CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Dibutyltin oxide

(DBTO)

EC Number: **212-449-1**

CAS Number: **818-08-6**

Index Number: **-**

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Version number: 02 **Date: August 2020**

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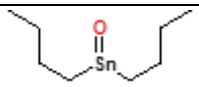
ABBREVIATIONS

DBTA	Dibutyltin di(acetate)
DBTC	Dibutyltin dichloride
DBTE	Dibutyltin bis(2-ethylhexanoate)
DBTL	Dibutyltin dilaurate
DBTM	Dibutyltin maleate
DBTO	Dibutyltin oxide
DBTP	Dibutylbis(pentane-2,4-dionato-O,O´)tin
GC-MS	Gas chromatography–mass spectrometry
GC-FPD	Gas chromatography-Flame Photometric Detector
HCL	Hydrochloric acid
HCE	Human corneal epithelial cells
HPLC-UV	High-Performance Liquid Chromatography-Ultraviolet
MTT	[3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide;
NMR	Nuclear magnetic resonance
PNDT	Prenatal developmental toxicity
SIDS	Screening Information Dataset
TSCA	Toxic Substances Control Act

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	dibutyl(oxo)stannane
Other names (usual name, trade name, abbreviation)	di-n-butyltin oxide dibutyloxostannane, dibutyloxide of tin, dibutyloxotin, dibutylstannane oxide, dibutylstannium oxide, stannane, dibutyloxo- DBTO
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	212-449-1
EC name (if available and appropriate)	dibutyltin oxide
CAS number (if available)	818-08-6
Other identity code (if available)	--
Molecular formula	$C_8H_{18}OSn$
Structural formula	
SMILES notation (if available)	[Sn](CCCC)(CCCC)=O
Molecular weight or molecular weight range	248.9392
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	>= 92 - <= 100 % (w/w)[1]

[1] *according ECHA dissemination site, https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14790/1 accessed 08/2020*

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
Dibutyltin oxide EC 212-449-1		-	Acute Tox 3, H301 Skin Irrit 2, H315 Skin Sens 1, H317 Eye Dam 1, H318 Muta 2, H341 Repr 1A, H360 STOT SE 1, H370 (thymus) STOT RE 1, H372 (thymus) Aquatic Chronic 2, H411

Concentration ranges of the constituent vary between registrants. Impurities are confidential and not relevant for this dossier which refers to the pure substance.

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Not relevant				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not relevant					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5: Proposed harmonised classification

	Index No	Chemical Name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	-	-	-	-	-	-	-	-	-	-	-
Dossier submitters proposal	TBD	Dibutyltin oxide	212-449-1	818-08-6	**Add:** Muta. 2 Repr. 1B Acute Tox. 3 STOT RE 1 Skin Corr. 1 Eye Dam. 1	**Add:** H341 H360FD H301 H372 (immune system) H314 H318	**Add**: GHS08 GHS06 GHS05 Dgr	**Add:** H341 H360FD H301 H372 (immune system) H314		**Add**: oral: ATE =172 mg/kg bw	
Resulting Annex VI entry if agreed by RAC and COM	TBD	Dibutyltin oxide	212-449-1	818-08-6	Muta. 2 Repr. 1B Acute Tox. 3 STOT RE 1 Skin Corr. 1 Eye Dam. 1	H341 H360FD H301 H372 (immune system) H314 H318	GHS08 GHS06 GHS05 Dgr	H341 H360FD H301 H372 (immune system) H314		oral: ATE =172 mg/kg bw	

Table 6: Reason for not proposing harmonised classification and status under standard consultation

Hazard class	Reason for no classification	Within the scope of consultation
Explosives	*hazard class not assessed in this dossier*	No
Flammable gases (including chemically unstable gases)	*hazard class not assessed in this dossier*	No
Oxidising gases	*hazard class not assessed in this dossier*	No
Gases under pressure	*hazard class not assessed in this dossier*	No
Flammable liquids	*hazard class not assessed in this dossier*	No
Flammable solids	*hazard class not assessed in this dossier*	No
Self-reactive substances	*hazard class not assessed in this dossier*	No
Pyrophoric liquids	*hazard class not assessed in this dossier*	No
Pyrophoric solids	*hazard class not assessed in this dossier*	No
Self-heating substances	*hazard class not assessed in this dossier*	No
Substances which in contact with water emit flammable gases	*hazard class not assessed in this dossier*	No
Oxidising liquids	*hazard class not assessed in this dossier*	No
Oxidising solids	*hazard class not assessed in this dossier*	No
Organic peroxides	*hazard class not assessed in this dossier*	No
Corrosive to metals	*hazard class not assessed in this dossier*	No
Acute toxicity via oral route	Acute Tox. 3, H301	Yes
Acute toxicity via dermal route	*data conclusive but not sufficient for classification*	Yes
Acute toxicity via inhalation route	*data lacking*	No
Skin corrosion/irritation	Skin Corr. 1, H314	Yes
Serious eye damage/eye irritation	Eye Dam. 1, H318	Yes
Respiratory sensitisation	*data lacking*	No
Skin sensitisation	*hazard class not assessed in this dossier*	No
Germ cell mutagenicity	Muta. 2, H341	Yes
Carcinogenicity	*hazard class not assessed in this dossier*	No
Reproductive toxicity	Repr. 1B, H360 FD	Yes
Specific target organ toxicity-single exposure	*data conclusive but not sufficient for classification*	Yes
Specific target organ toxicity-repeated exposure	STOT RE 1, H372 (immune system)	Yes
Aspiration hazard	*hazard class not assessed in this dossier*	No
Hazardous to the aquatic environment	*hazard class not assessed in this dossier*	No
Hazardous to the ozone layer	*hazard class not assessed in this dossier*	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Dibutyltin oxide (DBTO) has no harmonized classification and labelling.

In 2006 the European Chemicals Bureau´s Technical Committee on Classification and Labelling (TC C&L) accepted the industry proposal to classify DBTO for Repr. Cat. 2; R60-61, Muta Cat. 3; R68, T; R25-R48/25, Xi; R41 and recommended its inclusion with the next ATP. However, this classification has not been included in the legislation.

This proposal to classify DBTO for Muta. 2; H341, Repr. 1B; H360FD and STOT RE; 1 H372 (immune system) is based on a category approach which is described in detail in Chapter 9.2. The underlying hypothesis is that the substances in the category have the same hydrolytic behaviour and are hydrolysed to dibutyltin dichloride (DBTC) (or derivates thereof). The same toxophor is responsible for the toxicological effects after oral administration.

The category approach has already been used for other category members in order to propose harmonised classification and labelling for Repr. 1B; H360FD, STOT RE 1 and also for Muta 2. [Dibutyltin dilaurate (DBTL), Dibutylbis(pentane-2,4-dionato-O,O´)tin (DBTP), Dibutyltin di(acetate) (DBTA), Dibutyltin bis(2-ethylhexanoate) (DBTE)].

Beside these endpoints the C&L proposal also covers the following endpoints: acute toxicity (oral, dermal route), skin corrosion/irritation, serious eye damage/eye irritation and STOT SE.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

[A.] There is no requirement for justification that action is needed at Community level.

DBTO is a presumed mutagen and reproductive toxicant and therefore fulfils the requirements according Article 36, CLP Regulation.

[B.] Justification that action is needed at Community level is required.

REACH notifiers differ in their self-classification for these endpoints as well as for other human health endpoints. For DBTO wide dispersive use can be assumed and consumer use is registered. Therefore, to ensure a high level of protection, also acute toxicity endpoints as well as effects after repeated exposure have been investigated in addition to mutagenicity and reproductive toxicity.

5 IDENTIFIED USES

Table 7: The following uses are indicated at ECHA dissemination site (accessed October, 2019):

	Use(s)	Technical function
Manufacture	Manufacture of the substance	-
Formulation	Formulation of preparations and articles Industrial use containing substance as catalyst and process regulator PC 1: Adhesives, sealants PC 9a: Coatings and paints, thinners, paint removes PC 21: Laboratory chemicals	Catalyst, process regulator

	PC 23: Leather treatment products PC 26: Paper and board treatment products PC 32: Polymer preparations and compounds PC 34: Textile dyes, and impregnating products PC 0: Other	
Uses at industrial sites	Use as intermediate PC 19: intermediate Industrial use of products containing substance as a catalyst/process regulator PCs see above SU 5: Manufacture of textiles, leather, fur SU 6a: Manufacture of wood and wood products SU 6b: Manufacture of pulp, paper and paper products SU 9: Manufacture of fine chemicals SU 11: Manufacture of rubber products SU 16: Manufacture of computer, electronic and optical products, electrical equipment SU 17: General manufacturing, e.g. machinery, equipment, vehicles, other transport equipment SU 19: Building and construction work Industrial waste disposal by incineration	Catalyst, process regulator
Uses by professional workers	Professional use of products containing substance as a catalyst/process regulator; product categories: adhesives, sealants; coatings and paints, thinners, paint removes PC 1: Adhesives, sealants PC 9a: Coatings and paints, thinners, paint removes SU 19: Building and	Catalyst, process regulator

	construction work Widespread indoor and outdoor use of processing aids in open systems Widespread indoor and outdoor use resulting in inclusion into or onto a matrix PROCs 10, 11	
Consumer Uses	Consumer use of products containing substance as a catalyst/process regulator PC 1: Adhesives, sealants PC 9a: Coatings and paints, thinners, paint removes Widespread indoor and outdoor use of processing aids in open systems Widespread indoor and outdoor use resulting in inclusion into or onto a matrix Widespread indoor and outdoor use of long-life articles and materials with low release	Catalyst, process regulator
Article service life	Industrial use, professional use and consumer use of products containing substance as a catalyst/process regulator AC 1: Vehicles AC 2: Machinery, mechanical appliances, electrical/electronic articles AC 3: Electrical batteries and accumulators AC 5: Fabrics, textiles and apparel AC 6: Leather articles	-

6 DATA SOURCES

The information included in this CLH report originates from the registration dossiers of DBTO and category members (DBTC, DBTL, DBTP, DBTA, DBTE) submitted to ECHA and disseminated on ECHA website [https://echa.europa.eu/de/information-on-chemicals; accessed October 2019].

The following sources for DBTO and category members have been considered:

- OECD SIDS dossier DBTO (OECD, 2008).
- CLH Report for DBTL (ECHA, 2014)
- CLH Report for DBTP (ECHA, 2016)
- RAC Opinion for DBTP (ECHA, 2017)
- CLH Report for DBTA (ECHA, 2019A)
- CLH Report for DBTE (ECHA, 2019B)
- OECD SIDS Initial Assessment Profile for dibutyltin dichloride and selected thioglycolate esters (2006) (OECD, 2006)

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	powder	REACH registration	-
Melting/freezing point	105 °C	REACH registration	differential thermoanalysis method
Boiling point	161.9 °C	REACH registration	differential thermal analysis
Relative density	1.50 g/cm³ at 20 ºC.	REACH registration	From MSDS
Vapour pressure	$4x10^{-6}$ Pa at 25 ºC	REACH registration	OECD Guideline 104
Surface tension	-	-	-
Water solubility	2.55 ± 0.16 mg a.i./L (20°C)	REACH registration	OECD Guideline 105
Partition coefficient n-octanol/water	5.33 (20°C)	-	QSAR
Flash point	-	-	-
Flammability	non flammable	REACH registration	EU Method A.10
Explosive properties	-	-	-
Self-ignition temperature	-	-	-
Oxidising properties	-	-	-
Granulometry	< 10.0 µm = 6.45 x 10^{-2} % < 5.5 µm = 4.89 x 10^{-2} %	REACH registration	OECD Guideline 110
Stability in organic solvents and identity of relevant degradation products	-	-	-
Dissociation constant	-	REACH registration	study technically not feasible
Viscosity	-	REACH registration	study technically not feasible

8 EVALUATION OF PHYSICAL HAZARDS

Not addressed in this dossier.

9 TOXIKOKINETICS AND CATEGORY APPROACH

9.1 Toxikokinetics (absorption, metabolism, distribution and elimination)

Toxicokinetic information supporting the category approach is listed below. The category approach is described in more detail in Chapter 9.2.

The available studies, except the study of Umweltbundesamt (2019), have also been considered for the category member DBTP and have been described previously (e.g. ECHA, 2016, Annex I). Study details are also presented in Annex I to the present CLH report.

Table 9: Summary table of toxicokinetic studies*

Method	Results	Remarks	Reference*
Simulated gastric hydrolysis studies			
Simulated hydrolysis (GC-FPD detection) The dibutyl organotin compounds were individually tested under low pH conditions (0.07 N HCL) at 37°C in order to simulate mammalian gastric conditions. The degree of hydrolysis was studied by determination of DBTC formed after 0.5, 1.0, 2.0 and 4.0 hours, using GC-FPD	DBTO hydrolyzed to 87.3% after 4 hours, with a half-life at 3.5 hours. The formation of DBTM and DBTL to DBTC plus the ligands was rapid. The calculated percentages of hydrolysis were 100.1 % after 0.5 hours for DBTM and 87.8% after 2 hours for DBTL. The half-life of DBTM and DBTL under simulated gastric hydrolysis conditions was < 0.5 hours. The hypothesis was that in the hydrochloric acid solution the tin-ligand bond breaks, leading to formation of the corresponding alkyltin chloride and simultaneous liberation of the ligand.	Reliability 2 (reliable with restrictions) Key study Test material: **DBTO** Purity: 98.2% **DBTL** Purity: 98.2% **DBTM** Purity: 99.65%	Schilt & Zondervan-van den Beuken, 2004 [Annex I, 1.1.1]
Simulated gastric hydrolysis (119 Sn NMR detection), pH 1.2, 40°C Time points: 72 hrs	DBTM is hydrolysed to the dimer distannoxane ClBu2SnOSnBu2Cl under acidic conditions. After 72 hrs the substance was completely hydrolysed to the dimer distannoxane.	Study to support read across Test material: **DBTM** Purity: 95%	Umweltbundesamt, 2019 [Annex I, 1.1.2]
Simulated gastric hydrolysis (^{119}Sn NMR detection), pH 1.2, 37°C	DBTP is rapidly hydrolyzed to the dimeric stannoxane ClBu2SnOSnBu2Cl under conditions representative for the mammalian stomach. After 2 hours almost all DBPT hydrolysed to the dimeric stannoxane, only 2 mol% of DBTC was also detected.	Study to support read across Test material: **DBTP** Purity: >90 %	Naßhan, 2015 [Annex I, 1.1.3]
Simulated gastric hydrolysis (^{119}Sn NMR detection), pH 1.2, 37°C Time points: 30s, 1hr, 4 hrs	DBTC is rapidly hydrolysed to the dimer stannoxane ClBu2SnOSnBu2Cl under gastric conditions. The degree of hydrolysis was reported as approximately 70, 85 and 90 % after 30 seconds, 1 hour and 4 hours respectively (not corrected for trace impurities of tributyltinchloride). The impurity tributyltin chloride remains unchanged during the hydrolysis.	Study to support read across Non-guideline study Test material: **DBTC** Purity: >90 % (Tributyltin chloride	Naßhan, 2016 [Annex I, 1.1.4]

Method	Results	Remarks	Reference*
		(TBTC) was identified as impurity in small amounts)	
Combination *in vitro* and *in vivo* study			
Microsomal metabolism *in vitro* and *in vivo* metabolism in swiss webster mice. *In vitro:* The metabolic fate of dibutyltin acetate was examined in a microsomal monooxygenase metabolism system (MO) derived from either rat or rabbit livers. Also other alkyltins were assessed in the MO system. Concentration tested: 0.003 µmol of [14C]butyltin derivative, 0.5 µmol of unlabeled compound *In vivo:* Groups of mice were gavaged with a single oral dose of 1.1 mg/kg bw with 14C-butyl labelled dibutyltin (di)acetate. The urine and faeces were investigated for metabolites. Tissue levels were also investigated at 138 hours after dosing.	*In vitro*: DBTA was metabolised to dibutyl and monobutyl species. *In* vivo: Data indicate partial absorption of DBTA, faeces contained a proportion of non-metabolised DBTA and dibutyltin derivatives. Extensive cleavage of the tin-carbon bond, with further metabolism of the liberated butyl group to exhaled carbon dioxide and small quantities of butene. Study results show that DBTA is metabolized to unidentified dibutyltin compound and liberation of the acetate moieties, which are further transformed and incorporated into normal cellular metabolism.	Reliability: 2 (reliable with restrictions) supporting study; non-guideline study published in a peer-reviewed journal Test material: **DBTA** Purity: >99%	Kimmel EC, Fish RH & Casida JE, 1977 [Annex I, 1.1.5]
In vivo study			

Method	Results	Remarks	Reference*
Metabolism of DBTC in male Wistar rats *in vivo* Intraperitoneal injection (4 mg/kg bw) Time points: 6-168 hours Samples: blood, urine, liver, kidney, spleen and brain Method: HPLC/MS	The half-life of DBTC in liver, kidney and blood was 3-5 days. DBTC was metabolised to butyl(3-hydroxybutyl)tin dichloride, butyl(4-hydroxybutyl)tin dichloride and butyltin trichloride. Highest concentrations of DBTC were found in the liver and kidneys (compared to brain and blood).	Reliability: 2 (reliable with restrictions) supporting study; non-guideline study published in a peer-reviewed journal Test material: **DBTC**	Ishizaka et al., 1989 [Annex I, 1.1.6]

* Further study details (except Umweltbundesamt, 2019) are provided in Annex I.

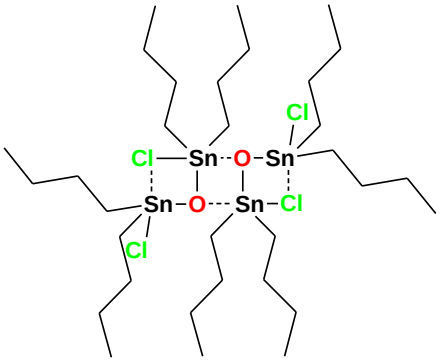
9.1.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification

The category approach (see Chapter 9.2) is predominantly based on the hypothesis that for all substances falling into the same category the same intermediates and metabolites are formed during the metabolism in mammals.

For DBTO only limited toxicokinetic data are available. All studies exept one gastric simulation study (Schilt & Zondervan-van den Beuken (2004)) were carried out with other category members.

In the study of Schilt & Zodervan von den Beuken it is demonstrated that DBTO and category members (DBTL, DBTM) form DBTC under simulated gastric conditions (0.07 N HCL) at 37°C. The degree of the hydrolysis was studied by determination of DBTC formed after 0.5, 1.0, 2.0 and 4.0 hours. DBTO was reported to hydrolyse to DBTC with a half-life of 3.5 hours (87% yield after 4 hours). A faster hydrolysis (half-life < 0.5 hours) was reported for DBTM and DBTL to form DBTC (95% and 87% yields, after 4 hours). The used method to detect and quantify DBTC was GC-FPD, the liberated ligands (maleic acid, lauric acid) were analysed using HPLC-UV and GC-MS, respectively. The findings provide information that the dibutyltin compounds (DBTO, DBTM, DBTL) are converted to DBTC under simulated gastric conditions, however, an unambiguous assignment of the structure of the common metabolites has not been made.

Further *in vitro* gastric hydrolysis studies carried out with DBTM (Umweltbundesamt, 2019), DBTP (Naßhan, 2015) and DBTC (Naßhan, 2016) using the ^{1}H, ^{13}C and ^{119}Sn NMR analytical methods demonstrate that under simulated gastric condition (ph 1.2, 37-40°C) dimer distannoxane $(ClBu2SnOSnBu2Cl)_2$ are formed (see Figure 1). These studies further demonstrate that the same DBTC derivates are formed under acidic conditions.



DBDTC dimer
CAS: 33194-92-2

Figure 1: Dimer distannoxane (ClBu2SnOSnBu2Cl)2

For category members DBTA and DBTC *in vivo* studies are available. The *in vivo* study performed with DBTC (Ishizaka et al., 1989), in which male wistar rats received an intraperitoneal injection of 4 mg/kg bw DBTC, indicates that the substance is metabolised to butyl(3-hydroxybutyl)tin dichloride, butyl(4-hydroxybutyl)tin dichloride and butyltin trichloride (detection method HPLC and MS). After 6 hours DBTC was detected in the liver and kidneys, but had been metabolised to some extent. The accumulation of DBTC in brain was slower than in the other organs investigated; the highest concentration was observed after three days and concentrations were lower than those in other organs (approximately one fifth of the concentration found in the liver and kidneys). The half-life of DBTC in the liver, kidney and blood was found to be between 3-5 days. It is suggested that butyl(3-hydroxybutyl)tin dichloride may be formed in the liver. DBTC and butyl(3-hydroxybutyl)tin dichloride are excreted into the bile and may be involved in the induction of biliary and hepatic lesions. The generation of monobutyltin derivatives from DBTC is also shown in microsomal preparations *in vitro* (Kimmel et al., 1977).

A further *in vivo* study has been carried out with DBTA, in which mice were given an oral dose of 1.1 mg/kg DBTA (Kimmel et al., 1977). The results indicate hydrolysis of DBTA, forming of an unidentified dibutyltin compound and also liberation of acetate moieties. These moieties are further transformed and are incorporated into normal cellular metabolism. A non-metabolised DBTA portion and other dibutyltin derivates were found in the faeces. And the study indicates that there is extensive cleavage of the tin-carbon bond.

9.2 Category approach

9.2.1 Category definition and its members

9.2.1.1 Category background

A category for dibutyltin chloride and selected thioglycolate esters has been proposed already in 2006 (OECD, 2006). In the more recent described category approach (ECHA, 2014, 2016, 2019A and B) dibutyl compounds containing labile ligands, e.g. chlorides or carboxylates, are considered together.

However, dibutyltin compounds containing thioglycolate ligands - e.g. dibutyltin bis(2-ethylhexylthioglycolate) DBT(EHTG) - are not anymore included, since recent hydrolysis studies carried out under REACH indicated that distinct hydrolysis behaviour may be associated with the thioglycolate ligands.

In the present category approach the following category members, DBTC, DBTO, DBTM, DBTA, DBTL, DBTP, are included. The category approach has been built up previously in the course of proposals for harmonised classification and labelling based on Regulation No 1272/2008 (CLP Regulation, Annex VI, Part

2) for category members DBTL (ECHA, 2014), DBTP (ECHA, 2016) and more recently for DBTA (ECHA, 2019A, under evaluation) and DBTE (ECHA, 2019B, under evaluation).

The underlying hypothesis is that these category members form identical hydrolysis products. This has been demonstrated by gastric hydrolysis studies.

DBTO is a member of the category. For DBTM a further member of the category a CLH report will be submitted at the same time.

9.2.1.2 Category hypothesis

The category members are chemically comparable since the substances contain a common functional dibutyltin (Bu2Sn-) group. The dibutyltin (Bu2Sn-) group is considered to be the toxic component.

