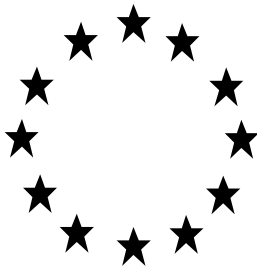
Regulation (EU) no 528/2012 concerning the making available on the market and use of biocidal products

Evaluation of active substances

Assessment Report



DBDCB

(1,2-Dibromo-2,4-dicyanobutane)

Product-type 6

December 2015

Czech Republic

CONTENTS

1 STATEMENT OF SUBJECT MATTER AND PURPOSE

1.1 Procedure followed

This report has been established as a result of the evaluation of **1,2-Dibromo-2,4-dicyanobutane** (DBDCB) as product-type 06 (Tektamer 38), carried out in the context of the work programme for the review of existing active substances provided for in Article 89 of Regulation (EU) No 528/2012, with a view to the possible approval of this substance.

1,2-Dibromo-2,4-dicyanobutane (CAS no. 35691-65-7) was notified as an existing active substance, by *LANXESS Deutschland GmbH*, hereafter referred to as the applicant, in product-type 06.

On 31 July 2007, the CZ competent authorities received a dossier from the applicant. The Rapporteur Member State accepted the dossier as complete for the purpose of the evaluation on 31 October 2007.

On 21 January 2009 the Rapporteur Member State submitted to the Commission and the applicant a copy of the evaluation report, hereafter referred to as the competent authority report.

In order to review the competent authority report and the comments received on it, consultations of technical experts from all Member States (peer review) were organised by the Commission and the Agency. Revisions agreed upon were presented at the Biocides Technical Meeting and at Biocidal Products Committee and its Working Group meetings and the competent authority report was amended accordingly.

1.2. Purpose of the assessment report

The aim of the assessment report is to support the opinion of the Biocidal Products Committee and a decision on the approval of 1,2-Dibromo-2,4-dicyanobutane as product-type 06, and, should it be approved, to facilitate the authorisation of individual biocidal products. In the evaluation of applications for product-authorisation, the provisions of Regulation (EU) No 528/2012 shall be applied, in particular the provisions of Chapter IV, as well as the common principles laid down in Annex VI.

For the implementation of the common principles of Annex VI, the content and conclusions of this assessment report, which is available from the Agency web-site shall be taken into account.

However, where conclusions of this assessment report are based on data protected under the provisions of Regulation (EU) No 528/2012, such conclusions may not be used to the benefit of another applicant, unless access to these data for that purpose has been granted to that applicant.

2 OVERALL SUMMARY AND CONCLUSIONS

2.1 Presentation of the Active Substance

2.1.1 Identity, Physico-Chemical Properties & Methods of Analysis

IDENTITY, PROPERTIES

Active substance is 1,2-Dibromo-2,4-dicyanobutane (CAS no. 35691-65-7), with molecular formula $C_6H_6Br_2N_2$ and relative molecular mass of 265.9.
The identity of impurities in the active substance is confidential. This information is provided separately in the confidential part of the dossier. None of the manufacturing impurities in typical concentrations is considered to be of potential concern.

1,2-Dibromo-2,4-dicyanobutane (further on DBDCB) is yellowish white granular solid with melting temperature 50.3 °C. Between 10 °C and 30 °C solubility in water increases from 1 to 2.6g/L, and solubility in n-octanol from 10 to 30g/L. Octanol/water partition coefficient of 10 (logPow about 1) indicates a preference of the hydrophobic compartments. Low values of vapour pressure (3.8×10^{-3} Pa at 20 °C) and of Henry's law constant (6.0×10^{-04} Pa m^3 mol^{-1} at 20 °C and pH 7) indicate slow evaporation and leakage from water solution. DBDCB is not surface active: surface tension 73 mN/m at 20 °C.

DBDCB is not highly flammable. It does not liberate flammable gases in hazardous amounts, it has no pyrophoric properties and does not undergo spontaneous combustion. The active substance contains no functional groups, which may indicate explosive or oxidising properties.

Further identification characteristics and physico-chemical properties of DBDCB are given in Chapter 1 of the List of endpoints.

ANALYSIS

The analytical methods for the determination of the active substance (RP18-HPLC with UV detection) and impurities in the active substance as manufactured have been validated.

The analytical method for the determination of active substance residues in water (HPLC-MS/MS) has been validated and shown to have sufficiently low limit of quantification (LOQ = 0.0001mg/L) with respect to the levels of concern. For the use as in-can preservative, the occurrence of DBDCB in air or soil is not expected.

Classification of DBDCB as toxic is based on the local irritation of airways rather than on systemic effects. The molecule of DBDCB has not been detected in blood or tissues even immediately after iv administration, and neither DBDCB nor 2-MGN have been detected in blood or tissues after dermal exposure. Determination of conjugated 2-MGN in urine or monitoring of bromine in blood or urine using standard laboratory techniques might be informative, but hardly so at expected levels of exposure.

Regarding the absence of systemic toxicity, determination of DBDCB concentrations in body fluids and tissues is not necessary.

Further details on analytical methods are given in the Chapter 2 of the List of endpoints.

2.1.2 Intended Uses and Efficacy

Intended uses

The representative biocidal product Tektamer 38 is identical with the active substance. It is used as an antimicrobial preservative (product type 6 of the EU Biocidal Product Directive) for water based paints intended for decorative brush/roller-painting indoors. Thus, bio-spoilage during the shelf life of the product is avoided. Tektamer is incorporated into the paint to be preserved homogeneously either directly or pre-dispersed in water or pre-dissolved in organic solvents.

Examples of products to be protected: water based paints for decorative brush/roller-painting indoors.

Efficacy

DBDCB molecule contains several highly electrophilic centres making the compound strongly reactive with nucleophilic groups in the microbial cell. The consequence is a broad activity spectrum covering bacteria, yeasts, fungi and algae. No time delay is expected.

Genetic barriers to resistance have not been studied. Relative resistance should be expected in microorganisms capable of producing higher amounts of nucleophilic compounds or in media containing such compounds. Development of resistance has not been observed during 20 years of use in cosmetic products. Nevertheless, applying DBDCB concentrations on the margin of efficacy should be avoided.

In efficacy tests the broad efficacy of DBDCB was confirmed against bacteria (minimum inhibitory concentrations, MIC <1 to 150 mg/L), yeasts (MIC 2 to 100 mg/L) and mould fungi (MIC 10 to 500 mg/L). The highest value of MIC corresponds to a concentration of 0.05% w/V. Efficacy tests in 3 types of water- based products have shown that the efficacy was good at slightly higher concentrations. On the basis of the evaluation of the summary data provided in support of the efficacy of the accompanying product, the product may be expected to be efficacious in concentrations 0.08 – 0.15%.

The intended uses of the substance are listed in the List of endpoints, Appendix 1, chapter 1.

2.1.3 Classification and Labelling

Classification and labelling for the active substance DBDCB and biocidal product Tectamer 38

In acute experimental tests, DBDCB irritated the gastrointestinal system if ingested and the respiratory system when inhaled, caused injury to eyes and sensitisation of skin. DBDCB appeared toxic by inhalation, harmful if swallowed and very toxic to aquatic organisms. The physical and chemical properties of DBDCB do not fulfil the criteria for a classification according to Council Directive 67/548/EEC.

Proposed classification and labelling for the active substance DBDCB and biocidal product Tektamer 38 according to Regulation 1272/2008

Hazard category:	Acute Tox. 4 (oral) ; H302 Acute Tox. 2 (inhalation) ; H330 Skin sens. 1 ; H317 Eye Dam. 1; H318 Aquatic Chronic 2; H411
Pictogram:	 *GHS05*  *GHS06*  *GHS09*
Signal word:	Danger
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 organisms with long lasting effects
Precautionary statements:	P260: Do not breathe dust. P273: Avoid release to the environment. P280: Wear protective gloves and eye protection / face protection. P304 + P340: IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P310: Immediately call a POISON CENTER or doctor/physician.

2.2 Summary of the Risk Assessment

2.2.1 Human Health Risk Assessment

2.2.1.1 Hazard identification

Toxicokinetics and metabolism

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

Similar absorption may be assumed after **inhalation** of aerosols containing low, non-irritating concentrations of DBDCB. At an airborne DBDCB concentration of 1 mg.m^{-3} the absorbed dose may be expected to be somewhere between 1 mg and 2 mg per hour.

Dermal absorption of DBDCB in rats following a 24 h exposure to a 20 mg/mL solution in acetone led to a systemic absorption of 22 %. When rats were exposed to a dose of 25 mg/kg bw DBDCB in 0.5 ml of ethanol / propylene glycol, 3:7 (v/v) for 72 h, an average percutaneous absorption of 6.8 % was found. On the basis of an *in vitro* study, human skin is estimated to absorb 19% of DBDCB from a water solution which is used in the risk characterization for the a.s. in the CAR. At the product authorisation stage EFSA guidance shall be used to evaluate dermal absorption rate relevant for uses to be authorised.

As an intact upper epidermal layer is a prerequisite for the skin to function as a chemical barrier, lesions of the upper epidermal layers would modify dermal absorption in an unpredictable way; testing of dermal absorption and toxicity of more concentrated solutions is thus not warranted.

Tissue distribution studies 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 main excretory path is via urine (65-88 %).

DBDCB is totally debrominated prior to systemic distribution, and converted to 2-methyleneglutaronitrile (2-MGN) by reaction with sulfhydryl groups. Two bromide ions are released from one molecule of DBDCB: absorbed dose of 1 mg of DBDCB contributes approximately 0.6 mg bromide to body load; for comparison, 6 to 60 mg of bromine is normal range of amount excreted daily by a man.

Unlike other cyano-containing compounds, DBDCB does not liberate cyanide ions. 2-MGN is further metabolized, and its metabolites are then excreted into the urine. The primary route of biotransformation for 2-MGN is glutathione conjugation, which results in *N*-acetyl-*S*-(2,4-dicyanobutane)-L-cysteine as the principal urinary metabolite.

Tissue exposure to intact DBDCB appears to be exceedingly low regardless of route of administration, incl. intravenous injection. A small amount of 2-MGN was present in the blood following oral administration (C_{max} 0.32 µg/ml).

With respect to dominant route of exposure, blood and tissue kinetics of DBDCB and its active metabolites following dermal exposure is of principal importance. Measurable radioactivity appeared in urine after 6 hours of topical exposure of rats; maximum cumulative radioactivity, corresponding to approximately 5 % of the dose, was attained after 24 hours, indicating that a portion of the dose was absorbed percutaneously from the ethanol / propyleneglycol solution. The average rate of percutaneous penetration may be estimated to be 0.0043 mg cm^{-2} h^{-1}(supporting the reliability of the estimate of the maximum dermal absorption rate calculated for water solutions, i.e., 0.005 mg cm^{-2} h^{-1}). At this absorption rate, neither DBDCB nor its metabolites were detected in the

blood, at any time point of exposure from 15 minutes through 72 hours, obviously due to a slow absorption of DBDCB and rapid conjugation of 2-MGN.

