CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Substance Name: 2-bromo-2-(bromomethyl)pentanedinitrile

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

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

Index Number: **-**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	**2-bromo-2-(bromomethyl)pentanedinitrile (DBDCB)**
EC number:	***252-681-0***
CAS number:	***35691-65-7***
Annex VI Index number:	**-**
Degree of purity:	***≥ 98 % w/w***
Impurities:	***See annex III (confidential)***

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	None
Current proposal for consideration by RAC	Acute Tox. 4 ; H302 Acute Tox. 2; H330 Skin Sens. 1 ; H317 Eye Dam. 1; H318 Aquatic Chronic 2, H411
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None.			Conclusive but not sufficient for classification.
2.2.	Flammable gases	None.			Conclusive but not sufficient for classification.
2.3.	Flammable aerosols	None.			Conclusive but not sufficient for classification.
2.4.	Oxidizing gases	None.			Conclusive but not sufficient for classification.
2.5.	Gases under pressure	None.			Conclusive but not sufficient for classification.
2.6.	Flammable liquids	None.			Conclusive but not sufficient for classification.
2.7.	Flammable solids	None.			Conclusive but not sufficient for classification.
2.8.	Self-reactive substances and mixtures	None.			Conclusive but not sufficient for classification.
2.9.	Pyrophoric liquids	None.			Not relevant.
2.10.	Pyrophoric solids	None.			Conclusive but not sufficient for classification.
2.11.	Self-heating substances and mixtures	None.			Conclusive but not sufficient for classification.
2.12.	Substances and mixtures which in contact with water emit flammable gases	None.			Conclusive but not sufficient for classification.
2.13.	Oxidising liquids	None.			Conclusive but not sufficient for classification.
2.14.	Oxidising solids	None.			Conclusive but not sufficient for classification.
2.15.	Organic peroxides	None.			Conclusive but not sufficient for classification.
2.16.	Substance and mixtures corrosive to metals	None.			Conclusive but not sufficient for classification.
3.1.	Acute toxicity - oral	Acute Tox. 4, H302			
	Acute toxicity - dermal	None.			Conclusive but not sufficient for classification.
	Acute toxicity - inhalation	Acute Tox. 2, H330			

3.2.	Skin corrosion / irritation				Conclusive but not sufficient for classification.
3.3.	Serious eye damage / eye irritation	Eye Dam. 1, H318			
3.4.	Respiratory sensitisation				Conclusive but not sufficient for classification.
3.4.	Skin sensitisation	Skin Sens. 1, H317			
3.5.	Germ cell mutagenicity				Conclusive but not sufficient for classification.
3.6.	Carcinogenicity				Conclusive but not sufficient for classification.
3.7.	Reproductive toxicity				Conclusive but not sufficient for classification.
3.8.	Specific target organ toxicity –single exposure				Conclusive but not sufficient for classification.
3.9.	Specific target organ toxicity – repeated exposure				Conclusive but not sufficient for classification.
3.10.	Aspiration hazard				Conclusive but not sufficient for classification.
4.1.	Hazardous to the aquatic environment	Aquatic Chronic 2, H411			
5.1.	Hazardous to the ozone layer				Conclusive but not sufficient for classification.

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Danger

Hazard pictograms:
GHS05
GHS06
GHS09

Hazard statements:
H302: Harmful if swallowed.
H330: Fatal if inhaled.
H317: May cause an allergic skin reaction.
H318: Causes serious eye damage.
H411: Toxic to aquatic life with long lasting effect.

Proposed notes assigned to an entry: none

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

DBDCB is not listed in *Annex VI to Regulation (EC) No 1272/2008 and its current classification is based on the available data*.

DBDCB has been assessed as an active substance for biocidal products according to the directive 98/8/EC. Based on this assessment performed by the Czech CA the harmonized classification is now proposed.

2.2 Short summary of the scientific justification for the CLH proposal

The evaluation of the available data submitted in support of inclusion of DBDCB into the list of approved active substances of Regulation (EC) No. 528/2012 result in the above proposed classification for the following reasons:

LD50 (oral) values of 640 mg/kg bw and 514 mg/kg bw observed in rats trigger classification as Acute Tox. 4; H302. LC50 (inhalation, rat) of 0.264 mg/l triggers classification as Acute Tox. 2; H330.

DBDCB caused strong irritation of the eye in a test on albino rabbits fulfilling the criteria for classification as Eye Dam. 1; H318: Causes serious eye damage.

The weight of the evidence based on both on animal studies and those with humans led to DBDCB classification as Skin Sens.1; H317: May cause an allergic skin reaction.

According to Regulation (EC) No. 1272/2008 the substance is not classified for acute aquatic toxicity. For chronic toxicity the most sensitive species is the freshwater algae *Desmodesmus subspicatus* with an E_rC_{10} (72 h) of 0.20 mg a.s./L. Moreover, the substance is considered to be not rapidly degradable. Thus, according to Regulation (EC) No. 286/2011 of 10 March 2011, Table 4.1.0 (Classification categories for hazardous to the aquatic environment) amending Regulation (EC) No. 1272/2008, the substance needs to be classified as Aquatic Chronic 2; H411.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

No harmonised classification for DBDCB is available according to Regulation (EC) No 1272/2008.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

No harmonised classification for DBDCB is available according to Regulation (EC) No 1272/2008.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Hazard pictograms:
GHS05
GHS06
GHS07
GHS09

Danger! According to the classification provided by companies to ECHA in **CLP notifications** this substance is toxic to aquatic life with long lasting effects, causes severe skin burns and eye damage, is harmful if swallowed, causes serious eye damage, causes skin irritation, may cause an allergic skin reaction and may cause respiratory irritation. For more details the reader is referred to the ECHA website.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Because the proposal is for classification of a biocidal active substance no justification is required.

Part B.

scientific evaluation of the data

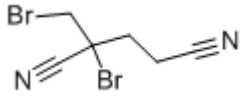
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	252-681-0
EC name:	2-bromo-2-(bromomethyl)pentanedinitrile
CAS number (EC inventory):	35691-65-7
CAS number:	35691-65-7
CAS name:	Pentanedinitrile, 2-bromo-2-(bromomethyl)-
IUPAC name:	2-bromo-2-(bromomethyl)pentanedinitrile
CLP Annex VI Index number:	-
Molecular formula:	$C_6H_6Br_2N_2$
Molecular weight range:	265.9 g/mol

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
DBDCB EC:252-681-0	Min. 98 %		

Current Annex VI entry: none

Impurities (non-confidential information)

Confidential information, see annex III.

Additives (non-confidential information)

Confidential information, see annex III.

1.2.1 Composition of test material

Confidential information, see annex III.

1.3 Physico-chemical properties

Table 6: Summary of physico - chemical properties

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 102 (DTA)
Boiling point	Up to the exothermic decomposition no boiling point could be observed	3.1/02	OECD 103 (DTA)
Relative density	1.918 at 20 °C	3.1/03	OECD 109 (pycnometer method)
Vapour pressure	3.81 x 10^{-03} Pa at 20 °C 7.77 x 10^{-03} Pa at 25 °C	3.2/01	OECD 104 (gas saturation method)
Surface tension	72.99 mN/m at 20 °C	3.13/01	OECD 115 (ring tensiometer) concentration 1g/L
Water solubility	<u>Results at pH 5:</u> 1.03 g/L at 10°C 1.68 g/L at 20°C 2.62 g/L at 30°C <u>Results at pH 7:</u> 1.05 g/L at 10°C 1.70 g/L at 20°C 2.62 g/L at 30°C <u>Results at pH 9:</u> 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 guideline 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	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).

	°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.		
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
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.	-	-

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant.

2.2 Identified uses

DBDCB is used in a wide range of products for consumers and occupation use, e. g. dishwashing liquid, household cleaning products and other detergents, car care products, wax and other polishing preparations for floors, adhesives, paints, and metal working fluids. In addition, it is used in veterinary products (e. g. in dog shampoos) and as a preservative in cosmetic products at a maximum authorised concentration of 0.1%.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 7: Summary table for relevant physico-chemical studies

No classification for physico - chemical properties is proposed. The relevant physico- chemical properties are provided in table 6 of section 1.3 above.

3.1.1 Summary and discussion of classification for physico-chemical properties

During Differential Thermal Analysis (DTA) measurement endothermic melting from 44 to 90 °C was observed. Exothermal decomposition starts at 140 °C.

During Isotherm Step Thermal Analysis (ISTA) measurement endothermic melting from 50 to 55 °C was observed. Exothermal decomposition starts at 120 °C.

The active substance DBDCB was found to be stable when subjected to accelerated storage at 50 °C for 30 days.

DBDCB is 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.

The active substance contains none of the functional groups which may indicate explosive or oxidising properties. It can therefore be concluded that DBDCB does neither present any risk for explosion nor oxidising properties.

No classification for physico - chemical properties is proposed.

3.1.2 Comparison with criteria

The physical and chemical properties of DBDCB do not fulfil the criteria for a classification set in Regulation (EC) No 1272/2008.

3.1.3 Conclusions on classification and labelling

No classification or labelling is therefore proposed for physico-chemical properties.

4 HUMAN HEALTH HAZARD ASSESSMENT

Human health hazard of DBDCB has been assessed during the process of its inclusion into the Union list of approved active substances according to Regulation (EC) No. 528/2012. The text of this section is lifted for the draft final Competent Authority Report where more detailed information can be found.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Table 8: Toxicokinetic and metabolism studies with DBDCB

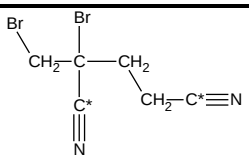
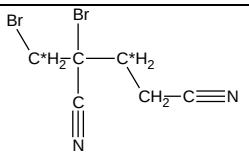
Route	Method Guideline GLP Reliability	Species Strain Sex No/group	Label	Dose levels	Reference
Gavage	Absorption, distribution, metabolism, excretion No guideline, but ≅ OECD 417 GLP- yes Reliability - 2 (The nature of radioactivity in faeces was not elucidated)	Rat, SD ♂+♀, 5/sex/group		Oral: 5, 200 mg/kg bw single low dose, single high dose, post-exposure 168 hours 15x low dose I.v.: 5 mg/kg bw single dose Post exposure =168 hours Dermal: 5mg/kg bwbw=5 mg/kg bw ≅ 2.5 mg/cm² Treatment duration = 24hours Post exposure period = 96 hours	C. (1990, 6.2/01) **KEY STUDY**
Gavage	Absorption, distribution, metabolism, excretion No guideline GLP –No Reliability- 2	Rat, SD ♂+♀, 5/sex/group		Oral: 80 mg/kg, single dose post-exposure 72h I.v.: 8 mg/kg bw, single dose	S. (1998, 6.2/02) **KEY STUDY**
Diet	Absorption No guideline GLP-No	Rat, CD® ♂, 4/group	^{14}C-DBDCB, position of label not specified	4.55 - 6.80 mg/kg bw, single dose	T& B, (1994, A6.2)

Table 8: Toxicokinetic and metabolism studies with DBDCB

Route	Method Guideline GLP Reliability	Species Strain Sex No/group	Label	Dose levels	Reference
Gavage	Absorption, distribution, excretion No guideline Non-GLP	Rat, CD® ♂, 4/group	^{14}C-DBDCB, position of label not specified	50 mg/kg bw, single dose	A. (A6.2)

4.1.2 Human information

No data available.

4.1.3 Summary and discussion on toxicokinetics

DBDCB is rapidly converted into 2-methyleneglutaronitrile (2-MGN) by reaction with sulfhydryl groups of plasma proteins. DBDCB is completely debrominated prior to systemic distribution, and tissue exposure to intact DBDCB seems to be low regardless of route of administration.

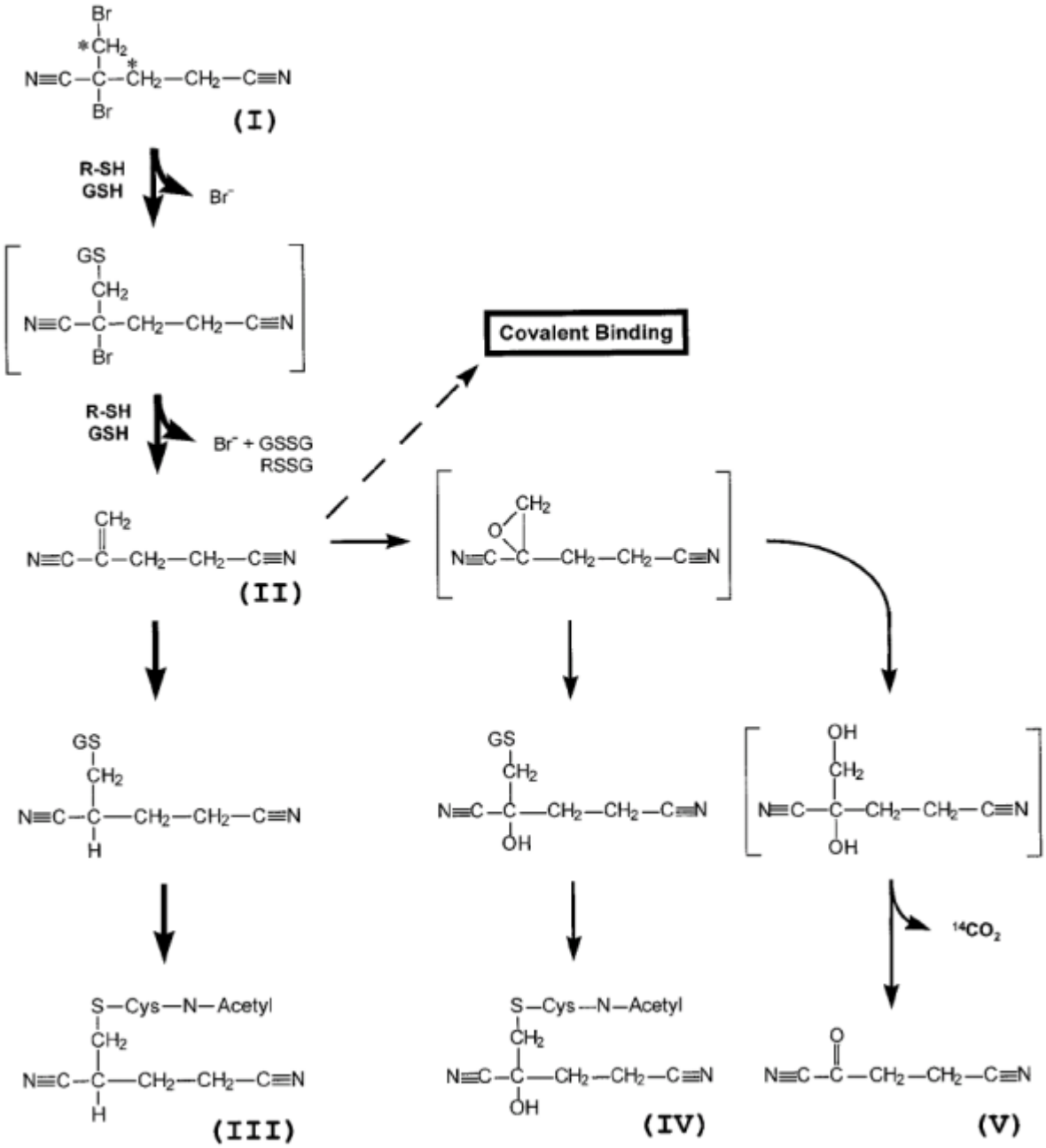
Radiolabelled components derived from ^{14}C-DBDCB appeared to be well absorbed (80-90%) following **oral administration**.

The main excretory path is via urine (65-88%). The pattern of excretion was similar following repeated oral administration of test material (at 5 mg/kg bw/day) to that following a single oral dose (5 mg/kg bw). At the high dose level (200 mg/kg) apparently almost complete absorption was observed via the oral route, as evidenced by high urinary excretion (mean of 87.9% of administered radioactivity).

Tissue distribution studies at the low dose level indicated that at 8 h and 48 h following oral administration radioactivity was distributed throughout the body with highest levels detected in organs of elimination such as liver, kidney and GI tract, and in lung and whole blood. Levels of radioactivity in other tissues were generally lower than in plasma.

The excretion pattern of i.v. administered 2-MGN (main metabolite) was similar to that following i.v. administration of DBDCB. The radioactivity associated with the urine was 86.9 ± 7.3% of the dose and 2.4 ± 0.1% with the faeces, and 2.9 ± 0.3% was bound to the blood at 48 h.

Figure 1 **Metabolic scheme proposed for DBDCB in the male Fischer 344 rat (S. (1998, 6.2/02).**



1,2-Dibromo-2,4-dicyanobutane (I), 2-methyleneglutaronitrile (II), N-acetyl-S-(2,4-dicyanobutane)-L-cysteine (III), N-acetyl-S-(2,4-dicyanobutan-2-ol)-L-cysteine (I.V.), and propanoic-1,3-dicyanide (V).
*, location of ^{14}C label; GS, glutathione conjugate; S-Cys-N-Acetyl, mercapturate conjugate; GSH, glutathione; R-SH, free sulfhydryl; GSSG, glutathione disulfide; RSSG, mixed disulfide.

