA 84-4 ≅ OECD 482 GLP – yes Reliability- 2 (The test substance is not characterised in terms of purity and appearance. This has no influence on the outcome of this study.)	IMR-90 human fibroblasts	–S9: 0.1-10 µg/mL +S9: 1-100 µg/mL	neg.	neg	Cytotoxicity: –S9: at ≥ 0.1µg/mL +S9: at 100 µg/mL	R., 1983b 6.6.2/02 **KEY STUDY**
HGPRT mutation assay No guideline, but ≅ OECD 476 GLP- yes, Reliability-2 (The test substance is not characterised in terms of purity and appearance. This has no influence on the outcome of this study)	V79 cells	Cytotoxicity assay: –S9: 0.03-10 µg/mL +S9: 10-900 µg/mL Mutagenicity test: –S9: 0.3-1.0 µg/mL +S9: 10-50 µg/mL	neg.	neg.	Cytotoxicity: –S9: at ≥ 0.1 µg/mL +S9: at ≥ 70 µg/mL	R., 1985, 6.6.3 **KEY STUDY**
$TK^{+/-}$ mutation assay No guideline, but ≅ OECD 476	L5178Y cells	-S9: 0.027-2.0 µg/ mL +S9: 0.67-50 µg/ mL	neg.	neg.	Cytotoxicity: –S9: at ≥ 0.2 µg/mL +S9: at ≥ 20 µg/mL	K., 1982, A6.6.3

Test system, Method, Guideline GLP Reliability	Organism/ strain(s)	Concentrations tested	Result		Remark	Reference
			– S9	+ S9		
Mammalian cell transformation No guideline, but ≅ EC Method B.21	BALB/c 3T3	Cytotoxicity assay: +S9: 16-125 µg/mL Mutagenicity test: +S9: 18-83 µg/mL	/	neg.	Cytotoxicity: +S9: at ≥ 50 µg/mL	M., 1984, A6.6.2
Mammalian cell transformation No guideline, but ≅ EC Method B.21	BALB/c 3T3	Cytotoxicity assay: 19-320 µg/mL Mutagenicity assay: –S9: 0.6-1.6 µg/mL +S9: 17-25 µg/mL	neg.	neg.	Cytotoxicity: –S9: at ≥ 1.3 µg/mL +S9: at 25 µg/mL	P., 1990, A6.6.2
Micro–nucleus assay US EPA 84-2 ≅ OECD 474 GLP- yes, Reliability = 1	Mouse ICR ♂ + ♀ 5/sex/ group	Single dose, i.p.	24, 48, 72 h	7.5, 15, 30 mg/kg bw/day	Lethargy in all animals treated with 15 or 30 mg/kg. Reduced proportion of PCEs. No increase in micronucleus frequency.	P.,1995, 6.6.4 **KEY STUDY**
Chromosome aberration assay US EPA 84-2 ≅ OECD 475	Rat, SD ♂ + ♀ 5/sex/ group	Single dose, oral gavage	8, 12 h	100 mg/kg bw/day	No increase in chromosomal aberration frequency.	P.& Y., 1991, A6.6.4
Chromosome aberration assay US EPA 84-2 ≅ OECD 475	Rat, SD ♂ 5/ group	5 doses on 5 consecutive days, oral gavage	24 h	5, 17, 50 mg/kg bw/ day	1/5 mortality at 50 mg/kg/day No increase in chromosomal aberration frequency.	P., 1982, A6.6.4
Dominant-lethal test No guideline but ≅ OECD 478, GLP- yes Reliabilty = 2-3 (The failure to induce dominant lethal mutations with the positive control is a deficiency. However, this might be a result of the rather low dose used (0.05 mg/kg bw/day, oral). Normally, single i.p. doses of around 0.3 mg /kg bw are used. These doses reliably increase the number of resorptions, dead implants etc. in rodents)	Mouse, Ham/ICR Swiss ♂+♀ 10 ♂/ 40 ♀	8 wks, diet (♂ only)	2 matings/ wk for 2 wks	0, 83.5, 500, 3 000 ppm ≅ 13, 75, 450 mg/kg/ day	Pregnancy rates, incidences of resorption, foetal death, dead implantations, and foetal viability were not affected by treatment with DBDCB.	W., 1980, 6.6.6 **KEY STUDY**

DBDCB was non-mutagenic in all bacterial and mammalian gene mutation tests, with and without metabolic activation. However, an *in-vitro* chromosomal aberration assay showed an increased frequency of aberrant metaphases – with and without metabolic activation – at concentrations that did not fulfil the cytotoxicity criteria of OECD Guideline 473. Thus, confirmatory *in vitro* cell transformation and UDS assays were performed. These assays were negative, with and without metabolic activation.

