SUBSTANCE EVALUATION REPORT

Public Name: N,N-dicyclohexylbenzothiazole-2-sulphenamide (DCBS)

EC Number(s): 225-625-8

CAS Number(s): 4979-32-2

Submitting Member State Competent Authority:

Federal Institute for Occupational Safety and Health (BAuA)
Division 5 "Federal Office for Chemicals, Authorisation of Biocides"
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Year of evaluation (as given in the CoRAP): 2013

Year of follow-up evaluation: 2017

VERSION NUMBER: 2.0 DATE: 23 July 2018

Conclusions of the most recent evaluation step*	Tick relevant box
Concern not clarified; Need to request further information from the Registrant(s) with the draft decision	
Concern clarified; No need of further risk management measures	
Concern clarified; Need for risk management measures: vPvB properties clarified – RMO analysis to be performed	X

**Include details in the executive summary.*

Executive summary

Grounds for concern

- N,N-dicyclohexylbenzothiazole-2-sulphenamide (DCBS) was considered as a potential PBT-/vPvB-substance. The vB property is clearly fulfilled. The registrants screened according to Annex XIII 3.1.1 and 3.1.3 the P, vP and T properties of DCBS. The results are suggesting that DCBS is persistent or very persistent under relevant environmental conditions and appearance of toxic properties cannot be excluded.

- Based on read across approach with the structure analogues: N-cyclohexyl-2-benzothiazole sulphenamine (CBS), N-(1,3-benzothiazol-2-ylsulfanyl)-2-methylpropan-2-amine (TBBS) and 2-(morpholin-4-ylsulfanyl)-1,3-benzothiazole (MBS),a skin sensitizing potential of DCBS in humans is suggested.

- Overall picture of toxicity to reproduction that is observed at relatively higher doses (476 mg/kg bw) in a non-guideline study.

- In a non-guideline study sarcomas observed at the port of entrance (subcutaneous application). However, no clear evidence of carcinogenicity based on the absence of genotoxic potential in vivo and no observation of pre-neoplastic lesions in an oral repeated dose toxicity study (subchronic).

- High exposure to workers. Also consumer exposure possible.

- During the evaluation of available human health information, an additional concern for prenatal developmental toxicity arose.

Procedure

Environment:

- Given the available information in the registration dossier under 2.3 PBT-assessment, 5 environmental fate pathways and 6 ecotoxicological information were evaluated. Moreover, information on environmental exposure in the CSR was checked for plausibility. For the hydrolysis study (TL, 1997) the DT_{50} values were recalculated using the software package ModelMaker 4. A theoretical assessment of potential biodegradation pathways and potential biodegradation metabolites of DCBS was performed using the University of Minnesota Biocatalysis/Biodegradation Prediction System (UM-PPS[1]). The assessment of the PBT-properties of DCBS was discussed at the fourth meeting of the ECHA PBT expert group from 28th to 29th of May 2013. Four tests on inherent biodegradation (Currenta 2013a, b, c, d) that were delivered during the SEV procedure in October 2013 were assessed and recalculated. Hence, it is concluded that simulation testing according to Annex XIII 3.2.1 needs to be provided including the fate and properties of potential transformation products of DCBS. Since information and assumptions underlying environmental exposure

[1] http://umbbd.ethz.ch/predict/ (accessed 19.09.2013)

assessment for DCBS were partly missing or not plausible it was requested to submit missing information and plausible data which are necessary to estimate environmental releases.

Human Health:

- The data supplied by the lead registrant in the updated dossier version of September 30th, 2013, was evaluated by the eMSCA. The scientific literature was evaluated for DCBS and the relevant endpoints.

Worker/Consumer Exposure

- In terms of consumer exposure the data provided by the lead registrant in the updated dossier version of September 30th, 2013, was evaluated by the eMSCA. The scientific literature, product registers and databases were evaluated for information on exposure.

Conclusions

Environment

- Persistency: Information on hydrolytic degradability of DCBS indicates that the substance is hydrolytically degradable to a certain degree. The hydrolytic transformation products of DCBS are MBT (CAS 149-30-4) and Dicyclohexylamine (CAS 101-83-7). However, hydrolysis rates are rather low and do not significantly influence the persistency of DCBS in the environment under relevant environmental conditions (temperature, pH, etc.). Screening information is suggesting that the substance is very persistent according to Annex XIII 3.1.1.

- In a test according to OECD 307 Aerobic and Anaerobic Transformation in Soil 14C-DCBS did not degrade in 120 days and at 12 °C. Maximum mineralization of DCBS was 3.2 % and the shares of NER were up to 19.4 %. DCBS quickly dissipated from the water compartment by adsorption to the sediment. A single first order model describes half-life best and results in a DT50 of 314.8 to 614.5 days depending on the soil considered. Thus, DCBS meets the specifications for the half-life in soil given for very persistent substances in REACH Regulation Annex XIII 1.2.1. Persistence. DCBS is very persistent in soil.

- Bioaccumulation: DCBS has a log K_{ow} of 5.95 indicating a high bioaccumulation potential. This is confirmed by available bioconcentration tests using *Cyprinus carpio*. In dependence of the used test concentrations the lipid normalized considered steady-state BCFs ranged between 3663 and 12821 L/kg. As reliable experimental BCF values of DCBS lay above the vB criterion (BCF > 5000) of Annex XIII, the substance fulfills the vB criterion.

- Ecotoxicity: Based on provided screening information DCBS does not fulfil the screening criterion for T properties (EC50/LC50 < 0.1mg/L) or the criterion for T assessment (NOEC < 0.01mg/L). As the criteria for B and P properties are proven to be fulfilled it is necessary for the PBT assessment to conduct the chronic fish test (Fish early-life stage toxicity test, OECD 210, Annex IX 9.1.6.1). For the reason of animal welfare no test on chronic toxicity to fish is requested as the substance is proven to be very persistent and very bioaccumulative and consequently fulfils the criteria for a SVHC already.

- Environmental exposure: Information and assumptions underlying environmental exposure assessment for DCBS have been sufficiently updated to finalise the evaluation. Consequently, no further information and plausible data to estimate environmental releases

is requested and assessed at this stage of the regulatory process. Furthermore DCBS is now proven to be very persistent and very bioaccumulative (vPvB substance) and consequently fulfils the criteria for a SVHC.

Human Health

- According to the Registrant(s) no evidence of pre-neoplastic lesions was found in a subchronic feeding study using male and female SD rats. There was no evidence of any gross pathological and histopathological finding associated to dosing with DCBS up to the highest dose group evaluated. Additionally, it was shown that DCBS is not genotoxic in an *in vivo* bone marrow chromosome aberration assay. Based on the absence of a genotoxic potential *in vivo* and no observations of pre-neoplastic lesions in an oral subchronic repeated dose toxicity study, no evidence was found for a carcinogenic potential of DCBS.

- The developmental toxicity of DCBS was evaluated in a repeated dose study with reproduction/developmental toxicity screening test (OECD TG 422, administration via gavage). In dams at 400 mg/kg bw/d severe maternal toxic effects were observed such as mortality and clinical signs like decreased body weights, changes in blood chemistry and/or histopathology. All dams lost their litters at delivery or by day 4 of lactation at this dose.

  In a two-generation study (OECD TG 416) no substance-related mortality and clinical signs were observed across generations up to the highest concentration evaluated (416 mg/kg bw/d in F0 females, administration via the diet), whereas reduction of body weight gain in parental animals and offspring was consistently observed throughout the generations in that dose group. In F1 females of the mid-dose group delays in vaginal opening and worse performance in water T-maze were observed and confirmed in the highest dose group. In F2 females of the mid-dose group a reduced uterine weight was observed and confirmed in the highest dose group. No malformed F1 pups were found in any groups.
  Another study was performed according to OECD TG 414 in rabbits to investigate the prenatal developmental toxicity of DCBS after oral administration via gavage. No maternal toxicity and no developmental toxicity was observed in the 100, 300 and 1000 mg/kg bw/d groups. The concern of developmental toxicity has been clarified. The eMSCA is of the opinion that the results of the study do no indicate the need to implement further risk management measures beyond those which are already in place with regard to the protection of human health.

- The skin-sensitization potential of DCBS was evaluated in a Guinea pig maximization test and found negative. However, in a read across approach with the structural analogues CBS, TBBS and MBS similarities in several parameters were noted. The structural analogues CBS, TBBS and MBS induced positive skin reactions in human volunteers and accordingly, CBS and MBS received harmonized classification as Skin Sens. according to Regulation (EC) no. 1272/2008, whereas TBBS was self-classified as Skin Sens.1. Based on a read-across approach to the structural analogues CBS, TBBS and MBS, a skin sensitizing potential of DCBS in humans is suggested; the self-classification of DCBS as skin sensitizer is considered as sufficient. No further action is recommended by the eMSCA.

Worker/Consumer Exposure

- During the course of the evaluation, the Registrant(s) stated that consumer exposure to DCBS is not expected, for the following reasons:
  - During the vulcanization process DCBS is completely consumed so that none remains in the final rubber products.
  - Consumer contact is unlikely since most of the DCBS tonnage is used in tyre production, where DCBS is found mainly in the interior parts of tyres. Even during tyres change consumers do not come into contact with these interior parts.
  - Lower amounts of DCBS are used in general rubber goods that involve special high-quality rubbers for industrial applications where consumer contact is not intended.
  - EU-RAR (2008) reported that no indication of exposure to CBS was found in all relevant databases. CBS is the most widely used representative of the group of sulfenamides as vulcanizer/accelerator through the use of gloves, rubber, toys and household products.

In addition, the evaluating MSCA noted the following findings:

- Spin-Database (Database on the use of Substances in Products in the Nordic Countries that is based on data from the Product Registries of Norway, Sweden, Denmark and Finland) shows only hints in ‘adhesives, binding agents, vulcanizing agents’ resulting in ‘probable consumer exposure’.
- No hints of consumer exposure in product register of Germany.
- No hints of consumer exposure in product register of Switzerland.
- No hints of consumer exposure in the database of the Federal Office of Consumer Protection and Food Safety (BVL) in the monitoring programme by the Federal States (Bundesländer) of Germany.

Consequently, the evaluating MSCA considers that consumer exposure may currently be minimal.

No in-depth evaluation of the exposure assessment for workers was conducted fol-lowing the evaluation of the toxicological intrinsic properties of DCBS. An evaluation of the worker exposure was performed in the EU-RAR (2008).

CONTENTS

TABLES

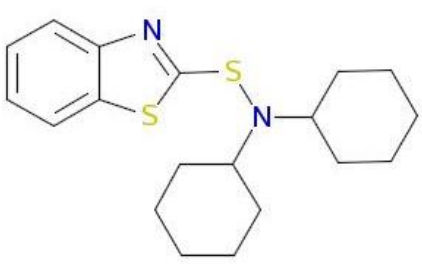
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Table 1: Substance identity

Public Name:	N,N-dicyclohexylbenzothiazole-2-sulphenamide
EC number:	225-625-8
EC name:	N,N-dicyclohexylbenzothiazole-2-sulphenamide
CAS number (in the EC inventory):	4979-32-2
CAS number:	4979-32-2
CAS name:	2-Benzothiazolesulfenamide, *N*,*N*-dicyclohexyl-
IUPAC name:	N-(1,3-Benzothiazol-2-yl-sulfanyl)-N-benzyl-1-phenylmethanamine
Index number in Annex VI of the CLP Regulation	not listed in Annex VI of the CLP Regulation
Molecular formula:	$C_{19}H_{26}N_2S_2$
Molecular weight range:	346.5531 g/mol
Synonyms:	DCBS N,N-dicyclohexyl-2-benzothiazolesulfenamide 2-Benzothiazolesulfenamide, N,N-dicyclohexyl- Rubenamid DS Vulkacit DZ Vulkacit DZ/EG-C Accelerator DZ

Structural formula:



1.2 Composition of the substance

Name: N,N-dicyclohexylbenzothiazole-2-sulphenamide

Description: mono constituent substance

Degree of purity: *confidential annex*

1.3 Physico-chemical properties

Table 2: Overview of physicochemical properties

Property	Value	Remarks
Physical state at 20°C and 101.3 kPa	white solid	-
Melting/freezing point	101 – 102 °C	Experimental data from: Torii et al., J. Org. Chem., 43, 3223 (1978). Literature values point to a melting point in this range. Akasaka, Shuichi; Journal of Applied Polymer Science 2006, V99(6), P2878-2884 CAPLUS; SRC online Data base, searched in January 2004
Boiling point	DCBS was decomposed at 300°C at 1013 hPa	OECD SIDS;
Vapour pressure	< 0.00001 Pa at 25°C	-
Surface tension		According to REACH regulation Annex VII 7.6. column 2, the test does not need to be conducted, because the water solubility of DCBS is below 1 mg/l at 20°C.
Water solubility	1.9 µg/L	LOD of column elution method
Partition coefficient n-octanol/water (log value)	Log Pow = 5.95	Estimated by using KOWWIN program v. 1.54; no pH and temperature reported.
Flash point	-	-
Flammability	-	-
Explosive properties	-	-
Self-ignition temperature	-	-
Oxidising properties	-	-
Granulometry	mass fraction of 1.68 % <100µm	<table><tr><td></td><td>spherical volume</td><td>cubical volume</td></tr><tr><td>particle size</td><td>mass fraction</td><td>mass fraction</td></tr><tr><td>< 4µm</td><td>0.87 %</td><td>0.76 %</td></tr><tr><td>4 - 10 µm</td><td>7.79 %</td><td>6.35 %</td></tr><tr><td>10 - 100 µm</td><td>91.34 %</td><td>92.89 %</td></tr><tr><td>median diameter</td><td>28 µm</td><td>30 µm</td></tr></table>
Stability in organic solvents and identity of relevant degradation products	-	-
Dissociation constant	-	Since the water solubility is low, dissociation constant is unnecessary.
Viscosity	-	In accordance with the REACH information requirements R.7A, R.7.1.18.4, viscosity testing is not relevant for substances that are not a liquid at room temperature. Therefore, no testing for viscosity is required.
Auto flammability	-	-
Reactivity towards container material	-	-
Thermal stability	-	-

2 MANUFACTURE AND USES

2.1 Quantities

Table 3: Aggregated tonnage (per year)

1 – 10 t	10 – 100 t	100 – 1000 t	1000- 10,000 t	10,000-50,000 t
50,000 – 100,000 t	100,000 – 500,000 t	500,000 – 1000,000 t	> 1000,000 t	Confidential

2.2 Identified uses

2.2.1 Uses by workers in industrial settings

- vulcanizer / vulcanization accelerator in rubber production

2.2.2 Use by professional workers

- tyre manufacturing
- general rubber products
- wide dispersive use

3 CLASSIFICATION AND LABELLING

3.1 Harmonised Classification in Annex VI of the CLP Regulation

N, N-dicyclohexyl-2-benzothiazolesulfenamide (DCBS) has currently no entry in Annex VI of the CLP regulation and, as a result, is not classified in a harmonised way.

3.2 Self classification

Table 4: Self classification

Classification		**Labelling**		**Specific Concentration limits, M- Factors**
Hazard Class and Category Code(s)	**Hazard Statement Code(s)**	**Hazard Statement Code(s)**	**Supplementary Hazard Statement Code(s)**	
Skin Irrit. 2	H315	H315		
Skin Sens. 1	H317	H317		
Eye Irrit. 2	H319	H319		
STOT SE 3	H335	H335		
Aquatic Ac. 1	H400	H400		
Aquatic Chr.1	H410	H410		
Aquatic Chr.4	H413	H413		

Signal Words: Warning **Pictograms:** GHS07, GHS09

4 ENVIRONMENTAL FATE PROPERTIES

4.1 Degradation

4.1.1 Abiotic degradation

4.1.1.1 Hydrolysis

For DCBS there are three studies on hydrolysis in the registration dossiers available.

Method	Reference
OECD 111	TL 1997
Peer-reviewed data from OECD SIDS 2004	CERI 2001
Experimental data	Bayer 1988

The registrants summarized: "*Hydrolysis half-lives were determined to be 57, 53 and 48 hours at pH 4, 7 and 9 (TL 1997).*" However, transformation products were not identified in this study. The registrants evaluate the peer-reviewed data from OECD SIDS 2004 only as supporting information and states: "*Although the study of CERI (2001) was used as critical study for SIDS endpoint, it is not used here as key study due to lack of detailed information and extrapolation of hydrolysis half-lives from 50, 60 and 70 °C to 25 °C.*" However, in the OECD-SIDS Initial Assessment Report (2004) Dicyclohexylamine and 2-mercaptobenzothiazole (MBT) were identified as major hydrolysis products. For the third experimental data from Bayer 1988 no results are documented in the registration dossiers but the study is summarized: "*DCBS hydrolyzed slowly at a temperature of 100 degree C, 2-mercaptobenzothiazole (MBT), MBT-sulfonic acid, 2-hydroxybenzothiazole and dicyclohexylamine were identified as decomposition products. Addition of strong acids accelerated the reaction."*

In the OECD-SIDS Initial Assessment Report (2004) it is stated: "*It was reported that this chemical was hydrolyzed at pH 4 (half-life time: 4.92 days at 25 °C), 7 (half-life time: 18.6 days at 25 °C) and 9 (half-life time: 112 days at 25 °C). Although the study was well conducted and measurement conditions were reliable, the initial concentration was above the water solubility and the actual degradation rates might therefore be faster than the reported values. As a result of the hydrolysis, N,N-Dicyclohexyl-2-benzothiazolesulfenamide seems to be converted into two major daughter chemicals which are possibly dicyclohexylamine and 2-mercaptobenzthiazole based on mass spectrometry (CERI, 2001). It is assumed that other metabolites can also be formed as a result of hydrolysis."*

However, the results of the three studies contravene to each other and other degradation results strongly. First of all, DCBS has a low water solubility and a high tendency for adsorption with the estimated adsorption coefficient (log K_{OC}) of 4.70 – 4.92. It is necessary to evaluate the hydrolysis rate in presence of suspended particles. It is known, that hydrolysis can be inhibited by adsorption of the substance (Boethling et al. 2009) causing persistency. In addition, the initial substance concentration has to be evaluated, because it plays a significant role on the test results. The CERI (2001) study shows an acceleration of strong acids by over 95% while one study (TL 1997) shows a

deceleration by 19 %. This contravenes each other as the unclear influence of high temperature does.