4.2 Acute toxicity

Table 9: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference (DOCIII A)
LD_{50} test US EPA 81-1 ≅ OECD 401 GLP - Yes Reliability -3 (LD50 for males was extrapolated; higher sensitivity of female animals is remarkable and does not permit to estimate one LD50 value, mortality data were probably distorted by aspiration of the DBDCB suspension.)	640 mg/kg bw (♂+♀)	Rat Wistar ♂+♀, 5/sex **Oral**	6.1.1 **key study**
LD_{50} test No guideline Non-GLP	541 mg/kg bw (♂+♀)	Rat BLU:(SD)BR ♀+♂ 10/sex/group **Oral**	6.1.1
Limit test US EPA 81-2 ≅ OECD 402 GLP- yes Reliability- 1	LD_{50} > 2000 mg/kg bw (♂+♀)	Rabbit NZW ♂+♀ 5/sex **Dermal**	6.1.2 **key study**
LC_{50} Test OECD 403 GLP- yes Reliability -1	LC_{50} = 0.265 mg/l (♂+♀)	Rat Wistar ♂+♀ 5/sex/group **Inhalation**	6.1.3 **key study**
LC_{50} Test US EPA 81-3 ≅ OECD 403	LC_{50} > 13.09 mg/l(♂+♀)	Rat SD ♂+♀, 5/sex/group **Inhalation**	6.1.3

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

In the study 6.1.1 five male and five female Wistar rats per dose level received 492, 553, 622, 700 mg/kg bw as a 50% suspension in corn oil by single-dose oral gavage.

Two, 5, 5, and 6 animals died in the 492, 553, 622 and 700 mg/kg.bw groups, respectively.

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.

Necropsy results of survivors were normal with the exception of one male dosed at 622 mg/kg, which exhibited adhesions in the peritoneal cavity.

Body weight changes of survivors were generally normal. One female survivor of the 622 mg/kg bw group lost weight during the second week of the study, and one male survivor of the 553 mg/kg bw group appeared emaciated during the first week of the study.

4.2.1.2 Acute toxicity: inhalation

In the study summarized in 6.1.3 four groups of rats were nose-only exposed to mean aerosol concentration of 0.217*,0. 226, 0.272*, and 0.239 mg/l air. The concentrations denoted with asterisk were generated using the non-micronized test article, whilst in all other cases the micronized test article was used.

Mortality occurred at 0.217 mg/l and above. Based on gross necropsy findings, mortality is causally related to an acute alveolar oedema. The following clinical signs were observed: bradypnoea, tachypnoea, laboured breathing pattern, irregular breathing pattern, dyspnoea, breathing sounds, nasal discharge (serous), nose: reddened, nose: red encrustations, muzzle; red encrustations, stridor, nostrils: red encrustations, 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 up to mid-term of the second post-exposure week. Necropsy findings were unremarkable in all surviving rats, whilst in those who succumbed displayed the following major changes: lungs less collapsed and with discolorations/white foci, firm consistency; watery to yellowish/red discharge from nose or content in the nasal cavities, nose/nostrils with red encrustations; trachea with foamy content; corneal opacity; gastrointestinal tract bloated and yellowish content in lumen, mucosa reddened; discolorations of parenchymatous organs.

With regard to the respirability of the aerosol generated internationally recognized recommendations such as of SOT (1992) were fulfilled, i.e. the MMAD (at the level of the LC50 of the micronized and non-micronized test article was MMAD 3.5 µm (GSD 2) and MMAD 6.8 µm (GSD 2.4). respectively. A conclusive dependence of the LC50 on the particle-size was not observed.

In summary, the test substance (solid aerosol) proved to have high acute inhalation toxicity to rats. The lethal mode of action is the elicitation of an acute lung oedema. The 4-h LC50 is 0.265 mg/l air at which no systemic toxicity, as evidenced by the necropsy finding of severe respiratory irritation and the clinical signs corresponding to the local effects in respiratory tract, was observed .

4.2.1.3 Acute toxicity: dermal

DBDCB was applied dermally to 5 male and 5 female New Zealand White rabbits at a dose of 2000 mg/kg bw.

The test article was applied on the abraded abdomen and the site occluded for 24 h.

No deaths resulted from the treatment at the dermal limit dose.

The principle toxic sign noted during the observation period was diarrhoea. Moderate erythema 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; 2 animals had crusted skin at the treated area.

4.2.1.4 Acute toxicity: other routes

No data available.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

DBDCB was of moderate toxicity to rats via the oral route of administration. The toxicity via the dermal route is low which is indicative of low dermal penetration.

Two 4-h inhalation toxicity studies with DBDCB in the rat are available. The more recent study by P. (2003, 6.1.3) found high mortality induced by inhalation of dust (LC_{50} = 0.264 mg/l), whereas the study by W (1992, 6.1.3) found no appreciable toxicity via this route. The latter study produced inconsistent results with 3/5 male and 2/5 female animals succumbing during exposure to DBDCB at a concentration of 4.76 mg/l, whereas no mortality was observed at concentrations of 8.31 and 13.09 mg/L. LC_{50} of 0.264 mg/l does not indicate systemic toxicity as evidenced by the necropsy finding of severe respiratory irritation and the clinical signs corresponding to the local effects in respiratory tract.

The respirability of the aerosol in the more recent study appeared to be better than in the older study, although P. (2003, 6.13) did not observe a conclusive effect of particle size on the inhalation toxicity exerted by DBDCB.

Necropsy observations made by P. (2003, 6.13) 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 to be expected.

4.2.4 Comparison with criteria

According to the criteria of Regulation (EC) no 1272/2008 DBDCB LD50 (oral) values of 640 mg/kg bw and 514 mg/kg bw observed in rats trigger classification as Acute tox 4, H302 as they fall in the interval of (300 mg/kg bw - 2000 mg/kg bw) specified in the CLP as criteria for this category. LC50 (inhalation, rat) of 0.264 mg/l triggers classification as Acute Tox 2, H330 as this value falls into the interval of 0.05 - 0.5 mg/l specified in the CLP as criteria for this category.

4.2.5 Conclusions on classification and labelling

According to the criteria of Regulation (EC) No. 1272/2008 DBDCB is classified as follows: Acute Tox. 2; H330: Fatal if inhaled; Acute Tox. 4; H302: Harmful if swallowed.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

Necropsy observations made by P. (2003, 6.13) 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 to be expected.

Comparison with criteria

Classification as STOT SE1 H370: Causes damage to respiratory system on inhalation is not justified as according to section 3.8.1.1. of the CLP Regulation specific target organ toxicity on single exposure is defined as a specific, non lethal target organ toxicity. Since mortality was induced classification as Acute Tox. 2, H330 Fatal if inhaled is triggered covering this endpoint.

4.3.2 Conclusions on classification and labelling

No classification or labelling is proposed for Specific Target Organ Toxicity – single exposure.

4.4 Irritation

4.4.1 Skin irritation

Table 10: Summary table of relevant skin irritation studies

Method , GLP, Reliability	**Results**	**Remarks**	**Reference DOCIIIA**
US EPA 81-5 GLP- yes Reliability -2 (Test substance was poorly characterised. However, the presence of any impurities is not expected to alter the outcome of this study towards non-irritancy)	Slightly irritating	Species: rabbit, Effects: reversible Average score 24, 48, 72 h Erythema 1.56 Oedema 0.78	6.1.4/ 01 **Key study**

4.4.1.1 Non-human information

Aliquots of 0.5 g DBDCB, moistened with distilled water, were applied for 4 hours to the intact skin on the back of six female New Zealand White rabbits under semi-occlusive conditions.

Skin reactions were scored at 30-60 minutes 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 method used in this study is in accordance with the US-EPA guidelines of the US EPA 81-5. The method is comparable to OECD guideline 404.

Erythema was very slight (1) to well-defined (2) at 30-60 minutes after removal of the test substance and at 24, 48, and 72 h thereafter. Erythema was absent on day 7. Oedema was absent (0) to well-defined (2) 30-60 minutes after removal of the test substance and at 24 and 48 h thereafter. Oedema was very slight at 72 h and absent on day 7.

The relevant average scores for erythema and oedema are 1.56 and 0.78, respectively. The scores for individual animals are summarized

OBSERVATION TIME	**RABBIT NO.**											
	C9642		**C9613**		**C9619**		**C9629**		**C9624**		**C9635**	
	E*	**O***	**E**	**O**	**E**	**O**	**E**	**O**	**E**	**O**	**E**	**O**
24 H	2	2	1	1	1	1	2	0	2	2	2	2
48 H	2#	1	1	1	1	0	2	0	2#	1	2	2
72 H	1	0	1	0	1	0	2	0	1	0	2	1
7D	0	0	0	0	0	0	0#	0	0	0	0#	0
MEAN VALUE 24 +48 + 72 H	1.67	1.00	1.00	0.67	1.00	0.33	2.00	0.00	1.67	1.00	2.00	1.67
REVERSIBLE	**Yes**											

* E: erythema, O: oedema (according to Draize Score)
# Animal re-clipped

4.4.1.2 Human information

No data available.

4.4.1.3 Summary and discussion of skin irritation

DBDCB caused only slight irritation on the skin. The results do not fulfil the criteria of the Regulation (EC) no 1272/2008 for classification as a skin irritant.

4.4.1.4 Comparison with criteria

According to the CLP Regulation the criteria for classification are fulfilled when: mean value falls into the interval of < 2.3 ;4.0 > for erythema/eschar or edema in at least 2 of 3 animals tested from gradings at 24, 48 and 72 hours after patch removal; inflammation persists to the end of the observation period of 14 days in at least 2 animals; in some cases, very definite positive effects related to chemical exposure occur . Since no animal scored more than 2 at any observation time and all effects disappeared within 7 days following exposure it follows that the criteria for classification are not fulfilled.

4.4.1.5 Conclusions on classification and labelling

No classification or labelling is proposed with respect to skin irritation.

4.4.2 Eye irritation

Table 11: Summary table of relevant eye irritation studies

Method, GLP, Reliability	Results	Remarks	Reference DoC iIIA
Section 1500.42, Federal Hazardous Substances Act, 16 CFR, p 125 OECD Guideline 405 GLP - yes Reliability 1	Serious eye damage	Average score 24, 48, 72 h Cornea 4 Iris 2.0 Conjunctiva Redness 2.0 Chemosis 4.0 irreversible	6.14 / 02 **Key study**

4.4.2.1 Non-human information

The eye irritation potential of DBDCB was tested in six young adult New Zealand White rabbits.

A 0.1 g aliquot was instilled into the conjunctival sac. 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 at the end of study on day 21. The results of the study are summarized in the following table:

	Cornea	Iris	Conjunctiva	
			Redness	Chemosis
Score (average of animals investigated)	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*	n	n	n	n
Average time for reversion	–	–	–	–
* *c : completely reversible* *n c : not completely reversible* *n : not reversible*				

4.4.2.2 Human information

No data available.

4.4.2.3 Summary and discussion of eye irritation

DBDCB caused strong irritation of the eye in a test on albino rabbits. Damage of the cornea and iris as well as conjunctival redness and chemosis, all of which persisted through the observation period, were noted.

4.4.2.4 Comparison with criteria

According to the CLP Regulation if a substance, when applied to the eye, produces in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse within 21 days and/or at least in 2 of 3 tested animals a positive response of corneal opacity ≥ 3 and/or irritis > 1.5 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material. Since the observed effects did not reverse in 21 days following installation and exceed the above values even when averaged over all animals it follows that the criteria for classification as Eye Dam. 1, H318: Causes serious eye damage according to Regulation (EC) no 1272/2008 are met.

4.4.2.5 Conclusions on classification and labelling

According to the criteria of Regulation (EC) No. 1272/2008, DBDCB has to be classified with Eye Dam. 1; H318: Causes serious eye damage.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

Table 12

Method, GLP, reliability	Results	Remarks	Reference DOCIIIA
LC_{50} Test OECD 403 GLP- yes Reliability -1	LC_{50} = 0.264 mg/l (♂+♀)	Rat Wistar ♂+♀ 5/sex/group **Inhalation**	6.1.3 Key study

4.4.3.2 Human information

No data available.

4.4.3.3 Summary and discussion of respiratory tract irritation

The information on the potential of DBDCB to cause irritation of the respiratory tract comes from a study on acute toxicity via inhaltion (6.1.3). 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 to be expected.

4.4.3.4 Comparison with criteria

Classification as STOT SE1 H370: Causes damage to respiratory system on inhalation is not justified as according to section 3.8.1.1. of the CLP Regulation (Regulation (EC) no 1272/2008) specific target organ toxicity on single exposure is defined as specific, non lethal target organ toxicity. Since mortality was induced classification as Acute Tox. 2, H330 Fatal if inhaled is triggered covering this endpoint. Similar argumentation applies to classification as Stot SE3, H335: May cause respiratory irritation.

4.4.3.5 Conclusions on classification and labelling

No classification or labeling is proposed according to the CLP Regulation.

Corrosivity

4.4.4 Non-human information

Table 13

Method	Results	Remarks	Reference DOCIIIA
US EPA 81-5 GLP – yes Reliability -2 (Test substance was poorly characterised. However, the presence of any impurities is not expected to alter the outcome of this study towards non-irritancy)	Slightly irritating	Species: rabbit, Effects: reversible Average score 24, 48, 72 h Erythema 1.56 Oedema 0.78	6.1.4

4.4.5 Human information

No data available.

4.4.6 Summary and discussion of corrosivity

DBDCB is not corrosive.

4.4.7 Comparison with criteria

According to the CLP Regulation skin corrosion means the production of irreversible damage to the skin, namely, visible necrosis through the epidermis and into the dermis, following the application of a test substance for up to 4 hours. Since the test on skin irritation/corrosion resulted in slight irritation only, the substance is not considered as corrosive.

4.4.8 Conclusions on classification and labelling

No classification or labelling with respect to skin corrosivity is proposed.

4.5 Sensitisation

4.5.1 Skin sensitisation

Table 14: Summary table of relevant skin sensitisation studies

Human data

Method, GLP, reliability	Results	Remarks	Reference DOCIIIA
Repeat Insult Patch Test No guideline Non-GLP	Sensitizing	Induction/challenge with 5000 ppm in petrolatum: 8%	6.1.5

		positive reactions Induction/challenge with 5000 ppm in ethanol:14% positive reactions Humans	
Repeat Insult Patch Test No guideline Non-GLP	Weakly sensitizing	Induction/challenge with 2500 ppm in water: 1% positive reactions Humans	6.1.5
Photoallergy test (RIPT with exposure to natural sunlight) Non-GLP	Photo- sensitizing	Induction/challenge with 2000 ppm in water: 6/24 positive reactions Humans	6.1.5
Photoallergy test (RIPT with exposure to UVA/UVB) Non-GLP	sensitizing	Induction/challenge with 2000 ppm in water: 11/26 positive reactions. Presence or absence of irradiation with UV light had no effect. Humans	6.1.5
Photoallergy test (RIPT with exposure to UVA/UVB) Non GLP	Weakly photo-sensitizing	Induction/challenge with 800 ppm in water: 2/50 positive reactions. Presence or absence of irradiation with UV light had no effect. Humans	6.1.5

Table 15: **Summary table of relevant skin sensitisation studies**

Animal data

Method, GLP, reliability	**Results**	**Remarks**	**Reference**
Magnuson-Kligman Maximzation method Guideline- The method used essentially follows the procedure for the Guinea Pig Maximization Test) (GMPT) aid down in OECD 406. GLP -yes Reliability -2 (The topical induction was not performed on irritated skin. The test substance is not sufficiently characterised. However, the GPMT is sufficiently sensitive to state that 5% solutions of DBDCB	Not sensitizing	Number of animals sensitized/ total number of animals 24 h: 0/20 48 h: 0/20 Guinea pig Induction - 5% solution in 80/20 ethanol/water Challenge -5% solution in 80/20 ethanol/water	6.1.5/01 **Key study**

are not sensitising)			
Freunds Complete Adjuvant test No guideline Non-GLP Reliability -2-3 (The test substance is not sufficiently described. The test system was not checked for reliability. The method was not in accordance with accepted guidelines. However, since a positive result was obtained, the result is considered valid.)	Weakly to moderately sensitizing	Number of animals sensitized/ total number of animals Induction with cca 0.2% Challenge -0.1 and 0.3% 24 h: 2/20 48h: 3/20 72h: 3/20 Guinea pig	6.1.5/02 1993 **Key study**
Ritz-Buehler-Method Guideline- no GLP- yes	Not sensitizing	Number of animals sensitized/ total number of animals 24 h: 0/10 48 h: 0/10 Guinea pig Induction -5% in 80/20 ethanol water Challenge- 5% in in 80/20 ethanol water	6.1.5
Ritz-Buehler-Method No guideline GLP- yes	Not sensitizing	Number of animals sensitized/ total number of animals 24 h: 0/20 48 h: 0/20 Guinea pig Induction – 75%w/v in acetone Challenge -5%w/v in acetone	6.1.5

4.5.1.1 Non-human information

DBDCB was tested for its skin sensitization potential in adjuvant and non-adjuvant tests on guinea pigs. All animal studies contain some deficiencies when judged according to the latest version of OECD guideline 406.

The GMP test (G., 1982b, 6.1.5/01) was performed in compliance with the OECD 406 guideline with the following deviations (1992): 1-chloro-2,4 dinitro benzene (DNCB) was used as a positive control (moderate sensitizer required); the test substance was not characterised; a pre-test to identify the lowest irritating/highest non-irritating concentration was not performed; the vehicle control group was not challenged with the test material; the topical induction was not preceded by creating a local irritation; duration of challenge exposure was 21 h, scoring was performed 24 and 48 h after the beginning of challenge exposure resulted in no effects.

In this test none of the guinea pigs induced with DBDCB showed a skin reaction 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 induction phase of the first (G.,1982 c, A6.1.5) Ritz-Buehler Test (Current Concepts in Cutaneous Toxicity, Academic Press,1980), consisted of application of 0.4ml of 5% test substance solution in ethanol/water 80:20 to intact skin sites of the test animals for 6 hours three times weekly per 3 consecutive weeks (10 applications in total). The scores for irritation were recorded and ranged from 0 (no irritation) to (strong erythema, with or without edema) observed for the applications 8-10. The challenge application, as described for the induction phase, took place at a skin site differing from the original application site after two weeks following the termination of the induction phase. The study gave negative results.