Three i*n vivo* assays for micronucleus formation or cytogenicity in bone marrow cells were performed in mice and rats. The doses were sufficient to exert systemic toxicity. No increases in the frequency of micronucleated polychromatic erythrocytes (PCEs) or chromosomal aberrations in bone marrow metaphases were seen. Since the findings in the various ADME studies demonstrated high levels of DBDCB-derived radioactivity in the blood it is likely that the bone marrow was reached by the substance or its metabolite.

Based on negative results in the available *in vitro* and *in vivo* genotoxicity studies the DS concluded that the criteria for classification to germ cell mutagenicity are not fulfilled and DBDCB should not be classified to this hazard class.

Comments received during consultation

One MSCA supported the proposal to not classify DBDCB for mutagenicity.

Assessment and comparison with the classification criteria

The germ cell mutagenicity potential of DBDCB has been assessed in relevant *in vitro* and *in vivo* tests.

DBDCB was negative in all tested assays *in vitro* and *in vivo*, except in one *in-vitro* chromosomal aberration assay showing an increased frequency of aberrant metaphases (with and without metabolic activation), however this was not confirmed in other *in vitro* and *in vivo* assays. RAC therefore agrees with the DS that **no classification for germ cell mutagenicity is warranted**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Despite long and widespread use of DBDCB in exposure-intensive applications, e.g. in cosmetics and toiletry articles, there are no reports on adverse effects, apart from contact dermatitis, in humans in the published literature.

The DS provided results of carcinogenicity studies in rats and mice treated with DBDCB via the dermal route (NTP technical report 555, June 2010).

In the rat study, solutions containing DBDCB in 95 % ethanol were applied to the backs of the animals five times per week for 2 years. Groups of 50 male and female rats received 2, 6, or 18 mg of DBDCB/kg bw/day, and similar groups of male and female mice received 0.6, 2, or 6 mg DBDCB/kg bw/day. Groups of 50 animals receiving just the ethanol solution served as controls. Tissues from more than 40 sites were examined for each animal.

Survival of animals exposed for 2 years to DBDCB was the same as for the controls in both rats and mice, but rats exposed to the highest concentrations weighed on average 7 % less than the controls. In the rat study, local effects observed at the site of application primarily included hyperkeratosis of the epidermis at the two highest doses in both males and females, and incidences of minimal to mild inflammation in the dermis of males (the two highest doses) and females (all 3 doses). At the highest dose, epidermal necrosis at the site of application was significantly increased in females. In the study with mice, local effects at the site of application were minimal to mild hyperplasia of the epidermis at the two highest doses in males and in all dosed groups of females. Minimal to mild chronic active inflammation in the dermis was significantly increased in all dosed groups of females.

There was no association between dermal exposure to DBDCB and any increase in the incidence of systemic non-neoplastic or neoplastic lesions in male or female rats (daily doses up to 18 mg/kg bw) or mice (daily doses up to 6 mg/kg bw), at the highest dose levels tested.

Based on the above data the DS concluded that DBDCB does not require classification for carcinogenicity

Comments received during consultation

One MSCA FR supported proposal to not classify DBDCB for carcinogenicity.

Assessment and comparison with the classification criteria

The DS presented the results of two reliable chronic toxicity/carcinogenicity studies via the dermal route of exposure in rats and mice (NTP technical report, June 2010). None of the studies showed any indication of increased tumour incidence.
Based on two negative and valid carcinogenicity studies, supported by a lack of genotoxicity, RAC agrees with the DS that **no classification of DBDCB for carcinogenicity is warranted due to inconclusive data**. RAC notes that no oral or inhalation carcinogenicity studies were provided.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

The DS evaluated the effects of DBDCB on sexual function and fertility based on a 90-day feeding study in rats combined with a one-generation reproduction study (W.,1980a, 6.4.1/01), the dominant-lethal mutation study in mice (W., 1980, 6.6.6) and repeated dose toxicity studies in dogs (R.,1994, A6.4.1; W., 1980b)

In the 90-day feeding study in rats combined with a one-generation reproduction study, no effects on litter size and sex ratio were found. A selected part of the F1 offspring was then subjected to subchronic dietary exposure as in a conventional subchronic study according to OECD TG 408. In the F1 generation, there were no macroscopic or microscopic findings in the reproductive organs (testes/epididymis, prostate gland, uterus, ovaries). The LOAEL in this study was based on reduced body weights and splenic haematopoiesis was 240/317 mg/kg bw/day (males/females). The corresponding NOAEL was 34/39 mg/kg bw/day (males/females).

In the dominant-lethal study in mice (W., 1980, 6.6.6; see Section 4.8.1.2, p.47) male mice were exposed to the substance throughout the premating period of 8 weeks (one spermatogenic cycle). No effects were noted at any dose of DBDCB on pregnancy rates, incidences of resorptions, foetal deaths, dead implantations or foetal viability. The reproductive NOAEL for male mice from this study is equivalent to 450 mg/kg bw/day (i.e. the top dose).

