Committee for Risk Assessment

RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

Dibutyltin oxide

EC Number: 212-449-1
CAS Number: 818-08-6

CLH-O-0000007033-84-01/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

16 September 2021

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: **-**

Contact details for dossier submitter:

Environment Agency Austria, Spittelauer Lände 5, A-1090 Vienna

on behalf of the Austrian Competent Authority (Austrian Federal Ministry for Climate Action, Environment, Energy, Mobility, Innovation and Technology, Radetzkystraße 2, 1030 Vienna, Austria

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

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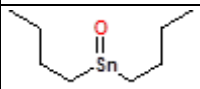
ABBREVIATIONS

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.

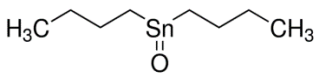
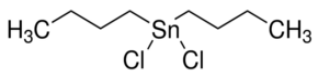
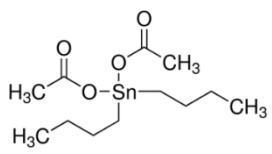
The category approach has already been used for other category members in order to propose harmonised classification and labelling for Repr. 1B; H360FD, STOT RE 1 and also for Muta 2. [Dibutyltin dilaurate (DBTL), Dibutylbis(pentane-2,4-dionato-O,O´)tin (DBTP), Dibutyltin di(acetate) (DBTA), Dibutyltin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

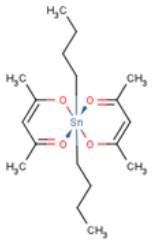
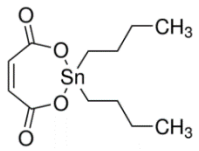
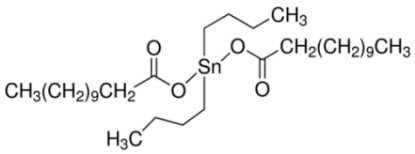
Beside these endpoints the C&L proposal also covers the following endpoints: acute toxicity (oral, dermal route), skin corrosion/irritation, serious eye damage/eye irritation and STOT SE.

RAC general comment

The dossier submitter (DS) proposed to classify dibutyltin oxide (abbreviated throughout this document as DBTO) for acute oral toxicity, skin corrosion, serious eye damage, mutagenicity, STOT RE and reproductive toxicity. In addition to studies performed with DBTO itself, reference was made to studies performed with the following substances as part of a read across, category approach: DBTC, DBTM, DBTA, DBTL and DBTP (see Table below for the full substance names and structures).

***Table**: Substance characteristics*, adapted from Table 10 in the CLH report*

Substance	EC # / CAS #	Structure	Purity (studies)	Purity / Impurity details (REACH Dossier)
Dibutyltin oxide (DBTO)	212-449-1 / 818-08-6		Not reported	>97.5% No further details (monoconstituent substance)
Dibutyltin dichloride (DBTC)	211-670-0 / 683-18-1		96-99.7% where reported for studies	93-100% Monoconstituent substance; tributyltin chloride (0.25-1%) in some sources
Dibutyltin (di)acetate (DBTA)	213-928-8 / 1067-33-0		Not reported	No further details (monoconstituent substance)

Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	245-152-0 / 22673-19-4		>92%	>92% No further details (monoconstituent substance)
Dibutyltin maleate (DBTM)	201-077-5 / 78-04-6		Not reported	No further details (monoconstituent substance)
Dibutyltin dilaurate (DBTL)	201-039-8 / 77-58-7		Not reported	95-100% Monoconstituent substance; potenital presence of tributyl(lauryloxy) stannane

* The structures are arbitrary as tin(IV) compounds may adopt various geometries and coordination numbers depending on the ligands.

The DS proposed to form this category for read across purposes based on the common hydrolytic behaviour of its members. According to the DS proposal, the result of the hydrolysis is a common tin compound that is responsible for the toxic effects observed. In addition, since all category members hydrolyse at neutral or low pH, it demonstrates that systemic exposure to the intact substances, following oral administration, was unlikely.

In the initial hydrolytic studies, an indirect detection method was used that could not determine the exact tin species that was formed; therefore, it was thought that dialkyltin compounds form DBTC after hydrolysis. However, recent *in vitro* hydrolysis studies which used 119Sn-NMR spectroscopy showed that both DBTC and the related compounds DBTP and DBTM form the distannoxane $ClBu_2SnOSnBu_2Cl$.

The CLH dossier of DBTO includes one *in vitro* gastric simulation study, performed with GC-FPD, which showed a conversion under simulated gastric conditions to DBTC. It is noted that this detection method cannot distinguish whether the distannoxane $ClBu_2SnOSnBu_2Cl$ is formed, however considering the behaviour of the other category members, this is highly likely.

Moreover, the available developmental toxicity studies with DBTO itself shows effects very similar to those induced by other category members, and in particular DBTC.

Considering the metabolism studies and similar toxicological profiles the RAC agrees with the read across approach proposed by the DS. In accordance with this approach, the classification proposal for DBTO for mutagenicity, STOT RE, and reproductive toxicity is mainly based on studies performed with DBTC, and supported by studies with related dibutyltin compounds. This is also consistent with the RAC opinions of dibutyltin dibutyltin di(acetate) (DBTA), dibutyltin maleate (DBTM), dilaurate (DBTL), dibutylbis(pentane-2,4-dionato)-OO')tin (DBTP) and dibutyltin bis(2-ethylhexanoate) (DBTE).

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 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	Catalyst, process regulator
Uses at industrial sites	Use as intermediate PC 19: intermediate Industrial use of products containing substance as a catalyst/process regulator	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	
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 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	Catalyst, process regulator
Consumer Uses	Consumer use of products containing substance as a catalyst/process regulator PC 1: Adhesives, sealants PC 9a: Coatings and paints,	Catalyst, process regulator

	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	
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)

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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 (TBTC) was identified as impurity in small amounts)	Naßhan, 2016 [Annex I, 1.1.4]
Combination *in vitro* and *in vivo* study			

Method	Results	Remarks	Reference*
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			
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.

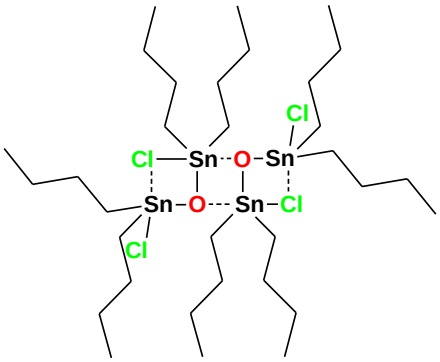
9.1.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification

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

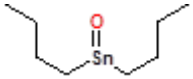
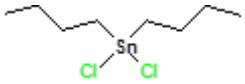
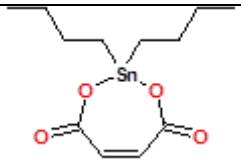
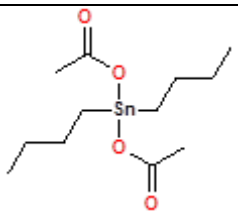
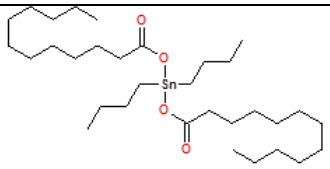
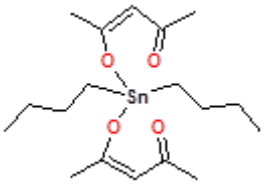
The read-across approach is limited to endpoints where toxicological data generated in experimental animal species *in vivo* by oral administration (e.g., *in vivo* mutagenicity, repeated dose toxicity, reproductive toxicity) are available. It is not applicable to studies using dermal or inhalation exposures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

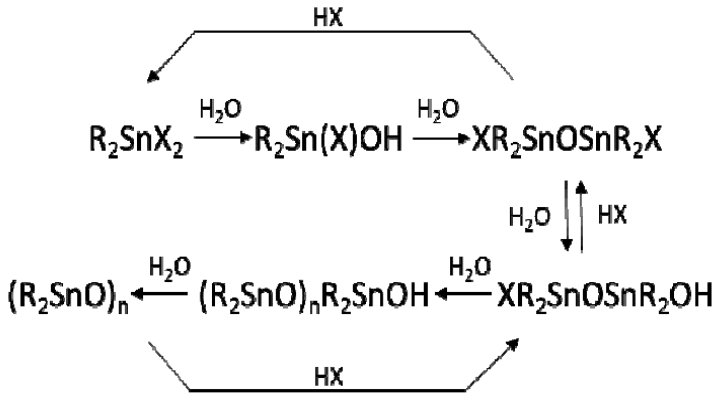


Figure 2. Simplified hydrolysis scheme for dialkyltins (Davies, 2004; Aylett et al., 1979) (reaction scheme as depicted in ECHA, 2016).

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).

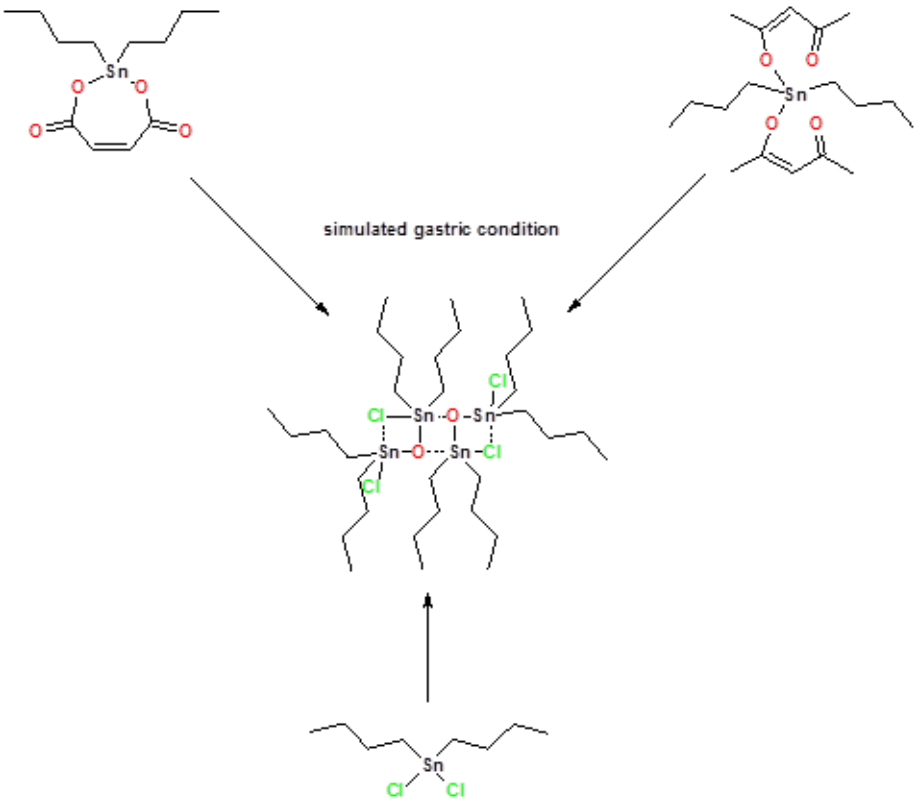


Figure 3. Overview of the hydrolysis of DBTM, DBTP and DBTC as determined in recent studies (Naßhan, 2015, 2016, Umweltbundesamt, 2019), which is in accordance with well established tin chemistry (Davies, 2004; Aylett et al., 1979).

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 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					Corrosive *in vivo*	
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	Muta. 2; H341 Repr. 1B; H360FD	Adopted RAC opinion (ECHA, 2017)	No harmonised Classification; CLH

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIBUTYLTIN OXIDE

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)**
			Acute Tox. 2*, H330 Skin Corr. 1B, H314 Muta. 2, H341 Repr. 1B, H360FD STOT RE 1, H372 (immune system) Aquatic Acute 1, H400 Aquatic Chronic 1, H410	STOT RE 1; H372 (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.	proposal under 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.

RAC evaluation of acute toxicity - oral

Summary of the Dossier Submitter's proposal

There are 10 acute oral toxicity studies included in the CLH dossier, all performed with DBTO itself. One study was a modern OECD test guideline (TG) 423 study (GLP), four were OECD TG 401 studies performed in the period 1978-1983 and five were older non-guideline studies.

The OECD TG 423 study resulted in an LD_{50} of 500 mg/kg bw (Anonymous, 2019). Two of the older, non-guideline 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). Two OECD TG 401 studies reported lower LD_{50} values of 172 (121-240) mg/kg bw and 260 (209-311) mg/kg bw, respectively (Anonymous, 1983 and Anonymous, 1978). Higher values were reported in Anonymous (1980a, b) with a value of > 794 mg/kg bw and Anonymous (1971) with a value of > 10000 mg/kg bw.

No explanation for the difference in outcomes was provided in the dossier. There was no relationship with sex of the animals, or the vehicle used. It was also noted that for none of the presented studies detailed information on the test substance, or on impurities that could explain the differences in outcome, was available.

Due to these considerations, the DS proposed to use the lowest LD_{50} value of 172 (121-240) mg/kg bw (males (m)/females (f)) justifying classification as Acute Tox. 3; H301, with an ATE value of 172 mg/kg bw.

Comments received during consultation

Two comments were received from Member State Competent Authorities (MSCAs) who agreed with the proposed classification and ATE. Six comments submitted by industry representatives disagreed with the classification in Category 3 and instead considered that classification in Category 4 based on the most recent OECD TG 423 study was more appropriate. The reasoning was that the impurities in the test material of the Anonymous (1983) study were unknown and the newer study is performed and reported according to modern standards. For these reasons, all studies except Anonymous (2019) and Anonymous (1980b) were given Klimisch scores of 4 by the registrants.

The DS replied that the OECD TG 401 study used as key study in the classification proposal was a well-documented guideline study. The study was also judged as valid and used as key study in the OECD SIDS Dossier for DBTO (2008). Moreover, the purity of the test substance was missing for all studies, including the recent OECD TG 423 study. For these reasons the DS disagreed with the registrants and considered that the outcome of the OECD TG 401 study cannot be dismissed.

Assessment and comparison with the classification criteria

A large amount of data is available on the acute oral toxicity of DBTO. Of the ten studies, five were performed according to OECD TGs. The LD_{50} values reported span a wide range of 172 to >794 mg/kg bw, excluding the oldest non-guideline studies, or 60-10 000 mg/kg bw if all studies are included. Gross necropsy revealed severe damage to the digestive system in deceased animals.

There was no clear link between the outcome of the studies and the rat strain or vehicle used. Both male and female rats were included and there was no notable difference in sensitivity. As no information was given on the impurities in the test material for any of the studies including the more recent study, it cannot be evaluated whether this was a factor of influence or not.

The guideline study with the lowest LD_{50} of 172 mg/kg bw (m/f) was an OECD TG 401 study in Tif:RA1f (SPF) rats, with six doses ranging from 50 to 525 mg/kg, with 5 animals/sex/dose.

RAC agrees with the DS that the limitations in reporting on the test material are not sufficient reasons to discard the outcome of this study. For all other parameters, the study was well reported and showed a very consistent pattern over the entire dose range.

In conclusion, considering the LD_{50} of 172 mg/kg bw (m/f), **RAC considers that classification of DBTO as Acute Tox. 3; H301 (Toxic if swallowed) is warranted** rounding down the **ATE value to 170 mg/kg bw**.

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

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.

RAC evaluation of acute toxicity - dermal

Summary of the Dossier Submitter's proposal

Two acute dermal toxicity studies were performed with DBTO, an OECD TG 402 study in rats (Anonymous, 2010a) and an older non-guideline study in rabbits (Anonymous, 1975). In both studies, local irritation was observed, but there were no signs of systemic toxicity or deaths up to the top dose of 2000 mg/kg bw. The DS proposed no classification for acute dermal toxicity.

Comments received during consultation

One MSCA expressed support for no classification.

Assessment and comparison with the classification criteria

There is one guideline study in rats and a non-guideline study in rabbits available investigating acute dermal toxicity of DBTO. Both studies were performed with a top dose of 2000 mg/kg bw and no mortality was observed in either one.

As the criteria for classification are not fulfilled, **RAC considers that no classification for Acute toxicity by the dermal route is warranted**.

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	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	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
			Only 4h exposure: DBTO was moistened with deionized water Removal: wiped with tap water	Not fully reversible within 14 days Edema score 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.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The CLH report includes three skin irritation studies, an OECD TG 404 study, a non-guideline study and a report from the OECD SIDS (OECD Screening Data Set). In addition, the findings from the two acute dermal toxicity studies were used as supporting evidence.

The OECD TG 404 study (Anonymous, 1994) in rabbits was negative for corrosivity under occlusive conditions for 3 minutes and 1 hour. After exposure for 4 hours under semi-occlusive conditions, DBTO induced erythema and oedema with mean (24, 48, 72h) scores of 1.78 and 0.72 for erythema and oedema, respectively. Maximum scores for both effects were 2. Erythema was not fully reversible within the observation period of 14 days as very slight to slight erythema were present on all sites.

In the non-guideline study (Anonymous, 1975) with three rabbits exposed for 24 hours, DBTO induced third-degree burns in two animals and second-degree burns in one animal. Mean scores of 4 were assigned for both erythema and oedema.

In the study cited by OECD SIDS (2008), four rabbits were exposed for 3-4 hours under semi-occlusive conditions, resulting in reddening and swelling with induration of the skin in all animals on days 5-9 and induration, demarcation and skin necrosis on days 6-10.

Based on the severe irritation, including skin burns, observed in the skin irritation study by Anonymous (1975) and in the acute dermal toxicity studies, the DS proposed classification of DBTO as Skin Corr. 1.

Comments received during consultation

Comments were received from six industry representatives and two MSCAs. All industry representatives objected to the proposed classification in Category 1 as no corrosion was observed in the OECD TG 404 study and the older skin irritation study, and also since the acute dermal toxicity studies used 24-h rather than 4-h exposure.

One MSCA preferred a classification in Category 2 rather than Category 1 as corrosion was only observed after 24-h occlusive exposure, which is not in line with the CLP criteria.

The other MSCA supported the proposed classification and suggested that as the effects in Anonymous (1994) occurred following 4-h exposure sub-category 1C could be considered.

The DS replied that although the OECD TG 404 study supports classification in Category 2, the other studies provide sufficient evidence for Category 1. In particular it was noted that as rats are less sensitive than rabbits, skin corrosivity in a rat dermal toxicity test indicates that a Category 1 classification is justified. It was noted that as the only study with 4-h exposure did not show corrosivity, it cannot be used to derive a sub-categorisation.

Assessment and comparison with the classification criteria

Three skin irritation and/or corrosion studies in rabbits are available, all studies performed with DBTO. The skin irritation studies included an OECD TG 404 study (Anonymous, 1994) and two older studies, for one of which there was only a secondary source. Of the two acute toxicity studies, one was performed in rats and one in rabbits. The studies are summarised in the table below.

***Table**: Overview of the results from the skin irritation and dermal toxicity studies. All studies were performed with DBTO.*

Method, test guideline, deviations if any	**Species, strain, sex, no/group**	**Dose levels, duration of exposure**	**Results -Observations and time point of onset -Mean scores/animal -Reversibility**	**Reference**
OECD TG 404 GLP	Rabbit, New Zealand White N=6 (3m+3f)	0.5 g/side Duration: 3 min / 60 min: for corrosion testing, occlusive 4 h: for irritation testing, semi-occlusive 4 h-exposure: DBTO was moistened with deionised water Purity: 98.5% Removal: wiped with tap water	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. Oedema score: 24h (mean 1, max 2) 48h (mean 0.17, max 1) 72h (mean 0, max 0) Fully reversible	Anonymous, 1994
Primary skin irritation study	Albino rabbits	500 mg un-diluted Exposure: 24 h Occluded, abraded and intact skin Observation: 24 h, 72 h	Extremely irritating. Third-degree chemicals burns (24/48h): 2/3 Second-degree chemical burns (24/48h): 1/3	Anonymous, 1975 (OTS0570737)
-	Rabbit N=4	500 mg / 0.19 mL peanut oil	Irritating. Reddening and swelling with	OECD SIDS, 2008

GLP	(2m+2f)	Semi-occlusive Exposure 3-4 h	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.	Secondary source
OECD TG 402 GLP	Rat, Wistar N=5m/5f per dose	2000 mg/kg bw Semi occlusive, 24 h Removal of residual test material with arachis oil (vehicle)	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	200, 500, 2000 mg/kg bw slurry in 3% (w/v) aqu. methylcellulose Abraded skin	24 h: red, well defined erythema, moderate to severe oedema and second-degree burns (all animals) Day 7: well defined erythema, mild oedema, escharosis, and wrinkling Day 14: escharosis, and severe desquamation	Anonymous, 1975 (OTS0570737)

According to the CLP criteria, all existing human and animal data should be considered, including acute dermal toxicity studies provided that the dilutions used, and species tested, are equivalent. In the evaluation, the method of application, exposure time, and species should be considered due to the differences between studies performed under different guidelines.

For the OECD TG 404 study with six rabbits (Anonymous, 1994), the following criteria apply with regard to severity:

a. Classification as skin corrosive – Category 1 if destruction of skin tissue (visible necrosis through the epidermis and into the dermis) occurs in at least one animal after exposure up to 4 hours.

b. Classification as skin irritant – Category 2 if at least 4 out of 6 rabbits show a mean score per animal of ≥ 2.3 ≤ 4.0 for erythema/eschar or for oedema.