The second Ritz-Buehler tests was performed with concentrations specified in the above table. The doses used are not specified in the report. The study gave negative results, however, it is unclear whether the induction concentrations were sufficiently high to elicit a mild skin irritation.

A published study (H.,1993, 6.1.5/02) using three intradermal induction treatments with Freunds Complete Adjuvant (FCA, 6 injections of 0.1-0.15mL) showed a concentration-dependent response to the challenge exposure. A DBDCB elicitation concentration of 0.3 % (0.05mL) caused moderate reactions (distinct erythema restricted to application area) in 1, 2 and 1 animal in 24, 48 and 72 hours, respectively. The remaining animals showed either a weak reaction (slight, spotted, erythrema) in 6, 5 and 6 animals in 24, 48 and 72 hours, respectively, or no reaction at all.

A DBDCB elicitation concentration of 0.1 % (0.05 ml) caused moderate reactions in 1, 1 and 2 animals in 24, 48 and 72 hours, respectively. The remaining animals showed either a weak reaction (slight spotted erythrema) 1, 2 and 3 animals in 24, 48 and 72 hours, respectively, or no reaction at all.

Apart from the studies summarized in the above table various studies on this substance were performed. Some of these studies are summarized 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 sensitizing potential is shown in non-adjuvant tests. Regarding the adjuvant test, it is concluded that in these tests no, or only a 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), provided also by the applicant)

similar to adjuvant method which showed positive skin sens. reactions. In addition summaries of 3 local lymph node assay (LLNA) test provided in the report showed positive reactions.

4.5.1.2 Human information

In the Repeat Insult Patch Test (RIPT) (M., 1982, A6.1.5) study, 100 human subjects were repeatedly exposed to 0.2 ml of the test solution (0.5 % DBDCB) by means of 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 hours following the application. The elicitation with a solution of the same DBDCB concentration took place approx. two weeks after the induction phase. A positive response was observed in 8-14% of the treated subjects. 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.
In a further RIPT (M., 1984, A6.1.5), induction and challenge of *ca.* 100 human subjects with 2500 ppm DBDCB in water produced only one positive response.

Photoallergy tests on human volunteers are not a data requirement for this endpoint. However, due to the widespread use of DBDCB in cosmetic products, testing on this endpoint was performed.

Clear positive results were obtained using induction and challenge treatments with 2000 ppm DBDCB each followed by exposures to natural sunlight (W., A6.1.5, 1983,) or artificial UVA/UVB radiation (B., 1988, A6.1.5). A weaker, yet detectable response was seen when 800 ppm solutions were used for induction and challenge (K., 1993, A6.1.5). These findings may serve as supplementary information.

Apart from the studies summarized in the above table various studies on this substance were performed. Some of these studies are summarized in the Existing Chemical Hazard Assessment Report on DBDCB (NICNAS report) compiled by the Department of Health and Ageing of Australian Government in 2009 (www.nicnas.gov.au). This report contains summaries of studies performed on both naive and pre- sensitized persons. The latter in principle cannot be used to assess a substance potential to bring about sensitization and therefore, are not mentioned in the following text. It is also noted that this report contains summaries of studies with EUXYL K 400, a mixture containing 20% DBDCB and 80% phenoxyethanol. The latter component is reported to have caused sensitization in at least one study ([redacted], as cited in the NICNAS report). Therefore, the mixture is not considered a suitable surrogate for DBDCB and the studies with this mixture are not taken into account. 0.5% of subjects in the study performed in the Netherlands 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 3 DBDCB concentrations (i.e. 0.05%, 0.1% and 0.3% w/w) were observed in 4% (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 1019 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 for the same DBDCB concentration. Hasan et al

(2005, as cited in the NICNAS report) reported a statistically insignificant increase in sensitivity to DBDCB (0.1% w/w) from 1% (1995-1997) to 1.5% (2000-2002). McFadden et al (2005, as cited in the NICNAS report) reported a statistically insignificant increase in sensitivity to DBDCB (0.3% w/w) from 0.4% (1989-1993) to 0.6% (1994-1999). Wilkinson et al. (2002, as cited in the NICNAS report) reported increase in allergic reactions towards common preservatives including 0.2% MDBGN in petrolatum with incidences from 0.7% in 1991 to 3.5% in 2000.

Other studies cited in the above report gave similar outcomes and the reader is referred to the NICNAS report for further information.

4.5.1.3 Summary and discussion of skin sensitisation

The animal data provided by the applicant indicate weak or no potential for sensitization. The studies cited in the NICNAS report support this conclusion; however exceptions including all the 3 LLNA studies are noted. The only study giving positive results for which a robust study summary is available in the DBDCB CAR is the study by H. (1993 6.1.5/02). The CLP does not provide criteria for this non-guideline study making a straight forward decision on classification impossible. It is noted that the weak reactions described as slight, spotted erythema, observed in the study do not fulfill the condition of discrete or patchy erythema (Magnusson and Kingman scale =1). The authors also argue, based on at least 30 articles published between 1980-1992 that the FCA method they used is more sensitive than the GPMT. This further confirms that the CLP criteria for the GPMT are not relevant for this study. Overall, it is concluded that the study results suggest that DBDCB should be classified as a weak or moderate skin sensitizer corresponding to category Skin Sens. 1 B.

In the weight evidence approach, higher weight is attributed to the studies where the details are available (studies provided by the applicant). This approach leads to an overall conclusion to classify DBDCB as Skin Sens. 1 B.

The human studies provided by the applicant (M. 1982, 1984, Doc III A, 6.1.5) confirm DBDCB potential to cause a sensitizing reaction. The relatively high percentage of subjects showing positive reaction can partly be attributed to relatively high exposure (permanent exposure during the 3 weeks of the induction phase). This would lead to classification as Skin Sens 1 without subcategorization.

This is supported by some of the studies in the NICNAS report. However, most of the NICNAS report studies could indicate classification as Skin Sens 1A due to the percentage of the persons with positive reactions. On the other hand, most of these studies were conducted with subjects suspected of contact dermatitis and neither the doses used in the studies nor the severity of the effects are sufficiently specified in the report. Since the studies included no induction phase, the persons reacting must have been pre-sensitized either to DBDCB or other substance (cross sensitization) or sensitizing reactions were confused with irritation common in contact dermatitis patients. The conclusion that the patients in the cited studies were pre–sensitized is supported by the outcome of the studies by Hasan et al (Patch test reactions to cosmetic allergens in 1995-1997 and 200-2002 in Finland, Contact Dermatitis , 53, 40-45, 2005), McFadden et al (Increased rate of patch test reactivity to methyldibromo glutaronitrile, Contact Dermatitis, 42: 54-55, 2000) and Wilkinson et al. (Monitoring levels of preservative sensitivity in Eorope., Contact Dermatittis, 46: 207-210

:2002) where the increasing trend in the percentage of individuals with positive reaction over the time rather indicates that pre-sensitized individuals were involved. Since DBDCB had been used in products applied daily on human skin (e.g. leave on cosmetics) it is assumed that frequent and long term exposure lead to pre- sensitization in patients with positive reaction. Such conclusion is also supported by the medical surveillance examinations performed on regular basis in the DBDCB manufacturing site in the USA. No cases of skin sensitization were reported while minor skin irritation was observed when proper skin protection was not used (Medical Statement Laxness Corporation US, 2015). This indicates that when exposure is infrequent, DBDCB does not provoke sensitizing reactions as expected from a strong sensitizer.

According to the cited CLP guidance care should be taken when classifying into category 1B when category 1A cannot be excluded. In such a case classification into category 1 should be considered. Thus, judging DBDCB classification based solely on human data classification into 1B is excluded.

According to the same guidance human data should be incorporated with animal data when deciding on the subcategorization. It is noted that according to the same guidance animal data are considered more reliable than human data. As the data are conflicting classification as **Skin Sens 1 without subcategorization is proposed.**

4.5.1.4 Comparison with criteria

According the Regulation (EC) No. 1272/2008 (CLP), substances shall be classified as skin sensitizers (category 1) where data are not sufficient for subcategorization if there is evidence in humans that the substance can lead to sensitization in a substantial number of persons or, if there are positive results from an appropriate animal test. The above weight of evidence approach leads to the conclusion that subcategorization into category 1B is not possible since, category 1 A cannot be excluded. As the data are conflicting classification as Skin Sens 1 without subcategorization is proposed.

4.5.1.5 Conclusions on classification and labelling

Proposed classification according to CLP: Classification as Skin Sens. 1; H317 “May cause an allergic skin reaction” is proposed.

4.5.2 Respiratory sensitisation

4.5.2.1 Non-human information

No data available. It is noted that according to Regulation (EC) No. 1272/2008 (table 3.4.1.) at present, recognized and validated animal models for testing of respiratory hypersensitivity are not available. It is of note that neither in the available acute nor in the repeated dose toxicity studies in animals any findings were made which would have indicated a respiratory sensitization potential of DBDCB.

4.5.2.2 Human information

No epidemiological studies are available for this endpoint despite the fact that DBDCB has been manufactured for several decades. This endpoint is not mentioned in the Proposed Acceptability for Continuing Registration (PACR2004-29) by Pest Management Regulatory Agency, Canada or in the Reregistration Eligibility Decision List B Case 2780 by USEPA. This suggests that the substance does not cause respiratory sensitization 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 sensitization were reported. (Medical Statement Lanxess Corporation US, 2015)

4.5.2.3 Summary and discussion of respiratory sensitisation

Though its structure suggests potential for macromolecular crosslinking and formation of immune-reactive adducts, there is no epidemiological or other evidence of respiratory tract sensitisation. In addition, neither in the available acute nor in the repeated dose toxicity studies in animals any findings were made which would have indicated a respiratory sensitization potential of DBDCB.

4.5.2.4 Comparison with criteria

According to the CLP Regulation a substance is to be classified if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity and/or there are positive results from an appropriate animal study. Since there is no such evidence in humans, nor is there an appropriate animal study available since there is no recognized and validated animal model for testing, the criteria of Regulation (EC) No. 1272/2008 for classification as a respiratory sensitizer are not fulfilled.

4.5.2.5 Conclusions on classification and labelling

No classification or labelling is proposed.

4.6 Repeated dose toxicity

Table 16: Summary table of relevant repeated dose toxicity studies

Route	**Duration of study, Method, Guideline GLP reliability**	**Species, Strain, Sex, no/group**	**Dose levels, Frequency of application**	**Results**	**LO(A)EL NO(A)EL**	**Reference DOC III A**
Dermal	21 days US-EPA 82-2 ≅ OECD 410 GLP- yes Reliability- 1	Rat Wistar (♂+♀) 5/sex	1000, 2000, 4000 mg/kg/day Once daily, 7 days per week,	At ≥ 1000 mg/kg bw/day: local irritation No systemic effects in any dose group	Systemic LOAEL and NOAEL could not be determined	**6.3.2 KEY STUDY**

			6 h/day			
Oral, feed	*In utero* (21 d), during lactation (21 d) and 13 wk post weaning US-EPA 82-1 ≅ OECD 408 GLP- yes Reliability-1	Rat, SD ♂+♀ 20/sex	83.5, 500, 3000 ppm, equiv. to ♂: 5.7, 33.8, 239.8 mg/kg/day ♀: 7.0, 39.3, 317.3 mg/kg/day	At 3000 ppm: body weight ↓, splenic haematopoiesis	LOAEL = 240 / 317 mg/ kg bw/day (♂/♀) NOAEL = 34 / 39 mg/kg bw/day (♂/♀)	6.4.1/01 **KEY STUDY**
Oral, feed	13 weeks Method ≅ OECD 409 GLP- yes Reliability -1	Dog Beagle ♂+♀ 4/sex	167, 1000, 4000 ppm, equiv. to ♂: 4.7, 28.9, 101.5 mg/kg/day ♀: 5.3, 37.7, 109.8 mg/kg/day	At 4000 ppm: clinical signs, bw gain ↓, haematological effects, and thyroid hyperplasia	LOAEL = 102/110 mg/ kg bw/day (♂/♀) NOAEL = 30/38 mg/kg bw/day (♂/♀)	6.4.1/02 **KEY STUDY**
Oral, feed	13 weeks Special study on thyroid hormones No guideline	Dog Beagle ♂+♀ 4/sex	167 ppm, equiv. to 5.9 / 5.7 mg/ kg/day (♂/♀)	No effects on basal and stimulated T3/T4 levels. No effects on thyroid histomorphology.	LOAEL > 5.7/5.8 mg/kg (♂/♀) NOAEL = 5.7/5.8 mg/kg bw/day (♂/♀)	6.10
Oral, feed	13 weeks US EPA 82-1 ≅ OECD 409	Dog Beagle ♂+♀ 4/sex	10, 100, 4000 ppm, equiv. to ♂: 0.29, 3.1, 102 mg/kg/day ♀: 0.33, 3.1, 119 mg/kg/day	At 4000 ppm: clinical signs, haematological and biochemical effects, bw ↓, thyroid enlargement	LOAEL = 102/119 mg/ kg (♂/♀) NOAEL = 3.1 mg/kg bw/day (♂+♀)	6.4.1
Dermal	2 years	Rats F344/N Mice B6C3F1 50M+50F	2,6,18 0.6, 2, 6 mg/kg.day in ethanol	18: lower body weight No systemic in any dose group. Local necrosis.	LOAEL=18mg/kg bw/day NOAEL=6mg/kg bw/day LOAEL=18mg/kg bw/day	NTP study, Technical report 2010 Summary in DOCIIA Appendix 2

4.6.1 Non-human information

The dermal subacute study in the **rat** is reflective of the apparent low percutaneous absorption of DBDCB (C., 1992, 6.3.2). Only severe topical effects were noted. No systemic effects were observable up to and including the highest dose level tested (4000 mg/kg bw/day). Therefore, the systemic NOAEL after dermal application is greater than 4000 mg/kg bw/day.

A **subchronic feeding** study in **rats** was designed so that the dietary exposure of weaned animals was preceded by dietary exposure of the parental animals prior to mating (one week) and of the mothers (throughout gestation and lactation) (W., 1980a, 6.4.1/01). Thus, when weanlings were first exposed via diet, they had already been potentially exposed to DBDCB or its metabolites *in utero* or via nursing. That way, the study also investigated potential effects on reproductive function and pre-natal development.

At the highest dose, the offspring showed lower birth weights and an 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 34 / 39 mg/kg bw per day (♂/♀).

Subchronic dietary exposure of **dogs** to DBDCB caused clinical signs of toxicity (diarrhea and/or soft stool, feed emesis and ataxia) at the highest dose level of 102 / 110 mg/kg bw per day (♂/♀) (W., 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 is concluded that the subchronic NOAEL for this dog study is 30 / 38 mg/kg bw per day (♂/♀).

The thyroidal effects seen in the study by W.(1980b, 6.4.1/02) were re-investigated in a special 13-week feeding study (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 (♂/♀).

A more recent 13-week feeding study (followed by a 3 months recovery period) in dogs (R. et al. 1994, A 6.4.1) revealed identical effects at the same LOAEL of ~100 mg/kg bw/day as were seen in the study by W.(1980b, 6.4.1/02) However, due to a poor choice of dose levels (40-fold difference between the mid- and high-dose level), the NOAEL in this study was very low at 3.1 mg/kg bw/day. Thus, the older dog study of W.(1980b, 6.4.1/02) is given preference for the derivation of an overall NOAEL for subchronic oral studies in the dog. The study by R.(1994, A6.4.1) was performed on groups of beagle dogs of 4-6 at 3 dose levels of approximately -0.3, 3.1 and 102 mg/kg.bw. Only in the highest dose group adverse effects were observed. The clinical signs included food like emesis, thin or weak appearance, diarrhea, prostration, trembling, reduced food consumption and body weight. Hematological changes included increased white blood cell counts and decrease in red blood cell count, hemoglobin concentration and hematocrit, increase in MCV and decrease in MCHC. Reticulocyte counts, platelet counts and segmented neutrophil counts were increased. In both male and female dogs effects on bone marrow including hypercellularity in erythropoietic cells and decrease in total myelogenous cells were observed. Biochemical changes in serum included decreases in calcium, phosphorus, alkaline phosphatase, albumin, glucose, alanine aminotransferase and total protein (females only) and slight increase of globulin in males. Urology revealed decrease in pH. All the differences in biochemical, hematological and urological parameters reversed within the 3 months of recovery period. The thyroid enlargement is attributed to the decreases in the thyroid hormones T3 and T4. On necropsy, effects on 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.

The authors of the study ascribed most of these finding to the thyroid gland hypofunction. Regarding anemia they used Direct Coombs test to exclude autoimmunity as the cause and make a reference to a study "Neuromuscular abnormalities associated with hypothyroidism and lymphocytic thyroiditis in three dogs" by Indrieri RJ, Whalen LR, Cardinet GH, Holliday TA. (J Am Vet Med Assoc. 1987 Mar 1;190(5):544-8,1987) to support the interpretation that hypothyroidism is its actual cause. The effects on bone marrow and hematological parameters were considered as secondary to anemia. The causal relation between thyroid hypo-function and the adverse effects on CNS is supported by referencing to the Handbook of Toxicologic Pathology, Chapter 21, Endocrine System by Capen, C.C et. al. (1991, Academic Press, New York, pp 675-760). The conclusion that the observed effects on testes were due to hypothyroidism are supported by citing Capen (Capen C.C. et.al. 1985 ,Chapter 3, The Endocrine Glands, In Pathology of Domestic Animals,3rd edition, Jubb, K.V.F. et al eds, Academic Press, New York, pp. 238-297) concludes that : " lack of libido, reduction in sperm count may occur in males and the spermatogenic epithelium in the testis is markedly atrophic in long-standing cases of hypothyroidism".