In a repeated dose study, Beagle dogs (4/sex/dose) were fed a diet containing DBDCB at concentrations of 10, 100 and 4 000 ppm for 13 weeks, leading to a daily dose of 0.29, 3.1 and 102 mg/kg/day for males and 0.33, 3.1 and 119 mg/kg/day for females, respectively, followed by a 3 month recovery period. The study was conducted according to OECD TG 409, reliability = 1 (R. *et al.* 1994, A 6.4.1). The effects on testes (immature appearance, mild degeneration of

the seminiferous tubules) were observed in the highest dose group (4 000 ppm). According to the DS, this effect was likely to be secondary to the overt toxicity observed in this dose group (weight loss and mortality) and the effects on the thyroid gland brought about by bromide released from DBDCB. Other male reproductive organs were unaffected by treatment. Female dogs showed no effect of the test substance on their reproductive organs at any dose.

Developmental toxicity

The DS evaluated the effects of DBDCB on developmental toxicity based on a developmental toxicity range finding study in rats (H., 1982, A6.8.1), a developmental toxicity study in rats conducted under GLP and according to US-EPA 83-3(a), which is similar to OECD TG 414, with reliability = 1 (H, 1982, 6.8.1/02, Key study) and a developmental toxicity study in rabbits, also conducted under GLP and according to US-EPA 83-3(a), with reliability = 1.

In the rat study (H., 1982, 6.8.1/02), data for prenatal measures of toxicity were not significantly different between the control and treated groups, except for embryo lethality. This finding was difficult to interpret because in the two high dose groups, resorptions were clustered in two litters with > 7 resorptions in each. In addition, the number of resorptions observed in the control group was at the low end of the normal range for rats. In the dose-range finding study preceding this main study, two control dams with 7 and 8 resorptions were noted, respectively. This indicates that the incidences of clustered resorptions within a litter are unlikely to be a compound-related effect.

The increase in the incidence of the skeletal variation, rudimentary ribs, was statistically significant; however, at the two high dose levels there was a decreased incidence compared to the control group. There was a numerical increase in runts at 175 mg/kg, but the parameter was not found to be statistically significant.

The data indicates that there may be a slight increase in prenatal toxicity at 175 mg/kg due to significant embryo lethality. However, in the absence of other conventional signs of embryotoxicity, i.e., malformations and foetal weight reduction, this finding should not be considered biologically significant.

In the rabbit teratogenicity study (D., 1994, 6.8.1/01), no treatment-related teratogenic/ embryo toxic effects were observed.

In summary, the DS concluded that DBDCB does not produce treatment-related and/or substance-specific reproductive/developmental effects and does not require classification for reproductive toxicity.

Comments received during consultation

One MSCA noted that in the key rat developmental toxicity study, there was a significant difference in weight change in the dams between treated and control groups. It was also noted that an increased number of resorptions was observed already in the mid dose group. However, no quantitative information was presented. These findings might be related. The reduction in weight change in the dams may not be due to maternal toxicity, as the DS suggested, but instead attributable to reduced growth of the developing foetuses and resorptions.

If there is any information on body weight gain with/without the uterus weight, this should be taken into account. According to the MSCA, the information might be indicative of a developmental effect and should be considered for classification.

Further, the MSCA noted the following:

- The statement in the section on toxicokinetics in the CLH report (also referred to under "STOT RE", above) that "DBDCB is completely debrominated prior to systemic distribution".
- In addition they quoted page 49 (section 4.10.1.1) of the CLH-report, which states as follows: "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 intakes of 36-150 mg/kg bw. LOAEL/ NOAEL values of 1 200/300 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 4 800 mg bromide/kg of diet (approx. 300 mg bromide/kg bw per day). Exposure to DBDCB at the NOAEL level corresponds to a daily bromide intake of 18 mg/kg bw." This was understood by the MSCA to mean that 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.
- Previously, RAC agreed on the classification of ammonium bromide[1] for the endpoint reproductive toxicity as Repr. 1B (H360FD) and Lact. (H362).

The comment noted that, when considering the bromide ion in relation to reproductive toxicity, the level of exposure has been taken into account by the DS, but, since the criteria in CLP are based upon the presence of a hazard rather than risk, this should therefore not be driven by the exposure level, but instead by considering the hazard of the bromide ion.