The registrants do not address the obvious controversy that neither in the aquatic BCF-study nor in the ready biodegradability study the hydrolysis plays a dominant role. If DCBS really would quickly undergo hydrolysation in aqueous media and under environmental relevant conditions than this would cause a low BCF-value and a quick degradation rate in the ready test. Neither is the case which leads to the conclusion that the hydrolysis rates under environmental conditions are much longer then evaluated by the registrants.

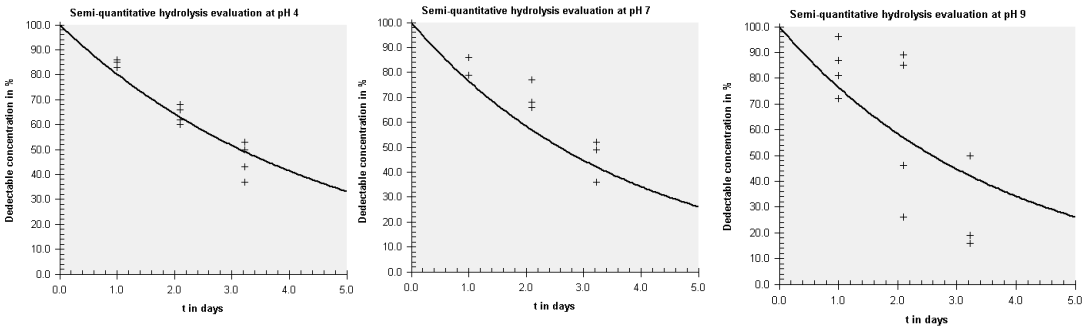
None of the three studies is documented in the registration dossier in detail and basic data and information on the three studies are missing in the registration dossier. Consequently, it is not possible to evaluate the three studies just from the content of the registration dossier.

The German CA asked the lead registrant for the three cited studies in original. One study (TL 1997) was transferred. The study CERI (2001) was transferred in the original Japanese version with all relevant numbers blackened, only. The study Bayer (1988) was not transferred at all.

For one study (TL 1997), DT_{50} values were recalculated using the software package ModelMaker 4. DCBS was spiked into the three buffer solutions at pH 4, 7 and 9 with concentrations between 3.57 and 4.84 mg/L and samples have been taken at four time steps, t = 0, 24, 50.45, 77.3 h. Measured concentration were normalized to 100% at t = 0 for all tested solutions separately.

		Solution 1		Solution 2		Solution 3		Solution 4	
		mg/l	%	mg/l	%	mg/l	%	mg/l	%
pH 4	0 hrs	4.07	100.00	3.57	100.00	3.79	100.00	3.60	100.00
	24 hrs	3.54	86.97	3.04	85.15	3.27	86.27	3.02	83.88
	50.45 hrs	2.48	60.93	2.23	62.46	2.52	66.49	2.46	68.33
	77.3 hrs	1.79	43.98	1.92	53.78	1.44	37.99	1.83	50.83
pH 7	0 hrs	3.93	100.00	4.24	100.00	4.08	100.00		
	24 hrs	3.38	86.00	3.39	79.95	3.51	86.02		
	50.45 hrs	2.71	68.95	2.84	66.98	3.15	77.2		
	77.3 hrs	1.42	36.13	2.1	49.52	2.16	52.94		
pH 9	0 hrs	4.84	100.00	4.36	100.00	4.53	100.00	3.62	100.00
	24 hrs	3.52	72.72	3.54	81.19	3.95	87.19	3.5	96.68
	50.45 hrs	2.27	46.9	1.17	26.83	3.87	85.43	3.25	89.77
	77.3 hrs	0.93	19.21	0.72	16.51	2.27	50.11	1.82	50.27

In a first step the Single First Order Kinetic model was fitted to all data points from the four (pH 7: three) solutions at once and one DT_{50} value was calculated. The result is shown in the following graphs.



In a second step the Single First Order Kinetic model was fitted to the data of each solution separately and the mean DT_{50} value was calculated. Both results are presented in the following table.

		k [h^{-1}]				$t_{1/2}$ [h]		
pH	*Solution*	*TL 1997*	*UBA*	*χ^2 Error*		*TL 1997*	*UBA*	
4	1	0.0117	0.0096	4.12		59	72.2	
	2	0.0124	0.0083	2.26		56	83.5	
	3	0.0117	0.0098	6.25		59	70.7	
	4	0.0131	0.0081	1.69		53	85.6	
	mean	0.0122	0.0090	3.26	0.00924	57	77.0	75
7	1	0.0127	0.0098	7.41		55	70.8	
	2	0.0136	0.0087	1.52		51	79.7	
	3	0.0131	0.0069	3.99		53	100.5	
	mean	0.0131	0.0085	4.42	0.00856	53	81.5	81
9	1	0.0152	0.0166	6.47		46	41.8	
	2	0.0140	0.0195	14.07		50	35.5	
	3	0.0143	0.0064	7.55		48	108.3	
	4	(0.0117)	0.0056	9.56		(59)	86.6	
	mean	0.0145	0.0120	4.26	0.01180	48	57.8	62

As the visual fit and the Chi square error show all SFO fits are acceptable. Based on the data from the (TL 1997) study, a hydrolysis DT_{50} of up to 86 hrs for pH 4, 101 hrs for pH 7 and 108.3 hrs for pH 9 was calculated for DCBS. This is up to 125% higher than the originally calculated value by (TL 1997). In average half-lives of 77 hrs for pH 4, 81.5 hrs for pH 7 and 58 hrs for pH 9 were calculated. This means the hydrolysis half-lives are significantly longer than originally calculated by the study authors.

Conclusion

DCBS is hydrolytically degradable. Hydrolysis half-lives by (TL 1997) were recalculated and they are up to 125% higher than the originally calculated values. The study of CERI (2001) reported that DCBS was hydrolyzed at 25°C 4.92 days for pH 4, 18.6 days for pH 7 and 112 days for pH 9 and Bayer (1988) even reported slow hydrolysis at 100 °C. None of the three studies is documented in the registration dossier in detail and basic data and information on the three studies are missing in the registration dossier. The results of the three studies contravene to each other strongly. The

hydrolysis of DCBS was discussed at the fourth meeting of the ECHA PBT expert group at 28th to 29th of May 2013. The hydrolysis rates are not quick enough to significantly decrease the persistency of DCBS in the environment. Based on the chemical structure the hydrolysis is caused by acid catalysis and neutral and alkaline pH-values, like they are relevant for environmental conditions, may hinder the hydrolysis of DCBS. Because of the high adsorption behaviour of DCBS it is necessary to measure the hydrolysis rate in presence of suspended particles. It is known, that hydrolysis can be inhibited by adsorption of the substance (Boethling et al. 2009) causing persistency. The hydrolysis products of DCBS are MBT (CAS 149-30-4) and Dicyclohexylamine (CAS 101-83-7). The cleavage of the covalent bond between the Dicyclohexylamine moiety and the MBT moiety is only possible by hydrolysis caused by acid catalysis. The identification of MBT (CAS 149-30-4) and Dicyclohexylamine (CAS 101-83-7) as primary degradation products is a proof for degradation via hydrolysis.

For DCBS three studies on hydrolysis were assessed. The reliability was given according to Klimisch et al. (1997).

Reference	Study available?	Results	Remarks	Reliability according to Klimisch et al. (1997)
TL 1997	Yes	Half life (DT_{50}) at 25°C: t1/2 (pH 4): 77 h t1/2 (pH 7): 82 h t1/2 (pH 9): 58 h	Recalculation was necessary	2 (reliable with restrictions)
CERI 2001	Only in Japanese with all numbers blackened	Half life (DT_{50}) at 25°C: t1/2 (pH 4): 4.92 d t1/2 (pH 7): 18.6 d t1/2 (pH 9): 112 d	Transformation products: Dicyclohexylamine, 2-mercaptobenzothiazole (MBT)	2 (reliable with restrictions)
Bayer 1988	No	Slow hydrolysis at 100°C	Transformation products: 2-mercaptobenzothiazole (MBT), MBT-Sulfonic acid, 2-hydroxybenzothiazole, Dicyclohexylamine	4 (not assignable)

4.1.1.2 Phototransformation/photolysis

4.1.1.2.1 Phototransformation in air

For DCBS there is one study on Phototransformation available in the registration dossiers. The reliability was given according to Klimisch et al. (1997)

Method	Result	Reliability according to Klimisch et al. (1997)	Reference
Indirect photolysis estimated by AOPWIN v 1.92, 2000 Estimated by calculation	Degradation: 50 % after 3.38 h	1 (reliable without restrictions)	Currenta 2010d

In the atmosphere a half-life of 3.38 hours for DCBS due to reaction with photochemically produced hydroxyl radicals is estimated by AOPWIN v1.92 with a rate constant of 1.13 *10-10cm3/(molecule*sec), considering an OH-concentration of 500,000 radicals/cm³ as a 24-h average (Currenta, 2010). The estimated half-life in air of DCBS is much shorter than 48 hours and hence no potential for long-range transport of DCBS in air is expected.

However, the rate of phototransformation in air has no relevance for the PBT-/vPvB-assessment.

4.1.1.2.2 Phototransformation in water

The endpoint was not evaluated.

4.1.1.2.3 Phototransformation in soil

The endpoint was not evaluated.

4.1.2 Biodegradation

4.1.2.1 Biodegradation in water

4.1.2.1.1 Estimated data

No results and no (estimated) half-lifes from simulation tests on biodegradation in water are available in the registration dossiers.

DT_{50} values were calculated based on inherent biodegradability tests (Currenta, 2013a & Currenta, 2013c). For Currenta 2013c a DT_{50} of 321.9 days and a DT_{90} of 860.9 days and for Currenta 2013a a DT_{50} of 196.1days and a DT_{90} of 635.0 days at 12 °C were determined. Although the tests are considered not reliable due to experimental shortcomings (for details see 4.1.2.1.2) and the calculation of DT_{50} values from an inherent test is not in accordance with Annex XIII, it is indicated that despite optimised test conditions DCBS might be vP under relevant environmental conditions.

4.1.2.1.2 Screening tests

Results of two screening tests on ready biodegradation are available in the registration dossiers. A summary is given in the Table. The reliability was given according to Klimisch et al. (1997)

Method	Result	Reliability according to Klimisch et al. (1997)	Reference
EU Method C.4-f (MITI-I Test, OECD 301 C Directive 84/449/EEC, C.7)	2% after 28 days	1 (reliable without restrictions)	TL, 1989
MITI-I (OECD 301 C)	0% in 28 days	1 (reliable without restrictions)	MITI-List 2011, SIDS Initial Assessment Report, 2004

In both screening tests, no or negligible biodegradation was observed. Therefore, DCBS is considered as “not readily biodegradable”.

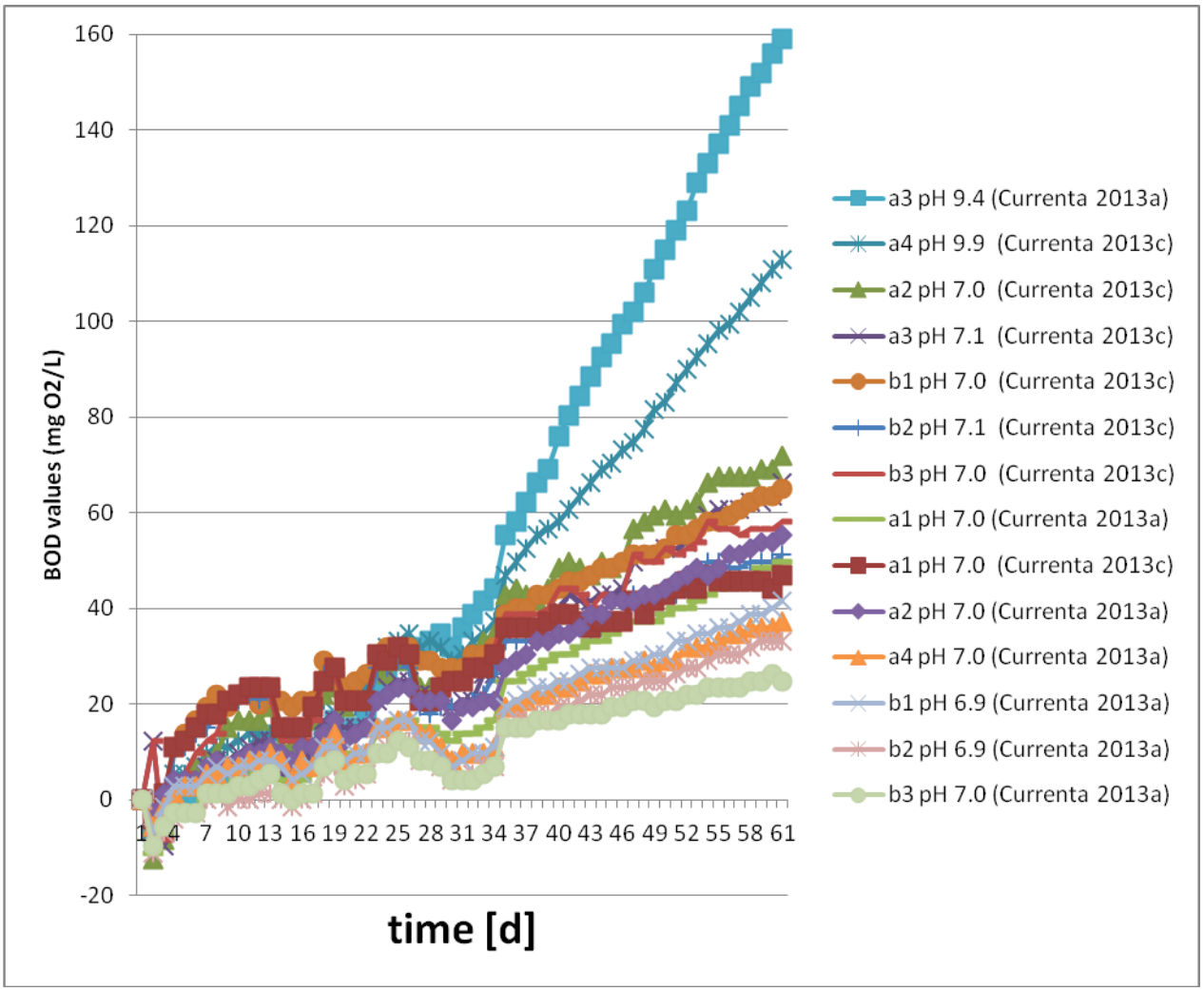
Four screening tests on inherent biodegradability in water were submitted in October 2013 during the process of substance evaluation (Currenta 2013a, Currenta 2013b, Currenta 2013c, Currenta 2013d).

Two of the tests (Currenta 2013a and Currenta 2013c) using O_2 consumption as indication of the degradation of the test substance are listed below.

Method	Reference
OECD Guideline 302 C (Inherent Biodegradability: Modified MITI Test (II)) equivalent or similar to EU Method C.4-D (Determination of the "Ready" Biodegradability - Manometric Respirometry Test) degradation was followed by continuous automated BOD determinations	Currenta (2013a)
OECD Guideline 302 C (Inherent Biodegradability: Modified MITI Test (II)) equivalent or similar to EU Method C.4-D (Determination of the "Ready" Biodegradability - Manometric Respirometry Test) degradation was followed by continuous automated BOD determinations	Currenta (2013c)

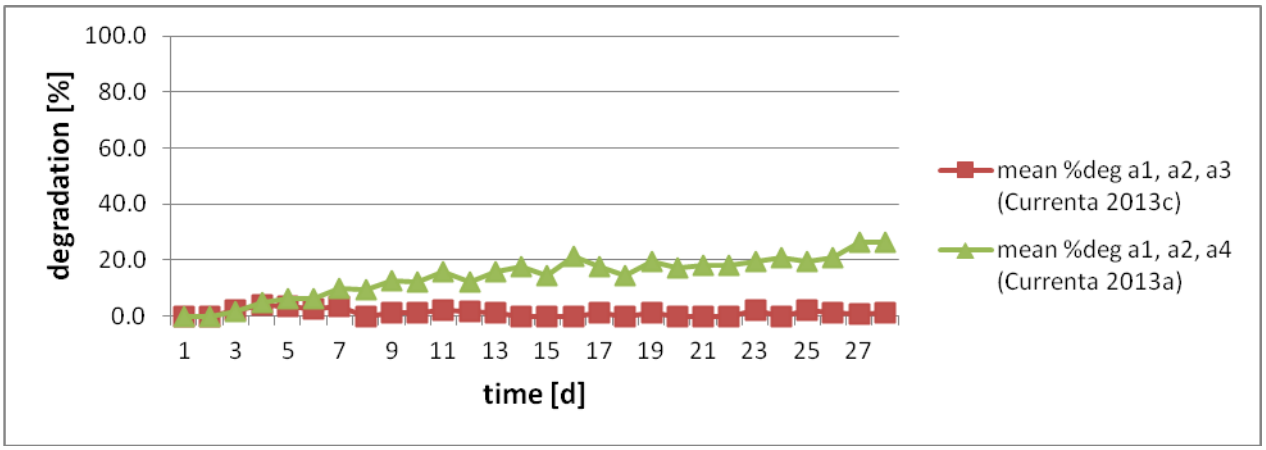
Both tests are not reliable and not valid, because they use 20 % sludge of an industrial sewage treatment plants (STP). This is not in accordance with test guidelines and assessment guidelines, because this type of inoculum may be pre-adapted to degradation of the test substance.