Mechanisms of toxicity

DBDCB molecule contains several highly electrophilic centres making the compound strongly reactive with nucleophilic groups. This reaction is probably the mechanism of local toxic reactions at the sites of contact and entrance, involving sensory irritation, inflammation and sensibilization. In the potency of macromolecular cross-linking and formation of immunoreactive adducts (haptens) DBDCB is similar to diisocyanates.

The submitted toxicokinetic *in vivo* study in rats indicates that DBDCB is rapidly debrominated and metabolized to 2-methyleneglutaronitrile – regardless of the route of administration. The debromination and transformation to methyleneglutaronitrile is so rapid – also *in vitro* in blood – that only the metabolites are disposed to the tissues, and determine probably the systemic toxic potential of DBDCB. The principal metabolite, and probably other intermediates, display high potency of covalent binding to macromolecules in blood and tissues, and are slowly eliminated, permitting effective cumulation. Structure activity analysis indicates mutagenicity, carcinogenicity and teratogenicity of alpha-beta unsaturated nitrile fragment (in 2-methyleneglutaronitrile) and oxirane fragment (in 2-methyleneglutaronitrile oxide).

Cumulation of bromine (bromide ion) might have some role in subchronic toxicity of DBDCB. The potency of bromide ion to replace other halogens is suspected to be responsible for experimental toxicity of high bromine doses, especially in animals on low chlorine diet. Bromide is a normal constituent of the human diet with daily intake ranging from 2 to 8 mg. Oral exposure to daily DBDCB doses in excess of 100 mg/kg bw corresponds to daily bromine intake higher than 60 mg/kg bw that is expected to have systemic effects, namely on thyroids.

The prediction of actual toxicity of DBDCB, 2-methyleneglutaronitrile and excess bromine for the particular human exposure scenario depends critically on the balance between absorption and disposal rates.

Acute and subacute toxicity

DBDCB was of moderate **oral toxicity** to rats (LD_{50} = 500 – 600 mg/kg in corn oil); necropsy findings indicated severe irritation of stomach and intestinal mucosa, and macroscopic changes in liver, lungs and kidneys. (Repeated daily doses near to the LD_{50} level were tolerated for many weeks when administered in diet.)

The toxicity via the **dermal route** was low, both in single dose study in rabbits (LD_{50} > 2000 mg/kg bw) and subacute study in the rat (1 to 4 $g.kg^{-1}.d^{-1}$). In the subacute study, systemic effects (decrease in haematocrit, haemoglobin and red blood cell values, increased relative weights of liver and kidneys) were significant but of low intensity in all dose groups and all values remained within the range of reference values, rendering the biological significance of these observations unclear. At the same time, local dermal reactions were severe at all tested levels of DBDCB. Such epidermal lesions would have modified dermal absorption in an unpredictable way rendering estimation of LOAEL and NOAEL values impossible.

Two 4-h **inhalation toxicity** studies with DBDCB dust in the rat have shown high toxicity with an LC_{50} of 265 mg/m^3 for both sexes. The toxicity pattern involved marked airway irritation resulting in fatal acute lung edema. Lung injury persisted for at least 2 weeks in survivors. Gastrointestinal mucosa displayed also signs of irritation. Of concern is corneal opacity in animals exposed to dust in high concentration of 200 mg/m^3.

DBDCB caused slight **irritation** of the skin not fulfilling the criteria for classification as a skin irritant, but caused strong irritation of the eye in tests on albino rabbits, and in rats severe irritation of airways resulting in acute lung edema. Low intensity of skin irritation induced by a single dose of solid DBDCB is clearly an artefact of testing procedure and contrasts with severe local dermal reactions to repeated dosing in the subacute dermal toxicity test on rats.

DBDCB was tested for its skin **sensitisation** potential on guinea pigs as well as in repeated insult patch tests on humans. One test on guinea pigs was positive with weak to moderate reaction observed in 7/10 animals.

Tests on human volunteers were clearly positive. Photoalergy test with 0.08 % DBDCB solution for induction and challenge was weakly positive. The exposure to a rinse-off product containing 0.1 % DBDCB elicited an allergic response in pre-sensitized individuals while similar product containing only 0.02 % DBDCB (calculated dose of 0.016 mg/cm^2 of skin) did not. The weight of the evidence is sufficient to conclude that DBDCB has a weak but distinct skin sensitising and photosensitising potential.

In the potency of macromolecular crosslinking and formation of immunoreactive adducts DBDCB could be considered similar to diisocyanates, but there is no epidemiological evidence of respiratory sensitisation. Therefore, DBDCB is not to be classified as potential resp. sensitizer.

Subchronic toxicity

A **subchronic feeding** study in **rats** (approximate daily doses 0, 6, 35 and 240 mg/kg) where 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). Thus, when weanlings were first exposed via diet, they had already been potentially exposed to DBDCB or its metabolites *in utero* or via nursing. 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. Only slight, non-significant deviations compared to control groups were observed in groups administered 34 and 39 mg/kg bw/day in male and female rats, respectively.

Subchronic dietary exposure of **dogs** to DBDCB (approximate daily doses 0, 5, 35 and 120 mg/kg) caused clinical signs of toxicity (diarrhoea, emesis and ataxia) at the highest dose level. Feed consumption and body weight development were also depressed in this dose group. Extramedullar haematopoiesis, significant changes in haematological values (decreased haematocrit, erythrocyte count) and significantly increased thyroid weights with glandular hyperplasia were noted at the top dose. Slight increase in the weight of thyroid was found also in dogs of the medium dose group.

Cumulation of bromine might have some role in subchronic toxicity of DBDCB. Exposure to DBDCB at LOAEL level corresponds to daily bromine intake of 144/191 mg/kg bw in male/female rats and of 61/66 mg/kg bw in dogs: daily doses above 60 mg/kg bw are expected to have systemic effects, namely on thyroids.

The thyroidal effects seen in the main subchronic dog study were re-investigated in a special 13-week feeding study. No effects were noted on basal or TSH-stimulated levels of serum T3/T4 concentrations. The histomorphological appearance of thyroids was not affected by a dose of 5.7 mg/kg bw/day: this dose corresponds to daily bromine intake of 3.8 mg/kg bw. (For comparison, LOAEL 9 mg/kg bw per day has been determined for effects of bromine on thyroids in human subjects.)

Both subchronic studies were performed with sufficient numbers of animals and broad spectrum of endpoints, and yielded consistent evidence of toxicity of high oral doses (extramedullar haematopoiesis, thyroid hyperplasia, weight changes of other endocrine glands). The interpretation of findings is, however, ambiguous. No truly systemic toxic effects, apart from effects of bromine on thyroid gland at the LOAEL dose, were identified. As the concentration of the irritating active substance in the diet increased proportionally to the dose, the lowered food consumption and body weight in animals exposed to oral doses on the level of LOAEL and higher were most likely secondary to local irritation of the gastrointestinal tract, and effects related to haematopoiesis were observed only in animals repeatedly administered high daily oral doses known to cause impaired digestion and malabsorption of nutrients.

The effects of daily dose 28 – 39 mg/kg bw were of marginal intensity and transient (weight gain, erythrocyte count, plasma proteins, thyroids, organ weights). They were mostly statistically not significant. No effects at all were observed at doses 6 and 5 mg/kg for rats and dogs, respectively.

Genotoxicity

The submitted data include protocols of four *in vitro* genotoxicity assays: Bacterial Ames test, Mammalian cell gene mutations (in hamster lung cells), UDS in human fibroblasts, and Chromosomal aberrations in hamster ovarian cells, and protocols of *in vivo* Micronucleus assay in bone marrow cells and Dominant lethal test in mice.

DBDCB was non-mutagenic in bacterial and mammalian gene mutation tests, with and without activation. However, *in-vitro* chromosomal aberration assay showed an increased frequency of aberrant metaphases –with and without activation– at concentrations that did not fulfil the cytotoxicity criteria of OECD Guideline 473. Due to extremely steep increase in cytotoxicity with concentration of DBDCB all evaluated doses were in the range of marked toxicity.

Confirmatory *in vitro* UDS assay in cultured human fibroblasts was negative, with and without activation. *In vivo* assay for micronucleus formation in bone marrow cells in mice was negative for doses sufficient to exert systemic toxicity. Dominant-lethal assay in mice demonstrated that DBDCB is not a germ cell mutagen.

Overall, the tested substance is not considered to be genotoxic.

Long-term toxicity and carcinogenicity

Long-term toxicity study has been performed as part of carcinogenicity testing and is summarised in Appendix 2 to DOC IIA. In the main part of the 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 (0.5 ml/kg of the solution), and similar groups of male and female mice received 0.6, 2, or 6mg DBDCB per kg (in 2 ml/kg bw). Groups of 50 animals receiving just the ethanol solution served as controls. Tissues from more than 40 sites were examined for every animal. Survival by animals exposed for 2 years to 1,2-dibromo-2,4-dicyanobutane was the same as for the controls, but rats exposed to the highest concentrations weighed less than the controls. Dermal exposure to DBDCB was not associated with any increase in the incidence of 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), except local skin lesions at the site of application..

NOAEL for systemic effects (incl. body weight) is 6 mg/kg bw.

The outcome of this study is further supported by toxicological and exposure considerations.

Products containing DBDCB in concentrations similar to those proposed for use as in-can preservatives have been in widespread use by the general public for many years. Reports on adverse effects were limited to contact dermatitis. Increased tumour incidences have neither been reported nor suspected in connection with the use of DBDCB in biocidal products.

The predicted genotoxic potential of DBDCB is low when considering the negative outcome of all but one *in vitro* genotoxicity assays, the latter using cytotoxic concentrations.

Subchronic studies in rats and dogs yielded no indication for any pre-neoplastic lesions or any other condition that poses a risk for non-genotoxic tumorigenesis, e.g., inflammatory reactions. Adverse effects observed included haematological alterations, signs of extramedullary haematopoiesis and mild thyroidal hyperplasia in animals of the top dose groups (100 – 240 mg/kg per day).

With respect to dominant route of exposure, blood and tissue kinetics of DBDCB and its active metabolites following dermal exposure is of principal importance. Measurable radioactivity appeared in urine after 6 hours of topical exposure of rats to labelled substance in a dose of 25 mg/kg bw in ethanol/propyleneglycol solution; at the same time neither DBDCB nor 2 -methyl glutaronitrile were detected in the blood, at any time point of exposure from 15 minutes through 72 hours, obviously due to a slow absorption and instant conjugation of 2-MGN.