In their response, the DS pointed out that in the developmental toxicity study in rats, the early resorptions were primarily clustered in two animals. One animal at the high dose (175 mg/kg bw/day) accounted for 13 out of 26 early resorptions, and one animal given the 100 mg/kg bw dose accounted for the other 14 out of 28 early resorptions in this dose group. Unfortunately, no historical control data were available on early resorptions in the 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 twofold higher than the 10 % in the highest dose group in the main study. No significant difference in the live foetus body weights were identified among the control and any of the treatment groups. Furthermore, reduced growth of foetuses is, in their opinion, usually linked to late organogenesis which can hardly be linked to early resorptions. However, they agreed that early resorptions and reduced growth can involve the same pathway and therefore such effects would be probably accompanied by other adverse effects (e.g. malformations).

As additional information from the report from the main study, the DS provided values of the actual body weight gain (g) from days 6-20 were 86.0±15.10, 88.7±14.47, 75.0±28.64 and 70.1±22.8 for the doses (mg/kg.bw/d) of 0, 25,100 and 175, respectively. The values of the body weight gain corrected for the gravid uterus (g) from days 6-20 were 25.8±7.32, 28.3±10.26, 16.8±21.34, and 13.7±19.58 for the doses (mg/kg.bw/d) of 0, 25, 100 and 175, respectively. Thus, the body weight change corrected for the uterus decreased as the dose increased (no statistical significance detected) which indicates that 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 (males) and 6.7, 41.5, 247.8 mg/kg bw/day (females) 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 significant difference from the control group on day 1 following parturition. Histopathology of the uterus did not reveal any adverse effects. This again indicates that the substance does not show any developmental toxicity in doses applied in the studies. Instead, 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 active substance resulting from the administration via gavage.

Regarding the "low dose", the DS noted that the dose was determined by the dose range finding study and the highest dose in the developmental study was based on the adverse effects (including mortality) beyond the MTD observed in the dams treated with 250 mg/kg bw/d. The DS therefore agreed with the highest dose of 175 mg/kg bw for DBDCB in the main developmental study.

A second MSCA supported the justifications proposed for not classifying DBDCB for reproductive/developmental toxicity. 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 the CNS in dogs. Similar effects in the thyroid (hypertrophy or hyperplasia), on the CNS (degeneration in brain), effects on the testis were observed in oral toxicity dog studies with methyldibromo glutaronitrile (MDBGN).

The MSCA noted that for DBDCB, in the summary of section 4.7.1 of the CLH report, the DS had stated that the guidance value for classification with STOT RE 2 is very close to 100 mg/kg bw/d for both rats and dogs. They also argued against the use of allometric scaling for STOT RE classification (discussed under the STOT RE section).

In their response, the DS argued for no-classification. Their reasoning was that it is generally known that physiological rates are higher in smaller animals when normalised per body weight. In this case faster elimination of Br- from rats as opposed to dogs is predicted.

Assessment and comparison with the classification criteria

Fertility

No effect of DBDCB at doses of 34/240 mg/kg bw/day in males and of 39/317 mg/kg bw/day in females on fertility parameters such as litter size, sex ratio, macroscopic or microscopic findings in testes/epididymis, prostate gland, uterus, ovaries were reported from the 90-day feeding study in rats combined with a one-generation reproduction study (W.,1980a, 6.4.1/01).

No effects of DBDCB at doses of 13, 75 and 450 mg/kg/day in the dominant-lethal study in mice (W., 1980, 6.6.6) were noted on pregnancy rates, incidences of resorptions, foetal death, dead implantations, and foetal viability.

The effects of DBDCB at 102 mg/kg/day on testes, such as immature appearance and mild degeneration of the seminiferous tubules was observed in a repeated dose toxicity study in Beagle dogs (R. *et al*. 1994, A 6.4.1). This effect could be due to a delay in maturation of the dogs used in the study, or, as proposed by the DS, could be secondary to the overt toxicity observed in this dose group (weight loss and mortality). However, in the repeated dose toxicity section of the CLH report it is also reported that DBDCB at 102 mg/kg/day caused in this study a degeneration of the seminiferous tubules, hypospermia (2/4 males) and prostatic atrophy. All of the test article related effects partially or totally receded within the 3 month recovery period. Female dogs showed no effect of the test substance on their reproductive organs at any dose (R. *et al*. 1994, A 6.4.1).

RAC notes that no detailed data on fertility parameters and histopathology of reproductive organs in animals were provided which makes the assessment of the effects more difficult.

Noting that the reported data do not provide clear-cut evidence that DBDCB may affect fertility in rats, mice and dogs, therefore RAC considers that **no classification for effects on sexual function and fertility is warranted**.