With such a test design it is of major importance that the test vessels with the blank test and with the test substance are treated exactly the same. However, in both studies this is not the case. One test vessel with test substance in both studies has a significant higher pH value (> pH 9.4) than all the other test vessels (pH < 7.1). The author reports as reason “*Some of the adsorbing sodium hydroxide solution dropped into the test flask resulting in a high pH value.*” The following figure shows the significantly different behaviour of these two test vessels (a3 pH 9.4 and a4 pH 9.9) compared to nine other test vessels.



One can clearly see that all test vessels with comparable pH-value < pH 7.1 show comparable development of BOD values. Only the two test vessel with significant higher pH value > 9.4 show after day 28 a complete different development of BOD values. This cannot be explained by a faster degradation of the test substance since in these test vessels the test substance would be completely degraded after day 28. One possible explanation is the activation of the inoculum and a larger production of BOD values by the higher pH value. Consequently these two test vessels must not be compared to the blank test vessels and have to be removed from any further assessment. The author of both studies failed to do so.

The mean value of the degradation (%) of the remaining three test vessels for both studies was calculated separately following the procedure of calculation by the author of the studies. The mean degradation reached after 28 days is 3.3 % for Currenta 2013c and 28.7 % for Currenta 2013a. The following figure shows the development of the mean degradation for up to 28 days.



Both tests were prolonged to 60 days. The mean degradation reached is 31.3 % for Currenta 2013c and 55.0 % for Currenta 2013a after 60 days.

time [d]	*recalculated mean degradation (%) a1, a2, a3 (without a4) (Currenta 2013c) DCBS applicated on silica gel as carrier*	*recalculated mean degradation (%) a1, a2, a4 (without a3) (Currenta 2013a) DCBS applicated directly into medium*
0	0.0	0.0
7	0.0	9.3
14	0.0	14.7
21	0.0	18.0
28	3.3	28.7
35	9.7	26.3
42	10.7	39.0
49	19.7	46.0
56	27.0	51.3
60	31.3	55.0

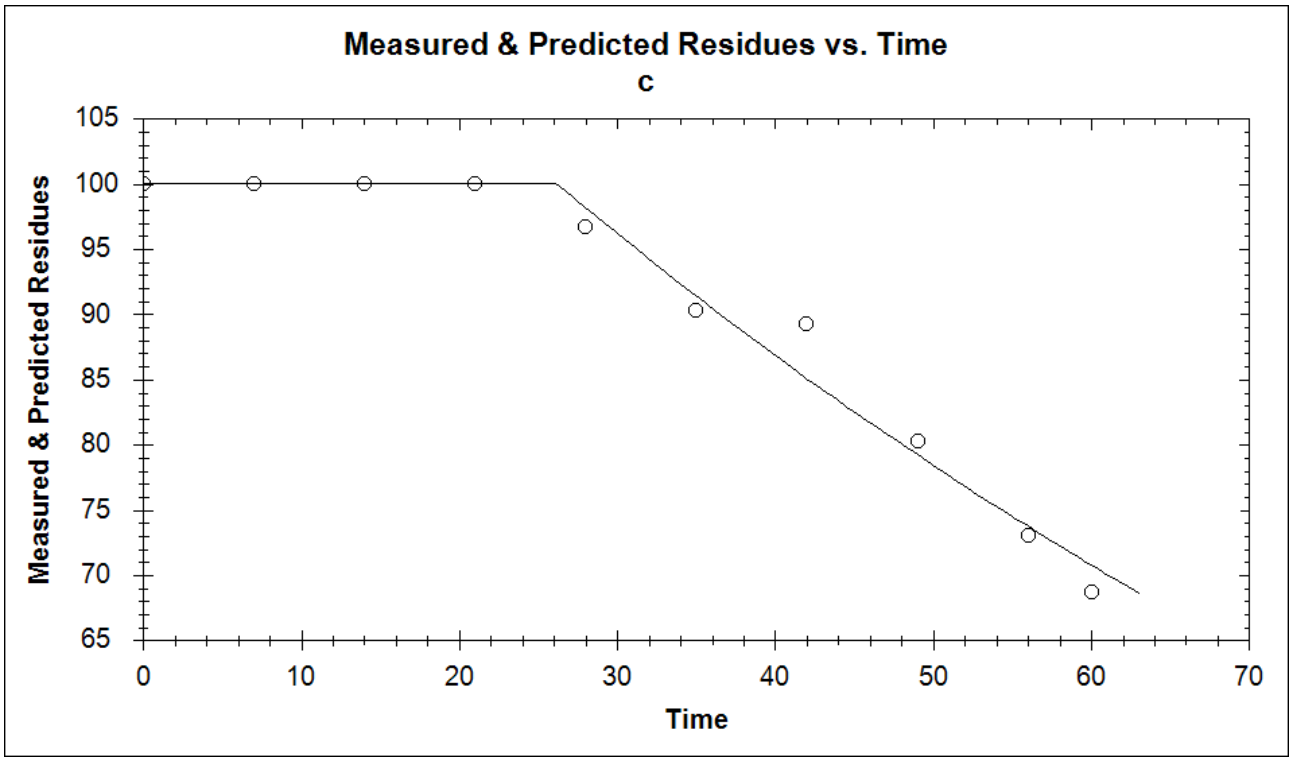
By comparing the recalculated mean degradation (%) of Currenta 2013c and Currenta 2013a one can clearly see that by applying DCBS on silica gel as carrier the start of the degradation is extremely delayed with a lag phase of 28 days and is still significantly reduced after 60 days. This supports the concern that DCBS might be persistent in the sediment and soil compartment, where similar adsorption phenomena can occur.

In addition, the experiments were run under best case conditions for degradation. The temperature was up to 27 °C, the amount of inoculum was increased and the test was prolonged. Even with these optimisations complete degradation of DCBS was not reached. Currenta 2013c and Currenta 2013a clearly show that DCBS is not inherently biodegradable, reaching after 14 days a degradation rate of 0 % and 14.7 %.

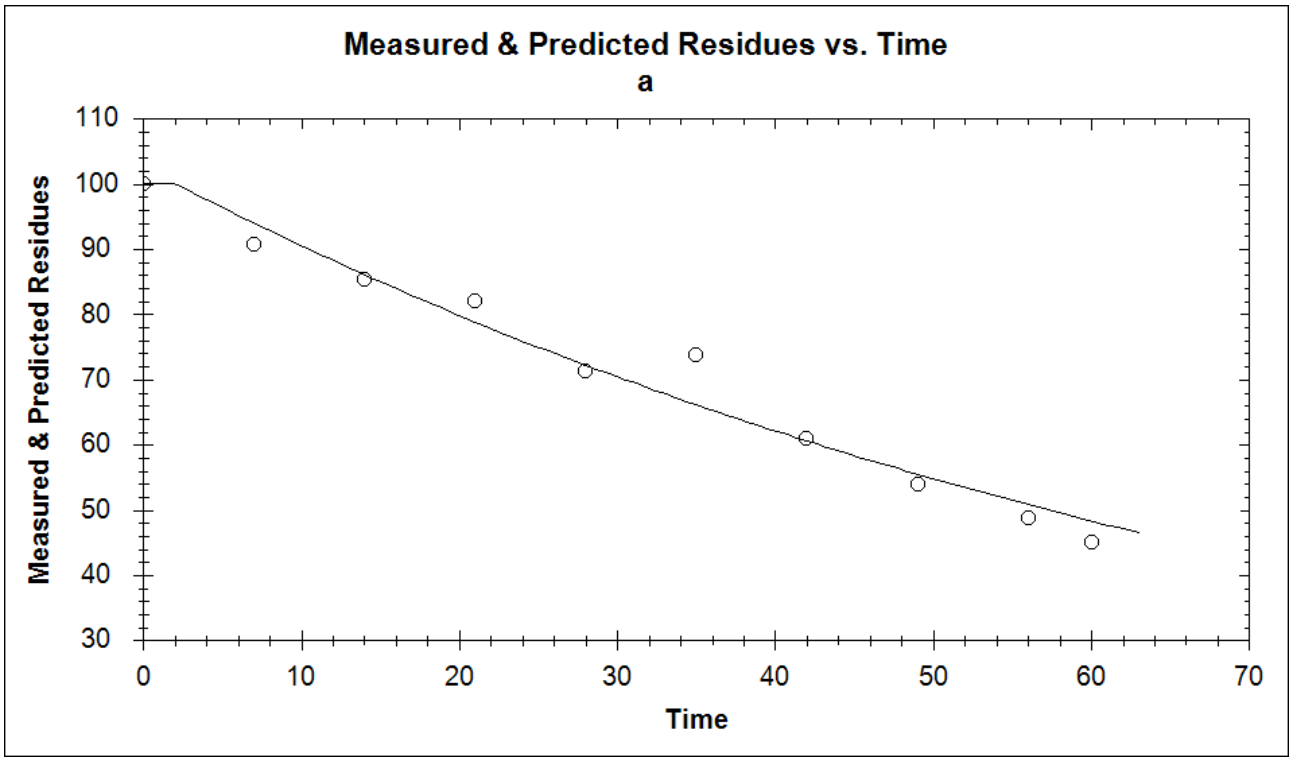
In general, it is not in accordance to Annex XIII to calculate DT_{50} values from an inherent test system. The test conditions are artificial and do not represent degradation condition in relevant environmental compartments. The registrants calculated DT_{50} in an update of the CSR. Consequently, these DT_{50} values for the studies Currenta 2013c and Currenta 2013a were recalculated using the software package ModelMaker 4. Because of the lag-phase the recommendation of FOCUS Deg. Kin. (2006) was followed and a modified hockey-stick kinetic

where the degradation rate before the breakpoint is set to zero was applied. The experiments were run under best case conditions for degradation including a temperature up to 27 °C. To allow a comparison with the P and vP criteria in Annex XIII 1.1.1 and 1.2.1 temperature was normalised to 12 °C by using the Arrhenius equation and a Q10 value of 2.58. The following table and the figure show the result.

		25 °C	12 °C	Annex XIII criteria for P in fresh water sediment	Annex XIII criteria for vP in fresh water sediment
Currenta 2013c	Kinetic model HS M0 = 100.0 kone = 0 ktwo = 0.01024 tb = 26.23 Chi2Err% 1.549	DT_{50}: 93.9 d DT_{90}: 251.1 d	DT_{50}: 321.9 d DT_{90}: 860.9 d	half-life > 120 d	half-life > 180 d



		25 °C	12 °C	Annex XIII criteria for P in water	Annex XIII criteria for vP in water
Currenta 2013a	Kinetic model HS M0 = 99.99 kone = 0 ktwo = 0.0126 tb = 2.083 Chi2Err% 3.695	DT_{50}: 57.2 DT_{90}: 185.2	DT_{50}: 196.1 DT_{90}: 635.0	half-life > 40 d	half-life > 60 d



The tests on inherent biodegradation (Currenta, 2013a & Currenta, 2013c) measure biological degradation via the O2 consumption, consequently it is possible to calculate DT_{50} values. For Currenta 2013c a DT_{50} of 321.9 days and a DT_{90} of 860.9 days and for Currenta 2013a a DT_{50} of 196.1 days and a DT_{90} of 635.0 days at 12 °C were determined. Although the tests are considered not reliable due to experimental shortcomings (for details see 4.1.2.1.2) and the calculation of DT_{50} values from an inherent test is not in accordance with Annex XIII, it is indicated that DCBS might be P or vP under relevant environmental conditions when considering the vP criteria of fresh water sediment (180 days) and the vP criteria of water (60 days).

The assessment of Currenta, 2013a and Currenta, 2013c is summarized in the following Table. The reliability was given according to Klimisch et al. (1997).

Reference	Study available?	Results	Remarks	Reliability according to Klimisch et al. (1997)
Currenta (2013a)	Yes	DCBS NOT inherently biodegradable DCBS according to R.11 P because of lack of degradation DCBS potentially vP	population of aquatic microorganisms from industrial STP (activated sludge) may be pre-adapted log phase of 3 days	3 (not reliable)
Currenta (2013c)	Yes	DCBS NOT inherently biodegradable DCBS according to R.11 P because of lack of degradation DCBS potentially vP	population of aquatic microorganisms from industrial STP (activated sludge) may be pre-adapted log phase of 28 days	3 (not reliable)

Two further screening tests on inherent biodegradability in water (Currenta 2013b and Currenta 2013d) were submitted in October 2013 during the process of substance evaluation.

Method	Reference
OECD Guideline 302 C (Inherent Biodegradability: Modified MITI Test (II)) equivalent or similar to EU Method C.4-D (Determination of the "Ready" Biodegradability - Manometric Respirometry Test) degradation was followed by continuous analytic determinations performed by HPLC-MS/MS analysis	Currenta (2013b)
OECD Guideline 302 C (Inherent Biodegradability: Modified MITI Test (II)) equivalent or similar to EU Method C.4-D (Determination of the "Ready" Biodegradability - Manometric Respirometry Test) degradation was followed by continuous analytic determinations performed by HPLC-MS/MS analysis	Currenta (2013d)

The two optimized screening tests on inherent biodegradability in water were performed by applying continuous analytic determinations using HPLC. Currenta (2013b) with low concentrations of DCBS added within 2,2,2-Trifluoroethanol (CAS 75-89-8) into the medium (67 µg/L) and Currenta (2013d) with low concentrations of DCBS applied adsorbed to silica gel (50 µg/L). Both tests are not reliable and not valid, because they use 20 % sludge of an industrial sewage treatment plants (STP). This is not in accordance with test guidelines and assessment guidelines, because this type of inoculum may be pre-adapted to degradation of the test substance.

In addition, both studies are of poor scientific quality and fail to provide necessary and relevant information needed to assess the measurements. Without any further explanation and amendment both studies are invalid and must not be used to assess the biodegradability of DCBS as explained in the following:

The amount of the test substance DCBS itself and the amount of transformation products at time point 0 (start of the experiment) is scientifically and chemically implausible. The theoretical concentrations of DCBS were 67 µg/L and 50 µg/L, however the measured concentration were 14,3 µg/L / 10,6 µg/L and 12,8 µg/L / 13,7 µg/L. The author does not give any explanation for these extremely low values of DCBS at time 0. At the same time point 0 the concentration of the two hydrolysis transformation products MBT and Dicyclohexylamine are many times higher than theoretically possible. MBT is measured at time 0 as 117 µg/L / 56,95 µg/L and 64,7 µg/L / 106 µg/L. These values do not correspond with the amount of DCBS applied theoretically and surely do not correspond to the amount of DCBS measured at time point 0. The mass balance at the beginning of the experiment is wrong. The author does not give any explanation for these extremely high values of MBT at time 0. The values give profound scientifical doubts that the purity of DCBS at the start of the experiment was 96.1 % as stated by the author.

The author failed to measure a chromatogram of the application solution. This would allow to judge on the purity and amount of DCBS in the application solution and would prove if any transformation products were present already in the application solution.

The amount of DCBS and transformation products at time point 0 also prove that degradation of DCBS started already before the beginning of the experiments. However, by looking at the measured transformation products this degradation must be abiotic and is very likely hydrolysis.

Under 4.1.2.2.2 the biotic degradation pathways of DCBS with the University of Minnesota Biocatalysis/Biodegradation Prediction System (UM-PPS[2]) were theoretically simulated. The occurrence of MBT (CAS 149-30-4) and Dicyclohexylamine (CAS 101-83-7) as primary degradation products is a proof for an abiotic degradation process. The covalent bond between the Dicyclohexylamine moiety and the MBT moiety is unlikely to be directly broken by a biotic degradation process. From a chemical point of view it is obvious that this bond cleavage is only possible via abiotic degradation processes caused by acid catalysis, most likely hydrolysis. Only the occurrence of hydroxylated transformation product of DCBS as primary degradation product would be an indicator for a biotic degradation process.

In both tests Currenta (2013b) and Currenta (2013d) DCBS was pre-treated e.g. with acetone or with 2,2,2-Trifluoroethanol (CAS 75-89-8). It remains unclear if the pre-treatment procedure supported the acid catalysis and the bond cleavage between the Dicyclohexylamine moiety and the MBT moiety. Also the author fails to report the pH values of each test vessel. They have been reported in Currenta (2013a) and Currenta (2013c) but not in these two studies. No reason is given. It is well known that acidic conditions accelerate the acid catalysis and the hydrolysis rate.

Overall, both tests Currenta (2013b) and Currenta (2013d) do not prove that DCBS is biologically degradable but that DCBS has the potential to quickly hydrolyse at high temperature (up to 27°C) and low pH values. The occurrence of MBT (CAS 149-30-4) and Dicyclohexylamine (CAS 101-83-7) as primary degradation products indicates an abiotic degradation process. Consequently, the degradation of DCBS can only be explained by hydrolysis and any DT_{50} value calculated from Currenta (2013b) and Currenta (2013d) represents a hydrolysis DT_{50}.

The assessment of Currenta 2013b and Currenta 2013d is summarized in the following Table. The reliability was given according to Klimisch et al. (1997).

Reference	Study available?	Results	Remarks	Reliability according to Klimisch et al. (1997)
Currenta (2013b)	Yes	No result, test not reliable and not valid. Without any further explanation and amendment must not be used to assess the biodegradability of DCBS.	population of aquatic microorganisms from industrial STP (activated sludge) may be pre-adapted degradation was not followed by continuous automated BOD determinations mass balance at the beginning of the experiment for DCBS and transformation products is wrong Purity of DCBS is suspected to be lower than 95% and is not proven by a chromatogram of the application solution degradation of DCBS started already before the beginning of the experiments the degradation products prove that the test measures hydrolysis of DCBS and not	3 (not reliable)

[2] http://umbbd.ethz.ch/predict/ (accessed 19.09.2013)

			biodegradation	
Currenta (2013d)	Yes	No result, test not reliable and not valid. Without any further explanation and amendment must not be used to assess the biodegradability of DCBS.	population of aquatic microorganisms from industrial STP (activated sludge) may be pre-adapted degradation was not followed by continuous automated BOD determinations mass balance at the beginning of the experiment for DCBS and transformation products is wrong Purity of DCBS is suspected to be lower than 95% and is not proven by a chromatogram of the application solution degradation of DCBS started already before the beginning of the experiments the degradation products prove that the test measures hydrolysis of DCBS and not biodegradation	3 (not reliable)

4.1.2.1.3 Simulation tests (water and sediments)

No simulation test on biodegradation in water and sediment is available in the registration dossiers. The simulation test (water and sediments) was waived for exposure considerations. The registrants support their assumption that DCBS degradation and/ or environmental exposure is negligible by Japanese monitoring studies in which DCBS was not detected (MOE 1998, MOE 2009, MOE 2010). However, as reasoned in 9.2.6 these studies are not suitable to show that DCBS does not persist in the environment.