The hypothesis for the category approach is that, following oral administration, substances within the category behave in a similar manner. The compounds will hydrolyse with the generation of DBTC (or derivatives thereof). Thus, systemic exposure will be to the same substance regardless of the substance administered. Therefore it is considered that the systemic toxicity which is due to intermediate compounds is comparable.

9.2.1.3 Applicability domain

Substances with the generic formula Bu2SnL2 (L is a labile ligand) as well as DBTO (shown to form DBTC in gastric simulation studies) are included in the category. Category members have been chosen based on structural similarity and comparable hydrolytic behaviour. Substances with non-labile ligands e.g. DBT(EHTG) are not included. It is noted that more substances than actually listed in the category might be included, however since those substances do not have any toxicological data for the endpoints considered in the CLH proposal the substances have not been considered.

9.2.1.4 List of endpoints covered

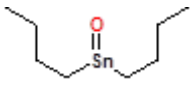
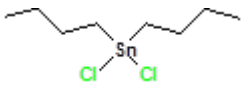
The read-across approach is limited to endpoints where toxicological data generated in experimental animal species *in vivo* by oral administration (e.g., *in vivo* mutagenicity, repeated dose toxicity, reproductive toxicity) are available. It is not applicable to studies using dermal or inhalation exposures or *in vitro* studies. *In vitro* studies in the mutagenicity section have been inserted only as supportive evidence.

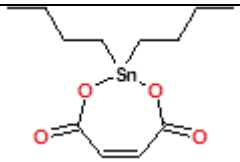
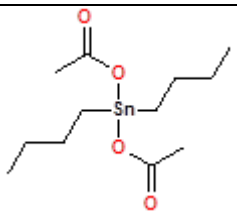
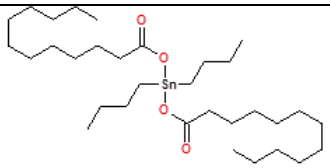
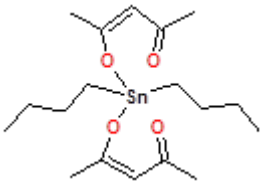
The following CLP hazard classes are covered by the read across: germ cell mutagenicity (see Chapter 9.10), reproductive toxicity (see Chapter 9.12), specific target organ toxicity - single exposure (Chapter 9.13), specific target organ toxicicity - repeated exposure (Chapter 9.14).

9.2.1.5 Category members

The table below summarises the proposed category members: DBTO, DBTC, DBTM, DBTA, DBTL, DBTP.

Table 10: Category members (Bu2Sn-) compounds (adapted from ECHA, 2016)

Substances	CAS	Structure	Purity/Impurity details (REACH dossier)
Dibutyltin oxide (DBTO)	818-08-6		>97.5% No further details (mono-constituent substance)
Dibutyltin dichloride (DBTC)	683-18-1		93-100% (mono-constituent substance) tributyltin chloride (0.25-1%) in some

Substances	CAS	Structure	Purity/Impurity details (REACH dossier)
			sources
Dibutyltin maleate (DBTM)	78-04-6		No further details (mono-constituent substance)
Dibutyltin (di)acetate (DBTA)	1067-33-0		No further details (mono-constituent substance)
Dibutyltin dilaurate (DBTL)	77-58-7		95-100% Mono-constituent substance; potential presence of tributyl(lauryloxy) stannane
Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	22673-19-4		>92% No further details (mono-constituent substance)

9.2.2 Category justification

Chemical similarities - hydrolytic behaviour

Dialkyltin compounds, which contain labile ligands (e.g. chlorides or carboxylates) undergo hydrolysis in aqueous solution with the formation of various oxide/hydroxide species at room temperature. The hydrolysis reactions have been studied previously. Depending on different conditions various reaction products are formed. The partly hydrolysed distannoxane (XR2SnOSnR2X) is frequently detected (Beckmann et al., 2002; Davies, 2004).

The mechanistic pathway is depicted in Figure 2 where the composition at equilibrium will depend on factors such as the medium used and the ionic strength. The reactions are reversible and the equilibria may be shifted by (strong) acids to favour the dimeric/monomeric structures (Davies, 2004; Aylett et al., 1979).

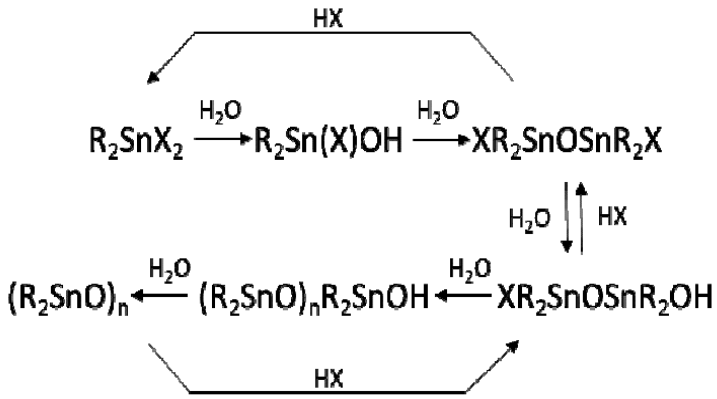


Figure 2. Simplified hydrolysis scheme for dialkyltins (Davies, 2004; Aylett et al., 1979) (reaction scheme as depicted in ECHA, 2016).

An important common property for these substances is the chemical behaviour at low pH. At pH 1-2, under simulated gastric conditions, compounds in the category behave in the same way and rapidly hydrolyse to form the same product.

Schilt & Zonder van der Beuken (2004) reported that DBTO forms DBTC with a half-life of 3,5 hours (87% after 4 hours) under gastric conditions. The category members DBTM and DBTL also formed DBTC. They hydrolysed under simulated stomach conditions very rapidly (half-life < 0.5 hours) (95% and 87%, respectively after 4 hours). DBTC was detected and quantified with GC-FPD using prepared stock solutions of DBTC while the liberated ligands (maleic acid and lauric acid) were analysed using HPLC-UV and GC-MS respectively. The results demonstrate that the substances are hydrolysed and converted to DBTC under gastric conditions, but an unambiguous assignment of the structure of the common intermediate has not been made.

Recent simulated gastric conditions studies using ^{1}H, ^{13}C and ^{119}Sn NMR spectroscopy demonstrate that DBTC and also the category member DBTP (Naßhan, 2015, 2016, cited in ECHA, 2017) and DBTM (Umweltbundesamt, 2019) form distannoxane (dimer) at pH 1.2. Minor amounts of DBTC after the reaction were also also detected (10 mol% study with starting marterial DBTC after 4 hours, 2 mol% with starting material DBTP after 4 hours, and no DBTC with starting material DBTM after 72 hours. The direct analytical method (with much higher substance concentrations) allow in contrast to gastric simulation studies of Schilt & Zonder van der Beuken (2004) a specific assignment of the formed substance.

These observations that distannoxane ClBu2SnOSnBu2Cl dimer is formed is in accordance with the well established chemistry of dialkyltin substances, some of which indicate that DBTC and the distannoxane is in a pH dependant equilibrium (see Figure 3) (Davies, 2004; Aylett et al., 1979).

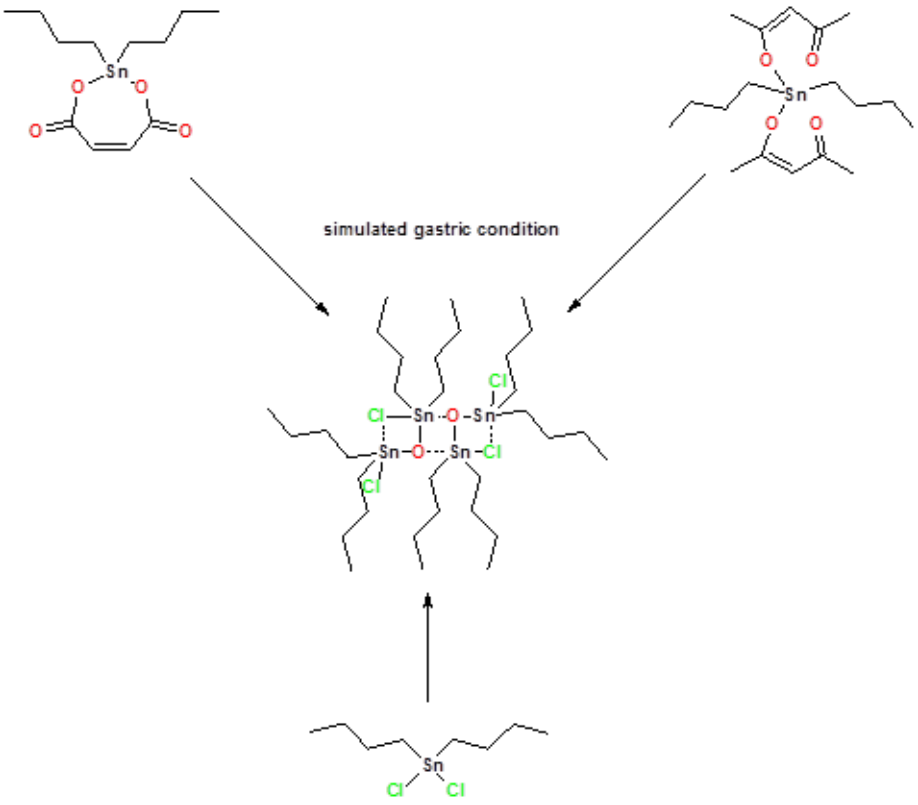


Figure 3. Overview of the hydrolysis of DBTM, DBTP and DBTC as determined in recent studies (Naßhan, 2015, 2016, Umweltbundesamt, 2019), which is in accordance with well established tin chemistry (Davies, 2004; Aylett et al., 1979).

Under neutral condition, however, the water solubility of category members is low according to REACH registration dossiers (ECHAs dissemination site, 2019). According to REACH registration DBTO has a water solubility of 2.55 ± 0.16 mg a.i./L. In the "OECD SIDS Initial Assessment Profile for dibutyltin dichloride and selected thioglycolate esters" (OECD, 2006), it is stated, however, that DBTC, DBTL and DBTM rapidly form oxides/hydroxides in contact with water, as expected due to the lability of the ligands.

The hydrolytic behaviour of the substances in the category (DBTO, DBTC, DBTM, DBTL, DBTA and DBTP) at neutral and low pH supports the category approach and demonstrates that systemic exposure to the intact substances following oral administration is very unlikely.

The category members will hydrolyse under gastric conditions with the generation of DBTC and/or derivatives there of. There are common intermediates at low pH, which may vary depending on the experimental conditions (e.g. solvent, temperature, pH, concentration).

Toxicokinetic and toxicological properties

A key study is the study of Schilt & Zodervan-van den Beuken (2004), in which DBTO and category members (DBTL, DBTM) form under simulated gastric conditions (0.07 N HCL at 37°C) DBTC. A limitation of the study is, that no unambiguous assignment of the structure is possible.

Further gastric simulation studies have been carried out with category members (DBTC, DBTM, DBTP). The used method (^{119}Sn NMR) allows to identify the structure of the DBTC derivates. The studies demonstrate that after hydrolysis of the category members dimeric stannoxanes ClBu2SnOSnBu2Cl are formed.

Data indicate that gastric hydrolysis is expected to be extensive for all substances in the category, therefore following absorption no toxikokinetic differences is expected for category members.

The comparative developmental study by Noda et al. (1993) demonstrates that category members (DBTO, DBTC, DBTA, DBTM and DBTL) have the same toxic effect on the developing foetus, which further stubstantiates that substances have similar toxicokinetic behaviour.

Based on the similar toxicokinetic behaviour it is plausible that upon exposure the same biological targets are effected by all members of the category (i.e. thymus, the developing embryo/foetus, implantation, fertility, genetic material). Comparison of available toxicity data therefore supports the category approach for mutagenicity, reproductive toxicity and STOT SE/RE.

Available data for DBTO are shown in the table below and are compared with data for the other category members in a data matrix (see Table 11).

Classification

Two of the category substances (DBTC and DBTL) are already harmonised classified and included in Annex VI of CLP Regulation. DBTC is further included in the candidate list for SVHC (toxic for reproduction).

A RAC opinion has already been adopted for the category member DBTP (ECHA, 2017) and for DBTA a CLH proposal is currently under evaluation (ECHA, 2019A)

For DBTM a CLH proposal will be submitted by AT at the same time as for DBTO. Self-classification in the REACH dossiers for these substances is comparable to the harmonised classification for DBTC and for DBTL (for those hazard classes assessed by RAC).

It is notable that harmonised or self-classification for mutagenicity (Category 2; H341), reproductive toxicity (Category 1B; H360FD) and for STOT RE (Category 1; immune system (thymus)) is the same for all members in this category. The comparable classifications of category members indicate similar toxicological properties and further support the category justification.

Physicochemical properties

The category members are either solid or liquid at room temperature and pressure.

DBTO is a powder (at 20°C and 101.3 kPA) and is reported to have a melting point of 105°C. The category substances have molecular weights in the range of 304-632 g/mol due to differences in the groups linked to the dibutyltin moiety.

Category substances possess a low water solubility. Physicochemical properties are not critical to the inclusion of substances in the category, but relevant properties are comparable.

9.2.3 Data matrix

Table 11: Summary of phys-chem and toxicological properties of category members (adopted from ECHA, 2016, CLH report of DBTP)

Substance	Dibutyltin oxide (DBTO)	Dibutyltin maleate (DBTM)	Dibutyltin dichloride (DBTC)	Dibutyltin dilaurate (DBTL)	Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	Dibutyltin (di)acetate (DBTA)
CAS no	818-08-6	78-04-6	683-18-1	77-58-7	22673-19-4	1067-33-0
EC no	212-449-1	201-077-5	211-670-0	201-039-8	245-152-0	213-928-8
MW	249	347	304	632	431	351
Physical-chemical properties						
Physical state	Solid	Liquid	Solid	Liquid	Liquid	Liquid
Water solubility	2.55 mg/L	Sparingly soluble	Study technically not feasible. Hydrolysis on contact with water.	Insoluble	Study technically not feasible. Hydrolysis on contact with water.	Insoluble
Hydrolysis, low pH (GC-FPD detection)	Formation of DBTC in gastric simulation studies: 43% in 0.5h, 65% in 1h, 90% in 2h, 87% in 4h	Formation of DBTC in gastric simulation studies: 100% in 0.5h, 97% in 1h, 98% in 2h, 95% in 4h	Not relevant	Formation of DBTC in gastric simulation studies: 82% in 0.5h, 78% in 1h, 88% in 2h, 87% in 4h	No data	No data
Hydrolysis, low pH (119Sn NMR detection)	No data	Formation of ClBu2SnOSnBu2Cl under gastric simulation studies: 100% in 72hrs	Formation of ClBu2SnOSnBu2Cl under gastric simulation studies: ~70% in 30s, ~85% in 1h, ~90% in 4hrs	No data	Formation of $ClBu_2SnOSnBu_2Cl$ under gastric simulation studies: close to quantitative in 2 hours (2 mol% of DBTC also detected)	No data
Toxicological data						
Oral LD50 (mg/kg bw)	172 (121-240)	510 (263-777)	219	2071 (1207-5106)	1864 (1039-3344)	1070
Dermal LD50 (mg/kg bw)	>2000	>2000	No data	>2000	>2000	No data
Skin corrosion/irritatio	Corrosive *in vivo*	Corrosive *in vivo*	Corrosive *in vivo*	Corrosive *in vivo*	Irritant but not corrosive *in vitro.* Corrosive *in vivo*	Corrosive *in vitro*

Substance	Dibutyltin oxide (DBTO)	Dibutyltin maleate (DBTM)	Dibutyltin dichloride (DBTC)	Dibutyltin dilaurate (DBTL)	Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	Dibutyltin (di)acetate (DBTA)
n						
Serious eye damage/eye irriation	Irritant *in vivo*	Serious eye damage *in vivo*	Serious eye damage *in vivo*	Irritant *in vivo*	Serious eye damage *in vitro*	No data
Germ cell mutagenicity	Only Ames test, negative	Only Ames test, negative	Positive *in vivo* somatic cell mutagenicity test, as well as support from positive results from *in vitro* mutagenicity/ genotoxicity tests.	Only Ames test, negative	Only Ames test, negative	Only Ames test, negative
Reproductive toxicity – adverse effects on sexual function and fertility	No data	No data	Large increase in pre-implantation loss in studies in the rat, mouse & monkey	No data, read across	No data, read-across	No data
Reproductive toxicity – adverse effects on the development of the offspring	Characteristic external and skeletal foetal malformations, largely affecting the jaw/skull New prenatal developmental toxicity indicates higher post implantation loss, no malformations detected	Characteristic external and skeletal foetal malformations, largely affecting the jaw/skull	Characteristic external and skeletal foetal malformations, largely affecting the jaw/skull	Characteristic external and skeletal foetal malformations, largely affecting the jaw/skull	No data, read-across	Characteristic external and skeletal foetal malformations, largely affecting the jaw/skull
Repeated dose toxicity	No data	No data	Marked reduction in thymus size & cellularity; similar effects on the spleen and lymph nodes	No data, read-across	No data, proposed read-across	No data, proposed read-across
Harmonised classification	No harmonised classification	No harmonised classification	Acute Tox. 3*, H301 Acute Tox. 4*, H312 Acute Tox. 2*, H330	Muta. 2; H341 Repr. 1B; H360FD STOT RE 1; H372	Adopted RAC opinion (ECHA, 2017)	No harmonised Classification; CLH proposal under

Substance	Dibutyltin oxide (DBTO)	Dibutyltin maleate (DBTM)	Dibutyltin dichloride (DBTC)	Dibutyltin dilaurate (DBTL)	Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	Dibutyltin (di)acetate (DBTA)
			Skin Corr. 1B, H314 Muta. 2, H341 Repr. 1B, H360FD STOT RE 1, H372 (immune system) Aquatic Acute 1, H400 Aquatic Chronic 1, H410	(immune system) Based mainly on read-across from DBTC.	Repr. 1B; H360FD STOT RE 1; H372 (immune system) Based mainly on read-across from DBTC.	evaluation: Repr. 1B, H360FD; STOT RE 1, H372 (immune system) Muta. 2 (H341): Based mainly on read-across from DBTC.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

For the evaluation of acute oral toxicity ten animal studies are available. One recent study was assessed by registrants with reliability of 1. The study of Anonymous (1980b) was rated with a reliability of 2. The others were assigned to a reliability of 4 due to limited documentation and information on the test material. All studies are presented in the following chapter.

Table 12: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 423 (acute toxic class method) GLP	Rat, Sprague-Dawley Females (n=3 per step)	DBTO Oral, gavage Vehicle: corn oil (suspension)	300 and 2000 mg/kg bw Observation: 14d	LD_{50} (f) = 500 mg/kg bw Mortalities: 300 mg/kg bw: 0/6 2000 mg/kg bw: 6/6	Anonymous, 2019
OECD 401	Rat, Tif:RAlf (SPF), N= 5/sex/dose	DBTO Oral, gavage Vehicle: Polyethylene glycol 400	50, 100, 150, 225, 350, and 525 mg/kg Observation: 14d	LD_{50} (m/f) = 172 (121 -240) mg/kg bw Mortalities: 50 mg/kg: 0/10 100 mg/kg: 4/10 (2 m, 2 f) 150 mg/kg: 4/10 (1 m, 3 f) 225 mg/kg: 5/10 (2 m, 3 f) 350 mg/kg: 8/10 (5 m, 3 f) 525 mg/kg: 10/10	Anonymous, 1983
Similar to OECD 401	Rat, Wistar strain albino rats N= 5/sex/dose	DBTO (Thermolite* 15, JCRDY-611K, 4201) Oral, gavage Vehicle: corn oil	180, 250, 350, and 500 mg/kg Observation: 14d	LD_{50} (m/f) = 260 (209-311) mg/kg bw (m/f) Mortalities: 180 mg/kg: 2/10 250 mg/kg: 4/10 350 mg/kg: 7/10 500 mg/kg: 10/10	Anonymous, 1978
-	Rat, Wistar N= 5/sex/dose	DBTO Oral, gavage Suspension in carboxymethyl	250, 350, 500, 700, 1000, and 1400 mg/kg	LD_{50} (m/f) = 487 (294 – 691) mg/kg bw Mortalities: 250 mg/kg: 2/10	Anonymous, 1971

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
		cellulose	Observation: 14d	(2 m) 350 mg/kg: 5/10 (2 m, 3 f) 500 mg/kg: 5/10 (4 m, 1 f) 700 mg/kg: 5/10 (3 m, 2 f) 1000 mg/kg: 8/10 (3 m, 5 f) 1400 mg/kg: 9/10 (5 m, 4 f)	
OECD 401	Rat N= 5/sex/dose	DBTO Vehicle: corn oil	315, 397, 500, 630 or 794 mg/kg Observation: 24d	LD_{50} (m/f) > 794 mg/kg bw Mortality: 630 mg/kg: 1/10	Anonymous, 1980a
OECD 401	Rat, Sprague-Dawley N= 5/sex/dose	DBTO Vehicle: corn oil (10% w/v) Control: Methylcellulose or water, 1 % Methocel®	0, 315, 397, 500, 630 or 794 mg/kg Observation: 24d	LD_{50} (m/f) > 794 mg/kg bw Mortality: 500 mg/kg: 2/10	Anonymous, 1980b
Acute oral toxicity study	Albino rats, female N=1/dose	DBTO 1% (w/v) suspension in aqu. methylcellulose 30% (w/v) suspension in aqu. methylcellulose for two highes dose levels	30, 100, 300, 1000, 3000, 10,000 mg/kg bw	Deaths: 10,000 mg/kg bw 1/1 Normal body weight increase for females of other dose levels	Anonymous, 1975 (OTS0570737)
-	Rat, Tif:RAI N= 5/sex/dose	DBTO (TK-11285) Oral (unspecified) Vehicle: carboxymethyl cellulose	6,000 and 10,000 mg/kg Observation: 7d	LD_{50} (m/f) > 10 000 mg/kg bw	Anonymous, 1972
-	Rat	DBTO Oral, gavage Vehicle: oil solution and Tylosesuspension	No data	LD_{50} (m) = 520 mg/kg bw in oil solution LD_{50} (m) = 800 mg/kg bw In Tylosesuspension	Klimmer, 1969
-	Rat	DBTO, suspended in propylene gycol,	0, 25, 50, 100, 250 mg/kg bw	LD_{50} ~60 mg/kg bw	Anonymous, 1950 (OTS0571954)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
		gavage		Mortalities: 0 mg: 0/6 25 mg: 1/6 50 mg 2/6 100 mg: 6/6 250 mg: 6/6	

10.1.1 Summary and overall relevance of the provided information on acute oral toxicity

For the acute tox class method (OECD 423) three female Sprague-Dawley rats were exposed to a starting dose of 300 mg/kg bw (selected from the fixed dose levels of 5, 50, 300 and 2000 mg/kg bw) via gavage and dose volume was 10 mL/kg bw. No mortaliy, but clinical signs of toxicity were observed. A confirmation was conducted after approximately 48h observation using three more rats (f) administering a single dose of 300 mg/kg bw. Again no mortalities but clinical signs of toxicity were observed. Clinical signs in both steps (diarrhoea 1h-4h after exposure, wet perineum on day 2) were reversible on day 4 of observation. After 48h three animals were exposed to a single dose of 2000 mg/kg bw. A confirmation was conducted using three more female rats after approximately 24 hours of observation by administering a single dose at 2000 mg/kg bw. High dose animals revealed clinical signs like lethargy, diarrhoea, wet perineum and nasal discharge followed by death. Diarrhoea was observed 1-3h after exposure; diarrhoea and wet perineum was observed at 4h check. On day 2 and 3 clinical signs like wet perineum and nasal discharge were observed. On day 4 clinical signs like lethargy, wet perineum and nasal discharge were revealed and one was found dead. On day 5 four animals died and on day 6 the last one died. In dead animals external gross changes like wet perineum (6/6) and internal gross change like stomach haemorrhage (5/6) and autolysis (1/6) were observed. An LD_{50} of 500 mg/kg bw was derived (Anonymous, 2019).