Developmental toxicity

In **the prenatal developmental toxicity study in rats** (H., 1982, 6.8.1/02), DBDCB was given orally by gavage at doses of 25, 100, 175 mg/kg/ day from 6 to 15 day of gestation. The maternal toxicity occurred as mild to severe dyspnoea observed in 6 dams at 175 mg/kg/day. This symptom was also seen in one dam at 100 mg/kg/day. No maternal deaths and no apparent differences between groups were observed in terms of the number or percent of dams pregnant. There was a significant difference observed for the dam weight change (day 6 to day 20) between the control and treated groups. There were no significant differences between the control and treated groups for the mean number of corpora lutea. There were no differences between the control and treated groups in the percentage of live foetuses, or mean sex ratio. There was an apparent increase in the number of resorbed foetuses at 100 and 175 mg/kg bw/day dose levels. Inspection of the individual litter data revealed that two litters at 100 mg/kg bw/day accounted for 13 of the 26 resorptions seen in that group. It is noted that resorptions in these high dose groups were clustered in two litters with > 7 resorptions each. The DS also reported that in the dose-range finding study preceding this main study, 7 and 8 resorptions were found in two control dams. Thus, since such similar incidences of resorptions were found in control dams, this increase in two high dose groups without a dose response relationship may not be related to treatment with DBDCB.
No significant differences were observed for the mean number of implantations, litter size or foetal body weights. Four foetuses (4/262) in the high-dose group were classified as runts. This finding was not found in the other groups. The incidence was not statistically significant. Visceral variations were observed in all dose groups, with the exception of the 25 mg/kg dose. Hydroureter and renal cavitation were the two visceral variations observed. Analysis of the hydroureter was not significantly different.
Several types of variations associated with skeletal ossification patterns were observed in the control and treated groups. The only variation showing statistical significance was the incidence of rudimentary ribs, however, inspection of the data showed that the two high dose groups had fewer rudimentary ribs compared to the control group. The data from this study did not provide evidence for developmental toxicity in rats.

In **the prenatal developmental toxicity study in rabbits** (H., 1982, 6.8.1/02) DBDCB was given orally by gavage at doses of 10, 30, 60 mg/kg bw day from 6 to 18 day of gestation. Transient reductions in absolute (g/day) and relative (g/kg feed) consumption values occurred in the 60 mg/kg/day dosage group on days 6 to 9 and 9 to 12 of gestation. The litter averages for corpora lutea, implantations, litter sizes, live foetuses, early and late resorptions, foetal body weights, percent male foetuses and percent resorbed conceptuses were comparable among the four dosage groups and did not significantly differ; all values were within the ranges observed historically. One doe in each of the 10, 30 and 60 mg/kg/day dosage groups had a resorbed litter. There were no dead foetuses. No gross external, soft tissue or skeletal malformations or variations in the foetuses were considered as effect of exposure to DBDCB.

Taking into account that results of reported prenatal toxicity studies do not provide evidence that DBDCB may impair *in utero* development of rats and rabbits, RAC is of the opinion that **no classification of the substance is warranted for developmental toxicity**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The DS reported evidence that DBDCB is not rapidly degradable and has no potential to bioaccumulate in aquatic species. According to the available aquatic toxicity data, the DS proposed no classification for acute aquatic toxicity and a classification as **Aquatic Chronic 2 (H411**) based on the 72 h E_rC_{10} of 0.20 mg/L for the green alga *Desmodesmus subspicatus*.

Environmental degradation and distribution

Hydrolysis

A hydrolysis study performed according to US-EPA guideline 161-1 using radiolabelled DBDCB was presented (7.1.1.1.1/01, 1996). The substance was tested for 30 days in four aqueous buffer solutions at pH 5 to 9 and 25±1 °C using a nominal test concentration of 10 ppm. DBDCB did not hydrolyse at pH 5, hydrolysed slowly at pH 7 (DT_{50} = 51.6 and 96.3 days corresponding to 146.4 and 273.3 days at 12 °C) and was subject to base-catalysed hydrolysis at pH 9 (DT_{50} = 9.1 days corresponding to 25.8 days at 12 °C). The major hydrolysis products were the *E-Z* isomers of 1-bromo-2,4-dicyano-1-butene and 2-methyleneglutaronitrile (2-MGN), exceeding 10 % of the initial measured dose (IMD) at pH 9 and 7, respectively. The product 2-MGN belongs to the class of the nitriles for which a hydrolytic decomposition is very likely to occur. However, this decomposition pathway is not relevant for pH 7 and 9, as resulting from the hydrolysis study with DBDCB which indicated that 2-MGN is hydrolytically stable at (between) pH 7 and pH 9.