The NOAEL of the study of by R. (1994, A6.4.1) was the second highest dose of 3.1 mg/kg bw/day. The conclusion that the hypothyroidism brings about adverse effect on testes has been widely recognized since the performance of this study. This is confirmed by various studies some of which were reviewed by Wagner MS, Wajner SM and Maia AL.(Clinical implications of altered thyroid status in male testicular function, Arq Bras Endocrinol Metab. 2009;53/8) who wrote in the conclusion of their review: " In the past decades, it has become clear that thyroid hormone plays an important role in Sertoli and Leydig cell proliferation and function, also influencing spermatogenesis, sperm motility and ultimately fertility. Disturbance of the normal euthyroid state affects the morphological and functional development of the testis".

4.6.1.1 Repeated dose toxicity: oral

See above under "4.6.1 Non-human information".

4.6.1.2 Repeated dose toxicity: inhalation

No data available.

4.6.1.3 Repeated dose toxicity: dermal

See above under "4.6.1 Non-human information".

4.6.1.4 Repeated dose toxicity: other routes

No data available.

4.6.1.5 Human information

No data available.

4.6.1.6 Other relevant information

No data available.

4.6.1.7 Summary and discussion of repeated dose toxicity

No primary systemic adverse effects were observed as all the systemic effects are considered as secondary to local effects (GI tract irritation) or the effect of bromide on the thyroid gland.

4.7 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.7.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to the CLP Regulation

The guidance value for classification with STOT RE 2 is 100 mg/kg bw/day for rats. The effects on the thyroid were observed at 102 mg/kg bw/day in dogs. These effects are likely to be due to bromide effect on the thyroid. To decide 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 publically available sources were compared. The T_3/T_4 half lives are 6 hours/ 12-24 hours in rats and 6 hours/ 10-16 hours in dogs (Neepa Y. Chksi et al, Birth Defects Research (Part B) 68:479–491 (2003); Traon G.LE, 2007; De Clementi C, 2001). The half life of bromide in dogs ranges between 15- 46 days whereas that in rats ranges from 3 to 8 day.(Hope E. Baird-Heinz et al , 2012 in Journal of the American Veterinary Medical Association 240(6):705-15 ·; MARCH P.A. et al , 2002 in J. vet. Pharmacol. Therap.25,425–432, 2002; Pavelka S in Appl Radiat Isot. 2009 Dec;67(12):2110-2. doi: 10.1016/j.apradiso.2009.05.001. Epub 2009; Pavelka S., 2005 in Biological Trace Element Research January 2005, Volume 103, Issue 1, pp 49–58|). Thus in terms of T_3/T_4 half-lives, rats and dogs are comparable, whereas in terms of bromide accumulation dogs are more prone to adverse effects by 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). As a result, 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. This conclusion is further supported by the fact that 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. Therefore, a classification with STOT RE 2 is not justified.

4.7.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

The guidance value for classification with STOT RE 2 is 100 mg/kg bw/day for rats. This value is exceeded and it is therefore concluded that the criteria for a classification with STOT RE are not fulfilled.

4.7.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

No classification or labelling with respect to STOT RE effects is proposed

4.8 Germ cell mutagenicity (Mutagenicity)

Table 17: Summary table of relevant in vitro and in vivo mutagenicity studies

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 – 1000 µg/ plate (prelim. test) 0.2-100 µg/ plate (main test)	neg.	neg.	Cytotoxicity: –S9: ≥ 50 µg/plate +S9: 1000 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- 2500 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**

Test system, Method, Guideline GLP Reliability	**Organism/ strain(s)**	**Concentrations tested**	**Result**		**Remark**	**Reference**
			– S9	**+ S9**		
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 is not influential 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 is not influential 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
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**

Test system, Method, Guideline GLP Reliability	Organism/ strain(s)	Concentrations tested	Result		Remark	Reference
			– S9	+ S9		
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, 3000 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**

4.8.1 Non-human information

4.8.1.1 In vitro data

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.

4.8.1.2 In vivo data

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.

This reduces the need for further *in vivo* testing in somatic tissues other than bone marrow. It is proposed that the *in vitro* UDS assay in human fibroblasts is of adequate detection power to cover the formal BPD requirements for a confirmatory assay. A detailed justification for the non-submission of further *in-vivo* studies is provided in the biocides dossier.

A dominant-lethal assay in mice demonstrates that DBDCB is not a germ cell mutagen.

4.8.2 Human information

No data available.

4.8.3 Other relevant information

No data available.

4.8.4 Summary and discussion of mutagenicity

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.

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This reduces the need for further *in vivo* testing in somatic tissues other than bone marrow. It is proposed that the *in vitro* UDS assay in human fibroblasts is of adequate detection power to cover the formal BPD requirements for a confirmatory assay.

A dominant-lethal assay in mice demonstrates that DBDCB is not a germ cell mutagen.

4.8.5 Comparison with criteria

According to the CLP Regulation the classification for mutagenicity is based on positive evidence on humans or on positive evidence obtained from experiments in mammals and/or from in vitro experiments. For more details on the CLP criteria see section 3.5.2 therein. Since no positive evidence was obtained in the available studies the criteria for classification with respect to germ cell mutagenicity are not fulfilled.

4.8.6 Conclusions on classification and labelling

No classification for mutagenicity is proposed.

4.9 Carcinogenicity

Table 18: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
Carcinogenicity study , dermal route	No neoplastic or non-neoplastic lesions observed	50 ♂ and 50 ♀ rats per dose 50 ♂ and 50 ♀mice per dose	NTP TR 555, Tox. and Carc. Studies of DBDCB, NIH Publication 10-5896

4.9.1 Non-human information

4.9.1.1 Carcinogenicity: oral

No study performed.

4.9.1.2 Carcinogenicity: inhalation

No study performed.

4.9.1.3 Carcinogenicity: dermal

Carcinogenicity testing of DBDCB via the dermal route has been conducted in rats and mice (NTP technical report 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 per kilogram of body weight, and similar groups of male and female mice received 0.6, 2, or 6mg DBDCB per kg. Groups of 50 animals receiving just the ethanol solution served as controls. Tissues from more than 40 sites were examined for each animal.

In the study with mice, the dosing was 0.6, 6 and 6 mg/kg bw of DBDCB provided in 95% ethanol 5 days per week. The number of animals per dose /per sex was 50.

Survival of animals exposed for 2 years to 1,2-dibromo-2,4-dicyanobutane was the same as for the controls in both rats and mice, but rats exposed to the highest concentrations weighed less by 7% 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 the 3 doses). At the highest dose epidermal necrosis at the site of application was significantly increased in females. These effects correlate with the above decrease in the weight of the top-dosed animals, and confirm that the dosing was properly selected for the study as higher doses could lead clinical signs or lesions, other than systemic neoplastic lesions, that could result in shortening the lifespan of the tested animals.
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. Like in the rat, these effects confirm that the dosing was correctly selected, as higher doses could lead to clinical signs or lesions, other than systemic neoplastic lesions, that could interfere with the study goal via shortening the lifespan of the tested animals.
Dermal exposure to DBDCB was not associated with 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), the highest dose levels tested.

4.9.2 Human information

It is noteworthy that despite its long and widespread use in exposure-intensive applications, e.g. in cosmetics and toiletry articles, there are no reports on adverse effects, besides contact dermatitis, of DBDCB in humans in the published literature.

4.9.3 Other relevant information

DBDCB has been extensively tested for potential genotoxic effects in a battery of *in vitro* and *in vivo* studies. A single *in vitro* chromosome aberration test was positive, albeit at concentrations at which cytotoxic effects of DBDCB on the indicator cells became evident. However, all confirmatory assays, including micronucleus and chromosomal aberration assays in rodents, were clearly negative. Thus, the overall conclusion is that DBDCB does not possess a mutagenic potential.

4.9.4 Summary and discussion of carcinogenicity

The negative results of the above dermal carcinogenicity studies, the conclusion that DBDCB is not mutagenic, the absence of pre-neoplastic lesions in the available subchronic studies in rats and dogs as well as the absence of reports of relevant adverse effects in humans strongly indicate that DBDCB is not carcinogenic.

4.9.5 Comparison with criteria

According to the CLP Regulation a substance is classified for carcinogenicity on the basis of evidence obtained from human and /or animal studies. The strength of evidence then determines the category to which the substance is placed. More details on the criteria are provided in the section 3.6. of the cited regulation. No such evidence was obtained in humans or from available animal studies the criteria for a classification with respect to carcinogenicity are considered not to be fulfilled.

4.9.6 Conclusions on classification and labelling

No classification or labelling is proposed for this endpoint.

4.10 Toxicity for reproduction

Table 19: Summary table of relevant reproductive toxicity studies

Route of exposure	**Test type, Method, Guideline**	**Species, Strain, no/group**	**Exposure Period**	**Doses**	**Critical effects 1) dams 2) foetuses**	**NOAEL / LOAEL Maternal toxicity**	**NOAEL / LOAEL Teratogenicity, Embryo-toxicity,**	**Reference**
Oral	Develop-mental toxicity Range finding study No guideline	Rat Sim:(SD)fBR ♀ 8/group	Day 6-15 of gestation	50, 125, 175, 250 mg/kg/day	1) At 250 mg/kg bw/day: mortality 3/8 2) At 250 mg/kg bw/day: viability ↓, bw ↓, dead/resorbed foetuses ↑	NOAEL = 175 mg/kg bw/day LOAEL = 250 mg/kg	NOAEL = 175 mg/kg LOAEL = 250 mg/kg	H., 1982, A6.8.1
Oral	Develop-mental toxicity US-EPA 83-3(a) ≅ OECD 414 GLP-yes Reliabilty - 1	Rat Sim:(SD)fBR ♀ 28-30/group	Day 6-15 of gestation	25, 100, 175 mg/kg/day	1) At 175 mg/kg: bw gain ↓ 2) No significant effects	NOAEL = 100 mg/kg bw/day LOAEL = 175 mg/kg bw/day	NOAEL = 175 mg/kg LOAEL > 175 mg/kg	H, 1982, 6.8.1/02 **KEY STUDY**
Oral	Develop-mental toxicity US-EPA 83-3 ≅ OECD 414 GLP-yes Reliabilty - 1	Rabbit NZW 20/group	Days 6-18 of gestation	10, 30, 60 mg/kg	1) At 60 mg/kg: bw gain ↓, feed consumption ↓ 2) No significant effects	NOAEL = 30 mg/kg bw/day LOAEL = 60 mg/kgbw/day	NOAEL = 60 mg/kg LOAEL > 60 mg/kg	D., 1994 6.8.1/01 **KEY STUDY**

4.10.1 Effects on fertility

4.10.1.1 Non-human information

A two-generation reproduction study in rats has not been conducted. Instead, the design of the 90-day feeding study in rats was combined with a one-generation reproduction study to allow an assessment of reproductive effects and effects of prenatal exposure to DBDCB (W.,1980a, 6.4.1/01,). This design comprised an initial one-generation reproduction study similar to OECD Guideline 415. The parental generation was fed the test compound for one week and then mated for a period of two weeks. The study is comparable with the OECD extended one generation reproductive toxicity study protocol in regulatory risk assessment. It differs from the guideline by the pre-mating exposure period of only one week compared to two weeks prescribed in the guideline and the number of parent animals of 10 per sex (the guideline recommends 20). According to the guideline dosing during the premating period should be sufficiently long to achieve a steady state level of the substance, but should take at least two weeks. Within one week of premating 90% of steady state concentration is reached thus the second condition of two weeks premating exposure duration is not fulfilled. However, this deviation from the guideline is covered by the weight of evidence drawing on the relevant endpoints in the other studies provided below.

There were no effects on litter size and sex ratio. A selected part of the F1 offspring was then subjected to subchro nnic dietary exposure as in a conventional subchronic study according to OECD Guideline 408. In the F1 generation, there were no macroscopic or microscopic findings in the reproductive organs (testes/epididymis, prostate gland, uterus, ovaries). Thus none of the triggers for the assessment of the second generation listed in the OECD guideline 117 are present. Then LOAEL based on reduced body weights and splenic hematopoiesis was 240/317 mg/kg bw/day (♂/♀). The corresponding NOAEL was 34/39 mg/kg bw/day (♂/♀).

An indication of the absence of effects on male reproductive performance can be extracted from the dominant-lethal study in mice (W., 1980, 6.6.6; see Section 4.8.1.2, p.47). Unlike in the above 90 days study with prenatal exposure, in this study male mice were exposed to the substance throughout the premating period of 8 weeks (one spermatogenic cycle). No effects at any dose level of DBDCB were noted on pregnancy rates, incidences of resorptions, foetal death, dead implantations, and 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 the subchronic dog studies (R.,1994, A6.4.1; W., 1980b, 6.4.1/02; see Section 4.6.1, pp. 42-43), effects on testes (immature appearance, mild degeneration of the seminiferous tubules) were observed in the highest dose group (4000 ppm). This effect is likely to be secondary to the overt toxicity observed in this dose group (weight loss and mortality) and the effects on 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 studies in rats and rabbits were submitted (H., 1982, A6.8.1.,; D., 1994

6.8.1/01).

In this study, maternal toxicity included 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 ($p<0.01$). Further analyses using the Dunnett test showed that the 175 mg/kgbw/day group was significantly different from the control group. There were no significant differences between the control and treated groups for the mean number of corpora lutea ($p=0.389$).

Regarding prenatal phase there were no noticeable differences between the control and treated groups in the percent 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.

Similarly, at 175 mg/kg bw/day two dams had 7 resorptions each thus amounting to 14 of the 28 resorptions observed. No significant difference was observed for embryolethality (number dead plus number resorbed divided by the number of implantations) using the Kruskal-Wallis test ($p=0.169$). However, a significant difference was observed for this parameter using the modified Jonckheere test ($p=0.024$). No significant differences were observed for the mean number of implantations ($p=0.939$) litter size ($p=0.638$) or foetal body weights ($p=0.364$). There was an apparent numerical increase in the percent dead and resorbed foetuses (see Table A6_8_1-2).

Malformations: Four foetuses (4/262) in the high-dose group were classified as runts (category: external malformation). This finding was not made in the other groups. The incidence was not statistically significant ($p=0.116$).

Variations: Visceral variations were observed in all groups, with the exception of the 25 mg/kg dose level. Hydroureter and renal cavitation were the two visceral variations observed. Analysis of hydroureter was not significantly different ($p=0.158$).

Several types of variations associated with skeletal ossification patterns were observed in the control and treated groups. The most frequent observations involved absent or incompletely ossified sternebrae (primarily numbers 5 and 6), and rudimentary ribs (number 14). Both categories were statistically analyzed, as was the classification "All Skeletal Variations Combined". The only classification showing significance was Rudimentary Ribs (= 0.008, Kruskal-Wallis test). Both the 100 and 175 mg/kg bw/day dose levels were significantly different from the control group by the Gladen test, however, inspection of the data showed that the two high dose groups had fewer rudimentary ribs compared to the control group. (see Table A6_8_1-3)

In the rabbit study (D., 1994, 6.8.1/01) two control group does aborted on days 24 and 28 of gestation. One 30 mg/kg/day dosage group doe was found dead on day 25 of gestation, and one 60 mg/kg/day dosage group doe was sacrificed for moribund. The moribund condition of doe was considered unrelated to the test substance and the sequelae of an intubation accident. The abortions and death were also considered unrelated to the test substance because the incidences were not dosage-dependent. No other does died, were sacrificed moribund or aborted, and no does prematurely delivered a litter.

All clinical and necropsy observations were considered unrelated to the test substance, including those in the does that were found dead, sacrificed moribund or aborted, because: 1) the incidences were not dosage-dependent; 2) the incidences were not statistically significant; 3) the observation occurred in only one doe; or 4) the observation was common in this species.

Transient weight loss occurred in the 60 mg/kg/day dosage group on days 6 to 9 of gestation; the control group and the two other groups given the test substance gained weight during this period. When calculated for the entire dosage period (days 6 to 19 of gestation), comparable maternal body weight gains occurred in the four dosage groups. No statistically significant differences occurred in maternal body weight gains or body weights during the dosage period.

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. When calculated for the entire dosing period (days 6 to 19 of gestation), these parameters were unaffected by dosages of the test substance as high as 60 mg/kg/day. No remarkable differences occurred in feed consumption during the post-dosage period (calculated as days 19 to 29 of gestation), and there were no statistically significant differences in the values for the four dosage groups during the dosage or post-dosage periods.

Caesarean-sectioning and litter parameters were unaffected by dosages of the test substance as high as 60 mg/kg/day. 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 effects of the test substance because: 1) the observations are frequent in this strain of rabbit; 2) the incidences were within the ranges observed historical1y 3) the incidences were not statistically significant; and/or 4) the alterations occurred in only one high dosage group foetus.

Summary of reproductive toxicity

The 90-day feeding study in rats combined with a one-generation reproduction study (W., 6.4.1/01 1980a, see Section 4.6.1, p.41) revealed no substance related reprotoxicity.

The absence of effects on male reproductive performance is supported by the outcome of the dominant-lethal study in mice (W., 1980, 6.6.6; see Section 4.8.1.2, p.47) where no effects at any dose level of DBDCB were noted on pregnancy rates, incidences of resorptions, fetal death, dead implantations, and fetal viability.

The subchronic dog studies (R., 1994, A6.4.1; W., 1980b, 6.4.1/02), reported no effects on male or female reproductive organs apart from the effects on the testis at the highest does considered to be secondary to general toxicity and effect on thyroidea.

Maternal toxicity, evident as impaired body weight gain, was the only significant and relevant effect of DBDCB in the main developmental toxicity studies in rats and rabbits.

In the rat (H., 1982, 6.8.1/02), data for prenatal measures of toxicity were not significantly different between the control and treated groups, with the exception of embryolethality. This finding was difficult to interpret because at the two high dose groups, resorptions were clustered in two litters

with >7 resorptions 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 skeletal variation, rudimentary ribs, was significant; however, the two high dose levels were found to have 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 significant.