Conducting further long-term cancer bioassays appears not justified because of the low exposure that can be expected. None of the products is related to food and beverage consumption. Several exposure scenarios for the products envisaged have been assessed using realistic worst-case assumptions. As the maximum levels of human exposure predicted by calculation are by approximately three orders of magnitude lower than the lowest extrapolated LOAEL – NOAEL margin for long-term exposures in animals, also the epigenetic carcinogenicity of DBDCB and 2-MGN seems highly improbable.

Reproductive toxicity

No human data are available. A two-generation reproduction study in rats has not been conducted. Instead, a study with rats exposed prenatally, during lactation and for further 13 weeks to daily DBDCB doses up to 240 mg/kg comparable with the OECD extended one generation reproductive toxicity study protocol in regulatory risk assessment was performed. The relatively short period of premating of one week was justified by the substance virtually reaching a steady within one week of exposure and by the outcome of other studies concerning the relevant endpoints. In this study no macroscopic or microscopic findings in the reproductive organs (testis/epididymis, prostate gland, uterus, and ovary) were detected. No triggers for test on the second generation were observed leading to a conclusion that performing a two generations study is redundant.

Furthermore, three other tests indicate no specific toxicity of DBDCB on reproduction functions. Dietary exposure of male mice to daily DBDCB doses up to 450 mg/kg for 8 weeks before mating had no effect on number and successfulness of pregnancies. Maternal toxicity, evident as impaired body weight gain, was the only significant and relevant effect of DBDCB in developmental toxicity studies in rats and rabbits. The overall NOAEL for maternal toxicity was 30 mg/kg bw per day, based on maternal toxicity in the rabbit study. Since there were no specific effects on developmental parameters in either species, an overall developmental NOAEL was 60 mg/kg bw per day.

Bromide in daily oral doses of 15 mg/kg bw had no effects on fertility and reproduction in a 3-generation study in rats; this result supports NOAEL of DBDCB of 30 mg/kg bw.

Epidemiological and medical data

Results of patch tests and use tests with DBDCB-containing preparations confirm that DBDCB has a distinct skin sensitising potential in humans. Experience with 0.1 % DBDCB as preservative in cosmetic products indicates the rising incidence of contact allergy to the compound.

Other/special studies

The thyroidal effects are discussed as part of subchronic toxicity.

b) Physical-chemical hazards

Given the physicochemical properties DBDCB is not hazardous with respect to flammability, explosivity and oxidative properties**.**

2.2.1.2 Effects assessment

Summary of toxicological data:

DBDCB causes slight irritation of the skin, strong irritation of the eye and has a weak but distinct skin sensitising potential. Local irritation at the place of entrance is probably the cause of moderate acute toxicity by oral route and high toxicity of inhaled dust aerosol.

DBDCB is easily absorbed from the gastrointestinal tract. Swallowing inhaled substance and secondary absorption from the gastrointestinal tract should be expected. Skin penetration from the deposited water solution is very limited; on the other hand, dermal absorption from solvent-based preparations is considerable.

No subchronic systemic toxic effects of concern were identified. Lowered food consumption and body weight in animals exposed to oral doses on the level of LOAEL and higher were clearly secondary to local irritation of the gastrointestinal tract. Effects related to haematopoiesis were observed only in animals repeatedly administered high daily oral doses known to cause impaired digestion and malabsorption of nutrients. Experimental studies indicate possible effects of the substance on the thyroid gland. No lesions in male or female reproduction organs, no impairment of fertility and no developmental toxicity were observed at toxic doses. No carcinogenic potential is predicted for relevant exposure levels.

Subchronic exposure to DBDCB at LOAEL level corresponds to daily bromine doses exceeding 60 mg/kg bw, doses known to have systemic effects, namely on thyroidal and other endocrine functions. No effects are expected at NOAEL levels.

Based on available data, irritation of eyes and respiratory tract (at higher concentrations) and skin sensitizing potential of DBDCB are the critical types of effect.

Reference values for <u>systemic effects</u>

Sec. 2 2.1 **Tab. 1: Summary of LOAEL and NOAEL values**

Study	Species	End point	LOAEL [mg/kg bw/day]	NOAEL [mg/kg bw/day]
21-day **dermal** 6.3.1.2.01 DOC IVA, 1992	Rat	No true systemic effects		
		Dermal effects	< 1000	< 1000
13-week **feeding** 6.4.1(01) DOC IVA, 1980a	Rat	Lower body weight, splenic haematopoesis	240/317 (♂/♀)	34/39 (♂/♀)
13-week **feeding** 6.4.1.(02a) DOC IV A, 1980b	Dog	Clinical signs, lower bw gain, haematological effects, and thyroid hyperplasia	102/110 (♂/♀)	30/38 (♂/♀)
Teratogenicity, **oral, gavage** 6.8.1. (02a) DOC IV A, 1994	Rabbit	Lowered maternal bw gain and feed consumption	60	30
		No reproductive toxicity	> 60	60

The acceptable exposure levels for short-term and long-term exposures are based on the overall NOAEL of **30 mg/kg bw** per day derived from the subchronic dog feeding study and the gavage teratology study in rabbits. As no true systemic effects of concern were identified in animals exposed to repeated oral doses on the level of LOAEL, and effects of higher doses were clearly secondary to local irritation of the gastrointestinal tract, a standard assessment factor of 100 is considered to be justified for the assessment of occupational and short-term (intermittent) residential exposures and factor of 200 for chronic exposures.:

$AEL_{short-term}$ = 30 mg/kg bw x 10^{-2} = **0.3 mg/kg bw/day**

$AEL_{intermediate}$ = 30 mg/kg bw x 10^{-2} = **0.3 mg/kg bw/day**

$AEL_{long-term}$ = 30 mg/kg bw x 10^{-3} = **0.03 mg/kg bw/day** *

*At product authorization stage AEL long term value based on the new ECHA guidance shall be used

Reference values for <u>local effects</u>

According to guidance document on human health risk characterization for local effects is triggered only when the product is correspondingly classified. The active substance is classified as Skin sens. 1, which should trigger only qualitative risk assessment. However, for information quantitative reference values are provided:

Paints/products containing DBDCB shall be classified or labelled depending on the harmonised classification to be in force before the product authorisation stage.

Safe levels with respect to respiratory tract irritation may be suggested also for the mixtures that are not classified for local effects, based on occupational exposure limit values for model respiratory irritants. For information, medium 8-hour time-weighted average (TWA) and Short-term Exposure Limit (STEL) values for irritant dusts or aerosols are **1** and **3 mg/m^3**, respectively.

2.2.1.3 Exposure assessment:

The production of active substance and the formulation of the biocidal product takes place outside the EU.

Systemic exposure

Primary systemic exposure occurs during addition of the neat active substance to a pre-mix tank. For pouring of the solid substance, deposition rate of 3 mg/min and airborne dust concentration of 5 mg/m^3 are realistic estimates. This task is performed by professional personnel wearing mandatory protective clothing (respiratory protection, gloves, coverall and goggles), decreasing both deposition on skin and inhalation exposure by 90 %.)

For task duration of 10 minutes per day and dermal absorption of 19 %, the total systemic daily doses of 0.051 and 0.0025 mg of the active substance were calculated for dermal and respiratory routes (see Tab. 2).

Secondary systemic exposure (i.e primary exposure to the treated paint) occurs when the treated paint containing 0.1 % or less of DBDCB is used by professional or residential handler. Brush painting is used as a typical example of activity leading to secondary exposure. Ingestion of paint chips by small children and domestic inhalation are also considered as possible exposure routes.

Table 2. Systemic doses resulting from primary and secondary exposure

Exposure scenario	Exposed population	Systemic daily dose [mg/kg bw]			
		Oral	Dermal	Inhalation	Total
Preparing DBDCB premix	Worker with PPE	/	0.051	0.0025	**0.053**
Brush painting, 0.1% DBDCB in paint	Amateur without PPE	/	0.0068	0.0000849	**0.0069**
	Professional without PPE	/	0.0163	0.000204	**0.0165**
Brush painting, 0.1% DBDCB in paint	Professional with gloves, coated coverall	/	0.0047	0.0002	**0.0049**
Ingestion of paint chips, 0.1% DBDCB in paint	Children	0.0008	/	/	**0.0008**
Ingestion of paint chips, shortly after painting	Children	0.004	/	/	**0.004**
Domestic inhalation	toddler	/	/	0.0026	0.00266

Local exposure

According to guidance document on human health a risk characterization for local effects is triggered only when the product is correspondingly classified. Classification triggers qualitative risk assessment as quantitative risk assessment for local effects is problematic. Therefore, local exposure was quantified for information only, giving the following values:

Primary local exposure The estimated local dose of DBDCB at manual pouring without gloves is **0.036 mg/cm^2** of skin.

Respiratory tract:

Realistic estimate for short-term airborne concentration of DBDCB dust at manual pouring of the biocidal product into the mixing vessel is **5 mg/m^3**. Mandatory respiratory protection with a minimum protection factor 10 would decrease the inhaled concentration to **0.5 mg/m^3**

Secondary local exposure (i.e primary exposure to the treated paint) occurs when the product (e.g., paint) containing 0.1 % or less of DBDCB is used by professional or residential handler.

Skin: The estimated local dose of DBDCB at brush painting without gloves is **0.0043** and **0.022 mg/cm^2** of skin for amateur and professional painters, respectively.

Respiratory tract: Realistic estimate for airborne concentration of DBDCB at painting is **0.00033** and **0.00089 mg/m^3** for amateurs and professionals.

2.2.1.4 RISK CHARACTERISATION FOR HUMANS

Systemic effects (Table 3)

The comparison of the estimated exposure with the relevant long-term AEL demonstrates that the addition of DBDCB as in-can preservative to water-based paints is safe for professional operators using PPE.

The highest potential exposure is predicted for professional painters using water-based paints containing DBDCB at the highest intended concentration. Even this use without PPE reaches only 55% of the long term AOEL and is considered as safe in terms of systemic effects.

Predicted systemic doses for all considered residential exposure scenarios are safely below AELs.

Combined primary + secondary (resident) exposure would lead to lower daily dose than secondary exposure of professional users.

Table 3. **Risk characterisation for systemic effects of primary and secondary exposure**

Local effects

Exposure scenario	Exposed population	Systemic daily dose [mg/kg bw]	*%AEL*
Preparing DBDCB premix	Worker with PPE	0.053	***17.6*** (intermediate)
Brush painting, 0.1% DBDCB in paint	Amateur without PPE	0.0069	***2.30*** (short term)
	Professional without PPE (long term)	0.0165	***55*** (long term)
Brush painting, 0.1% DBDCB in paint	Professional with PPE (gloves, coated coverall)	0.0049	*16.3* (long term)
Ingestion of paint chips, 0.1% DBDCB in paint	toddler	0.008	2.66 (short term)
Ingestion of paint chips, shortly after painting	toddler	0.004	***1.33*** (intermediate)
Domestic inhalation	toddler	0.00266	***0.8*** (short term)

According to guidance document on human health risk characterization for local effects is triggered only when the product is correspondingly classified. Classification triggers qualitative risk assessment as quantitative risk assessment for local effects is considered

problematic. However, in addition to qualitative risk assessment, its quantitative version is also provided, albeit for information only:

Qualitative risk assessment

The active substance is classified for as Skin sens 1. Therefore appropriate PPE, including gloves must be used by industrial workers when handling it. Use of RPE is recommended if exposure on inhalation is envisaged. The same applies to the product classified for local effects.