Photolysis

The photodegradation of DBDCB in water was assessed in a 30-day study conducted according to EPA-FIFRA N-161-2. ^{14}C-radiolabelled DBDCB was tested at a concentration of 10 µg/L at 25±1 °C in aqueous solution buffered at pH 5 (7.1.1.1.2/01, 1992). A decline from 100 % at day 0 to 67.2 % was observed at day 30. Analytical data revealed the formation of the photodegradation products (*E*)- or (*Z*)-1-bromo-2,4-dicyano-1-butene isomers (via dehydrohalogenation) and 2-MGN (28.5 % after 30 days) (via debromination), the latter showing a continuous increase up to 28.5 % at day 30. According to the Tier-1 'Theoretical Screening' in OECD TG 316, a UV-Spectrum was determined for 2-MGN. No direct UV-absorption occurred, indicating that there is no hint for a photolytically induced decomposition of 2-MGN.
The photodegradation of DBDCB in air was estimated by AOPWIN model (v. 1.91, 2000) (7.3.1/01, 2006). The software calculated a tropospheric half-life of 27.107 days with a degradation rate of 0.5919×10^{-12} cm^3 $molecule^{-1}$ s^{-1}.

Ready biodegradability

A study carried according to OECD TG 301D (closed bottle test) was performed by incubating DBDCB with a STP effluent inoculum for 28 days at 20±1 °C (7.1.1.2.1/01, 1995). DBDCB showed a -35 % degradation as measured by the dissolved oxygen depletion. Based on the significantly lower data measured in inhibition control (1.6 mg BOD/L after 28 days) compared to positive control (2.6 mg BOD/L), a potential inhibition of microbial action by the test substance is reported by the DS as the possible reason for the negative (-35 %) value of percent degradation measured in test vessel.

Inherent biodegradability

DBDCB was investigated for its inherent biodegradability in a Zahn-Wellens/EMPA Test (OECD TG 302B) at 21 °C and pH 7.2-7.6 over a period of 28 days (7.1.1.2.2/01, 2007). Based on the test parameter DOC, the test item was found to undergo a very limited ultimate inherent biodegradation under the test condition (2 % at 28 d). The test substance was subjected to primary inherent degradation with a clear disappearance with time (around 65 %) corresponding to an increase in the concentration of the degradation product 2-MGN (48 % at 28 days).

2-MGN Biodegradation

Based on the above data, an estimation of the biodegradability for 2-MGN made using EPISuite Biowin v4.10 was presented by the DS. A prediction in favour of a fast biodegradation was reported based on by Biowin1 and Biowin2 outputs. Biowin3 (ultimate survey model) output predicted a ready biodegradation of 2-MGN in "weeks" or faster, as confirmed by the BIOWIN5 (MITI Linear Model) output of 0.651 (cut-off level ≥ 0.5).

Adsorption/desorption in soil

An adsorption/desorption study (7.2.3.1/01, 1990) was conducted with radiolabelled DBDCB according to the US-EPA 163-1 guideline on four soils (sand, sandy loam, clay loam and silt loam) using the batch equilibrium method. The K_{oc} values obtained for the adsorption ranged from 33.4 to 528 mL/g, the mean being 64.7 mL/g (n = 3, the results of sandy soil were excluded due to its carbon content 0.05 % organic below the 0.3 % criteria set out in OECD TG 106). The major degradation products observed in the adsorption phase were the *E-Z* isomers of 1-bromo-2,4-dicyano-1-butene. The K_{oc} value for desorption was not calculated due to the low amount of test compound adsorbed.

Volatilisation

According to the EPIWIN model, the DBDCB was estimated to have a vapour pressure = 3.81 × 10^{-3} Pa (at 20 °C) and Henry´s Law Constant = 3.99 × 10^{-5} Pa at 25 °C; therefore, the DS concluded that the air is no compartment of concern for the substance.

Aerobic aquatic degradation

A water/soil aerobic transformation study was performed according to US-EPA guideline N162-4 using ^{14}C-radiolabelled DBDCB as test substance (7.1.2.2.1/01, 1990). The test was conducted under dark conditions at a temperature of 25±1 °C on a sandy soil flooded with blended water and dosed at 10.9 ppm test substance (measured). Residues of the parent compound in soil and test water decreased from 79.8 % of the IMD at day 0 to 0.410 % at day 7, and no measurable amounts were observed thereafter. Using first-order kinetics, a whole-system DT_{50} of 0.874 days was calculated for DBDCB. The metabolite 2-MGN, which accounted for 13.2 % of the IMD at day 0, increased to 34.1 % at day 3 and then decreased to 0.798 % at day 30. The formation of $^{14}CO_2$ was also monitored during the study and resulted in 10 % of the IMD after 30 days.

Anaerobic degradation in soil

A 1-year study investigating the behaviour of ^{14}C-radiolabelled DBDCB (nominal concentration of 10.0 ppm) in a flooded sandy loam soil under anaerobic aquatic conditions is available. The study was conducted for 365 days in an environmental chamber regulated at 25±1 °C. A significant degradation of the parent compound occurred during the test period, with a half-life of 0.495 days calculated using first-order degradation kinetics. Volatilization and/or

mineralization (to $^{14}CO_2$ and organic volatiles) was observed at appreciable amounts during the study (10.1 % of the applied radioactivity). The major degradation product was 2-MGN, which further degraded to a polar (not-characterized) degradation product that accounted for ≤ 25 % of the applied radioactivity at 12 months.