4.1.2.2 Biodegradation in soil

4.1.2.2.1 Estimated data

Reference	Study available?	Results	Remarks	Reliability according to Klimisch et al. (1997)
TL 2017a	Yes	The half-life of DCBS in soils at 12°C is greater than the criteria set in Annex XIII of REACH for very persistence.	none	1

This study was designed to determine the rate of degradation of DCBS in soil under aerobic conditions at 12°C.

4 samples were analysed, representative for agriculturally used soils. For soil samples a constant moisture content of 50% WHC (max) was maintained. Sterile samples were applied under sterile

conditions. Each pre-incubated non-sterile soil sample was treated with 50 µg and 346 kBq per soil sample, taking into account the specific radioactivity of the test item of 6.92 MBq/mg. The requirement of the OECD test guideline 307 that the microbial biomass should be at least 1 % of the total organic carbon was met.

The study duration was 120 days. The test consisted of a flow-through apparatus.

The total recoveries ranged from 92.5 to 106.0 % AR and are well within the range of 90-110 % AR required by the test guideline. The total extractable radioactivity ranged from 82.2 to 101.3 % AR and decreased only slightly over time, remaining < 90% AR for most individual samples throughout the test. Non-extractable residues (NER), determined by combustion analysis of dried soil samples after extraction, increased throughout the study and reached a range of 11.7 (Lufa 2.4, soil no. #1) to 19.4 (Refesol 03-G, soil no. #3) % AR at end of the test.

Only low amounts of radioactivity evolved from the incubation flasks as volatile degradation products. Only a low amount of $^{14}CO_2$ was found, increasing slightly throughout the study to a range of 1.9 to 3.2 % AR at the end of the study.

For the analysis of the combined organic soil extracts (extracts 1-3) the radio-HPLC method was applied as primary method. For all samples, with few exceptions, which are caused by artefacts during the sample processing, no residues other than the parent DCBS could be identified by radio-HPLC. However, during the study the occurrence of transformation products was observed for some samples. Aliquots of these extracts were then subjected to repetition of sample concentration. For most samples the analysis of the repeatedly concentrated extract samples resulted in a complete reversal of the distribution observed for the first concentrate with no observed transformation products and 100% parent in the respective radio-HPLC chromatograms. Thus, it was concluded that the appearance of transformation products was most likely induced by the sample concentration step.

The degradation rate ($DegT_{50}$) of DCBS in four different soils at 12 °C was determined by means of KinGUI (version 1.1). First, kinetic analyses were performed using all available kinetic models, namely single first order (SFO), first order multi compartment (FOMC), hockey stick (HS), and double first order in parallel (DFOP). The calculation was conducted using the fractions of the parent obtained from radio- HPLC analyses of the respective samples.

$DegT_{50}$ and $DegT_{90}$ values for DCBS in four soils at 12 °C are:

Soil	DegT50 [days]	DegT90 [days]
Soil no. #2 (Refesol 02-A)	462.2	>1000
Soil no. #3 (Refesol 03-G)	314.8	>1000
Soil no. #4 (Refesol 04-A)	528.2	>1000
Soil no. #1 (Lufa 2.4)	614.5	>1000

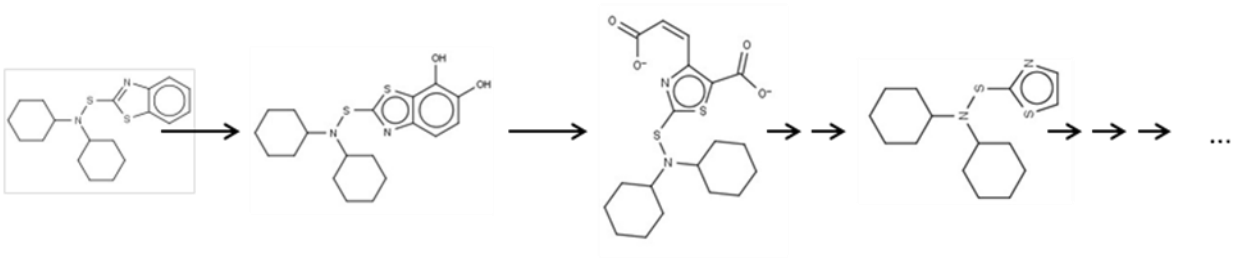
Since the Annex XIII criteria refer to half-lives at 12 °C, only the SFO $DegT_{50}$ values are presented here. The half-life of DCBS in soils at 12 °C is greater than the criteria set in Annex XIII of REACH for very persistent (vP) substances.

4.1.2.2.2 Theoretical Assessment using UM-PPS

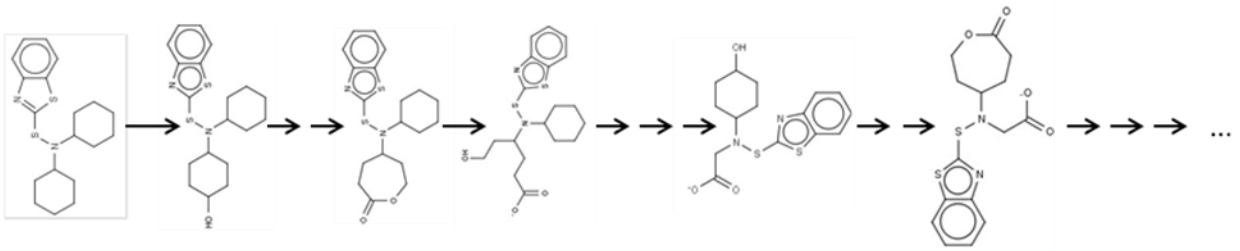
To theoretically assess the potential of DCBS for biodegradation and which biodegradation products should be expected the potential biotic degradation pathways of DCBS were simulated

with the University of Minnesota Biocatalysis/Biodegradation Prediction System (UM-PPS[3]). This web application is a rule-based system currently encompassing 250 microbial biotransformation rules based on over 1350 microbial catabolic reactions and about 200 biodegradation pathways. The system compares the organic functional groups of the entered molecules with its set of rules and shows all possible degradation steps. The reaction steps are colour coded according to the likelihood that the respective reaction is catalysed by certain bacteria in water, soil or sediment. An overview of the system can be found in two recent publications by Ellis et al. (2008) and Gao et al. (2011). Please note that it is not possible to predict rate constants with this system. Also there is no defined applicability domain for this rules based system.

For DCBS generally two idealized reaction pathways are possible.



The first reaction pathway starts with the formation of two vicinal hydroxyl groups at the phenolic ring of the MBT-moiety. This structure allows the cleavage of the phenolic ring. Also these first two reactions are likely to happen they are theoretically followed by a not likely and complex series of oxidative reaction steps involving the formation of primary alcohols, aldehydes and carboxylic acids. Finally, a thiazol remains for further degradation.



In the second reaction pathway a hydroxyl group is formed in position 4 of one of the cyclohexyl rings. This is the first step of a Baeyer-Villiger-type oxidation leading to the cleavage of the ring. This is assessed by UM-PPS as not likely and kinetically slow reaction. Afterwards the remaining aliphatic side chains are degraded, following the usual pattern of alcohol, aldehyd, carboxylic acid. After approximately 20 reaction steps the degradation stops and the second cyclohexyl ring is attacked.

4.1.3 Summary and discussion on degradation

DCBS is hydrolytically degradable (for details see 4.1.1.1). The hydrolysis rates are rather low and do not significantly influence the persistency of DCBS in the environment under relevant environmental conditions (temperature, pH, etc.). The hydrolytical transformation products of DCBS are MBT (CAS 149-30-4) and dicyclohexylamine (CAS 101-83-7).

[3] http://umbbd.ethz.ch/predict/ (accessed 19.09.2013)

According to structure activity assessments (for details see 4.1.2.2.2.), biological degradation of DCBS – if possible at all – might require several complex steps. Some of these include unlikely reaction steps (kinetically slow reaction steps). It is also expected that the covalent bond between the dicyclohexylamine moiety and the MBT moiety is unlikely to be biologically degraded. It is obvious that this bond might only be cleaved by an abiotic degradation process, most likely hydrolysis caused by acid catalysis. The degradation products observed in degradation studies might indicate whether abiotic or biotic degradation is taking place. If MBT (CAS 149-30-4) and dicyclohexylamine (CAS 101-83-7) are identified as primary degradation products this is suggesting that degradation is dominated by abiotic processes, i.e. hydrolysis. If hydroxylated transformation products of DCBS might be identified as primary degradation products this would be an indicator for a biotic transformation process.

DCBS is not readily biodegradable based on screening information on biodegradation according to Annex XIII 3.1.1 (TL, 1989 & MITI-List 2011, OECD SIDS Initial Assessment Report, 2004). Screening tests on inherent biodegradability (Currenta, 2013a, Currenta, 2013b, Currenta, 2013c, Currenta, 2013d) are also available. However, these tests are not reliable because pre-adapted industrial inoculums were used, the purity was unclear, the mass balance at the start of the experiment was not confirmed, and pH values are partly implausible or missing. Nevertheless, MBT (CAS 149-30-4) and dicyclohexylamine (CAS 101-83-7) were identified as primary degradation products suggesting that hydrolysis is the dominant degradation process in these tests.

Currenta, 2013a and Currenta, 2013c measure at 14 days a mean biodegradation of only 0% and 14.7%. Biological degradation was determined via O_2 consumption under optimized and enhanced conditions. This does not allow the calculation of DT_{50} values that may be compared to the assessment criteria of Annex XIII 1.1.1 and 1.2.1. However, if calculated anyway (for details see 4.1.2.1.2) for Currenta 2013c a DT_{50} of 321.9 days and for Currenta 2013a a DT_{50} of 196.1 days at 12 °C have been determined. Although the tests are considered not reliable (for details see 4.1.2.1.2) and the calculation of DT50 values from an inherent test is not in accordance with Annex XIII, it is indicated that despite optimised test conditions DCBS might be vP under relevant environmental conditions.

In a test according to OECD 307 Aerobic and Anaerobic Transformation in Soil ^{14}C-DCBS did not degrade in 120 days and at 12 °C. Maximum mineralization of DCBS was 3.2 % and the shares of NER were up to 19.4 %. DCBS quickly dissipated from the water compartment by adsorption to the sediment. A single first order model describes half-life best and results in a DT_{50} of 314.8 to 614.5 days depending on the soil considered. Thus, DCBS meets the specifications for the half-life in soil given for very persistent substances in REACH Regulation Annex XIII 1.2.1. Persistence. DCBS is very persistent in soil.

4.2 Environmental distribution

4.3 Bioaccumulation

4.3.1 Aquatic bioaccumulation

The estimated log K_{ow} of 5.95 (see section 1.3, Table 5) indicates a strong potential for bioaccumulation due to the high hydrophobicity.

The MITI database provides experimental bioconcentration factors (BCF) for DCBS determined for *Cyprinus carpio* using a standard test system (Table 3.4). It is unclear if the weight of the fish at the beginning and end of the test was used to corrected the BCFs for growth during the test. Six different test concentrations were used and BCF analyses were done every two weeks within a timeframe of 6 to 10 weeks. The reported BCF values widely range from 15 to 80 l/kg for the first test concentration (1000 µg/l) and from 2800 to 7700 l/kg for the last one (0.01 µg/l). The reason therefore is that the chosen test concentration in the first three tests (1000, 100, 10 µg/l) exceeds the water solubility of DCBS (1.9 µg/l, OECD SIDS 2004). As only the dissolved part of the test concentration can be taken up over the fish gills, a test concentration above the water solubility of the substance falsifies the BCF values. Therefore these test concentrations are not reliable and consequently are not considered during the assessment of the bioaccumulation potential of DCBS.

Table 3.4: Available bioconcentration factors (BCF) for DCBS

Organism	Exposure [µg/L]	Exposure [d]	BCF whole body [l/kg]	Steady-state BCF [l/kg]	Lipid content [%]	BCF 5% lipids [l/kg]	Reference	Rel.
Fish:								
Cyprinus carpio	(i) 1000*	70	15-80		3.9		MITI 2005	3
	(ii) 100*	56	74-316		3.9			3
	(iii) 10*	56	331-916		4.0			3
	(iv) 1	56	1150-3820	2930**	4.0	3663**		2
	(v) 0.1	56	3380-7310	6605**	3.7	8926**		2
	(vi) 0.01	42	2800-7700	6000	2.34	12821		2

* test concentration above water solubility of DCBS (1.9 µg/l)
** considered steady-state

A steady-state BCF (6000, lipid normalized: 12821) could only be determined in the last test with the lowest test concentration of 0.01 µg/l (Table 3.4, Figure 3.1). For the two other reliable tests (test concentration (iv), (v)) the average BCF at week eight is considered as steady-state BCF (Table 3.4, Figure 3.1). The average BCFs at week eight was 3663 l/kg (lipid normalized) for the test concentration (iv) of 1 µg/l and 8926 l/kg (lipid normalized) for the test concentration (v) of 0.02 mg/l.

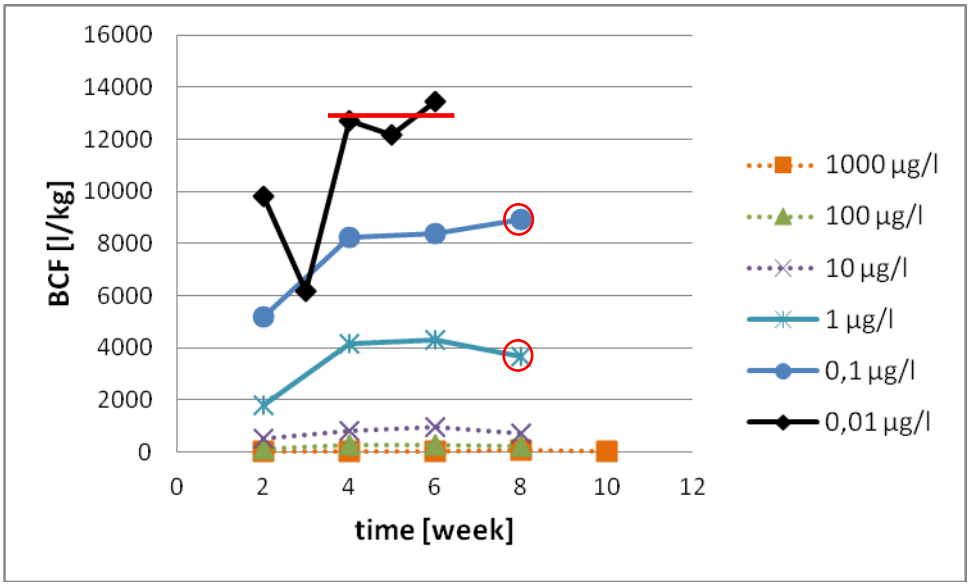


Figure 3.1: Time courses of the lipid normalized BCFs from the available MITI tests for DCBS (MITI 2005). The steady-state BCF of the test concentration 0.01 µg/l is marked with a red line. The considered steady-state BCFs for the test concentration 0.1 µg/l and 1 µg/l are marked with a red circle.

The aquatic bioaccumulation of DCBS shows a clear dose dependency. In the lowest test concentration an outlier at week 3 does not influence the reliability. The test with the lowest test concentration provides a steady state BCF of 6000 l/kg (lipid normalized: 12821 l/kg). The considered steady-state BCF values of the tests with test concentrations of 1 and 0.1 µg/l range from 2930 to 6605 (lipid normalized: 3663 and 8926).

4.3.2 Terrestrial bioaccumulation

No information available.

4.3.3 Summary and discussion of bioaccumulation

DCBS has a log K_{ow} of 5.95 indicating a high bioaccumulation potential. This is confirmed by available bioconcentration tests using *Cyprinus carpio*. The test with the lowest test concentration provides a steady state BCF of 6000 l/kg (lipid normalized: 12821 l/kg). The considered steady-state BCF values of the tests with test concentrations of 1 and 0.1 µg/l range from 2930 to 6605 (lipid normalized: 3663 and 8926). Three additional bioconcentration tests are available, which are not reliable due to used test concentrations above the water solubility of DCBS.

4.4 Secondary poisoning

n.a.

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

According to the registration dossier no study data are available on toxicokinetics of the test substance N, N-dicyclohexyl-2-benzothiazolesulfenamide (DCBS).

DCBS is a solid powder with a low vapour pressure < 7.0 x 10^{-5}Pa at 100 °C (OECD SIDS 2004); therefore inhalation exposure to the vapour might be negligible.

DCBS is practically insoluble in water (1.9 µg/l at 25 °C). However, the molecular mass of 346.59 g/mol and the n-octanol/water partition coefficient (log Pow of > 4.80 at 25 °C) suggest intestinal absorption subsequent to oral ingestion.

The results of an oral gavage study (Ema 2007b) (OECD TG 422) in Sprague-Dawley (SD) rats demonstrated systemic absorption of DCBS since 3 females of the 400 mg/kg bw group died on the expected day of parturition or on the following day.

5.1.1 Non-human information

No information available.

5.1.2 Human information

No information available.

5.1.3 Summary and discussion on toxicokinetics

No experimental data are available. However, the results of oral repeated dose toxicity studies pointed to systemic absorption of DCBS as serious effects were observed in the highest dose group.