In the short exposure periods of 3 min and 1 h, no corrosion was observed. No individual scores after 4 hr exposure were included in the CLH report, but the max scores were 2 for both erythema and oedema. This means that the severity criterium for irritation was not met.

Classification for irritation can also be warranted due to persistence of the effects:

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

Erythema and desquamation persisted over the duration of the study of 14 days, while oedema was reversible after 72 h. Although no individual data are given it was described by the DS as "*very slight to slight erythema were present on all sites at study termination on day 14."* Considering the erythema persisted to the end of the observation period, the criterium for

classification based on irreversibility is met.

The second study was an older non-guideline skin irritation study (Anonymous, 1975) in which three rabbits were exposed for 24 h, on occluded, abraded and intact skin. All three rabbits showed chemical burns and scores of 4 for erythema and oedema after 24 h and 72 h.

The interpretation of this study is more difficult, as the exposure is longer and it takes place under more severe conditions than in the guideline studies. The Guidance on the application of the CLP criteria (CLP guidance, ECHA, 2017) states the following on the interpretation of such studies: "*Calculation of mean scores should normally be restricted to the results obtained from intact skin. In case of pronounced responses at the 72 h-time point an expert judgement is needed as to whether the data is appropriate for classification.*" It is not entirely clear from the available information whether one or two rabbits with intact skin were included. On the other hand, all rabbits showed strong irritation reactions. Overall, this study supports classification for irritation, but is of insufficient quality to be used as the key study.

The third irritation study was cited from a secondary source (OECD SIDS, 2008). Four rabbits were exposed for 3-4 h under semi-occlusive conditions. No scores were given for erythema or oedema, but these effects were described after 5-9 days, increasing in severity at days 6-10 to induration, demarcation and skin necrosis. The effects were no longer observed after 30 days. Due to the limitations in the available information, it is difficult to judge the reliability of this study. Remarkable in this case is the apparent delay in the induction of irritation. Although this was not noted in the other irritation studies with DBTO, two dermal irritation studies in the CLH dossier for DBTM showed a very similar delay. Again, this study can only be used as supportive information as it is currently presented.

In addition to the skin irritation studies, the effects observed in the two acute dermal toxicity studies in rats and rabbits are relevant for the evaluation of skin irritation. Both studies reported clear effects indicative of irritation and/or corrosion after 24-h exposure. It should be noted that the rabbit skin was abraded, which resulted in effects indicative of corrosion. Unfortunately, no scoring was provided and there is no information on the effects after 4 h of exposure.

In conclusion, only one guideline study with 4 h exposure is available, which indicates some, but not severe skin irritation, that did not fully recover within 14 days. A secondary source noted more severe effects after 4 h, but the information was very limited and there was a delay in effects not observed in any other study. Several studies with longer exposure times showed strong irritation/corrosion but did not include shorter observation periods and some used abraded skin. Taking into account these factors, **RAC considers that classification as Skin Irrit. 2; H315 (Causes skin irritation) is warranted**.

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	Rabbit,	DBTO	0.1 ml (93 mg)	Corrosive	Anonymous,

GLP	New Zealand White N=1		Test material was not rinsed off	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	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 (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 (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.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

Three *in vivo* eye irritation tests in rabbits were included as well as one *in vitro* SkinEthic Reconstituted Human Corneal model.

In an OECD TG 405 (Anonymous, 2010b), corneal opacity, blepharitis, and redness, chemosis and discharge of the conjunctiva were seen in one rabbit after administration of 93 mg DBTO to one eye. The effects increased in severity over time and were not reversible within 14 days.

A second non-guideline *in vivo* study in three rabbits (Anonymous, 1975) reported chemical burns and corrosion in all animals after exposure to 100 mg, without reversibility in 14 days. 29.7

The third study was a description from the SIDS dossier (2008), in which 72 mg was instilled into the eyes of six rabbits. The maximum average score was 19.5 (of 110 possible) at 72 h. Secondary effects like corneal oedema, hypopyon, corneal neovascularisation (including pannus) and the formation of scleral vesicles were described. At 21 days, corneal irritation in two animals was still present.

The *in vitro* study (Anonymous, 2010c) was based on transformed human keratinocytes that form a corneal epithelial tissue. 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). Exposure was 10 min with 30 mg DBTO. The resulting tissue viability was 93.6% vs 100% in the negative control. As the threshold for irritation is 60%, DBTO was not irritating. It was noted that the test was not in line with the OECD TG 492, and amongst others the exposure time was too short.

As DBTO induced irreversible, worsening effects on cornea, iris and conjunctiva in a recent guideline study with mean scores (24, 48, 72 h) of 0.67, 1, 2, 2.33 for cornea opacity, iris, conjunctival redness and chemosis, respectively. Severe eye effects were also observed in an older eye irritation study and a secondary source.

Based on these effects, the DS proposed to classify DBTO as Eye Dam 1. It was noted by the DS that due to their proposed classification for Skin Corr. 1, classification for Eye Dam. 1 is already implicit.

Comments received during consultation

Two MSCAs and one industry representative commented specifically on the proposal for eye damage. All agreed that the available data support classification in Category 1. However, one MSCA and the industry representative disagreed with the classification for Skin Corr. 1 and noted that if DBTO is not classified for skin corrosion, the automatic classification for Eye Dam. 1 should be removed.

Assessment and comparison with the classification criteria

There are three *in vivo* studies in rabbits available, including one guideline study, which consistently show that DBTO induces irreversible eye damage.

According to the criteria, a substance should be classified in Category 1 if it 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 h after installation of the test material.

Only the study cited in the OECD SIDS had an observation period of 21 days, in which no full reversion was observed in two animals. However, the other two studies (Anonymous, 1975 and Anonymous, 2010b) showed such severe effects after 14 days that reversibility was deemed highly unlikely. The effects observed in Anonymous (1975) also met the (b) criterium for severity.

As both criteria are met, **RAC concludes that DBTO warrants classification as Eye Dam. 1; H318 (Causes serious eye damage)**.

As no classification for skin corrosion is proposed, a separate classification for eye damage is necessary.

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.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Only one *in vitro* gene mutation study in bacteria has been performed with DBTO itself, and it was negative. The evaluation of mutagenicity was thus based on studies with DBTC as well as on one study with DBTDL. These studies have been evaluated previously for other category members, most recently for DBTA (RAC opinion, 2020). Apart from the bacterial reverse mutation assay, no new information was added in this evaluation.

The study with DBTDL investigated *in vivo* DNA damage in rat cerebral cortical cells and found a significant, dose-dependent increase (Jin *et al*., 2012).

Twelve *in vitro* studies and two *in vivo* studies with DBTC are presented in the CLH dossier. A GLP-compliant (similar to OECD TG 473) *in vitro* mammalian chromosome aberration test (± S9-mix) was reported with positive results (Anonymous, 1990a). Two bacterial reverse mutation tests were reported with one demonstrating positive results (no metabolic activation applied) (Hamasaki *et al*., 1993) and the other presenting negative results (± S9-mix) (Anonymous, 1979). A CHO/HGPRT gene mutation assay (non–guideline, GLP not specified; Li *et al*., 1982) showed positive results (no metabolic activation applied), whereas an OECD TG 476-compliant *in vitro* mammalian cell gene mutation test using Chinese hamster lung fibroblasts (V79) showed negative results (± S9-mix) (Lang and Schmitt , 1989). Furthermore, a study with bacterial SOS-assay and a bacterial rec-assay (Hamasaki *et al*., 1992) showed positive results from both assays (no metabolic activation applied).

In addition, various non-guideline, non-GLP studies were included in the CLH dossier, reporting both positive and negative results. DBTC was shown to induce breakage of naked λ-DNA (Hamasaki *et al*., 1995), to form condensates with DNA (Piro *et al*., 1992), and to affect spindle structure during mitosis in V79 Chinese hamster cells (Jensen *et al*., 1991a), but did not affect chromosomal length in human peripheral lymphocytes (Jensen *et al*., 1989), nor did DBTC induce hyperdiploid cells (aneuploidy) in human peripheral lymphocytes (Jensen *et al*., 1991b).

In the OECD TG 474 and GLP-compliant *in vivo* micronucleus study, mice received DBTC via single oral gavage. Dose levels of 2, 10 or 50 mg DBTC/kg bw were applied (vehicle: corn oil). A statistically significant increase in the incidence of micro-nucleated polychromatic cells was observed in bone marrow 48 h and 72 h after exposure of mice to DBTC at 50 mg/kg bw, with effects more clearly seen in female compared to male animals. No positive result was obtained upon DBTC-exposure at the post-treatment time-interval of 24 h.

The positive mutagenic result for DBTC was not confirmed in a second *in vivo* mouse micronucleus study. Mice received a single oral gavage exposure of DBTC of 0, 50, 100 or 200 mg/kg bw (vehicle: arachis oil). In this second micronucleus test DBTC did not show any evidence of mutagenic potential up to the (toxic) dose level of 200 mg/kg bw as measured at 24 h, 48 h and 72 h post-treatment.

Overall, for DBTC there was a mixed outcome both for *in vitro* and *in vivo* studies, but in

general most studies were positive.

The DS concluded that given the absence of germ cell mutagenicity studies for DBTO or other members of its category, there is insufficient evidence to warrant classification in Category 1B. There is a positive *in vivo* somatic cell mutagenicity test as well as supportive evidence from positive results from *in vitro* mutagenicity/genotoxicity tests with DBTC, which has been previously classified as Muta. 2.

The DS proposed to classify DBTO also as Muta. 2 based on a category approach.

Comments received during consultation

Three MSCAs expressed their support for the proposed classification for Muta. 2 based on the category approach.

Six industry representatives commented. They presented arguments against the use of the category approach and using read across between category members, arguing that new hydrolysis studies show that a dimer (DBDTC distannoxane dimer) is formed rather than DBTC itself (Ghobrial *et al*., 2019; Munschi *et al*., 2010; Patel *et al*., 2009; Alwyn, 2009). According to the comments, the dimeric structure is the only metabolite of the gastric hydrolysis and has a molecular weight of 1089.4 Dalton. With this high molecular weight, the dimeric distannoxane is by far too heavy to be biologically active and have a low reaction potential because the molecules are too large to pass through biological membranes, limiting their bioavailability. They also noted that further testing related to the read across is planned. The DS replied that the available information indicates that common metabolites/intermediates are formed in vivo, and affect common biological targets, which supports the applicability of a read across from category members (e.g., DBTC) to DBTO for oral toxicity studies (mutagenicity, toxicity to reproduction, specific target organ toxicity repeated exposure, specific target organ toxicity single exposure). The DS concluded, that based on available information, the read across is robust and it is valid to consider data of category members to read across between these endpoints. The DS also took note of the new studies.

Assessment and comparison with the classification criteria

The classification proposal for mutagenicity is based solely on the category approach, as the only available study with DBTO itself is a negative *in vitro* gene mutation study in bacteria. This result is in line with results of category members, which do not indicate mutagenic effects in bacterial mutagenicity assay either. RAC agrees with the DS replies on the use of the category approach. As DBTO forms at least in part the same metabolite as DBTC, RAC considers the proposed read across valid for germ cell mutagenicity. Also see 'RAC general comment'.

Overall, the results of the *in vitro* tests performed with DBTC were variable with both positive and negative results. Additionally, two *in vivo* mouse micronucleus studies with DBTC are presented in the CLH dossier. One study showed positive effects at the highest dose only (50 mg/kg bw) (Anonymous, 1991), whereas a similar study did not show positive effects at doses up to 200 mg/kg bw (Anonymous, 1991).

Both mouse micronucleus studies included a sufficient number of animals. Positive as well as negative controls were included with appropriate results in both studies, and toxicity was observed in both studies. After full evaluation, no clear explanation could be found for the discrepancy in results. Without any reason to discard one of the two *in vivo* mouse

micronucleus studies, the positive result of the first study is taken forward for the evaluation.

In vivo mammalian germ cell mutagenicity tests are not available for DBTO or DBTC. However, a positive result was obtained from a well-performed OECD TG- and GLP-compliant *in vivo* mouse micronucleus test with DBTC. The positive result is supported by indications from one *in vivo* test with DBTDL (*in vivo* Comet assay, non-GLP). Further, the formation of micronuclei in the bone marrow suggests systemic availability.

Although distribution into testes/ovaries can be expected, no experimental evidence is available which demonstrates a direct interaction of the substance or its metabolite with the genetic material of germ cells. Therefore, RAC considers classification in Category 1B not appropriate.

Taking all available data into account, RAC concludes that **DBTO warrants classification for germ cell mutagenicity as Muta. 2; H341 (Suspected of causing genetic defects).**

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	Unpulished report, 2003 (REACH registration, DBTO) [Annex I, 2.2.1.1]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		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)	
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]
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	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	Ema et al., 2003 [Annex I, 2.2.1.4]

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
injection of 2 mg progesterone GD 0-8			without progesterone ; 10.5%, 25.9% and 60.6% with application of progesteron	
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) Sprague Dawley (25 females/group)	DBTO Purity: > 97% 0, 0.75, 3 and 6 mg/kg bw/d GD 0-19, gavage	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. 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	Unpublished report, 2017 [Annex I, 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%)	Noda et al., 1993 [Annex I, 2.2.1.6] (REACH registration, DBTO)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		was observed. A NOAEL cannot be determined for this study.	
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 mg/kg bw/d) LOAEL (reproduction): 200 ppm, (12.0-15.4 mg/kg bw/d)	Unpublished report, 2003 [Annex I, 2.2.1.1] (REACH registration, DBTO)
OECD 414 (Prenatal development 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	Farr et al., 2001 [Annex I, 2.2.1.8]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		toxicity)	
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 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)	Ema et al., 1991 [Annex I, 2.2.1.9] (REACH registration, DBTO)
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.	Ema et al., 1992 [Annex I, 2.2.1.10] (REACH registration, DBTO)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		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)	
Wistar rat (10 females/group) Non-guideline conform study Oral gavage	DBTC purity not reported 0, 10, 15 mg/kg bw/d GD 7-8	Total resorptions at 10 (2/10) and 15 mg/kg bw/d (4/10); increased post-implantation loss 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)	Ema et al., 1995b [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	DBTC	Total resorption was seen at 7.6 mg/kg bw (3/16) and 15.2 mg/kg bw (14/16); post-	Ema & Harazono,

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
(16-19 females/group) Non-guideline conform study Oral gavage	97% purity 0, 3.8, 7.6, 15.2 mg/kg bw/d GD 4-7	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)	2000 [Annex I, 2.2.1.2] (REACH registration, DBTO)
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	Noda et al., 2001 [Annex I, 2.2.1.15]

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

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
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	DBTC: 98% purity 2.5, 3.8 mg/kg bw/d GD 20-50 (period of organogenesis) Pregnancy outcome was	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	Ema et al., 2007b [Annex I, 2.2.1.16]

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
nasogastric intubation	determined on GD 100	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)	
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	Ema et al., 1995a [Annex I, 2.2.3.1]

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			concentration, statistically significant for embryos exposed to 10 and 30 ng/mL)	
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 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.	Ema et al., 1996a [Annex I, 2.2.3.2]
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.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

No data are available for DBTO; thus, the evaluation was based on studies with DBTC. This evaluation has been made previously for several category members, including DBTA (RAC opinion, 2020), DBTP (RAC opinion, 2017) and DBTDL (RAC opinion, 2015.

The OECD TG 421 study in rats with DBTC (Unpublished report, 2003) showed body weight effects in both females and males at the high dose (200 ppm, 12.0-15.4 mg/kg bw/d). In female rats, reduced weight gain was observed over the pre-mating, gestation, and lactation periods at the higher dose level. The corpora lutea numbers were not measured in this study. No reproductive toxicity was observed in males. There was a significant increase in the incidence of ovarian cysts in the high-dose females. Furthermore, the number of pregnant females was reduced in mid (30 ppm, 1.7-2.4 mg/kg bw/d) and high dose groups (7/12 in

both mid and high dose group vs. 9/12 in the controls) and only 3/7 pregnant high dose females delivered offspring. This resulted in a reduction in the number of live pups (10 vs. 101 in controls).

A fertility study with DBTC (Ema & Harazono, 2000) was reported in which female rats were exposed via oral gavage to DBTC in olive oil (0, 3.8, 7.6 and15.2 mg/kg bw/d) on GD 0-3 or GD 4-7. In addition to a control group (olive oil), also a pair-fed group (feed restricted to same amounts as high dose DBTC-group) was included. A significantly higher number of non-pregnant dams was observed in the mid and high dose exposed on GD 0-3 (number of pregnant dams in high dose: 2/16, mid dose: 11/16, low dose: 16/16, control: 19/19, pair-fed: 16/19). Further, a reduced number of implantations (number of implantations in high dose: 1.8±4.8, mid dose: 10.1±7.1, low dose: 15±1.5, control: 15±1.4, pair-fed: 13.4±4.3) and increased incidences of pre-implantation loss (high dose: 87.9%, mid dose: 35.6%, low dose: 4.1%, control: 2.7%, pair-fed: 16.4%) was observed.

In a developmental toxicity study in the CD1 mice (Ema *et al*., 2007a), DBTC (in olive oil) was administered by gavage to pregnant females at dose levels of 0 (vehicle control), 7.6, 15.2 or 30.4 mg/kg bw on GD 0-3 or GD 4-7. Mortality occurred in all treated groups but without a dose-response relationship. Other signs of toxicity (vaginal discharge, hypoactivity, hypothermia) were also observed at all dose levels and jaundice was seen in the mid and high dose groups. Body weight and food consumption were also affected negatively. Regarding the number of pregnant females, there was an increase in the pre-implantation loss in dams treated on GD 0-3 with the dose administered (29.7% at 7,6 mg/kg bw, 34.0% at 15.2 mg/kg bw, 58.3% at 30.4 mg/kg bw) that was statistically significant in the high dose group. An increase in pre-implantation loss was seen in the high dose group also in dams treated on GD 4-7; however, not statistically significant. Post-implantation losses increased with the dosing and the effect at the mid dose (15.2 mg/kg bw) was also statistically significant (see 'Adverse effects on development').

A supportive mechanistic study explored the effect of progesterone on implantation failure induced by DBTC in rats. DBTC administration (7.6 and 15.2 mg/kg bw/d on GD 0-3 or GD 4-7) lead to reduced uterus weight and serum progesterone levels in dams treated GD 0-3 and GD 4-7. Oestradiol levels and corpora lutea numbers were unaffected by treatment. Administration of progesterone reversed the suppression of uterine decidualisation (Harazono & Ema, 2003).

Administration of progesterone caused slightly lower pre-implantation loss after exposure on GD 0-3, but offered no complete protection (8.6%, 62.8%, 81.3% at dose levels of 0, 7.6, 15.2 mg/kg bw, respectively, without progesterone; 10.5%, 25.9% and 60.6%, respectively, with application of progesterone) (Ema *et al*., 2003).

Based on the increased number of non-pregnant females among successfully mated females, the reduced number of implantations, and the increased pre-implantation losses and increased early total resorptions, as well as the previous harmonised classification of DBTC as Repr. 1B for adverse effects on sexual function and fertility, the DS considered that DBTO should have the same classification as DBTC. The DS therefore proposed Repr. 1B; H360F for adverse effects on sexual function and fertility for DBTO.

Adverse effects on development

There is one developmental toxicity study available with DBTO (Unpublished report, 2017), as well as a large number of studies with DBTC or DBTA and one study performed with DBTDL, DBTM and DBTA in addition to DBTO and DBTC. All studies except the one performed with

DBTO have been evaluated previously for category members. The CLH dossier divides the studies into three groups: studies with DBTO, studies with DBTC, and studies with DBTA.

Studies with DBTO

The study with DBTO (Unpublished report, 2017) was an OECD TG 414 study in which 25 SD rats/dose were exposed on GD 0-19 at dose levels of 0, 0.75, 3, and 6 mg/kg bw/d. The selection of the top dose was based on a range finding study in which 40% of the dams had to be euthanised at 9.5 mg/kg bw/d. Effects were only observed at the high dose. Maternal effects consisted of lower body weights (-8% at GD 18, -9% at GD 20), lower body weight gain, and lower food consumption and clinical findings (low body carriage, red material around the nose, thin appearance, loss of skin elasticity, and pale body colour). Two dams were euthanised in extremis due to general toxicity; these dams were not pregnant. An overview of the developmental effects observed in this study is given in the table below.

***Table**: 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. non-pregnant	1	1	2	3
Pregnancy index (%)	96.0	96.0	92.0	88.0
No. of females with total resorption	0	0	0	4
No. of females with viable foetuses GD 20	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 foetuses No. per animal	12.5 ± 1.96	14.1 ± 2.36	13.5 ± 1.78	9.7 ± 5.42
Post-implantation loss % per animal	5.40 ± 5.626	1.46 ± 2.952	4.89 ±5.687	25.70 ± 39.37 (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)*

An increase in post-implantation loss was observed in the high dose, in particular in four females that resorbed all of their foetuses. Two of these displayed signs of maternal toxicity, while the other two did not. Another female with 75% post-implantation loss also showed no clinical signs or altered body weight. No other effects on the developing foetus were observed in this study.