Conclusion on the rapidly degradable property of DBDCB

The DS concluded that based on CLP Regulation criteria, Annex I, section 4.1.2.9.5, DBDCB **is not rapidly degradable**, because:

a) It is not readily biodegradable.
b) A value of BOD/COD is not available.
c) The results of a hydrolysis study show that the longest DT_{50} (pH range 4-9) is > 16 days.

In the aerobic aquatic degradation study, it cannot be demonstrated that the DT_{50} for DBDCB is < 16 days. Data from an anaerobic degradation study cannot be used in order to decide if a substance should be considered as rapidly degradable because the aquatic environment is generally aerobic.

Bioaccumulation

A bioconcentration factor (BCF) of 10 was calculated using the measured log K_{ow} of 2.0, indicating a negligible potential for DBDCB to bioaccumulate in fish. As a supporting evidence, results from a non-GLP bioconcentration test on carps (*Cyprinus* sp.), performed according to a Japanese standard method (reference of the method not clear), were presented. The test was conducted under flow-through conditions for 8 weeks, at 25±1 °C using 0.005 and 0.05 mg/L DBDCB as test concentrations and resulted in a BCF below 2.5 for both treatments.

The DS concluded that since the available measured and calculated BCFs are ≤ 10, DBDCB has no potential to bioaccumulate in aquatic species according to the criteria of the CLP Regulation (log K_{ow} ≥ 4; BCF ≥ 500).

Aquatic toxicity

The relevant ecotoxicity data presented by the DS on DBDCB (and 2-MGN) are displayed in the table below.

Method/Species	Results	Remarks	Reference
Fish			
US-EPA guideline 72-1, Oncorhynchus mykiss	**LC_{50} (96 h) = 1.26 mg/L***	Measured	7.4.1.1/01, 1997a
US-EPA guideline 72-1, Lepomis macrochirus	LC_{50} (96 h) = 2.73 mg/L	Measured	7.4.1.1, 1997b
US-EPA guideline 72-4, Oncorhynchus mykiss	NOEC (81 d) = 0.75 mg/L	Mean measured Most sensitive endpoint: survival	7.4.1.2, 1991
Aquatic invertebrates			
US-EPA guideline 72-2, Daphnia magna	EC_{50} (48 h) = 4.83 mg/L	Measured	7.4.1.2/01, 2006
US-EPA guideline 72-4, Daphnia magna	NOEC (21 d) = 1.4 mg/L	Mean measured Most sensitive endpoint: first generation survival	7.4.1.2, 1991,

Algae			
Method C.3 (2009), Desmodesmus subspicatus	E_rC_{50} (72 h) = 5.4 mg/L **E_rC_{10} (72 h) = 0.20 mg/L**** NOEC (72 h) = 0.017 mg/L	Geometric mean measured values	7.4.1.3/02, 2011
Method C.3 (2009), Desmodesmus subspicatus, Test substance: 2-MGN	E_rC_{50} (72 h) > 100 mg/L NOEC (72 h) ≥ 100 mg/L	Nominal values	7.4.1.3/03, 2011
Microorganisms			
OECD TG 209, Activated sludge	EC_{50} (3 h) = 34 mg/L	Nominal value	7.4.1.3/01, 1995
Method C.11 (2008), Activated sludge Test substance: 2-MGN	EC_{50} (3 h) > 1 000 mg/L EC_{10} (3 h) = 789.4 mg/L	Nominal values	7.4.1.4/02, 2011

*** -** key value for acute aquatic toxicity; ****** - key value for chronic aquatic toxicity

Information on DBDCB acute and chronic aquatic toxicity were reported for the three main trophic levels (fish, invertebrates, and algae). Toxicity data on microorganisms were presented as additional information because of the biocidal properties of the Substance. Generally, all L(E)C_x / NOEC values reported for the 2-MGN were equal to or higher than 100 mg/L and were thus not considered further in the classification process.

Acute aquatic toxicity

All acute toxicity values (LC_{50} or EC_{50}) for DBDCB were higher than 1 mg/L. Among tested endpoints, fish survival was the most sensitively affected, with the lowest median lethal concentration of 1.26 mg/L (96 h) being reported in the rainbow trout *Oncorhynchus mykiss*. On this basis, the DS concluded that DBDCB does not fulfil the criteria for acute aquatic classification.