The data indicates that there may be a slight increase in prenatal toxicity at 175 mg/kg due to significant embryolethality. However, in the absence of other conventional signs of embryotoxicity, i.e., malformations and foetal weight reduction, this finding should be considered biologically insignificant.

The assessment of reproductive toxicity of DBDCB should take into account the effects of bromide ion released from the DBDCB molecule and cumulating in tissues at higher daily intakes. Exposure to DBDCB at LOAE levels of 60 – 250 mg/kg bw corresponds to daily bromide intake of 36 - 150 mg/kg bw. LOAEL/ NOAEL values of 1200/300 mg bromide/kg of diet determined in a 3-generation test in rats (cited in JMPR, 1988) correspond approximately to 72/18 mg bromide/kg bw per day. Fertility and the viability of the offspring were significantly reduced at 4800 mg bromide/kg of diet (approx. 300 mg bromide/kg bw per day). Exposure to DBDCB at NOAE level corresponds to daily bromide intake of 18 mg/kg bw.

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

In summary, it is concluded that DBDCB does not produce treatment-related and/or substance-specific reproductive/developmental effects.

4.10.1.2 Human information

No data available.

4.10.2 Developmental toxicity

4.10.2.1 Non-human information

See above under “4.10.1.1 Non-human information”.

4.10.2.2 Human information

No data available.

4.10.3 Other relevant information

No data available.

4.10.4 Summary and discussion of reproductive toxicity

No classification for reproductive/developmental toxicity and teratogenicity is justified.

4.10.5 Comparison with criteria

According to the CLP Regulation a substance is classified as reproductive toxicants based on evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development. The strength of such evidence then determines the category to which the substance is placed. No such evidence is provided in the available studies and therefore, the criteria, specified in detail in section 3.7 of the cited regulation, for a classification with respect to reproductive toxicity are considered not to be fulfilled.

4.10.6 Conclusions on classification and labelling

No classification or labelling with respect to reproductive/developmental toxicity and teratogenicity is proposed.

4.11 Other effects

4.11.1 Non-human information

4.11.1.1 Neurotoxicity

DBDCB bears no structural similarity to organophosphates, carbamates or other known inducers of delayed neurotoxicity. Studies in several species did not indicate occurrence of neurotoxic effects.

4.11.1.2 Immunotoxicity

The available data do not suggest potential for immunotoxicity. Studies in several species did not indicate the occurrence of immunotoxic effects. The only genuine adverse systemic effect observed was the effect on thyroid gland due to bromide released from DBDCB molecule. All other adverse effects were considered as either secondary to this effect or to the local effects (e.g. GI tract irritation).

4.11.1.3 Specific investigations: other studies

No data available.

4.11.1.4 Human information

No data available.

4.11.2 Summary and discussion

DBDCB is neither considered as neurotoxic nor immunotoxic.

4.11.3 Comparison with criteria

The criteria include evidence from humans and observations from experimental animals. The dose at which the effect takes place is a decider regarding placing the substance into the category. The criteria are not fulfilled and the substance is not classified for other effects.

4.11.4 Conclusions on classification and labelling

No classification for other effects such as neurotoxicity or immunotoxicity is proposed.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 20: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Stability			
Hydrolysis, U.S. EPA, 161-1	pH 5 Acetate: DT_{50} = 3884 days pH 7 HEPES: DT_{50} = 51.6 days (146.4 days at 12ºC) pH 7 TRIS: DT_{50} = 96.3 days(273.3 days at 12ºC pH 9 Borate: DT_{50} = 9.10 days (25.8 days at 12ºC) (25 ± 1 °C, Duration 30 days) E-Z isomers and 2-methyleneglutaronitrile (2-MGN): > 10% of the initial measured dose	Measured.	G., W. ,1996 7.1.1.1.1/01
Photolysis in water, EPA-FIFRA N-161-2	Photolytic half-life in aqueous buffered solution (pH 5): DT_{50} experimental: 54 days (exposed) and 381 days (non-exposed), first order kinetics (25 °C, 30 days irradiation) According to OECD TG 316 the DT_{50} for irradiated samples corrected for degradation in non-irradiated control is 62 days. One major metabolite > 10% was formed: 2-methyleneglutaronitrile (28.5% after 30 days)	Measured.	S.; K. W (1992, 7.1.1.1.2/01
Photolysis in air, QSAR-calculation, AOPWIN, v. 1.91, 2000	DT_{50}= 27.107 days	Estimated value.	F., 2006, 7.3.1/01
Biodegradation			
Ready biodegradability, OECD 301D	Not readily biodegradable according to Closed Bottle Test; 0% biodegradation after 28 days (3 mg a.s./L)	Measured.	M. & R, 1995, 7.1.1.2.1/01
Inherent biodegradability, Zahn Wellens ≅ OECD 302B	2% ultimate biodegradation Degree of a. s. degradation: 65% (primary degradation)	Measured.	R., 2007, 7.1.1.2.2/01

5.1.1 Stability

Hydrolysis

- **DBDCB**

The hydrolysis of radiolabelled DBDCB (1,2-dibromo-2,4-dicyano-butane,) was studied by G. and W. (1996, 7.1.1.1.1/01) in four aqueous buffer solutions at a nominal test concentration of 10 ppm at 25 ± 1 °C according to U.S. EPA guideline 161-1. The buffers used were: pH 5, 0.01 M acetate

buffer; pH 7, 0.01 M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer; pH 7, 0.01 M Tris(hydroxyl-methyl) aminomethane (TRIS) buffer; and pH 9, 0.01 M borate buffer. The concentrations of DBDCB in each test sample and its hydrolysis products were determined using HPLC. Liquid scintillation counting was used to determine the total radioactivity of the test substance at each sample point.

During the 30 day study period, DBDCB did not hydrolyze at pH 5 and was demonstrated to hydrolyse slowly at pH 7 (DT_{50} = 51.6 and 96.3 days corresponding to 146.4 and 273.3 days at 12 ^{o}C). DBDCB was subject to base-catalyzed hydrolysis at pH 9 (DT_{50} = 9.1 days corresponding to 25.8 days at 12^{o}C). The major hydrolysis products were the E-Z isomers of 1-bromo-2,4-dicyano-1-butene as well as 2-methyleneglutaronitrile (2-MGN), exceeding 10% of the initial measured dose at pH 9 and 7, respectively.

At neutral and acidic pH DBDCB is considered to be hydrolytically stable; hydrolysis will only contribute to some extent to the degradation of the active substance in basic pH.

- **2-MGN**

With respect to the hydrolysis of 2-MGN it can be concluded from its specific structure belonging to the chemical class of nitriles that a hydrolytic decomposition is very likely to occur. The decomposition pathway of nitriles is described in literature (March 1985), which proceeds in a stepwise mode via formation of carboxylic acid amides $RC(=O)NH_2$ and then carboxylic acids RCOOH or carboxylic acid salts.

This decomposition pathway of 2-MGN, is not, however relevant for pH 7 and 9. This can be concluded from the results of the hydrolysis study with DBDCB which strongly indicates that 2-MGN is hydrolytically stable at (between) pH 7 and pH 9. No conclusion can be made on hydrolysis at pH 5 as no significant amounts of 2-MGN are formed due to the hydrolytic stability of the parent compound.

Photolysis in water

- **DBDCB**

A photolysis study was conducted with radiolabelled DBDCB at 25 ± 1°C in aqueous solution buffered at pH 5. The study was performed according to EPA-FIFRA N-161-2, using a test concentration of 10 µg/L. Samples were exposed to a xenon arc lamp or placed in the dark in a temperature controlled environmental chamber. Quantification of DBDCB and characterisation of photolysis products was conducted by TLC/autoradiography, LSC and HPLC.

The parent compound was subject to photodegradation during the course of this experiment. Its amount in the pH 5 buffered aqueous medium declined from 100% at day 0 to 67.2% of the initial dose at day 30. In the non-exposed system, still 95.8% of the initially applied dose was present at day 30.
Photolysis rate constants and half-life values were 0.0129 $days^{-1}$ (DT_{50} = 54 days) for the exposed samples and 0.0018 $days^{-1}$ (DT_{50} = 381 days) for the non-exposed samples (first order kinetics). A correction of the photolysis half-life for hydrolysis processes (attributing to degradation in dark control samples), yields a half-life of 62 days (according OECD TG 316).

The primary product of photolysis was 2-methyleneglutaronitrile (28.5% after 30 days), due to debromination (-Br_2) of DBDCB. The (E)- and (Z)-1-bromo-2,4-dicyano-1-butene isomers, formed via dehydrohalogenation (-HBr), were found only at one sampling event and at low amounts (2.1% at day 14 in exposed samples).
A correlation (r) of 0.994 for the exposed system confirmed the first order photodegradation rate for DBDCB.
The results of the study reveal that DBDCB is susceptible to photodegrade in aqueous media and that this reaction follows first order kinetics. The compound 2-methyleneglutaronitrile is the main photoproduct.

- **2-MGN**

The primary photodegradate of DBDCB was 2-MGN with 15.8% of the applied radioactivity (AR) at day 14 increasing to 28.5% AR at day 30, due to debromination (-Br_2) of DBDCB. The (E)- and (Z)-1-bromo-2,4-dicyano-1-butene isomers, formed via dehydrohalogenation (-HBr), were found only at one sampling event and at low amounts (2.1% at day 14 in exposed samples) and are therefore not discussed in the following.

For characterization of the environmental fate of 2-MGN, a UV-Spectrum for the wavelength 200-800 nm was experimentally determined, in accordance with the Tier-1 ‘Theoretical Screening’ in OECD TG 316. No direct UV-absorption occurred in the environmentally relevant wavelength between 290 and 750 nm indicating that a half-life ≤ 30 days due to direct photolysis is not applicable for 2-MGN. According to the decision scheme in the OECD TG 316 no Tier-2 photolysis study is required in this case. The implication that 2-MGN is stable to photolytic decomposition is supported by the results from the photolysis study with DBDCB. Analytical data in this study shows a continuous increase of 2-MGN until study termination at day 30. Thus, there is no hint for a photolytically induced decomposition of 2-MGN.

Photolysis in air

The tropospheric half-life of DBDCB was estimated using the AOPWIN program (v. 1.91, 2000) (F., 2006, 7.3.1/01). The software is based on a quantitative structure analysis developed by Atkinson. The Atkinson calculation method sums up the reactivity towards OH radicals of all structural elements. Using a mean daily OH concentration in air of 0.5 x 10^6 OH radicals per cm^3, a half-life of DBDCB in the troposphere was calculated to be 27.107 days with a degradation rate of 0.5919 x 10^{-12} cm^3 * $molecule^{-1}$ * s^{-1}.

DBDCB fulfils with a half–life of 27.107 days in air one POP – criterion of the Stockholm – Convention on Persistent Organic Pollutants, but due to the usage of the substance for conservation of packed products, and to its low vapour pressure (at 20 °C: 3.81x10^{-3} Pa) and Henry´s Law constant (3.99 x 10^{-5} Pa x m^3 x mol^{-1} at 25 °C, calculated using EPI WIN), its occurrence in air is not to be expected.

DBDCB is restrictedly susceptible to photolytic processes in air. However, the air is no compartment of concern for DBDCB due to its limited potential for volatilisation (vapour pressure = 3.81 x 10^{-3} Pa (at 20 °C) and Henry´s Law Constant = 3.99 x 10^{-5} Pa at 25 °C (EPIWIN calculation)).

5.1.2 Biodegradation

- **DBDCB**

Ready biodegradability

The test on ready biodegradability of DBDCB was performed by M.,R.,(1995, 7.1.1.2.1/01) according to OECD Guideline for Testing of Chemicals 301D, Closed Bottle Test (1992), and the Official Journal of the European Communities, Part VI, Method C.4-E (1992). The test substance was incubated with a STP effluent inoculum for a period of 28 days at 20 ± 1°C. Sampling was conducted at appropriate intervals to include the 10-d window.

DBDCB showed a -35% degradation after 28 days as measured by the dissolved oxygen depletion. The Positive Control, sodium benzoate, yielded 87% of the theoretical dissolved oxygen during the course of the test.

The potential for microbial inhibition by the test article was assessed in a series of Inhibition Control BOD bottles. In this experiment, the Inhibition Control (1.6 mg BOD/L after 28 days) was significantly lower than the Positive Control (2.6 mg BOD/L), indicating an inhibition of microbial action, which occurred especially during the last week. This inhibition caused the test vessels to respire less than the blank vessels (organisms' endogenous respiration) and led to a negative value for percent inhibition. The negative value is physically not significant and biodegradation should be interpreted as zero percent.

Inherent biodegradability

DBDCB was investigated for its inherent biodegradability in a Zahn-Wellens/EMPA Test (OECD test guideline 302B) at 21 °C and pH ranging from 7.2-7.6 over a period of 28 days. The biodegradation was determined by following the DOC (Dissolved Organic Carbon) of the test item in the incubation flasks during exposure. In addition, the test item concentration was measured in specimens by HPLC methods at appropriate sampling dates.

According to the test guideline for OECD 302 B in order to achieve a sufficient carbon concentration for the DOC analysis this study was performed with a DBDCB concentration which is inhibitory to bacteria. Based on the test parameter DOC, the test item was found to undergo a limited ultimate inherent biodegradation under the test condition (2% at termination of the test). The test item's specific analysis showed a reduction to 35% (mean) of the initial concentration after 28 days of incubation (65% degradation). Hence, the chemical analysis showed a clear disappearance of DBDCB with time, which can be considered as primary degradation, yielding one or more degradation products. Since the hydrolysis rate at the test pH (7.2 -7.6) is not determined it is impossible to quantify the relative contributions of hydrolysis and biodegradation to the degradation process under the test conditions. The low hydrolysis rate determined at neutral pH rather indicates that the degradation of the DBDCB under the test conditions can be primarily attributed to biodegradation.

2-Methyleneglutaronitrile (2-MGN, degradation product of DBDCB) was analysed in parallel. It was found that the 2-MGN amount increased with decreasing DBDCB concentration. After 28 days, the 2-MGN concentration was about 48% (mean).

The sum of DBDCB and 2-MGN concentrations at day 28 was about 154 mg/L (mean). Compared to the starting concentration which was calculated to be about 192 mg/L (mean), this means a loss of 38 mg/L, corresponding to about 20% of the initial concentration. Taking into consideration 2% ultimate biodegradation, the loss of DBDCB and 2-MGN can be attributed to a further transformation into not identified degradation products.

The reference item diethylene glycol was sufficiently degraded by mean 98% after 14 days, and to 100% after 28 days of incubation, thus confirming the suitability of the used activated sludge inoculum.

- **2-MGN**

In the aerobic aquatic degradation study of DBDCB, the metabolite 2-MGN increased from 13.2% of the initial measured dose at day 0 to 34.1% at day 3. Afterwards, 2-MGN amounts decreased to 0.798% until termination of the test at day 21 with a minimum of 0.499%. In parallel, CO_2 formation increased. The decrease of 2-MGN during the course of the study proves that 2-MGN is an unstable breakdown product of DBDCB, and is of transient character. This is supported by the modelling output on ready biodegradability from BIOWIN v4.10 model calculations gained with the EPI Suite Tool developed by the U.S. Environmental Protection Agency in addition to the physico-chemical properties of 2-MGN.

When comparing the numeric and time-indicating results from the different Biowin Models 1 to 7 for the main metabolite 2-MGN, a clear prediction in favour of a rapid (Biowin1 and Biowin2) and ultimate biodegradation (Biowin3) of the substance is given. Supportive data for the assumption of a fast biodegradation of 2-MGN are the high water solubility (35.8 g/L) and the low Koc value (72.24 L/kg), which indicate that the substance will stay in the water phase and will thus be bio-available for microbial populations. Moreover, the results from the aerobic aquatic metabolism study prove that the concentration of 2-MGN rapidly declines from the maximum concentration at study day 3 (34.1% of initially measured dose) to less than 1% at study termination. Between study day 3 and study day 14 the measured concentration of 2-MGN already fell from 34.1% to 3.01% of IMD representing a reduction of about 91% within 11 days. Assuming first order kinetics this corresponds to a rate constant of 0.22 $days^{-1}$ from which a DT_{50} of 3.1 days is calculated at 25°C temperature of the study. This corresponds to a DT_{50} of 8.77 days at 12°C strongly indicating that the substance is not persistent in fresh water. Furthermore, the outcome of the hydrolysis study on DBDCB shows that 2- MGN is not hydrolysed at pH 7 and 9 and therefore contribution of hydrolysis to 2-MGN decomposition in the aerobic aquatic degradation study is considered negligible. Hence, 2-MGN DT_{50} is indicative of DT_{50} of biodegradation.

Table 21: Probability of Rapid Biodegradation of 2-MGN using BIOWIN 4.10

Method	Value
Biowin1 (Linear Model)	**1.3110** [1]
Biowin2 (Non-Linear Model)	1.0000 [1]
Expert Survey Biodegradation Results:	

Biowin3 (Ultimate Survey Model)	2.7999 weeks [2, 5]
Biowin4 (Primary Survey Model)	3.5642 days-weeks [3]
MITI Biodegradation Probability (only for degradation in the OECD 301-C test):	
Biowin5 (MITI Linear Model)	0.6509 [4, 5]
Biowin6 (MITI Non-Linear Model)	0.6997 [4]
Anaerobic Biodegradation Probability:	
Biowin7 (Anaerobic Linear Model)	0.7410 [1]
Ready Biodegradability Prediction:	**YES** [5]

[1] Fast degradation is defined as predicted probability > 0.5

[2] Expected time for complete mineralisation

[3] Expected time frame for primary biodegradation

[4] The biodegradation probability is defined positive when the value is >= 0.5

[5] YES = readily biodegradable (if the Biowin3 (ultimate survey model) result is "weeks" or faster AND the Biowin5 (MITI linear model) probability is >= 0.5, then the prediction is YES; if this condition is not satisfied, the prediction is NO).