A comparative study with DBTO, DBTC, DBTA, DBTM, and DBTL (Noda *et al*., 1993) using a single gavage administration of 80 µmoL/kg bw on GD 8 (20 mg/kg bw DBTO), showed a comparable spectrum of effects for all substances, in the absence of maternal toxicity. For DBTO, external malformations were observed in 20.7% of the pups (n=28), mainly consisting of cleft mandible, cleft lower lip, ankyloglossia or schistoglossia and exencephaly. Skeletal malformations had an incidence of 26.2% (n=30), of which anomaly of mandibular fixation, fused ribs, absent ribs, and fused thoracic arches were significantly increased. Treatment showed a comparable incidence and type of foetal malformations for all organotin substances.

Studies with DBTC

The developmental toxicity effects of DBTC observed in the OECD TG 421 (Unpublished report, 2003) included an increase in the number of dams with post-implantation loss, a reduction in the number of live pups and a reduction in the gestation index.

An OECD TG 414 study (Farr *et al*., 2001) with DBTC reported severe malformations in four pups at 10 mg/kg bw/d, including anasarca, ankyloglossia, hydrocephaly, agnathia and other skeletal defects. Maternal toxicity at this dose level consisted of reduced weight gain and food consumption.

In a supportive rat developmental toxicity study, rats were exposed during the gestation period (GD 7-15) via oral gavage to DBTC in olive oil (0, 2.5, 5, 7.5, 10 mg/kg bw/d) (Ema *et al.*, 1991). Clear maternal toxicity was observed at the two highest dose levels and effects included significantly higher mortality in dams (5/10 and 9/10 dams died in the 7.5 and 10 mg/kg bw/d dose groups, respectively) with stomach haemorrhages observed in dead animals. In the 7.5 and 10 mg/kg bw/d dose groups, total resorptions were observed in the remaining 5/10 and 1/10 pregnant rats, respectively. *In utero* exposure of foetuses resulted in developmental effects such as increased incidences of external and skeletal malformations, with cleft jaw and ankyglossia being the most frequently observed type of malformations. Although observed at the two highest dose levels in the presence of clear maternal toxicity, these developmental effects were also observed at the dose level of 5 mg/kg bw/d (i.e., without the presence of maternal toxicity).

Three additional studies on potential developmental toxicity in relation to the most sensitive window for exposure to DBTC indicated that DBTC-induced teratogenic effects were observed following exposure on GD 7-8 and were most pronounced when dams were exposed on GD 8 (Ema *et al.*, 1992, 1995, 1996). Embryo-lethality was observed at all tested time-points for exposure during gestation.

The sensitivity of the rat foetus to DBTC was confirmed by several *in vitro* studies (Ema *et al*., 1995a, 1996a; Yonemoto *et al*., 1993).

A single study performed in CD1 mice (Ema et al., 2007b) found a clear increase in post-implantation loss, up to 100% at 30.4 mg/kg bw/d. No significant increase in foetal malformations was found, however this is unsurprising considering the small number of

foetuses investigated.

Two studies in cynomolgus monkeys gave unclear results (Ema et al., 2007b, 2009).

Studies with DBTA

The three studies performed by Noda *et al*. (1992a, 1992b, 2001) with DBTA had as main purpose to characterise the critical parameters of DBTA-induced teratogenicity. In particular the critical window of exposure was investigated. It was observed that three days of exposure to 15 mg/kg bw on GD 7-9 resulted in a clear rise in resorbed embryo's and skeletal and external malformations. The malformations included cleft mandible, cleft lower lip, ankyloglossia or schistoglossia, exencephaly, anomaly of mandibular fixation, cranial hypoplasia, and fused ribs. Experiments with single doses showed that GD 8 was the critical window of exposure for these effects.

Noda *et al*. (1992b) also reported maternal effects after exposure to DBTA during GD 7-17, which consisted of reduced weight gain, albeit not in dams with living foetuses, and dose-related thymus atrophy with statistical significance at 5 mg/kg bw/d and above. The developmental effects observed were an increase in early resorptions, increases in external and skeletal malformations and a decrease in foetal weight.

The third study by Noda *et al*. (2001) applied single doses of 0 (vehicle control), 7.5, 10, 15 or 22 mg/kg bw on GD 8 and investigated the effect of the age of the dams at the time of mating on the susceptibility to DBTA toxicity. In the group with 7.5-month-old dams, maternal body weight gain, but not adjusted body weight gain, was statistically significantly decreased at the top dose. The effects on the pups were similar to the previous studies and included post-implantation loss, reduced pup weight, and external and skeletal malformations. The LOAEL for external malformations was the lowest dose of 7.5 mg/kg bw. There was no clear relationship between the age the dams and DBTA effects, mainly because the implantation loss in older dams (12 months) was very high in all groups.

Based on the clear and consistent 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, the DS proposed classification of DBTO as Repr. 1B; H360D.

Comments received during consultation

Three MSCAs agreed with the proposed classification for reproductive toxicity, based on the data from category members and the OECD TG 414 study with DBTO.

Six industry representatives commented. They disagreed with the category approach and using read across between category members, arguing that new hydrolysis studies show that a dimer (DBDTC distannoxane dimer) is formed rather than DBTC itself (Ghobrial *et al*., 2019; Munschi *et al*., 2010; Patel *et al*., 2009; Alwyn, 2009). According to the comments, the dimeric structure is the only metabolite of the gastric hydrolysis and has a molecular weight of 1089.4 Dalton. With this high molecular weight, the dimeric distannoxane is by far too heavy to be biologically active and have a low reaction potential because the molecules are too large to pass through biological membranes, limiting their bioavailability. It was also commented that the rejection of the category approach is supported by absence of teratogenic effects in the OECD TG 414 study with DBTO. They also noted that further testing related to the read across is planned. The DS replied that the available information indicates that common

metabolites/intermediates are formed in vivo, and affect common biological targets, which supports the applicability of a read across from category members (e.g., DBTC) to DBTO for oral toxicity studies (germ cell mutagenicity, toxicity to reproduction, specific target organ toxicity repeated exposure, specific target organ toxicity single exposure). The DS concluded, that based on available information, the read across is robust and it is valid to consider data of category members to read across between these endpoints. They also noted that the OECD TG 414 study referred to by Industry representatives demonstrates that DBTO has an adverse impact on thymus integrity, which is a target organ for category members, further supporting the validity of the category approach. In the study, reduced thymus weights were observed (relative and absolute, up to -38-44%) in a dose-dependent manner and also an increased incidence of small thymus was observed in dams at the highest dose applied. The DS also took note of the new studies.

Assessment and comparison with the classification criteria

RAC agrees with the DS's replies and concludes that the read across is robust and valid and that data from the category members can be used to assess reproductive toxicity. Also see 'RAC general comment'.

Adverse effects on sexual function and fertility

The effects of DBTC on sexual function and fertility have been investigated in a reproduction/developmental toxicity screening study in rats and two studies with exposure in early pregnancy in respectively rats and mice. The studies showed consistent decreases in the number of pregnant dams and number of implantations. Maternal toxicity in the form of reduced body weight gain and food consumption was observed, but mainly at the high dose, while reproductive effects also appeared at the mid dose levels, in particular in the rat studies. Moreover, the pair-fed group (Ema & Harazono, 2000) confirmed that the reproductive effects could not be explained by reduced food consumption.

Considering that several studies consistently showed fertility effects (non-pregnant dams, reduced number of implantations), at doses with limited or no maternal toxicity, that supportive studies indicate that DBTC has an adverse effect on progesterone levels and that there is no basis to question the human relevance of these effects, RAC considers that there is clear evidence of an adverse effect on fertility upon exposure to DBTC. This was also concluded in the RAC opinion for DBTC itself.

Given that both DBTO and DBTC are hydrolysed to the same distannoxane, RAC is of the opinion that data on DBTC can be used for classification of DBTO for effects on sexual function and fertility (see also 'RAC general comment').

Specific concentration limit

Setting of an SCL is not considered necessary for adverse effects on sexual function and fertility, given that (cf. section 3.7.2.5 of the CLP guidance, ECHA, 2017) ED_{10}-values for DBTO fall within the ranges of a medium potency group (i.e., 4 mg/kg bw/d < ED_{10} < 400 mg/kg bw/d) and modifying factors which might change the potency group are considered not needed, resulting in the GCL of 0.3%.

Altogether, RAC supports the conclusion of the DS that **DBTO warrants classification for adverse effects on sexual function and fertility as Repr. 1B; H360F (May damage**

fertility).

Adverse effects on development

There are two developmental toxicity studies with DBTO available. The first was an OECD TG 414 study (Unpublished report, 2017) that showed an increase in post-implantation loss at the highest dose of 6 mg/kg bw/d. At this dose also maternal toxicity occurred, consisting of lower body weights (-8% at GD 18, -9% at GD 20), lower body weight gain, lower food consumption and clinical findings. Two dams in the high dose group were sacrificed in extremis on GD 9 and 12, respectively. However, it was noted that three dams with high resorption rates did not show clear signs of toxicity.

The second study with DBTO was the study by Noda *et al*. (1993), in which single doses of several dibutyltins were given on the critical day for organotin toxicity, namely GD 8. DBTO showed a statically significant higher incidence of in foetal malformations similar to those induced by other category members. There was no maternal mortality or signs of general toxicity.

In addition to these studies with DBTO, there are numerous studies with DBTC and DBTA that consistently show dose-dependent increases in foetal effects (malformations, post-implantation loss and weight reduction). Maternal effects were minimal or absent at the lowest doses that induced foetal effects. It should be noted that it is highly likely that the reduced maternal body weight gain at higher doses was caused by the sharp increase in post-implantation loss, as dams with live foetuses at the same dose did not show this effect. Moreover, dose-related foetal toxicity was observed even after single exposure and has a clear critical window, which makes it very unlikely that there is a causative relationship with maternal effects. There is no basis to question the human relevance of these effects, and RAC considers that there is clear evidence of an adverse effect on development upon exposure to DBTO.

Specific concentration limit

Setting of an SCL is not considered necessary for adverse effects on development, given that the ED_{10}-values fall within the range of the medium potency group (i.e., 4 mg/kg bw/d < ED10 < 400 mg/kg bw/d) and modifying factors which might change the potency group are considered not needed, resulting in the GCL of 0.3% (cf. section 3.7.2.5 of the CLP guidance, ECHA, 2017).

Altogether, RAC supports the conclusion of the DS that **DBTO warrants classification for adverse effects on development as Repr. 1B; H360D (May damage the unborn child)**.

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.

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

Summary of the Dossier Submitter's proposal

There are two non-guideline studies with DBTC that investigated the effects of a single dose on the thymus of rats (Snoeij *et al*., 1989) and SCID-hu mice (SCID mice engrafted with human foetal thymus and liver tissue fragments; de Heer *et al*., 1995), respectively.

In the rat study, a single dose of 15 mg/kg bw was given by gastric intubation. DBTC induced rapid but reversible atrophy of the thymus in the 9-d observation period.

Lower doses of 0.03 and 1.0 mg/kg bw were given intraperitoneally to SCID-hu mice engrafted with human thymus and liver tissue fragments. DBTC induced a reduction in thymus cortex size.

Although effects occurred after single application below the guidance value for STOT SE Category 1 of 300 mg/kg bw, both studies are non-standard mechanistic studies with few animals and limitations in the description of the results. In addition, thymus effects were according to the DS already covered by the proposed STOT RE 1 classification. Hence, the DS proposed no classification for STOT SE.

Comments received during consultation

Four comments were received for STOT SE; three of these came from industry representatives and were general objections to the category approach.

One MSCA disagreed with the proposal for no classification and argued that classification as STOT SE Category 1 was warranted. The reasoning presented was that although both studies

had limitations, their results were very much in line with those from repeated dose studies, including both target organ and effective dose range. It was also stated that reversibility and a proposed classification for STOT RE were no valid justifications to forfeit classification for STOT SE.

The DS replied that these studies had been previously considered for other category members, such as DBTP (Dibutylbis(pentane-2,4-dionato-O,O')tin), which then did not result in classification. It was also considered that the evidence for STOT RE was more comprehensive; hence, this classification was preferred for thymus toxicity.

The Industry representatives presented arguments against the use of the category approach and using read across between category members, arguing that new hydrolysis studies show that a dimer (DBDTC distannoxane dimer) is formed rather than DBTC itself (Ghobrial *et al*., 2019; Munschi *et al*., 2010; Patel *et al*., 2009; Alwyn, 2009). According to the comments, the dimeric structure is the only metabolite of the gastric hydrolysis and has a molecular weight of 1089.4 Dalton. With this high molecular weight, the dimeric distannoxane is by far too heavy to be biologically active and have a low reaction potential because the molecules are too large to pass through biological membranes, limiting their bioavailability. They also noted that further testing related to the read across is planned. The DS replied that the available information indicates that common metabolites/intermediates are formed in vivo, and affect common biological targets, which supports the applicability of a read across from category members (e.g., DBTC) to DBTO for oral toxicity studies (germ cell mutagenicity, reproductive toxicity, specific target organ toxicity repeated exposure and specific target organ toxicity single exposure). The DS concluded, that based on available information, the read across is robust and it is valid to consider data of category members to read across for these endpoints. The DS also took note of the new studies.

Assessment and comparison with the classification criteria

RAC agrees with the replies by the DS regarding the use of the category approach and considers that based on the available data, read across between category members can used for STOT SE. Also see 'RAC general comment'.

Two studies are presented that specifically addressed the potential of DBTC to induce thymus toxicity after single exposure. Both studies have been included previously for category members as mechanistic evidence for the assessment of STOT RE. No additional evidence is available from the acute toxicity studies. Repeated dose studies consistently showed thymus toxicity but did not include examinations after one day.

The effect observed after a single exposure consists of reversible thymus atrophy. Although also reversible effects should be considered for classification, RAC did not consider that there was 'clear evidence of marked organ dysfunction' in these single exposure studies as required for the STOT SE classification. In addition, one of the studies was conducted intraperitoneally which is a less relevant route for STOT SE.

RAC also considered the classification for Acute Tox. 3 (oral). As the ATE value is with the guidance value range for STOT SE 1, classifying for both endpoints would result in double classification.

For these reasons, **RAC considers that no classification is warranted for STOT SE**.

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.

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

Summary of the Dossier Submitter's proposal

There is one prenatal developmental toxicity study (PNDT, OECD TG 414) available with DBTO itself. In addition, a category approach, supported on the basis of the toxicokinetic and hydrolytic behaviour of the substances in the category, was used by the DS to justify that studies on DBTC can be taken into consideration when classifying DBTO for this hazard class.

In the PNDT study in rats, a significant reduction in both absolute and relative thymus weights was observed at GD 20 after exposure to 0.75, 3 and 6 mg/kg bw/d DBTO. The weight reduction showed a clear dose-response relationship (Unpublished report, 2017).

Only one 90-d study is available, and it was performed with DBTC. This feeding study in rats (0, 10, 20, 40, 80 ppm DBTC in diet, corresponding to 0, 0.5, 1, 2, 4 mg/kg bw/d) indicated some slight effects such as reduced food consumption and body weight and mild anaemia at the highest dose (Gaunt *et al*., 1968). No abnormalities were seen at autopsy or histology (including the thymus).

A reproductive/developmental toxicity screening study according to OECD TG 421 (diet) with DBTC in rats (Unpublished report, 2003) showed a reduced relative thymus weight and moderate to severe lymphoid depletion in dams exposed to 1.7-2.4 mg/kg bw/d after ~41 days exposure. A dose of 6.2-15.4 mg/kg bw/d induced reduced absolute and relative thymus weight and severe to very severe lymphoid depletion in dams.

A 28-d rat/mouse immunotoxicity study with doses of 0, 50 and 150 ppm DBTC in the diet (corresponding to 0, 2.5, 7.5 mg/kg bw/d for rats and 0, 7.1, 21.4 mg/kg bw/d for mice) was included in the CLH dossier (Seinen & Vos, 1977). No treatment-related effects were observed in mice. In rats, mortality was observed in the 7.5 mg/kg bw/d group (4/10 females and 2/10 males). Further, clear dose-dependent effects on the thymus were observed. Reductions in relative organ weights were noticed for the thymus (2.5 mg/kg bw/d: 53%; 7.5 mg/kg bw/d: 68-72%), but also the spleen (2.5 mg/kg bw/d: 16%; 7.5 mg/kg bw/d: 33%) and popliteal lymph nodes (2.5 mg/kg bw/d: 16%; 7.5 mg/kg bw/d: 28%). A pronounced reduction in size of the thymus was found in all DBTC-treated animals. The most important effect observed was lymphocyte depletion in lymphoid organs, which was most pronounced in the thymic cortex of DBTC-treated animals. At the 7.5 mg/kg bw/d level, the thymic cortex was almost completely depleted, although no signs of cell destruction were observed. Lymphocyte depletion was also present in the thymus-dependent areas of the spleen and popliteal lymph nodes.

An additional 2-week rat feeding study (0, 50, 150 ppm DBTC in diet, corresponding to 0, 2.5, 7.5 mg/kg bw/d) confirmed previous findings of clear effects on thymus (Penninks & Seinen, 1982). Relative thymus weight was reduced (<30% of control group at 7.5 mg/kg bw/d), and lymphocyte depletion was observed in thymus (mainly in the thymic cortex and in thymus-dependent lymphoid areas of the spleen).

An old 6-month non-guideline study in rats showed reduced weight gain, food consumption and mortality, with a LOAEL of 2.5 mg/kg bw/d (Barnes and Stoner, 1958).

Two OECD TG 414 studies in rats (both oral gavage; 0, 1, 2.5, 5, 10 mg/kg bw/d) showed clear maternal toxicity (Study Report, 1994; Farr *et al*., 2001). Effects included reduced bw gain (10 mg/kg bw/d), reduced food consumption (10 mg/kg bw/d) and significantly increased number of animals with thymus atrophy (≥ 2.5 mg/kg bw/d). Maternal toxicity was not observed at a dose of 1 mg/kg bw/d.

Further investigation of the effects of DBTC on the immune system was reported by DeWitt *et al.* (2005, 2006), both in rats. Both dams and offspring were exposed to relatively low levels of DBTC (up to 5 mg/kg bw/d for direct exposure of offspring) via oral route but no effects on the immune system were reported.

Several mechanistic immunotoxicity studies were included in the CLH dossier. In general, these studies suffered from limitations including too low doses, the use of a single dose level or single exposure. However, the results confirmed that the thymus is a target organ of DBTC.

The DS considered the 28-d study (Seinen & Vos, 1977), 14-d study (Penninks & Seinen, 1982) and reproduction/developmental screening study (Unpublished report, 2003) to be the key studies. All three studies showed thymus toxicity at low dose levels. As DBTO showed similar potency to DBTC in the study by Noda *et al*. (1993), no adjustment was proposed for molecular weight. The effective doses from the 28-d and 56-d studies (see RAC note on the 56-d study further down) were extrapolated to the 90-d equivalents of 0.8-1.25 mg/kg bw/d, which are clearly within the guidance value range for STOT RE 1. The DS concluded that the data supported classification for specific target organ toxicity following repeated exposure as STOT RE 1 with the immune system as the target organ.

Comments received during public consultation

Three comments from MSCAs were received, all indicating support for the category approach, and all were in favour of the proposed classification as STOT RE 1.

Seven industry representatives commented, presenting arguments against the use of the category approach and using read across between category members, arguing that new hydrolysis studies show that a dimer (DBDTC distannoxane dimer) is formed rather than DBTC itself (Ghobrial *et al*., 2019; Munschi *et al*., 2010; Patel *et al*., 2009; Alwyn, 2009). According to the comments, the dimeric structure is the only metabolite of the gastric hydrolysis and has a molecular weight of 1089.4 Dalton. With this high molecular weight, the dimeric distannoxane is by far too heavy to be biologically active and have a low reaction potential because the molecules are too large to pass through biological membranes, limiting their bioavailability. They also noted that further testing related to the read across is planned. The DS replied that the available information indicates that common metabolites/intermediates are formed in vivo, and affect common biological targets, which supports the applicability of a read across from category members (e.g., DBTC) to DBTO for oral toxicity studies (germ cell mutagenicity, toxicity to reproduction, specific target organ toxicity repeated exposure, specific target organ toxicity single exposure). The DS concluded, that based on available information, the read across is robust and it is valid to consider data of category members to read across between these endpoints. The DS also took note of the new studies.

Assessment and comparison with the classification criteria

Given that both DBTO and DBTC are hydrolysed to the same distannoxane, RAC is of the opinion that data on DBTC can be used for classification of DBTO for STOT RE. RAC agrees with the DS's arguments and concludes that the read across is robust and valid and that data from the category members can be used to assess STOT RE. Also see 'RAC general comment'.

The results of the studies with DBTC consistently showed that the immune system, in particular the thymus, was the target organ after repeated oral exposure. Effects included reduced thymus weight, thymus atrophy, and severe lymphoid depletion. At higher doses, also effects on liver, bile duct and pancreas have been reported.

The only study with DBTO itself that used multiple dose levels as well as a repeated dosing regime is the OECD TG 414 study (Unpublished report, 2017). Mean absolute thymus weights were 19%, 34%, and 44% lower than the mean control value in the 0.75, 3.0, and 6.0 mg/kg bw/day dose groups, respectively, and relative to the adjusted GD 20 body weights, they were 20%, 35%, and 37% lower, respectively (see the table below).