5.2 Acute toxicity

5.2.1 Non-human information

5.2.1.1 Acute toxicity: oral

Table 5: Compilation of experimental studies on acute toxicity after oral administration according to CSR of the lead registrant/registration dossier

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels (mg/kg bw)	LD_{50} (mg/kg bw)	Remarks	Reference

Oral (gavage) Method: according to EPA OPP 81-1 Acute oral toxicity study with 14-day post-treatment observation period	Rat Sprague-Dawley Male/female N=5 (per sex and dose)	5000 test substance suspension (SANTOCURE powder) in 1% methocel A15C	>5000 (♂/♀) No mortality. Clinical signs: Some animals exhibited decreased food consumption, several were hypoactive and a few exhibited other abnormalities (e.g. nasal discharge)	Key study	TL (1985a)
Oral (gavage) Method: other acute toxicity study with 14-day post-treatment observation period	Rat Young albino rats from the Institute´s colony (Wistar derived) Dose range finding: 2 per dose and sex Main experiment: 10 per dose and sex	Dose range finding: 4000 6000 10000 Main experiment 10000 Test material in a 40% W/v suspension in polyethylene glycol. (25ml/kg bw)	10000 (♂/♀) Deaths occurred from day s 3 - 8 posttreatment	Supporting study	De Groot (1975)
Oral (gavage) Method: OECD 401 with 14-day post-treatment observation period	Rat (Crj:CD(SD)) 5 animals per dose and sex	1077 1401 1821 2367 4000 Test material (99.2% purity) in sesame oil	Mortality occured: ≥ 1401 (♀) (1/5) ≥ 2367 (♂) (2/5) Death occurred from the first to third posttreatment day.	Supporting study	TL (1998a)

No data Method: acute toxicity study	Mouse No data	No data	8500	Supporting study	Vorobeva (1968)

In a GLP and EPA guideline study with male and female CD rats no mortality was observed after treatment with 5000 mg/kg bw DCBS. Three of the five treated females exhibited slight weight loss at day 7 but all treated animals gained weight between day 7 and termination of the study (day 14). Most animals were free of abnormalities on the day of dosing. However, after 24 hours all animals exhibited decreased food consumption, several were hypoactive and a few exhibited other abnormalities (nasal discharge, oral discharge, wet rales, ocular discharge, urinary straining, unthrifty coat, soft stool, and eyes closed). In addition, no treatment-related gross pathology findings were noted. Based on these findings, the authors concluded that the LD50 of DCBS in rats is greater than 5000 mg/kg bw (TL 1985).
This finding confirmed an earlier acute oral toxicity study performed in rats, which identified an oral LD50 of 10000 mg/kg bw (de Groot 1975).
In another acute oral toxicity study no dose-related increases in mortality were observed. Male and female Crj: CD (SD) rats received 1077, 1401, 1821, 2367, 3077 and 4000 mg DCBS /kg bw by gavage. Mortality occurred in males at doses of 2367 (2/5) mg/kg bw and higher (2367 mg/kg bw (2/5), 3077 mg/kg bw (2/5), 4000 mg/kg bw (1/5)) and in females at doses of 1401 mg/kg bw (1/5) and higher (2367 mg/kg bw (4/5), 3077 mg/kg bw (1/4), 4000 mg/kg bw (4/5)). Clinical signs such as tremor, convulsion, decreased locomotor activity, deep respiration, piloerection, chromodacryorrhea and perigenital region solid with urine, as well as low body weight in comparison to control were observed in both sexes. Based on the absence of a dose-response relationship of mortality the author assessed that the LD50 value for male rats is higher than 1821 mg/kg bw and for female rats higher than 1077 mg/kg bw (TL 1998).
Moreover, in a poorly documented publication, the LD50 for mice was estimated to be 8500 mg/kg bw (Vorobeva 1968).

5.2.1.2 Acute toxicity: inhalation

No data presented by the registrant.

5.2.1.3 Acute toxicity: dermal

Table 6 Presentation of experimental studies on acute toxicity after dermal administration according to CSR of the lead registrant/registration dossier

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels (mg/kg bw)	LD_{50} (mg/kg bw)	Remarks	Reference
Dermal (24 h occlusive) Method: EPA OPP 81-2 (acute dermal	Rabbit New Zealand White Male/female	5000 (SANTOCURE powder) without vehicle,	>5000 (♂/♀) No mortality. Clinical signs: Several animals	Key study	TL (1985b)

toxicity) Acute dermal toxicity study with 14-day post-treatment observation period	N=5 (per sex and dose)	moistened with 0.9% saline	exhibited nasal discharge		

The acute dermal toxicity of the test substance DCBS was evaluated in New Zealand White rabbits in a GLP and EPA guideline study. Moistened test substance was applied for 24 h directly to the clipped intact skin of New Zealand White rabbits at a dose of 5000 mg/ kg bw (5 males and 5 females.). A 14-day observation period followed application. No mortality was observed. Most animals were free of signs of systemic toxicity, although several occurrences of nasal discharge were seen, primarily in a single animal, and a few occurrences of ocular irritation were noted in another animal. Gross postmortem observations were similar to those seen in control animals in this laboratory or were considered to represent normal physiological variation. Based on these findings the authors concluded that the dermal LD50 is greater than 5000 mg/kg bw (TL 1985).

5.2.1.4 Acute toxicity: other routes

No data available from the CSR of the lead registrant/registration dossier.

5.2.2 Human information

No data available from the CSR of the lead registrant/registration dossier.

5.2.3 Summary and discussion of acute toxicity

Data for evaluating acute oral and acute dermal toxicity of DCBS were obtained from animal testing in rats, mice and in rabbits. Some of the studies were performed according to test guidelines for acute toxicity testing and the overall available information is sufficient to conclude that the acute toxicity of DCBS is low. An **oral LD50 of >5000 mg/kg bw** was determined in the key study in male/female rats (TL 1985a) and a **dermal LD50 of > 5000 mg/kg bw** was determined from a study in male/female rabbits (TL 1985b).

Based on the available data, it is concluded that DCBS does not require classification for acute toxicity according to Regulation (EC) No. 1272/2008.

5.3 Irritation

5.3.1 Skin

Table 7 Overview of experimental studies on skin irritation according to the registration dossier

Method/ Guideline	Species, Strain, Sex, No/group	Average score 24, 72 h	Reversibility yes/no	Results	Remarks	Reference
other: according to EPA OPP 81-5 (Acute dermal irritation) Coverage: (semi)occlusive shaved), treatment: 4 h (semi-occlusive) and 24 h (occlusive), observation: up to 72 h Santocure (DCBS), 0.5 g per site, moistened with 0.5 ml 0.9 % saline	Rabbit, New Zealand White, 6 animals	Overall irritation score: 0 of max. 8 (mean) after 4 h treatment Time point: 24 h, 48 h, 72 h: score of 0.1 of max. 8 after 24 h.	Fully reversible within 7 d	not irritating	Key study	TL (1985c)

In a GLP and EPA Guideline study with New Zealand White rabbits, the dermal application of 0.5 g of the solid test substance DCBS to 6 animals for 4 and 24 hours was non-irritating to the skin. The only sign of irritation was transient very slight (barely perceptible) erythema in one animal at the sites exposed for 24 hours under occlusive covering. This animal was free of irritation at both sites by day 7. The primary irritation index for the 4 h-hour exposure was 0 and for the 24 -hour exposures 0.1 (TL 1985c).

Human information: No data available.

5.3.2 Eye

Table 8 Overview of experimental studies on eye irritation according to the registration dossier

Method/ Guideline	Species, Strain, Sex, No/group	Average score 24, 48, 72 h	Reversibility yes/no	Results	Remarks	Reference
other: according to	Rabbit,	Overall	fully	not	Key	TL

EPA OPP 81-4 (Acute eye irritation) treatment: 24 h, observation: 7 days Santocure (DCBS), application of 0.1 cc of the test substance	New Zealand White, 6 animals	irritation score: 0 of max. 4 (mean) and 2 (iris score) DCBS showed slight effects 1 h after treatment in the conjunctivae score and 1 of 6 animals showed (+) grade at 24 and 48 h in iris score.	reversible within 72 h	irritating	study	(1985d)

In a GLP and EPA guideline study with six New Zealand White rabbits, a single application of 100 mg DCBS into the lower conjunctivial sac of the right eye produced only mild and transient ocular irritation, consisting primarily of mild conjunctival irritation (redness, chemosis and/or discharge) and iridial changes, with most severe effects occurring one hour after application. All animals were free of ocular irritation within 24 hours to 7 days after instillation of the test material (TL 1985).

Human information: No data available.

5.3.3 Respiratory tract

No data available.

5.3.4 Summary and discussion of irritation

Classification as a skin, an eye or respiratory tract irritant is not warranted under Regulation (EC) 1272/2008 on classification and labelling of substances and mixtures (CLP).

5.4 Corrosivity

No corrosive effects were seen in the skin/eye irritating tests.

5.5 Sensitisation

5.5.1 Skin

Table 9 Overview of experimental studies on skin sensitisation according registration dossier

Method/ Guideline	Species, Strain, Sex, No/group	Number of animals sensitised/total number of animals	Results	Remarks	Reference
OECD Guideline study: Guinea pig maximisation test according to OECD 406 Induction: intradermal (5 %) and epicutaneous (25 %) Challenge: epicutaneous, occlusive (25 %) Santocure (DCBS) Vehicle: peanut oil	guinea pig, Hartley strain, female treatment group n=20 solvent control group n=10 positive control n=10	1st reading: 0/20 (test group), 24 h after challenge; dose: 25 % 2nd reading: 0/20 (test group); 48 h after challenge; dose: 25 %	Not Sensitising	Key study	TL (1985e)

The skin-sensitization potential of the test substance DCBS was evaluated in a Guinea pig maximization test. The intradermal induction was performed using 5% test substance, and topical induction was performed with 25% test substance concentration. The challenge using 25 % test substance formulation led to very slight skin reactions in the treatment group similar to the negative control group (erythema average score 24 h: 0.15 treatment group, 0.15 negative control; 48 h: 0.13 treatment group, 0.15 negative control). In contrast, all positive control animal exhibited a positive reaction (erythema average score 24, 48 h: 1.9, 1.9). In summary, by comparing the results in the treatment group and in the negative control group the test substance did not show a skin-sensitization potential (TL 1985).

Table 10 Human data on skin sensitisation

Method/ Guideline	population	Results	Test material	Remarks	Reference
Repeated Insult Patch Test (modified Shelanski (4x4 method))	General population Dermal administration 51 volunteers	5/51 individuals were sensitized	CAS-No 95-33-0 N-cyclohexyl-2-benzothiazole sulphenamine **(CBS)** 200 mg of the test material was placed for 24 h on the back as a 70% preparation in petrolatum (4 days/week for 3 weeks). After a two week rest period the subjects received an additional 24 h treatment, the application site was examined and scored at 24, 48 and 72 h following removal.	Supporting study	TL (1982a)
Repeated Insult Patch Test (modified Shelanski (4x4 method))	General population Dermal administration 54 volunteers	45 subjects completed the study. During induction period, 11 subjects demonstrated intense irritation. Finally, in 13/54 subjects a sensitization was observed.	CAS-No 95-31-8 N-(1,3-benzothiazol-2-ylsulfanyl)-2-methylpropan-2-amine **(TBBS)** For the description of the method see precedent study. 200 mg were tested as a 60% preparation in petrolatum	supporting study	TL (1983)

Repeated Insult Patch Test (modified Shelanski (4x4 method)) Accord. to GCP	General population Dermal administration 49 volunteers	Irritation was observed in 31/49 individuals Sensitisation was observed in 24/49 individuals	CAS-No 102-77-2 2-(morpholin-4-ylsulfanyl)-1,3-benzothiazole **(MBS)** For the description of the method see precedent study. 200 mg were tested as a 75% preparation in petrolatum	supporting study	TL (1982b)

In several well documented Repeated Insult Patch tests performed in human volunteers a skin sensitizing potential of the structural analogues CBS, TBBS and MBS were noted. The substances CBS, TBBS and MBS were classified as skin sensitizers in humans. In a read-across approach, where physicochemical data and toxicological findings of DCBS, CBS, TBBS and MBS were compared, consistencies in acute toxicity (oral and dermal), skin and eye-irritation, as well as repeated dose toxicity were found. However, CBS and TBBS showed no positive response in Buehler assays with guinea pigs but positive responses in humans. In light of this observation, it is questioned whether the negative response of DCBS noted in a guinea pig maximization test reflect the human situation. Based on the read-across approach with data from human volunteers treated with the CBS, TBBS or MBS a skin sensitizing potential of DCBS in humans is suggested.

5.5.2 Respiratory system

No data available.

5.5.3 Summary and discussion on sensitisation

The skin-sensitization potential of DCBS was evaluated in a Guinea pig maximization test and found negative. However, in a read across approach with the structural analogues CBS, TBBS and MBS similarities in several parameters were noted. The structural analogues CBS, TBBS and MBS induced positive skin reactions in human volunteers and accordingly, CBS and MBS received harmonized classification as Skin Sens. according to Regulation (EC) no. 1272/2008, whereas TBBS was self-classified as Skin Sens.1.

Based on a read-across approach to the structural analogues CBS, TBBS and MBS, a skin sensitizing potential of DCBS in humans is suggested; the self-classification of DCBS as skin sensitizer is considered as sufficient. No further action is recommended by the eMSCA.

5.6 Repeated dose toxicity

5.6.1 Non-human information

5.6.1.1 Repeated dose toxicity: oral

Table 11 Presentation of experimental studies on acute toxicity after oral administration according to CSR of the lead registrant/registration dossier

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels (mg/kg bw/day)	NOAEL/LOAEL (mg/kg bw/day)	Remarks	Reference
Method: OECD 408 (Repeated Dose 90-Day oral Toxicity in Rodents)	Rat, Sprague-Dawley Male/female N=10 (per sex and dose)	500 ppm 2500 ppm 5000 ppm (SANTOCURE in feed) Corresponding to 37 177 343	NOAEL = 37 LOAEL = 177 Slight decrease in body weight gain compared to control (males - 6.4%, females - 10%). No mortality.	Key study	TL (1989)
Method: OECD 407 (Repeated Dose 28-Day oral Toxicity in Rodents)	Rat, Sprague-Dawley Male/female N=5 (per sex and dose)	2000 ppm 3000 ppm 5000 ppm 7500 ppm 10000 ppm (SANTOCURE in feed) Corresponding to 186 m, 202 f 282 m, 293 f 457 m, 474 f 701 m, 715 f	LOAEL = 186 m LOAEL = 202 f Reduced body weight gain and food consumption	Supporting study	TL (1988)

		933 m, 946 f			
Method: OECD 422 (Combined Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test) Oral: Gavage Daily treatment	Rat, Crj:CD (SD) Male/female N=10 (per sex and dose) Males: 44 days including 14 days before mating Females: From 14 days before mating to day 3 of lactation.	6 25 100 400 DCBS in sesame oil	NOAEL = 100 m+f LOAEL = 400 m+f Death in females, decreased body weights and changes in urinalysis (increase in urinary ketones in ♂ at 400 mg/kg bw), blood chemistry, and/or histopathology in both sexes Hyaline droplets were observed in the proximal tubular epithelium in the kidney at 100 and 400 mg/kg bw/d in males.	Supporting study	Ema (2007b) TL (1998b) OECD SIDS (2004) MHW Japan (1994a,b)
Method: OECD 416 (Two-Generation Reproduction Toxicity Study) Oral: via feed	Rat, Crj:CD (SD) Male/female N=24 (per sex and dose) F0: start dosing: 10 weeks before mating, age at scheduled terminal sacrifice: males: 19-20 wks, females: 21-22 wks;	80 ppm 600 ppm 4500 ppm DCBS in feed F0 males: 5.2, 39, 291 F0 females: 7.2, 54, 416 F1 males: 5.9, 44, 331 F1 females:	NOAEL = 39 m NOAEL = 54 f No adverse effects No mortality in F0 or F1. Body weight and body weight gain: F0 (♂/♀) 4500 ppm significant decrease throughout the dosing period.	Supporting study	Ema (2008) Ema (2007)

	F1: start: Postnatal day (PND) 21-25 (day 0 of dosing), starting dosing: 10 weeks prior mating, continuing throughout the matting period, administration was continued throughout gestation and lactation	7.4, 55, 417	Food consumption: F0 (♂): 4500 ppm significant decrease during weeks 1-8 and 13-14 F0 (♀): 4500 ppm significant decrease during weeks 1 and days 14-21 of lactation F1 (♂): 80 ppm significant decrease during weeks 4and 7, 600 ppm during week 6, and 4500 ppm during week 4 Organ weights: F0 (♂): 4500 ppm significant lower abs organ weights: spleen, adrenal gland; increase in relative weight of: brain, thyroid, liver, kidney and testis F0 (♀): significant increase in absolute weights of: brain (80 ppm, 600 ppm), pituitary (80 ppm); decrease in relative weights: spleen (80 ppm and 600 ppm); significant decrease in absolute weight of spleen and increase in rel weights of:		

			brain, kidney, adrenal gland at 4500 ppm.		
Other: Screening Assay for the evaluation of reproductive and developmental toxicity) Oral: via feed	Rat, Crj:CD (SD) Male/female N=6 (per sex and dose) Males: 57 days (beginning 16 days before mating) Females: 61 to 65 days (from 16 days before mating to day 21 of lactation)	1500 ppm 3000 ppm 6000 ppm 10000 ppm DCBS in feed Males: 83 172 343 553 Females: 126 264 476 707	NOAEL = 83 m NOAEL = 126 f No adverse effects No mortality	Supporting study	Ema (2007a)

5.6.1.2 Repeated dose toxicity: inhalation

No valid data available.