5.1.3 Summary and discussion of degradation

DBDCB was not “readily biodegradable” under the conditions of the test (OECD guideline 301D) due to microbial inhibition, as observed in the Inhibition Control. DBDCB failed to qualify for classification as “inherently biodegradable” according to the definitions given by the OECD guideline 302B. However, DBDCB is primary inherently biodegradable. DBDCB was reduced to 35% of the initial concentration (65% degree of a. s. degradation) and transformed to 2-methyleneglutaronitrile and other degradation products.

The data on the (bio-) degradability of 2-methyleneglutaronitrile show that it undergoes rapid biodegradation in the fresh water.

During the 30 day study period, DBDCB did not hydrolyze at pH 5 and was demonstrated to hydrolyse slowly at pH 7 (DT_{50} = 51.6 and 96.3 days corresponding to 146.4 and 273.3 days at 12 ^{o}C). DBDCB was subject to base-catalyzed hydrolysis at pH 9 (DT_{50} = 9.1 days corresponding to 25.8 days at 12 ^{o}C). The major hydrolysis products were the E-Z isomers of 1-bromo-2,4-dicyano-1-butene as well as 2-methyleneglutaronitrile (2-MGN), exceeding 10% of the initial measured dose at pH 9 and 7, respectively.

Taking into account the results of the experimental ready biodegradation and inherent biodegradation studies, DBDCB cannot be considered as rapidly biodegradable for classification purposes according to Regulation (EC) No. 1272/2008.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Adsorption / desorption in soil

Screening test

An adsorption/desorption OECD screening test using High Performance Liquid Chromatography (HPLC) is not considered as being relevant due to the results of a reliable batch equilibrium study conducted with DBDCB (see below).

Adsorption and desorption in four soil types

The adsorption/desorption behaviour of radiolabelled DBDCB has been investigated on four soils (sand, sandy loam, clay loam and silt loam), using the batch equilibrium method. The test was conducted according to the U.S. EPA, 163-1 guideline.

The adsorption phase was carried out by shaking pre-equilibrated air dried soil (3 g soil to 10 mL solution) with DBDCB at concentrations of 1.0, 0.75, 0.5 and 0.01 g a.s./mL in darkness at 25 ± 1 °C for 4 hours (equilibrium time). 0.01 M aqueous $CaCl_2$ solution was used as equilibration solution (soil/solution ratio: 3/10). The desorption phase was carried out by treating pre-adsorbed soil samples with fresh 0.01 M aqueous $CaCl_2$ solution. The aqueous supernatants were separated by centrifugation and the DBDCB residues in the supernatant were analysed by liquid scintillation counting (LSC). Samples at the highest concentration for adsorption were analysed by TLC methods in order to detect potential degradation products.

Information on the soils used and measured parameters as the adsorption coefficients K_d (in this study corresponding to K_F values) and K_{OC}, line equations and correlation coefficients obtained for the determined isotherms of each soil type, are presented in table below.

Table 22: Parameters for the adsorption of DBDCB obtained with the U.S. EPA guideline 163-1

Soil	**Adsorption**				
	% Organic carbon	**K_d[1)] [mL/g]**	**K_{oc} [mL/g]**	**Line Equation**	**r^2**
Sand	0.05	0.264	528	y = 1.138 X -1.331	0.9965
Sandy Loam Soil	0.40	0.351	87.8	y = 1.110 X -1.046	0.9951
Clay Loam Soil	0.65	0.474	72.9	y = 0.9963 X -0.770	0.9978
Silt Loam Soil	2.1	0.701	33.4	y = 1.086 X -0.3559	0.9979
Arithmetic mean[2)]		**0.509**	**64.7**		**0.9969**

[1)] Corresponding to the K_F value of the Freundlich isotherm

[2)] The sand soil is not taken into account for the mean calculation due to its organic carbon content being below the 0.3% OC criteria set in the OECD TG 106.

The adsorption/ desorption properties can be characterised by the Freundlich isotherm:

$x/m = K_d \times C_e \times (1/n)$ or $\ln x/m = \ln K_d + 1/n \times \ln C_e$

where,
x = the amount of chemical adsorbed in µg
m = mass of adsorbent in grams

when in equilibrium with an aqueous solution, where
C_e = concentration of chemical in aqueous solution
K_d = adsorption coefficient
n = a constant

Calculations were made of x/m, ln x/m and ln C_e. From these data, ln C_e was plotted versus ln x/m and equations for the adsorption isotherms for each soil type were determined.
The adsorption constant K_{oc} for each soil type was determined by using the equation:

$K_{oc} = K_d \times 100 / \%$ O.C.,

where % O.C. = organic carbon content of the soil.

The K_{OC} values obtained for the adsorption ranged from 33.4 to 528 mL/g. The results from the sand soil were excluded from the mean calculation due to its organic carbon content of 0.05% being below the 0.3% criteria as set in the OECD TG 106. According to the guideline, soils with less than 0.3% organic carbon content may disturb the correlation between the organic matter content and adsorption. Therefore the arithmetic mean of K_{OC} for adsorption is 64.7 mL/g (n = 3). Due to the low amount of test compound adsorbed, K_{OC} values for the desorption could not be calculated. The major degradation products observed in the adsorption phase were the EZ isomers of l-bromo-2,4,dicyano-l-butene and amounted to 6.16%, 23.9%, 22.5% and 75.9% for sand, sandy loam, clay loam and silt loam. Due to the instability of the parent compound (as a result of hydrolysis and ionic interactions), the sorption parameters determined reflect sorption characteristics of the parent and its degradation products.

Conclusion

According to Briggs (Proc. 7th British Insecticide and Fungicide Conference, Nottingham, UK, 83-86, 1973), DBDCB can be classified as intermediate mobile substance: 64.7 mL/g (mean K_{OC} for adsorption), range 33.4-87.8 mL/g, n = 3.

5.2.2 Volatilisation

The air is no compartment of concern for DBDCB due to its limited potential for volatilisation (vapour pressure = 3.81×10^{-3} Pa (at 20 °C) and Henry´s Law Constant = 3.99×10^{-5} Pa at 25 °C (EPIWIN calculation)).

5.2.3 Distribution modelling

- **DBDCB**

Aerobic aquatic degradation

DBDCB as active substance within PT 6 products may enter surface water bodies indirectly via sewage treatment plants. The TNsG on Data Requirements for active substances and biocidal products proposes two kinds of studies designed for fulfilling demands on biodegradation in freshwater. An aerobic degradation study is requested, whereas the point 7.1.2.2.2 DOC IIIA refers to water/sediment degradation studies. The design of the latter study is recommended especially for substances with a certain sorption potential to sediment. Therefore, a water/sediment study (in this case a water/soil study) has been submitted for DBDCB.

The data summarised below show that the a.s. was primarily present in the water phase during the course of the study. Therefore, the whole system half-life is taken as indicative of the water phase DT_{50} and the aerobic aquatic degradation study is not required.

A 30-day aerobic aquatic metabolism study with radiolabelled DBDCB was conducted by S. (1990, 7.1.2.2.1/01) following the general data requirements of the US EPA, Pesticides Assessment Guidelines, Subdivision N, Section 162-4 as required by 40 CFR, 158.130.

Test vessels, containing a sandy loam soil flooded with blended water and the test substance at a concentration of 10.9 ppm were kept at 25 ± 1 °C under dark conditions. Soil samples and test water were collected regularly, worked up und analysed for radioactivity by LSC as well as for residues of DBDCB and its degradation products with TLC and HPLC methods.

Extractable soil residues decreased from 10.2% of the initial measured dose (IMD) at day 0 to 4.06% after 1 month, with a maximum of 10.4% after 7 days. Residues in the test water decreased from 89.4% of the IMD at day 0 to 80.5% after 1 month, with a minimum of 78.8% at day 21. Volatile residues increased to 10.3% of the IMD until termination of the test. The majority of these residues (10.0% of the IMD) were confirmed to be $^{14}CO_2$. Bound residues increased from 0.777% of the IMD at day 0 to 8.88% after 1 month. Mean mass balance accountability for the study was 96.9%.

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. No measurable amounts of the parent compound were observed after day 7. However, already in the samples taken immediately after the application (0 hours) the amount of the parent was as low as 79.8%. This early decrease can most likely be attributed to the rapid hydrolysis of DBDCB in basic media.

A degradation product identified as 2-methyleneglutaronitrile (2-MGN) increased from 13.2% of the IMD at day 0 to 34.1% at day 3, then decreased to 0.798% after 1 month, with a minimum of 0.499% at day 21. An unknown degradation product I increased from 0.559% of the IMD at day 0 to 1.39% at day 2, then decreased to 0.119% until termination. A significant amount of unresolved activity was observed at and slightly above the origin. This activity increased from 5.54% of the IMD at day 0 to 83.0% after 30 days, with a peak of 83.4% at day 21. No activity was observed on TLC autoradiograms corresponding to the (E)- and (Z)-isomers. Analysis of selected test water samples by HPLC, however, did reveal the presence of minimal amounts of this degradation

product at day 0 (0.439 of IMD in water) and day 2 (0.246% of IMD in water), but none was observed in the 1 month test water sample.

A summary of the distribution and nature of radioactivity for the incubation period and the derived half-live of DBDCB under aerobic aquatic conditions is presented in table below.

Table 23: Aerobic aquatic degradation of radiolabelled DBDCB

Guideline/Test method	**Compartment characteristics**		**DT_{50} of DBDCB**	**Other results (%initial measured dose, IMD)**	**Reference**
	Conditions	**Water / Soil**			
US-EPA (162-4) OECD Guide-line: no	For 30 d --- 10.9 ppm --- Dark --- 25 ± 1 °C	Water: pH 8.2 Soil: Sandy loam pH 6.5; 0.47% oc	whole system: 0.874 d	➢ CO_2: 10.0% (30 d) ➢ Bound residues: 8.88% (30 d) ➢ Tektamer 38 (soil and water): n.d. (30 d) ➢ 2-MGN (soil and water): 0.798 (30 d); 34.1% (3 d) ➢ (E)- and (Z)-isomers (water): n.d. (30 d); 0.439% (0 d)	S., 1990, *7.1.2.2.1/01*

n.d. = not detectable

Due to the basic character of the water/soil test system (pH 8.2 in the water phase), hydrolysis may contribute to the dissipation of DBDCB from the water phase. However, the inherent catalytic nature of the soil may also contribute to the rapid decline. The soil is characterised by total bacterial plate counts of $1.07*10^6$ to 1.04×10^7 cfu/g on the first and last test day, respectively. The fungal counts are $1.11*10^6$ to 1.25×10^5 cfu/g on the first and last test day, respectively. Since the hydrolysis rate constant (k_h = 0.0763, at pH 9, 25 °C, Hydrolysis DBDCB) is known, the contribution of biotic degradation to the dissipation of DBDCB in the total system can, however, be estimated by applying the Wegscheider principle assuming first order kinetics for all side reactions ($C = C_0 \exp^{-kt}$). The Wegscheider principle, introduced by Rudolf Wegscheider in 1901, describes the relations between kinetic rate constants that follow from the principle of a detailed balance. Following this principle, the biotic degradation rate constant (k_b) is derived by using the aerobic aquatic half-life of the total system with DT_{50} = 0.874 d.

In a first step the degradation rate constant for the total system is calculated:

$\exp^{-kt} = 0.5 \Leftrightarrow -kt = \ln 0.5 = -0.693$

$-kt = -0.693 \Leftrightarrow k = 0.693/t$

With $t = 0.874 \Leftrightarrow k = 0.693/0.874 = 0.793$ days $^{-1}$ rate constant for the total system.

When subtracting the hydrolysis rate constant from the rate constant of the total system, the biotic degradation rate constant (k_b) is derived in the following:

$k_b = 0.793 – 0.0762 = 0.71$

From the degradation rate constant, the half-life time due to biotic degradation (t_b) can be estimated:

$k_b = k/t_b \Leftrightarrow 0.71 = 0.693/t_b \Leftrightarrow t_b = 0.693/0.71 = 0.976$ days (25 °C)

Conversion to 12 °C:

DT_{50} (12 °C) = 2.76 days for biotic degradation.

The biotic half-life DT_{50} of 2.76 days (12 °C) is in good agreement with the estimated degradation half-life value for the total system and indicates a quick dissipation of DBDCB in the soil phase.

Conclusion

DBDCB undergoes a rapid degradation under aerobic aquatic conditions, with conversion to several metabolic products, including a considerable mineralization to CO_2. The half-life of DBDCB was calculated to be 0.874 days using first-order degradation kinetics.

DBDCB is known to be susceptible to hydrolysis, especially in basic medium. It can be assumed that the pH of the test water used (8.2), and the inherent catalytic nature of the soil, contributed to the rapid decline. This is supported by the estimation of a half-life time due to biotic degradation according to the *Wegscheider Principle*.

Major single degradation product was 2-MGN (2-methyleneglutaronitrile). Its amounts increased through the first 2-3 days of the study, but decreased to < 1% of the IMD by the end of the study. Therefore, 2-MGN would not be expected to persist under aerobic aquatic conditions. Residues of the (E)- and (Z)-isomers were observed in the day 0 and day 2 water samples by HPLC and in traces, only. Several degradation products were found at negligible concentrations throughout the course of the study.

Amounts of CO_2 were formed at an increasing rate as the study progressed. It can be concluded, that one or more degradation products were further degraded and converted to CO_2.

Based on the results of the study it can be concluded, that DBDCB, when entering the aerobic aquatic environment, will dissipate quickly. The compound 2-MGN is the major single breakdown product; however, its nature is transient. As a final mineralisation product CO_2 is formed.

Summary of degradation in water

DBDCB will be significantly degraded via hydrolysis in basic aquatic media. The hydrolysis at pH 9 was more extensive than hydrolysis at neutral pH (7). The parent compound was subject to photodegradation during the course of this experiment. Photolysis rate constants and half-lives were 0.0129 $days^{-1}$ (DT_{50} = 54 days) for the exposed samples and 0.0018 $days^{-1}$ (DT_{50} = 381 days) for the non-exposed samples (first order kinetics), corresponding to a photolytic half-life of 62 days when corrected for degradation in dark control samples (according OECD TG 316). The primary product of photolysis was 2-methyleneglutaronitrile (2-MGN; 28.5% after 30 days).

DBDCB was not “readily biodegradable” under the conditions of the test (OECD guideline 301D) due to microbial inhibition, as observed in the Inhibition Control. DBDCB failed to qualify for classification as “inherently biodegradable” according to the definitions given by the OECD

guideline 302B. However, DBDCB is primary inherently biodegradable. DBDCB was reduced to 35% of the initial concentration (65% degree of a.s. degradation) and transformed to 2-methyleneglutaronitrile and other (minor) degradation products.

It can be concluded that abiotic degradation processes will significantly contribute to the overall degradation of DBDCB in aquatic systems.

From the aerobic aquatic degradation study it can be concluded that DBDCB undergoes rapid degradation with a half-life for the total system calculated to be 0.874 days. The hydrolytic degradation products 2-MGN (2-methyleneglutaronitrile) and (E)- and (Z)-isomers which have also been detected in the aqueous photodegradation study, decreased to < 1% of the initial measured dose (IMD) by the end of the study. Therefore, they are not considered as being relevant in water / soil systems because they appear only temporary and in small amounts.

Aerobic degradation in soil

Due to the exclusive indoor application of DBDCB, potential direct contamination of the environment via the soil pathway is considered negligible. The only possibility of DBDCB entering the soil compartment is via STP sludge application onto soil surfaces. STP sludge might contain very low concentrations of the active substance.

Conclusion

No key study has been carried out. However, it can be concluded from the aerobic aquatic metabolism study which had been performed with viable soil (sandy loam) that once DBDCB is released onto soil it will be rapidly degraded (DT_{50} of DBDCB in soil / water system was < 1 day), particularly in wet soils. This is supported by the fact that DBDCB is well soluble is water. Furthermore, photolytic processes interacting directly after the application might contribute to the overall degradation of DBDCB.

Anaerobic degradation in soil

Due to the exclusive indoor application of DBDCB, a potential direct contamination of the environment via the soil pathway is considered negligible. It can be assumed that DBDCB will not be exposed to anaerobic soil conditions according to the use pattern. The entry of DBDCB to soils will predominately proceed via sewage sludge applications. Further tests are therefore not considered as being relevant.

However, a study investigating the behaviour of radiolabelled DBDCB (nominal concentration of 10.0 ppm) in a flooded sandy loam soil under anaerobic aquatic conditions is available (A7.1.2.2.1/01). 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. The half-life was calculated to be 0.495 days (r = -0.938) 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), indicating that this was a significant pathway for the loss of DBDCB under anaerobic aquatic conditions. Chromatographic analysis of the test water and soil extracts revealed the presence of DBDCB, and the major degradation product 2-methyleneglutaronitrile (2-MGN). 2-MGN was subsequently degraded partially to a polar (not-

characterized) degradation product that accounted for ≤ 25% of the applied radioactivity at 12 months. This component was also present in the aerobic aquatic metabolism study.

Conclusion

No key study has been carried out. However, DBDCB has been shown to degrade quickly under anaerobic aquatic conditions in a flooded sandy loam soil and thus, can be expected to dissipate when unintentionally reaching the soil compartment (estimated laboratory half-life of 0.495 days).

Field degradation

Due to the exclusive indoor application of DBDCB a potential direct contamination of the environment via the soil pathway is considered negligible. Potential residues could enter the soil compartment indirectly, via the application of sewage sludge. However, as described above, DBDCB dissipates quickly in biological systems. This property prevents the compound from being accumulated or transported into deeper soil layer. Further tests are not considered as being reasonable.