***Table**: Thymus weight, and thymus weight adjusted for bw on GD20, 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 weight (g)	0.239 ± 0.062	0.193* ±0.042	0.158* ± 0.043	0.134* ±0.046
Thymus weight, adjusted GD 20 (g)	0.0891 ± 0.0192	0.0716* ± 0.0123	0.0581*± 0.0143	0.0558* ± 0.0108

**statistically significantly different compared to control values (p<0.01)*

No histopathology was performed on the thymus in this study, which increases the uncertainty on the severity of this effect. On the other hand, similar reductions in thymus weight were observed in the studies with DBTC and were accompanied by lymphoid depletion. In particular Seinen & Vos (1977) noted reductions of 53% at 2.5 mg/kg bw/d and 68-72% at 7.5 mg/kg bw/d after 28 days of exposure, which were accompanied by marked lymphocyte depletion. The Unpublished report (2003) showed a reduced relative thymus weight and moderate to severe lymphoid depletion in dams exposed to 1.7-2.4 mg/kg bw/d after ~41 days exposure (in the CLH report the exposure length is indicated as 56 days but it is not clear to RAC how this was calculated).

The outcome of these studies confirms that DBTO has similar toxicity compared to DBTC as well as similar potency.

Overall, RAC considers the effects on the immune system as sufficiently severe to fulfil the classification criteria for STOT RE. The effects on the immune system include morphological changes that provide clear evidence of marked organ dysfunction and are considered as significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination.

Effective dose levels for DBTC are within the extrapolated guidance value ranges for classification as STOT RE 1 (i.e., 10, 30 and 60 mg/kg bw/d for a 90-d, 28-d and 14-d study, respectively). As there is only a small difference in molecular weight between DBTC (303.84 g/M) and DBTO (248.92 g/M), this applies to the equivalent values of DBTO as well.
Setting of a specific concentration limit (SCL) is not considered necessary, given the small margin between the effective dose levels and the guidance values for STOT RE.

RAC therefore supports the conclusion of the DS that **DBTO warrants classification as STOT RE 1; H372 (Causes damage to the immune system through prolonged or repeated exposure).**

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

Annex I to the 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: **Not applicable**

Contact details for dossier submitter:

Environment Agency Austria, Spittelauer Lände 5, A-1090 Vienna

on behalf of the Austrian Competent Authority (Austrian Federal Ministry for Climate Action, Environment, Energy, Mobility, Innovation and Technology Radetzkystraße 2, 1030 Vienna, Austriaon

Version number: 1 **Date: August 2020**

CONTENTS

In the following a detailed study description is provided for studies cited and refered in the CLH report for dibutyltin oxide (DBTO).

The studies described in the section Toxicokinetic (Chapter 1) and Reproductive Toxictity (Chapter 2.2) and Specific Target Organ Toxicity (Chapter 2.3) below (exept unpublished report, 2017) have been described in the CLH-dossier for DBTP (EC 245-152-0, CAS 22673-19-4) and assessed by RAC in 2017.

The studies decribed in the section germ cell mutagenicity (Chapter 2.1) have been described in the CLH dossier for Dibutyltin di(acetate) (DBTA) (EC 213-928-8, CAS 1067-33-0) which is currently under evaluation.

Where the description of the studies below are in quotes the text is a verbatim rendering of the text in the mentioned CLH-dossier for DBTP and DBTA.

Only the simulated gastric hydrolysis study carried out by Umweltbundesamt in 2019 (Chapter 1.1.2) and the prenatala developmental toxicity study with DBTO (unpublished study, 2017) are described for the first time in detail.

1 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

The studies described below have been described in the CLH-dossier for DBTP (EC 245-152-0) and assessed by RAC in 2017. Where the description of the studies below are in quotes the text is a verbatim rendering of the text in the mentioned CLH-dossier.

1.1 Simulated gastric hydrolysis

Reference	”Schilt R & Zondervan-van den Beuken EK (2004). Dibutyltin dilaurate (DBTL, CAS #77-58-7), Dibutyltin maleate (DBTM, CAS #78-04-6), Dibutyltin oxide (DBTO, CAS #818-08-6) and Dioctyltin oxide (DOTO, CAS #870-08-6): simulated gastric hydrolysis. TNO Nutrition and Food Research, Zeist, The Netherlands. TNO Report V5047.
Guideline	None followed
Reliability	Klimisch 2: reliable with restrictions (non-guideline study)
Species/strain	Not relevant: *in vitro* study
Test material	**DBTL** CAS 77-58-7 EC 201-039-8 Purity 98.2% **DBTM** CAS 78-04-6 EC 201-077-5 Purity 99.65% **DBTO** CAS 818-08-6 EC 212-449-1 Purity 99.2%
Study design	Gastric hydrolysis studies were performed under the auspices of the Organotin Environmental Programme (ORTEP) Association Stabilizer Task Force. Simulated gastric reaction studies were performed using dibutyltin dilaurate (DBTL), dibutyltin maleate (DBTM) and dibutyltin oxide (DBTO) at approximate concentrations of 0.015-0.040 mM. The extent of hydrolysis was assessed under low pH (1-2) conditions (0.07 N HCl) at 37°C, simulating mammalian gastric contents. The

degree of hydrolysis was measured by determination of the amount of DBTC formed after 0.5, 1, 2, and 4 hours, using GC-FPD.

Findings Simulated gastric hydrolysis studies indicate that dibutyltin substances undergo rapid conversion to dibutyltin chloride species when exposed to conditions representative of the mammalian stomach.

Conversion of dibutyltin compounds to DBTC

Time	DBTL	DBTM	DBTO
0.5 h	82%	100%	43%
1 h	78%	97%	65%
2 h	88%	98%	80%
3 h	-	-	-
4 h	87%	95%	87%

Conclusion DBTL, DBTM and DBTO are shown to be rapidly converted to dibutyltin chloride species under conditions representative of the mammalian stomach. The generation of a common intermediate supports the read-across approach and the formation of a category for these substances and for dibutylbis(pentane-2,4-dionato-O,O')tin."

1.2 Simulated gastric hydrolysis

Reference Umweltbundesamt (2019). NMR based investigation of the hydrolysis of DOTE and DBTM

Report No 0709, Vienna 2019

Guideline None followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study)

Species/strain Not relevant: *in vitro* study

Test material **Dibutyltin maleate (DBTM)**

CAS 78-04-6

EC 201-077-5

Purity >95 %

Study design Hydrolysis study was performed using dibutyltin maleate

The extent of hydrolysis was assessed under low pH conditions (0.1 mol/L of aqeous HCL, 72h, 40°C). The degree of hydrolysis was measured after workup with dichloromethane-D_2 by ^{119}Sn NMR.

Findings Hydrolysis studies demonstrate that DBTM is hydrolysed to the dimeric stannoxane $ClBu_2SnOSnBu_2Cl$.

Conclusion Dibutyltin maleate is shown to be converted to $ClBu_2SnOSnBu_2Cl$ under acidic conditions. The generation of a common intermediate, identical to the hydrolysis product of DBTC (see 1.4) and also to DBTP (see 1.3), supports the read-across approach for the involved substances in the category including DBTO and DBTM.

1.3 Simulated gastric hydrolysis

Reference "Naßhan H (2015). Dibutylbis(pentane-2,4-dionato-O,O´)tin [DBTAcAc) CAS number: 22673-19-4. In-vitro Metabolism Study

Galata Chemicals GmbH, Chemiestrasse 22, 68623 Lampertheim, Germany

Guideline None followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study)

Species/strain Not relevant: *in vitro* study

Test material **Dibutylbis(pentane-2,4-dionato-O,O´)tin**

CAS 22673-19-4

EC 245-152-0

Purity >90 %

Study design Simulated gastric hydrolysis studies were performed using dibutylbis(pentane-2,4-dionato-O,O´)tin.

The extent of hydrolysis was assessed under low pH (1.2) conditions at 37°C at a concentration of 23.2 mM. The degree of hydrolysis was measured after workup in hexane by ^{119}Sn NMR in toluene-d^8 which allowed positive identification of the hydrolysis product. Any remaining tin-residues (decomposition products and/or water soluble substances) was analysed by atomic absorption spectrometry (AAS).

Findings Simulated gastric hydrolysis studies demonstrate that dibutylbis(pentane-2,4-dionato-O,O´)tin rapidly form the dimeric stannoxane $ClBu_2SnOSnBu_2Cl$ (^{119}Sn-NMR: δ (ppm) -91, -144) in almost quantitative yield when exposed to conditions representative of the mammalian stomach. Minor amounts (~2 mol%) of non-hydrolyzed DBTC was also detected.

Conclusion Dibutylbis(pentane-2,4-dionato-O,O´)tin is shown to be rapidly converted to $ClBu_2SnOSnBu_2Cl$ under conditions representative of the mammalian stomach. The generation of a common intermediate, identical to the hydrolysis product of DBTC (see 1.4) and DBTM (see 1.2). “

1.4 Simulated gastric hydrolysis

Reference ”Naßhan H (2016). Dibutyltin dichloride [DBTC] CAS number: 683-18-1. In-vitro Metabolism Study. Galata Chemicals GmbH, Chemiestrasse 22, 68623 Lampertheim, Germany

Guideline None followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study)

Species/strain Not relevant: *in vitro* study

Test material **Dibutyltin dichloride**

CAS 683-18-1

EC 211-670-0

Purity >90 % (Tributyltin chloride (TBTC) was identified as impurity in small amounts)

Study design Simulated gastric hydrolysis studies were performed using dibutyltin dichloride.

The extent of hydrolysis was assessed under low pH (1.2) conditions at 37°C at a concentration of 33 mM. The degree of hydrolysis was measured after 30 s, 1 h, and 4 h respectively, after workup in hexane by ^{119}Sn NMR in toluene-d^8 which allowed positive identification of the hydrolysis product.

Findings Simulated gastric hydrolysis studies demonstrate that dibutyltin dichloride rapidly form the dimeric stannoxane $ClBu_2SnOSnBu_2Cl$ (^{119}Sn-NMR: δ (ppm) -91, -144) as the only observed hydrolysis product when exposed to conditions representative of the mammalian stomach. Minor amounts (~6 mol%) of DBTC remains after 4 hours. The impurity tributyltin chloride remains unchanged during the hydrolysis. The recovery of total tin (as calculated from the isolated product mass) ranged from 80-97%.

Conversion of DBTC to $ClBu_2SnOSnBu_2Cl$

Time	DBTC	$ClBu_2SnOSnBu_2Cl$	TBTC

30 s	25 mol%	70 mol%	5 mol%
1 h	11 mol%	85 mol%	4 mol%
4 h	6 mol%	90 mol%	4 mol%

Conclusion Dibutyltin dichloride is shown to be rapidly converted to $ClBu_2SnOSnBu_2Cl$ under conditions representative of the mammalian stomach. The generation of a common intermediate, identical to the hydrolysis product of dibutyltin maleate (see 1.2) and dibutylbis(pentane-2,4-dionato-O,O´)tin (see 1.3), supports the read-across approach for the involved substances in the category."

1.5 Toxicokinetics in the mouse

Reference "Kimmel EC, Fish RH & Casida JE (1977)
Bioorganotin Chemistry. Metabolism of Organotin Compounds in Microsomal Monooxygenase Systems and in Mammals
Journal of Agriculture & Food Chemistry 25 (1):1-9.

Guideline None

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species/strain Mouse (Swiss Webster)

Test material **Dibutyltin (di)acetate**
CAS 1067-33-0
EC 213-928-8
Radiochemical purity >99%

Study design In an *in vivo* phase, groups of mice (group size not specified) were gavaged with a single oral dose of 1.1 mg/kg bw ^{14}C-butyl labelled dibutyltin (di)acetate (in methoxytriglycol). Urine and faeces were investigated for metabolites. Tissue levels of radioactivity were investigated at 138 hours following dosing.
In an i*n vitro* phase, the metabolites of ^{14}C butyl labelled dibutyltin (di)acetate were investigated in rat liver microsomal systems. The metabolism of unlabelled dibutyltin dichloride was also investigated.

Findings *In vitro*, rat microsomal systems were shown to generate ^{14}C butyl labelled dibutyltin (di)acetate to dibutyl and monobutyl species by both nonenzymatic destannylation and by a- and β-carbon hydroxylation and decomposition of the hydroxy derivatives.
The results of the *in vivo* phase indicate partial absorption of dibutyltin (di)acetate in the mouse following oral gavage; the faeces contained a proportion of non-metabolised test material and some non-labelled dibutyltin derivatives. Extensive cleavage of the tin-carbon bond was also indicated, with further metabolism of the liberated butyl group to (exhaled) carbon dioxide and small quantities of butene.

Conclusion The results of this study show that oral administration of dibutyltin (di)acetate to the mouse results in hydrolysis of the test material to form an unidentified dibutyltin compound and liberation of the acetate moieties, which are further transformed and incorporated into normal cellular metabolism."

1.6 Toxicokinetics in the rat

Reference "Ishizaka T, Suzuki T & Saito Y (1989)
Metabolism of Dibutyltin Dichloride in Male Rats
Journal of Agricultural and Food Chemistry 37(4): 1096-1101.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species/strain	Rat (Wistar)
Test material	**Dibutyltin dichloride (DBTC)** CAS 683-18-1 EC 211-670-0
Study design	The metabolism of DBTC was investigated in male Wistar rats following a single intraperitoneal administration at a dose level of 4 mg/kg bw. Rats were terminated at time points of 6-168 hours after administration. Blood and urine samples were collected and the liver, kidneys and brain were removed and analysed for the presence of DBTC and its metabolites.
Findings	DBTC and its metabolites were detected in the liver, kidney and spleen at 6 hours after administration. The half-live of DBTC in the liver, kidney and blood was calculated to be between 3-5 days. The accumulation of DBTC in the brain was found to be relatively slow compared to the other tissues investigated in this study. The highest concentration of DBTC in brain was observed three days after administration and corresponded to one fifth of the concentration found in the liver and kidneys. Butyl(3-hydroxybutyl)tin dichloride, butyl(4-hydroxybutyl)tin dichloride and butyltin trichloride were detected by HPLC and MS. The authors suggest that butyl(3-hydroxybutyl)tin dichloride may be formed in the liver and accumulate in the kidney. DBTC and butyl(3-hydroxybutyl)tin dichloride were shown to be excreted into the bile. The concentration of DBTC in the blood was about 1/20 of the concentration in the liver and kidneys."

2 HEALTH HAZARDS

2.1 Germ cell mutagenicity

Detailed summaries of studies relevant to classification for mutagenicity are presented in this section.

The studies described below have been described in the CLH dossier for Dibutyltin di(acetate) (DBTA) (EC 213-928-8) which is currently under evaluation. Where the description of the studies below are in quotes the text is a verbatim rendering of the text in the CLH-dossier for DBTA.

2.1.1 Micronucleus assay (chromosome aberration). Key study.

Reference	"Anonymous, 1991 (*in vivo* mammalian somatic cell study: cytogenicity / erythrocyte micronucleus, registration dossier for DBTA on ECHAs dissemination site)
Guideline	Performed according to OECD guideline No. 474 (Mammalian Erythrocyte Micronucleus Test). Conducted according to GLP.
Reliability	Klimisch 2: reliable with restrictions. Key study.
Species/strain	Bone marrow erythrocytes from male and female mice (ICR).
Test material	**Dibutyltin dichloride (DBTC)** CAS 683-18-1 EC 211-670-0 Purity 97.7%
Study design	Male and female mice were given a single oral dose of DBTC (in corn oil) at 2, 10 or 50 mg/kg. Five males and five females from each group were scheduled for termination 24 hours after treatment; further lots of five males and five females, given DBTC at 50 mg/kg bw or the vehicle control, were scheduled for termination 48 and 72 hours after treatment. Dose selection was based on a preliminary toxicity test using DBTC dosages of 62.5, 125.0, 250.0 and 500.0 mg/kg. All animals dosed with DBTC at 125, 250 and 500 mg/kg bw showed adverse reactions to treatment (severe rales, piloerection, immobility, hunched posture and uneven respiration) and all were killed in extremis 4 hours (500 mg/kg) or 23 hours (125 and 250 mg/kg) following dosing. All animals dosed at 62.5 mg/kg bw showed piloerection on the day following dosing, males were hunched and lethargic from day 3 until termination and all animals lost weight over the 72 hour period. Slides were prepared and stained for all animals. Examination of slide preparations showed evidence of bone marrow toxicity (depression in bone marrow proliferation) in individual animals

dosed at 62.5, 125.0 or 250.0 mg/kg. After consideration of these data, the highest DBTC dosage selected for the main micronucleus test was 50 mg/kg.

Dose groups consisted of 5 male/5 female in the 2 and 10 mg/kg bw groups and 15 males/15 females in 50 mg/kg bw and control group. Control animals were given corn oil at 10 ml/kg bw. The positive control group (5 male/5 female) were given Chlorambucil orally (30 mg/kg in aqueous 10% ethanol). The mice were housed in single sex groups of two or five.

After sacrifice, bone marrow erythrocytes were isolated from the marrow canal in femurs. Smears of cells were fixed and stained on slides. At least one slide from each animal was randomly coded. A total of at least 2000 erythrocytes per animal were examined. Each erythrocyte scored was classed as polychromatic or mature: polychromatic cells stain blue/pink and the older cells stain red/pink. At least 1000 cells of each type were scored from each animal where possible, but where there was an appreciable deviation from unity in the ratio of polychromatic to mature erythrocytes, scoring continued until a minimum of 2000 of the predominant cell type were counted. In addition each erythrocyte scored was examined for the presence or absence of micronuclei. The frequencies of micronucleated cells per 1000 erythrocytes were then calculated. The ratio of polychromatic to mature cells was also determined; a decrease in this may indicate inhibition of cell division following treatment, and the incidence of micronuclei in the mature cell population 24 hours after treatment reflects the pretreatment situation, since most of these cells were produced before treatment. The frequency of micronuclei in polychromatic cells provides an index of induced genetic damage.

Findings Positive - a biologically and statistically significant increase in the incidence of micro-nucleated polychromatic 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 such effect was apparent for any group treated with DBTC and killed 24 hours later. Statistically significant increases over controls were also seen in positive control group animals given chlorambucil at 30 mg/kg bw ($p<0.01$).

Other toxicities: at a dosage of 2 mg/kg, no animal showed reactions to treatment. At 10 mg/kg, 3 males showed hunched posture and piloerection on the day of dosing only: no signs were observed in females. No marked incidences of weight loss were apparent in animals of either group. At 50 mg/kg, one male was killed in extremis approximately 2 hours after dosing (as a result of inactivity, unstable gait, slow respiration and piloerection). All but one of the remaining animals showed reactions to treatment including hunched posture, piloerection, inactivity, rales, closing of one or both eyes, and yellow staining of the coat. In addition, one female was found dead at termination, although it was seen to be alive 2 hours previously. At the 24 hour termination time, 5 animals had lost weight and one had failed to gain weight. At the 48 hour termination time all animals were seen to have lost weight, and all but two animals had lost weight at the 72 hour termination time. All weight losses recorded at 48 and 72 hours were marked. Of the ten mice given chlorambucil, the positive control agent, seven lost weight during the 24 hour period before termination."

2.1.2 Micronucleus assay (chromosome aberration)

Reference **"**Anonymous, 1990 (in vivo mammalian somatic cell study: cytogenicity / erythrocyte micronucleus, registration dossier for DBTA on ECHAs dissemination site)

Guideline Internal Method No. 185.3, Experimental Toxicology+.
References:
- Schmid, W., The micronucleus test for cytogenetic analysis In: Hollaender, A. (ed.) Chemical Mutagens, vol. 4, Plenum Press, New York, 1976, p.31-53.
- Schmid, W., The micronucleus test In: Handbook of mutagenicity test procedures B.J. Kilbey et al. (eds.), Elsevier, Amsterdam, New York, Oxford, 1977, p.235-242.
Study did not identify if it was conducted in accordance with Good Laboratory Practices (GLP), however, quality assurance was equivalent.

Reliability Klimisch 2: reliable with restrictions.

Species/strain Male and female mice (NMRI)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design 0, 50, 100, and 200 mg/kg bw by gavage, single exposure. DBTC was dissolved in arachis oil. 5 males and 5 females from each of the negative control and the test material groups were killed by cervical dislocation 24, 48 or 72 hours after treatment. The positive control animals were killed 24 hours after treatment.

A range-finding study was not performed. Doses were based on findings in a preceeding acute toxicity study where toxic effects were seen at 200 mg/kg bw.

Negative control and test groups consisted of 15 males and 15 females (30 in total) with an additional 3 reserve animals of each sex in the high-dose group. Control animals were given the vehicle (arachis oil) at 10 ml/kg bw. The positive control was triaziquone (0.15 mg/kg bw; single i.p. treatment) given to 5 males and 5 females.

After sacrifice, bone marrow erythrocytes were isolated from both femurs. Smears of cells were fixed and stained on slides. The slides were coded and analyzed "blind" in random order.