Quantitative risk assessment (for information)

The estimated local skin dose of DBDCB at manual transferring DBDCB solid to pre-mix tank (with gloves) is **0.0036 mg/cm^2**, lower than the safe dose of 0.016 mg/cm^2. At professional painting (without gloves), the cumulative whole-shift dose is predicted to be **0.022 mg/cm^2.** Deposition of the cumulative dose is spread over the whole shift, and thus cannot be directly compared with the safe dose of 0.016 mg/cm^2 administered instantly.

The estimated airborne concentration of DBDCB of **0.5 mg/m^3** in the breathing zone of workers equipped with respirators at manual dosing of the solid biocidal product into the mixing vessel is lower than the occupational level for short-term exposure to respiratory irritants (3 mg/m^3). Predicted airborne concentrations at brush painting are <**0.001 mg/m^3**, respectively, and are safely below the reference limit for 8-hour exposure to respiratory irritants (1 mg/m^3).

Conclusions for toxicity

The primary exposure of professionals to DBDCB during industrial formulation of water-based products is considered to be acceptable provided appropriate PPE is worn.

There is no primary exposure to non-professionals in this product type (PT6).

The secondary exposure of professional or residential handlers to the products containing 0.1 % or less of DBDCB is considered to be acceptable.

The potential combination of primary and secondary exposure is considered to be acceptable.

This risk assessment for the use of DBDCB in a Product Type 6 application demonstrates that there are no toxicological concerns over human health following primary or secondary exposure.

ASSESSMENT OF RELIABILITY OF ASSUMPTIONS:

1) ***Reference values*** are considered to be sufficiently protective. Regarding the absence of true systemic toxicity of DBDCB, AEL values for systemic effects are considered to overestimate the toxic hazard.
2) ***Dermal absorption*** *of 19%* of DBDCB from water based solutions was determined in an *in vitro* experiment. . At the product authorisation stage EFSA guideline shall be used to evaluate dermal absorption due to uses to be authorised

3) The ***models used for estimation of human exposure*** are considered conservative and yet the risk characterisation for the use of DBDCB shows that there is no concern over primary or secondary human exposure, despite the worst-case assumption is consistently used.

CONCLUSION

The use of DBDCB as in-can preservative for water based paints to be used for decorative brush/roller paintings indoors is safe for all individuals that might be exposed during application and all downstream processes.

When handling the neat active substance, the following **risk reduction measures** are required to prevent eye damage, airway irritation and skin sensitisation:

1) Safety goggles must be worn.
2) Respiratory protection with a minimum protection factor 10 must be worn.
3) Impermeable clothing must be worn.

Professionals using water based products containing over 0.1 % of DBDCB on a daily basis should wear gloves and body protection, decreasing dermal deposition by at least 90%.

2.2.2 Environmental Risk Assessment

The biocidal active substance DBDCB is intended to be used for the in-can conservation (PT06) of aqueous systems such as polymer emulsions, coating compositions, adhesive materials, inks, and similar products prepared on aqueous basis.

The biocidal active substance was assessed for the use to treat paints for indoor decorative painting. For the active substance evaluation, the applicant has submitted the required studies on the environmental fate and behaviour of the active substance DBDCB as well as additional studies on the major metabolite 2-methyleneglutaronitrile as decided in the TM IV 2010.

*2.2.3 **Fate a**nd distribution in the Environment for DBDCB

Abiotic degradation

HYDROLYSIS

DBDCB will be significantly degraded via hydrolysis in basic aquatic media (pH 9, DT_{50} = 9.1 days, corresponding to 25.8 days at 12°C). At environmentally relevant pH value of 7, DBDCB is considered to be hydrolytically stable (DT_{50} = 51.6 – 96.3 days corresponding to 146.4 - 273.3 days at 12°C); hydrolysis will only contribute to some extent to the degradation of the active substance. Main hydrolytic transformation products (>10% of the initial measured DBDCB) are 2-methyleneglutaronitrile at the environmentally relevant pH 7 and E-Z isomers of 1-bromo-2,4-dicyano-1-butene at pH 9, respectively.

PHOTOLYSIS IN WATER

DBDCB is susceptible to photodegradation in aqueous media at pH 5 and 25 °C. An experimental half-life of 54 days was determined for the irradiated sample. The primary product of photolytic decomposition was 2-methyleneglutaronitrile (28.5 % after 30 days). Isomers (E)- or (Z)-1-bromo-2,4-dicyano-1-butene, formed at dehydrohalogention (-HBr) were found only in small amount in one sample (2.1 % after 14 days of exposure).

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). According to OECD TG 316, the DT_{50} for irradiated samples corrected for degradation in non-irradiated controls is 62 days.

PHOTOTRANSFORMATION IN AIR

DBDCB is restrictedly susceptible to photolytic processes in air (DT_{50} = 27.107 days using a 24-hours day and a mean daily OH concentration in air of $0.5x10^{-6}$ OH radicals per cm^3). However, the air is no compartment of concern for DBDCB due to its limited potential for volatilisation, due to its low vapour pressure ($3.81x10^{-3}$ Pa at 20 °C) and the low Henry's law constant ($3.99x10^{-5}$ Pa x m^3 x mol^{-1}, calculated using EPI WIN).

Biodegradation

READY AND INHERENT BIODEGRADABILITY

DBDCB was not readily biodegradable and it did not fulfil the criteria of inherent biodegradability in a Zahn – Wellens test. This was demonstrated by the specific tests (OECD guideline 301D and OECD guideline 302B).

However, DBDCB is primary inherently biodegradable. DBDCB was degraded by 65 % of the initial concentration and transformed into 2-methyleneglutaronitrile and other minor unidentified degradation products.

AEROBIC DEGRADATION IN SOIL

It can be concluded from the aerobic aquatic metabolism study, which had been performed with viable soil (sandy loam, see above) that once DBDCB is released into soil, it will be rapidly degraded (DT_{50} of DBDCB in soil / water system was 0.874 day corresponding to 2.76 days at 12°C*), 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

DBDCB has been shown to degrade quickly under anaerobic aquatic conditions in a flooded sandy loam soil and thus, can be expected to dissipate quickly under anaerobic conditions in soil when unintentionally reaching the soil compartment (estimated laboratory half-life of 0.495 days corresponding to 1.35 days at 12°C*). Volatilization and/or mineralization were observed at appreciable degree during the study, 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-Methyleneglutaronitrile 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.

*However, due to the deficiences in the water/soil degradation study in the risk assessment of the substance a DT50 value of DBDCB in soil of 1000 days, as agreed at BPC 12, was used, representing a worst-case. This resulted in a PEC/PNEC ratio less than 1. Hence, even under this unrealistic and stringent assumption, a risk for soil is considered as acceptable. The DT50 of 1000 days was also used to assess the risk to ground water, the outcome is provided in 2.2.7. At product authorisation stage DT50 value of 1000 days shall be used unless new, reliable data are provided allowing derivation of DT50 value for soil.

Distribution

ADSORPTION ONTO / DESORPTION FROM SOIL

A batch equilibrium study allows deriving an organic carbon-water coefficient (K_{OC}) value of 64.7 $L.kg^{-1}$ (arithmetic mean K_{OC} value of n=3 soils). Due to the low amount of test compound adsorbed, K_{OC} values for 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, which amounted to 6.16 %, 23.9 %, 22.5 %, and 75.9 % for sand, sandy loam, clay loam, and silt loam, respectively. Due to the instability of the parent compound (because of hydrolysis and ionic interactions), the sorption parameters determined reflect sorption characteristics of the parent compound and its degradation products.

Bioaccumulation

For DBDCB, a log K_{OW} of 2.0 has been determined (at 25 °C). The BCF was estimated on the basis of this partition coefficient n-octanol/water according to the TGD on risk assessment (log BCF = 0.85 x log K_{OW} – 0.70) resulting in a value of BCF = 10 for DBDCB.

Hence, no food chain concern is expected.

Fate and distribution of the major metabolite 2-methyleneglutaronitrile

Abiotic degradation

With respect to the hydrolysis of 2-methyleneglutaronitrile, due to its specific structure belonging to the chemical class of nitriles hydrolytic decomposition is very likely to occur. The decomposition pathway of nitriles is described in literature (March 1985[1]), 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. No direct UV-absorption occurred in the environmental relevant wavelength between 290 and 750 nm indicating that the metabolite is not susceptible to be degraded by direct photolysis in aqueous medium.

Similar to the parent DBDCB, the metabolite 2-methyleneglutaronitrile was considered to be restrictedly susceptible to photolytic processes in air. However, a vapour pressure of 7.08 Pa at 25 °C (Mean vapour pressure of Antoine & Grain methods) and a Henry´s Law Constant of 1.20 x 10^{-2} Pa x m³/mole at 25 °C (HENRYWIN v3.20, Bond method) was estimated. Therefore it can be concluded that due to the low Henry's Law Constant, the metabolite 2-methyleneglutaronitrile can be considered as non-volatile and the air is not a compartment of concern.

Biodegradation

In the aerobic aquatic degradation study of the parent DBDCB, the metabolite 2-methyleneglutaronitrile was shown to be an unstable breakdown product with a transient character (decrease from 33% at maximum on day 2 to 0.499% until termination of the test at day 21). 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. Rapid and ultimate biodegradation of 2-methyleneglutaronitrile were clearly predicted. In addition, the physico-chemical properties of 2-methyleneglutaronitrile such as the high water solubility (35.8 g/L) and the low Koc value (72.24 L/kg) indicate that the substance will stay in the water phase and will thus be bio-available for microbial degradation.

Distribution

The distribution of 2-methyleneglutaronitrile 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. To indicate the reliability of the applied methods, 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 and a good relationship between the experimentally derived and calculated K_{OC} values was shown for the MCI method.

A K_{OC} of 17.76 mL/g was calculated (by the MCI method) for 2-methyleneglutaronitrile indicating a low sorption potential to soil.

1 March, J. (1985): Advanced Organic Chemistry (3rd Edition), Reactions, Mechanisms, and Structure. John Wiley & Sons, New York, Chichester, Brisbane, Toronto, Singapore.