In an OECD 401 study rats were exposed to concentrations of 50, 100, 150, 225, 350, and 525 mg/kg DBTO in Polyethylene glycol 400 via gavage (Anonymous, 1983). 5 male and 5 female animals were used per dose and they were observed for 14 days after administration. Deaths occured on day 1 to 13. All animals in the 525 mg/kg bw group died within five days after dosing. Motalities for all dose groups are presented in Table 13.

Table 13: Rat mortalities after 14day oral exposure to DBTO (Anonymous, 1983).

		50 mg/kg bw	100 mg/kg bw	150 mg/kg bw	225 mg/kg bw	350 mg/kg bw	525 mg/kg bw
males	# deaths	0/5	2/5	1/5	2/5	5/5	5/5
	% deads	-	40	20	40	100	100
females	# deaths	0/5	2/5	3/5	3/5	3/5	5/5
	% deads	-	40	60	60	60	100

Signs of toxicity observed included dyspnoea, exophthalamus, ruffled fur and curved body position. Also slight sedation of inconstant duration occurred in four of the six dose groups (no further details given). Surviving animals recovered within 14 days. Gross necropsy showed dilation of parts of the digestive system (stomach, small and large intestines) in almost all animals dying prior to the end of observation period. Based on the method of Berkson (1944) an LD_{50} (m/f) of 172 mg/kg bw was calculated.

Another study similar to OECD 401 (Anonymous, 1978) derived an LD_{50} of 260 mg/kg bw based on mortalities seen in males and females (see Table 12) using the method of Miller (1944). Common signs of effects during this study included ataxia, red nasal discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy and fecal staining. Gross necropsy showed red nasal and clear oral discharge, urinary

staining of the abdomen, red faecal staining of the abdomen, pronounced blood vessels in the intestines, liver mottled and 10% white, lungs mottled with dark red patches, liver light brown discoloring black patches on liver, chromodacryorrhea, one lobe of the liver yellow, kidneys tan in color.

In the toxicity study by Anonymous (1971) wistar rats were exposed to DBTO concentrations of 250, 350, 500, 700, 1000 and 1400 mg/kg bw. The volume of the test substance administered exceeded 1 mL/100 g body weight for dose levels greater than 500 mg/kg. Signs of toxicity were matted fur, increased abdominal swelling, and cachexia. In addition apathy within one hour of dosing continuing sometimes for several days was observed. Mortalities are shown in Table 12. Spontaneous death occurred on day 4-12 after administration. Necropsy showed hyperemia, bleeding and hemorrhagic erosion of the gastric mucosal glands, gastorenteritis, bleeding in the small intestines, emphysema, and passive hyperemia in lungs. An LD_{50} of 487 mg/kg bw was derived (Probit method).

Anonymous (1980a) exposed in an OECD 401 study 5 animals/sex/dose to 315, 397, 500, 630 or 794 mg DBTO/kg bw. Animals were observed for 24 days. One male rat exposed to 630 mg/kg bw died on day 2 exhibiting weight loss before death. All other animals survived and exhibited body weight gains over the 24 day observation period. Signs of toxicity were soft stool, faecal staining, motor activity decrease and ungroomed or unthrifty appearance. The acute oral LD_{50} of the test material has been considered to be > 794 mg/kg bw under the given test conditions.

In a similar study (Anonymous, 1980b) Sprague-Dawley rats (5/sex/dose) were exposed to 0, 315, 397, 500, 630 or 794 mg DBTO/kg bw. Signs of toxicity were recorded 1, 2 and 4 hours after dosing and daily thereafter for 24 days. Soft stool, faecal staining and an ungroomed or unthrifty appearance were seen in some rats at all dosages. Other signs were noted (not further specified). Most animals at all doses were free of significant in-life observations within 14 days after dosing. Two of ten animals at 500 mg/kg bw exhibited weight loss and died, one male on day 5 and one female on day 6. The majority of animals in this study exhibited body weight gains over the 24 day observation period. The LD_{50} of DBTO was considered to be > 794 mg/kg bw.

In another acute toxicity study (Anonymous, 1975, TSCA submission, OTS0570737) female rats (one per dose) were exposed to 30, 100, 300, 1000, 3000 or 10000 mg DBTO/kg bw. The female in the highest dose group died within the first 22h. Animals from 1000 mg/kg bw upwards showed reactions like hypoactivity, ptosis and ruffeld fur, which were reversible within 22h. At the highest dose in addition muscular weakness and prostration were described.

In a not specified test (Anonymous, 1972) rats (5/sex/dose) were exposed to high concentrations of DBTO (6000 and 10000 mg/kg bw). Animals were observed within 2 hours after treatment and for 5 to 7 additional days. Within 2 hours the rats in both dosage groups showed sedation, dyspnoea, exophthalmus, curved position and ruffled fur. The animals recovered within 5 to 7 days. No mortalities were documented. The study was assessed as non reliable by the registrants.

In a publication by Klimmer (1969) for male rats LD_{50} values of 520 mg/kg bw (in oil solution) and 800 mg/kg bw (in tylose suspension) are stated. No further information is available.

One old study with limited information is documented in TSCA submission (Anonymous, 1950). An LD_{50} of 60 mg/kg bw is reported based on the exposure of 6 animals/dose to concentrations of 0, 25, 50, 100 or 250 mg/kg bw.

LD_{50} values of 683 mg/kg bw and ~500 mg/kg bw for rats are reported in OECD (2008) (secondary source; Yamamoto, 1992 and Worden, 1957). An LD_{50} value in the range of 310-600 mg/kg bw is also reported for mice (OECD, 2008 - Yamamoto, 1992).

10.1.2 Comparison with the CLP criteria

According to Table 3.1.1 of Regulation (EC) No. 1272/2008 a substance shall be classified as

- Acute Tox 4 (oral) if the LD_{50}/ATE values are > 300 and ≤ 2000 mg/kg bw.

- Acute Tox 3 (oral) if the LD_{50}/ATE values are > 50 and ≤ 300 mg/kg bw.

One recent well reported guideline study (GLP) resulted in an LD_{50} of 500 mg/kg bw (Anonymous, 2019). Two other studies gave LD_{50} values in the same order of magnitude, namely 487 mg/kg bw (Anonymous, 1971) and 520 mg/kg bw (Klimmer, 1969). Anonymous (1983) and Anonymous (1978) report lower LD_{50} values of 172 (121 -240) mg/kg bw and 260 (209-311) mg/kg bw respectively; reporting deficiencies especially on the used test material (physical form of the substance, purity, technical grade) in older studies are highlighted by the registrants. Higher values are also reported in Anonymous (1980a,b) with a value of > 794 mg/kg bw and Anonymous (1971) with a value of > 10000 mg/kg bw. It also has to be noted that for none of the presented studies detailed information on the test substance is availabe.

For evaluation of the most relevant data the guidance on the application of CLP criteria (ECHA, 2017) has been applied. The purity of the substance used in the available studies could not be evaluated due to missing data. The age of animals is only stated in 2 studies (9 weeks in Anonymous, 2019; 7-8 weeks in Anonymous, 1983). The observation period has been 14d or even 24 days for two studies (Anonymous, 1980a and b). Anonymous (1972) had a shorter observation periode of 7 days and Klimmer (1969) did not give any information. The sex of the animals or the strain used did not show any clear trend of specific sensitivity. Also the use of different vehicles showed no trend. Therefore it is recommended to use the lowest available value for classification.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the lowest LD_{50} value of 172 (121 -240) mg/kg bw (m/f) available (Anonymous, 1983) a classification as Acute Tox 3, H301 is indicated.

An ATE value of 172 mg/kg bw has to be assigned.

10.2 Acute toxicity - dermal route

Table 14: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance	Dose levels duration of exposure	Value LD_{50}	Reference
OECD 402 GLP	Rat, Wistar N=5m/5f per dose	DBTO Vehicle: arachis oil	2000 mg/kg bw Semi occlusive, 24h Removal of residual test material with arachis oil	> 2 000 mg/kg bw Dermal reactions (erythema,very slight oedema, haemorrhage of dermal capillaries, small superficial scabs, glossy skin)	Anonymous, 2010a
Acute dermal toxicity study	Albino rabbit N=1/dose	DBTO slurry in 3% (w/v) aqu. methylcellulose	200, 500, 2000 mg/kg bw Abraded skin	> 2 000 mg/kg bw Severely irritating	Anonymous, 1975 (OTS0570737)

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

In the available OECD 402 study (Anonymous, 2010a) a group of five male and five female rats was treated with DBTO at a dose level of 2000 mg/kg (vehicle arachis oil). Approximately 10% of the total body surface area were exposed (semi occlusive) for 24h. The test substance was removed after 24h. No signs of systemic toxicity or deaths were observed. All males showed expected bodyweight gains over the study period. Bodyweight loss was noted in all females during the first week with expected gain in bodyweight during the second week. At the test sites of all animals well-defined erythema and very slight oedema were

noted. In addition the following dermal reactions are documented: haemorrhage of dermal capillaries, loss of skin elasticity and flexibility, small superficial scattered scabs, hardened light brown coloured scab, scab lifting to reveal glossy skin, scab undulating, scab cracking, glossy skin. The LD_{50} was found to be greater than 2000 mg/kg bw.

In another acute dermal toxicity study (Anonymous, 1975) rabbits (one per dose, abraded skin) were exposed to 200, 500 or 2000 mg/kg bw. No animal died and no systemic toxic symptoms were exhibited by any rabbit. The test material was severely irritating. Skin changes at 24h were characterized by red, well defined erythema, moderate to severe edema and second degree burns in all animals. At day 7 red, well defined erythema, mild edema, escharosis and wrinkling were observed. At day 14 escharosis and severe desquamation were documented.

10.2.2 Comparison with the CLP criteria

According to Table 3.1.1 of Regulation (EC) No. 1272/2008 a substance shall be classified as

- Acute Tox 4 (dermal) if the LC_{50}/ATE values are > 1000 and ≤ 2000 mg/kg bw
- Acute Tox 3 (dermal) if the LC_{50}/ATE values are > 200 ≤ 1000 mg/kg bw

For the evaluation of acute dermal toxicity one GLP guideline study is available (Anonymous, 2010a). The second study supports these results. The LD_{50} was found to be greater than 2000 mg/kg bw.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the CLP classification critera and the available studies with an LD_{50}>2000 mg/kg bw no classification for acute dermal toxicity is proposed.

10.3 Acute toxicity - inhalation route

No data available.

10.4 Skin corrosion/irritation

Table 15: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
OECD Guideline 404 GLP	Rabbit, New Zealand White N=6 (3m+3f)	DBTO (98.5 %)	0.5 g/side Duration: 3 min, 60 min – for corrosion testing, occlusive 4h – for irritation testing, semi-occlusive Only 4h exposure: DBTO was moistened with	Corrosion (3min/60 min exposure): no effects Irritation (4h exposure): Erythema score 24h (mean 1.83, max 2) 48h (mean 1.67, max 2) 72h (mean 1.83, max 2) 14d (mean 1.83, max 2), desquamation Not fully reversible within 14 days Edema score	Anonymous, 1994

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
			deionized water Removal: wiped with tap water	24h (mean 1, max 2) 48h (mean 0.17, max 1) 72h (mean 0, max 0) Fully reversible	
Primary skin irritation study	Albino rabbits	DBTO N=3	500 mg un-diluted Exposure 24h Occluded, abraded and intact skin Observation: 24h, 72h	Extremely irritating Third degree chemicals burns (24/48h): 2/3 Second degree chemical burns (24/48h): 1/3	Anonymous, 1975 (OTS0570737)
- GLP	Rabbit N=4 (2m+2f)	DBTO	500 mg / 0.19ml peanut oil Semiocclusive Exposure 3-4h	Irritating Reddening and swelling with induration of the skin in all animals on days 5-9 Induration, demarcation and skin necrosis at days 6-10. No local effects at day 30.	OECD SIDS, 2008 Secondary source

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

In an OECD 404 study (Anonymous, 1994) six rabbits were exposed to 0.5g DBTO/site for an exposure periode (occlusive) of 3 min and 60 min to test possible corrosivity of the substance. There was no evidence of corrosivity. In addition rabbits were exposed to 0.5g DBTO/site, moistened with water (semi-occlusive), for 4h and scored according to Draize. The mean scores are presented in Table 15. No individual animal data are available. DBTO induced slight to moderate erythema and slight edema on skin of all rabbits following the 4h-exposure. All sites had desquamation by day 11. Edema completely subsisted within 72 hours. Very slight to slight erythema were present on all sites at study termination on day 14. The calculated mean (24, 48,72h) scores are 1.78 and 0.72 for erythema and edema respectively.

In the study by Anonymous (1975) three albino rabbits were exposed for 24h to 500 mg DBTO (applied to pre-moistened skin). Two rabbits showed third degree chemical burns at the 24h and 72h observation (abraded and intact skin). One rabbit showed second degree chemical burns. Study authors assigned erythema and edema scores of 4 for all animals at all timepoints resulting in mean scores (24h, 72h) of 4.

In another study, cited from a secondary source (OECD, 2008), 4 rabbits were exposed to 500 mg DBTO under semiocclusive conditions. Effects observed included reddening and swelling with induration of the skin in all animals on days 5-9 and induration, demarcation and skin necrosis at days 6-10. No local effects were seen at day 30.

In dermal acute toxicity studies presented in Chapter 9.4 also irritant effects have been observed. In the study by Anonymous (2010a) rats were exposed to 2000 mg DBTO/kg bw for 24h. At the test sites of all animals

well-defined erythema and very slight oedema were noted. In addition haemorrhage of dermal capillaries, loss of skin elasticity and flexibility, small superficial scattered scabs, hardened light brown coloured scab, scab lifting to reveal glossy skin, scab undulating, scab cracking and glossy skin were described. In the second study (Anonymous, 1975) rabbits were exposed to 200, 500 or 2000 mg DBTO/kg bw for 24h. The test material was severely irritating. Skin changes at 24h were characterized by red, well defined erythema, moderate to severe edema and second degree burns in all animals. At day 7 red, well defined erythema, mild edema, escharosis and wrinkling were observed. At day 14 escharosis and severe desquamation were documented.

10.4.2 Comparison with the CLP criteria

Category 1	Destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least one tested animal after exposure ≤ 4 h
Category 2	In the case of testing six animals a classification for Irritation Category 2 applies if: (1) Mean score of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 4 of 6 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or (2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or (3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

In the available OECD guideline study (GLP) (Anonymous, 1994) six rabbits were used and the mean scores (24, 48, 72h) for erythema and edema were 1.78 and 0.72. Maximum scores for both effects were 2. Erythema were not fully reversible within the observation periode of 14 days as very slight to slight erythema were present on all sites.

However in another study with limited information (Anonymous, 1975) skin burns of grade two and three were described after 24h and 72h. Mean scores for erythema and edema were assigned to be 4.

In two acute dermal toxicity studies with dosing up to 2000 mg/kg bw for 24h severe irritating effects with for example haemorrhage, scrabs, severe edema, second degree burns, escharosis and severe desquamation over time were described.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Exposure to moistened DBTO in an OECD 404 study resulted in slight erythema and edema in skin irritation studies which were not fully reversible within the observation periode of 14 days. Based on these data a classification as Skin Irrit 2, H315 is indicated. However, in a primary skin irritation study and in acute dermal toxicity studies severe irritating effects have been described. A scoring of effects is missing but the effects indicate corrosive properties of the substance.

Based on the available data a classification as Skin Corr 1, H314 is proposed.

10.5 Serious eye damage/eye irritation

Table 16: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
OECD 405 GLP	Rabbit, New Zealand White N=1	DBTO	0.1 ml (93 mg) Test material was not rinsed off	Corrosive cornea opacity score: 0.67 (max 1), not reversible cornea opacity score: 2.67 (max 4), not reversible iris score: 1 (max 1), not reversible conjunctivae score: 2 (max 2), not reversible chemosis score: 2.33 (max 3), not reversible killed for humane reasons on day 14	Anonymous, 2010b
Eye irritation test	Albino rabbit N=3	DBTO	100 mg, undiluted Observation at 1h, 24h, 48h, 72h, 7d, 14d	Extremely irritating	Anonymous, 1975 (OTS0570737)
OECD 405 GLP	Rabbit N=6	DBTO	72 mg, undiluted	Irritating MAS was 19.5/110 at 72h Effects: corneal edema, hypopyon, corneal neovascularization (incl. pannus), scleral vesicles Corneal irritation irreversible at day 21	OECD SIDS (2008) Secondary source

Table 17: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
SkinEthic Reconstituted Human Corneal model (not in line with OECD 492, but similar to SkinEthic™ HCE EIT*) GLP	DBTO	transformed human keratinocytes of the cell line HCE 30mg DBTO applied exposure duration: 10 min, washing cell viability testing after treatment with MTT	Not irritating Viability (%) DBTO: 93.6% Neg. control: 100% Pos. Control: 38.5%	Anonymous, 2010c

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

For the available *in vivo* study (Anonymous, 2010b) the right eye of one rabbit was exposed to 0.1 ml DBTO (equal to 93 mg). Assessment was made approximately 1, 24, 48 and 72 hours following treatment. To assess reversibility additional observations were made on days 7 and 14. The determined Draize scores are presented in Table 18. Scattered or diffuse corneal opacity was noted at 48 and 72 h as well as on day 7. Due to adverse ocular reactions accurate evaluation of the cornea at 14 days was not possible. Blepharitis was noted in the treated eye at 72 h. Off white appearance of the majority of the nictitating membrane and lower conjunctival membrane was noted on day 7 and 14. Pannus formation, over the whole of the cornea, and blood stained discharge were noted in the treated eye at day 14. Accurat evaluation of the cornea and iris at 14d was not possible due to advers occular reactions. The rabbit was killed for humanity reasons on day 14 due to worsening reactions and evidence of irreversibility.

Table 18: Draize scores of the DBTO-exposed rabbit (Anonymous, 2010b).

	1h	24h	48h	72h	Mean score (24/48/72h)	7d	14d
Cornea							
Degree of opacity	0	0	1	1	0.67	1	Adverse ocular reactions, evaluation not possible
Area of cornea involved	0	0	4	4	2.67	4	Adverse ocular reactions, evaluation not possible
Iris	0	1	1	1	1	1	Adverse ocular reactions, evaluation not possible
Conjunctiva							
Redness	2	2	2	2	2	2 (off white appearance)	2 (off white appearance, blood stained discharge)
Chemosis	2	2	2	3 Blepharitis	2.33	3	3
Discharge	2	3	3	3	3	3	3

In a second *in vivo* study eyes of three rabbits were exposed to 100 mg DBTO. DBTO was assigned to be extremely irritating. Results are presented in Table 19. The scoring was done according to Draize (1944) and the MAS (Maximum average score)-system (highest score 110). No further information available. For all three animals a MAS of 110 was documented at 7d and 14d observation. Chemical burns and corrosion are reported for all animals. No reversibility was seen.

Another study, shortly described in an OECD SIDS dossier (2008), investigated the effect of 72 mg DBTO instilled into the eyes of 6 rabbits. The maximum average score was 19.5 (of 110 possible) at 72h. Secondary effects like corneal edema, hypopyon, corneal neovascularization (including pannus) and the formation of scleral vesicles were described. At 21 days corneal irritation in two animals was still present.

Table 19: Eye Irritation study results (Anonymous, 1975) according to Draize (MAS-score)*.

Animal No	effects	1h	24h	48h	72h	7d	14d
1	Cornea	20 (1-4)	#	40 (2-4)	40 (2-4)	80 (4-4)	80 (4-4)

	(opacity-area involved)					blister, vascularization, hypopyon	Corrosion, loss of lenses
	Iris	5	#	10 blanched	10 blanched	10 blanched	10
	Conjunctiva (redness-chemosis-discharge)	16 (2-3-3)	20 (3-4-3), chemical burn	20 (3-4-3), chemical burn, free blood	20 (3-4-3), chemical burn, free blood	20 (3-4-3), chemical burn	20 (3-4-3), chemical burn
	MAS	41	20	70	70	110	110
2	Cornea (opacity-area involved)	20 (1-4)	#	40 (2-4)	40 (2-4)	80 (4-4) blister, vascularization, hypopyon	80 (4-4) Corrosion, loss of lenses
	Iris	5	#	10 blanched	10 blanched	10 blanched	10
	Conjunctiva (R-S-D)	16 (2-3-3)	20 (3-4-3), chemical burn	20 (3-4-3), chemical burn, free blood	20 (3-4-3), chemical burn, free blood	20 (3-4-3), chemical burn	20 (3-4-3), chemical burn
	MAS	41	20	70	70	110	110
3	Cornea (opacity-area involved)	20 (1-4)	#	40 (2-4)	40 (2-4)	80 (4-4) blister, vascularization, hypopyon	80 (4-4) Corrosion, loss of lenses
	Iris	5	#	10 blanched	10 blanched	10 blanched	10
	Conjunctiva (R-S-D)	16 (2-3-3)	20 (3-4-3), chemical burn	20 (3-4-3), chemical burn, free blood	20 (3-4-3), chemical burn, free blood	20 (3-4-3), chemical burn	20 (3-4-3), chemical burn
	MAS	41	20	70	70	110	110

evaluation not possible, large amount of edema

* Cornea score=(opacity x area involved x 5); iris score=value x 5; conjunctivae=(redness + chemosis + discharge) x 2

In an *in vitro* study the SkinEthic Reconstituted Human Corneal model was used to determine the eye irritation potential of DBTO after a treatment periode of 10 min (Anonymous, 2010c). The model consists of transformed human keratinocytes of the cell line HCE that form a corneal epithelial tissue (mucosa), devoid of stratum corneum, resembling, histologically, the mucosa of the human eye. The test is based on the hypothesis that irritant chemicals are able to penetrate the corneal epithelial tissue and are sufficiently cytotoxic to cause cell death (measured by a reduction of MTT (3[4,5dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide). For the main test, triplicate SkinEthic tissues were treated with 30 mg of the test material for 10 minutes. As negative control served triplicate tissues treated with 30 µl of Solution A (not further specified). Triplicate tissues treated with 30 µl of 1% w/v sodium dodecyl sulphate served as positive control. At the end of the exposure period (10 min) each tissue was rinsed. Two per group were taken for MTT loading. The remaining tissues were retained for possible histopathology. Following MTT loading the reduced MTT was extracted from the tissues. After extraction the absorbency of triplicate aliquots of the extracted MTT solution for each SkinEthic tissue was measured. The optical density was measured at 540

nm (OD540). Data are presented in the form of percentage viability (MTT conversion relative to negative controls) in Table 20.