5.6.1.3 Repeated dose toxicity: dermal

Table 12 Presentation of experimental studies on acute toxicity after dermal administration according to CSR of the lead registrant/registration dossier

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels (mg/kg bw/day)	NOAEL/LOAEL (mg/kg bw/day)	Remarks	Reference
Method: OECD 410 (Repeated Dose Dermal	Rabbit, New Zealand White	125 500	NOAEL = 2000 No major signs of local or	Key study Read across from	TL (1981a)

Toxicity: 21/28 Day Study) Exposure: 21d (5 days per week)	Male/female N=10 (per sex and dose) (5 per dose and sex inctact skin) (5 per dose and sex abraded skin)	2000 Ground test substance was applied onto skin, moistened and covered with semiocclusive dressing	systemic toxicity noted in rabbits treated with CBS at dosage of 125, 500, or 2000 mg/kg bw/day	supporting substance CAS-No: 95-33-0 **(CBS)**	
Method: OECD 410 (Repeated Dose Dermal Toxicity: 21/28 Day Study) Exposure: 21d (5 days per week)	Rabbit, New Zealand White Male/female N=10 (per sex and dose) (5 per dose and sex inctact skin) (5 per dose and sex abraded skin)	125 500 2000 Ground test substance was applied onto skin, moistened and covered with semiocclusive dressing	NOAEL (systemic) > 2000 NOAEL (local) = 500 LOAEL (local effects) Acanthosis, hyperkeratosis, and dermal inflammatory cell infiltration in the treated skin of rabbits from the 2000 mg/kg group	Key study Read across from supporting substance CAS-No: 95-31-8 **(TBBS)**	TL (1981)
Method: OECD 410 (Repeated Dose Dermal Toxicity: 21/28 Day Study) Exposure: 21d (5 days per week)	Rabbit, New Zealand White Male/female N=10 (per sex and dose) (5 per dose and sex inctact skin) (5 per dose and sex abraded skin)	125 500 2000 Ground test substance (purity: 95.6%) was applied onto skin, moistened and covered with semiocclusive dressing	NOAEL > 2000 no major signs of local or systemic toxicity were noted	Key study Read across from supporting substance CAS-No: 102-77-2 **(MBS)**	TL (1981b)

5.6.1.4 Repeated dose toxicity: other routes

No data available.

5.6.2 Human information

No data available.

5.6.3 Summary and discussion of repeated dose toxicity

Several repeated dose studies and reproduction/developmental toxicity studies were performed to evaluate the repeated dose toxicity of DCBS. The consistent finding in all studies is a reduction of body weight, body weight gain and/or food consumption in treated animals. With the exception of the sex-specific hyaline droplet nephropathy in male rats no further target organ of toxicity was identified. Overall, the lowest NOAEL of 37 mg/kg bw/d reported in the subchronic study should be taken as starting point for oral risk assessment. Based on a read-across approach with the structural analogues CBS, TBBS and MBS a starting point of 2000 mg/kg bw/d is used for dermal risk assessment.

Classification is not warranted under Regulation (EC) 1272/2008 on classification and labelling of substances and mixtures (CLP).

5.7 Mutagenicity

5.7.1 Non-human information

5.7.1.1 In vitro data

Table 13 Presentation of in-vitro-studies on mutagenicity testing according to CSR of the lead registrant/registration dossier

Method/ Guideline	Test organism, Strain, Dose levels	Results	Remarks	Reference
Method: OECD 471 (Bacterial Reverse Mutation Assay) Test substance: Santocure (DCBS)	Salmonella typhimurium (TA 100, TA 1535, TA 1537, TA 1538, TA 98) With and without metabolic activation Pre-experiment:	The test substance was negative in the Ames assay.	Key study	TL (1984)

	0, 3, 12, 60, 300, 800, 2500 µg/plate Main experiment: 0, 0.3, 1.2, 6, 30, 100, 300 µg/plate			
Method: Japanese Guideline for Screening Mutagenicity testing of chemicals Test substance: Santocure (DCBS)	Salmonella typhimurium (TA 98, TA 100, TA 1535, TA 1537,), E. coli (WP2uvrA) With and without metabolic activation 0, 312.5, 625, 1250, 5000 µg/plate	The test substance was negative.	Supporting study	TL (1998c) OECD SIDS (2004)
Method: OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test) Test substance: DCBS	Chinese hamster ovary cells (CHO-K1-BH4) With and without metabolic activation Main study with 0.5% S9: 5, 50, 100, 250, 500 µg/ml	the test substance was negative in the CHO/HGPRT assay	Supporting study	TL (1985f)
in vitro mammalian chromosome aberration test Method: Japanese Guideline for Screening Mutagenicity testing of chemicals Test substance: DCBS	Chinese hamster lung (CHL/IU) cells With and without metabolic activation -S9 (continuous treatment) 0, 0.21, 0.41, 0.82 mg/ml; -S9 (short-term treatment) 0, 0.9, 1.8, 3.5 mg/ml; +S9 (short-term treatment) 0, 0.9, 1.8, 3.5 mg/ml	ambiguous without metabolic activation	Key study	TL (1998d) MHW Japan (1994c, 1994) OECD SIDS (2004)

Chromosome aberration Method: in vitro mammalian cell micronucleus test Test substance: DCBS	Chinese hamster lung (CHL/IU) cells Without metabolic activation 0, 0.21, 0.41, 0.82 µg/ml	Test results: Positive	Supporting study	OECD SIDS (2004)
Method: OECD Guideline 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro) Test substance: DCBS	Primary rat hepatocytes With metabolic activation Up to and including 50 µg/ml	Test results: negative	Supporting study	TL (1985g)

5.7.1.2 In vivo data

Table 14 Presentation of in-vivo-studies on mutagenicity testing according to CSR of the lead registrant/registration dossier

Method/ Guideline	Species, Strain, No. of animals Dose levels	Results	Remarks	Reference
Method: OECD Guideline 475 (Mammalian Bone Marrow Chromosome Aberration Test) Test substance: Santocure (DCBS) Oral: gavage	Rat, Sprague-Dawley Male/female 15/sex and dose Single administration 1000 mg/kg bw	Genotoxicity: negative.	Key study	TL (1985h)

5.7.2 Human information

No data available.

5.7.3 Summary and discussion of mutagenicity

Data from the bacterial mutation assays indicated no genotoxic potential of DCBS. These results were confirmed by the results from a mammalian cell mutation assay and an UDS assay using primary rat hepatocytes. An in vitro chromosome aberration assay with CHL cells revealed no clastogenic effect of DCBS; but an increase of polyploidy was noted in the long-term treatment without metabolic activation. Beside the fact that according to the recommendation given in the OECD guideline 473 this test system is not designed to measure numerical aberrations and should not routinely be used for that purpose, no dose-response relationship was noted and thus the increase in polyploidy was considered as ambiguous. However, in a limited documented in vitro micronucleus assay an increase in micronuclei and multiple nuclei was noted.

The potency to induce genotoxicity could not be confirmed under in vivo conditions. In an in vivo bone marrow chromosome aberration assay with Sprague-Dawley rats DCBS did not induce a biologically relevant increase in aberrant cells and no statistically significant differences in the mean chromosome numbers of the test group compared to the control.

In summary, the weight of evidence indicates a low or even a non-genotoxic potential of DCBS in vitro and no genotoxity in vivo.

Classification is not warranted under Regulation (EC) 1272/2008 on classification and labelling of substances and mixtures (CLP).

5.8 Carcinogenicity

5.8.1 Non-human information

5.8.1.1 Carcinogenicity: oral

No data available.

5.8.1.2 Carcinogenicity: inhalation

No data available.

5.8.1.3 Carcinogenicity: dermal

No data available.

5.8.1.4 Carcinogenicity: other routes

Table 15 Overview of experimental studies on carcinogenicity after s.c. administration according to CSR of the lead registrant/registration dossier

Method/Guideline	Route of exposure, duration	Species, strain, sex, no/group	Dose levels (mg/kg bw/week)	Results main effects/target organs/tumours	Remarks	Reference
Carcinogenicity study with subcutaneous application DCBS of technical (purity: 98.6 %) and analytical grade was tested (purity: 99%)	Subcutaneous Once a week 413 days Vehicle: Physiol. saline	Rat (Wistar) m/f 20 per dose/sex Control: 25 per sex	0 1000 20000 (total amount)	No signs of systemic toxicity were reported, there was no difference between the survival of the control group and the dose group, an increased number of sarcomas located at the injection site was observed in all dose groups	Supporting study	Bayer AG (1975b)

The study is very old and does not comply with current guidelines. The number of animals is insufficient, the time of treatment is too short and the route of application is unusual. Furthermore, the information about dosage is unclear: DCBS has a very low solubility in water, it is difficult to comprehend in which way a dose of 1000 mg/kg bw/week could be dissolved in physiological saline. The dosage of "20000 mg/kg bw (total amount)" as stated in the registration dossier remains unclear, it is too high for a weekly single dose. However, it is low compared to a weekly dose of 1000 mg/kg bw for 59 weeks (corresponding to 413 days) yielding in a total dose of 59000 mg/kg bw.

5.8.2 Human information

No data available.

5.8.3 Summary and discussion of carcinogenicity

No reliable data are available. According to the registrant no evidence of pre-neoplastic lesions was found in a subchronic feeding study using male and female SD rats. There was no evidence of any

gross pathological and histopathological finding associated to dosing with DCBS up to the highest dose group evaluated.

Additionally, it was shown that DCBS is not genotoxic in an in vivo bone marrow chromosome aberration assay.

In conclusion, based on the absence of a genotoxic potential in vivo and no observations of pre-neoplastic lesions in an oral subchronic repeated dose toxicity study, no evidence was found for a carcinogenic potential of DCBS.

Classification is not warranted under Regulation (EC) 1272/2008 on classification and labelling of substances and mixtures (CLP).

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

5.9.1.1 Non-human information

Table 16 Presentation of experimental studies on fertility after oral administration according to CSR of the lead registrant/registration dossier

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels (mg/kg bw/day)	NOAEL/LOAEL (mg/kg bw/day)	Remarks	Reference
Method: OECD 416 (Two-Generation Reproduction Toxicity Study) Oral: in the feed	Rat, Crj:CD (SD) Male/female N=24 (per sex and dose) F0: start dosing: 10 weeks before mating, age at scheduled terminal sacrifice: males: 19-20 wks, females: 21-22 wks; F1: start: Postnatal day (PND) 21-25	80 ppm 600 ppm 4500 ppm DCBS in feed F0 males: 5.2, 39, 291 F0 females: 7.2, 54, 416 F1 males: 5.9, 44, 331 F1 females: 7.4, 55, 417	NOAEL (maternal) = 54 NOAEL (foetal) = 291	key study	Ema (2008) Ema (2007)

	(day 0 of dosing), starting dosing: 10 weeks prior mating, continuing throughout the mating period, administration was continued throughout gestation and lactation				
Method: OECD 422 (Combined Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test) Oral: Gavage Daily treatment	Rat, Crj:CD (SD) Male/female N=10 (per sex and dose) Males: 44 days including 14 days before mating Females: From 14 days before mating to day 3 of lactation.	6 25 100 400 DCBS in sesame oil	NOAEL (P)= 100 NOAEL (F1) = 100 3/10 F in the 400 mg group with live born pups 3/10 F in the 400 mg group died pregnant	Supporting study	Ema (2007b)
Other: Screening Assay for the evaluation of reproductive and developmental toxicity) Oral: DCBS via feed	Rat, Crj:CD (SD) Male/female N=6 (per sex and dose) Males: 57 days (beginning 16 days before mating) Females: 61 to 65 days (from 16 days before mating	1500 ppm 3000 ppm 6000 ppm 10000 ppm DCBS in feed Males: 83 172 343 553	NOAEL (effects on reproduction) = 264 (No adverse effects on reproduction) NOAEL (maternal toxicity) = 126 (No adverse effects) LOAEL (maternal toxicity) = 264	Supporting study	Ema (2007a)

	to day 21 of lactation)	Females: 126 264 476 707	(decreased body weight gain)		

5.9.1.2 Human information

No data available.

5.9.2 Developmental toxicity

5.9.2.1 Non-human information

Table 17 Presentation of experimental studies on developmental toxicity after oral administration according to CSR of the lead registrant/registration dossier

Method/ Guideline	Species, Strain, Sex, No/group	Dose levels (mg/kg bw/day)	NOAEL/LOAEL (mg/kg bw/day)	Remarks	Reference
Method: OECD 416 (Two-Generation Reproduction Toxicity Study) Oral: via feed	Rat, Crj:CD (SD) Male/female N=24 (per sex and dose) F0: start dosing: 10 weeks before mating, age at scheduled terminal sacrifice: males: 19-20 wks, females: 21-22 wks; F1: start: Postnatal day (PND) 21-25 (day 0 of dosing),	80 ppm 600 ppm 4500 ppm DCBS in feed F0 males: 5.2, 39, 291 F0 females: 7.2, 54, 416 F1 males: 5.9, 44, 331 F1 females: 7.4, 55, 417	NOAEL (maternal) = 54 NOAEL (foetal) = 7.2 Delayed vaginal opening in F1 F at 600 and 4500 ppm Worse performance in water T-maze in F1 F at 600 and 4500 ppm Reduced uterine weight in F2 F at 600 and 4500 ppm.	key study	Ema (2008) Ema (2007)

	starting dosing: 10 weeks prior mating, continuing throughout the matting period, administration was continued throughout gestation and lactation				
Method: OECD 414 (Prenatal Developmental Toxicity Study) Oral: Gavage Daily treatment between day 6 and day 28 of gestation Purity of test substance: 93.9%	Rabbit, New Zealand White 22 Females per dose	0 100 300 1000 (DCBS suspended in 1% aqueous carboxymethyl-cellulose with 0.1% Tween-80 Controls (0) received the vehicle only	NOAEL (maternal toxicity) = 1000 NOAEL (fetotoxicity) = 1000	Key study	TL (2016)
Method: OECD 422 (Combined Repeated Dose Toxicity Study with the Reproduction/ Developmental Toxicity Screening Test) Oral: Gavage Daily treatment	Rat, Crj:CD (SD) Male/female N=10 (per sex and dose) Males: 44 days including 14 days before mating Females: From 14 days before mating to day 3 of	6 25 100 400 DCBS in sesame oil	NOAEL (maternal toxicity) = 100 NOAEL (fetotoxicity) = 100 No adverse effects	Supporting study	Ema (2007b) TL (1998b) OECD SIDS (2004) MHW Japan (1994a,b)

	lactation.				

5.9.2.2 Human information

No data available.

5.9.3 Summary and discussion of reproductive toxicity

The developmental toxicity of DCBS was evaluated in a repeated dose study with reproduction/developmental toxicity screening test (OECD 422). In dams at 400 mg/kg bw/d severe maternal toxic effects were observed such as mortality and clinical signs like decreased body weights, changes in blood chemistry and/or histopathology. All dams lost their litters at delivery or by day 4 of lactation at this dose (Ema 2007b). Therefore, a NOAEL of 100 mg/kg bw/d was derived from this study.

Another study was performed in rabbits to investigate the prenatal developmental toxicity (according to OECD 414) of DCBS after oral administration. No maternal toxicity and no developmental toxicity was observed in the 100, 300 and 1000 mg/kg bw/d groups. Thus, an NOAEL of 1000 mg/kg bw/d was derived from this study for developmental effects and general toxicity.

In a two-generation study (OECD 416) no substance-related mortality and clinical signs were observed across generations up the highest concentration evaluated (416 mg/kg bw/d in F0 females), whereas reduction of body weight gain in parental animals and offspring was consistently observed throughout the generations in that dose group. In F1 females of the mid-dose group delays in vaginal opening and worse performance in water T-maze were observed and confirmed in the highest dose group. In F2 females of the mid-dose group a reduced uterine weight was observed and confirmed in the highest dose group. No malformed F1 pups were found in any groups (Ema 2008).

An NOAEL of 7.2 mg/kg bw/d was derived from this study.

Classification is not warranted under Regulation (EC) 1272/2008 on classification and labelling of substances and mixtures (CLP).

5.10 Endocrine disrupting properties

No data available.

5.11 Other effects

No data available.

5.12 Combined effects

No data available.

5.13 Derivation of DNEL(s) / DMEL(s)

No DNEL/DMEL values were derived due to the fact that no exposure was noted for consumers.

6 ENVIRONMENTAL HAZARD ASSESSMENT

6.1 Aquatic compartment (including sediment)

The aquatic toxicity of DCBS was mostly investigated by the Ministry of the Environment of Japan and some business concerns. The acute aquatic toxicity was determined by use of fish, Daphnia and algae. The chronic toxicity of DCBS was only determined in Daphnia and algae. The chronic toxicity to fish of DCBS itself was not determined (MBT was used as read-across substance by the registrants).

In addition the toxicity of three degradation products were examined: Benzothiazole-2-thiole (MBT, CAS: 149-30-4, not readily biodegradable), Dicyclohexylamine (CAS: 101-83-7, readily biodegradable). Benzothiazole-2-thiole and Dicyclohexylamine were chosen because they are major transformation products. They are evaluated in spite of the considerable persistence of the substance because partly the substance may hydrolyse after a certain time and then the hydrolysis products occur in the environment. Furthermore both substances are formed during vulcanisation process.

There are no experimental data available for the sediment or soil for DCBS and degradation products. The equilibrium partitioning method (EPM) was used by the registrants to obtain data.

There are some additional data from publications in order to supplement the existing information.

6.1.1 Toxicity data

According to Annex XIII DCBS does not fulfill the T-screening (EC/LC50 < 0.1mg/L) and T-criteria (NOEC < 0.01mg/L). The reliable studies on chronic toxicity to Daphnia (OECD 211, National Institute of Technology and Evaluation 2001; OECD SIDS,2004) and the test on toxicity to algae (OECD 201, National Institute of Technology and Evaluation 2001; OECD SIDS, 2004) showed no effects, other studies showed effects far above water solubility. DCBS is a poorly water soluble substance (water solubility: 1.9 µg/L).