Accumulation in soil

The active substance is not directly released to soils following exclusive indoor application and the intended use pattern of DBDCB. It can be assumed that soil will not be a compartment of concern for DBDCB residues. A soil accumulation test is not considered as being reasonable.

- **2-MGN**

The distribution of 2-MGN was described by the K_{OC} value which was estimated using both the MCI method and the Kow method of the KOCWIN v2.00 model. Furthermore, the K_{OC} value of DBDBC was estimated with the same methods and was compared to the experimentally derived mean K_{OC} from the OECD TG 106 study to indicate the reliability of the applied methods.

A good relationship between the experimentally derived (mean 64.7 mL/g) and calculated K_{OC} values (50.71 mL/g) of the parent DBDCB was shown for the MCI method. The Kow method was shown to overestimate the experimentally derived K_{OC} values by one order of magnitude (654.2) and was therefore disregarded. Nevertheless, Kanazawa (1989) showed a significant correlation between the log Kow and log K_{OC} and proposed the following equation for calculating the K_{OC} by using log K_{OC} = 0.402*log Kow + 1.071 for various pesticide substances. When applying this equation for DBDCB (log Kow 2.00, experimentally) and 2-MGN (log Kow 0.27, KOWWIN estimate), a log K_{OC} of 1.88 and 1.18 was calculated, respectively. The good relationship between the outcome of the MCI method and the experimental data of the parent DBDCB validates the calculation output for the metabolite 2-MGN. Accordingly, a K_{OC} of 17.76 mL/g was calculated (by the MCI method) for 2-MGN indicating a low sorption potential to soil.

Table 24: Comparison of experimentally derived K_{OC} values for DBDCB and EPI Suite estimated K_{OC} values for DBDCB and 2-MGN

KOCWIN v2.10 method	**DBDCB**		**2-MGN**
	Experimentally determined	**EPI Suite Output**	**EPI Suite Output**
	K_{OC} (log K_{OC}) [L/kg]		
MCI method	64.7 (1.81)	50.71 (1.705)	17.76 (1.250)
Kow method	64.7 (1.81)	654.2 (2.816)	72.24 (1.859)

Conclusion on the rapidly degradable property of DBDCB

According to CLP Regulation criteria, Annex I, section 4.1.2.9.5, DBDCB is not rapidly degradable, because:

a) DBDCB is not readily degradable.

b) Value of BOD/COD is not available.

c) Results of hydrolysis are following:

During the 30 days study period, DBDCB did not hydrolyse at pH 5 and was demonstrated to hydrolyse slowly at pH 7 (DT50 = 51.6 days and 96.3 days corresponding to 146.4 days and 273.3 days at 12°C). DBDCB was subject to base – catalysed at pH 9 (DT50 = 9.1 days corresponding to 25.8 days at 12°C). To take into account data on hydrolysis for classification purposes the longest DT50 (pH range 4-9) must be shorter than 16 days. This criterion is clearly not fulfilled in this case.

Aerobic aquatic degradation: In the available study it cannot be demonstrated that the DT50 for DBDCB is < 16 days.

Anaerobic degradation data: Data investigating the anaerobic degradation cannot be used in order to decide if a substance should be considered as rapidly degradable because the aquatic environment is generally aerobic.

5.3 Aquatic Bioaccumulation

Table 25: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Calculation based on measured log K_{ow} value	Based on a log Kow value of 2.0, obtained from an experimental study, a BCF of 10 is obtained. According to this value bioaccumulation of DBDCB in fish can be considered negligible.	This statement is consistent with the experimentally derived BCF value, which was obtained in an experimental test (Anonymous, 1982) and accounts for < 2.5.	F, 2007, 7.4.2/01)
Japanese standard	BCF = 0.5 - 0.7 for 0.05 mg /L BCF = < 2.5 for 0.005 mg /L	Supportive information.	A, 1982, 7.4.3.3.1/01

5.3.1 Aquatic bioaccumulation

The BCF was calculated using the measured log K_{ow} value of 2.0 and resulted in a low bioaccumulation factor of 10. This result confirms a negligible potential of the test substance to bioaccumulate.

Furthermore, a test on the bioconcentration of DBDCB is available. Since relevant details are missing and the study was not performed according to GLP, the results are considered as supportive information for the expected bioaccumulation in aquatic organisms. The test was conducted under flow-through conditions for 8 weeks, at 25 ± 1 °C, according to a Japanese standard method (reference of the method not clear) to evaluate the bioconcentration of DBDCB in carps (*Cyprinus* sp). An unspecified number of fish per treatment, with a mean body length of 10.2 cm, a body weight of 29.4 g and a mean fat content of 5.4%, were given 0.005 and 0.05 mg/L DBDCB in the water. The water tank was made of glass with a capacity of 100 L. The amount of running water was 1158 L/day. Only uptake was investigated. Concentrations in water were measured every two weeks after the start of the test. For both treatments, the BCF was below 2.5. This indicates that bioaccumulation in fish is negligible.

5.3.2 Summary and discussion of aquatic bioaccumulation

The available measured and calculated steady-state bioconcentration factors (BCF) are ≤ 10. Therefore, a significant bioaccumulation of DBDCB in aquatic organisms is not to be expected.

DBDCB has no potential to bioconcentrate with regard to classification purposes according to Regulation (EC) No. 1272/2008 (cut-off value: BCF ≥ 500).

5.4 Aquatic toxicity

Table 26: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
Fish			
US-EPA guideline 72-1, *Oncorhynchus mykiss* (Rainbow Trout)	LC_{50} = 1.26 mg/L (96 hours) NOEC = 0.726 mg/L (96 hours)	Measured.	B.K.W, 1997a, 7.4.1.1/01
US-EPA guideline 72-1, *Lepomis macrochirus* (Bluegill Sunfish)	LC_{50} = 2.73 mg/L (96 hours) NOEC = 1.43 mg/L (96 hours)	Measured.	B.K.W, 1997b, 7.4.1.1
US-EPA guideline 72-4, *Oncorhynchus mykiss* (Rainbow Trout)	NOEC = 0.75 mg/L (81 days)	Mean measured concentrations. Most sensitive endpoint: survival.	W.B., 1991, 7.4.1.2
Aquatic invertebrates			
US-EPA guideline 72-2, *Daphnia magna*	EC_{50} = 4.83 mg/L (48 hours)	Measured.	W.,K.,B. 2006, 7.4.1.2/01
US-EPA guideline 72-4, *Daphnia magna*	NOEC = 1.4 mg/L (21 days)	Mean measured concentrations. Mortality was most sensitive parameter.	W.B., 1991, 7.4.1.2
Algae			
Method C.3 (2009), *Desmodesmus subspicatus*	NOEC = 0.017 mg/L E_rC_{50} =5.4 mg/L (72 hours)	Geometric mean measured values.	R., 2011, 7.4.1.3/02
Method C.3 (2009), *Desmodesmus subspicatus*, Test substance: **2-MGN**	NOEC ≥ 100 mg/L E_rC_{50} > 100 mg/L (72 hours)	Nominal values.	R., 2011, 7.4.1.3/03
Microorganisms			
OECD 209, Activated sludge	EC_{50} = 34 mg/L (3 hours)	Nominal value.	W., M.,B., 1995, 7.4.1.3/01
Method C.11 (2008), Activated sludge Test substance: **2-MGN**	EC_{50} > 1000 mg/L EC_{10} = 789.4 mg/L (3 hours)	Nominal values.	7.4.1.4/02 , R, 2011

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

The key study presents the lowest value obtained from tests available with fish species. Since this test was performed with analytical monitoring, the results are based on mean measured concentrations (nominal concentrations were 0.42, 0.72, 1.2, 1.8, and 3.0; recovery rates were

between 97 - 104%). The most sensitive endpoint was mortality but sublethal effects also occurred. Sublethal effects observed as erratic swimming and lethargy were observed in several fish exposed to 1.75 and 2.91 mg/L DBDCB after 24 hours of exposure. At 48, 72, and 96 hours, several fish exposed to 1.19 and 1.75 mg/L exhibited a change in coloration and lethargy. The validity criteria and the reporting of the test are adequate, therefore the test is considered as valid.

5.4.1.2 Long-term toxicity to fish

No chronic toxicity study in juvenile fish was undertaken with DBDCB since a fish early life stage toxicity test is available.

Long-term toxicity of DBDCB to fish was determined in a fish early life-stage toxicity test according to US-EPA guideline 72-4 in the rainbow trout (*Oncorhynchus mykiss*), found to be the most sensitive species tested in the acute toxicity tests in fish.

The test was performed under flow-through conditions, with five concentrations of test substance, a dilution water control, and a solvent control at a mean temperature of 10.2°C. Nominal concentrations of DBDCB were: 0.0 mg/L (control and solvent control), 0.38, 0.60, 1.0, 1.5, and 2.5 mg/L. Mean measured concentrations were: not detected (<0.1 mg/L; control and solvent control), 0.37, 0.75, 1.0, 1.5, and 2.8 mg/L of DBDCB.

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 the non-treatment control and solvent control. The survival (the most sensitive measured biological parameter) of rainbow trout after 81 days of exposure was significantly reduced in comparison to the control at all concentrations above 0.75 mg/L DBDCB. The time to hatch, the time to swim-up, the total length and wet weight of surviving fish, and the occurrence of sublethal effects were not different to the control at 1.5 mg/L DBDCB, the highest concentration that did not cause the complete mortality of exposed organisms during the 81 day test.

Table 27: The time to hatch and the time to swim – up during the early life stage toxicity test

Mean measured concentration (mg/L)	Average time to hatch (days)	Average time to swim – up (days)
0.37	32.5	49
0.75	32.8	49
1.0	33.2	49
1.5	40.0	51
2.8	*	*

Parameters at concentration marked with * are significantly different than the control at the 95% confidence level.

Therefore, the exposure of embryos, larvae, and juvenile fish to DBDCB resulted in a lowest observed effect level (LOEL) of 1.0 mg/L, a no observed effect level (NOEL) of 0.75 mg/L, and a

maximum acceptable toxicant concentration (MATC), expressed as the geometric mean of the LOEL and NOEL, of 0.87 mg/L**.**

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

The key study is a valid test performed according to an US-EPA test guideline for 48 hours. The test was conducted under flow-through conditions with five concentrations of DBDCB. Nominal concentrations were 0 (control), 1.6, 2.5, 4.0, 5.9, and 10 mg/L, and mean measured concentrations were none detected at the quantitation limit of 0.0250 mg/L; control (ND), 1.77, 2.71, 4.46, 6.16, and 10.9 mg/L. The results are based on the number of surviving organisms and the occurrence of sublethal effects after 48 hours.

5.4.2.2 Long-term toxicity to aquatic invertebrates

The chronic toxicity of DBDCB to the daphnid (*Daphnia magna)* was investigated. Forty daphnids were exposed for up to 21 days under flow-through conditions to five concentrations of test substance, a solvent control, and a dilution water control.

Nominal concentrations of the active ingredient were: 0 (control and solvent control), 1.5, 2.4, 4.0, 6.0, and 10.0 mg/L. Mean measured concentrations were: ND (<0.6 mg/L; control and solvent control), 1.4, 2.6, 4.3, 6.9, and 11 mg/L of DBDCB.

The test was performed according to the US-EPA guideline 72-4. Measured biological parameters were: 1. survival of first generation daphnids, 2. dry weight of the first generation daphnids at the conclusion of the test, 3. time to first brood, and 4. production of young by the first generation daphnids.

The most sensitive biological endpoint, when statistical comparisons between the treatments of DBDCB and the control are made, is survival after 21 days. Exposure of daphnids to DBDCB resulted in a no observed effect level (NOEL) of 1.4 mg/L, a lowest observed effect level (LOEL) of 2.6 mg/L, and a maximum acceptable toxicant concentration (MATC) of 1.9 mg/L. The 21 day median effective concentration (EC_{50}) was 2.8 mg/L.

The NOEC for Daphnia magna exposed to DBDCB was **1.4 mg a.s./L** based on the most sensitive parameter, survival.

5.4.3 Algae and aquatic plants

The influence of **DBDCB** on the growth of the green algae *Desmodesmus subspicatus* was investigated in a 72 hours static test according to Method C.3, Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2009) which is equivalent to OECD guideline 201 (2006). This key study was performed with six concentrations of test substance and a dilution water control at a temperature of 21 - 24 °C. Nominal concentrations of DBDCB were: 0 mg/L (control), 0.1, 0.32, 1.0, 3.2, 10 and 32 mg/L. The maintenance of the test item concentrations was proved by analytical measurements (HPLC-MS/MS) at 0 and 72 hours (for all concentrations). The results are expressed in terms of geometric mean measured concentrations, i.e. 0.009, 0.017, 0.301, 2.644, 9.886 and

34.15 mg/L. Cell density measurements were made at 24, 48 and 72 hours using a microcell counter. The criteria of adverse effects were the item-induced inhibition of yield and growth rate of the algal population.

The results for DBDCB achieved in the key study test were the growth rate based E_rC_{50} of 5.4 mg a.s./L and a NOEC of 0.017 mg a.s./L.

In addition an E_rC_{10} (72 h) of 0.20 mg/L was calculated. As the reduction of growth rate instead of yield is the preferred endpoint according to the OECD guideline 201, this value will be taken into consideration for classification purposes.

In this study at the second lowest test concentration of 0.017 mg/L (NOEC) an inhibition of 1.0% of the growth rate after 72 h has been determined and at 0.30 mg/L an inhibition of 13.5% occurred. It is stated by various algal test guidelines (e.g. DIN 38 412 part 33) that only effects of > 10% are considered as biologically relevant. This means, that after 72 h test duration, 10% effect regarding growth rate is effectuated by a concentration > 0.017 mg/L and < 0.30 mg/L (13.5% inhibition).

In general, EC_{10} values should be preferred for classification purposes. According to the ECHA Guidance on the Application of the CLP Criteria (Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Version 4.1, November 2015) the following is stated for the Classification of substances hazardous to the aquatic environment: "if a NOEC or EC_x value is available, preference is given to EC_{10}" (see OECD (2006): Series on Testing and Assessment Number 54, Current approaches in the statistical analysis of ecotoxicity data: a guidance to application. ENV/JM/MONO(2006)18).

Thus, the classification will be based on the **E_rC_{10} (72 h) of 0.20 mg a.s./L** from the most sensitive aquatic species which is the freshwater algae *Desmodesmus subspicatus*.

The influence of the metabolite **2-methyleneglutaronitrile (2-MGN)** on the growth of the green algae *Desmodesmus subspicatus* was investigated in a 72 hours static test according to Method C.3, Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2009) which is equivalent to OECD guideline 201 (2006).

The test was performed with three concentrations of test substance and a dilution water control at a temperature of 21 - 24 °C. Nominal concentrations of 2-MGN were: 0 mg/L (control), 10, 32 and 100 mg/L. The maintenance of the test item concentrations was proved by analytical measurements (HPLC-MS/MS) at 0 and 72 hours (for all concentrations). Effective concentrations ranged from 105 % to 110 % of nominal values at 0 hours, and from 107 % to 119 % of nominal values at 72 hours. Thus, the results are expressed in terms of nominal concentrations. Cell density measurements were made at the beginning and end of the test. Inhibition of the algal population was measured as reduction in growth rate, relative to control cultures grown under identical conditions.

The results for the metabolite 2-MGN achieved in the algae test were the growth rate based **E_rC_{50} of > 100 mg 2-MGN/L and a NOEC of ≥ 100 mg 2-MGN/L**. This value based on the maximum test concentration for which no growth inhibition on algae was shown. Therefore it was concluded that the metabolite 2-methyleneglutaronitrile is not toxic for the aquatic compartment.

5.4.4 Other aquatic organisms (including sediment)

5.4.4.1 Inhibition of microbial activity (aquatic)

- **DBDCB**

The inhibition of activated sludge respiration by DBDCB was investigated according to OECD test guideline 209 to determine the 3 hour median effective concentration (EC_{50}) of the test substance to aquatic microorganisms

The test was performed at 20 ± 2°C with a control and five concentrations of test substance (0, 10, 32, 100, 320, and 1000 mg/L). Nominal concentrations of DBDCB were used for all calculations. Activated sludge was obtained from the wastewater treatment plant in Newburyport, Massachusetts.

The test substance inhibited activated sludge respiration in the tested range, resulting in the following respiration rates after 3 hour incubation period: 47 mg/L/hr for the control and at the 10 mg/L treatment, 31 mg/L/hr at 32 mg/L, 0 mg/L/hr at 100 and 320 mg/L, and finally -6 mg/L/hr at 1000 mg/L. Exposure of activated sludge to DBDCB resulted in a 3 hour EC_{50} value of 34 mg/L with a 95% confidence interval of 10 to 100 mg/L.

DBDCB showed inhibitory effects on activated sludge. Fifty percent inhibition (EC_{50}) of microbial activity was determined at 34 mg a.s./L.

- **2 – MGN**

The inhibition of activated sludge respiration by the metabolite 2-methyleneglutaronitrile (2-MGN) was investigated according to Method C.11, Biodegradation: Activated Sludge Respiration Inhibition Test (2008) equivalent to OECD Guideline 209 (1984), to assess the toxicity to aquatic microorganisms. The activated sludge was exposed to 2-MGN at nominal concentrations of 10, 100 and 1000 mg/L at a test temperature of 20 ± 2 °C. The effect value relates to nominal concentrations, since no analytical monitoring was included. Two untreated controls and a toxic reference item were run in parallel. The respiration rate of each mixture was determined after aeration periods of 3 hours.
Activated sludge was obtained from a domestic sewage treatment plant in Cologne, Germany.