Chronic aquatic toxicity

Chronic aquatic toxicity values for DBDCB spanned from 0.017 to 1.4 mg/L. The key study was a 72-h growth test performed according to method C.3 (equivalent to OECD TG 201) on the freshwater green microalga *Desmodesmus subspicatus,* which resulted in a NOEC of 0.017 mg/L and an E_rC_{10} of 0.20 mg/L. According to the CLP Guidance, "if a NOEC or EC_x value is available, preference is given to EC_{10}". Hence, given the classification criteria for not rapidly degradable substances, the DS proposed to classify DBDCB as Aquatic Chronic 2, H411 based on the 72 h-E_rC_{10} of 0.20 mg/L measured in *D. subspicatus*.

Comments received during consultation

Two comments were received by one Member State (MS) and one National Authority (NA). The MS agreed with the DS's classification proposal. The NA noted that the chronic fish NOEC based on survival is in the same concentration range (0.1-1 mg/L) as the key algal chronic endpoint, and thus supports the proposed chronic classification. Given the importance of these two endpoints, the NA asked the DS to confirm whether the OECD TG 210 and the OECD TG 201 validity criteria were met in these fish and algal studies, and to provide EC_{10} values for the chronic fish study if these are available and reliable, noting that these are preferred over NOEC for the purpose of hazard classification.

The DS responded that 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. For the fish early life-stage toxicity test (US-EPA guideline 72-4, equivalent to OECD TG 210) the DS states

that EC_{10} values were not provided and that methodological deviations from the test guideline were implemented to improve the quality of data and to allow additional 3-8 weeks to the test duration, as follows:

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

Regarding the results and validity criteria, the DS reported that:

i) Control and solvent control survival rates were 96.7-100 %,
ii) 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,
iii) The relative standard deviation of the weights for surviving fish in the control test chambers was less than 40 %,
iv) Water quality parameters were within acceptable limits throughout the test,
v) Mean measured concentrations were in good agreement with the nominal concentrations, and that NOEC and LOEC of 0.75 mg/L and 1.0 mg/L, respectively, were thus calculated based on mean measured concentrations.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS that DBDCB is **not rapidly degradable** according to CLP criteria based on the following evidence:

- The substance was not found to be readily biodegradable or inherently biodegradable under OECD TG 301D and OECD TG 302B test guideline conditions, respectively.
- The longest 30-day half-life for DBDCB hydrolysis determined using US-EPA guideline 161-1 at pH range 5-9 is > 16 days.
- It was not demonstrated that DBDCB is ultimately degraded with a half-life < 16 days under aerobic aquatic conditions.

Bioaccumulation

RAC agrees with the DS that DBDCB has a **low potential to bioaccumulate** in aquatic organisms:

- Both the calculated and measured (*Cyprinus* sp.) bioconcentration factors in fish are ≤ 10, i.e., below the cut-off value of 500.
- The measured Log K_{ow} of 2.0 is lower than the cut-off value of 4.

Aquatic toxicity

Aquatic acute classification

RAC agrees with the DS that DBDCB does not fulfil the criteria for acute aquatic classification. All reliable median effect/lethal concentrations for fish, invertebrates and algae were greater

than the cut-off threshold of 1 mg/L, the lowest being the LC_{50} of 1.26 mg/L reported in the rainbow trout (*O. mykiss*).

Aquatic chronic classification

RAC notes that chronic toxicity values lower than 1 mg/L are available for two trophic levels: algae (*D. subspicatus*) and fish (*O. mykiss*).

The lowest values (NOEC = 0.017 mg/L and ErC_{10} = 0.20 mg/L) were obtained in a 72-h growth inhibition test performed on *D. subspicatus*. The test was performed under GLP according to the Method C.3, Freshwater *Alga* and *Cyanobacteria*, Growth Inhibition Test (2009) which is equivalent to OECD TG 201 (2006). The methodology was consistent with test guideline specifications for what concerns experimental design, physico-chemical conditions and data report. All validity criteria according to the test guidelines were fulfilled. The influence of the metabolite 2-MGN on the growth/yield of *D. subspicatus* was also investigated using the same test method (non-GLP), resulting in a NOEC ≥ 100 mg/L.

According to the CLP Guidance, if a NOEC or ECx value is available, preference is given to EC_{10}. Therefore, considering the classification criteria for not rapidly degradable substances, RAC agrees with the DS proposal to classify DBDCB as **Aquatic Chronic 2, H411,** based on the 72 h-E_rC_{10} of 0.20 mg/L for *D. subspicatus* ($0.1 < NOEC/EC_x \leq 1$ mg/L).

RAC evaluation of hazards to the ozone layer

Summary of the Dossier Submitter's proposal

No data on the hazards posed by DBDCB to the ozone layer were presented in the CLH report and a DS assessment based on CLP criteria is missing. No classification is proposed.

Comments received during consultation

No comments have been received

Assessment and comparison with the classification criteria

RAC notes that the information on the hazards posed by DBDCB to the ozone layer is missing in the CLH report and agrees with the DS that **no classification is warranted due to a lack of data**.

ANNEXES:

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

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