The hydrolysis product MBT (2-Mercaptobenzothiazole, CAS 149-30-4) was shown to be very toxic to aquatic organisms. The lowest acute value was LC50 0.73mg/L (96h, fish). Other acute tests with reliability 4 showed effects at lower concentrations. The chronic fish test showed a NOEC of 0.041 mg/L (reliability 4 as there were no data about used concentrations). However, the T-screening criterion and T-criterion are not fulfilled. Another hydrolysis product Dicyclohexylamine (CAS 101-83-7) showed no elevated acute effects on fish, Daphnia and algae. In contrast the chronic effects on Daphnia and algae (both NOECs 0.016mg/L) were very near to the T-criterion but did not fulfill it.

From the results above it is unlikely that DCBS shows considerable toxicity in the chronic fish test. And the chemical structure does not indicate existence of a specific mode of action.

In the following an overview of the effects on aquatic organisms (and in-vitro tests) is given.

Table 6: Summery of effects on the aquatic compartment

Author	Test organism Guideline	Analysis	Performance, Duration of exposure	Concentration	Effect value (Information about measurement)	Reliability	Test substance
Short-term toxicity to fish							
National Institute of Technology and Evaluation; OECD SIDS Initial Assessment Report 2004	O. latipes OECD 203	yes	semistatic Limittest 96 h	0.04 mg/L (n) WR: 84%	LC 50 >0.033 mg/L (m)	2	DCBS (CAS: 4979-32-2)
TL 1988	D. rerio n.s.	DOC was measured	static Limittest 96 h	1000mg/L (n), after filtration: DOC= 15 mg/L	NOEC 15 mg/L (based on DOC) NOEC 22.8mg/L (calculated from DOC)	4 (far above water solubility)	DCBS (CAS: 4979-32-2)
Ueda 1992	O. latipes OECD 203	no	semistatic 96 h	no data	LC50 > 1000mg/L (n)	4 (data lacking)	DCBS (CAS: 4979-32-2)
Short-term toxicity to invertebrates							
National Institute of Technology and Evaluation 2001; OECD SIDS Initial Assessm. Report 2004	D. magna OECD 202	yes	semi-static, 48 h	0.00323, 0.00523, 0.00925, 0.0190, and 0.0314 mg/l (m)	EC50 > 0.031 mg/L (m)	2	DCBS (CAS: 4979-32-2)
Environmental Agency Japan 1994	D. magna OECD 202 (1984)	no	static, 24 h	no data	EC50 > 1000mg/L (n)	3 (only 24 h exposure, no analysis)	DCBS (CAS: 4979-32-2)
Long-term toxicity to invertebrates							
National Institute of Technology and Evaluation 2001; OECD SIDS Initial Assessm. Report	D. magna OECD 211	yes	semi-static 21 d	0.00134, 0.00265, 0.00589, 0.0141, 0.0331 mg/l (m)	NOEC ≥ 0.033 mg/L (m) Basis for effect: reproduction	2	DCBS (CAS: 4979-32-2)

Author	Test organism Guideline	Analysis	Performance, Duration of exposure	Concentration	Effect value (Information about measurement)	Reliability	Test substance
2004							
Environmental Agency Japan 1994	D. magna OECD 202 (extended)	no	semi-static 21 d	no data	NOEC 10 mg/L (n), LOEC 18 mg/L (n), EC50 40 mg/L (n)	4 (data lacking)	DCBS (CAS: 4979-32-2)
Toxicity to algae							
National Institute of Technology and Evaluation 2001; OECD SIDS Initial Assessment Report 2004	P. sub-capitata OECD 201	yes	Limittest, 72 h	0.04 mg/L (n), 0.012mg/L (m)	ErC50>0.012mg/L (m), NOErC ≥ 0.012mg/L (m)	2	DCBS (CAS: 4979-32-2)
Ueda et al. 1992	P. sub-capitata OECD 201	no data	72 h	no data	EC50 16mg/L NOEC 10mg/L	4 (data lacking)	DCBS (CAS: 4979-32-2)
In-vitro tests Additional information							
He, Guochun et al. 2011	Mouse hepatoma clonal cell-lines and see column 'concen-tration'		Incubation duration: 4h	Effect on the aryl hydrocarbon receptor is described. Gene expression after stimulation by test substances was carried out with recombinant mouse hepatoma clonal cell-lines	MBT: partial aryl hydrocarbon receptor agonist. At 100µM it showed half of the activity of 1nM TCDD. OBT was more efficacious. At 4µM the activity was 50% of the activity of TCDD. DCBS: low activity MeSBT: no activity	2 (no test guideline but well documented)	DCBS, MBT, OBT (2-hydroxy-Benzothiazole; metabolite from MBT), MeSBT (2-methyl-Thiobenzo-Thiazole; another metabolite of DCBS)

Author	Test organism Guideline	Analysis	Performance, Duration of exposure	Concentration	Effect value (Information about measurement)	Reliability	Test substance
				(contain a luci-ferase plasmid). Gene expression was measured by luziferase activity.			
He, Guochun et al. 2011	Guinea pig hepatic cytosol and see column ' concen-tration'		Incubation duration: 2 h	Substance concentration in each case: 200µM	Stimulation of AhR (aryl hydrocarbon receptor) transformation and DNA binding; DCBS formed approx. 40% of amount formed by 20nM TCDD. MBT formed approx. 50% of 20nM TCDD. OBT formed approx. 40%.	2 (no test guideline but well documented)	DCBS, MBT (2-Mercapto-benzothiazole), OBT (2-hydroxy-Benzothiazole, metabolit from MBT), MeSBT (2-methyl-Thiobenzo-Thiazole, another metabolite of DCBS)
Kusakabe et al. 2002	Chinese hamster lung cells (CHL/IU)		exposure 48 h	5 mg/mL	DCBS induced polyploid cells at the frequency 6%, however with significant difference from controls ($p < 0.01$)	2	DCBS (CAS: 4979-32-2)

6.1.1.1 Fish

6.1.1.1.1 Short-term toxicity to fish

The short-term toxicity to fish was determined in three tests with DCBS. The first study (National Institute of Technology and Evaluation, 2001; OECD SIDS 2004) was a semistatic limittest with 96 h duration and reliability Klimisch 2. The test was performed according OECD TG 203. The nominal concentration was 0.04 mg/L. All fish (O.latipes) survived at the measured concentration of 0.033 mg/L. Therefore the LC50 was > 0.033 mg/L.

The second test (TL 1988) was a static limit test with nominal concentration of 1000 mg/L. The filtrate was used for exposition and DOC measured. No effects occurred; therefore the NOEC for D. rerio was 15 mg/L DOC. The test was stated with Klimisch 4.

The third test (Ueda, 1992; EA Japan, 1994) was also stated with Klimisch 4, no analysis was done, concentration was far above the solubility and a high amount of solvent was used. Nevertheless the LC50 for O.latipes was > 1000 mg/L.

Summary for short-term toxicity to fish:

The LC50 value for DCBS is higher than the T-screening value of 0.1 mg/L.

6.1.1.1.2 Long-term toxicity to fish

There are no data available for DCBS.

MBT (2-mercaptobenzothiazole, CAS 149-30-4) was used by the registrants as read across substance to DCBS for this test. MBT represents only a part of the whole DCBS molecule (DCBS consists of the parts 2-mercaptobenzothiazole and Dicyclohexylamine (CAS 101-83-7)) and the other moiety Dicyclohexylamine showed also considerable toxicity in different chronic ecotoxicity tests. Furthermore, also the physicochemical properties of MBT deviate significantly from DCBS. So is MBT a very well soluble substance (118mg/L) and DCBS has a very low solubility (1.9µg/L). A read-across for such different substances is not possible. For these reasons it is not possible to use MBT as a read-across substance.

Summary for long-term toxicity to fish:

There are no data available for DCBS.

6.1.1.2 Aquatic invertebrates

6.1.1.2.1 Short-term toxicity to aquatic invertebrates

The effect on acute toxicity to Daphnia magna was determined in a semi-static test (National Institute of Technology and Evaluation, 2001; OECD SIDS, 2004) at the duration of 48h according OECD TG 202. Up to the concentration of 0.031 mg/L no immobilisation of Daphnia occurred. Consequently, the EC_{50} is > 0.031 mg/L.

Another test (Environmental Agency Japan, 1994) was done with exposure of Daphnia for 24 h. No analysis was done and very high test concentrations were used. As information about used concentrations was not shown and the testing duration was only 24h, the test is stated with Klimisch 3. At higher concentrations (560 and 1000 mg/L) effects occurred, but distinction between physical and toxicological effects was not possible. The EC_{50} was > 1000 mg/L.

Summary for short-term toxicity to aquatic invertebrates:

No effects appeared in the acute Daphnia test with DCBS. Consequently, the EC_{50} is higher than 0.031mg/L (maximum solubility in test medium).

6.1.1.2.2 Long-term toxicity to aquatic invertebrates

A chronic test (National Institute of Technology and Evaluation, 2001; OECD SIDS, 2004) on D. magna was conducted according OECD guideline 211, concentrations were measured; the test type was semi-static and the duration 21 d (Klimisch 2). At the highest concentration of 0.033 mg/L no effects occurred, therefore the NOEC is ≥ 0.033 mg/L.

Another test (Environmental Agency Japan, 1994) with duration of 21 d similar to OECD guideline 202 (extended) was conducted. The test was assessed with Klimisch 4 due to insufficient documentation. The test type was semi-static and concentrations were not measured. The exposure concentration was extremely higher than the water solubility. The NOEC was 10 mg/L and the LOEC 18 mg/L. The 21d-EC50 (immobility): 140mg/L. Physical effects cannot be ruled out.

Summary for long-term toxicity to aquatic invertebrates:

DCBS does not fulfil the T-criterion as there are no effects up to solubility limit in the test medium (NOEC ≥ 0.033 mg/L).

6.1.1.3 Algae and aquatic plants

The toxicity of DCBS to algae was examined in a limit test (National Institute of Technology and Evaluation, 2001; OECD SIDS, 2004) according to OECD guideline 201. The tested organism was P. subcapitata. No effects were determined and therefore the NOEC based on growth rate was >= 0.012 mg/l and the EC50 > 0.012 mg/L. The test was assessed with Klimisch 2.

Another test (Ueda, S. et al., 1992; EA Japan, 1994) for the determination of toxicity of DCBS to algae exists. For this test also P. subcapitata was used, concentrations were not analysed, no data about concentrations exists at all. The test was assessed with Klimisch 4. The NOEC based on biomass was 10 mg/L and the EC50 16 mg/L.

Summary for toxicity to algae:

No effects appeared during exposure to DCBS up to solubility limit in the test medium. So the NOEC is ≥ 0.012 mg/L (Klimisch 2). The T-criterion is not fulfilled. Another test with DCBS was assessed with Klimisch 4 and showed higher effect concentrations (NOEC 10 mg/L).

6.1.1.4 Sediment organisms

Sediment tests were not conducted by the registrants because the risk assessment based on the equilibrium partitioning method does not indicate a concern for the relevant compartment (PECsediment/PNECsediment ratio < 1).

6.1.1.5 Other aquatic organisms

6.1.2 Calculation of Predicted No Effect Concentration (PNEC)

PNECs were not calculated as they are not relevant for PBT and vPvB evaluation.

6.2 Terrestrial compartment

There is only additional information on toxicity to rats and two in-vitro studies in section 7.2.1.4.

The other sections were not evaluated.

6.2.1 Toxicity test results

6.2.1.1 Toxicity to soil macro organisms

6.2.1.2 Toxicity to terrestrial plants

6.2.1.3 Toxicity to soil micro-organisms

6.2.1.4 Toxicity to other terrestrial organisms

Cf. Chapter 5.2.1 for an assessment of the available *in vivo* data from repeated dose studies on rats.

***In vitro* studies**

Mouse hepatoma clonal cell-lines and Guinea pig hepatic cytosol (DCBS and MBT and other benzothiazoles)

A publication by He et al. (2011) describes the effects of DCBS, MBT (2-mercaptobenzothiazole), OBT (2-hydroxybenzothiazole), and other benzothiazoles on the aryl hydrocarbon receptor (AhR).

Firstly the authors conducted cell-based bioassays demonstrating the induction of AhR-dependent reporter gene activity. Analysis of different benzothiazoles for their ability to stimulate AhR-dependent gene expression was carried out using recombinant mouse hepatoma cell-based clonal cell lines (CALUX = chemically activated luciferase expression). The cell lines contain a luciferase plasmid whose luziferase induction varies as a result of differences in the intracellular localization and stability of the luziferase gene product. The luziferase activity was measured in comparison to

TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin) in a concentration of 1nM. The incubation duration was 4h and 24h.

MBT was identified as a partial aryl hydrocarbon receptor agonist. It showed at 100µM approximately 50% of the activity produced by TCDD. OBT was relatively efficacious. It demonstrated a concentration-dependent effect on luziferase activity: At approximately 4 µM the activity was 50% compared with TCDD. The activity at 100µM was more than 100 % activity of TCDD. DCBS had low activity.

A second test (DNA-binding assay) with guinea pig hepatic cytosol was done. There the ability of substances to stimulate AhR transformation and DNA binding was determined. Thereby the amount of formed AhR-DRE [DRE: dioxin responsive element (a piece of DNA); AhR-DRE: the formed complex between AhR (protein) and DRE] was measured. The used concentration for substances was 200µM respectively.

It was shown that DCBS formed about 40% of the amount of TCDD (20nM), MBT formed approx. 50%. Furthermore OBT formed about 40% and CBS nearly 50%. (These values were derived from a chart.) The results give evidence that these substances are able to stimulate AhR transformation and bind to DNA in vitro.

Citation from He et al. 2011: "The ability …to stimulate guinea pig AhR transformation and DNA binding in vitro … was relatively consistent with the mouse cell induction for compounds ....., N,N-Dicyclohexyl-2-benzothiazolesulfenamide, ..., indicating that they can directly activate the AhR….".

Chinese hamster lung cells

Kusakabe et al. 2002: The chromosome aberration (consisting of structural chromosome aberration and polyploidy) testing of industrial chemicals was conducted by Kusakabe et al. (2002). The chemicals were tested using Chinese hamster lung cells (CHL/IU) at an exposure concentration of 5mg/mL (personal communication).

DCBS was determined to cause no structural chromosome aberration but polyploidy at a frequency of 6% at the mid (mean) concentration 0.41mg/mL. The results were significant compared with the controls ($p < 0.01$). The authors concluded that "…these frequencies may not appear to be clearly positive, but lend some difficulty in evaluating the long-term genetic hazard from exposure to these chemicals" (here DCBS). They assumed that substances causing polyploidy could interfere with the mitotic apparatus during cell division (mitose) in the case of numerical aberrations of chromosomes.

Citation from Kusakabe et al. 2002: "N,N-Dicyclohexyl-2-benzothiazolesulfenamide …did not induce structural CA (chromosome aberrations) but did induce polyploid cells at low frequencies (6.0 %...) with significant difference (Fisher's exact probability test, $P < 0.01$) from the number of polyploid cells scored in solvent/vehicle control ….. These frequencies may not appear to be clearly positive, but lend some difficulty in evaluating the long-term genetic hazard from exposure to these chemicals."

Summary for *in vitro* tests:

Mouse hepatoma clonal cell-lines and Guinea pig hepatic cytosol

- A Cell-based bioassay was used to demonstrate the induction of AhR-dependent reporter gene activity: MBT was identified as a partial aryl hydrocarbon receptor agonist. It showed at

100µM approximately 50% of the activity produced by TCDD (reference substance). DCBS showed low activity.
- DNA-binding assay: There was determined the ability of substances to stimulate AhR transformation and DNA binding. Both DCBS and MBT stimulated AhR transformation and DNA binding (DCBS formed about 40% of the amount of TCDD, MBT formed approx. 50%).

Chinese hamster lung cells

- DCBS caused the statistically significant effect polyploidy however at a low frequency. The authors assumed that substances causing polyploidy could interfere with the mitotic apparatus during cell division (mitose) in the case of numerical aberrations of chromosomes.

6.2.2 Calculation of Predicted No Effect Concentration (PNEC soil)

The PNEC was not evaluated.

7 PBT AND VPVB ASSESSMENT

7.1 Assessment of PBT/vPvB properties – Comparison with the criteria of Annex XIII

7.1.1 Persistence assessment

7.1.1.1 Screening Information

Information on hydrolytical degradability of DCBS (4.1.1) indicates that the substance is hydrolytically degradable to a certain degree. However, hydrolysis rates are rather low and do not significantly influence the persistency of DCBS in the environment under relevant environmental conditions (temperature, pH, etc.).

As shown in 4.1.2 screening information on biodegradability is suggesting that DCBS is very persistent according to Annex XIII 3.1.1. However, due to experimental shortcomings, the information seems not to be reliable enough to allow an assessment of the persistency.

7.1.1.2 Assessment Information

In a test according to OECD 307 Aerobic and Anaerobic Transformation in Soil ^{14}C-DCBS did not degrade in 120 days and at 12 °C. Maximum mineralization of DCBS was 3.2 % and the shares of NER were up to 19.4 %. DCBS quickly dissipated from the water compartment by adsorption to the sediment. A single first order model describes half-life best and results in a DT_{50} of 314.8 to 614.5 days depending on the soil considered. Thus, DCBS meets the specifications for the half-life in soil given for very persistent substances in REACH Regulation Annex XIII 1.2.1. Persistence. DCBS is very persistent in soil.

7.1.2 Bioaccumulation assessment

DCBS has a log K_{ow} of 5.95 indicating a high bioaccumulation potential. This is confirmed by available bioconcentration tests using *Cyprinus carpio*. In dependence of the used test concentrations the reliable and lipid normalized (considered) steady-state BCFs ranged between 3663 and 12821 L/kg. As reliable experimental BCF values (8926 and 12821 for test concentration 0.1 and 0.01 µg/l) of DCBS lay above the vB criterion (BCF > 5000) of Annex XIII, the substance fulfills the vB criterion.

7.1.3 Toxicity assessment

With the provided information DCBS does not fulfill the T criterion (NOEC <0.01mg/L for chronic aquatic toxicity).