Table 20: SkinEthic Reconstituted Human Corneal model – viability of tissue after treatment (Anonymous, 2010c).

	Mean tissue viability	Mean OD_{540}	Viability (%)
Neg. control	0.844	0.924	100
	1.003		
Pos Control	0.368	0.356	38.5
	0.344		
DBTO, 30 mg	0.829	0.865	93.6
	0.865		

For evaluation the following critera were used:

- relative mean tissue viability ≥60%: the test material was considered to be non irritant
- relative mean tissue viability <60%: the test material was considered to be an irritant

The relative mean viability of the test material treated tissues after a 10 minute exposure was 93.6% and according to the above mentioned criteria DBTO was considered to be not irritating to eyes. However the used test method is not in line with the OECD 492 test guideline (adopted 2015) as duration of treatment was only 10 min, sodium dodecyl sulphate was used as positive control and the post-exposure procedure (rinsing, post-exposure immersion) is not described in detail.

10.5.2 Comparison with the CLP criteria

A substance has to be classified for serious eye damage (category 1) or eye irritation (category 2) according to the following criteria:

Category 1:	A substance that produces: (a) in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or (b) in at least 2 of 3 tested animals, a positive response of: (i) corneal opacity ≥ 3 and/or (ii) iritis > 1,5 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material.
Caegory 2:	Substances that produce in at least in 2 of 3 tested animals, a positive response of: (a) corneal opacity ≥ 1 and/or (b) iritis ≥ 1, and/or (c) conjunctival redness ≥ 2 and/or (d) conjunctival oedema (chemosis) ≥ 2 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material, and which fully reverses within an observation period of 21 days

DBTO elicidated irreversible effects on cornea, iris and conjunctiva in the observation period of 14d in one recent guideline study. Mean scores (24, 48, 72h) were 0.67, 1, 2, 2.33 for cornea opacity, iris, conjunctival redness and chemosis respectively. Worsening of effects over time was observed. Scoring on day 14 was not possible due to adverse ocular reactions. The animal was killed on day 14 due to humanity reasons.

In a second study eyes of three rabbits were exposed to DBTO and to all of them the highest possible scores were assigned on observation day 7 and 14. Chemical burns, corrosion, blister formation and loss of lenses were reported.

Both studies showed delayed effects.

A third study showed irreversible corneal irritation on day 21 and severe secondary effects.

In the available *in vitro* study with a short exposure periode of 10 min DBTO did not reduce viability of transformed human keratinocytes and was therefore assessed to be not irritating.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

Based on the irreversibility of effects seen in rabbits and the worsening of effects over time a classification as Eye Dam 1, H318 is proposed.

DBTO showed corrosive effects in skin irritation studies and is therefore proposed to be classified as Skin Corr. 1, H314 (see Chapter 9.6). According to the CLP guidance (ECHA, 2017) serious damage to eyes is implicit.

10.6 Respiratory sensitisation

No data available.

10.7 Skin sensitisation

Not evaluated.

10.8 Germ cell mutagenicity

Information on germ cell mutagenicity of DBTO and category member DBTC has been retrieved from REACH registration dossier of DBTO and from the CLH dossier of category member DBTA (currently under evaluation (ECHA, 2019A)).

The read across approach is dedicated to the data generated in experimental animal species *in vivo* by oral administration. The read across is not applicable to *in vitro* studies.

In the following tables (Table 21, 22) *in vitro* data of DBTO but also of the read across substance DBTC are summarised. This information is provided as additional information. Study details also of *in vitro* data have been summarised previously (ECHA, 2019A) in the course of the CLH process of category member DBTA. Details of *in vivo* studies are also provided in Annex I of the present CLH report (Chapter 2.1).

Table 21: Summary table of mutagenicity/genotoxicity tests *in vitro* with DBTO and DBTC (DBTO registration, ECHA, 2019A).

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Bacterial reverse mutation assays				
Bacterial reverse mutation assay (gene mutation) (with and without metabolic activation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100, E. coli WP2 uvr A OECD Guideline 471 GLP	DBTO Purity: not indicated on dissemination site	Assay 1: 7, 21, 62, 185, 556, 1667, 5000 µg/plate Assay 2: 1.25, 2.5, 5, 10, 20 µg/plate Cytotoxic concentration: 21-62 µg/plate	**Negative** in absence and presence of S9-mix and with all strains tested - TA 1535, TA 1537, TA 98 and TA 100)	Krul (2002) Key study (ECHA dissemination site, REACH registration dossier DBTO)
Bacterial reverse mutation assay (gene mutation) (with and without metabolic activation) OECD Guideline 471 S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 Pre-dates GLP	DBTC	Doses ranged between 0.5 and 1000 µg/plate in the first test and between 1 and 100 µg/plate in the second test.	**Negative.** The test material did not demonstrate genetic activity in any of the assays	Anonymous, 1979 (ECHA dissemination site, REACH registration dossier DBTC)
In vitro mammalian cell assays				
Mammalian cell gene mutation assay Chinese hamster lung fibroblasts (V79) (gene mutation) (with and without metabolic activation) OECD Guideline 476 GLP	DBTC	Test concentration: -S9: 0.000001 to 0.000060 µl/ml, +S9: 0.00020 to 0.00050µl/ml The test compound was strongly toxic at 0.0005 µl/ml with metabolic activation therefore 0.0005 µl/ml was chosen as the highest final concentration	**Negative.** The test material did not show a mutagenic potential in the HGPRT/V79 gene mutation test neither - nor + S9 mix in two independently performed experiments.	Lang R. and Schmitt R. (1989) Key study (ECHA dissemination site, REACH registration dossier DBTO)
Mammalian chromosome aberration test Human lymphocytes: whole blood culture OECD guideline No. 473 GLP	DBTC	Assays -S9 mix; 1st assay: 0.001 - 3.0 µg/ml; 2nd assay: 0.006 - 0.4 µg/ml. +S9 mix: 1st assay: 0.050 - 7.5 µg/ml; 2nd assay: 0.05 - 3.0 µg/ml	**Positve.** The study indicates a clastogenic potential of the test material in the human lymphocyte test in vitro at cytotoxic concentrations. From the four assays (two assays +S9, two assays – S9), one assay without and one with S9 mix gave statistically significant (P	Reimann R & Gramlich U (1990) Key study (ECHA dissemination site, REACH registration dossier DBTO)

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
			< 0.05) increases in the frequency of chromosomal aberrations at the highest concentrations. The other two assays were borderline negative. The test material was tested up to cytotoxic concentrations (reduction of the mitotic index).	
In vitro lymphocyte toxicity Lymphocytes from Fischer 344 rats No guideline, GLP not specified	DBTC	Test concentration: 9 to 75 µg/mL (without metabolic activation)	**Positive.** The LC50 for lymphocytes as determined by dye-exclusion was approximately 50 µg/ml (0.16 mM). At the same concentration of DBTC, the number of antibody-forming cells (AFC) was reduced to approximately 10 % of the control.	Li AP et al., (1982) (ECHA dissemination site, REACH registration dossier DBTO)
Mammalian cell gene mutation assay Chinese hamster Ovary (CHO) No guideline, GLP not specified	DBTC	Test concentration: 0.05 to 0.3 µg/ml (without metabolic activation)	**Positive.** DBTC induced mutations at the HGPRT gene locus in CHO cells. The LC50 value of DBTC for CHO cells, as determined by cloning efficiency, was approximately 0.35 µg/ml (1.12 µM). The mutant frequency increased with dose up to 0.2 µg/ml (0.66 µM) for DBTC. A decrease in mutant frequency was observed at higher concentrations.	Li AP et al., (1982) (ECHA dissemination site, REACH registration DBTO)

Further information on the *in vitro* mutagenic activity of DBTC have been summarised in the CLH dossier of the category member DBTA (ECHA, 2019A).

Table 22: Summary table of *in vitro* mutagenicity/genotoxicity tests with DBTC (from ECHA, 2019A).

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Breakage of naked λ-DNA (±H2O2) Non-guideline, non-	DBTC	Purchased λ-DNA (0.5 µg, double-stranded) was incubated with DBTC at	**Negative.** DBTC did not induce dsDNA breaks in the presence or absence of	Hamasaki et al., 1995

GLP		37°C for 2 h.	H2O2.	
Bacterial reverse mutation assay Non-guideline, non-GLP	DBTC	Doses ranged between 0.1 and 10 µg/tube.	**Positive** without metabolic activation.	Hamasaki et al., 1993
Bacterial SOS chromotest and rec-assay Non-guideline, non-GLP	DBTC	SOS chromotest (sfi A induction; a SOS system related gene) with E. coli PQ37 and rec-assay with Bacillus subtilis (H17 Rec+ and M45 Rec-).	**Positive** without metabolic activation.	Hamasaki et al., 1992
Condensate formation with DNA Non-guideline, non-GLP	DBTC	DBTC was added to calf thymus DNA to give molar ratios r of 0.48-1.00 (test 1) and 2.40 (test 2), followed by analysis of pellet formation.	**Positive.** DBTC formed pellets (condensates/solid phases) with DNA in both experiments.	Piro et al., 1992
Effect on spindle structure in V79 Chinese hamster cells Non-guideline, non-GLP	DBTC	V79 Chinese hamster cells were treated with 10-8 - 10-4 M DBTC for 30 min at 37°C	**Positive.** In general, loss of stainable spindle could be demonstrated at slightly higher concentrations than c-mitosis (DBTC also induced c-mitosis).	Jensen et al., 1991a
Aneuploidy in human peripheral lymphocytes Non-guideline, non-GLP	DBTC	Human lymphocytes were treated with 10^{-8} - 10^{-6} M DBTC for 48 h. After fixation, 100 metaphases were selected randomly, photographed and the chromosomes were counted.	**Negative.** No significant induction of hyperdiploid cells (aneuploidy) was observed	Jensen et al., 1991b
Effect on spindle-inhibition as chromosomal contractions in human lymphocytes Non-guideline, non-GLP	DBTC	Lymphocyte cultures were exposed to 10^{-9} - 10^{-3} mol dm^{-3} DBTC for 24 h. After fixation, the length of chromosome No. 1 was determined in 100 metaphases.	**Negative.** No effect on average chromosome length was seen in the range of 10^{-9} - 3 x 10^{-7} mol dm^{-3} DBTC versus control. No results were obtained at higher concentrations (≥1 x 10-6 mol dm-3) due to toxicity of treatment.	Jensen et al., 1989

Table 23: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo* with DBTC or DBTL (DBTO registration, ECHA, 2019A)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Micronucleus assay (chromosome aberration) mouse (ICR) male/female oral: gavage OECD Guideline 474	DBTC	2, 10, 50 mg/kg bw (actual ingested); oral single dose Five mice/sex/group were terminated 24, 48 and 72 hours after treatment. (doses selected based on preliminary toxicity test)	**Positive.** A statistically significant increase in the incidence of micronucleated poly-chromatic cells was observed in the bone marrow of mice treated with DBTC at 50 mg/kg bw and killed 48 and 72 hours later ($0.01<p<0.05$): this effect was seen more clearly in females than in males. No effect was apparent for any group killed 24 hours after DBTC treatment.	Anonymous (1991) Key study (ECHA dissemination site, REACH registration dossier DBTO) [Annex I, 2.1.1]
Micronucleus assay (chromosome aberration) mouse (NMRI) male/female oral: gavage, Non OECD guideline, GLP not stated	DBTC	50, 100, 200 mg/kg bw (actual ingested), oral single dose Five mice/sex/group were terminated 24, 48, 72 hours after treatment (no range finding study, dose selection based on acute toxicity tests)	**Negative.** Test material failed to produce any increase in the number of micronucleated polychromatic erythrocytes in male and female mice and so failed to show any evidence of mutagenic potential up to 200 mg/kg bw. After application of the high dose four males and one female died; after application of the mid dose, one male died. More than half of the animals of the two highest dose groups showed signs of toxicity (e.g. apathy, eyelid closure, ruffled fur).	Anonymous (1990) [Annex I, 2.1.2]
DNA damage in rat cerebral cortical cells Single cell gel electrophoresis assay (SCGE, comet assay) was performed Non-guideline, non-GLP	DBTL	0, 5, 10 and 20 mg/kg bw/day for 5 days/week for 7 weeks 10 rats/dose group were gavaged with DBTL (vehicle: corn oil)	**Positive.** A significant and dose-dependent increase in DNA damage was seen in rat cerebral cortical cells. Also other toxic effects such as right parietal cortex cell cycle disturbance and increased apoptosis	Jin et al., 2012 [Annex I, 2.1.3]

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			was observed.	

Table 24: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

With DBTO itself only a bacterial reverse mutation assay according to OECD TG 471 has been carried out. In both the absence and the presence of S9-mix and in all strains, DBTO did not cause any mutagenic effects. This result is in line with results of category members, which do not indicate mutagenic effects in bacterial mutagenicity assay.

For this endpoint a read across (see Chapter 9.2) is justified based on same toxicokinetic behaviour and toxicological effects of category members and therefore data from category members are considered. Relevant data are available with DBTC and DBTL. There are three *in vivo* experiments available (two carried out with DBTC and one study with DBTL).

In a well conducted GLP and guideline conform (OECD TG 474) study DBTC was applied via gavage to male and female mice (ICR) (single dose: 2, 10 and 50 mg/kg bw). A statistically significant increase ($p<0.05$) in the incidence of micronucleated poly-chromatic cells was observed in the bone marrow of mice treated with DBTC at 50 mg/kg bw and killed 48 and 72 hours later. The effect was seen more clearly in females than in males. In the study no effect was apparent in rodents killed after 24 hours (Anonymous, 1991).

In a similar *in vivo* study (a mammalian erythrocyte micronucleus test in mice) in which DBTC was tested up to dose level of 200 mg/kg bw (single dose: 50, 100 and 200 mg/kg bw, gavage), no mutagenic effect were detected in any of the treated groups (Anonymous, 1990). No clear explanation for the distinct findings can be provided. Different mice strains were treated with DBTC and in the second study the GLP status was not identified (similar quality assurance is assumed). Both studies are considered as reliable with restrictions (Klimisch 2).

In the third *in vivo* study in which rats were treated with DBTL (5, 10 and 20 mg/kg bw/day) for 5 days/week for 7 weeks increased DNA damage was seen in rat cerebral cortical cells (Jin et al., 2012). The study is published in a peer reviewed journal but the reporting is low and no guideline has been followed. Thus, the study is considered as not reliable.

Positive *in vitro* mutagenicity and genotoxicity tests of category members (e.g., Li et al. 1982; Reimann and Gramlich,1990, Hamasaki et al., 1993, Hamasaki et al., 1992) have been summarised (ECHA, 2019A). Some of the available assays indicate clastogenicity (Reimann and Gramlich, 1990; Anonymous, 1991) and effects on spindle formation during mitosis (Jensen, 1991a). On the other hand, some *in vitro* mammalian mutagenicity and genotoxicity tests (e.g. Lang and Schmitt, 1989) are not indicating any effect, but overall most studies are positive.

The genotoxic mechanism is presently not known, but has been suggested to involve penta-coordinate organotin-DNA structure formation leading to DNA condensation (Li et al., 1982; Pagliarani et al., 2013), which was shown to occur at high DBTC to DNA ratios (Piro et al., 1992) (as stated in ECHA, 2019A).

The studies performed with DBTC demonstrate variable results for *in vitro* and *in vivo* studies, but overall most studies are positive.

10.8.2 Comparison with the CLP criteria

According to CLP Regulation for the purpose of classification for mutagenicity, substances are allocated to one of two categories (Table 3.5.1., CLP Regulation).

Category 1	Substances known to induce heritable mutations or to be regarded as if they induce heritable mutations in the germ cells of humans Substances known to induce heritable mutations in the germ clls of humans
Subcategory 1A	The classification in Cat. 1A is based on positive evidence form human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans.
Subcategory 1B	The classification in Category 1B is based on: — positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or — positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or — positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people.
Category 2	Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans. The classification in Category 2 is based on: — positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from: — somatic cell mutagenicity tests in vivo, in mammals; or — other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays.

No epidemiological studies are available for DBTO and/or for category members and thus no classification in Cat. 1A is warranted. There is no *in vivo* heritable germ cell mutagenicity tests available, which allows classification into Cat. 1B.

For the classification proposal for DBTO read across to category members (DBTC and DBTL) is applied, for which (in addition to information from *in vitro* cell mammalian studies) *in vivo* studies with laboratory rodents are available. The *in vivo* animal studies are considered for read across to DBTO.

For the category member DBTC there is a well conducted reliable GLP compliant *in vivo* somatic cell mutagenicity test (MN test) available which demonstrates mutagenic properties (Anonymous, 1991). Furthermore, there is evidence from *in vitro* studies that DBTC interacts with the gene material.

Category members DBTC and DBTL are harmonised classified for Muta. 2; H341. Currently there is a CLH proposal under evaluation to classifiy the category member DBTA as Muta. 2 (ECHA, 2019A).

It is justified that DBTO is classified in the same way as category members based on the category approach (as described in Chapter 9.2).

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Based on the mutagenic effects observed with category member DBTC a classification of DBTO as Muta. 2. H341 is warranted.

10.9 Carcinogenicity

Not evaluated in this CLH report.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

For the endpoint sexual function and fertility reference is made to studies with DBTC as part of the category (see details Chapter 9.2). The studies listed in the table below have been either considered in the registration of DBTO and/or in frame of harmonised classification proposal of category members (e.g. ECHA, 2016, ECHA 2019A and B). The studies described below have been already described in the CLH dossier for DBTP (ECHA, 2016) and assessed by RAC in 2017.

Details of individual studies are provided in Annex I of the present CLH report.

An overview of the studies considered relevant for fertility endpoint is listed below.

Table 25: Summary table of animal studies on adverse effects on sexual function and fertility (adopted from ECHA, 2016)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
OECD 421 Reproduction/Developmental Toxicity Screening Test) Wistar rat (12/sex) Oral: feed No significant deviations	DBTC Purity: 98.57% 0, 5, 30, 200 ppm (diet); Administration for four weeks (males) or for two weeks prior to mating and to day 4 or 6 post partum (females).	200 ppm diet: reduced maternal weight gain (values not reported). Reduced litter size (6.0 compared to 11.3); reduced numbers of foetuses (10 compared to 101 in controls). Gestation index: 43% vs 100% (high dose vs control) Post-implantation loss: 87.6% vs 13.4% (high dose vs control). Gross necropsy and histopathology in males did not reveal any effects of treatment on the reproductive tract. NOAEL (for general toxicity) = 5 ppm (0.3-0.4 mg/kg bw/day) (thymus effects) NOAEL(reproduction) = 30 ppm (1.7-2.4 mg/kg bw/d) LOAEL (reproduction) = 200 ppm, (12.0-15.4 mg/kg bw/d)	Unpulished report, 2003 (REACH registration, DBTO) [Annex I, 2.2.1.1]
Wistar rat (16-19 female/group) non-guideline study	DBTC Purity: 97% 0, 3.8, 7.6, 15.2 mg/kg bw/d, GD 0-3 (and GD 4-7) Termination: GD 20	Maternal toxicity at ≥3.8 mg/kg bw/d (clinical signs), weight loss during early gestation at 3.8 (-2 g), 7.6 (-14 g) and 15.2 mg/kg bw/d (-20 g); reduced food consumption (≥3.8 mg/kg bw/d) Increased pre-implantation loss at 7.6 (35.6%) and 15.2 mg/kg bw/d (87.9%), compared to 2.7% in controls. LOAEL =3.8 mg/kg bw/d NOAEL <3.8 mg/kg bw/d	Ema & Harazono, 2000 [Annex I, 2.2.1.2]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
CD1 mouse (12 females/group) non-guideline study	DBTC Purity: 99.5% 0, 7.6, 15.2, 30.4 mg/kg bw/d GD 0-3 (or GD 4-7)	Increased pre-implantation loss at 7.6 (29.7%), 15.2 (34.0%) and 30.4 mg/kg bw/d (58.3%) compared to 9.7% in controls (GD 0-3). Maternal toxicity: mortality, clinical signs, reduced weight gain GD 0-3 (-82%) at 30.4 mg/kg bw/d reduced food consumption) at 7.6 (- 18%), 15.2 (-8%) and 30.4 mg/kg bw/d (- 19%). LOAEL =7.6 mg/kg bw/d NOAEL <7.6 mg/kg bw/d	Ema et al., 2007a [Annex I, 2.2.1.3]

Table 26: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data are available.				

Table 27: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Mechanistic in vivo study Wistar rats (14-15 female/group) 0, 7.6, 15.2 mg/kg bw/day (with and without progesterone) subcutaneous injection of 2 mg progesterone GD 0-8	DBTC Purity: 98%	Investigation of the effects of progesterone on implantation failure.	Administration of progesterone on GD 0-8 offered some protection against implantation failure in Wistar rats treated with 7.6 or 15.2 mg/kg bw/d DBTC on GD 0-3. Pre-implantation losses were 8.6%, 62.8%, 81.3% at dose levels of 0, 7.6, 15.2 mg/kg bw without progesterone ; 10.5%, 25.9% and 60.6% with application of progesteron	Ema et al., 2003 [Annex I, 2.2.1.4]
Mechanistic 0, 3.8, 7.6, 15.2 mg/kg bw Pseudopregnat Wistar rats	DBTC: purity not reported	Investigation of the effects of DBTC on decidual cell response in pseudopregnant rats Uterine weight was used as an index of uterine decidualisation.	DBTC administration (7.6 and 15.2 mg/kg bw/d on GD 0-3 or GD 4-7) reduced uterus weight and serum progesterone levels. Oestradiol levels and corpora lutea numbers were unaffected by treatment. Administration of progesterone reversed the suppression of uterine decidualisation.	Harazono & Ema, 2003 [Annex I, 2.2.1.5]

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

For the endpoint sexual function and fertility reference is made to studies performed with dibutyltin dichloride (DBTC), which is part of the category approach (see details Chapter 9.2). A recently conducted prenatal developmental toxicity (PNDT) study carried out with DBTO according to OECD TG 414 is available which is in more detail described in Chapter 9.12.4 (Unpublished report, 2017).