2.2.4 Environmental Effects Assessment

Effects assessment for DBDCB

Aquatic organisms

ACUTE AND CHRONIC TOXICITY TO FISH

Short time and chronic tests of DBDCB on trout (*Oncorhynchus mykiss),* which shows the highest sensitivity towards this substance, are supposed to be key studies for evaluation DBDCB effects.

Short time (acute) toxicity was determined according to US EPA guideline 72-1, equivalent to OECD 203, in flow arrangement with measurements of concentrations. From the results of these experiments, the following values were calculated for DBDCB: 96h-LC_{50} = 1.26 mg.l^{-1} a NOEC = 0.726 mg.l^{-1}.

Long-term toxicity of DCDCB to fish was determined in a fish early life-stage toxicity test according to US-EPA guideline 72-4, equivalent to OECD 210, in rainbow trout. The test was performed under flow-through conditions with measurement of concentrations. The overall NOEC was determined to be 0.75 mg.l^{-1} and the overall LOEC to be 1.0 mg.l^{-1}, based on mean measured concentrations. Most sensitive endpoint was mortality.

ACUTE AND CHRONIC TOXICITY TO INVERTEBRATES

The effects of DBDCB in short time and chronic study on invertebrates were evaluated from tests on *Daphnia magna.*

In the study according to the procedure of US-EPA guideline 72-2, which is analogous to OECD 202, the following values were determined: 48 h-EC_{50} = 4.83 mg.l^{-1} and NOEC = 2.71 mg.l^{-1}.

By carrying out the 21-days chronic reproduction study with Daphnia according to US-EPA guideline 72-4, for DBDCB the value of NOEC for reproduction and growth of Daphnia was found to be 1.4 mg.l^{-1}.

GROWTH INHIBITION ON ALGAE

The effects on algae growth were evaluated based on the test carried out according to Method C.3 (2009) with the green algae *Desmodesmus subspicatus.*

Medium value of effective concentration determined based on growth rate was found to be 72 h- E_rC_{50} = 5.4 mg.l^{-1}. The value of NOErC = 0.017 mg.l^{-1} was determined.

Determination of predicted no effect concentrations for aquatic compartment ($PNEC_{water}$)

The lowest NOEC value was that of the algae with 0.017 mg.l^{-1} and this value was used for the PNEC calculation. Since long-term NOECs are available for all three trophic levels, an assessment factor of 10 was applied to the lowest of three long-term NOEC values.

$PNEC_{water}$ in this assessment has been calculated as

$PNEC_{WATER}$ = 0.0017 mg.L^{-1}.

Inhibition of activated sludge respiration rate

The effects on microorganisms were studied using the test of inhibition of breathing of activated sludge according to OECD 209 methodology. At the concentration of DBDCB 10 mg.l-1, no inhibition was observed. The 3h EC50 value was determined to be 34 mg.l-1.

Determination of predicted no effect concentrations for STP micro-organism

In agreement with EU TGD on Risk Assessment it is possible to determine PNEC microorganism from NOEC or EC50 values obtained in the test of inhibition of respiration of the activated sludge using estimation factors 10 and 100, respectively.

For NOEC 10 mg.l-1 the result was PNECmicroorganism = 1 mg.l-1.

For EC50 34 mg.l-1 the result was PNECmicroorganism = 0.34 mg.l-1.

The PNEC derived based on NOEC is preferred in the risk characterization, therefore:

PNECmicroorganism = 1 mg a.s./L.

Sediment

For DBDCB estimated log K_{OW} = 2.0 was calculated. Based on the measurement with four types of model soil samples, K_{OC} = 64.7 (ranged from 33.4 to 87.8 mL/g) was estimated. According to EU TGD on Risk Assessment, for substances with log K_{OC} or log K_{OW} < 3 it is not necessary to evaluate the risk for organisms living in the sediment. Further supporting argument justifying not verifying experimentally the effects of DBDCB on organisms living in the sediment was the fact that biocide formulations of PT06 type are not directly discarded into surface waters.

Determination of predicted no effect concentrations for freshwater sediment (PNECsediment)

There are no experimental results available for characterization of effects of DBDCB on organisms living in the sediment. However, high-quality data are available on the effects on aquatic organisms. Therefore, the value of $PNEC_{sediment}$ was calculated using equilibrium partitioning method according to equation (70) from Part II of EU TGD on Risk Assessment.

For K_{OC} = 64.7, $PNEC_{water}$ = 1.7 µg.l^{-1} and bulk density (wet) suspended matter = 1150 kg.m^{-3} is **$PNEC_{sediment}$ = 3.7 µg.kg^{-1}** suspended sediment.

Terrestrial compartment

The effects of DBDBC on soil were not experimentally verified. DBDCB used in biocide formulation of PT06 type is not emitted directly into or onto soil. Both log K_{OW} and log K_{OC} are lower than 3. According to EU TGD on Risk Assessment, the calculation based on equilibrium partitioning method can be used to evaluate effects of DBDCB on soil organisms similarly to the evaluation of effects on aquatic organisms.

Above the framework of requirements of TNsG, the applicant submitted results of acute toxicity study of DBDCB for wild duck (*Anas platyrhynchos*) and the results of 8 days feeding study for bobwhite quail (*Colinus virginianus*). Both studies were carried out more than 30 years ago by the procedure based on no standardized methods and not carried out under GLP regime. However, the description of the used methods and obtained results is so detailed, that it enables to use the test results as the basis for risk evaluation of DBDCB for birds.

From the experiment with one-time supplied DBDCB, the values of LD_{50} = 1064 mg.kg bw^{-1} and of NOEL = 464 mg.kg bw^{-1} were calculated.

From the results of 8 days feeding experiment in which DBDCB was given for 5 days to 14 days old birds of bobwhite quail and for further 3 days the exposed birds were observed, the values of LC_{50} = 4042 mg/kg of feed and of NOEC = 2150 mg/kg of feed were calculated.

Determination of predicted no effect concentrations for terrestrial compartment

Since no experimental tests are available with DBDCB, according to Chapter 3 of the TGD for Risk Assessment (EC, 2003) the PNEC derivation can be conducted on the basis of the equilibrium partition method, TGD-formula No. 72. This method uses the $PNEC_{water}$ for aquatic organisms and the soil water partitioning coefficient as inputs.

This results in a **$PNEC_{soil}$ = 0.0024 mg.kg^{-1} (dry weight)**.

Effect assessment for the major metabolite 2-methyleneglutaronitrile

Aquatic organisms

The effects of the metabolite 2-methyleneglutaronitrile on algae growth were evaluated based on the test carried out according to Method C.3 (2009) with the green algae *Desmodesmus subspicatus.*

Medium value of effective concentration determined based on growth rate was found to be 72 h- E_rC_{50} > 100 mg.l^{-1}. A NOE_rC value of ≥ 100 mg.l^{-1} was determined. 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.

Inhibition of activated sludge respiration rate

The effects on microorganisms were studied using the test of inhibition of respiration of activated sludge according to Method C.11 (2008). The EC_{10} concentration of 2-methyleneglutaronitrile was determined to be 789.4 mg.l^{-1}. At the highest test concentration of 1000 mg.L^{-1} the activated sludge respiration rate was not significantly inhibited. Thus, the EC_{50} value could not be determined and it is therefore given as 3-h EC_{50} = > 1000 mg.l^{-1}.

Overall evaluation for the major metabolite 2-methyleneglutaronitrile

Based on the above test results, we conclude that 2-methyleneglutaronitrile is not toxic to the aquatic compartment.

2.2.5 PBT and POP assessment

According to Annex XIII of REACH Regulation, substances are classified when they fulfil the criteria for all three inherent properties Persistent (P), Bioaccumulable (B), Toxic (T).

Evaluation of P

The interaction of abiotic (photodegradation) and biotic degradation prove a rapid dissipation of DBDCB within a time frame of a few days. This is substantiated by the outcome of aerobic aquatic degradation and primary inherent biodegradation.

DBDCB and its metabolites' affinity to organic carbon is rather limited and therefore the difference in org. carbon content in soil or sediment appears to be irrelevant. If it is normal to assume first order kinetics for water/sediment systems then the quantity of microbes is also irrelevant (small amount would lead to zero order kinetics at least at the beginning of the study). What could matter then is the ability of the microbes to degrade the substance. This can differ from sediment to sediment or from soil to soil and between soil and sediment. Then using two sediments gives more certainty then using one sediment only, yet uncertainty still remains.

If we multiply the DT_{50} of 2.76 days (derived from the aerobic aquatic degradation test) by an assessment factor of 10 to cover the uncertainty, we get the half -life of 27.6 days which is still less than 40 days which would trigger P.

DBDCB is considered as being potentially P considering all the studies available.

To clarify of the P – criterion new data will need to be requested for product authorisation if other uses are proposed.

Evaluation of B

DBDCB has a log Kow of 2 and an estimated BCF of 10, which suggest that this active substance would not bioaccumulate in the environment and there does not fulfil the "B" criteria.

Evaluation of T

DBDCB is not classified as proven carcinogenic, mutagenic, or toxic for reproduction substance. Based on the long-term chronic NOEC for algae and the avian sub-chronic NOEC, DBDCB does not fulfil the criterion of toxicity for PBT substance.

Overall evaluation

On the basis of the characteristics of the substance, DBDCB can not be considered as a PBT nor vPvB substance because it does not fulfil criteria for all the three inherent properties - **persistence, bioaccumulation and toxicity.**

Conclusion for the POP characterisation:

The substance does not fulfil the POP criterion for bioaccumulation. The potential for persistence could not be fully excluded as the data available on biodegradation of DBDCB in surface water are not sufficiently reliable. As the bioaccumulation criterion is not fulfilled the substance cannot be considered a persistent organic pollutant (POP).

2.2.6 Environmental exposure assessment

DBDCB is used for in-can conservation (PT06) of aqueous systems such as polymer emulsions, coating compositions, adhesive materials, inks, and similar products prepared on aqueous basis. The active substance is used under the commercial name Tektamer 38 as the biocidal product. The exemplary use of in-can preservation of paints and coatings (PT06.02) has been suggested in the human and environmental risk assessment for worst case considerations.

The consumption based assessment involves the formulation of the biocide containing product (addition of biocide in products to be preserved). The assessment of emissions is

based on the common practice for the formulation stage as reported by the applicant. Furthermore, input values of US-EPA and IC-14 have been used. Primary receiving compartment is STP.

The tonnage based approach assesses the life cycle phases of both the formulation of the biocide containing paint (addition of biocide in products to be preserved) and the application of the biocide containing paint (use of preserved product). The emissions factors being relevant for the formulation of biocide containing paints are presented by the IC-14 emission scenario (TGD, part IV, 2003). The use of paint could be seen as the disposal phase of DBDCB since it is no longer required to prevent organisms growing in the container of the product. Among the fields of application as displayed in the TGD IC-14 document, DBDCB release estimates were selected from the "Water-borne wood lacquer" scenario with a fraction of 0.25% being released to waste water (fwaste water) for water borne systems. This conservative approach does not take into account the common practice of re-using paint residues containing wash water during the paint formulation process. Therefore, the emissions into waste water from the paint formulation were evaluated with a tiered approach by using fwaste water = 0.25%. The results from the tonnage approach are provided in the confidential part. Primary receiving compartment is STP.