The slides were examined for the incidence of micronucleated cells per 2000 polychromatic (PCE) and 1000 normochromatic (NCE) erythrocytes per animal. The ratio of polychromatic to normochromatic erythrocytes was calculated on the basis of 1000 NCE scored.

Any toxic effect of the test material on the immature nucleated cells may lead either to a reduction in cell division or to cell death. These effects in turn lead to a reduction in cell numbers and to compensate for this, peripheral blood is shunted into the bone marrow. Therefore, a decrease in the frequency of polychromatic erythrocytes is taken as being indicative of toxicity. A statistical analysis was conducted for each of the following variables: proportion of micronucleated PCE, proportion of micronucleated NCE and ratio of PCE/NCE.

Findings Negative - the test material failed to show any evidence of mutagenic potential when administered by gavage up to the toxic dose level of 200 mg/kg. Triaziquone, the positive reference, gave the expected mutagenic response.

Other toxicities: three days after application of 100 mg/kg one male died; after application of the high dose (200 mg/kg) three males died two days after application, one male and one female after three days. More than half of the animals of the two highest dose groups showed signs of toxicity (predominantly apathy, eyelid closure, ruffled fur)."

2.1.3 DNA damage in rat cerebral cortical cells (single cell gel electrophoresis)

Reference **"**Jin M, Song P, Li N, Li X and Chen J (2012). A plastic stabilizer dibutyltin dilaurate induces subchronic neurotoxicity in rats. Neural Regen. Res., 7, 2213-2220.

Guideline Non-guideline. Non-GLP.

Reliability Klimisch 3: reliable with restrictions (non-guideline study published in a peer-reviewed journal, but of low quality and with major deviations particularly regarind methods and results).

Species/strain Wistar male/female rats

Test material **Dibutyltin dilaurate (DBTDL)**

EC number: 201-039-8

CAS Number: 77-58-7

Purity not reported

Study design Animals (40 in total, 10 rats/dose group) were gavaged with DBTDL in corn oil at dose levels of 0, 5, 10 and 20 mg/kg bw/day for 5 days/week for 7 weeks. The single cell gel electrophoresis assay (Comet assay) was performed by the modified Singh method (Singh NP et al (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res., 175, 184-191). 50 ethidium bromide stained cells were scored per slide and the DNA damage was divided into 5 levels (0 – 4). The method of isolating cerebral cortical cells from brain tissue appears not to have been specified.

Findings Positive – a significant 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 was observed."

2.2 Reproductive toxicity

Detailed summaries of studies relevant to classification for reproductive toxicity (adverse effects on sexual function and fertility, adverse effects on development of the offspring, effects on or via lactation) are presented in this section.

The studies described below have been described in the CLH-dossier for DBTP (EC 245-152-0) and assessed by RAC in 2017. Where the description of the studies below are in quotes the text is a verbatim rendering of the text in the mentioned CLH-dossier.

2.2.1 Animal data

2.2.1.1 Reproductive/developmental toxicity screening study in the rat

Reference	"Unpublished report (2003) TNO, The Netherlands. TNO Report V4906. [Study summary included in the publically disseminated REACH Registration Dossier for dibutyltin dichloride]
Guideline	OECD 421
Reliability	Klimisch 2: reliable with restrictions (Guideline and GLP-compliant study, full report not available)
Species / strain	Rat (Wistar)
Test material	**Dibutyltin dichloride (DBTC)** CAS 683-18-1 EC 211-670-0 98.57% purity
Study design	Groups of 12 Wistar rats/sex were administered the test material in the diet at concentrations of 0, 5, 30 or 200 ppm for four weeks (males) or for two weeks prior to mating and to Day 4 or 6 *post partum* (females). Animals were observed daily for mortality and clinical signs. Bodyweights were measured pre-test and on Days 0, 7 and 14 of the pre-mating period. Bodyweights of males were measured on Days 21 and 28; bodyweights of females were measured during mating (Days 0, 7 and 14); on Days 0, 7, 14 and 21 of gestation; on Days 1 and 4 of lactation and at termination. Food consumption was measured for both sexes during the pre-mating period (Days 0-7, 7-13); for males during the post-mating period (Day 21-28); for females during the gestation period (Days 0-7, 7-14, 14-21) and during the lactation period (Days 1-4). At termination, weights of the kidney liver, spleen, testes, thymus and brain were recorded for parental animals. Gross necropsy was performed on all parental animals; the prostate, seminal vesicles and testes of male animals were investigated. The ovaries, uterus (including counting of implantation sites) and thymus were investigated in females. Pups were assessed for gross abnormalities.
Findings	Mean male bodyweights were significantly lower from Day 14-28 of the study at the highest dose level. Weight gains by males at the highest dose level were significantly lower over the study period; weight gain by males at 30 ppm was also reduced from Day 14-21. There was no effect of treatment on weight gain in males administered 5 ppm DBTC. Weight gain by females at the highest dose level was reduced over the premating, gestation and lactation periods; mean bodyweights of females in this group were significantly lower at Day 14 of the premating period, over the entire gestation period and during the lactation period. Food consumption by males at the highest dose level was reduced; significantly at Day 7-14. Food consumption by females in this group was significantly reduced during the pre-mating, gestation and lactation periods. Food consumption by females in the other treatment groups was comparable to controls. The number of pregnant females was comparable in all groups. A marked increase in post-implantation loss was seen at 200 ppm; only three females in this group had live offspring. Pup weight at birth and Day 4 at the highest dose level was also significantly lower than controls. Pup mortality in this group was markedly increased (50%) compared to controls (5%). One pup at the highest dose level had a missing tail tip.

Reproductive parameters

Dietary concentration (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%

Gross necropsy and histopathology of males did not reveal any effects of treatment on the reproductive tract. Thymuses of males were not investigated histopathologically. Severe or very severe thymic lymphoid depletion was observed in females at 200 ppm; moderate to severe lymphoid depletion was seen in the majority of the pregnant (but not in the non-pregnant) females at 30 ppm. 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.

Conclusion Administration of DBTC in the diet at a concentration of 200 ppm caused an increase in post-implantation loss. The NOAEL for effects on reproduction for this study is therefore 30 ppm (equivalent to 1.7-2.4 mg/kg bw/d in females)."

2.2.1.2 Developmental toxicity study in the rat

Reference "Ema M & Harazono A (2000). Adverse effects of dibutyltin dichloride on initiation and maintenance of rat pregnancy. Reproductive Toxicology 14: 451-456.

Guideline No guideline followed. The study was designed to assess the effects of exposure to the test material on post-implantation loss following exposure of female rats during the early gestation period.

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

97% purity

Study design Mated female Jcl:Wistar rats (16-19/group) were gavaged with the test material (in olive oil) at dose levels of 0 (vehicle control), 3.8, 7.6 or 15.2 mg/kg bw/d on Gestation Day 0-3 or Gestation Day 4-7. Groups of food-restricted rats were provided with the same amount of diet as consumed by rats administered the test material at 15.2 mg/kg bw/d on GD 0-3 or on GD 4-7.

Rats were observed for mortality and signs of toxicity. Bodyweights and food consumption were measured daily. Female rats were terminated on Gestation Day 20 and the uterus assessed. Corpora lutea and implantation numbers were reported. Foetuses were assessed for viability, sexed, weighed and investigated for gross external malformations and malformations of the oral cavity.

Findings No deaths were seen in females of any group. After administration of the test material on GD 0-3, female rats in the treated groups showed signs of toxicity (chromodacryorrhoea, piloerection and diarrhoea); the incidence of clinical signs increased in a treatment-related manner. Bodyweight gains on Days 0-4 were significantly reduced in all treated groups; weight loss was seen. Bodyweight gains on Days 4-20 and adjusted weight gains were significantly lower in females administered 7.6 and 15.2 mg/kg bw/d. Food consumption on Days 0-4 and Days 4-20 were significantly reduced at

≥3.8 mg/kg bw/d and at ≥7.6 mg/kg bw/d respectively. The proportion of non-pregnant females and the incidence of pre-implantation loss were both significantly higher at 7.6 mg/kg bw/d (compared to controls) and at 15.2 mg/kg bw/d (compared to the control and pair-fed groups). Only two dams at the highest dose level had litters with viable foetuses. In females with implantations, the numbers of implantations and live foetuses and the incidence of post-implantation loss in treated groups were comparable to controls. Mean foetal weights in treated groups were comparable to controls. Pair-fed controls showed a comparable weight loss to the highest dose level dams on GD 0-4; weight gain on GD 0-20 was less than controls but was notably higher than at the highest dose level. A slight increase in pre-implantation loss was seen in pair-fed controls, but not to the extent seen at the highest dose level; post-implantation loss was significantly higher than controls. Mean foetal weight was significantly reduced in the pair-fed controls.

Summary of findings: rats exposed GD 0-3

Group	Control	3.8	7.6	15.2	Pair-fed control
Mated (#)	19	16	16	16	17
Pregnant (#)	19	16	11*	2*	16
Non-pregnant (#)	-	-	5*	14*	1
Weight gain (g) D0-4	6	-2*	-14*	-20*	-20*
Weight gain (g) D4-20	100	104	74*	27*	75*
Adjusted weight gain (g)	35	29	16*	-5*	12
Food consumption (g) D0-4	51	35*	16*	13*	12*
Food consumption (g) D4-20	288	280	237*	197*	200*
Implantations (#)	15.0	15.0	10.1*	1.8*	13.4
Pre-implantation loss (%)	2.7	4.1	35.6*	87.9*	16.4
Litters (#)	19	16	11	2	16
Total resorption (#)	-	-	1	-	3
Corpora lutea (#)	15.0	15.6	15.6	14.5	16.2
Early resorptions (#)	1.0	1.0	3.0	1.0	4.3*
Late resorptions (#)	-	-	-	-	-
Post-implantation loss (%)	6.7	6.8	21.3	7.1	32.1*
Litter size (#)	14.1	14.0	11.6	13.0	10.0*
Foetal weight M (g)	3.42	3.50	3.48	3.25	3.09*
Foetal weight F (g)	3.25	3.26	3.28	3.02	2.95*

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

After administration of the test material on Gestation Day 4-7, female rats in the treated groups showed signs of toxicity (chromodacryorrhoea, piloerection and diarrhoea); the incidence of clinical signs increased in a treatment-related manner. Weight gain over GD 4-8 was reduced in all treated groups, significantly at ≥7.6 mg/kg bw/d; food consumption over the same period was significantly reduced in all treated groups. Adjusted weight gain was significantly reduced in dams at 15.2 mg/kg bw/d. Pre-implantation loss was increased at 15.2 mg/kg bw/d; the number of total resorptions was significantly increased in this group and was slight increased at 7.8 mg/kg bw/d. Post-implantation loss was significantly increased in all treated groups, with a clear dose-response relationship. Litter size and mean foetal weights were significantly reduced at ≥7.6 mg/kg bw/d. Pair-fed controls also showed a significantly reduced weight gain over GD 4-8 and significantly reduced adjusted weight gain. A slight increase in post-implantation loss and significantly reduced mean foetal weights were also seen in this group.

Summary of findings: rats exposed GD 4-7

Group	Control	3.8	7.6	15.2	Pair-fed

Mated (#)	16	16	16	17	17
Pregnant (#)	16	16	16	16	17
Non-pregnant (#)	-	-	-	1	-
Implantations (#)	15.0	14.0	15.0	14.1	14.6
Weight gain (g) D0-4	12	11	9	10	9
Weight gain (g) D4-8	8	4	-2*	-14*	-15*
Weight gain (g) D8-20	227	228	226	228	224
Adjusted weight gain (g)	35	32	30	5*	0*
Food consumption (g) D0-4	68	68	64	65	66
Food consumption (g) D4-8	57	46*	34*	25*	25*
Food consumption (g) D8-20	219	213	210	158*	145*
Pre-implantation loss (%)	2.4	4.5	4.4	32.7	5.9
Litters (#)	16	16	16	16	17
Total resorption (#)	-	-	3	14*	2
Corpora lutea (#)	15.4	15.4	16.2	16.3	15.7
Early resorptions (#)	1.1	2.1	6.3*	13.6*	2.5
Late resorptions (#)	-	-	-	-	-
Post-implantation loss (%)	7.0	13.9*	39.9*	91.5*	18.3
Litter size (#)	13.9	12.6	9.3*	1.3*	12.1
Foetal weight M (g)	3.45	3.38	2.99*	2.62*	2.98*
Foetal weight F (g)	3.22	3.16	2.85*	2.74*	2.74*

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

In females with implantations, the numbers of *corpora lutea*, implantations, resorptions, dead and live foetuses, the incidence of totally resorption, the proportions of pre- and post-implantation loss were unaffected by treatment. Foetal bodyweight and sex ratio were comparable in all groups. No external foetal malformations were noted in any group.

Conclusion The results of this study show that the administration of DBTC at dose levels of ≥7.6 mg/kg bw during very early gestation (GD 0-3) causes an increase in pre-implantation loss, including a high incidence of total litter loss. Maternal toxicity (clinical signs, reduced weight gain and food consumption) was seen in all treated groups.

Administration of DBTC at dose levels of ≥3.8 mg/kg bw during early gestation (GD 4-7) causes an increase in post-implantation loss. Maternal toxicity (clinical signs, reduced weight gain and food consumption) was seen in all treated groups. Reductions in litter size and foetal weight were seen at ≥7.6 mg/kg bw/d. Pair-fed control groups included in the design of this study show that maternal toxicity (reduced food consumption and weight gain) caused by exposure to the highest dose level of DBTC resulted in some effects (increased post-implantation loss, reduced foetal weight), but not to the same extent as seen in the DBTC-treated groups. Exposure to DBTC on GD 0-3 or GD 4-7 did not result in teratogenicity (external malformations or malformations of the oral cavity). A NOAEL of ≤3.8 mg/kg bw/d can be determined for this study, based on the significantly increased incidence of post-implantation loss in dams administered DBTC on GD 4-7."

2.2.1.3 Developmental toxicity study in the mouse

Reference "Ema M, Fujii S, Ikka T, Matsumoto M, Hirose A & Kamata E (2007a). Early pregnancy failure induced by dibutyltin dichloride in mice. Environmental Toxicology 22(1):44-52.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Mouse (CRlj:CD1(ICR)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

99.5% purity

Study design The effects of oral administration of DBTC during early gestation were investigated in the mouse. Groups of mated female ICR mice were gavaged with DBTC (in olive oil) at dose levels of 0 (vehicle control), 7.6, 15.2 or 30.4 mg/kg bw on GD 0-3 or GD 4-7. Mice were observed at least daily for signs of toxicity. Maternal bodyweights were recorded daily; food consumption was measured at regular intervals. Mice were terminated on GD 18 and the uterine contents examined. The uterus was weighed and the number of corpora lutea recorded. The numbers of implantations, live and dead foetuses and resorptions were counted. The uteri were placed in 10% ammonium sulphide for confirmation of pregnancy status. Foetuses were sexed, weighed and inspected for external malformations and malformations of the oral cavity. Placental weight was also measured. Terminal blood samples were taken from dams of control and highest dose groups for the measurement of serum progesterone and 17β-oestradiol.

Findings In mice administered DBTC on GD 0-3, mortality occurred in each treated group but without a dose-response relationship. It is unclear, therefore, if deaths are related to treatment. Signs of toxicity (vaginal discharge, hypoactivity, hypothermia) were observed in all treated groups; jaundice was additionally observed at 15.2 and 30.4 mg/kg bw. Bodyweight gain and food consumption were reduced in the treated groups; significantly at the highest dose level.

Maternal findings: dosing on GD 0-3

Dose level (mg/kg bw)	**0**	**7.6**	**15.2**	**30.4**
Mated (#)	12	12	12	12
Mortality (#)	-	2	1	1
Weight gain (g) GD 0-4	1.7	0.6	1.2	0.3*
Weight gain (g) GD 4-8	2.9	2.5	2.1	1.6
Weight gain (g) GD 8-18	20.1	9.9	7.9	5.3
Adjusted weight gain (g)	8.9	9.9	7.9	5.3
Food consumption (g) GD 0-4	1.82	15.0*	16.7	14.8*
Food consumption (g) GD 4-8	22.9	22.0	21.7	20.9
Food consumption (g) GD 8-18	71.7	71.0	64.6	57.8*

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

The number of pregnant females was lower in al treated groups; significantly at 30.4 mg/kg bw and with a clear dose-response relationship; findings are associated with increased pre-implantation loss. Post-implantation loss was also increased in the treated groups, significantly at 15.2 mg/kg bw. Mean foetal weights were significantly reduced in all treated groups. There was no clear effect of treatment on the incidence of malformations. One foetus at 15.2 mg/kg bw showed findings characteristic of DBTC (cleft palate, kinked tail); however no findings were seen at the highest dose level (although the number of foetuses available for examination in this group was lower than other groups) and cleft palate was also seen in one control foetus.

Litter findings: dosing on GD 0-3

Dose level (mg/kg bw)	**0**	**7.6**	**15.2**	**30.4**
Mated (#)	12	12	12	12
Pregnant (#)	11	9	8	5*
Corpora lutea (#)	10.5	13.1	12.4	13.3
Implantations (#)	9.5	9.8	8.3	5.4

Pre-implantation loss (%)	9.7	29.7	34.0	58.3*
Total resorption (#)	-	-	1	1
Post-implantation loss (%)	10.1	14.1	41.3*	32.2
Live foetuses (#)	9.4	11.5	8.1	9.3
Foetal weight (M)	1.54	1.30*	1.14*	1.12*
Foetal weight (F)	1.42	1.28	1.08*	1.01*
Foetuses examined (#)	103	92	57	37
Malformations (#)	1 (1)	-	2 (1)	-
Cleft palate (#)	1 (1)	-	1 (1)	-
Kinked tail (#)	-	-	1 (1)	-

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

In mice administered DBTC on GD 4-7, one death occurred at 15.2 mg/kg bw. Signs of toxicity (vaginal discharge, hypoactivity, hypothermia) were observed in all treated groups; jaundice was additionally observed at 30.4 mg/kg bw. Bodyweight gain and food consumption were reduced in the treated groups.

Maternal findings: dosing on GD 4-7

Dose level (mg/kg bw)	**0**	**7.6**	**15.2**	**30.4**
Mated (#)	12	12	12	12
Mortality (#)	-	-	1	-
Weight gain (g) GD 0-4	1.6	1.9	1.2	1.6
Weight gain (g) GD 4-8	3.1	1.9	0.5*	-0.3*
Weight gain (g) GD 8-18	24.9	14.9*	2.9*	2.4*
Adjusted weight gain (g)	8.3	8.1	3.2*	3.8*
Food consumption (g) GD 0-4	18.5	18.9	18.4	18.8
Food consumption (g) GD 4-8	21.8	19.2	16.4*	15.6*
Food consumption (g) GD 8-18	74.5	67.7	55.2*	57.2*

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

The number of pregnant females was comparable in all groups. Pre-implantation loss was increased at 15.2 and 30.4 mg/kg bw. Total resorption was increased in all treated groups (significantly at 15.2 and 30.4 mg/kg bw) and with a clear dose-response relationship. Post-implantation loss was markedly increased in all treated groups and reached 100% at the highest dose level. Litter size was consequently reduced in all treated groups. Mean foetal weights were significantly reduced in all treated groups. There was no clear effect of treatment on the incidence of malformations. Two foetuses at 7.6 mg/kg bw showed malformations (omphalocoele, exencephaly); no malformations were seen at higher dose levels, however no foetuses were examined at 30.2 mg/kg bw/d and the numbers of foetuses examined at 15.2 was very low. A teratogenic effect of DBTC cannot therefore be excluded.

Litter findings: dosing on GD 4-7

Dose level (mg/kg bw)	**0**	**7.6**	**15.2**	**30.4**
Mated (#)	12	12	12	12
Pregnant (#)	11	11	10	11
Corpora lutea (#)	13.8	14.5	10.6	13.9
Implantations (#)	13.7	14.4	9.4	12.7
Pre-implantation loss (%)	8.9	8.9	24.7	18.3
Total resorption (#)	-	2	8*	10*

Post-implantation loss (%)	4.3	48.3*	94.4*	100*
Live foetuses (#)	13.1	7.2*	0.8*	-
Foetal weight (M)	1.45	1.23*	1.27	
Foetal weight (F)	1.39	1.18*	1.18	
Foetuses examined (#)	144	79	7	
Malformations (#)	-	2 (2)	-	
Omphalocoele (#)	-	1 (1)	-	
Exencephaly (#)	-	1 (1)	-	

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

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 (values represented graphically in the published paper).

Conclusion Administration of DBTC to pregnant mice during early gestation results in pregnancy failure, which is associated with reduced progesterone levels at high dose levels. Increased post-implantation loss was seen at all dose levels in this study, the NOAEL is therefore <7.6 mg/kg bw/d. There is no clear indication of teratogenicity in this study."

2.2.1.4 Mechanistic study in the rat

Reference "Ema M, Harazono A, Hirose A & Kamata E (2003). Protective effects of progesterone on implantation failure induced by dibutyltin dichloride in rats. Toxicology Letters 143(2):233-8.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species/strain Rat (Jcl:Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

98% purity

Study design Groups of 14-15 mated female Jcl:Wistar rats were gavaged with DBTC (in olive oil) at dose levels of 0 (vehicle control), 7.6, or 15.2 mg/kg bw on GD 0-3, with or without progesterone supplementation (subcutaneous injection of 2 mg progesterone GD 0-8. Maternal bodyweight and food consumption were measured. Pregnancy outcome was determined on GD 9 and reproductive outcome was investigated. Numbers of corpora lutea and implantations were measured.