In a guideline compliant (OECD 421) screening study (Unpublished report, 2003), administration of DBTC in the diet for two weeks prior to mating and to lactation (females) at a concentration of 200 ppm (12.0- 15.4 mg/kg bw/d) caused bodyweight effects in females (reduced weight gain over the pre-mating, gestation and lactation periods, resulting in significantly lower mean body weights at the end of the pre-mating period and during the gestation and lactation periods).

Only 3 of the 7 pregnant females at the highest dose level delivered live offspring. The number of pregnant females in this group (7/12) is lower than controls (9/12); however the numbers of pregnant females in the other treated groups are also low without a dose-response relationship. The gestation index is 43% in the highest dose group vs. 100% in the control group. Corpora lutea numbers were not measured in this study, therefore the extent of pre-implantation loss cannot be assessed.

An effect of treatment on fertility at the highest dose level, however, cannot be totally excluded. The full study report is not available, summary data are taken from the disseminated REACH registration dossier for DBTO, the CLH report for dibutyltin dilaurate (ECHA, 2014) and the CLH report for DBTP (ECHA, 2016). Complete details on the study methodology and findings are therefore not available. Notably, values for maternal bodyweight and also for bodyweight gain are absent from both sources; due to reporting deficiencies the extent of maternal toxicity seen at the highest dietary concentration of 200 ppm cannot be fully assessed.

Beside the OECD TG 421 conform study (Unpublished report, 2003), further studies carried out by Ema et al. (Ema & Harazono, 2000; Ema et al., 2007a, Ema et al., 2003, Harazono & Ema, 2003) are considered relevant for effects on sexual function and fertility. These studies used administration of DBTC during early gestation (prior to implantation). The studies do not fully comply with regulatory guidelines but are sufficiently robust to support the classification proposal as part of a weight of evidence.

In the study of Ema & Harazono (2000) DBTC (3.8, 7.6 and 15.2 mg/kg bw) was administered during very early gestation (GD 0-3) or early gestation (GD 4-7) to Wistar rats. The application of 7.6 and 15.2 mg/kg bw DBTC to rats resulted in a significantly increased level of pre-implantation loss (35.6% and 87.9%, respectively, compared to 2.7% in controls) and a corresponding reduction in the number of pregnant females of 11/16 (in the 7.6 mg/kg bw/d group) and 2/16 (in the 15.2 mg/kg bw/d group) in the GD 0-3 group. Findings were associated with maternal weight loss in all groups on GD 0-4, and in the mid and high dose group on GD 4-20. No effects on corpora lutea were seen.

Administration of 3.8, 7.6 and 15.2 mg/kg bw DBTC during early gestation (GD 4-7) resulted in a higher number of post-implantation loss (13.9%, 39.9% and 91.5%, respectively), accompanied with a reduced litter size (12.6, 9.3 and 1.3, respectively). Findings were associated with maternal weight loss only in the mid and high dose group on GD 4-8. No effects on corpora lutea were seen.

A further study by the same authors was conducted to investigate the effects of DBTC administration on very early and early gestation in CD1 mice (Ema et al., 2007a). The administration of DBTC in CD1 mice during GD 0-3 showed an increase in pre-implantation loss (and a corresponding reduction in the number of pregnant females) following treatment with ≥ 7.6 mg/kg bw/d on GD 0-3. Findings at this dose levels (≥ 7.6 mg/kg bw/d) were associated with maternal toxicity including mortality. A small number of deaths were seen in all treated groups in this study, but not in controls; however the absence of a dose-response relationship (mortality of 0/12, 2/12, 1/12 1/12 at 0, 7.6, 15.2 and 30.4 mg/kg bw/d, respectively) indicates that the deaths of dams may not be directly related to treatment with DBTC. Other signs of maternal toxicity seen in this study were clinical signs, and moderate reductions in food consumption and weight gain.

Pre-implantation loss in mice treated on GD 4-7 with different doses of DBTC was not statistically significant altered. There was an increase in post-implantation loss (4.3% (control group), 48.3% (7.6 mg/kg

bw), 94.4% (15.2 mg/kg bw) and 100% (30.4 mg/kg bw)). Findings were accompanied with reduced weight gain in dams (reduced weight gain on GD 8-18 in all treated groups, on GD 4-8 in mid and high dose group).

In the aforementioned studies in rats and mice no effects on corpora lutea after DBTC administration were detected.

The authors hypothesise that reduced serum progesterone is responsible for the pregnancy failure observed. Serum progesterone levels were significantly lower in mice administered DBTC at 30.4 mg/kg bw/d on GD 0-3 and GD 4-7 of pregnancy (Ema et al., 2007a).

Study outcome of a study in which some protection against the failure of implantation is afforded by the administration of progesterone during early gestation (Ema et al., 2003a) substantiates this hypothesis. Administration of DBTC on GD 0-3 caused a marked increase in pre-implantation loss at 7.6 mg/kg bw/d (62.8%) and at 15.2 mg/kg bw/d (81.3%) compared to controls (8.6%); progesterone treatment reduced the level of pre-implantation loss to 25.9% and 60.0% at 7.6 and 15.2 mg/kg bw/d DBTC, respectively.

Further mechanistic data indicate that DBTC may result in the failure of implantation due to a suppression of the decidual cell response and reduction in circulating progesterone levels (Harazono & Ema, 2003) in the rat. In the study no effect on number of corpora lutea or on serum oestradiol levels were detected.

10.10.3 Comparison with the CLP criteria

According to CLP Regulation for the purpose of classification for reproductive toxicity, substances are allocated to one of two categories (Table 3.7.1(a), CLP Regulation). Within each category, effects on sexual function and fertility, and on development, are considered separately. In addition, effects on lactation are allocated to a separate hazard category.

Category 1	Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).
Subcategory 1A	Known human reproductive toxicant The classification of a substance in Category 1A is largely based on evidence from humans.
Subcategory 1B	Presumed human reproductive toxicant The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.
Category 2	Suspected human reproductive toxicant Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or

	if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

The definition of reproductive toxicity in the CLP Regulation (Annex I: 3.7.1.1) includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. Adverse effects on sexual function and fertility are defined (Annex I: 3.7.1.3) as any effect of a substance that has the potential to interfere with sexual function and fertility including, but not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive system.

Data with category member DBTC clearly show that DBTC causes marked effects on fertility in studies in rats and mice through a reduction of implantations. The effects on post and pre-implantation losses were depended on the GD on which DBTC was applied. Mechanistic data suggest that the increased level of pre-implantation loss may be due to a reduction in circulating progesterone levels, which is also of relevance to humans.

Effects were seen at maternally toxic dose levels, including relatively high dose levels causing marked bodyweight effects, reduced food consumption, signs of toxicity and possible mortality.

However, at lower dose levels, where less marked maternal toxicity is observed, marked increases in the level of pre-implantation loss are still apparent. The data suggest, that the adverse effect on reproduction is not considered to be a secondary non-specific consequence of other toxicity.

Classification of DBTO for reproductive toxicity (adverse effects on sexual function and fertility) in Category 1B (H360F) is considered to be appropriate.

10.10.4 Adverse effects on development

A guideline conform study carried out recently according to OECD TG 414 (Prenatal developmental toxicity study) is available with DBTO (Unpublished report, 2017). Furthermore, with DBTO a non-guideline conform comparative study is available, which also investigates the effects of various category members (DBTC, DBTL, DBTA, DBTM) on reproductive parameters (Noda et al., 1993). All other available studies have been carried out with the category members DBTC or DBTA.

All studies lited below, except the recent prenatal developmental toxicity study (OECD 414) with DBTO, have been considered in the frame of harmonised classification proposals of category members (e.g. ECHA, 2016, ECHA 2019A and B) and have already been described in the CLH-dossier for DBTP (ECHA, 2016) assessed by RAC in 2017.

Details of individual studies are provided in Annex I of the present CLH report.

Table 28: Summary table of animal studies on adverse effects on development – rats (adopted from ECHA, 2016)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
DBTO			
OECD 414 (Prenatal development toxicity study)	DBTO Purity: > 97% 0, 0.75, 3 and 6 mg/kg	At 6 mg/kg bw dams with clinical signs, lower body weights, lower food consumption. Lower thymus weight in dams in all treated groups. Reduced pregnancy index at 6 mg/kg bw/day.	Unpublished report, 2017 [Annex I,

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Sprague Dawley (25 females/group)	bw/d GD 0-19, gavage	Significant increased post implantation loss. Four dams had resorbed foetuses (100%). No effect of DBTO on fetal sex ratio, fetal body weight, or fetal external and or skeletal examinations. NOAEL (maternal toxicity) = 3 mg/kg bw/d NOAEL (developmental toxicity) = 3 mg/kg bw/d	2.2.1.18]
Comparative study with different di-n-butyltin compounds			
Wistar rat (10 females /group) Single dose, gavage, 80 µmol/kg bw, GD 8 Non-guideline conform study	DBTO: purity not reported 80 µmol/kg bw (20 mg/kg bw), GD 8 DBTC:purity not reported 80 µmol/kg bw; (25 mg/kg bw), GD 8 DBTL: purity not reported 80 µmol/kg bw (50 mg/kg bw) GD 8 DBTA: purity not reported 80 µmol/kg bw (28 mg/kg bw), GD 8 DBTM: purity not reported 80 µmol/kg bw (28 mg/kg bw),GD 8	The nature of malformations was similar in all treatment groups. The di-n-butyltin moiety rather than the associated anionic group is therefore concluded to be responsible for teratogenicity. <u>External malformations:</u> cleft mandible, cleft lower lip, ankyloglossia, schistoglossia <u>Skeletal malformations:</u> anomalies of mandibular fixation, fused mandibula, cranial hypoplasia, fused ribs, fused vertebral arches and cleft maxilla were commonly observed. <u>Skeletal variations:</u> asymmetric/cleft sternebra and cervical rib. Maternal toxicity: No maternal mortality or signs of maternal toxicity in all treated groups. DBTO LOAEL = 20 mg/kg bw DBTC LOAEL = 25 mg/kg bw DBTL LOAEL = 50 mg/kg bw DBTA LOAEL = 28 mg/kg bw DBTM LOAEL = 28 mg/kg bw Comparable incidence of foetal malformations for DBTC (17.3%), DBTA (28.3%), DBTL (30.6%), DBTM (12.5%) and DBTO (20.7%) was observed. A NOAEL cannot be determined for this study.	Noda et al., 1993 [Annex I, 2.2.1.6] (REACH registration, DBTO)
DBTC			
OECD 421 Reproduction/Developmental Toxicity Screening Test) Wistar rat (12/sex) Oral: feed No significant deviations	DBTC Purity: 98.57% 0, 5, 30, 200 ppm (diet); Corresponds to 0, 0.3-0.4, 1.7 -2.4, 12.0-15.4 mg/kg bw Administration for four weeks (males) or for two weeks prior to mating and to day 4 or 6 post partum (females).	200 ppm diet: reduced maternal weight gain (values not reported). Reduced litter size (6.0 compared to 11.3); reduced numbers of foetuses (10 compared to 101 in controls),. Gestation index: 43% vs 100% (high dose vs control) Post-implantation loss: 87.6% vs 13.4% (high dose vs control) NOAEL (for general toxicity): 0.3-0.4 mg/kg bw/day (thymus effects) NOAEL(reproduction): 30 ppm (1.7-2.4	Unpublished report, 2003 [Annex I, 2.2.1.1] (REACH registration, DBTO)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		mg/kg bw/d) LOAEL (reproduction): 200 ppm, (12.0-15.4 mg/kg bw/d)	
OECD 414 (Prenatal developmental toxicity study) Wistar rat (25 females/group) Oral gavage No significant deviations	DBTC Purity: >98% 0, 1, 2.5, 5, 10 mg/kg bw/d GD 6-15	Incidence of foetuses with malformations increased at 10 mg/kg bw/d (4 foetuses from 3 litters, including anasarca, ankyloglossia, hydrocephaly, agnathia and other skeletal defects). Mean number of foetuses per litter was comparable in all groups. The fetal sex distribution was similar in all groups. Maternal toxicity: at 5 mg/kg bw/d (reduced weight gain) and 10 mg/kg bw/d (reduced weight gain and food consumption); values not reported. Thymus weight was significantly lower at 10 mg/kg bw/d. Thymus atrophy at 10 mg/kg bw, to a lesser extent at 5 and 2.5 mg/kg bw. LOAEL =10 mg/kg bw/d (developmental toxicity) NOAEL =5 mg/kg bw/d (developmental toxicity) LOAEL = 5 mg/kg bw (maternal toxicity) NOAEL = 2.5 mg/kg bw (maternal toxicity)	Study report, 1994 [Annex I, 2.2.1.7] (REACH registration, DBTO)
OECD 414 (Prenatal developmental toxicity study) Wistar rat (25 females/group) Oral gavage	DBTC purity not reported 0, 1, 2.5, 5, 10 mg/kg bw/d GD 6-15	Marginal increase in malformations (including single incidences of ankyloglossia, agnathia, mandibular defect at 10 mg/kg bw/d). Maternal toxicity (reduced weight gain (-17%) & reduced food consumption (-7%)) at 10 mg/kg bw/d. LOAEL = 10 mg/kg bw/d (reproductive toxicity) NOAEL = 5 mg/kg bw/d (reproductive toxicity)	Farr et al., 2001 [Annex I, 2.2.1.8]
Wistar rat (10-12 females/group) Non-guideline conform study Oral gavage	DBTC purity not reported 0, 2.5, 5.0, 7.5, 10 mg/kg bw/d GD 7-15	Increased resorptions at 7.5 (10.0%) and 10 mg/kg bw/d (5.3%) compared to controls (1.3%); increased post-implantation loss at 7.5 (77.0%) and 10 mg/kg bw/d (37.9%) compared to controls (10.2%). Reduced number of live foetuses at 7.5 mg/kg bw/d (3.6, compared to 11.8 in controls). Reduced foetal weight at 5 (~15%), 7.5 (~38%) and 10 mg/kg bw/d (~30%). Foetal malformations at ≥5 mg/kg bw/d, typically cleft jaw and related mandibular defects. Maternal toxicity: mortality at 7.5 (5/12) and 10 mg/kg bw/d (9/12), clinical signs, weight loss or reduced weight gain during the dosing period at 7.5 and 10 mg/kg bw/d (-9 g, 6 g	Ema et al., 1991 [Annex I, 2.2.1.9] (REACH registration, DBTO)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		respectively) & reduced food consumption during dosing at 7.5 (-43%) and 10 mg/kg bw/d (-39%). No maternal toxicity was apparent at 5 mg/kg bw LOAEL = 5 mg/kg bw/d (developmental toxicity) NOAEL = 2.5 mg/kg bw/d (developmental toxicity)	
Wistar rat (11 females/group) Non-guideline conform study	DBTC purity not reported 0, 20 mg/kg bw/d (GD 7-9, 10-12 or 13-15) 0, 20, 40 mg/kg bw/d (GD 6, 7, 8 or 9)	GD 7-9: increased resorption (9.9) compared to controls (1.3) and increased post-implantation loss (75.1% compared to 10.2%). Total resorption in 5/11 dams, resulting in low litter size (3.3 compared to 11.8 in controls). Mean foetal weight reduced (~40%). Increased malformations (largely omphalocoele and jaw defects) GD 10-12: reduced foetal weight (~15%); no malformations. GD 13-15: reduced foetal weight (~20%); no malformations. GD 6: increased post-implantation loss at 20 (18.9%) and 40 mg/kg bw/d (43.5%); total resorption at 20 (1/11) and 40 mg/kg bw/d (3/11). Marginal increase in malformations at 40 mg/kg bw/d. GD 7: increased post-implantation loss at 20 (24.6%) and 40 mg/kg bw/d (76.2%); total resorption at 20 (1/11) and 40 mg/kg bw/d (7/11). Increase in malformations at 20 and 40 mg/kg bw/d. GD 8: increased post-implantation loss at 20 (42.8%) and 40 mg/kg bw/d (79.7%); total resorption at 20 (3/11) and 40 mg/kg bw/d (7/11). Increase in malformations at 20 and 40 mg/kg bw/d. GD 9: increased post-implantation loss at 40 mg/kg bw/d (31.7%); total resorption at 40 mg/kg bw/d (3/11). Marginal increase in malformations at 20 mg/kg bw/d. Details on maternal toxicity not reported. The study demonstrates that the most sensitive period is GD 8. LOAEL =20 mg/kg bw/d (reproductive toxicity) NOAEL <20 mg/kg bw/d (reproductive toxicity)	Ema et al., 1992 [Annex I, 2.2.1.10] (REACH registration, DBTO)
Wistar rat	DBTC	Total resorptions at 10 (2/10) and 15 mg/kg bw/d (4/10); increased post-implantation loss	Ema et al., 1995b

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
(10 females/group) Non-guideline conform study Oral gavage	purity not reported 0, 10, 15 mg/kg bw/d GD 7-8	at 10 (53.9%) and 15 mg/kg bw/d (71.2%) compared to controls (11.8%). External and skeletal foetal malformations (typically exencephaly, cleft jaw, ankyloglossia and other mandibular defects) at 10 and 15 mg/kg bw/d. Maternal toxicity: reduced weight gain at 10 and 15 mg/kg bw/d (- 29% and -51% respectively), with initial weight loss (-5 g, -8 g, respectively). LOAEL =10 mg/kg bw/d (reproductive toxicity) NOAEL <10 mg/kg bw/d (reproductive toxicity)	[Annex I, 2.2.1.11] (REACH registration, DBTO)
Wistar rat (11-13 females/group) Non-guideline conform study Oral gavage	DBTC purity not reported 0, 50, 100 mg/kg bw/d GD 13-15	Reduced foetal weight at 50 (-29%) and 100 mg/kg bw/d (-34%). Increased post-implantation loss at 50 (22.0%) and 100 mg/kg bw/d (34.4%) compared to controls (9.8%). No clear increase in foetal malformations. Maternal toxicity at 50 and 100 mg/kg bw/d: mortality at 50 (1/11) and 100 mg/kg bw/d (3/13), reduced weight gain -70%, -88%). LOAEL =50 mg/kg bw/d (reproductive toxicity) NOAEL <50 mg/kg bw/d (reproductive toxicity)	Ema et al., 1996b [Annex I, 2.2.1.12] (REACH registration, DBTO)
Wistar rat (16-19 females/group) Non-guideline conform study Oral gavage	DBTC 97% purity 0, 3.8, 7.6, 15.2 mg/kg bw/d GD 4-7	Total resorption was seen at 7.6 mg/kg bw (3/16) and 15.2 mg/kg bw (14/16); post-implantation loss was increased at 3.8 (13.9%), 7.6 (39.9%) and 15.2 mg/kg bw (91.5%) compared to controls (7.0%). Foetal weight was decreased at 7.6 (~13%) and 15.2 mg/kg bw (~24%). No malformations were observed. Maternal toxicity: Exposure on GD 4-7 resulted in signs of maternal toxicity and weight loss during the dosing period at 7.6 mg/kg bw (-2 g) and 15.2 mg/kg bw (-14 g) DBTC casues pre- and post-implantation embryonic loss when adminsitert to maternal rats during early pregnany. LOAEL =3.8 mg/kg bw/d (reproductive toxicity) NOAEL <3.8 mg/kg bw/d (reproductive toxicity)	Ema & Harazono, 2000 [Annex I, 2.2.1.2] (REACH registration, DBTO)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
DBTA			
Wistar rat (9-10/group) Non-guideline conform study Oral gavage	DBTA purity not reported 0, 15, 30 mg/kg bw/d: GD 7-9 0, 5.0, 7.2, 10.5, 15.2, 22 mg/kg bw/d: GD 8	Foetal malformations: (mainly affecting the jaw: cleft mandible, cleft lower lip, ankyloglossia or schistoglossia; exencephaly) Details on maternal toxicity not reported. LOAEL =15.2 mg/kg bw (reproductive toxicity) NOAEL =10.5 mg/kg bw (reproductive toxicity) GD 8 is the critical period for the teratogenesis of DBTA.	Noda et al., 1992a [Annex I, 2.2.1.13]
Wistar rat (13-14 females /group) Non-guideline conform study; comparable to OECD 414 Oral gavage	DBTA purity not reported 0, 1.7, 5, 10, 15, 22 mg/kg bw/d GD 7-17 (comparative study betwenn DBTA and monobutyltin chloride)	Reduced numbers of dams with viable foetuses at 15 mg/kg bw (7/16) due to foetal loss and total resorption (9/16). Reduced foetal weight at 10 mg/kg bw (~18%) and 15 mg/kg bw (~26%. Foetal malformations (mainly cleft mandible, cleft lower lip, ankyloglossia and schistoglossia) increased at ≥5 mg/kg bw/d. Maternal toxicity (reduced weight gain) at 15 mg/kg bw/d. LOAEL =5 mg/kg bw/d NOAEL =1.7 mg/kg bw/d	Noda et al., 1992b [Annex I, 2.2.1.14]
Wistar rat (12-14 females/group) Non-guideline study Oral gavage	DBTA purity not reported 0, 7.5, 10, 15, 22 mg/kg bw/d GD 8	Implantation loss increased at 22 mg/kg bw in 3-month old (19.2%), 7.5 month-old (37.8%) and 12 month old dams (95.2%). Foetal malformations (typically cleft mandible, cleft lower lip, ankyloglossia, schistoglossia, fused ribs, fused cervical and vertebral arches) at ≥7.5 mg/kg bw/d. Reduced numbers of litters with viable foetuses (6/13) due to total resorption (5/13) at 22 mg/kg bw (7.5 month-old dams). Maternal toxicity: reduced maternal weight gain at 22 mg/kg bw in 7.5 month-old dams (-33%). NOAEL <7.5 mg/kg bw/d (reproductive toxicity) LOAEL =7.5 mg/kg bw/d (reproductive toxicity)	Noda et al., 2001 [Annex I, 2.2.1.15]

Table 29: Summary table of animal studies on adverse effects on development – mouse (adopted from ECHA, 2016)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
CD1 mouse (12 females/group) non-guideline study	DBTC Purity: 99.5% 0, 7.6, 15.2, 30.4 mg/kg bw/d GD 4-7 (or GD 0-3)	Increased post-implantation loss at all tested concentration; at 7.6 (48.3%), 15.2 (94.4%) and 30.4 mg/kg bw (100%). Total resorption at 7.6 (2/12), 15.2 (8/12) and 30.4 mg/kg bw (10/12); Marginal increase in malformations at 7.6 mg/kg bw (omphalocoele, exencephaly) but not at 15.2 mg/kg bw. Maternal toxicity: in mice exposed GD 4-7, maternal mortality was seen at 15.2 mg/kg bw (1/12) only. Reduced weight gain over the treatment period at 7.6 (+1.9 g), 15.2 (+1.2 g) and 30.4 mg/kg bw (- 0.3g) compared to +3.1 g in controls. Food consumption was reduced at 15.2 mg/kg bw (~25%) and 30.4 mg/kg bw (~28%). NOAEL <7.6 mg/kg bw (reproductive toxicity) LOAEL =7.6 mg/kg bw (reproductive toxicity)	Ema et al., 2007a [Annex I, 2.2.1.3]

Table 30: Summary table of animal studies on adverse effects on development – monkeys (adopted from ECHA, 2016)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Cynomolgus monkey (10-12 females/group) Non-guideline study nasogastric intubation	DBTC: 98% purity 2.5, 3.8 mg/kg bw/d GD 20-50 (period of organogenesis) Pregnancy outcome was determined on GD 100	Reduced foetal survival at 2.5 mg/kg bw/d (8/12 females with embryofoetal loss) and at 3.8 mg/kg bw/d (4/10 females with embryofoetal loss) compared to 18/12 controls. Maternal toxicity: clinical signs, reduced weight gain (-242±423g and -556 ± 526 g) on GD 20-51 compared to control (+ 57±237g) accompanied with reduced food consumption at 2.5 and 3.8 mg/kg bw/d; DBTC causes embryolethal effects, but no malformations were observed. LOAEL =2.5 mg/kg bw/d (reproductive toxicity) NOAEL <2.5 mg/kg bw/d (reproductive toxicity)	Ema et al., 2007b [Annex I, 2.2.1.16]
Cynomolgus monkey (5/group; 12 controls) Non-guideline study nasogastric intubation	DBTC: 98% purity 0, 7.5 mg/kg bw/d: GD 19-21, 21-23, 24-26, 26-28, 29-31, 31-33, 34-36	Embryofoetal loss (GD 19-21 (1/5), 24-26 (2/5), 34-36 (1/5) compared to 1/12 controls. Findings associated with maternal toxicity Maternal toxicity: vomiting, soft stool diarrhoea, body, marginally reduced weight gain). DBTC causes embryolethal effects, but no malformations were observed. LOAEL =7.5 mg/kg bw/d (reproductive toxicity) NOAEL <7.5 mg/kg bw/d (reproductive toxicity)	Ema et al., 2009 [Annex I, 2.2.1.17]

Table 31: Summary table of human data on adverse effects on development

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available				

The read across approach is dedicated to the data generated in experimental animal species *in vivo* by oral administration. The read across is not applicable to *in vitro* studies. Nevertheless, in the following table also *in vitro* data of the read across substance DBTC is summarised. This information is provided as additional information.