2-Methyleneglutaronitrile has been identified as major metabolite (> 10% of the initial applied DBDCB concentration (hydrolysis, photolysis, aerobic aquatic metabolism study, inherent biodegradability). The results from both experimental studies and modelling calculations indicate that 2-methyleneglutaronitrile is less ecotoxic than the parent compound and is of transient character. Therefore, the risk assessment of DBDCB covers the major metabolite 2-methyleneglutaronitrile. However, it should be noted that since DBDCB degrades in the aquatic compartment under environmental relevant conditions, only the remaining fraction of DBDCB is relevant for the environmental exposure.

2.2.7 Risk characterization for the environment

To allow for a quantitative assessment of a potential risk for the environment when DBDCB is applied as in can preservative, the calculated PEC values are compared to the respective PNEC values for the different compartments, resulting in the following PEC/PNEC ratios:

Table 2-1: Risk assessment for the use of DBDCB as preservative in paints

Compartment	STP	Surface water	Sediment	Soil	Ground water	Aquatic food chain
Tonnage approach						
Paint formulation	< 1					
Paint application	< 1					
Consumption approach						
Paint formulation	< 1				grassland: < 0.1 µg/L; agric. Soil: 0.08 – 0.29 µg/L	< 1

n.r. (not relevant)

As shown in Table 2-1, the PEC/PNEC ratios for DBDCB treatment are below 1 for all compartments, except the groundwater assessment for the consumption-based approach of the paint formulation (only for agricultural soils). However, predicted groundwater concentrations for the consumption approach are the outcome of modelling with a sequence of worst-case assumptions. As being proven by the FOCUS PEARL calculations based on tonnage, groundwater concentrations are far below 0.1 µg/L for all scenarios, with high margins of safety. These results indicate acceptable risk for sewage treatment plant, surface water, sediment, soil, groundwater and the aquatic food chain considering the intended use of DBDCB as in can preservative.

2.2.8 Assessment of endocrine disruptor properties

DBDCB is not included in the Commission staff working document on implementation of the Community Strategy for Endocrine Disruptors - a range of substances suspected of interfering with the hormone systems of humans and wildlife (COM (1999) 706). The evaluation of the human health-related data during the Annex I inclusion/Approval process does not suggest that DBDCB is to be considered as a potential endocrine disruptor. DBDCB is not classified for any adverse effects as a result of an interference with the endocrine system. Therefore, DBDCB is not to be considered an endocrine disruptor.

Overall conclusions

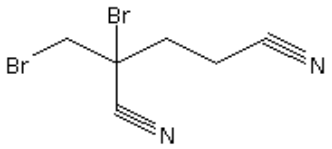
The outcome of the assessment for **1,2-Dibromo-2,4-dicyanobutane** in product-type 6 is specified in the BPC opinion following discussions at the 13th meeting of the Biocidal Products Committee (BPC). The BPC opinion is available from the ECHA website.

Appendix I: List of endpoints

Chapter 1: Identity, Physical and Chemical Properties, Classification and Labelling

Active substance (ISO Name)	1,2 dibromo 2,4 dicyanobutane
Product-type	PT 6

Identity

Chemical name (IUPAC)	2-Bromo-2-(bromomethyl)pentanedinitrile
Chemical name (CA)	Pentanedinitrile, 2-bromo-2-(bromomethyl)-
CAS No	35691-65-7
EC No	252-681-0
Other substance No.	Not allocated
Minimum purity of the active substance as manufactured (g/kg or g/l)	98%
Identity of relevant impurities and additives (substances of concern) in the active substance as manufactured (g/kg)	The identity of impurities and additives in the active substance as manufactured is confidential. This information is provided in the confidential part of the dossier.
Molecular formula	$C_6H_6Br_2N_2$
Molecular mass (g/mol)	265.9
Structural formula	

Physical and chemical properties (Annex IIA, point III, unless otherwise indicated)

Melting point (state purity)	50.3 °C (purity: 100.4%)
Boiling point (state purity)	Up to the exothermic decomposition no boiling point could be observed. (purity: 100.4%)

Temperature of decomposition	During DTA measurement endothermic melting from 44 to 90 °C was observed. Exothermal decomposition starts at 140 °C. During 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.
Appearance (state purity)	Yellowish white granular solid with slightly sweet odour (purity: 99.62%).
Density (state purity)	1.918 at 20 °C (purity: 100.4%)
Surface tension	72.99 mN/m at 20 °C, conc. 1g/L
Vapour pressure (in Pa, state temperature)	3.81×10^{-3} Pa at 20 °C 7.77×10^{-3} Pa at 25 °C
Henry's law constant (Pa m^3 mol^{-1})	Ratio between vapour pressure and water solubility: $6.03*10^{-04}$ Pa m^3 mol^{-1} at 20 °C and pH 5 $5.96*10^{-04}$ Pa m^3 mol^{-1} at 20 °C and pH 7 $1.28*10^{-03}$ Pa m^3 mol^{-1} at 20 °C and pH 9
	EPIWIN calculation: 3.99×10^{-05} Pa m^3 mol^{-1} at 25 °C (Bond method)
Solubility in water (g/l or mg/l, state temperature)	Results at pH 5: Results at pH 7: 1.03 g/L at 10°C 1.05 g/L at 10°C 1.68 g/L at 20°C 1.70 g/L at 20°C 2.62 g/L at 30°C 2.62 g/L at 30°C Results at pH 9: 0.42 g/L at 10°C 0.79 g/L at 20°C 2.09 g/L at 30°C

Solubility in organic solvents (in g/l or mg/l, state temperature) (Annex IIIA, point III.1)	Solubility in 1-octanol: 9.9 g/L at 10 °C 17.0 g/L at 20 °C 29.8 g/L at 30 °C Solubility in ethanol: 62.2 g/L at 10 °C 109 g/L at 20 °C 208 g/L at 30 °C Solubility in p-xylene: 168 g/L at 10 °C > 250 g/L at 20 °C and 30 °C Solubility in propylene glycol (CAS 57-55-6): 41.1 g/L at 10 °C 69.9 g/L at 20 °C 113 g/L at 30 °C Solubility in PEG 300 (CAS 24322-68-3): > 250 g/L at 10 °C, 20 °C and 30 °C
Stability in organic solvents used in biocidal products including relevant breakdown products (IIIA, point III.2)	Not applicable. The active substance as manufactured does not include an organic solvent.
Partition coefficient (log P_{ow}) (state temperature)	Experimental data: Log Pow = 2.0 (at 25 °C and pH 5, 7 and 9) Temperature dependence: Log Pow = 0.95 at 10 °C Log Pow = 0.96 at 20 °C Log Pow = 1.02 at 30 °C The log Pow is not influenced by pH or temperature.
Hydrolytic stability (DT_{50}) (state pH and temperature) (point VII.7.6.2.1)	pH 5 (25 °C): stable to hydrolysis pH 7 (25 °C): DT_{50} = 51.6 – 96.3 days (146.4 - 273.3 days at 12 °C); pH 9 (25 °C): DT_{50} = 9.1 days (25.8 days at 12°C) The major hydrolysis products were 2-methyleneglutaronitrile (MGN) at pH 7 and 1-bromo-2,4-dicyano-1-butene (E-Z isomers) at pH 9, exceeding 10% of the initial measured dose.
Dissociation constant (not stated in Annex IIA or IIIA; additional data requirement from TNsG)	DBDCB has no dissociation constant
UV/VIS absorption (max.) (if absorption > 290 nm state ε at wavelength).	At 290 nm: extinction = 0.0037; molar absorption coefficient ε = 2 No UV absorbance above 290 nm.

Photostability (DT_{50}) (aqueous, sunlight, state pH) (point VII.7.6.2.2)	DBDCB is susceptible to photodegeradation in aqueous media. The degradation follows first order kinetics. Irradiated: DT_{50} = 54 days k^c_p = 0.0129 $days^{-1}$ Non-irradiated: DT_{50} = 381 days k^c_p = 0.0018 $days^{-1}$ Irradiated samples corrected for degradation in dark controls: DT_{50} = 62 days k^c_p = 0.0111 $days^{-1}$ The major photoproduct is 2-methylene-glutaronitrile (MGN). Approx. 2% of the hydrolysis products (E)- and (Z)-1-bromo-2,4-dicyano-1-butene isomers were also identified, however they were found only at one sampling event.
Quantum yield of direct phototransformation in water at □ > 290 nm (point VII.7.6.2.2)	Not determined
Flammability	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.
Explosive properties	DBDCB does not present any risk for explosion.
Oxidising properties	DBDCB contains none of the functional groups which may indicate oxidising properties. It can therefore be concluded that DBDCB has no oxidising properties.
Auto-ignition or relative self-ignition temperature	EC method A.16 was used to show that DBDCB does not undergo auto-ignition

Classification and proposed labelling (Annex IIA, point IX)

with regard to physical/chemical data	No classification / labelling results from the physico-chemical properties.
with regard to toxicological data	Acute Tox. 2; H330 Acute Tox. 4; H302 Skin Sens. 1; H317 Eye Dam. 1; H318 STOT SE 3; H335
with regard to fate and behaviour data	No classification / labelling results from the environmental properties.
with regard to ecotoxicological data	N; R51/53; S61 Aquatic Chronic 2; H411

Chapter 2: Methods of Analysis

Technical active substance (principle of method) (Annex IIA, point 4.1)	RP18-HPLC using UV detection at 200 nm. Quantification is done by external standard method.
Impurities in technical active substance (principle of method) (Annex IIA, point 4.1)	Analytical methods for the determination of impurities in the active substance as manufactured are confidential. This information is provided in the confidential part of the dossier.

Analytical methods for residues

Soil (principle of method and LOQ) (Annex IIA, point 4.2)	Not relevant since DBDCB is not intended to be placed on, in or near soils. It is intended to be used as antimicrobial active substance in in-can preservatives (product type 6). Its occurrence in soil can therefore be excluded.
Air (principle of method and LOQ) (Annex IIA, point 4.2)	Not relevant since DBDCB is a non-volatile solid and it is not intended to be sprayed. It is intended to be used as antimicrobial active substance in in-can preservatives (product type 6). Its occurrence in air can therefore be excluded.
Water (principle of method and LOQ) (Annex IIA, point 4.2)	Method for quantitative analysis of 1, 2-dibromo-2,4-dicyanobutane in drinking water: HPLC-MS/MS; LOQ = 0.1 µg/L ; linear regression in the range 0.1 - 100 micrograms/L ; SD<16%.