Findings Marked weight loss and reduced food consumption were observed at both dose levels of DBTC. Effects at 7.6 mg/kg bw/d were reduced slightly by the administration of progesterone; however progesterone administration had little effect at 15.3 mg/kg bw/d.

Administration of progesterone alone had no effect on pregnancy rate or on the number of implantations. Both the pregnancy rate and the number of implantations were significantly lower in the groups administered DBTC; some reduction in pregnancy rate and the number of implantations were also seen in the groups administered progesterone and DBTC; although parameters were not affected to the same extent as in the groups administered DBTC alone.

Summary of findings [24]

Dose level (mg/kg bw/d)	**0**		**7.6**		**15.2**	
Progesterone +/-	**-P**	**+P**	**-P**	**+P**	**-P**	**+P**
Weight gain (g) D0-4	8	7	-24*	-24*	-31*	-28*
Weight gain (g) D4-9	12	14	-11*	-22*	-35*	-31*

Food consumption (g) D0-4	48	46	10*	9*	4*	3*
Food consumption (g) D4-9	80	78	25*	15*	2*	4*
Mated (#)	14	14	15	14	15	14
Pregnant (#)	14	14	7*	13	5*	9*
Implantations (#)	14.9	15.1	5.6*	11.6	2.9*	6.1*
Pre-implantation loss (%)	8.6	10.5	62.8*	25.9*	81.3*	60.0*

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

Conclusion The study confirms other data by the same authors which demonstrates an adverse effect of DBTC on pregnancy rate and implantation numbers when administered to pregnant rats during very early gestation. There is some indication for a protective effect of progesterone on implantation failure; the authors therefore propose that implantation failure due to DBTC is due to a decline in progesterone levels.

NOAELs of <7.6 mg/kg bw/d for maternal toxicity and developmental toxicity can be determined for this study."

2.2.1.5 Mechanistic study in the rat

Reference "Harazono A & Ema M (2003). Suppression of decidual cell response induced by dibutyltin dichloride in pseudopregnant rats: as a cause of early embryonic loss. Reproductive Toxicology 17(4):393-9.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

No purity details

Study design Groups of pseudopregnant female Wistar rats were administered DBTC by gavage at dose levels of 0 (vehicle control), 3.8, 7.6 or 15.2 mg/kg bw on pseudopregnant day (PPD) 0-3 or PPD 4-7. Decidual cell response was induced by bilateral uterine scratch on PPD 4. Uterine weight (PPD 9) was used as an index of uterine decidualisation.

Findings Uterine weight and serum progesterone levels on PPD 9 were significantly decreased after administration of DBTC at 7.6 and 15.2 mg/kg bw (PPD 0-3 and 4-7). Treatment with DBTC had no effect on the serum oestradiol levels or the number of corpora lutea. Administration of progesterone reversed the suppression of uterine decidualisation seen in rats administered DBTC on PPD 0-3.

Conclusion The authors conclude that DBTC administration to the pregnant rat suppresses the uterine decidual cell response and decreases progesterone levels. It is proposed that these effects may be factors involved in the induction of early embryonic loss resulting from exposure to DBTC."

2.2.1.6 Developmental toxicity study in the rat

Reference "Noda T, Morita S & Baba A (1993). Teratogenic effects of various di-n-butyltins with different anions and butyl(3-hydroxybutyl)tin dilaurate in rats. Toxicology 85: 149-60.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Dibutyltin di(acetate) (DBTA)

CAS 1067-33-0

EC 211-670-0

Purity not reported

Dibutyltin maleate (DBTM)

CAS 78-04-6

EC 201-077-5

Purity not reported

Dibutyltin dilaurate (DBTL)

CAS 77-58-7

EC 201-039-8

Purity not reported

Dibutyltin oxide (DBTO)

CAS 818-08-6

EC 212-449-1

Purity not reported

Study design Groups of 10 mated female Wistar rats were gavaged with a single dose (equivalent to 80 µmol/kg bw) of five dibutyltin substances (in olive oil) on Gestation Day 8. A concurrent control group received the dosing vehicle only. Dams were observed daily for clinical signs; bodyweights and food consumption were measured daily. Dams were sacrificed on Gestation Day 20 and the uterine contents investigated. Foetuses were weighed, sexed and were assessed for external malformations and for skeletal malformations following staining with Alizarin Red S.

Findings There was no maternal mortality or signs of toxicity. Maternal bodyweights and food consumption were unaffected by treatment. No significant effects of treatment were seen on implantation numbers, implantation losses, litter size or foetal weight.

A significantly higher incidence of external foetal malformations was observed in all the treated groups; the nature of malformations was similar in all groups. Findings consisted predominantly of exencephaly and mandible findings (cleft mandible, cleft lower lip, ankyloglossia, schistoglossia).

External malformations

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.

Skeletal malformations

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

Skeletal variations

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 ($p<0.05$); **$p<0.01$*

Conclusion The results of the study demonstrate that the di-*n*-butyltin compounds cause a similar spectrum of foetal malformations when administered during a sensitive period of gestation. The di-*n*-butyltin moiety rather than the associated anionic group is therefore concluded to be responsible for teratogenicity. A NOAEL cannot be determined for this study."

2.2.1.7 Developmental toxicity study in the rat

Reference "Study report (1994). Summary included in the publically disseminated REACH Registration Dossier for dibutyltin dichloride; the full study report is not available. Anonymous.

Guideline OECD 414; no deviations reported

Reliability Klimisch 2: reliable with restrictions (Guideline and GLP-compliant study, full report not available)

Species / strain Rat (Wistar) Crl:CD(Wi)BR

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

>98% purity

Study design Groups of 25 mated Wistar female rats were gavaged with the test material (in corn oil) at dose levels of 0, 1, 2.5, 5 or 10 mg/kg bw/d on Days 6-15 of gestation. Dams were investigated for mortality and clinical signs. Bodyweights and food consumption were recorded at regular intervals.

Rats were sacrificed on Day 20 of gestation and the uterine contents investigated. All foetuses were assessed for external findings. Foetuses were sexed and weighed. Approximately half of the foetuses from each litter were assessed for visceral findings; the remainder of the foetuses were assessed for skeletal findings following staining with Alizarin Red.

Dams were subject to gross necropsy; the thymus, liver, bile duct and mesenteric lymph nodes were investigated histopathologically.

Findings No deaths occurred and no signs of maternal toxicity were observed during the study period. Reduced weight gain and food consumption were seen at 10 mg/kg bw/d; weight gain was also slightly reduced at 5 mg/kg bw/d. Gross necropsy of dams did not reveal any treatment-related findings. Mean thymus weight was significantly lower in dams at 10 mg/kg bw/d. Histopathology of the thymus showed atrophy at 10 mg/kg bw/d and to a lesser extent at 5 and 2.5 mg/kg bw/d.

Mean litter size and foetal weights were comparable in all groups.

The incidence of foetuses with malformations was increased at 10 mg/kg bw/d; four foetuses from three litters had malformations. Oedema (anasarca) was observed in one foetus; the remaining three foetuses showed more severe malformations. One showed ankyloglossia, hydrocephaly,

anophthalmia and diaphragmatic hernia. A second foetus exhibited agnathia, absent mandibles and malformed zygomatic arches. A third foetus had a filamentous and curly tail, scoliosis and an absence of sacral and caudal vertebrae and sacral vertebral arches.

Conclusion A NOAEL for maternal toxicity of 1 mg/kg bw/d can be determined for this study based on thymic atrophy at dose levels of ≥2.5 mg/kg bw/d; reduced weight gain at ≥5 mg/kg bw/d. A NOAEL for developmental toxicity of 5.0 mg/kg bw/d can be determined for this study based on an increased incidence of skeletal malformations at 10 mg/kg bw/d."

2.2.1.8 Developmental toxicity study in the rat

Reference "Farr CH, Reinisch K, Holson JF & Neubert D (2001). Potential teratogenicity of di-n-butyltin dichloride and other dibutyltin compounds. Teratogenesis, Carcinogenesis & Mutagenesis 21(6):405-15.

Guideline OECD 414

Reliability Klimisch 2: reliable with restrictions (guideline study summary, published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design A developmental toxicity study was conducted in the rat according to OECD guidelines and GLP. Groups of 25 mated female Wistar rats were gavaged with DBTC (in olive oil) at dose levels of 0, 1, 2.5, 5 or 10 mg/kg bw on GD 6-15. Evaluation of pregnancy outcome was performed on day 20 of pregnancy.

Findings Maternal toxicity (reduced food consumption, bodyweight gain and reduced thymus weight) were seen at 10 mg/kg bw. No evidence of embryotoxicity as assessed by numbers of total resorptions, viable foetuses or foetal weight was noted in any treated group. **A slightly increased frequency of total malformations was seen at 10 mg/kg bw (4/262 foetuses) compared to the control group (1/269 foetuses).** The authors consider that the nature and pattern of malformations does not suggest any effect of treatment; however the nature of findings (including single incidences of ankyloglossia, agnathia, mandibular defect) are consistent with the results of other studies and therefore indicate a relationship to treatment with DBTC

Maternal findings

Dose level (mg/kg bw)	**0**	**1.0**	**2.5**	**5.0**	**10.0**
Inseminated females (#)	25	25	25	25	25
Pregnant females (#)	20	25	23	19	20
100% intrauterine deaths (#)	0	1	0	1	0
Females with viable foetuses (#)	20	24	23	18	20
Malformed foetuses (#)	1/269	0-343	0-292	1/224	4/262
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**
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

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

Conclusion A NOAEL of 5 mg/kg bw can be determined for teratogenicity and developmental toxicity, based on the slightly elevated incidence of characteristic foetal malformations at 10 mg/kg bw/d. A NOAEL of 5 mg/kg bw/d can be determined for maternal toxicity, based on reduced bodyweight gain, food consumption and reduced thymus weight at the highest dose level."

2.2.1.9 Developmental toxicity study in the rat

Reference "Ema M, Itami T & Kawasaki H (1991). Teratogenicity of di-n-butyltin dichloride in rats Toxicology Letters 58(3): 347-356.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design Groups of mated female Wistar rats were gavaged with DBTC (in olive oil) at dose levels of 0 (vehicle control), 2.5, 5.0, 7.5 or 10 mg/kg bw/d on Days 7-15 of gestation. Dose levels were based on the individual bodyweights at Day 0 of gestation and were not subsequently adjusted. Animals were observed daily for mortality and clinical signs. Bodyweights and food consumption were also measured daily. Dams were terminated on Gestation Day 20; the numbers of live and dead foetuses and resorptions were recorded. Foetuses were sexed, weighed and investigated for external malformations and for malformations of the oral cavity. Placental weight was measured. Approximately two thirds of the foetuses from each litter were assessed for skeletal findings following staining with Alizarin Red S. The remaining foetuses were fixed in Bouin's solution and examined for internal malformations following freehand serial sectioning.

Findings The majority of rats administered 7.5 and 10.0 mg/kg bw/d DBTC showed signs of toxicity including chromodacryorrhoea and piloerection. A high level of mortality was seen in rats administered 7.5 mg/kg bw/d (5/12) and at 10 mg/kg bw/d (9/12) groups; deaths occurred on average at 8 and 6 days after dosing with 7.5 and 10 mg/kg bw/d, respectively. Necropsy of the decedent females revealed haemorrhagic stomachs. Maternal bodyweight gain on Gestation Days 7-15, 15-20 and 0-20 were markedly (and generally significantly) lower at 7.5 and 10 mg/kg bw/d compared to controls; adjusted weight gain was also significantly lower in these groups. Food consumption over Gestation Days 7-15, 15-20 and 0-20 was significantly lower at 7.5 and 10 mg/kg bw/d compared to controls. No significant effects on maternal bodyweight or food consumption were seen at 2.5 or 5 mg/kg bw/d.

Maternal findings

Dose level (mg/kg bw/d)	**0**	**2.5**	**5.0**	**7.5**	**10**
Pregnant rats (#)	11	10	11	12	12

Deaths (#)	0	0	0	5*	9*
Weight gain (g) GD 0-7	25	21	26	25	21
Weight gain (g) GD 7-15	38	34	27	-9*	6*
Weight gain (g) GD 15-20	65	61	59	-17*	30
Weight gain (g) GD 0-20	128	116	112	-2*	58*
Adjusted weight gain (g)	56	46	50	-20*	14*
Food consumption (g) GD 0-7	129	105	127	114	131
Food consumption (g) GD 7-15	140	126	118	80*	85*
Food consumption (g) GD 15-20	107	100	108	39*	69
Food consumption (g) GD 0-20	376	331	353	232*	285*

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

Complete resorption was seen in at 7.5 mg/kg bw/d (5/7 surviving rats) and at 10 mg/kg bw/d (1/3 surviving rats); there were consequently only two dams with live foetuses at 7.5 and 10 mg/kg bw/d. Significantly higher numbers of resorptions and dead foetuses per litter, a significantly higher proportion of post-implantation loss and a significantly lower litter size were observed at 7.5 and 10 mg/kg bw/d. Mean foetal and placental weights were significantly lower at 5.0, 7.5 and 10 mg/kg bw/d.

Reproductive findings

Dose level (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)*

A dose-related increase in the incidence of foetuses with external malformations was observed at 5.0, 7.5 and 10 mg/kg bw/d. Craniofacial malformations predominated; most frequently cleft jaw and ankyloglossia. Cleft jaw varied in severity from mandibular hypoplasia and a small cleft on the midline of the lower jaw, to a large v-shaped cleft in the lower jaw. Mild findings were associated with fusion of the tongue at the midline of the lower lip; more sever cleft jaw was associated with ankyloglossia and/or cleft tongue. Micrognathia, cleft palate, omphalocoele, exencephaly, anal atresia, club foot and anomalies of the tail (vestigial, kinked and short tail) were also frequently observed in foetuses from the 5.0, 7.5 and l0 mg/kg bw/d dose groups. No external malformations were observed in the control or 2.5 mg/kg bw/d dose groups. In the 5.0 mg/kg bw/d group, 12% of the malformed foetuses had a single finding such as omphalocoele and exencephaly; 59% of the malformed foetuses had cleft jaw and ankyloglossia. The majority of affected foetuses in this group had a relatively slight cleft jaw. At 7.5 mg/kg bw/d, 12% of the malformed foetuses had micrognathia only. 61% of the malformed foetuses had cleft jaw, ankyloglossia and/or cleft tongue. At 10 mg/kg bw/d, all malformed foetuses showed multiple findings; 88% of the malformed foetuses had cleft jaw, ankyloglossia and/or cleft tongue, and also had other types of malformation. The cleft jaw seen at 7.5 and 10 mg/kg bw/d was more severe than that seen at 5.0 mg/kg bw/d.

Incidence of external malformations

Dose level (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 ($p<0.05$)*

A significant increase in the incidence of skeletal malformations was also observed at dose levels of 5.0 mg/kg bw/d and above. Defects of the mandible, fusion of the ribs and deformity of the vertebral column, including fusion and/or absence of the vertebral bodies and/or arches in the cervical and/or thoracic regions were significantly increased. Defects of the mandible were found in foetuses with cleft jaw. The severity of the mandibular defect reflected the severity of cleft jaw and varied from separation of the right and left mandibles to small/short mandible and a wide separation between right and left mandibles. The incidence of fused ribs and deformed vertebral column was significantly increased at dose levels of 5.0 mg/kg bw/d and above. A dose-related increase in the incidence of foetuses with skeletal variations was also observed in these groups.

Incidence of skeletal malformations

Dose level (mg/kg bw/d)	0	2.5	5.0	7.5	10
Examined (#)	84 (11)	80 (10)	83 (11)	16 (2)	18 (2)
Total malformations (#)	-	-	18 (5)*	13 (2)*	10 (2)*
Mandibular defect (#)	-	-	5 (2)	13 (2)*	10 (2*)
Cervical arches fused/absent (#)	-	-	4 (2)	7 (2)*	4 (1)
Thoracic arches/bodies fused/absent (#)	-	-	7 (2)	8 (2)*	9 (2)*
Lumbar arches/bodies fused/absent (#)	-	-	1 (1)	-	-
Fused ribs (#)	-	-	12 (4)*	10 (2)*	8 (1)
Absent ribs (#)	-	-	3 (2)	1 (1)	-
Cleft sternum (#)	-	-	-	3 (1)	-
Fused sternebrae (#)	-	-	3 (3)	-	-

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

Foetuses with internal malformations (undescended testis, hydrocephaly and microphthalmia) were observed at dose levels of 5.0 mg/kg bw/d and higher; findings were apparent in foetuses with external malformations.

Incidence of internal malformations

Dose level (mg/kg bw/d)	0	2.5	5.0	7.5	10
Examined	46 (11)	41 (10)	42 (11)	9 (2)	9 (2)
Total malformations	-	-	1 (1)	1 (1)	3 (1)
Undescended testes	-	-	1 (1)	-	-
Hydrocephaly	-	-	-	1 (1)	1 (1)
Microphthalmia	-	-	-	-	2 (1)

Conclusion Exposure to DBTC at dose levels of 5 mg/kg bw/d and above on Days 7-15 of gestation in the rat resulted in teratogenicity (predominantly craniofacial malformations). Dose levels of 7.5 and 10 mg/kg bw/d resulted in marked maternal toxicity (including mortality); however no maternal toxicity was apparent at 5.0 mg/kg bw/d. Administration of DBTC was also embryotoxic, resulting in complete resorption (at 7.5 and 10 mg/kg bw/d). Foetal weight was reduced at dose levels of 5.0 mg/kg bw/d and above; litter size was reduced at dose levels of 7.5 and 10 mg/kg bw/d.

Based on the results of this study, a NOAEL for developmental toxicity of 2.5 mg/kg bw/d can be determined. The NOAEL for teratogenicity is 2.5 mg/kg bw/d, based on increased incidences of craniofacial malformations at dose levels of 5.0 mg/kg bw/d and above. The NOAEL for maternal toxicity is 5.0 mg/kg bw/d, based on mortality and bodyweight effects at dose levels of 7.5 and 10 mg/kg bw/d."

2.2.1.10 Developmental toxicity study in the rat

Reference "Ema M, Itami T & Kawasaki H (1992). Susceptible period for the teratogenicity of di-n-butyltin dichloride in rats. Toxicology 73: 81-92.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design Groups of mated female Wistar rats were gavaged with DBTC (in olive oil) at a dose levels of 0 (vehicle control) or 20 mg/kg bw on Gestation Days 7-9, 10-12 or 13-15. Additional groups of mated female rats were gavaged with DBTC at dose levels of 0, 20 or 40 mg/kg bw on Gestation Days 6, 7, 8 or 9. Dose levels were based on bodyweights at Gestation Day 0 and were not subsequently adjusted. Dams were terminated on Gestation Day 20; the numbers of live and dead foetuses and resorptions were recorded. Foetuses were sexed, weighed and inspected for external malformations and malformations of the oral cavity. Approximately two thirds of the foetuses in each litter were examined for skeletal malformations following staining with Alizarin Red S. The remaining foetuses were fixed in Bouin's solution and assessed for visceral malformations following freehand serial sectioning.

Findings Dosing on Gestation Days 7-9, 10-12 or 13-15

Complete resorption was observed for five rats administered DBTC at 20 mg/kg bw/d on GD 7-9; six litters contained live foetuses. A significantly higher number of resorptions and dead foetuses, a lower number of live foetuses and an increased incidence of post-implantation loss were observed in this group. Mean foetal weights in all treated groups were significantly lower than controls. The numbers of live foetuses, dead foetuses and resorptions and the proportion of post-implantation loss in rats administered DBTC on GD 10-12 or GD 13-15 were comparable to control.

No foetuses with external malformations were found in the control groups or in the groups treated with DBTC on GD 10-12 or GD 13-15. Treatment with DBTC on GD 7-9 resulted in a significant

increase in the incidence of foetuses with external malformations; 26 of the 36 live foetuses in this group had external malformations. A significantly higher incidence of cleft jaw, ankyloglossia, omphalocoele, open eye, tail anomalies and club foot was seen, compared to controls. Of the 26 affected foetuses, one had a single malformation (omphalocoele), while the remainder had multiple findings. 54% of the malformed foetuses had omphalocoele and club foot. All foetuses with cleft jaw also showed ankyloglossia and/or cleft tongue. No skeletal malformations were observed in the control groups of the groups administered DBTC on GD 10-12 or GD 13-15. A significant increase in the incidence of foetuses with skeletal malformations was observed in the group treated with DBTC on GD 7-9; 14 of the 23 assessed foetuses had skeletal malformations. Deformity of the vertebral column including fusion and/or absence of the vertebral bodies and/or arches in the cervical and thoracic regions, fusion and/or absence of the ribs and cleft of the sternum were significantly increased in incidence. A significantly higher incidence of foetuses with visceral malformations was seen foetuses treated with DBTC on GD 7-9, but not in foetuses treated on GD 10-12 or GD 13-15. Eight of the 13 investigated foetuses showed internal malformations; the incidence of anophthalmia or microphthalmia was significantly increased. All internal malformations were found in foetuses also showing external malformations.