Table 32: Summary table of other studies relevant for developmental toxicity (adopted from ECHA, 2016)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
In vitro Cultured rat embryo study	DBTC	Wistar rat embryos were explanted on GD 8 and cultured for 68 hours in the presence of DBTC at concentrations of 0 (controls), 3, 10 or 30 ng/mL.	Embryos cultured in the presence of 30 ng/mL DBTC showed a marked and statistically significant reduction in the incidence of well-developed vascularization in the body and yolk sac. Reduced yolk sac diameter, crown-rump length and number of somite pairs at 30 ng/ml; decrease in the overall morphological score; increase in the incidence of embryos with anomalies (all concentration, statistically significant for embryos exposed to 10 and 30 ng/mL)	Ema et al., 1995a [Annex I, 2.2.3.1]
In vitro Cultured rat embryo study	DBTC purity not reported	Cultured GD 8.5, GD 9.5 or GD 11.5 embryos were cultured for 68, 46 or 48 hours and were exposure to DBTC concentrations for 24, 46 or 46 hours respectively.	In GD 8.5 embryos DBTC caused decreases in placental diameter (≥10 ng/mL) and the number of somite pairs and the morphological score (30 ng/mL). In GD 9.5 embryos, significant decreases in yolk sac diameter and crown-rump length (100 ng/mL, reduction in the number of somite pairs (≥50 ng/mL) and a reduction in the morphological score (≥30 ng/mL). No adverse effects were seen in GD 11.5 embryos. Dysmorphogenesis was seen in embryos from GD 8.5 (≥10 ng/mL), GD 9.5 (≥50 ng/mL) and GD 11.5 (300 ng/mL). Incomplete turning and	Ema et al., 1996a [Annex I, 2.2.3.2]

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			craniofacial defects in embryos cultured from GD 8.5 and GD 9.5 and defects of the forelimb buds and tail in embryos cultured from GD 11.5 were frequently observed.	
In vitro Cultured rat embryo study	DBTC purity not reported	Cultured rat embryo limb buds were used to assess the teratogenicity of DBTC.	DBTC showed very strong inhibition of cell differentiation (ID50 0.13-1.71 µM and cell proliferation (IP50 0.12-2.81 µM).	Yonemoto et al., 1993 [Annex I, 2.2.3.3]

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

A recently conducted PNDT study with DBTO according to OECD 414 was provided by REACH registrants (Unpublished report, 2017). The study is a guideline conform study carried out under GLP. DBTO was applied to 25 female Sprague Dawley rats via gavage on gestation day 0-19 at dose levels up to 6 mg/kg bw/day. The highest dose of 6 mg/kg bw/day was selected based on a dose range finding study in which 40% of the dams had to be euthanised at 9.5 mg/kg bw/day.

At 6.0 mg/kg/day, two animals were euthanized in extremis with clinical signs of toxicity, low body weights, low body weight change, and low food consumption No effect of DBTO at dose levels of 0.75 and 3.0 mg/kg/day were observed on gestation bodyweights and body weight change. At 6.0 mg/kg/day, mean body weights were statistically lower than mean control values on GD 18 (-8%) and 20 (-9%). At 6.0 mg/kg bw/day, maternal effects were apparent from clinical findings (low body carriage, red material around the nose, thin appearance, loss of skin elasticity, and pale body color) lower gestation body weights, lower body weight change, and lower food consumption.

These effects on gestation body weights and body weight change at 6.0 mg/kg/day were considered test substance related correlating with adverse pregnancy outcomes in some animals.

Pregnancy index was 96%, 96%, 92%,and 88% in the 0, 0.75, 3.0, and 6.0 mg/kg bw/day groups, respectively. There were one, one, two, and three nonpregnant females in the 0, 0.75, 3.0, and 6.0 mg/kg/day groups, respectively. Two of the non-pregnant females in the highest dose group were euthanized in extremis. Four females in the highest dose group (#4510, 4511, 4516, and 4524) had uterine implantations comprised entirely of resorbing fetuses (100% post-implantation loss). Overall, there were 24, 24, 23, and 18 litters with GD 20 fetuses for evaluation in the 0, 0.75, 3.0, and 6.0 mg/kg/day dose groups, respectively. The increased incidence of females with all resorption sites in utero is considered to be related to DBTO administration and adverse.

Severe maternal toxicity was present in two out of four animals which had 100% post-implantation loss (#4516, 4524). In the other two animals (#4510, 4511) with 100% post-implantation loss body weight was not affected by DBTO and no severe maternal toxicity (absence of clinical signs or only minor clinical signs) were observed. A further dam (#4520) with 75% post-implantation loss did also not indicate any clinical signs or altered body weight.

In 3 out of 5 dams with the highest increase of post-implantation loss (75-100%) no or marginal maternal toxicity was observed. Therefore considering individual data no strong correlation between maternal toxicity and adverse pregnancy outcome was present.

In the following table maternal and developmental observations at uterine examination are depicted.

Table 33: Maternal and developmental observations at uterine examination (Unpublished report, 2017)

Endpoint	**0 mg/kg bw**	**0.75 mg/kg bw**	**3.0 mg/kg bw**	**6.0 mg/kg bw**
No of dams	25	25	25	25
No not pregnant	1	1	2	3
Pregnancy index (%)	96.0	96.0	922.0	88.0
No. females with all resorptions	0	0	0	4
No. females with viable foetuses day 20 gestation	24	24	23	18
Endpoint	**0 mg/kg bw (Mean ± SD)**	**0.75 mg/kg bw (Mean ± SD)**	**3.0 mg/kg bw (Mean ± SD)**	**6.0 mg/kg bw (Mean ± SD)**
Corpora lutea No. per animal	15.4 ± 2.30	16.1 ± 2.02	16.0 ± 3.01	15.4 ± 2.43
Implantation sites No. per animal	13.2 ± 1.89	14.3 ± 2.35	14.2 ± 1.67	12.5 ± 2.42
Preimplantation loss % per animal	12.91 ± 14.05	11.03 ± 9.76	9.80 ± 10.11	14.66 ± 15.27
Viable fetuses No. per animal	12.5 ± 1.96	14.1 ± 2.36	13.5 ± 1.78	9.7 ± 5.42
Postimplantation loss % per animal	5.40 ± 5.626	1.46 ± 2.952	4.89 ±5.687	25.70 ± 39.370 (18.3 ± 32.7[b])
Litter size No. per animal	12.5 ± 1.96	14.1[a] ± 2.36	13.5 ± 1.78	9.7 ± 5.42
Resorptions: early + late No. per animal	0.7 ± 0.75	0.2[a] ± 0.41	0.7 ± 0.82	2.7 ± 4.26
Resorptions: early No. per animal	0.7 ± 0.75	0.2[a] ± 0.41	0.7 ± 0.83	2.7 ± 4.22

[b] *data without considering animal #4516 and 4524 (high toxicity observed in these dams)*

No effect of DBTO on fetal sex ratio, fetal body weight, fetal external and/or skeletal examinations. The increase of irregular palatal rugae pattern, a visceral variation, in the control, 0.75, and 3.0 mg/kg/day treated groups was 4.2%, 12.5%, and 26.1%, respectively. The higher incidence of this variation in these treated groups did not differ statistically from con-current controls and in the absence of a similar finding among fetuses in the 6.0 mg/kg/day group these observations were not considered test article related.

A comparative study with DBTO, DBTC, DBTA, DBTM and DBTL (Noda et al., 1993) using a single gavage administration on GD 8, demonstrates a comparable spectrum of effects for all substances, in the absence of maternal toxicity. The study used dose levels of 80 μmol/kg bw, equivalent to dose levels of 25 mg/kg bw (DBTC), 50 mg/kg bw (DBTL), 28 mg/kg bw (DBTM), 28 mg/kg bw (DBTA) and 20 mg/kg bw (DBTO). Treatment showed a comparable incidence of foetal malformations for DBTC (17.3%), DBTA (28.3%), DBTL (30.6%), DBTM (12.5%) and DBTO (20.7%) and that the di-n-butyltin compounds cause a similar spectrum of foetal malformations (external malformations: cleft mandible, cleft lower lip, ankyloglossia, schistoglossia, skeletal malformations: anomalies of mandibular fixation, fused mandibula, cranial hypoplasia, fused ribs, fused vertebral arches and cleft maxilla were commonly observed, skeletal

variations: asymmetric/cleft sternebra and cervical rib). The study is considered a key study in order to substantiate the category approach.

Details on external malformations, skeletal malformations and variations is provided in the tables below.

Table 34: External malformations (Noda et al., 1993)

Group	Control	DBTA	DBTC	DBTM	DBTO	DBTL
Foetuses examined (#)	126	133	107	124	129	130
Malformations (%)	-	28.3**	17.3**	12.5	20.7*	30.6*
Malformations (#)	-	37 (7)**	18 (6)**	16 (5)**	28 (6)**	37 (6)**
Cleft mandible, cleft lower lip, ankyloglossia or schistoglossia	-	37 (7)**	8 (4)**	13 (5)**	23 (6)**	33 (6)**
Micrognathia	-	2 (1)	1 (1)	-	-	2 (1)
Peaked mandible	-	-	1 (1)	-	1 (1)	-
Exencephaly	-	18 (6)**	9 (4)**	-	7 (6)*	16 (5)**
Cleft upper lip	-	3 (1)	1 (1)	5(2)*	2(2)	4 (3)
Cleft palate	-	1 (1)	-	-	1(1)	2 (2)
Facial cleft	-	-	2 (2)	-	-	-
Asymmetric face	-	1(1)	1 (1)	-	-	-
Omphalocoele	-	-	-	-	-	-
Kinked tail	-	-	1 (1)	-	-	-
Vestigial tail	-	-	-	-	-	-
Pes varus	-	-	1 (1)	-	-	-
Pes valgus	-	-	-	-	-	-
Scoliosis	-	-	3 (1)	-	-	-

**significantly different to controls ($p<0.05$); **$p<0.01$*

Skeletal malformations were also observed with significantly increased incidences in all treated groups. Anomalies of mandibular fixation, fused mandibula, cranial hypoplasia, fused ribs, fused vertebral arches and cleft maxilla were commonly observed.

Table 35: Skeletal malformations (Noda et al., 1993)

Group	Control	DBTA	DBTC	DBTM	DBTO	DBTL
Foetuses examined (#)	126	133	107	124	129	130
Malformations (%)	-	21.9**	29.2*	9.3	26.2 *	28.1*
Malformations (#)	-	29 (7)**	29 (5)**	12 (4)	30 (6)**	34 (6)**
Anomaly of mandibular fixation	-	17 (6)**	29 (5)**	11 (4)	18 (6)**	25 (6)**
Fused mandibles	-	1 (1)	2 (2)	-	1 (1)	1 (1)
Fused mandibles / micromandible	-	2 (1)	2 (1)	-	-	2 (1)

Cranial hypoplasia	-	12 (5)**	3 (3)	3 (2)	4 (4)	15 (5)**
Fused ribs	-	9 (2)**	10 (4)**	-	12 (3)**	7 (3)*
Absent ribs	-	2 (1)	25 (4)**	-	6 (2)*	-
Fused cervical arches	-	1(1)	16 (4)**	-	3 (1)	-
Fused thoracic arches	-	5 (1)	6 (2)**	-	8 (3)**	3 (2)
Fused lumbar arches	-	-	16 (4)**	-	-	-
Cleft maxilla	-	3 (1)	2 (1)	-	2 (2)	3 (3)
Vertebral agenesis	-	-	2 (2)	-	-	-
Leg bone agenesis	-	-	2 (2)	-	-	-

**significantly different to controls (p<0.05); **p<0.01*

The incidences of skeletal variations were also significantly increased in all treated groups; the most common findings were asymmetric/cleft sternebra and cervical rib.

Table 36: Skeletal variations (Noda et al., 1993)

Group	Control	DBTA	DBTC	DBTM	DBTO	DBTL
Foetuses examined (#)	126	133	107	124	129	130
Variations (%)	1.4	70.2**	95.9**	33.2**	66.7**	65.3**
Variations (#)	2 (2)	93 (8)**	103 (8)**	39 (9)**	83 (9)**	82 (8)**
Asymmetric/cleft sternebra	-	19 (6)**	23 (7)**	1 (1)	11 (4)**	11 (5)**
Cervical rib	2 (2)	90 (8)**	100 (8)**	37 (8)**	80 (9)**	76 (8)**
Lumbar rib	-	-	1 (1)	-	1 (1)	1 (1)
Rudimentary lumbar rib	-	4 (2)	4 (2)*	2 (1)	2 (2)	7 (5)*
Bifurcated cervical arch	-	8 (5)**	15 (6)**	1 (1)	14 (5)**	13 (5)**
Bifurcated thoracic vertebra	-	11 (2)**	32 (5)**	-	20 (3)**	13 (4)**
Variations in numbers of vertebrae	-	3 (1)	13 (4)**	-	6 (2)*	-
Occipital dysplasia	-	1 (1)	3 (1)	-	-	-
Short 13th rib	-	-	5 (2)*	-	3 (1)	-

**significantly different to controls (p<0.05); **p<0.01*

Category member DBTC: Guideline conform studies

In a guideline compliant (OECD 421) reproduction/developmental screening study (Unpublished report, 2003), administration of DBTC in the diet for two weeks prior to mating and to lactation at a concentration of 200 ppm (12.0-15.4 mg/kg bw/d) caused an increase in post-implantation loss (87.6% compared to 13.4% in controls). The application of DBTC caused bodyweight effects in females (reduced weight gain over the pre-mating, gestation and lactation periods, resulting in significantly lower mean bodyweights at the end of the pre-mating period and during the gestation and lactation periods), only 3 of the 7 pregnant females at the highest dose level delivered live offsprings.

The table below summarises information on the reproductive parameters and also on the post-implantation loss.

Table 37: Reproductive parameters (Unpublished report, 2003)

Dietary concentration DBTC (ppm)	**0**	**5**	**30**	**200**
Mated (#)	12	11	12	12
Pregnant (#)	9	8	7	7
Females with liveborn (#)	9	8	7	3
Gestation index	100%	100%	100%	43%
Live birth index	99%	99%	94%	56%
Litters with stillborn pups	1	1	3	3
Post-implantation loss	13.4%	7.5%	20.4%	87.6%

In a guideline-compliant (OECD TG 414) prenatal developmental toxicity study performed with DBTC at dose levels of 1, 2.5, 5 or 10 mg/kg bw/d (Study report, 1994), the incidence of foetuses with malformations was increased at 10 mg/kg bw/d (4 foetuses out of three litters). Oedema (anasarca) was observed in one foetus; the remaining three foetuses showed more severe malformations (including ankyloglossia, hydrocephaly, anophthalmia and diaphragmatic hernia, agnathia, absent mandibles and malformed zygomatic arches; filamentous and curly tail, scoliosis, absence of sacral and caudal vertebrae and sacral vertebral arches). Evidence of maternal toxicity was seen at 5 mg/kg bw/d (reduced weight gain) and at 10 mg/kg bw/d (reduced weight gain and food consumption). No deaths occurred. The original study report is not available; therefore full methodological details and tabulated results (including details of maternal toxicity) are not available (Annex I CLH report, ECHA, 2016).

In a further guideline and GLP conform study with DBTC (Farr et al., 2001) administration on GD 6-15 resulted in a slightly increased frequency of foetal malformations at the highest (and maternally toxic) dose level of 10 mg/kg bw/d (1.5% compared to 0.4% in controls). The authors conclude that the pattern of findings does not indicate any effect of treatment, however the nature of malformations seen at the highest dose level is consistent with the results of other studies. Therefore the effects are considered to be potentially related to treatment.

Table 38: Foetal malformations (Farr et al., 2001)

DBTC (mg/kg bw)	**0**	**1.0**	**2.5**	**5.0**	**10.0**
Total number	269	ns	ns	ns	262
Malformed foetuses (#)	1 (1)	-	-	1 (1)	4 (3)
Anasarca	-	-	-	1	1
Hydrocephaly	-	-	-	-	1
Ankyloglossia	-	-	-	-	1
Agnathia	-	-	-	-	1
Pulmonary valve atresia	1	-	-	-	-
Scoliosis	-	-	-	-	1
Anophthalmia	-	-	-	-	1
Mandible absent	-	-	-	-	1
Vertebrae / arches absent	-	-	-	-	1

n.s: not specified in available source

Category member DBTC: Supporting evidence

A number of published studies are also available with DBTC. The study protocols do not fully comply with OECD TG 414 but the investigations are considered to be sufficiently robust to support the classification proposal as part of a weight of evidence.

Ema et al. (1991) report increased foetal malformations (predominantly craniofacial malformations) following exposure to DBTC at dose levels of 5, 7.5 and 10 mg/kg bw/d on GD 7-15; no effects were seen at 2.5 mg/kg bw/d. No maternal toxicity was apparent at 2.5 mg/kg bw/d and at 5 mg/kg bw/d. Maternal toxicity was seen in this study at 7.5 and 10 mg/kg bw/d (mortality, clinical signs, reduced weight gain and food consumption). Increased resorption and post-implantation loss was seen at 7.5 and 10 mg/kg bw/d; mean foetal weight was reduced at ≥5 mg/kg bw/d.

Malformations seen in affected foetuses were mainly craniofacial (cleft jaw and ankyloglossia); micrognathia, cleft palate, omphalocoele, exencephaly, anal atresia, club foot and anomalies of the tail (vestigial, kinky and short tail) were also frequently observed. Although malformations at 7.5 and 10 mg/kg bw/d were associated with marked maternal toxicity, it is notable that the increased incidence of foetal malformations at 5 mg/kg bw/d occurred in the absence of overt maternal toxicity.

The following tables summarise reproductive findings and incidence of external malformations.

Table 39: Reproductive findings (Ema et al., 1991)

DBTC (mg/kg bw/d)	0	2.5	5.0	7.5	10
Litters (#)	11	10	11	7	7
Implantations (#)	13.1	14.4	13.8	13.6	14.3
Resorptions (#)	1.3	2.3	2.5	10.0*	5.3
Post-implantation loss (%)	10.2	16.3	18.9	77.0*	37.9
Total resorption (#)	0	0	0	5*	1
Live foetuses (#)	11.8	12.1	11.4	3.6*	9.0
Foetal weight (g) M/F	4.05/3.92	3.84/3.63	3.36*/3.38*	2.50*/2.47*	2.80*/2.84*
Placental weight (g)	0.50	0.50	0.38*	0.29*	0.32*

**significantly different to controls (p<0.05)*

Table 40: Incidence of external malformations (Ema et al., 1991)

DBTC (mg/kg bw/d)	0	2.5	5.0	7.5	10
Examined	130 (11)	121 (10)	125 (11)	25 (2)	27 (2)
Total malformations (#)	-	-	18 (5)*	18 (2)*	16 (2)*
Cleft jaw (#)	-	-	10 (4)*	11 (2*)	14 (2)*
Micrognathia (#)	-	-	1 (1)	7 (1)	3 (1)
Cleft lip (#)	-	-	2 (2)	-	3 (1)
Cleft palate (#)	-	-	1 (1)	3 (2)*	8 (1)
Ankyloglossia (#)	-	-	10 (4)*	12 (2)*	14 (2)*
Cleft tongue (#)	-	-	-	2 (1)	7 (1)
Omphalocoele (#)	-	-	2 (2)	5 (1)	6 (2)*
Exencephaly (#)	-	-	1 (1)	3 (1)	1 (1)

Ecephalocoele (#)	-	-	-	5 (1)	2 (1)
Open eye (#)	-	-	-	1 (1)	-
Anal atresia (#)	-	-	4 (2)	1 (1)	1 (1)
Anasarca (#)	-	-	-	1 (1)	-
Ectopia cordis (#)	-	-	-	3 (1)	-
Oligodactyly (#)	-	-	1 (1)	6 (1)	-
Club foot (#)	-	-	4 (2)	2 (1)	1 (1)
Tail anomaly (#)	-	-	3 (2)	2 (2)*	1 (1)

**significantly different to controls (p<0.05)*

Further work by Ema et al. (Ema et al., 1992b) using higher dose levels of 20 or 40 mg/kg bw/d, identify the sensitive period for DBTC teratogenicity to be GD 7 or 8, with a higher incidence of foetuses affected by administration on GD 8. Exposure at later time points resulted in increased post-implantation loss, reduced litter size and reduced foetal weight. The table below summarises the reproductive and foetal findings of GD 6, 7, 8 oder 9.