Body fluids and tissues (principle of method and LOQ) (Annex IIA, point 4.2)	While DBDCB is proposed to be classified as toxic by inhalation, the marked effects after inhalation exposure are expected to be mainly due to DBDCB's irritation potential to mucous membranes, which is a local effect. This irritant effect is reflected by the respective classification proposal with the R-phrase 37, "Irritating to respiratory system". The systemic toxicity is expected to play only a minor role in the observed effects that required a classification as 'toxic'. Therefore an analytical method for body fluids and tissues for monitoring of systemic exposure is considered not to be required.
Food/feed of plant/animal origin (principle of method and LOQ for methods for monitoring purposes) (Annex IIIA, point IV.1)	Not relevant since DBDCB or the material treated with it is not used in a manner which may cause contact with food or feedstuffs, or intended to be placed on, in or near soils in agricultural or horticultural use.
Food/feed of animal origin (principle of method and LOQ for methods for monitoring purposes) (Annex IIIA, point IV.1)	Not relevant since DBDCB or the material treated with it is not used in a manner which may cause contact with food or feedstuffs, or intended to be placed on, in or near soils in agricultural or horticultural use.

Chapter 3: Impact on Human Health

Absorption, distribution, metabolism and excretion in mammals (Annex IIA, point 6.2)

Rate and extent of of oral absorption:	Oral biovailability of DBDCB is 80-90%
Rate and extent of of oral absorption	19% (may not be applicable at product authorisation, where EFSA guidance will be used for dem. abs. assesemnet)
Distribution:	Highest levels detected in liver, kidney and GI tract, lung and whole blood. Levels of radioactivity in other tissues were generally lower than in plasma.
Potential for accumulation:	No evidence for accumulation in animal organs and tissues. Two bromide ions are released from one molecule of DBDCB and may cumulate in the organism till a steady state is reached.
Rate and extent of excretion:	Urine: 72.0%, faeces: 9.7%, CO_2: 7.5% of applied dose at 72 h after oral dose. Excretion was essentially complete after 24 h. Systemic body clearance: 11.5 ± 0.4 mL/kg × min
Toxicologically significant metabolite(s)	DBDCB is rapidly converted into 2-methylene-glutaronitrile (2-MGN) by reaction with SH groups of plasma proteins. DBDCB is totally debrominated prior to systemic distribution; the main metabolite in urine (46.4% of dose) was the mercapturic acid conjugate of 2-MGN. Two bromide ions are released from one molecule of DBDCB and may cumulate in the organism till a steady state is reached.
Toxicologically significant compounds (animals, plants and environment)	None (no relevant metabolite residues that were not also rat metabolites)

Acute toxicity (Annex IIA, point 6.1)

Rat LD_{50} oral	640 mg/kg
Rat LD_{50} dermal	> 2000 mg/kg bw
Rat LC_{50} inhalation	265 mg/m^3, severe injury to airways
Skin corrosion/ irritation	slightly irritating – no classification
Eye irritation	strongly irritating
Respiratory tract irritation	Strongly irritating

Skin sensitization (test method used and result)	RIPT on human volunteers: sensitising FCA test on guinea pigs: sensitising
Respiratory sensitisation (test method used and result)	No test performed, weight of evidence indicates no classification

Repeated dose toxicity (Annex IIA, point 6.3-6.5)

Subchronic

Target / critical effect	Dog: reduced body weight gain, thyroid hyperplasia
Lowest relevant oral NOAEL / NOEL	30 mg/kg./day (3 month dog feeding study)
Lowest relevant dermal NOAEL / NOEL	21-day rat dermal study resulted in severe local injury (dose level 4000 mg/kg bw/day) without systemic toxicity.
Lowest relevant inhalation NOAEL / NOEL	–

Genotoxicity (Annex IIA, point 6.6)	Not genotoxic, not mutagenic

Carcinogenicity (Annex IIA, point 6.7)

Target/critical effect	Skin
Lowest relevant NOAEL / NOEL	18mg/kg bw (rats/top dose) 6mg/kg bw (mice/top dose)
Carcinogenicity	No neoplastic lesions

Reproductive toxicity (Annex IIA, point 6.8)

Species/ Developmental target / critical effect	Rat: reduced body weight
Relevant maternal NOAEL	30 mg/kg bw
Relevant developmental NOAEL	60 mg/kg bw
Fertility	
Species/critical effect	Reduced body weight, splenic hematopoesisi
Relevant parental NOAEL	196/248 mg/kg bw (♂/♀).

Relevant offspring NOAEL	34/39 mg/kg bw (♂/♀).
Relevant fertility NOAEL	196/248 mg/kg bw (♂/♀)

Neurotoxicity **Neurotoxicity / Delayed neurotoxicity** (Annex IIIA, point VI.1)

Species/ target/critical effect	No effect observed	Delayed neurotoxicity	DBDCB bears no structural similarity to organophosphates, carbamates or other known inducers of delayed neurotoxicity. Studies in several species did not indicate the occurrence of neurotoxic effects.
Developmental Neurotoxicity			
Species/ target/critical effect	No effect observed		

Immunotoxicity

Species/ target/critical effect	No effect observed

Developmental Immunotoxicity

Species/ target/critical effect	No effect observed

Other toxicological studies (Annex IIIA, VI/XI)

The thyroidal effects seen in the main subchronic dog study were re-investigated in a special 13-week feeding study. 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 by a dose of 5.7 mg/kg bw/day.

Medical data (Annex IIA, point 6.9)

Patch tests have revealed a sensitisation rate towards DBDCB of 2.4% in dermatitis patients.

Summary (Annex IIA, point 6.10)[2]	**Value**	**Study**	**Safety factor**
ADI	0.15 mg/kg	Dog, oral subchronic NOAEL 30 mg/kg	200
short-term AEL	0.3 mg/kg/day	Dog, oral subchronic NOAEL 30 mg/kg	100
long-term AEL	0.15 mg/kg/day	Dog, oral subchronic NOAEL 30 mg/kg	200
Drinking water limit	450 µg/L	Dog, oral subchronic	200
ARfD (acute reference dose)	0.3 mg/kg/day	Rabbit, teratology, maternal NOAEL = 30 mg/kg/day	100

MRLs

Relevant commodities	Irrelevant for the proposed use

Reference value for groundwater

According to BPR Annex VI, point 68	0.1 ug/l

Dermal absorption

Study (*in vitro/vivo*), species tested	In vitro
Formulation (formulation type and including concentration(s) tested, vehicle)	Aqueous solution.
Dermal absorption values used in risk assessment	19%

Acceptable exposure scenarios (including method of calculation)

Primary exposure	**NOAEL [mg/kg/day]**	**Systemic exposure [mg/kg/day]**	**MoE**
Preparation of pre-mix tank TNsG Model 7 for Mixing & Loading, dumping of solids into system	30	0.053	566

2 The safety factor of 200 was agreed at the Human Health WG-I-2016 to be sufficient for the derivation of long-term AEL and the other reference values based on the same study. Only the LOEP was updated.

Secondary exposure			
Brush painting -Amateur	30	0.0069	4348
Brush painting – Professional, PPE	30	0.0049	6122
Child ingesting paint chips	30	0.004	7500
Domestic inhalation	30	0.00206	$>10^4$

Chapter 4: Fate and Behaviour in the Environment

Route and rate of degradation in water (Annex IIA, point 7.6, IIIA, point XII.2.1, 2.2)

Hydrolysis of active substance and relevant metabolites (DT_{50}) (state pH and temperature)	pH 5 Acetate: DT_{50} = 3884 days pH 7 HEPES: DT_{50} = 51.6 days (146.4 days at 12°C) pH 7 TRIS: DT_{50} = 96.3 days(273.3 days at 12°C pH 9 Borate: DT_{50} = 9.10 days (25.8 days at 12°C) (25 ± 1 °C, Duration 30 days) E-Z isomers and 2-methyleneglutaronitrile (MGN): > 10% of the initial measured dose
Degradation (hydrolysis) in seawater	No data provided, not required for the currently requested uses.
Photolytic / photo-oxidative degradation of active substance and resulting relevant metabolites	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)

Readily biodegradable (yes/no)	Not readily biodegradable according to Closed Bottle Test; 0% biodegradation after 28 days (3 mg a.s./L) <u>Inherent biodegradability</u> 2% ultimate biodegradation Degree of a.s. degradation: 65% (primary biodegradation)
Non-extractable residues	8.88% IMD at study termination (day 30)
Mineralisation	10.0% IMD at study termination (day 30)
Distribution in water / sediment systems (active substance)	<u>Aerobic aquatic metabolism study</u> DEGRADATION IN SOIL/WATER SYSTEM, AEROBIC: DT_{50} whole system = 0.874 days (2.76 days at 12°C) DISTRIBUTION IN SOIL/WATER SYSTEM, AEROBIC: Tektamer 38 (soil and water): 79.8% IMD at day 0 to 0.410% IMD at day 7 and n.d. at study termination (day 30)

Route and rate of degradation in water [cont.]

Distribution in water / sediment systems (metabolites)	Aerobic aquatic metabolism study DISTRIBUTION IN SOIL/WATER SYSTEM, AEROBIC: - 2-methyleneglutaronitrile (2-MGN) (soil and water): 13.2% IMD at day 0 to 34.1% IMD at day 3 and 0.798% IMD at study termination (day 30) with a low of 0.499% IMD at day 21 - Unknown I (soil and water): 0.559% IMD at day 0 to 1.39% IMD at day 2 and 0.119% IMD at study termination (day 30) - (E)- and (Z)-isomers (water): 0.439% IMD at day 0 and 0.246% IMD at day 2 and n.d. at study termination (day 30) IMD = initial measured dose

Route and rate of degradation in soil (Annex IIIA, point VII.4, XII.1.1, XII.1.4; Annex VI, para. 85)

Mineralization (aerobic)	Not relevant due to proposed use pattern and indoor application;
Laboratory studies (range or median, with number of measurements, with regression coefficient)	Not relevant due to proposed use pattern and indoor application;
Field studies (state location, range or median with number of measurements)	Not relevant

Route and rate of degradation in soil [cont.]