Reproductive and foetal findings in rats dosed on GD 7-9, 10-12 or 13-15

		Days of treatment		
	Controls	**GD 7-9**	**GD 10-12**	**GD 13-15**
Litters (#)	11	11	11	11
Implantations (#)	13.1	13.2	14.3	13.3
Resorptions (#)	1.3	9.9*	2.2	1.6
Post-implantation loss (%)	10.2	75.1*	15.4	14.0
Total resorption (#)	0	5*	0	0
Live foetuses (#)	11.8	3.3*	12.1	11.6
Foetal weight (g) M/F	4.05 / 3.92	2.43* / 2.38*	3.51*/ 3.29*	3.30* / 3.03*
External malformations				
Examined (#)	130 (11)	36 (6)	133 (11)	128 (11)
Malformations (#)	-	26 (6)	-	-
Skeletal malformations				
Examined (#)	84 (11)	23 (6)	87 (11)	85 (11)
Malformations (#)	-	14 (6)	-	-
Internal malformations				
Examined (#)	46 (11)	13 (5)	46 (11)	43 (11)
Malformations (#)	-	8 (4)*	-	-

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

Reproductive and foetal findings in rats dosed on GD 6, 7, 8 or 9

The incidence of total resorption was significantly increased in the groups treated with 40 mg/kg bw DBTC on Days 7 or 8; a significantly lower number of live foetuses per litter was also seen in these groups. An increased incidence of post-implantation loss was seen in the groups treated with DBTC on GD 6, 7, 8 or 9. Administration of 40 mg/kg bw DBTC on GD 6, 7 or 8 caused a significant increase in post-implantation loss; a similar effect was seen with 20 mg/kg bw only when administered on GD 8. A dose-related decrease in mean foetal weight was observed in the treated groups.

Treatment on GD 7 or 8 with DBTC at 20 or 40 mg/kg bw resulted in a significant and dose-related increase in the incidence of external foetal malformations. The highest incidence of malformations (14/95 foetuses at 20 mg/kg bw 23/34 foetuses at 40 mg/kg bw) was seen after treatment on GD 8.

21% (at 20 mg/kg bw) and 20% (at 40 mg/kg bw) of the malformed foetuses had a single malformation such as exencephaly, omphalocoele and encephalocoele following treatment with DBTC on GD 7. 50% (at 20 mg/kg bw) and 13% (at 40 mg/kg bw) of the malformed foetuses had a single malformation such as omphalocoele, club foot and exencephaly following treatment with DBTC on GD 8.

Treatment with 20 mg/kg bw DBTC on GD 7 or with 20 or 40 mg/kg bw DBTC on GD 8 resulted in a significantly increased incidence of foetuses with skeletal anomalies. The highest increase in the incidence of skeletal malformations resulted treatment with DBTC on GD8; 21 of the 63 foetuses at 20 mg/kg bw and 22 of 23 foetuses at 40 mg/kg bw showed malformations. Cleft sternum was the predominant finding in foetuses treated with 20 mg/kg bw on GD 7. Following treatment on GD 8, a dose-related increase in malformations of the cervical, thoracic and lumbar vertebrae; fusion and absence of the ribs and fusion of the sternebrae were observed.

A significantly higher incidence of visceral malformations was observed for groups treated with 20 or 40 mg/kg bw DBTC on GD 7 or GD 8. The predominant malformations were anophthalmia or microphthalmia and dilatation of the cerebral ventricles (treatment on GD 7), absence or hypoplasia of the kidney (treatment on GD 8).

Reproductive and foetal findings in rats dosed on GD 6 or GD 7

	Day of treatment			
	GD 6		**GD 7**	
Dose level (mg/kg bw)	**20**	**40**	**20**	**40**
Litters (#)	11	11	11	11
Implantations (#)	14.0	14.2	14.1	14.4
Resorptions (#)	2.5	6.1	3.5	10.6*
Post-implantation loss (%)	18.9	43.5*	24.6	76.2*
Total resorption (#)	1	3	1	7*
Live foetuses (#)	11.5	8.1	10.5	3.7
Foetal weight (g) M/F	3.78 / 3.59	3.57 / 3.38*	3.30* / 3.23*	3.41/ 3.22*
External malformations				
No. examined (#)	127 (10)	89 (8)	116 (10)	41 (4)
Total malformations (#)	0	2 (2)	14 (6)*	5 (4)*
Skeletal malformations				
No. examined (#)	85 (10)	59 (8)	78 (10)	27 (3)
Total malformations (#)	0	1 (1)	13 (6)*	1 (1)
Internal malformations				
No. examined (#)	42 (10)	30 (8)	38 (10)	14 (4)
Total malformations (#)	0	2 (2)	16 (7)*	6 (4)*

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

Reproductive and foetal findings in rats dosed on GD 8 or GD 9

	Day of treatment			
	GD 8		**GD 9**	
Dose level (mg/kg bw)	**20 mg/kg bw**	**40 mg/kg bw**	**20 mg/kg bw**	**40 mg/kg bw**
Litters (#)	11	11	11	11
Implantations (#)	14.6	13.3	14.1	14.2

Resorptions (#)	6.0	10.2*	1.3	4.0
Post-implantation loss (%)	42.8*	79.7*	8.6	31.7
Total resorption (#)	3	7*	0	3
Live foetuses (#)	8.6	3.1	12.8	10.2
Foetal weight (g) M/F	3.39*/ 3.26*	2.84*/ 2.49*	3.78 / 3.61	3.49* / 3.21*
External malformations				
No. examined (#)	95 (8)	34 (4)	141 (11)	112 (8)
Total malformations (#)	14 (6)*	23 (4)*	3 (2)	0
Skeletal malformations				
No. examined (#)	63 (8)	23 (4)	93 (11)	75 (8)
Total malformations (#)	21 (6)*	22 (4)*	3 (2)	5 (3)
Internal malformations				
No. examined (#)	32 (8)	11 (4)	48 (11)	37 (8)
Total malformations (#)	7 (4)*	7 (4)*	0	0

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

Conclusion The results of this study identity Gestation Day 7-8 as the critical period for DBTC-mediated teratogenicity in the rat; the most sensitive period was shown to be GD 8. Malformations were not induced following exposure on GD 6 or on GD 9 or later. Exposure at later time points resulted in post-implantation loss, reduced litter size and reduced foetal weight.

A NOAEL of <20 mg/kg bw can be determined for this study."

2.2.1.11 Developmental toxicity study in the rat

Reference "Ema M, Kurosaka R, Amano H & Ogawa Y (1995b). Comparative Developmental Toxicity of Butyltin Trichloride, Dibutyltin Dichloride and Tributyltin Chloride in Rats. Journal of Applied Toxicology 15(4): 297-302.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design Groups of 10 mated female STD:Wistar-ST rats were gavaged with DBTC (in olive oil) at dose levels of 0 (vehicle control), 10 or 15 mg/kg bw (based on GD 0 bodyweight) on Days 7-8 of gestation. Maternal bodyweights were recorded. Dams were sacrificed on Day 20 of gestation. The numbers of live and dead foetuses and resorptions were counted. Foetuses were sexed, weighed and inspected for external malformations and malformations of the oral cavity. Approximately two thirds of the foetuses in each litter stained with Alizarin Red S and examined for skeletal malformations. The remaining foetuses in each litter were fixed in Bouin's solution and examined for internal malformations by freehand serial sectioning.

Findings Significantly decreased maternal weight gains on GD 7-9 and GD 0-20 was observed in both treated groups, compared to controls. Total resorptions were observed in both treated groups; the incidence of total resorption was significantly higher at 15 mg/kg bw. A significantly higher incidence of post-

implantation loss, lower numbers of live foetuses and lower foetal weight were observed in both treated groups.

Maternal and litter findings

Dose level (mg/kg bw/d)	0	10	15
Pregnant (#)	10	10	10
Weight gain (g) GD 0-7	23	25	19
Weight gain (g) GD 7-9	8	-5**	-8**
Weight gain (g) GD 9-20	82	58	44
Weight gain (g) GD 0-20	113	78*	55**
Adjusted weight gain (g)	40	43	30
Total resorption (#)	-	2	4*
Post-implantation loss (%)	11.8	53.9**	71.2**
Litter size (#)	13.5	6.3*	4.4**
Foetal weight (g) M/F	3.88 / 3.74	3.20* / 2.87*	2.76* / 2.61*

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

Administration of DBTC resulted in a marked and statistically significant increase in the incidence of external foetal malformations; malformation incidences were 37/63 foetuses (59%) at 10 mg/kg bw and 27/44 at 15 mg/kg bw (62%). Significantly increased incidences of exencephaly, cleft jaw, cleft lip, ankyloglossia and club foot were apparent at 10 mg/kg bw; the incidences of exencephaly, cleft tongue and omphalocoele were additionally significantly increased at 15 mg/kg bw.

The incidences of foetal skeletal malformations were significantly increased after treatment with DBTC at 10 and 15 mg/kg bw; malformations were observed in 22/43 foetuses (51%) at 10 mg/kg bw and in 15/29 foetuses at 15 mg/kg bw (52%). Significantly increased incidences of the vertebral column deformity (cervical and thoracic regions) and ribs were observed in both treated groups; mandibular defects and fusion of the sternebrae were additionally observed at 15 mg/kg bw. A significantly increased incidence of foetal visceral malformations was also seen in the DBTC-treated groups; malformation incidences were 12/20 (60%) at 10 mg/kg bw and 10/15 (75%) at 15 mg/kg bw. The most frequent malformations were anophthalmia and microphthalmia.

Foetal malformations

Dose level (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*

Conclusion The results of this study demonstrate that the administration of DBTC to maternal rats at dose levels of 10 and 15 mg/kg bw on Days 7-8 of gestation results in embryolethality and teratogenicity. Findings were associated with maternal toxicity (reduced weight gain). Teratogenicity was characterised by increased incidences of external, skeletal and visceral malformations; malformations (predominantly exencephaly and mandibular defects) are characteristic of those induced by dibutyltin compounds in other studies. A NOAEL for teratogenicity of <10 mg/kg bw can be determined for this study."

2.2.1.12 Developmental toxicity study in the rat

Reference "Ema M, Kurosaka R, Amano H & Ogawa Y (1996b). Comparative Developmental Toxicity of Di-, Tri- and Tetrabutyltin Compounds after Administration during Late Organogenesis in Rats. Journal of Applied Toxicology 16(1): 71-76.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design Groups of mated female STD:Wistar-ST rats were gavaged with DBTC (in olive oil) at dose levels of 0 (vehicle control; 11 females), 165 (11 females) or 330 µmol/kg bw (13 females) on Days 13-15 of gestation (dose levels equivalent to 50 or 100 mg/kg bw/d). Maternal weight gain was measured on Days 13, 16 and 20. Dams were sacrificed on Day 20 of gestation. The numbers of live and dead foetuses and resorptions were counted. Foetuses were sexed, weighed and inspected for external malformations and malformations of the oral cavity. Approximately two thirds of the foetuses in each litter stained with alizarin red S and examined for skeletal malformations. The remaining foetuses in each litter were fixed in Bouin's solution and examined for internal malformations by freehand serial sectioning.

Findings Maternal deaths occurred in the low dose group (1/11) and in the high dose group (3/13). Weight gain and adjusted weight gains were significantly lower in both of the treated groups compared to controls. Post-implantation loss was also slightly (but not significantly) higher in the treated groups.

Mean foetal weights were significantly lower in the treated groups compared to controls. Three foetuses in the low dose group showed external (one foetus with cleft palate, one foetus with tail anomaly and anal atresia) or skeletal malformations (fuse sternebra). No malformations were observed in the control or high dose groups; the malformations observed in the low dose group are not considered to be related to treatment with DBTC.

Summary of findings

Dose level (µmol/kg bw)	0	165	330
Pregnant (#)	11	11	13
Deaths (#)	-	1	3
Weight gain (g) DG 0-13	47	46	50
Weight gain (g) DG 13-16	17	-13**	-13**
Weight gain (g) DG 16-20	40	0**	-22**
Weight gain (g) DG 0-20	104	31**	12**
Adjusted weight gain (g)	38	-13**	-26**
Implantations (#)	13.4	13.6	14.2
Total resorption (#)	-	-	2
Post-implantation loss (%)	9.8	22.0	34.4
Live foetuses (#)	12.1	10.5	9.1
Foetal weight (g) M/F	3.80 / 3.67	2.68**/2.43**	2.52**/2.19**

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

Conclusion In the absence of any foetal malformations in the high dose group, it can be concluded that maternal exposure to DBTC on Days 13-15 of gestation does not result in teratogenicity in the rat. Developmental effects clearly related to treatment were limited to reduced foetal weight, associated with reduced maternal weight gain. A NOAEL cannot be determined for this study due to findings at both dose levels investigated. The relevance of the study for the purposes of classification is limited by the level of mortality seen."

2.2.1.13 Developmental toxicity study in the rat

Reference "Noda T, Nakamura T, Shimizu M, Yamano T & Morita S (1992a). Critical gestational day of teratogenesis by di-n-butyltin (di)acetate in rats. Bulletin of Environmental Contamination & Toxicology 49(5):715-722.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin (di)acetate (DBTA)**

CAS 1067-33-0

EC 211-670-0

Purity not reported

Study design Groups of pregnant Wistar-rats were gavaged with dibutyltin acetate (DBTA) at a dose level of 15 mg/kg bw DBTA on 2 or 3 consecutive days of gestation or were gavaged with single doses of 15 and 30 mg/kg bw on three different days of gestation; or were gavaged with DBTA at dose levels of 5.0, 7.2, 10.5, 15.2 or 22.0 mg/kg bw on GD 8. DBTA was dissolved in olive oil. Maternal animals were observed for signs of toxicity; bodyweight and food consumption were measured. Rats were

sacrificed on GD 20 and were assessed for pregnancy status and foetal malformations.

Findings

Rats treated with DBTA at 15 mg/kg bw for 2 or 3 consecutive days were most susceptible to teratogenesis on GD 7-9 (higher number of resorptions and malformed foetuses were observed). Rats administered single doses of DBTA on GD 8 had the highest proportion of foetal malformations; treatment on GD 7 resulted in a lower frequency of malformations. The incidence of foetal malformations was significantly increased at the highest dose of DBTA. External malformations observed in the DBTA treated rats included cleft mandible, cleft lower lip, ankyloglossia, schistoglossia and exencephaly. Maternal thymus weights on GD 20 were unaffected by single doses of DBTA on GD 8.

External and skeletal foetal observations of foetuses from dams treated with DBTA on GD 8

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)*

Conclusion

The study demonstrates that the administration of DBTA to the rat on GD 8 results in a characteristic spectrum of external and skeletal foetal malformations. The authors conclude that the GD8 is the critical period for the teratogenesis of DBTA in the rat. A NOAEL of 10.5 mg/kg bw can be determined for this study, based on increased incidences of foetal malformations at dose levels of ≥15.2 mg/kg bw."

2.2.1.14 Developmental toxicity study in the rat

Reference "Noda T, Yamano T, Shimizu M, Saitoh M, Nakamura T, Yamada A & Morita S (1992b). Comparative teratogenicity of di-n-butyltin (di)acetate with n-butyltin trichloride in rats. Archives of Environmental Contamination & Toxicology 23(2):216-22.

Guideline Comparable to OECD 414

Species / strain Rat (Wistar)

Test material **Dibutyltin acetate (DBTA)**

CAS 1067-33-0

EC 213-928-8

Purity not reported

Study design Groups of 13-16 mated female Wistar rats were gavaged with DBTA (in olive oil) at dose levels of 0 (vehicle controls), 1.7, 5.0, 10.0 or 15.0 mg/kg bw on GD 7-17. Rats were observed daily for signs of toxicity; bodyweights and food consumption were also measured daily. Rats were terminated on GD 20 and pregnancy status assessed. Maternal thymus weight was reported. Foetuses were weighed, sexed and investigated for external and skeletal malformations.

Findings Reduced maternal weight gain during late gestation was observed at the highest dose level of 15 mg/kg bw/d; no effects of treatment were seen on food consumption. A single rat at 15 mg/kg bw/d showed piloerection and vaginal bleeding. Thymic atrophy of the pregnant rats was observed in a dose-dependent manner by DBTA treatment.

The incidences of dead or resorbed foetuses and total foetal resorption were increased at the highest dose level. The proportion of foetuses with external malformations (mainly cleft mandible, cleft lower lip, ankyloglossia and schistoglossia) was increased in a dose-dependent manner by DBTA treatment at dose levels of ≥5.0 mg/kg bw/d. The proportion of foetuses with skeletal malformations (anomalies of mandibular fixation, fused ribs, fused cervical vertebral arches and fused thoracic vertebral arches) was also increased at 10.0 and 15.0 mg/kg bw. No visceral malformations were observed in any group. Similar effects were not seen with monobutyltin chloride, a major metabolite of DBTA.

Summary of effects

Dose level (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)*

Conclusion The results of this study demonstrate that DBTA is teratogenic in the rat; the absence of similar effects with a metabolite indicate that teratogenicity is an effect of dibutyltin and not monobutyltin.

A NOAEL of 1.7 mg/kg bw can be determined for this study, based on increased incidences of foetal malformations at ≥5 mg/kg bw. A NOAEL for maternal toxicity of 10 mg/kg bw can be determined."

2.2.1.15 Developmental toxicity study in the rat

Reference "Noda T, Yamano T & Shimizu M (2001). Effects of maternal age on teratogenicity of di-n-butyltin (di)acetate in rats. Toxicology 167(3):181-9.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin (di)acetate (DBTA)**

CAS 1067-33-0

EC 213-928-8

Purity details not reported

Study design Groups of 12-14 mated female Wistar rats (aged 3, 7.5 or 12 months at mating) were gavaged with a single dose DBTA at dose levels of 0 (vehicle control), 7.5, 10, 15 or 22 mg/kg bw on GD 8. Maternal bodyweight and food consumption were measured daily. Dams were terminated on GD 20; uterus weights were recorded and the uterine contents examined following Caesarean section. Foetuses were weighed and sexed and were stained with Alizarin Red S for the assessment of skeletal findings.

Findings Maternal weight gain and gravid uterus weight decreased with age and were also significantly reduced by treatment with 22 mg/kg bw in 7.5 month old dams. The number of dams with viable foetuses was markedly reduced in the 12-month old group; reduced conception rat and increased total resorption were apparent. In 7.5 month-old dams, numbers of viable foetuses were reduced, foetal weight was reduced, resorption and implantation loss were increased at 15 and 22 mg/kg bw. In 3 month-old dams, increased implantation loss and resorption rate were observed only at 22 mg/kg bw.

Reduction in litter size was seen in all treated groups, most notably in the older dams. Death of most of the foetuses of the 12-month dams precluded accurate evaluation of malformation incidences. In litters from the 3-month old dams, external foetal malformations typical of DBTA (cleft mandible, cleft lower lip, ankyloglossia and/or schistoglossia) were observed at ≥15 mg/kg bw. Similar malformations were seen in the litters of 7.5-month old dams at dose levels of ≥10 mg/kg bw. The incidences of these malformations at 15 and 22 mg/kg bw were similar to those seen in litters from 3-month old dams.

Summary of maternal and litter findings

Dose level (mg/kg bw)		**0**	**7.5**	**10**	**15**	**22**
Weight gain (g)	**3M**	111	115	112	107	105
	7.5M	91	86	78	79	61*
	12M	36	40	36	39	23
Gravid uterus weight (g)	**3M**	72	73	71	68	61
	7.5M	56	54	47	52	31*
	12M	13	10	12	13	16
Adjusted weight gain (g)	**3M**	39	42	42	39	44
	7.5M	35	32	31	27	30
	12M	36	33	36	36	30

Mated (#)	**3M**	12	12	12	12	12
	7.5M	12	13	14	13	13
	12M	12	14	13	12	13
Pregnant (#)	**3M**	12	11	12	11	11
	7.5M	11	13	12	13	11
	12M	8	11	8	9	9
Litters with viable foetuses (#)	**3M**	12	11	12	11	11
	7.5M	11	13	12	13	6*
	12M	4	9	4	3	1
Total resorption (#)	**3M**	-	-	-	-	-
	7.5M	-	-	-	-	5*
	12M	4	2	4	6	8
Implantation loss (%)	**3M**	3.4	6.6	11.4	7.1	19.2*
	7.5M	16.7	20.1	27.6	14.2	37.8*
	12M	79.2	52.5	79.0	86.7	95.2
Foetal weight (g) M/F	**3M**	3.4/3.2	3.3/3.2	3.3/3.1	3.2/3.1	2.7*/2.7*
	7.5M	3.2/3.0	2.9/2.8	3.0/2.8	2.8*/2.6*	2.2*/2.2*
	12M	2.6/2.5	2.4/2.3	2.3/2.2	2.5/2.0	2.1/1.6

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

External and skeletal malformations (typically cleft mandible, cleft lower lip, ankyloglossia, schistoglossia, fused ribs, fused cervical and vertebral arches) were observed in foetuses from 3 month-old and 7.5 month-old females. The incidence of exencephaly was also markedly increased at 22 mg/kg bw. Malformations were observed only in a single foetus from 12 month-old females due to the high level of foetal mortality in this group.