7.1.4 Summary and overall conclusions on PBT and vPvB Properties

In a test according to OECD 307 Aerobic and Anaerobic Transformation in Soil ^{14}C-DCBS did not degrade in 120 days and at 12 °C. Maximum mineralization of DCBS was 3.2 % and the shares of NER were up to 19.4 %. DCBS quickly dissipated from the water compartment by adsorption to the sediment. A single first order model describes half-life best and results in a DT_{50} of 314.8 to 614.5 days depending on the soil considered. Thus, DCBS meets the specifications for the half-life in soil given for very persistent substances in REACH Regulation Annex XIII 1.2.1. Persistence. DCBS is very persistent in soil.

DCBS has a log K_{ow} of 5.95 indicating a high bioaccumulation potential. This is confirmed by available bioconcentration tests using *Cyprinus carpio*. In dependence of the used test concentrations the lipid normalized considered steady-state BCFs ranged between 3663 and 12821 L/kg. As reliable experimental BCF values of DCBS (lay above the vB criterion (BCF > 5000) of Annex XIII, the substance fulfills the vB criterion.

DCBS is vPvB since it meets the criteria set out in Annex XIII of REACH.

The available toxicity tests of DCBS do not fulfill the T-criterion (NOEC < 0.01mg/L for aquatic toxicity). However, for PBT assessment normally all three trophic levels should be tested (if P and B criteria are fulfilled). There is no test available on chronic toxicity to fish.

8 EXPOSURE ASSESSMENT

8.1 Human Health

8.1.1 Exposure assessment for worker

No further in-depth evaluation of the exposure assessment for workers was conducted following the evaluation of the toxicological intrinsic properties of DCBS. An evaluation of the worker exposure was performed by the eMSCA in the EU-RAR (2008).

8.1.2 Exposure assessment for consumer

8.1.2.1 Overview of uses and exposure scenarios

Only the scenario “Use of tyres and general rubber goods” in the CSR of the lead registrant covers consumer uses.

8.1.2.2 Scope and type of exposure

The lead registrant has stated: “Nevertheless, consumer are not exposed to DCBS for the following reasons:

- During the vulcanization process DCBS is consumed completely and no DCBS remains in the final rubber products
- Beside the fact that DCBS is completely consumed during vulcanisation, it is used in rubber types where consumer contact is unlikely:

The vast majority of the DCBS tonnage is used in the production of tyres. According to information from downstream user organisations, rubber produced with DCBS is found mainly (99.95%) in interior parts of tyres. Even during tyres change (typically twice per year) consumers do not come into contact with this interior parts.

- Lower amounts of DCBS are used in general rubber goods. These rubbers are special high-quality rubbers for industrial applications where consumer contact is not intended.

Hence, consumer exposure to DCBS through the use of tyres and general rubber goods is not expected. This is supported by EU-RAR (2008) which mentioned that no indication of exposure to CBS was found in all relevant databases. CBS is the most widely used representative of the group of sulfenamides as vulcanizer/accelerator through the use of gloves, rubber, toys and household products; therefore consumer exposure is thought to be minimal. “(page 135, CSR dated 2013-10-17)

Based on the statement mentioned above and the following findings no further assessment of consumer exposure was necessary:

- Spin-Database (= SPIN is a database on the use of Substances in Products in the Nordic Countries. The database is based on data from the Product Registries of Norway, Sweden, Denmark and Finland) shows only hints in “adhesives, binding agents, vulcanizing agents” resulting in „probable consumer exposure“.
- No hints of consumer exposure in product register of Germany
- No hints of consumer exposure in product register of Switzerland
- No hints of consumer exposure in the database of the Federal Office of Consumer Protection and Food Safety (BVL) in the monitoring programme by the Federal States (Bundesländer) of Germany

The eMSCA concludes that consumer exposure today is thought to be minimal. This assessment was supported in the past by EU-RAR (2008) too. Therefore no further assessment of consumer exposure during the substance evaluation was necessary.

8.2 Environmental exposure assessment

In the following paragraphs 8.2.1 -8.2.5 a general overview of the different exposure scenarios based on available information in the year 2014 is given. Since the main focus was always on the PBT/ vPvB assessment, environmental exposure has not been evaluated in detail. Information and assumptions underlying environmental exposure assessment for DCBS have been partly updated. It must be concluded that not all request from the decision have been fulfilled. However since at the end of the SEV process further updates have been announced by the registrants, the newly available information in the registration dossier has not been assessed in detail to avoid costly double work. Furthermore DCBS is now proven to be very persistent and very bioaccumulative (vPvB substance) and consequently fulfils the criteria for a SVHC. Consequently, no further information and plausible data to estimate environmental releases is requested and assessed at this stage of the regulatory process.

8.2.1 Scenario 1: Manufacture of the substance (ES 1)

ES 1 in the chemical safety report covers the industrial manufacturing of DCBS for which the Environmental Release Category 1 is assigned.

The annual amount used per site is reported to be greater than 1000 tonnes. Moreover, the registrants assume up to 365 emission days. No further specifications of this data are given to check whether assumptions and input data in this scenario are reasonable. The number of given emission days is not conclusive. According to REACH Guidance R.16.3.2.1 (Industrial setting scenario) for manufacture of substances produced in amounts 1,000-10,000 tonnes 100 release days per year are given as a default value which would lead to higher daily emissions. Therefore, emissions might be higher than assumed by the registrants.
The author of the chemical safety report state DCBS to be mainly released via the aquatic route. Waste water is treated in a physico-chemical treatment plant e.g. by oxidation or in an industrial mechanical-biological sewage treatment plant. However, it is not clear how much of the residual DCBS will be effectively removed since the registrants do not provide any information on the removal efficiency of waste water treatment. Therefore, the amount released to the environment cannot be estimated.
Waste gases are stated to be transferred directly to an incineration plant. The registrants recommend checking emissions from ventilation or working process equipment in order to comply with the

requirements of environmental protection legislation. Furthermore, they point out that in some cases fume scrubbers, filters or engineering modifications to the process equipment are necessary to reduce emissions to acceptable levels. It is not specified what “acceptable levels” exactly means in this context. For concerning risk management measures no removal efficiency is given. DCBS is a potential PBT-/vPvB-substance. In case this will be definitely confirmed, it is not possible to derive a safe level for DCBS emissions.
Concerning waste disposal and treatment the author of the chemical safety report state that organic solvent used for cleaning procedures undergoes incineration in a hazardous waste combustion unit. Furthermore, it is recommended to examine possibilities for reutilisation and instructions for waste disposal (e.g. how to handle uncleaned empty containers) are given. The registrants do not assume environmental exposure during waste treatment and therefore consider it negligible.

No quantitative data on releases and PECs for DCBS itself are given for the manufacturing scenario. Instead the registrants use a read-across approach with CBS (CAS 95-33-0), for which an EU RAR exists (EU RAR 2008). The registrants reason this method by the similar chemical structure and physico-chemical properties of both substances. DCBS and CBS are solid, show low volatility, a water solubility of less than 1 mg/L and a log P_{ow} greater 3. Furthermore, for both substances they assume a relatively fast hydrolysis, although the data are conflicting to some extent.
Both substances are manufactured at the same locations in the EU. Since the production volume of DCBS is stated to be lower than that of CBS, the registrants consider the use of measured release data of CBS as a worst case.
The read-across approach to CBS is acceptable. However, as outlined above at least assumptions for emission days and manufactured amounts should be specified even though operational conditions are stated to be the same for DCBS.
Regarding predicted environmental concentrations the author of the CSR additionally consider concentrations in sewage treatment plants and the aquatic pelagic compartment for the breakdown products of CBS, which are stated to be also relevant for DCBS.
Measured effluent concentrations of sewage treatment plants (90 %ile) are taken from the EU RAR for CBS and account for 0.006 mg/L and less than 0.1 mg/L. With regard to concentrations in the aquatic compartment a PEC regional of 0.011 µg/L and local concentrations of 0.00008 mg/L and <0.00013 mg/L are given in the EU RAR for CBS. The registrants assume the aquatic exposure to DCBS to be lower.
Estimated concentrations in sediments are missing for ES 1. The registrants argue that although the log Pow of 5.95 indicates relevant accumulation potential of DCBS in sediments, it is not expected due to fast hydrolysis. This argumentation is not acceptable since the exposure estimation should be carried out on a precautionary basis and as outlined in chapter 4.1.1.1 hydrolysis rates are rather low and do not significantly influence the persistency of DCBS under relevant environmental conditions.
The registrants exclude the likelihood of releases to soil and groundwater for the manufacturing scenario since sewage sludge is not applied to soil and waste gases are directly transferred to an incineration plant. It seems to be plausible that no exposure will occur by sludge application. However, the EU RAR states for CBS significant amounts of dust being released which might reach the soil by wet and/ or dry deposition. Since operational conditions are stated to be the same this could also be the case for DCBS.
Annual releases to air are reported to be less than 147 kg/a for one site cited in the EU RAR of CBS and for the other one 180 kg/a. For the latter value it is stated that it is updated data from manufacture but it is not specified whether this data is on CBS or DCBS. Finally, the registrants exclude emissions of DCBS to air due to implemented risk management measures; however no removal efficiency is reported.

The registrants assume no risk of secondary poisoning due to fast hydrolysis. As elucidated in chapter 4.1.1.1 hydrolysis rates are rather low and do not significantly influence the persistency of DCBS under relevant environmental conditions.

8.2.2 Scenario 2: Production of tyres and general rubber products (ES 2)

ES 2 covers the use of DCBS for production of tyres and general rubber products.

Four Environmental Release Categories have been assigned to that scenario:
ERC 3 (Formulation in materials),
ERC 5 (Industrial use resulting in inclusion into or onto a matrix),
ERC 6b (Industrial use of reactive processing aids), and
ERC 6d (Industrial use of process regulators for polymerization process)

It is not specified what products are exactly comprised by the term “general rubber products” and it is not differentiated between the amount used for tyre production and that for general rubber products. There is also no information provided on what contribution general rubber products have on the total usage of DCBS.

The annual amount used per site is not given due to several downstream users. However, the assumptions made in the exposure scenarios need to be further specified, not only as input data for possible calculations but also as a conclusive description of the conditions of use. Moreover, up to 365 emission days are reported by the registrants. This does not seem to be realistic for single downstream users.

The registrants list three different process temperatures: storage at room temperature, mixing is performed at temperatures up to 190 °C and curing up to 200 °C.

The registrants state that DCBS is physically bound in the matrix and contained in preparations up to 4 %. It is assumed that during vulcanization DCBS completely breaks down. Nevertheless, it cannot be excluded that the product still contains some residual DCBS or that DCBS will be emitted during the production of tyres and general rubber products.

Environment and waste related measures recommended are similar to that of ES 1. With regard to environmental exposure the registrants have reasoned a read-across approach with CBS as carried out for ES 1.

Except for the predicted environmental concentration in food for secondary poisoning which has been obtained for DCBS by using EUSES, all other PECs are based on CBS. Freshwater sediment shows the highest PEC of 8.97 µg/L (ww) followed by soil with 4.0 µg/L (ww).

8.2.3 Scenario 3: Tyre mounting/ dismounting and handling of technical rubber goods (ES 3)

ES 3 covers tyre mounting, dismounting and the handling of technical rubber goods. The corresponding Environmental Release Category is ERC 11a (Wide dispersive indoor use of long-life articles and materials with low release). The registrants consider DCBS emissions to water, air, and soil negligible which is reasonable since this use is limited to indoor and there are no abrasive processes involved release of DCBS from the articles to the environment is not very likely to occur during that life cycle step.

8.2.4 Scenario 4: Retreading (ES 4)

ES 4 covers retreading processes which the registrants consider similar to the ones identified for the production of tyres and which are assumed to take place under the same conditions. Moreover, the authors of the CSR consider retreading as more relevant for heavy and agricultural vehicles than for car tyres. Environmental Release Category 11b has been assigned to that use.

The concentration of DCBS in preparation and in article is up to 4%. Processes are performed at temperatures between 35 °C and 200°C.

Regarding environmental and waste related measures similar procedures are recommended as for ES1 and ES2. However, rubber "buffing" arises additionally from the retreading process as by-product. It is described as a fine type of crumb which is formed when the residual tread is ground off the old tyre. This crumb will be recycled or incinerated.

For predicted environmental concentrations the author of the CSR use exactly the same values derived from the EU RAR for CBS as for the tyre production scenario ES 2.Since the conditions of use are similar to that of tyre production, the main emission pathways are assumed to be the same. E.g. emissions to wastewater might occur during vulcanization and cleaning processes.

8.2.5 Scenario 5: Use of tyres and general rubber goods (ES 5)

ES 5 addresses the use of tyres and that of general rubber goods.

Following Environmental Release Categories have been assigned to that scenario:

ERC 10a (Wide dispersive outdoor use of long-life articles and materials with low release),
ERC 10b (Wide dispersive outdoor use of long-life articles and materials with high or intended release (including abrasive processing)),
ERC 11a (Wide dispersive indoor use of long-life articles and materials with low release).

Although the registrants consider exposure during use of general rubber goods and plastic goods negligible, it is not reasonable to summarize the listed ERCs in a single exposure scenario since the use categories include different emission factors.

As outlined in 8.2.2, there is only little information what "general rubber" products are. The article categories indicate uses in vehicles (AC 1), machinery, mechanical appliances, electrical/ electronic articles (AC 2), electrical batteries and accumulators (AC3) and rubber articles (AC 10).
Moreover, there is no information on what contribution general rubber products have on the total usage of DCBS.
The registrants assume the concentration of DCBS in articles to be 0% due to complete consumption during vulcanization. However, a concentration of 0% in tyres does not seem to be plausible when it is referred to a concentration of DCBS in preparations of up to 4% in ES 2 (Production of tyres and general rubber products) and ES 4 (Retreading), especially when process temperatures do not exceed 200 °C but according to the registrations the decomposition temperature of DCBS accounts for ≥ 300°C at 1013 hPa. It cannot be ruled out that residues of DCBS will be still contained in tyres and will be potentially released to the environment via abrasion during use and the following processes in the environment (leaching, degradation of particles, etc.). As already stated before, even the registrants did not perform exposure estimations for DCBS itself, the assumptions and (possible) input data need to be plausible. Moreover, a concentration of 0 % is not a worst case consideration.

The registrants assumed 400 tons/year of tyre wear particles to be released within the EU. Exposure, which the registrants relate to the transformation products of DCBS, occurs due to abrasion from tyres, whereas exposure during use of general rubber goods and plastic goods is considered negligible. Because the registrants expect DCBS to be completely consumed during vulcanization, PECs are only available for the breakdown products.

Regarding waste disposal used tyres are stated to be incinerated or recycled. Since recycled rubbers from waste/used tyres are regarded as a new product emission scenarios from uses of recycled rubber are not covered.

8.2.6 Likely routes of exposure and monitoring data

Overall, it is not possible to estimate environmental releases based on the data available in the exposure scenarios (8.2.1-8.2.5).

Emissions of DCBS to different environmental compartments may occur during different life cycle steps and processes.

For the exposure scenarios “manufacture of the substance”, “production of tyres and general rubber goods” as well as “retreading” included in the CSR it is stated that DCBS is mainly released via the aquatic route due to operational condition. Emissions into wastewater may e.g. arise during vulcanisation processes or cleaning processes (OECD ESD 2004). After being released to water it is very likely that DCBS adsorbs to sediment. This assumption is supported by distribution modelling (Mackay fugacity model I) where it is assumed that sediment is the main target compartment with 49.72 % (TL 2010b).

Soil is the other major target compartment for DCBS with 49.17 % (TL 2010b). However, for industrial uses this does not seem very likely since the registrants state that sewage sludge is not applied to soil but incinerated or disposed of according to national waste regulation. Emissions to soil are more likely to arise by the use of tyres which is considered wide dispersive. Although the registrants assume that DCBS will be completely consumed during vulcanization, it cannot be excluded that the tyres still contain residues of DCBS. Abrasion processes in combination with surface runoff could also lead to soil contamination.

Air emissions in form of dust could also lead to soil contamination by deposition, although this is not considered relevant by the registrants due to existing operational conditions and risk management measures. However, the EU RAR states for CBS significant amounts of dust being released which might reach the soil by wet and/ or dry deposition. Since operational conditions are stated to be the same this could also be the case for DCBS.

Monitoring data

The provided monitoring information from Japan on "Chemicals in the Environment" published by the Japanese Ministry of the Environment (MOE 1998, MOE 2009, MOE 2010) is not suitable to demonstrate the absence or no significant exposure. It is not reasonable to use this data according to Annex XIII of REACH for the following reasons:

In general, no information is available on environmental releases (release pathways and amounts and temporal course of releases). Moreover, environmental conditions in Japan are not directly transferable to that in Europe.

In more detail, in 1998, 39 water and bottom sediment samples were analyzed for DCBS. In the study carried out in 2009, 69 surface water samples (MOE 2009) and in 2010, 87 sediment and 33

biota samples (MOE 2010) were analyzed. DCBS was not found in any of those samples. However, the number of detection/ sampling stations ranged from 11-29. For the purpose of monitoring, sample size and amount of detection/ sampling stations are not representative to rule out persistency, especially when no information on environmental releases is available. Investigations on soil samples were not conducted. Therefore, soil as one of the main target compartments was not considered.

Overall, no proof for the absence of persistence is given if the substance of interest was not found in monitoring studies since other emission pathways or sinks might be relevant.

In addition, there is information available showing DCBS to be found in landfill effluents (18 and 19 ng/L) and in one deposition sample (53 ng/m² day) (Brorström-Lundén et al 2011). Although analytical recovery of the deposition sample was low the findings indicate that emissions of DCBS might occur.

9 RISK CHARACTERISATION

9.1 Human Health

Not available. Without consumer exposure, no risk characterization was necessary for DCBS.

9.2 Environment

Not available. For PBT and vPvB substances a safe concentration in the environment cannot be established with sufficient reliability for an acceptable risk to be determined in a quantitative way. Consequently a risk characterization cannot be performed. Respective risk management measures have to be implemented in order to minimise exposure and emissions.

10 REFERENCES

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