Anaerobic degradation	Not relevant due to proposed use pattern and indoor application; however, an anaerobic aquatic degradation study is available (see below). Anaerobic aquatic degradation study: DT_{50lab} (25 ± 1 °C): 0.495 days - 2-methyleneglutaronitrile (2-MGN) (soil and water): Up to 74% AR at day 1 to 32% AR at study termination AR = applied radioactivity
Soil photolysis	Not relevant due to proposed use pattern and indoor application (for aqueous photolysis, see above)
Non-extractable residues	Not relevant due to proposed use pattern and indoor application; an aerobic aquatic degradation study is available (results see above).
Relevant metabolites - name and/or code,% of applied a.i. (range and maximum)	Not relevant due to proposed use pattern and indoor application; an aerobic aquatic degradation study is available (results see above).
Soil accumulation and plateau concentration	Not relevant due to proposed use pattern and indoor application; an aerobic aquatic degradation study is available (results see above). Soil accumulation is not expected

Mobility in soil (Annex IIA, point 7.1.3, Annex IIIA, point 9.1.2)

Aged residues leaching	Not relevant due to proposed use pattern
Lysimeter/ field leaching studies	Not relevant due to proposed use pattern

Adsorption/desorption (Annex IIA, point XII.7.7; Annex IIIA, point XII.1.2)

Ka , Kd Ka_{oc}, Kd_{oc} pH dependence (yes / no) (if yes type of dependence)	Adsorption: K_d (parent and degr. products) = 0.351 to 0.701 mL/g (mean K_d: 0.509 mL/g, n = 3) K_{oc} (parent and degr. products) = 33.4 to 87.8 mL/g (mean K_{oc}: 64.7 mL/g, n = 3)

Fate and behaviour in air (Annex IIIA, point VII.3, VII.5)

Direct photolysis in air	Air will not be an environmental compartment of concern for DBDCB used in In-can preservatives, as DBDCB has a low vapour pressure and Henry's Law constant.
Quantum yield of direct photolysis	Not given
Photo-oxidative degradation in air	Chemical life-time in the troposphere: DT_{50} = 27.1 d (calculated with AOPWIN v1.91, ©US-EPA 2000) with a degradation rate of 0.5919×10^{-12} $cm^3 * molecule^{-1} * s^{-1}$
Volatilization	Insignificant due to low vapour pressure and Henry's Law constant.

Monitoring data, if available (Annex VI, para. 44)

Soil (indicate location and type of study)	Not available, not required.
Surface water (indicate location and type of study)	Not available, not required.
Ground water (indicate location and type of study)	Not available, not required.
Air (indicate location and type of study)	Not available, not required.

Chapter 5: Effects on Non-target Species

Toxicity data for aquatic species (most sensitive species of each group) (Annex IIA, point VII., Annex IIIA, point XIII.)

Species	Test substance	Time-scale	Endpoint	Toxicity
Fish				
Oncorhynchus mykiss	DBDCB	96 hours	Mortality	LC_{50} = 1.26 mg/L (m)
Oncorhynchus mykiss	DBDCB	81 days	Number of embryos hatched; time to hatch; time to swim-up; survival of embryos, larvae, and juveniles; length and weight of surviving fish; sublethal effects	NOEC = 0.75 mg/L (m)
Aquatic invertebrates				
Daphnia magna	DBDCB	48 hours	Immobility	EC_{50} = 4.83 mg/L (m)
Daphnia magna	DBDCB	21 days	Survival & reproduction	NOEC = 1.4 mg/L (m)
Algae				
Desmodesmus subspicatus	DBDCB	72 hours	Growth inhibition	NOEC = 0.017 mg/L (m) E_rC_{50} = 5.4 mg/L (m)
Desmodesmus subspicatus	2-MGN (metabolite)	72 hours	Growth inhibition	NOEC ≥ 100 mg/L (n) E_rC_{50} > 100 mg/L (n)
Microorganisms				
Activated sludge	DBDCB	3 hours	Inhibition of respiratory rate	EC_{50} = 34 mg/L (n) NOEC = 10 mg/L (n)
Activated sludge	2-MGN (metabolite)	3 hours	Inhibition of respiratory rate	EC_{50} > 1000 mg/L (n) EC_{10} = 789.4 mg/L (n)

n = nominal concentration
m = measured concentration

Effects on earthworms or other soil non-target organisms

Acute toxicity to earthworms (Annex IIIA, point XIII.3.2)	No data required
Acute toxicity to terrestrial plants (Annex IIIA, point XIII.3.2)	No data required
Reproductive toxicity to non-target organisms (Annex IIIA, point XIII.3.2)	No data required

Effects on soil micro-organisms (Annex IIA, point 7.4)

Nitrogen mineralization Carbon mineralization	No data required

Effects on terrestrial vertebrates

Acute toxicity to mammals (Annex IIIA, point XIII.3.3)	See above acute toxicity towards mammals: oral-LD_{50} = 640 mg/kg dermal-LD_{50}> 2000 mg/kg bw inhalation-LC_{50} = 265 mg/m^3
Acute toxicity to birds (Annex IIIA, point XIII.1.1)	*Anas platyrhynchos* LD_{50} = 1064 mg/kg bw (8 days, nominal)
Dietary toxicity to birds (Annex IIIA, point XIII.1.2)	*Colinus virginianus* LC_{50} = 4042 mg/kg food (5 days treatment, 3 days observation, nominal)
Reproductive toxicity to birds (Annex IIIA, point XIII.1.3)	No tests are available, not required

Effects on honeybees (Annex IIIA, point XIII.3.1)

Acute contact toxicity	No data required

Effects on other beneficial arthropods (Annex IIIA, point XIII.3.1)

Acute oral toxicity	No data required
Acute contact toxicity	No data required
Acute toxicity to	No data required

Bioconcentration (Annex IIA, point 7.5)

Bioconcentration factor (BCF)	BCF = 10 (calculated based on log Pow) Measured values: BCF = 0.5-0.7 at 0.05 mg/L BCF < 2.5 at 0.5 mg/L
Depuration time (DT_{50}) (DT_{90})	No data
Level of metabolites (%) in organisms accounting for > 10% of residues	Not determined

Chapter 6: Other End Points

None

Appendix II: List of Intended Uses

DBDCB (product Tektamer 38) is an antimicrobial preservative for aqueous products. The exemplary exposure assessment presented in this dossier covers the preservation of water-based paints. Typically, a 25% pre-mix of DBDCB in water is prepared on site so that the preservative can be added to the paint slurry in an automated pumping process. The product is added to the suspension at a final concentration of 0.01 – 0.1% DBDCB. Considering the possibility of resistance, the use of marginally effective concentrations should be discouraged.

Product type	**Field of use envisaged** **Water based product**	**Likely in-use concentration of a.s. in % (w/v)**
PT 6.02 In-can preservatives for products other than detergents	Preservation of water-based decorative paints	0.01 – 0.1

List of studies for DBDCB AR

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

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]	1988	Human Phototoxicity/ Photoallergy Study. Date: 1988-03-21	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	Non-key (A6.1.5)
[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)
Britton, J.E.R. *et al.*	2003	The British Standard Series of Contact Dermatitis Allergens: Validation in Clinical Practice and Value for Clinical governance.	Dept. of Dermatology, General Infirmary at Leeds, UK	*Br. J. Dermatol.* **148**(2): 259-264	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

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]	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
[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

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 a	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]	2007 b	Spectral data of DBDCB. Date: 2007-03-02	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	3.4/01
[redacted]	2007 c	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]	2007 d	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
[redacted]	2007 e	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

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	DBDCB. Calculation of the Bioconcentration Factor (BCF). Date: 2007-05-16	[redacted]	[redacted]	No	LANXESS Deutschland GmbH	7.4.2/01
[redacted]	1975 a	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]	1975 b	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
Hausen, B.M.	1993	The sensitizing potency of Euxyl® K 400 and its components 1,2-dibromo-254-dicyanobutane and 2-phenoxyethanol.	Department of Dermatology, University Hospital, Hamburg, Germany	*Contact Dermatitis* **28**: 149-153	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

Authors (s)	Year	Title	Testing Company	Report No.	GLP Study (Yes/No)	Data Owner	Section No. in Doc III-A/ Non.key study / Published
Jensen, J.D. *et al.*	2004	Methyldibromo-glutaronitrile in rinse-off products causes allergic contact dermatitis: an experimental study.	National Allergy Research Centre, Gentofte, Denmark	*Br. J. Dermatol.* **150**: 90–95	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
[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)
Kasting G.B., Smith R.I. and Cooper E.R	1987	Effect of lipid solubility and molecular size on percutaneous absorption	The Procter and Gamble Company, Cincinaty, Ohio and Alcon Lab., Inc, Forth Worth, Tex., USA	Pharmacol. Skin 1,138-153			Published

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	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)
[redacted]	1981 a	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)

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]	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)
Lederer et al.	1982	Cited in Paulus, W. (1993): Microbiocides for the protection of materials - A handbook. Chapman and Hall, London, UK, pp. 373-374Handbook of 1993 (s. below)	--	--	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)

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]	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)
[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

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].	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
Paulus, W.	1993	Microbiocides for the protection of materials - A handbook. Chapman and Hall, London, UK, pp. 373-374	--	--	--	--	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)
[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] R.R.	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

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	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]	2011 a	Alga, Growth Inhibition Test with Dibromodicyano butan (DBDCB). Date: 2011-07-25	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.1.3/02
[redacted]	2011 b	Alga, Growth Inhibition Test with 2-Methyleneglutaronitrile. Date: 2011-07-20	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	7.4.1.3/03
[redacted]	2011 c	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]	1983 a	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]	1983 b	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
Sauer, J.-M. *et al.*	1998	Metabolic and Dispositional Fate of 1,2-Dibromo-2,4-dicyanobutane in the Male Fischer 344 Rat. Date: 1997-10-02	Department of Pharmacology and Toxicology and the Center for Toxicology, Univ. of Arizona, Tucson, AZ, USA	*Drug Metabolism and Disposition* **26**(5), 429-436	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

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	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]	n.a.	n.a.	LANXESS Deutschland GmbH	Attached in section 13,IUCLID CLH dossier for DBDCB
[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)

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	Cytogenicity Study - Chinese Hamster Ovary Cells in Vitro. Date: 1982-09-22	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.6.2/01
Tosti, A., Vincenzi, C. and Smith, K.A.	2000	Provocative use testing of methyldibromo-glutaronitrile in a cosmetic shampoo	Dept. of Dermatology, University of Bologna, Italy Procter & Gamble Technical Centres Limited, Staines, UK	*Contact Dermatitis,* **42***,* 64–67	No	–	Non-key Published (A6.12.6)
[redacted]	1991 a	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]	1991 b	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)
[redacted]	1991 c	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)

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]	1991 d	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]	1991 e	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]	1982 a	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]	1982 b	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]	1982 c	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)
Williams, J.D. *et al.*	2007	Allergic contact dermatitis from methyl–dibromo-glutaronitrile in a sanitary pad and review of Australian clinic data.	Occupational Dermatology Research and Education Centre, Skin and Cancer Foundation, Melbourne, Victoria, Australia	*Contact Dermatitis* **56**: 164–167	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

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 a	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]	1980 b	Thirteen-Week Subchronic Dietary Administration in Dogs. Date: 1980-02-07	[redacted]	[redacted]	Yes	LANXESS Deutschland GmbH	6.4.1/02
[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)