Table 41: Reproductive and foetal findings in rats dosed on GD 6, GD 7, GD 8 and GD 9 (Ema et al., 1992b)

	Day of treatment							
	GD 6		**GD 7**		**GD 8**		**GD 9**	
DBTC (mg/kg bw)	**20**	**40**	**20**	**40**	**20**	**40**	**20**	**40**
Litters (#)	11	11	11	11	11	11	11	11
Implantations (#)	14.0	14.2	14.1	14.4	14.6	13.3	14.1	14.2
Resorptions (#)	2.5	6.1	3.5	10.6*	6.0	10.2*	1.3	4.0
Post-implantation loss (%)	18.9	43.5*	24.6	76.2*	42.8*	79.7*	8.6	31.7
Total resorption (#)	1	3	1	7*	3	7*	0	3
Live foetuses (#)	11.5	8.1	10.5	3.7	8.6	3.1	12.8	10.2
Foetal weight (g) M/F	3.78 / 3.59	3.57 / 3.38*	3.30* / 3.23*	3.41/ 3.22*	3.39*/ 3.26*	2.84*/ 2.49*	3.78 / 3.61	3.49* / 3.21*
External malformations								
No. examined (#)	127 (10)	89 (8)	116 (10)	41 (4)	141 (11)	112 (8)	141 (11)	112 (8)
Total malformations (#)	0	2 (2)	14 (6)*	5 (4)*	3 (2)	0	3 (2)	0
Skeletal malformations								
No. examined (#)	85 (10)	59 (8)	78 (10)	27 (3)	93 (11)	75 (8)	93 (11)	75 (8)
Total malformations (#)	0	1 (1)	13 (6)*	1 (1)	3 (2)	5 (3)	3 (2)	5 (3)

Internal malformations								
No. examined (#)	42 (10)	30 (8)	38 (10)	14 (4)	48 (11)	37 (8)	48 (11)	37 (8)
Total malformations (#)	0	2 (2)	16 (7)*	6 (4)*	0	0	0	0

**significantly different from controls (p <0.05)*

Further work carried out by Ema and coworkers are described below.

Ema et al. (1995b) clearly demonstrate that the administration of DBTC at dose levels of 10 and 15 mg/kg bw/d during a sensitive period (GD 7-8) results in teratogenicity. Significantly increased incidences of exencephaly, cleft jaw, cleft lip, ankyloglossia and club foot were apparent at 10 mg/kg bw/d; the incidences of exencephaly, cleft tongue and omphalocoele were additionally significantly increased at 15 mg/kg bw/d. Furthermore, significantly increased incidences of rib deformities and vertebral column were observed in the treated groups; in the 15 mg/kg bw group additionally mandibular defects and fusion of the sternebrae were observed. Incidences of anophthalmia and microphthalmia were also increased. Although maternal toxicity was observed in this study (initial slight weight loss, overall reductions in weight gain) at dose levels of 10 and 15 mg/kg bw/d, the severity of maternal toxicity is not considered to be sufficient to account for the level of foetal malformations seen at these dose levels. In the table below foetal malformations are summarised.

Table 42: Foetal malformations (Ema et al., 1995b)

DBTC (mg/kg bw/d)	**0**	**10**	**15**
Examined (#)	135 (10)	63 (8)	44 (6)
Total external malformations (#)	-	37 (8)**	27 (6)**
Exencephaly	-	25 (7)**	19 (6)**
Encephalocoele	-	8 (3)	4 (3)*
Spina bifida	-	1 (1)	-
Cleft jaw	-	14 (6)**	11 (4)**
Micrognathia	-	6 (3)	2 (1)
Cleft lip	-	11 (4)*	10 (5)**
Ankyloglossia	-	18 (5)**	7 (4)**
Cleft tongue	-	5 (3)	3 (3)*
Cleft palate	-	2 (2)	-
Omphalocoele	-	2 (1)	3 (3)*
Kinked tail	-	1 (1)	-
Club foot	-	10 (5)**	3 (3*)
Hind limb deformity	-	1 (1)	1(1)
Anasarca	-	-	3 (2)
Total skeletal malformations (#)	-	22 (7)**	15 (6)**
Mandibular defect	-	10 (3)	6 (5)**

Fused/absent cervical arch/body	-	13 (5)**	11 (6)**
Fused/absent thoracic arch/body	-	10 (4)*	9 (4)**
Fused/absent lumbar arch/body	-	2 (1)	-
Fused/absent ribs	-	14 (6)**	12 (5)**
Fused sternebrae	-	6 (3)	4 (3)*
Total visceral malformations (#)	-	12 (7)**	10 (4)**
Anophthalmia/microphthalmia	-	8 (5)**	9 (4)**

**significantly different to controls (p<0.05); **p<0.01*

In a further study by Ema et al. (1996) in which higher dose levels (50 and 100 mg/kg bw, oral gavage) were applied (but on GD 13-15), reduction in foetal weight but no evidence of embryofoetal mortality or malformations were observed. The dose levels cause significant maternal toxicity, including mortality, thereby limiting the relevance to the study for classification purposes. The absence of foetal malformations is consistent with other data, demonstrating that the dosing period (GD 13-15) does not cause malformations.

Ema & Harazono (2000) focussed on the effects of DBTC administration during early gestation in the rat. Treatment on GD 4-7 with DBTC at dose levels of 7.6 and 15.2 mg/kg bw/d resulted in increased post-implantation loss. No increase in foetal malformations was seen in this study following the administration of DBTC at dose levels of up to 15.2 mg/kg bw/d. Effects were associated with maternal toxicity (initial weight loss).

Category member DBTA

Further investigations using DBTA confirm that administration on GD 8 to female Wistar rats results in foetal malformations including cleft mandible, cleft lower lip, ankyloglossia, schistoglossia and exencephaly (Noda et al., 1992a). For further details see Table 43.

Table 43: External and skeletal foetal observations of foetuses from dams treated with DBTA on GD 8 (Noda et al., 1992a).

DBTA (mg/kg bw)	**0**	**5.0**	**7.2**	**10.5**	**15.2**	**22.0**
Foetuses/dams	115/9	140/10	138/10	120/10	117/10	103/9
External observations						
Foetuses with malformations (%)	0.9 (1)	-	0.6 (1)	-	1.9 (2)	26.3 (7)**
Foetuses with malformations (#)	1 (1)	-	1 (1)	-	2 (2)	18 (7)**
Cleft mandible, cleft lower lip, ankyloglossia or schistoglossia	-	-	-	-	2 (2)	14 (7)**
Exencephaly	-	-	-	-	-	8 (3)**
Cleft upper lip		-	-	-	-	4 (1)
Peaked mandible	9 (1)	-	-	-	-	0
Agnathia	-	-	-	-	-	1 (1)
Microcephaly	-	-	-	-	-	1 (1)

Vestigial tail	-	-	1 (1)	-	-	0
Club foot	-	-	-	-	-	1 (1)
Skeletal observations						
Foetuses with malformations (%)	0.8 (1)	0	1.2 (2)	0	0.7 (1)	22.4 (5)**
Foetuses with malformations (#)	1 (1)	0	2 (2)	0	1 (1)	13 (5)**
Anomaly of mandibular fixation	0	0	0	0	0	9 (5)**
Cranial hypoplasia	0	0	0	0	0	8 (3)**
Fused ribs	0	0	0	0	0	6 (1)*
Fused cervical or thoracic vertebral arches	0	0	0	0	0	5 (1)*
Fused mandibles	1 (1)	0	0	0	0	0
Agenesis of sacro-coccygeal or coccygeal vertebrae	0	0	2 (2)	0	1 (1)	0
No. of foetuses with cervical ribs	4 (4)	3 (2)	8 (6)	9 (4)	34 (8)**	62 (9)**

** significantly different from control (p<0.05); ** (p<0.01)*

Similar effects were seen following administration of DBTA at dose levels of 10 and 15 mg/kg bw on GD 7-17 (Noda et al., 1992b). In this study no effects were observed with monobutyltin chloride. Maternal toxicity was observed in this study at 15 mg/kg bw/d (reduced weight gain) but not at 10 mg/kg bw/d. Effects are summarised in the table below.

Table 44: Summary of effects (Noda et al., 1992b)

DBTA (mg/kg bw/d)	**0**	**1.7**	**5**	**10**	**15**
Mated (#)	14	13	14	14	16
Pregnant (#)	14	12	14	14	16
Dams with viable foetuses (#)	14	12	14	14	7**
Total resorption (#)	-	-	-	-	9**
Implants (#)	13.6	13.8	14.3	14.3	13.7
Early resorption (%)	5.9	4.6	2.9	10.7	69.5**
Late resorption (%)	-	-	0.4	2.1	4.9
Litter size (#)	12.9	13.3	14.0	12.8	4.3
Foetal weight (g) m/f	3.2/3.0	3.2/.9	3.0/2.8	2.6**/2.5**	2.3**/2.3**
External malformations (#)	-	-	2 (2)	43 (10)**	19 (7)**
External malformations (%)	-	-	1.0	25.1**	38.9**
Skeletal malformations (#)	-	-	-	20 (9)**	18 (7)**
Skeletal malformations (%)	-	-	-	22.7**	54.7**

***significantly different to controls (p<0.01)*

A further study by Noda et al. (2001) investigated the effects of maternal age on the teratogenicity of DBTA administered on GD 8 to female Wistar rats. Malformations were seen in foetuses from 3-month old dams at dose levels of ≥15 mg/kg bw and in foetuses from 7.5 month-old dams at ≥10 mg/kg bw. The observed

predominant malformations (cleft mandible, cleft lower lip, ankyloglossia and/or schistoglossia) were comparable in both groups. The foetal findings are summarised in the table below.

Table 45: Summary of foetal findings (Noda et al., 2001)

DBTA (mg/kg bw)		**0**	**7.5**	**10**	**15**	**22**
Foetuses examined (#)	3 months	166	155	166	148	139
	7.5 months	122	140	110	143	43
	12 months	8	14	8	8	3
External malformations (%)	3 months	-	-	-	28.4*	61.8*
	7.5 months	-	1.3*	7.9*	34.8*	64.0*
	12 months	-	5.6	12.5	8.3	-
Skeletal malformations (%)	3 months	-	-	-	30.2*	62.6*
	7.5 months	-	-	7.0	32.0*	81.3*
	12 months	-	-	-	8.3	-

**significantly different to controls (p<0.01)*

Other species than rats (category member: DBTC)

Ema et al. also investigated effects of DBTC in CD1 mice (Ema et al., 2007a) and cynomolgus monkeys (Ema et al., 2007b; Ema et al., 2009).

A study with DBTC in CD1 mice showed an increase in pre-implantation loss following treatment with ≥7.6 mg/kg bw/d on GD 0-3; findings were associated with marked maternal toxicity including mortality. Treatment on GD 4-7 resulted in a marked increase in post-implantation loss, which reached 100% at 30.4 mg/kg bw/d. There was no clear indication of teratogenic effects (Ema et al., 2007a).

A study with DBTC in cynomolgus monkeys (Emy et al., 2009) reports embryofoetal loss but no foetal malformations following treatment with 7.5 mg/kg bw/d (nasogastric intubation) between GD 19-36. Findings were associated with maternal toxicity (e.g. vomiting, diarrhea and slightly reduced weight gain). A further study in monkeys (Ema et al., 2007b) reports embryofoetal loss but no foetal malformations following treatment with dose levels of 2.5 and 3.8 mg/kg bw/d on GD 20-50. Findings were associated with signs of toxicity and weight loss. The dosing periods in these studies were designed to cover organogenesis (GD 20-50). Pregnancy outcome was determined at day 100 and foetuses were assessed for external, visceral and skeletal malformations/variations.

Studies in mice and monkeys are supportive for the embroylethal effects, however teratogenic effects seen in rat studies (characteristic pattern of external and skeletal malformations, predominantly affecting the skull and jaw) are not supported.

In vitro studies

The read across approach is dedicated to the data generated in experimental animal species *in vivo* by oral administration. *In vitro* data for source substance DBTC is provided as an additional information. Studies in cultured explanted rat embryos (Ema et al., 1995a, Ema et al., 1996a) demonstrate that DBTC causes craniofacial defects (as seen in *in vivo* studies), and also that the period of sensitivity was comparable to that seen in studies in the rat *in vivo*.

In vitro studies with cultured rat limb bud cells clearly demonstrate the potential of DBTC to inhibit cell differentiation and cell proliferation (Yonemoto et al., 1993).

10.10.6 Comparison with the CLP criteria

For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. Within each category, effects on sexual function and fertility, and on development, are considered separately. Effects on lactation are allocated to a separate hazard category.

Category 1	Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).
Subcategory 1A	Known human reproductive toxicant The classification of a substance in Category 1A is largely based on evidence from humans.
Subcategory 1B	Presumed human reproductive toxicant The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.
Category 2	Suspected human reproductive toxicant Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

In the recently conducted PNDT study with DBTO (according to OECD 414) pregnancy outcome parameters were adverse effected at a dose level of 6 mg/kg bw/day e.g. increased incidence of post-implantation loss was observed. At 6 mg/kg bw/day maternal toxicity characterised by reduced body weight gain and clinical signs such as hunched posture, discoloured skin, thin appeareance was observed. However, not all animals with higher incidence of post-implantation loss showed adverse toxicity signs. In three out of 5 animals with the highest incidence of post-implantation loss (75-100%) no clinical signs were detected and body weight was not affected. Therefore altered pregnancy outcome (e.g. higher incidence of post-implantation loss) can be regarded as an effect seen without severe maternal toxicity.

Increased incidence of post-implantation loss was also observed in previous developmental toxicity studies carried out with the category member DBTC (Unpublished report, 2003, Ema et al., 1991, 1992, 2000, 2003, 2007a).

In the studies of Ema et al. (e.g. 1991, 1992, 2000) effects of DBTC application at different time windows during gestation are studied. In these studies higher incidence of post-implantation loss was observed in

treatment groups depending on the gestation day at which DBTC was applied. Ema and co-workers could demonstrate that the sensitive window of DBTC application is during GD 7-8 in rats.

In the PNDT study with DBTC (according to OECD 414), in which female rats received DBTC at dose levels of 0, 1, 2.5, 5 and 10 mg/kg bw at GD 6-15 no effects were observed on the number of foetuses per litter.

No effect of DBTO on fetal sex ratio, fetal body weight, or fetal external and or skeletal examinations was detected (Unpublished report, 2017). An increase of irregular palatal rugae pattern, a visceral variation, in the control, 0.75, and 3.0 mg/kg/day treated groups was 4.2%, 12.5%, and 26.1%, respectively. The higher incidence of this variation in the low and mid dose group did not differ statistically from controls and in the absence of a similar finding among fetuses in the 6.0 mg/kg/day group the effect was not considered test article related. However, lack of effects at the highest dose group can be masked due to lower number of viable foetuses. Adverse effects on the jaw (e.g. cleft palate) are typical adverse effects for the present category of substances. However, it is also noted, that the irregular ossification of the palatine is higher in the control as in the treated groups (10, 1, 0, 0 fetuses affected in the control, 0.75, 3 and 6 mg/kg bw/day group).

In most of developmental toxicity studies carried out with DBTC Wistar rats have been used as model animal. Rat strain differences in the sensitivity towards category members are rather speculative but might have an impact.

Further evidence for adverse impact on the development comes from a comparative study with category members and from developmental toxicity studies carried out with category members (see below):

A comparative study carried out by Noda et al. (1993) in which a single oral dose (80 µmol/kg bw) of category members DBTO, DBTC, DBTA, DBTM and DBTL where applied to Wistar rats (10 females per group) demonstrated that all category members have comparable foetal malformations (external malformations: cleft mandible, cleft lower lip, ankyloglossia, schistoglossia, skeletal malformations: anomalies of mandibular fixation, fused mandibula, cranial hypoplasia, fused ribs, fused vertebral arches and cleft maxilla were commonly observed, skeletal variations: asymmetric/cleft sternebra and cervical rib). The study used dose levels of 80 µmol/kg bw, equivalent to dose levels of 20 mg/kg bw DBTO, 25 mg/kg bw DBTC, 50 mg/kg bw DBTL, 28 mg/kg bw DBTM and 28 mg/kg bw DBTA. No maternal toxic effects have been observed with any of the category members. The study clearly demonstrates that DBTO has same or similar effects as category members and thus further substantiates the category hypothesis.

Data with DBTC demonstrate consistently that DBTC has the potential to cause foetal malformations (a characteristic pattern of external and skeletal malformations, pre-dominantly affecting the skull and jaw) in studies in the rat, and that the sensitive period of exposure is gestation day 8. DBTC exposure is also shown to cause post-implantation loss (and subsequently a reduced litter size), as well as a reduction in foetal weight. Some studies used relatively high dose levels sufficient to cause also marked maternal toxicity. Nevertheless developmental effects are also apparent at dose levels not causing marked maternal toxicity. Interestingly, studies with mice and cynomolgus monkey demonstrate foetotoxicity and increased post-implantation loss but do not confirm the characteristic pattern of malformations seen consistently in studies in the rat. The lack of effects on malformation parameters might be masked by the relatively high level of post-implantation loss.

The characteristic foetal malformations including cleft mandible, cleft lower lip, ankyloglossia or schistoglossia and exencephaly and also reduced implantation loss have also been observed in studies in which the category member DBTA has been applied to Wistar rats.

In vitro and mechanistic data further substantiate *in vivo* findings and demonstrate the sensitivity of the rat foetus to malformations induced by DBTC.

The study outcome of the recent conducted PNDT study with DBTO (up to 6 mg/kg bw/day) substantiates the evidence from category members having an adverse impact on development. In the study a higher incidence of post-implantation loss was observed not attributable to maternal toxicity. No teratogenic effects were observed in this study. However, in the study of Noda et al.(1993) application of 20 mg/kg bw/day DBTO on GD 8 leads to category characteristic malformations of the jaw and skull (e.g. cleft mandible, cleft lower lip, ankyloglossia or schistoglossia) in Wistar rats. The comparative study of Noda et al. (1993)

indicate that category member have an identical toxicological pattern and therefore further studies of category members have been considered in the evaluation.

Based on the clear evidence of effects on the developing foetus (post-implantation loss, skeletal and external malformations) in rat studies with DBTO and with category members and in the absence of data indicating that effects are not relevant to humans, classification of DBTO for reproductive toxicity (adverse effects on development) in Category 1B (H360D) is considered appropriate.

10.10.7 Adverse effects on or via lactation

No data available.

10.10.8 Conclusion on classification and labelling for reproductive toxicity

Classification of DBTO for reproductive toxicity in Category 1B (H360FD: May damage fertility. May damage the unborn child) is warranted.

10.11 Specific target organ toxicity-single exposure

Table 46: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
Wistar rat; 3 males/group Thymus weights and body weights were measured 1, 2, 3, 4, 7 and 9 days after dosing. Further measurements: histopathology and incorporation of radiolabelled precursors into DNA, RNA and protein. No guideline study	DBTC >98% purity; Oral gavage; 0 or 15 mg/kg bw (single dose)	Rapid (from day 2, maximal at day 4) but reversible (by day 9) reduction in thymus weight. Reduced thymus cellularity, cell populations were normal at day 9. NOAEL <15 mg/kg bw	Snoeij et al., 1989 [Annex I, 2.3.1.1]
SCID mice engrafted with human foetal thymus and liver tissue fragments (SCD-hu mice) were exposed to a single intraperitoneal dose of DBTC (0, 0.03, 1.0 mg/kg bw). 36 female SPF derived homozygous C.B.17 SCID mice No guideline followed, mechanistic study	DBTC purity not reported DBTC (0, 0.03, 1.0 mg/kg bw).	Histopathology showed reduced thymus size and a reduction in the size of the thymic cortex following DBTC exposure. No bodyweight effects were observed.	de Heer et al., 1995 [Annex I, 2.3.3.1]

Table 47: Summary table of human data on STOT SE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No information available.				

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

No data are available with DBTO itself. Read across is applied to the source substance DBTC. In the study of Snoeij et al. (1989) rats received once 0 or 15 mg DBTC per kg body weight by gastric intubation. At day 1, 2, 3, 4, 7 and 9 after dosing, body and thymus weights of 3 rats per group were determined. On each day cell suspensions of each thymus gland were prepared. Total cell count and the percentage of small (volume < 130 µm^3), intermediate (volume between 130 and 225 µm^3) and large cells (volume > 225 µm^3) were determined. In addition, the incorporation of DNA, RNA and protein precursors into acid-precipitable material of isolated thymocytes was measured using radiolabelled thymidine, uridine and leucine.

A decrease in absolute and relative thymus weights from the second day after dosing was observed, with a maximum thymus weight reduction at day 4. These effect was shown to recover by day 9. No quantitative details on thymus weight reduction are presented in the publication of Snoeij (1989). The number of cells isolated from the thymus was significantly reduced at days 3, 4 and 7, with recovery by day 9. The number of large cells (volume >225 µm^3) was decreased at days 1 and 2, the numbers of small (volume <130 µm^3) and intermediate cells were not affected until day 3. Cell populations were normal by day 9. Details on number of cells are provided in the table below. The incorporation of radioactivity was reduced on days 1 and 2, but subsequently returned to control values.

Table 48: Influence of a single oral dose of DBTC on small, intermediate, large and total cell count (x10^7) per thymus at various days after administration (Snoeij et al., 1989)

	Small		Intermediate		Large		Total cells	
mg/kg bw	**0**	**15**	**0**	**15**	**0**	**15**	**0**	**15**
Day 1	14.0 ± 3.0	16.0 ± 2.0	3.5 ± 1.0	2.9 ± 0.2	1.0 ± 0.3	0.4 ± 0.1[a]	18.4 ± 4.3	19.4 ± 2.7
Day 2	19.6 ± 5.0	13.7 ± 5.0	4.2 ± 1.2	2.1 ± 1.2	1.1 ± 0.3	0.4 ± 0.2[a]	24.8 ± 7.5	16.2 ± 6.4
Day 3	20.7 ± 3.8	9.9 ± 4.5[a]	4.7± 0.3	2.5 ± 1.0[a]	1.2 ±0.2	0.6 ± 0.3[a]	26.6 ± 4.3	13.0 ± 5.8[a]
Day 4	26.0 ± 4.8	5.8 ± 2.7[s]	4.3 ± 0.2	2.6 ± 0.9[s]	1.1 ± 0.1	0.7 ± 0.3[s]	31.3 ± 4.8	9.1 ± 3.8[s]
Day 7	37.7±1.3	24.3±5.0[a]	6.7±0.4	4.1 ±0.4[a]	1.5 ±0.1	1.2±0.1[a]	45.9± 1.6	29.6±5.5[a]
Day 9	38.9±3.0	40.1±5.0	6.9±1.6	7.8 ± 1.7	1.4±0.3	1.6±0.3	47.2±3.1	49.4±5.7

[a] *$p \leq 0.05$*

In the study of Snoeij (1989) a single oral dose of 15 mg/kg bw DBTC induces a rapid but reversible atrophy of the thymus in the rat. Based on the thymus cell profile, the authors speculate that thymic atrophy is caused by the selective reduction of rapidly proliferating thymic lymphoblasts (which generate small cells populating the thymic cortex). Recovery is shown by a rise in the number of large cells and an increase in macromolecular synthesis.