The test substance inhibited activated sludge respiration in the tested range, resulting in the following respiration rates after 3 hour incubation period: 21.4 mg/L/hr for the control, 25.5 mg/L/hr at the 10 mg/L treatment, 22.5 mg/L/hr at 100 mg/L and 19.0 mg/L/hr at 1000 mg/L.

Exposure of activated sludge to 2-MGN resulted in a 3 hour EC_{50} value of > 1000 mg 2-MGN/L and in a 3 hour EC_{10} value of 789.4 mg 2-MGN/L representing the highest test concentration. At the highest test concentration of 1000 $mg.L^{-1}$ the activated sludge respiration rate was not significantly inhibited. Therefore it was concluded that the metabolite 2-methyleneglutaronitrile is not toxic for the aquatic compartment in terms of toxicity to microbes.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

According to Regulation (EC) No. 1272/2008 the substance is not classified for acute aquatic toxicity. For chronic toxicity the most sensitive species is the freshwater algae *Desmodesmus subspicatus* with an E_rC_{10} (72 h) of 0.20 mg a. s. /L. Moreover, the substance is considered to be not rapidly degradable. Thus, according to Regulation (EC) No. 286/2011 of 10 March 2011, Table 4.1.0 (Classification categories for hazardous to the aquatic environment) amending Regulation (EC) No. 1272/2008, the substance needs to be classified as Aquatic Chronic 2 (H411).

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

According the Regulation (EC) No. 1272/2008 the substance is classified as **Aquatic Chronic 2, H411; Toxic to aquatic life with long lasting effects.**

6 OTHER INFORMATION

None.

7 REFERENCES

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
[redacted]	1980	Pharmacokinetic and Metabolic Evaluation of ^{14}C-Tektamer®-38 in Male Rats. Date: 1980-05-19	[redacted]	–	Yes	LANXESS Deutschland GmbH	Non key (A6.2)
Anonymous	1982	Test on Bioconcentration in Fish with DBDCB. Date: 1982-01-05	Chemicals inspection Association, Japan	Test-No. 34453	No	LANXESS Deutschland GmbH	7.4.3.3.1/01
Anonymous	2003	Product specification Tektamer 38. Date: 2003-09-22	LANXESS Deutschland GmbH, Chemicals, Geschäftsfeld Materialschutz, Leverkusen, Germany	Art.-No. 06026664 Issue 001	No	LANXESS Deutschland GmbH	2.7/01
Australian government Department of Health and Aging	2009	NICNAS Existing Chemical Hazard Assessment Report Mythydibromo Glutaronitrile (MDBGN)	n.a.	n.a.	No	n.a.	Attached in section 13 of IUCLID CLH dossier for DBDCB
[redacted]	2007	1,2-Dibromo-2,4-dicyanobutane (DBDCB), Calculation of Henry's law constant. Date: 2007-05-25	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	3.2/02
[redacted]	1988	Human Phototoxicity/ Photoallergy Study. Date: 1988-03-21	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	Non-key (A6.1.5)

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
[redacted]	1997a	Tektamer 38: Acute Toxicity To The Rainbow Trout, *Oncorhynchus mykiss*. Date: 1997-02-05	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.1.1/01
[redacted]	1997b	Tektamer 38: Acute Toxicity To The Bluegill Sunfish, *Lepomis macrochirus*. Date: 1997-02-05	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.1.1 (Non-key study)
[redacted]	2003	The British Standard Series of Contact Dermatitis Allergens: Validation in Clinical Practice and Value for Clinical governance.	[redacted]	[redacted]	No	–	Non-key Published (A6.12.6)
[redacted]	1990	The Absorption, Distribution, Metabolism and Excretion of [^{14}C]-DBDCB in the Rat. Date: 1997-02-06	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.2/01
[redacted]	1991a	Tektamer 38 – Single Dose Oral Toxicity in Rats/LD50 in Rats. Date: 1991-02-21	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.1.1
[redacted]	1991b	Tektamer 38 – Primary Dermal Irritation in Albino Rabbits. Date: 1991-02-21	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.1.4/01
[redacted]	1992	21 Day Repeated Dose Dermal Toxicity Study in Rats. Date: 1992-04-16	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.3.2

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
[redacted]	1994	Developmental Toxicity (Embryo-Foetal Toxicity and Teratogenic Potential) Study of DBDCB Administered Orally via Stomach Tube to New Zealand White Rabbits. Date: 1994-05-23	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.8.1/01
[redacted]	2005	Validation of a HPLC-method for the determination of DBDCB and MGN in Tektamer 38. Date: 2005-12-01 **CONFIDENTIAL**	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	4.1/01
[redacted]	2007a	Physicochemical properties of DBDCB. Date: 2007-05-15	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	3.1/01 3.2/01 3.10/01 3.13/01
[redacted]	2007b	Spectral data of DBDCB. Date: 2007-03-02	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	3.4/01
[redacted]	2007c	Determination of the water solubility (flask method) of DBDCB at 10 °C, 20 °C, and 30 °C and at pH 5, pH 7 and pH 9. Date: 2007-03-13	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	3.5/01
[redacted]	2007d	Solubility of DBDCB in different organic solvents at 10 °C, 20 °C and 30 °C. Date: 2007-04-07	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	3.7/01

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
[redacted]	2007e	Partition coefficient of DBDCB at pH 5, pH 7 and pH 9 and temperature dependence. Date: 26-03-2007	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	3.9/01
[redacted]	2006	DBDCB. Calculation of indirect photo-degradation. Date: 2006-10-02	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	7.3.1/01
[redacted]	2007	DBDCB. Calculation of the Bioconcentration Factor (BCF). Date: 2007-05-16	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	7.4.2/01
[redacted]	1975a	MPXP-38: Acute oral LD_{50}-Mallard duck. Date: 1975-12-26	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	7.5.3.1.1/01
[redacted]	1975b	MPXP-38: Eight-day dietary LC_{50}-Bobwhite quail. Date: 1975-12-26	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	7.5.3.1.2/01
[redacted]	1982a	Tektamer 38 – Primary Eye Irritation – Rabbits. Date: 1982-06-24	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.1.4/02
[redacted]	1982b	Tektamer 38 – Guinea Pig Sensitization Study - Magnusson-Kligman Maximization Method. Date: 1982-01-26	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.1.5/01
[redacted]	1982c	Guinea Pig Contact Dermal Irritation/ Sensitization Ritz-Buehler Method. Date: 1982-01-02	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	Non-key (A6.1.5)

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
[redacted]	1996	Hydrolysis study of Tektamer 38 as a function of pH at 25 °C. Date: 1996-07-31	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.1.1.1.1/01
[redacted]	1978	Untitled report. Date: 1978-04-05	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	Non key (A6.1.1)
[redacted]	1982	A Dose Range-Finding Study of DBDCB Administered Orally via Stomach Tube to New Zealand White Rabbits. Date: 1982-05-21	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	Non key (A6.8.1)
[redacted]	1982	A Teratology Study of Tektamer 38 in Albino Rats. Date: 1982-09-16	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.8.1/02
[redacted]	1993	The sensitizing potency of Euxyl® K 400 and its components 1,2-dibromo-254-dicyanobutane and 2-phenoxyethanol.	[redacted]	[redacted]	No	–	6.1.5/02 Published
[redacted]	2007	Determination of safety relevant data of DBDCB. Date: 2007-01-30	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	3.11/01 3.15/01 3.16/01
[redacted]	2004	Methyldibromo-glutaronitrile in rinse-off products causes allergic contact dermatitis: an experimental study.	[redacted]	[redacted]	No	–	6.12.6 Published
[redacted]	1992	Determination of eleven product chemistry parameters for Tektamer 38. Date: 1992-11-20	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	3.3/01 3.6/01 3.10/01 3.17/01

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
[redacted]	1993	The Determination of the Photocontact Allergenic Potential of a Topically Applied Test Material (DBDCB) by Means of the Photocontact Allergenicity Test. Date: 1993-06-14	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	Non-key (A6.1.5)
[redacted]	1987	Effect of lipid solubility and molecular size on percutaneous absorption	[redacted]	[redacted]			Published
[redacted]	1982	Test for Chemical Induction of Mutation in Mammalian Cells in Culture – The L5178Y TK+/– Mouse Lymphoma Assay. Date: 1982-03-31	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	Non-key (A6.6.3)
[redacted]	1988	Delayed Contact Hypersensitivity Study in Guinea Pigs. Date: 1988-07-30	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	Non-key (A6.1.5)
[redacted]	2003	Determination of the antimicrobial effects of Tektamer 38 against bacteria and fungi.. Date: 2003-07-07	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	5.3.1
[redacted]	1977	Acute toxicity of MPXP-38 to the water flea (*Daphnia magna*). Date: May, 1977	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	7.4.1.2 (Non-key study)

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
[redacted]	1981a	Acute toxicity of Tektamer 38, A.D. to Bluegill (*Lepomis macrochirus*). Date: January, 1981	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	7.4.1.1 (Non-key study)
[redacted]	1981b	Acute toxicity of Tektamer 38, A.D. to Rainbow trout (*Salmo gairdneri*). Date: January, 1981	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	7.4.1.1 (Non-key study)
[redacted]	1981c	Acute toxicity of Tektamer 38, A.D. to the water flea (*Daphnia magna*). Date: January, 1981	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	7.4.1.2 (Non-key study)
[redacted]	1982	[redacted] Microbiocides for the protection of materials - [redacted]	--	--	No	--	5.3.1
[redacted]	1982	Modified Draize Skin Sensitization Study. Date:1982-04-19	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	Non-key (A6.1.5)
[redacted]	1984	Modified Draize Skin Sensitization Study. Date:1984-06-30	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	Non-key (A6.1.5)
[redacted]	1984	Evaluation of Tektamer 38 in the in Vitro Transformation of BALB/c-3T3 Cells Assay with S9 Activation. Date: 1984-12-17	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	Non-key (A6.6.2)

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
[redacted]	2007	Validation of an analytical method for the determination of DBDCB (1,2-dibromo-2,4-dicyanobutane) in drinking water. Date: 2007-04-24	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	4.2/01
[redacted]	1980	1,2-Dibromo-2,4-Dicyanobutane: Test for Acute Dermal Toxicity in Rabbits. Date: 1980-07-10	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.1.2
[redacted]	1995	Determination of ready biodegradability (biotic degradation) using the Closed Bottle Test OECD 301D. Date: 1995-07-28	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.1.1.2.1/01
[redacted]	1985	Microbiological Mutagen Test of 1,2-Dibromo-2,4-dicyanobutane. Date: 1985-01-28	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	6.6.1
[redacted]	2003	Study on Acute Inhalation Toxicity in Rats According to OECD No. 403. Date: 2004-04-16	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.1.3
[redacted].	1993	Microbiocides for the protection of materials - [redacted]	--	--	--	--	5.4.1
[redacted]	1982	Activity of Tektamer® 38 (T1752) in the In-Vivo Cytogenetics Assay in Sprague-Dawley Rats. Date: 1982-10-27	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	Non-key (A6.6.4)

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
[redacted]	1990	Morphological Transformation of BALB/3T3 Mouse Embryo Cells. Date: 1990-01-18, amended 1990-03-08	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	Non-key (A6.6.2)
[redacted]	1991	Acute in Vivo Cytogenetics Assay in Rats. Date: 1991-10-08	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	Non-key (A6.6.4)
[redacted]	1995	Micronucleus Cytogenetic Assay in Mice. Date: 1995-09-27	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.6.4
[redacted]	2007	Inherent Biodegradability of DBDCB (1,2-Dibromo-2,4-dicyanobutane) in a Zahn-Wellens/EMPA Test. Date: 2007-06-21	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.1.1.2.2/01
[redacted]	1994	Three Month Dietary Toxicity Study in Dogs with a Three Month Recovery. Date: 1994-11-08	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	Non-key (A6.4.1)
[redacted]	2011a	Alga, Growth Inhibition Test with Dibromodicyanobutan (DBDCB). Date: 2011-07-25	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.1.3/02
[redacted]	2011b	Alga, Growth Inhibition Test with 2-Methyleneglutaronitrile. Date: 2011-07-20	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.1.3/03
[redacted]	2011c	Activated Sludge, Respiration Inhibition Test with 2-Methyleneglutaronitrile. Date: 2011-05-31	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.1.4/02

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
[redacted]	1983a	Tektamer 38 – Microbial Mutagen Test with and without Rat Liver Enzyme Activation. Date: 1983-02-04	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	Non-key (A6.6.1)
[redacted]	1983b	Tektamer 38 – Measurement of Unscheduled DNA Synthesis in Human IMR-90 Fibroblasts. Date: 1983-02-04	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.6.2/02
[redacted]	1985	Tektamer 38 – V-79 Mammalian Cell Mutagenesis. Date: 1985-01-06	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.6.3
[redacted]	1998	Metabolic and Dispositional Fate of 1,2-Dibromo-2,4-dicyanobutane in the Male Fischer 344 Rat. Date: 1997-10-02	[redacted]	[redacted]	No	–	6.2/02 Published
[redacted]	1992	Determination of the photolysis rate of ^{14}C-Tektamer 38 in pH 5 buffered solution at 25 °C. Date: 23-07-1992	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.1.1.1.2/01
[redacted]	1990	Aerobic aquatic metabolism of ^{14}C-Tektamer 38 Date: 1990-08-27	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.1.2.2.1/01
[redacted]	1994	Anaerobic aquatic metabolism of ^{14}C-Tektamer 38. Date: 1994-04-27	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.2.2.4 (Non-key study)
[redacted]	2015	Medical Statement Lanxess Corporation US, 2015)	[redacted]	[redacted]	n.a.	LANXESS Deutschland GmbH	Attached in section 13,IUCLID CLH dossier for DBDCB

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
[redacted]	1994	Dibromodicyano-butane (DBDCB): Species Comparisons of *In Vitro* Skin Penetration Following a Single Application to the Excised Skin of Humans and Sprague Dawley Rats. Date: 1990-09-28	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	Non-key (A6.2)
[redacted]	1994	Dibromodicyano-butane (DBDCB): Bioavailability Following Diet Ingestion in the CD® Rat. Date: 1994-04-01	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	Non key (A6.2)
[redacted]	1978	MPXP-38 – Bacterial Mutagen Test (Ames Test). Date: 1978-11-29	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	Non-key (A6.6.1)
[redacted]	1982	Cytogenicity Study - Chinese Hamster Ovary Cells in Vitro. Date: 1982-09-22	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.6.2/01
[redacted]	2000	Provocative use testing of methyl-dibromo-glutaronitrile in a cosmetic shampoo	[redacted]	[redacted]	No	–	Non-key Published (A6.12.6)
[redacted]	1991a	Acute flow-through toxicity of Tektamer 38 to the sheepshead minnow, *Cyprinodon variegates.* Date: 1991-04-01	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.1.1 (Non-key study)
[redacted]	1991b	Acute flow-through toxicity of Tektamer 38 to the mysid, *Mysidopsis bahia.* Date: 1991-04-01	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.1.2 (Non-key study)

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
[redacted]	1991c	Static acute toxicity of Tektamer 38 to bivalve mollusc embryos and larvae. Date: 1991-04-02	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.1.2 (Non-key study)
[redacted]	1991d	Early life-stage toxicity of Tektamer 38 to the rainbow trout, *Oncorhynchus mykiss*. Date: 1991-06-26	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.3.2/01
[redacted]	1991e	Chronic Toxicity of Tektamer 38 to the Daphnid, *Daphnia magna*. Date: 1991-04-01	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.3.4/01
[redacted]	1995	Activated sludge Respiration Inhibition Test with DBDCB. Date: 1995-06-26	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.1.4/01
[redacted]	1995	Acute Toxicity of DBDCB to the Freshwater Alga, *Selenastrum capricornutum*. Date: 1995-06-26	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.1.3/01
[redacted]	1982a	Acute toxicity of Tektamer 38 to the sheepshead minnow, *Cyprinodon variegates.* Date: June, 1982	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	7.4.1.1 (Non-key study)
[redacted]	1982b	Acute toxicity of Tektamer 38 to grass shrimp (*Palaemonetes pugio*). Date: June, 1982	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	7.4.1.2 (Non-key study)

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
[redacted]	1982c	Acute toxicity of Tektamer 38 to blue carbs (*Callinectes sapidus*). Date: June, 1982	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	7.4.1.2 (Non-key study)
[redacted]	1996	Tektamer 38: Acute Toxicity To The Daphnid, *Daphnia magna*. Date: 1996-12-18	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.1.2/01
[redacted]	1992	Inhalation Toxicity in Rats. Date: 1992-10-09	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	Non key (A6.1.3)
[redacted]	1983	Photoallergy Test with Natural Sunlight. Date: 1983-01-19	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	Non-key (A6.1.5)
[redacted]	2007	Allergic contact dermatitis from methyl–dibromo-glutaronitrile [redacted]	[redacted]	[redacted]	No	–	Non-key Published (A6.12.6)
[redacted]	1990	Soil/sediment adsorption-desorption of Tektamer 38. Date: 1990-10-16	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.2.3.1/01
[redacted]	1980	Modified Dominant Lethal Evaluation in Mice. Date: 1980-01-14	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.6.6
[redacted]	1980a	Ninety-Day Feeding Study in Rats Exposed *In Utero* - Tektamer 38. Date: 1980-03-04	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.4.1/01
[redacted]	1980b	Thirteen-Week Subchronic Dietary Administration in Dogs. Date: 1980-02-07	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.4.1/02

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
[redacted]	1982	T3 and T4 Toxicity Study in Dogs – Tektamer®38. Date: 1982-09-17	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.10
[redacted]	2007	Statement	[redacted]	–	No	–	Non-key (6.12.1)

8 ANNEXES

Annex 1: NICNAS Existing Chemical Hazard Assessment Report Methydibromo Glutaronitrile (MDBGN)

Annex 2: Medical Statement Lanxess Corporation US, (2015)

Annex 3: Confidential information