In a further single dose study in which DBTC in dose levels of 0, 0.03, 1.0 mg/kg bw was applied intraperitoneal to SCID mice engrafted with human thymus and liver tissue fragments, effects on the thymus were observed. A reduction in thymus cortex size following treatment with DBTC was observed (de Heer et al., 1995).

10.11.2 Comparison with the CLP criteria

According to CLP Regulation for the purpose of classification for specific target organ toxicity – single exposure, substances are allocated to one of three categories (Table 3.8.1., CLP Regulation). Guidance values to assist in Category 1 and Category 2 are provided in the CLP Regulation (Table 3.8.2).

Category 1	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure. Substances are classified in Category 1 for specific target organ

	toxicity (single exposure) on the basis of: (a) reliable and good quality evidence from human cases or epidemiological studies; or (b) observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations. Guidance dose/ concentration values are provided below (see 3.8.2.1.9) to be used as part of weight-of- evidence evaluation.
Category 2	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following single exposure Substances are classified in Category 2 for specific target organ toxicity (single exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. Guidance dose/concentration values are provided below (see 3.8.2.1.9) in order to help in classification. In exceptional cases, human evidence can also be used to place a substance in Category 2 (see 3.8.2.1.6).
Category 3	Transient target organ effects This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function. Substances are classified specifically for these effects as laid down in 3.8.2.2.

No studies with DBTO are available. The category approach as described in Chapter 9.2 is applied. In the study of Soneij et al. (1989) application of a single dose of DBTC (15 mg/kg bw) via intubation (gavage) results in thymus weight and thymus cellularity reduction. These effects were present until day 9.

According to the guidance values for single exposure oral (rat), which is ≤ 300 mg/kg bw, the application of a single dose of DBTC of 15 mg/kg/bw which induces toxic effects is well below the guidance values. A further study with SCID mice engrafted with human foetal thymus and liver tissue fragments in which thymus effects appeared after intraperitoneal application of low amounts of DBTC (0, 0.3, 1 mg/kg bw) substantiates the single dose findings.

Based on the effects observed in the aforementioned studies with the category member DBTC a harmonised classification of DBTO for STOT SE Cat. 1 might be justified. Data indicate that single application has an adverse impact on the thymus, nevertheless the studies are non-standard mechanistic studies, with some limitations (e.g. no detailed result description, number of animals low). Furthermore, effects on the thymus are shown to be reversible (Snoeij et al., 1989) and therefore functional consequences are unclear. Since the substance is already proposed for classification for STOT RE 1 H372 (causes damage to the immune system), no further classification for STOT SE 1 H370 (causes damage to the immune system) is proposed.

10.11.3 Conclusion on classification and labelling for STOT SE

No harmonised classification of DBTO for specific target organ toxicity – single exposure - is proposed.

10.12 Specific target organ toxicity-repeated exposure

For evaluation of specific target organ toxicity – repeated exposure an OECD TG 414 (PNDT) study carried out with DBTO and further studies performed with DBTC (read across substance) as part of the category (see details Chapter 9.2) are considered. The studies listed in the table below (exept unpublished study, 2017) have been either included in the registration of DBTO and/or have been considered in the frame of harmonised classification proposal of category members (e.g. ECHA, 2016, ECHA 2019A and B). All of the

studies described below (except unpublished study, 2017) have been already described in the CLH-dossier for DBTP (ECHA, 2016) and assessed by RAC in 2017.

Details of individual studies are provided in Annex I of the CLH report. .

Table 49: Summary table of animal studies on STOT RE (adopted from ECHA, 2016)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
OECD 414 (Prenatal development toxicity study) Sprague Dawley (25 females/group)	DBTO Purity: > 97% 0, 0.75, 3 and 6 mg/kg bw/d GD 0-19, gavage	Lower maternal thymus weights were observed at all DBTO-treatment levels and an increased incidence of small thymus observed macroscopically in the 6.0 mg/kg/day animals. NOAEL (maternal toxicity) = 3 mg/kg bw/d NOAEL (developmental toxicity) = 3 mg/kg bw/d	Unpublished report, 2017 [Annex I, 2.2.1.18]
comparable to OECD 408 (Repeated dose 90 day oral toxicity study in rodents) Rat CFE (m, f) 16/sex	DBTC Purity: 99.7% Oral (dietary) 10, 20, 40, 80 ppm (approximately, 0.5, 1, 2 and 4 mg/kg bw) (for 90 days)	Reduced weight gain (~5%) at 80 ppm (significant in females). Marginally reduced Hb concentration at 80 ppm. No effects on the thymus. LOAEL >80 ppm (~4 mg/kg bw/d) NOAEL =80 ppm (~4 mg/kg bw/d)	Gaunt et al., 1968 (REACH registration, DBTO) [Annex I, 2.3.1.2]
OECD 421 Reproduction/ Developmental toxicity screening test Wistar rat 25 fem-ales/group	DBTC Purity: 98.75% Oral (dietary) 5, 30, 200 ppm (0.3-0.4, 1.7-2.4 and 12.0- 15.4 mg/kg bw) 2 (f) or 4 weeks (m) pre-mating to PND 4	Severe/very severe lymphoid depletion of the thymus at 200 ppm (F); moderate/severe lymphoid depletion at 30 ppm (F). Thymus was not investigated in males. Reduced weight gain, food consumption and mean bodyweight at 200 ppm (M, F); reduced weight gain at 30 ppm (M). LOAEL =30 ppm (1.7-2.4 mg/kg bw/day) (thyums effect) NOAEL =5 ppm (0.3-0.4 mg/kg bw/d) (thymus effect	Unpublished report, 2003 (REACH registration, DBTO) [Annex I, 2.3.1.3]

Comparable to OECD 407 (Repeated dose 28-day oral toxicity study in rodents) Wistar (WU-CPB) rat (m, f); 10/sex/group Swiss mouse (m) 10/sex/group	DBTC Purity: >98% Oral (diet); 50, 150 ppm (28 days) (approximately 2.5 and 7.5 mg/kg bw) Oral (diet); 50, 150 ppm (28 days) (approximately 2.5 and 7.5 mg/kg bw)	Reduced lymph node weights in males and females at 50 ppm (-22%, - 19%) and at 150 ppm (- 29%, -16%). Reduced thymus weight in males and females at 50 ppm (-55%, -52%) and at 150 ppm (-72%, -68%). Reduced spleen weight in males and females at 50 ppm (-17%, -25%) and at 150 ppm (-33%, -32%). Liver/bile duct pathology at 150 ppm. Lymphocyte depletion in the thymic cortex and PALS at 50 and 150 ppm Deaths at 150 ppm. LOAEL = 50 ppm (~2.5 mg/kg bw/d) NOAEL <50 ppm (~2.5 mg/kg bw/d) No effects of treatment	Seinen & Vos, 1977 Penninks & Seinen, 1982 (REACH registration, DBTO) [Annex I, 2.3.1.4, 2.3.1.5]
Rat (strain not reported) No guideline study	DBTC purity unknown Oral (diet); 20, 50, 75, 100 ppm (approximately 1, 2.5, 3.75 and 5 mg/kg bw) (periods of up to 6 months)	Reduced weight gain at 20 ppm (-11%), 50 ppm (-19- 22%), 75 ppm (-35%) and 100 ppm (-30-42%). Reduced food consumption at 50 ppm (-21-23%), 75 ppm (-26%) and 100 ppm (- 19-29%) following treatment for 54-55 days. Treatment for 6 months resulted in mortality (75 and 100 ppm), reduced weight gain and food consumption (≥50 ppm), bile duct and pancreas pathology (≥50 ppm). LOAEL =50 ppm (2.5 mg/kg bw/d) NOAEL = 20 ppm (1mg/kg bw/d)	Barnes & Stoner, 1958 (REACH registration, DBTO) [Annex I, 2.3.1.6]
Wistar rat (f) 25/group OECD 414 (Prenatal developmental toxicity study)	DBTC Purity: >98% Oral (gavage); 1, 2.5, 5, 10 mg/kg bw/d (GD 6-15)	Thymic atrophy at 10 mg/kg bw/d and (to a lesser extent) at 2.5 and 5 mg/kg bw/d. Reduced weight gain & food consumption at 10 mg/kg bw/d; slightly reduced weight gain at 5 mg/kg bw/d. LOAEL = 2.5 mg/kg bw/d (thymus effects, maternal toxicity) NOAEL < 2.5 mg/kg bw/d (thymus effects, maternal toxicity)	Study report, 1994 [Annex I, 2.3.1.7]
Wistar rat (f) OECD 414 (Prenatal developmental toxicity study)	DBTC purity not reported Oral (gavage); 0, 1, 2.5, 5, 10 mg/kg bw/d (GD 7-17)	Reduced thymus weight (-23%) at 10 mg/kg bw/d. Reduced maternal weight gain (~17%) & food consumption (~7%) at 10 mg/kg bw/d. LOAEL = 10 mg/kg bw/d NOAEL =5 mg/kg bw/d	Farr et al., 2001 [Annex I, 2.3.1.8]

CD rat (m, f) 8/sex No guideline study	DBTC Purity: 96% Oral (drinking water); 0, 0.9, 1.9 mg/kg bw/d initial study, 0, 1.0, 2.5 mg/kg bw/d confirmatory study	No effects on thymus weight, antibody production, DTH response or NK cell activity. No bodyweight effects. Reduced water consumption at 25 mg/L (M, F). No effects of treatment were observed NOAEL >2.5 mg/kg bw/d NOAEL =2.5 mg/kg bw/d	DeWitt et al., 2005 [Annex I, 2.3.1.9]
SD rats SD rat (f, maternal); pregnant rats SD rat (m, f; pups) No guideline study	DBTC purity not reported DBTC in drinking water at 0, 10 or 25 mg/L on GD 6-PND 21. (1 and 2.5 mg DBTC/kg bw during gestation, 2.0 and 4.4 mg DBTC/kg bw while nursing) DTH and NK response assessed in offspring at PND 42. Pups gavaged with DBTC at 0, 1.0 or 2.5 mg/kg bw from PND 3 (3/week). DTH and NK response assessed in offspring at PND 42.	No effects of treatment Reduced weight gain (2.5 mg/kg bw/d) No clear effects on immune parameters NOAEL = 2.5 mg/kg bw/d	DeWitt et al., 2006 [Annex I, 2.3.1.10]

Table 50: Summary table of human data on STOT RE

Type of data/report	Test substance	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 51: Summary table of other studies relevant for STOT RE (adopted from ECHA, 2016)

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
None	DBTC >98% purity	WU rat WAG rat Swiss mouse Dietary concentrations of 0 or 150 ppm; rats were sensitised after three weeks, hypersentitive response was tested after 5 or 6 weeks; Weights of the thymus, spleen, adrenals and lymph node were recorded; allograft rejection response measured in rats.	Allograft rejection was significantly delayed; other measures of immune function were unaffected by treatment.	Seinen et al., 1977 [Annex I, 2.3.1.11]

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

With DBTO a PNDT study according to OECD TG 414 has been considered, further studies are available with DBTC which are considered as part of the category approach. The studies have been submitted in the REACH registration of DBTO and/or have been described previously (e.g. ECHA, 2016) and have been already considered by RAC in 2017.

The most critical effect is the thymus toxicity of DBTC which has been observed in a number of studies intended to address repeated dose toxicity. Additional relevant information is available from developmental and/or reproductive toxicity studies, which include measurement of thymus weight or assessment of thymus histopathology.

In the guideline compliant GLP conform study (OECD TG 414, Unpublished report, 2017) with DBTO significant reduced thymus weights were observed at all DBTO treatment levels (see table below) and an increased incidence of small thymus observed macroscopically in the 6.0 mg/kg bw/day animals. No histopathological examinations have been performed in this study.

Table 52: Reduced thymus weight and thymus weight/adjusted GD20 body weight after treatment with DBTO (Unpublished report, 2017).

Endpoint	0 mg/kg bw/day (Mean ± SD)	0.75 mg/kg bw/day (Mean ± SD)	3 mg/kg bw/day (Mean ± SD)	6 mg/kg bw/day (Mean ± SD)
Thymus g	0.239 ± 0.062	0.193* ±0.042	0.158* ± 0.043	0.134* ±0.046
Thymus/adjusted GD 20 BWT %	0.0891 ± 0.0192	0.0716* ± 0.0123	0.0581*± 0.0143	0.0558* ± 0.0108

**statistically significant to control values (p<0.01)*

In a 90-day sub-chronic toxicity study performed at dietary concentrations of 0, 10, 20, 40 and 80 ppm DBTC (Gaunt et al., 1968) (approximately 0, 0.5, 1, 2, 4 mg/kg bw/d), reduced weight gain and food consumption and a marginal effect on haemoglobin concentration were seen at the highest dietary concentration. No effects on the thymus were reported in this study at the highest dietary concentration of 80 ppm (equivalent to approximately 4 mg/kg bw/d).

In a guideline compliant (OECD 421) screening study (Unpublished report, 2003), administration of DBTC in the diet for two weeks prior to mating and to lactation (females) at a concentration of 200 ppm (12.0- 15.4 mg/kg bw/d) caused bodyweight effects in females (reduced weight gain over the pre-mating, gestation and

lactation periods, resulting in significantly lower mean bodyweights at the end of the pre-mating period and during the gestation and lactation periods). At 200 ppm a severe to very severe lymphoid depletion has been observed and a moderate to severe lymphoid depletion at 30 ppm; findings at 30 ppm were apparent in the majority of pregnant females but were not observed in non-pregnant rats. Lymphoid depletion was characterised by decreased size of the thymic lobules due to extensive loss of cortical and medullary lymphocytes. The border between the thymus cortical and medullary areas was therefore indistinct. In the most severe cases, the cortex was very small or partially absent. The remaining cells visible in the cortical areas were mainly lymphoblasts and reticular epithelial cells. A NOAEL of 5 ppm (0.4 mg/kg bw/d) can be determined for thymus histopathology in this study.Thymus was not investigated in males.

In the 28-day study with DBTC application at a dietary concentration of 0, 50 and 150 ppm to rats and mice (Seinen & Vos., 1977) no effects were observed in treated mice. Mortality occurred in rats at 150 ppm. Thymus size, thymus and spleen weights were markedly reduced in rats at 50 and 150 ppm. Effects on the lymphoid organs were characterised by a marked degree of lymphocyte depletion, with no evidence of cell destruction. A NOAEL for immune system effects of <50 ppm (equivalent to approximately 2.5 mg/kg bw/d) can be determined for this study. Details on body weights and relative organ weights are listed in the table below.

Table 53: Body weight and relative organ weights (means ± SD) (rats) (Seinen & Vos., 1977)

Dietary level (ppm)	Body weight (g)	Liver (g/kg)	Thymus (g/kg)	Spleen (g/kg)	Popliteal lymph nodes (mg/kg)
Males					
0	115.3 ± 3.9	42.5 ± 0.9	3.77 ± 0.19	3.62 ± 0.20	73 ± 10
50	107.7 ± 2.4*	42.9 ± 0.7	1.70 ± 0.11*	3.01 ± 0.13*	57 ± 3*
150	92.1 ± 4.5*	49.3 ± 1.0*	1.04 ± 0.12*	2.41 ± 0.11*	52 ± 6*
Females					
0	106.4 ± 2.3	49.7 ± 0.9	3.76 ± 0.15	3.20 ± 0.12	62 ± 4
50	102.2 ± 0.9*	49.3 ± 1.3	1.79 ± 0.10*	2.39 ± 0.12*	50 ± 3*
150	86.0 ± 7.0*	50.8 ± 2.3	1.20 ± 0.18*	2.18 ± 0.08*	52 ± 6*

*Significantly different to controls, *p <0.001 Students t-test*

In an older study in which DBTC was applied to rats using exposure periods of up to 6 months (Barnes & Stoner, 1958) at dietary concentrations of up to 100 ppm, mortality was reported at 75 and 100 ppm (6 months administration). Pathology of the liver is reported in all treated groups; it is unclear whether the thymus or other immune tissues were investigated in this study. Reduced weight gain and food consumption were reported at all dietary concentrations (≥20 ppm) (details see Appendix I: Chapter 2.3.1.6).

Thymus parameters, such as thymus weight and histopathology were investigated in a guideline-compliant rat prenatal developmental toxicity study (Study report, 1994) using DBTC at concentrations of 0, 1, 2.5 5 and 10 mg/kg bw/d. Thymus weight was reduced at 10 mg/kg bw/d; histopathology showed atrophy of the thymus at 10 mg/kg bw/d and to a lesser extent at 2.5 and 5 mg/kg bw/d. A NOAEL of 1 mg/kg bw/d can therefore be determined for thymus effects in this study. Reduced weight gain and food consumption were observed at 10 mg/kg bw/d.

In an additional developmental toxicity study in the rat (Farr et al., 2001) maternal thymus weight was investigated at dose levels of 0, 1, 2.5, 5 and 10 mg/kg bw/d DBTC. Reduced maternal weight gain and food consumption were seen at the highest dose level of 10 mg/kg bw/d; reduced thymus weight was also seen in this group. Details are provided in the table below.

Table 54: Maternal weight gain, food consumption and maternal thumus weight (Farr et al., 2001)

Dose level (mg/kg bw)	0	1.0	2.5	5.0	10.0
Maternal weight gain (g) GD 6-16	67.2	67.3	64.9	67.4	55.7*
Maternal food consumption (g) GD 6-16	25.5	25.5	25.2	25.9	23.7*
Maternal thymus weight (mg)	371	366	409	339	287**

DeWitt et al. (2005) investigated the immune responses of DBTC exposure in drinking water (dose levels up to 2.5 mg/kg bw) in adult rats. No clear effects of treatment were seen on antibody production, DTH (delayed type hypersensitivity) response or NK cell activity. Different results for antibody responses in male rats were obtained in two experimental replicates. In the first replicate, IgG was elevated at the highest dose level whereas in the second replicate, IgM was suppressed. No statistically significant effects were seen on bodyweight. Absolute and relative thymus and spleen weights were unaffected by treatment.

In a further study by DeWitt et al. (2006) pregnant rats were given drinking water containing 0,10,25 mg/L of DBTC from GD 8 through weaning of pups, group of litters were gavaged with 0, 1.0, 2.5 mg/kg bw/d DBTC for 10 times. No effects were observed on DTH and antibody synthesis. NK cell activity in the 10mg/l DBTC maternal group was greater in male offspring than in female. Thus, the data of DeWitt suggest no immunological effects, however the dose levels used in these studies were relatively low (up to 2.5 mg/kg bw/d for direct exposure of offspring, and 4.4 mg/kg bw dams).

Further non-guideline conform studies were carried out in order to investigate the toxic mechanism of DBTC. A study in SCID mice engrafted with human thymus fragments (de Heer et al., 1995) shows a reduction in thymus cortex size following treatment with DBTC. Snoeij et al. (1989) demonstrated that a single gavage exposure of rats to 15 mg/kg bw DBTC is sufficient to result in a marked, but reversible reduction in thymus weight and cellularity (see also Chapter 9.13). Thymus weight reduction was apparent from day 2 following treatment. The reduction was most marked at day 4 but was reversible by day 9. In the same study the numbers of large cells were reduced from day 1 after DBTC application; whereas small and intermediate cells were reduced from day 3 following treatment. The cell populations had recovered by day 9. The incorporation of radioactivity into DNA, RNA and protein precursors was only reduced on days 1 and 2. The authors conclude that DBTC causes thymus atrophy due to a selective reduction in the number of rapidly proliferating lymphoblasts in the first 2 days after dosing.

In the study of Seinen et al. (1977) a significant delay in allograft rejection caused by administration of 150 ppm DBTC for six weeks was reported. No other measures of immune function were affected. The authors therefore conclude that DBTC has a selective inhibitory effect on T-lymphocyte activity.

The key studies for STOT RE classification are the guideline-comparable 28-day study (Seinen & Vos (1977; Peninks & Seinen (1982)) and the OECD 421 screening study (Unpublished report, 2003). An extrapolation of equivalent effective dose of toxicity studies is presented in Table 55.

It needs to be considered that all the studies were performed with DBTC. DBTO is hydrolysed in the mammalian stomach to form DBTC (see category approach Chapter 9.2). The toxicity of DBTO is comparable to DBTC as seen in the comparative toxicity study of di-n-butytins of Noda et al. (1993). Therefore, the guidance values for classification of DBTC can be taken as basis for classification of DBTO.

Table 55: Extrapolation of equivalent effective dose for toxicity of selected studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Seinen & Vos (1977) Penninks & Seinen (1982)	2.5 mg/kg bw/d (LOAEL)	28 days	0.8 mg/kg bw/d	STOT RE1
Unpublished report, (2003)	1.7-2.4 mg/kg bw/d (LOAEL)	~56 days	1.25 mg/kg bw/d	STOT RE1

10.12.2 Comparison with the CLP criteria

According to CLP Regulation for the purpose of classification for repeated dose toxicity, substances are allocated to one of two categories (Table 3.9.1., CLP Regulation). Guidance values to assist in Category 1 (Table 3.9.2) and Category 2 (Table 3.9.3) are provided.

Category 1	Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: — reliable and good quality evidence from human cases or epidemiological studies; or — observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.
Category 2	Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. In exceptional cases human evidence can also be used to place a substance in Category 2.

The repeated dose and other relevant rodent studies clearly demonstrate that DBTO and category member DBTC have the potential to cause severe effects on the thymus (lymphoid depletion) following single and repeated exposure (extrapolated effective dose of 90 day exposure: 0.8-1.25 mg/kg bw/d).

DBTO needs to be classified for STOT RE 1, since the effective dose levels are well below the guidance values (≤ 10 mg/kg bw/day) established for STOT RE 1 classification. Furthermore, a mechanistic study in SCID mice grafted with human thymus fragments also reported effects, indicating that DBTC is also likely to have similar effects in humans.

According to CLP Regulation the observed effects on the thymus are considered to represent a significant health effect.

10.12.3 Conclusion on classification and labelling for STOT RE

Based on the thymus effects seen in a study with DBTO and in several studies with DBTC (read across substance) classification for STOT RE in Category 1 (H372: causes damage to the immune system) is considered to be appropriate for DBTO.

10.13 Aspiration hazard

Not evaluated.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated.

13 ADDITIONAL LABELLING

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14 REFERENCES

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15 ANNEXES

Annex I – Detailed study descriptions

see separate document

Annex II – Confidential information on study references