Summary of foetal findings [18]

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

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

Conclusion The study confirms that GD 8 is the susceptible period for teratogenesis caused by DBTA. The spectrum of foetal malformations is comparable to that induced by DBTC. The results of this study also indicate an influence of maternal age on the susceptibility of the rat to the developmental toxicity of DBTA. Effects on foetal survival were more marked in older dams; results also indicate that teratogenicity may be more marked in older dams, although findings in the oldest (12 month-old)

dams may have been masked by the high level of foetal loss in this group.

A NOAEL of <7.5 mg/kg bw can be determined for this study, based on reduced litter size in all treated groups. Teratogenicity (increased incidences of craniofacial malformations) was seen at dose levels of ≥10 mg/kg bw."

2.2.1.16 Developmental toxicity study in the monkey

Reference "Ema M, Fukunishi K, Matsumoto M, Hirose A, Kamata E & Ihara T (2007b). Developmental toxicity of dibutyltin dichloride in cynomolgus monkeys. Reproductive Toxicology 23(1):12-19.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Cynomolgus monkey

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

98% purity

Study design Groups of cynomolgus monkeys were administered DBTC (in olive oil) by nasogastric intubation at dose levels of 0 (vehicle control), 2.5 or 3.8 mg/kg bw/d on GD 20-50 (the period of organogenesis). Maternal animals were observed for signs of toxicity; bodyweight and food consumption were measured. Pregnancy outcome was determined on GD 100. Foetuses were weighed, sexed and measured (crown-rump length and tail length); anogenital distance was also recorded. Foetuses were assessed for external, visceral and skeletal malformations and variations.

Findings Maternal toxicity (soft stool, yellowish stool and/or diarrhoea) was observed in females of both treated groups; a significant increase in the incidence of females exhibiting these symptoms was observed. Soft stool and/or diarrhoea were also observed in one control female. In both treated groups, yellowish stool was noted in 8 females and vomiting was observed in 3 females. Maternal weight gain was reduced at 3.8 mg/kg bw/d; food consumption was decreased in 2.5 and 3.8 mg/kg bw/d during the treatment phase. Higher plasma progesterone levels were observed in treated dams compared to controls, however the difference was not statistically significant and no differences in 17β-estradiol were observed. Foetal survival was decreased in both treated groups, significantly at 2.5 mg/kg bw/d. There was no effect of treatment on foetal weight, crown-rump length, tail length, sex ratio, anogenital distance or placental weight. No external, visceral or skeletal malformations were observed in any group; similarly there was no effect of treatment on the incidence of visceral variations, skeletal variations or on the extent of foetal skeletal ossification. A significant decrease in absolute brain and lung weight, and an increase in the relative spleen weight of male foetuses at 3.8 mg/kg bw; no significant difference in relative brain or lung weight or absolute spleen weight were detected. There were no other significant differences in absolute and relative foetal organ weights.

Maternal and reproductive findings

	Control	**2.5 mg/kg bw**	**3.8 mg/kg bw**
Pregnant females (#)	12	12	10
Soft stool/diarrhoea (#)	1	12*	10*
Yellowish stool (#)	0	8*	8*
Vomiting (#)	0	3	3
Weight gain (g) GD 0-20	76 ± 114	42 ± 160	73 ± 142
Weight gain (g) GD 20-51	57 ± 237	-242 ± 423	-556 ± 526*
Weight gain (g) GD 51-100	710 ± 162	755 ± 174	848 ± 263
Females with embryonic/foetal loss (#)	1	8*	4

Females with live foetuses (#)	11	4*	6
Live foetuses (#)	11	4*	6

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

Maternal food consumption

Food consumption (g/day)	**Control**	**2.5 mg/kg bw**	**3.8 mg/kg bw**
GD 20-21	99 ± 18	93 ± 23	76 ± 33
GD 23-24	91 ± 27	71 ± 31	55 ± 31*
GD 27-28	77 ± 28	47 ± 19*	37 ± 34*
GD 30-31	63 ± 32	33 ± 15*	22 ± 10*
GD 34-35	88 ± 25	53 ± 42	23 ± 17*
GD 37-38	86 ± 28	53 ± 42*	25 ± 24*
GD 41-42	87 ± 27	59 ± 59	36 ± 29*
GD 44-45	95 ± 22	62 ± 40	41 ± 31*
GD 48-49	98 ± 18	70 ± 48	59 ± 44
GD 51-52	94 ± 20	97 ± 24	71 ± 39
GD 55-56	102 ± 12	107 ± 2	100 ± 20
GD 58-59	106 ± 7	108 ± 0	104 ± 10
GD 62-63	106 ± 7	108 ± 0	106 ± 5
GD 80-81	108 ± 0	108 ± 0	108 ± 0
GD 90-91	106 ± 7	108 ± 0	108 ± 0
GD 99-100	108 ± 0	108 ± 0	108 ± 0

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

Conclusion The results of this study show that the administration of DBTC causes embryofoetal lethality, but not teratogenicity, in the monkey. The NOAEL for this effect is <2.5 mg/kg bw/d. Findings were associated with maternal toxicity (clinical signs, weight loss)."

2.2.1.17 Developmental toxicity study in the monkey

Reference "Ema M, Arima A, Fukunishi K, Matsumoto M, Hirata-Koizumi M, Hirose A & Ihara T (2009). Developmental toxicity of dibutyltin dichloride given on three consecutive days during organogenesis in cynomolgus monkeys. Drug & Chemical Toxicology 32(2):150-7.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Cynomolgus monkey

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

98% purity

Study design Groups of Cynomolgus monkeys were administered DBTC (in olive oil) by nasogastric intubation at a dose levels of 7.5 mg/kg bw on GD 19-21, 21-23, 24-26, 26-28, 29-31, 31-33 or 34-36. Control data (animals administered olive oil on GD 20-50) were available from a recent previous study (see Study 21 below). Maternal animals were observed for signs of toxicity; bodyweight and food consumption were measured. Pregnancy outcome was determined on GD 100. Foetuses were

weighed, sexed and measured (crown-rump length and tail length). Foetuses were assessed for external, visceral and skeletal malformations and variations.

Findings

Maternal toxicity (vomiting) was observed in all treated groups. Soft stool and/or diarrhoea were observed in all groups including the control. Significant increases in the incidence of females showing soft stool and/or diarrhoea after administration of DBTC on GD 19-21, 21-23, 24-26 or 26-28 were noted. Significant increases in the incidence of vomiting after administration of DBTC on GD 19-21 were noted. Maternal body weight gain was reduced over days 20-51 in dams given DBTC on GD 24-26, 26-28, 29-31 and 34-36, however differences were not statistically significant. A significant reduction in food consumption was observed on days 27-28 in the dams administered DBTC on GD 26-28; no other effects on food consumption were observed. Embryofoetal loss was observed in one female given DBTC on GD 19-21, in two females given DBTC on GD 24-26 and one female given DBTC on GD 34-36. There were no effects of treatment on developmental parameters in surviving foetuses, including foetal weight, crown-rump length, tail length or placental weight. No external, visceral or skeletal malformations were observed in any group. Treatment with DBTC similarly did not affect the incidence of skeletal variations or the level of skeletal ossification.

Reproductive findings

	Control	**7.5 mg/kg bw DBTC**						
GD dosing	**20-50**	**19-21**	**21-23**	**24-26**	**26-28**	**29-31**	**31-33**	**34-36**
Pregnant (#)	12	5	5	5	5	5	5	5
Embryofoetal loss (#)	1	1	0	2	0	0	0	1
Females with live foetuses (#)	11	4	5	3	5	5	5	4
Foetal weight (g)	126	122	124	100	110	117	111	124

Conclusion

The results of this study show that the administration of DBTC causes embryofoetal lethality, but not teratogenicity, in the monkey. The NOAEL for this effect is 7.5 mg/kg bw/d."

2.2.1.18 Developmental toxicity study in the rat

Reference

Unpublished report (2017). Dibutyloxide: an oral prenatal developmental toxicity study in rats

Guideline

OECD 414

Reliability

Klimisch 1: reliable without restrictions (Guideline and GLP-compliant study, full report available)

Species/strain

Hsd: Sprague Dawley® SD®

Test material

Dibutyltin Oxide (DBTO)

CAS 818-08-6

EC 212-449-1

Purity 97.3%

Vehicle

Peanut oil

Dose levels Study design

0, 0,75, 3 und 6 mg/kg bw/day

Groups of 25 mated female SD rats were gavaged with DBTO (in peanut oil) at dose levels of 0, 0.75, 3 and 6 mg/kg bw/d on days 0-19 of gestation. Dams were investigated for clinical signs and mortality. Body weights and food consumption were recorded at regular intervals. Rats were sacrified at day 20 and ovarian and uterine were examined. Foetuses were individually weighted sexed, tagged, and examined for external malformations and variations.

Approximately half of the foetuses from each litter were assessed for visceral findings; the remainder of the foetuses were assessed for skeletal findings following staining with Alizarin Red.

Dams were subject to gross necropsy. Emphasis was placed on structural abnormalities or pathologic changes that may have influenced the pregnancy. Thymus gland from each animal was collected, weighted,

and preserved for possible further evaluation.

Findings

No death occurred in the control and in the 0,75 and 3 mg/kg bw group. Two animals in the highest dose group were euthanised *in extremis* on GD 12 and 9. Animals had clinical signs of toxicity that included decreased activity, low body carriage, red material around the nose/mouth, hunched posture, pale body collar, and thin unkempt appearance accompanied by body weight loss and low food consumption. These effects are considered test article related. In the 0.75 and 3 mg/kg bw/d group no adverse effects were observed. In these dose groups some animals showed also red material around the nose in the early phase of the study (week 1). In the 6 mg/kg bw/d group clinical signs that included low body carriage, red material around the nose, thin appearance, loss of skin elasticity and pale body color were considered adverse and substance related.

No effect of DBTO at dose levels of 0.75 and 3.0 mg/kg/day was 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%).

Gestation body weight values (n = 22-24)

Dose group/ Study Interval (d)	**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)**
0	187.7 ± 13.87	188.1 ± 14.45	187.3 ± 14.93	186.3 ± 13.16
3	205.0 ± 15.09	205.2 ± 13.75	206.1 ± 16.25	199.5 ±15.07
6	215.4 ± 14.72	216.3 ± 14.32	218.3 ± 17.18	210.0 ± 16.60
9	232.4 ± 15.62	232.5 ± 14.84	234.7 ± 18.18	221.1 ± 22.80
12	248.2 ± 16.57	247.2 ± 17.27	250.4 ± 20.35	233.6 ± 29.55
15	265.5 ± 16.66	265.7 ± 17.17	270.0 ± 21.28	248.5 ± 38.22
18	303.9 ± 17.65	310.0 ± 20.51	311.5 ± 26.81	280.3[a] ± 51.34
20	334.4 ± 19.82	344.6 ± 24.61	344.9 ± 30.34	305.4[a] ± 62.95

[a] *significantly different from control (p<0.05)*

The mean body weight change in this group was lower than mean control values over much of gestation and statistically lower over GD 0 to 3 (-24%), GD 6 to 9 (-34%) and over the entire GD 0 to 20 treatment period (-19%). Considerable variability in gestation body weight change was observed in the 6.0 mg/kg/day dose group.

This changes are attributed to low weight gain and/or weight loss in four animals (#4510, 4511, 4516, and 4524) that failed to retain pregnancies with fetuses and at GD 20 had uterine implantations comprised entirely of resorbing fetuses (100% post-implantation loss). These effects on gestation body weights and body weight change at 6.0 mg/kg/day were considered test article related correlating with adverse pregnancy outcomes in several animals.

The individual gestation body weight values (g) and the individual gestation body weight change values (g) of these four animals receiving 6 mg/kg bw/day on GD 0 and GD 20 are depicted in the table below.

Animal Number #	**GD 0**	**GD 20**	**GD 0-20**
	Individual gestation body weight values		**Individual gestation body weight change values**
4510	176g	238g	62 g
4511	189g	255g	36 g
4516	165g	145g	-20 g
4524	181g	151g	-30 g

Mean gravid uterine weights, adjusted GD 20 body weights and adjusted weight change GD 0 to 20 in the

DBTO-treated groups were comparable to mean control values.

The adjusted final body weight (GD 20 body weight minus gravid uterine weight) in the control group is 260.7 ± 15.5 and adjusted body weight change (GD 0 to 20) is 73.0 ± 7.98.

A comparison of data of the four animals with control data indicates that for animal #4510 and 4511 no significant difference is between gestation body weight values (below 10%), whereas for animals #4516 and 4524 no increase in gestation body weight was observed.

No clinical or minor clinical observations (material around mouth, red thin) were detected for #4510, 4511. Whereas, #4516 and 4524 were in bad conditions and clinical signs such as loss of skin elasticity, posture hunched, scabbed area, skin discolored and thin were detected observed mainly between GD 9-20.

Summary of gravid uterine weight and ajusted body weight/body weight change values is provided in the following table:

Endpoint	**0 mg/kg bw/d (Mean ± SD) n=24**	**0.75 mg/kg/bw/d (Mean ± SD) n=24**	**3.0mg/kg bw/d (Mean ± SD) n=23**	**6 mg/kg bw/d (Mean ± SD) n=18**
Gravid Uterine Weight, g	73.7 ± 10.76	81.9 ± 11.93	80.1 ± 12.17	68.8 ± 15.14
Final Body Weight, g	334.4 ± 19.82	344.6 ± 24.61	344.9± 30.34	331.1 ± 25.13
Adjusted Final Body Weight, g	260.7 ± 15.50	262.7 ± 16.49	264.8 ± 22.03	262.3 ± 15.88
Adjusted Weight Change from Day 0, g	73.0 ± 7.98	74.6 ± 10.45	77.4 ± 12.28	74.1 ± 11.11

Reduced thymus weights were observed at all DBTO treatment levels 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.

In the following table reduced thymus weight and thymus weight/adjusted GD20 body weight is depicted

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^{x} ±0.042	0.158^{x} ± 0.043	0.134^{x} ±0.046
Thymus/adjusted GD 20 BWT, %	0.0891 ± 0.0192	0.0716^{x} ± 0.0123	0.0581^{x} ± 0.0143	0.0558^{x} ± 0.0108

^{x}statistically significant form control values ($p<0.01$)

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 nonpregnant 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) characterised by early resorptions. 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.

No substance related effects were observed on GD 20 uterine implantation parameters at 0.75 and 3.0 mg/kg/day.

In the 6 mg/kg bw/day group notable increases in mean postimplantation loss (25.70%) and in mean number of resorption sites (total and early)/dam (2.7) relative to controls (5.40% and 0.7, respectively) were observed in this group along with a decrease in number of viable fetuses/dam (9.7 vs. 12.5 in controls). Changes in these parameters in the 6.0 mg/kg/day dose group were also outside the range of recent historical control data for the laboratory (maximum study values were 5.35% for postimplantation loss, 0.7 resorption sites (total and early)/dam and 13.5 viable fetuses/dam) and these changes were largely attributable to the four females in the group with all resorption sites in utero (100% postimplantation loss).

These effects are considered substance related and adverse.

Maternal and developmental observations at uterine examination

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 (Percent)	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 was observed on fetal sex ratio, fetal body weight, or fetal external, visceral, or skeletal examinations.

Although no dose-related effect of DBTO was observed from the fetal visceral examinations it is noteworthy, that the litter incidence of an 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 controls and in the absence of a similar finding among fetuses in the 6.0 mg/kg/day group, was not considered test article related.

In the following table individual visceral observation are summarized:

Endpoint	**0 mg/kg bw (Mean ± SD)**	**0.75 mg/kg bw (Mean ± SD)**	**3.0mg/kg bw (Mean ± SD)**	**6.0 mg/kg bw (Mean ± SD)**
No. of litters evaluated	24	24	23	18
No. of foetuses evaluated	150	169	154	106
Head - Retina folded	0 (0)	0 (0)	1 (4.3)	0 (0.0)

No. of litters (%) No. of foetuses (%)	0 (0)	0 (0)	1 (0.6)	0 (0.0)
Mouth – Palate, rugae irregular No. of litters (%) No. of foetuses (%)	 1 (4.2) 3 (2.0)	 3 (12.5) 3 (1.8)	 6 (26.1) 6 (3.9)	 0 (0) 0 (0.0)
Thyroid, smaller than normal No. litters (%) No. foetuses (%)	 0 (0.0) 0 (0.0)	 0 (0.0) 0 (0.0)	 0 (0.0) 0 (0.0)	 1 (5.6) 1 (0.9)

Maternal thymus weight (absolute and relative) was reduced at GD 20. Mean absolute thymus weights were 19%, 34%, and 44% lower than the mean control value in the 0.75, 3.0, and 6.0 mg/kg/day dose groups, respectively and relative to the adjusted GD 20 body weights, were 20%, 35%, and 37% lower, respectively. The differences are statistically significant and are considered as test article related. An increased incidence of small thymus were observed macroscopically in the 6 mg/kg/d animals. No histopathological examination has been carried out, therefore the significance of this decrease is unclear.

Conclusion A NOAEL for maternal toxicity and developmental toxicity of 3 mg/kg bw/day DBTO can be determined for the study. At a dose level of 6 mg/kg bw dams had clinical signs and lower body weights, lower gestation body weights and lower food consumption. Lower thymus weights were observed in all substance treated groups and an increased incidence of small thymus were observed macroscopically in the 6 mg/kg/d animals. At a dose level of 6 mg/kg bw/day 4/23 femals at GD 20 had uterine implantation sited comprised entirely of resorbing foetuses (100% postimplantation loss). This observation is considered adverse and substance related. At dose levels of 0.75, 3.0, and 6.0 mg/kg/day DBTO was not teratogenic in the rat. An irregular palatal rugae pattern - a visceral variation- was dose dependent increased up to the dose level of 3 mg/kg bw/day. No findings of the variations was found at the 6 mg/ kg bw/group. Thus, study authors consider the variation as not treatment related. No effect of DBTO was observed on fetal sex ratio, fetal body weight, or fetal external and or skeletal examinations.

2.2.2 Human data

No human data are available.

2.2.3 Other data (e.g. studies on mechanism of action)

2.2.3.1 Cultured rat embryo study

Reference "Ema M, Iwase T, Iwase Y & Ogawa Y (1995a). Dysmorphogenic effects of di-n-butyltin dichloride in cultured rat embryos. Toxicology In Vitro 9(5):703-9.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

No purity details

Study design 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. At the end of the culture period the embryos were examined for the development of body and yolk sac vascularisation; yolk sac diameter, crown-rump length and the number of somite pairs were measured. Foetuses were given a morphological score

and external anomalies were recorded.

Findings 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. Yolk sac diameter, crown-rump length and number of somite pairs were also reduced at this concentration. A concentration-dependent decrease in the overall morphological score and an increase in the incidence of embryos with anomalies were observed at all concentrations; differences compared to controls were statistically significant for embryos exposed to 10 and 30 ng/mL DBTC. The observed anomalies were mainly open anterior neuropore and craniofacial abnormalities.

Conclusion The study indicates that exposure of explanted GD 8 rat embryos to DBTC *in vitro* at concentrations of ≥3 ng/mL causes dysmorphogenesis."

2.2.3.2 Cultured rat embryo study

Reference "Ema M, Iwase T, Iwase Y, Ohyama N & Ogawa Y (1996a). Change of embryotoxic susceptibility to di-n-butyltin dichloride in cultured rat embryos. Archives of Toxicology 70(11):742-8.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain [*In vitro* study]

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

No purity details

Study design Rat embryos explanted on GD 8.5, 9.5 or 11.5 were cultured for 68, 46 and 48 hours and were exposed to a range of DBTC concentrations for the first 24, 46 and the last 46 hours of culture, respectively.

Findings In GD 8.5 embryos, exposure to DBTC resulted in significant decreases in placental diameter (at concentrations of ≥10 ng/mL) and in the number of somite pairs and the morphological score (at 30 ng/mL). In GD 9.5 embryos, significant decreases in yolk sac diameter and crown-rump length were seen at 100 ng/mL, a reduction in the number of somite pairs was seen at ≥50 ng/mL and a reduction in the morphological score was seen at ≥30 ng/mL. No adverse effects on these parameters were detected in embryos cultured from GD 11.5, even at the highest concentration tested of 300 ng/mL. Dysmorphogenesis was seen in embryos cultured from GD 8.5 (≥10 ng/mL), GD 9.5 (≥50 ng/mL) and GD 11.5 (300 ng/mL). Incomplete turning and 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 most frequently observed.

Conclusion The study shows that exposure to DBTC interferes with normal embryonic development during three different stages of organogenesis, and that susceptibility to the embryotoxicity and dysmorphogenic potential of DBTC varies with developmental stage."

2.2.3.3 Cultured rat embryo limb bud study

Reference "Yonemoto J, Shiraishi H & Soma Y (1993). *In vitro* assessment of teratogenic potential of organotin compounds using rat embryo limb bud cell cultures. Toxicology Letters 66(2):183-91.

Guideline No guideline followed

Reliability Klimisch 4: not assignable (insufficient experimental detail)

Species / strain [*In vitro* study]

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

No purity details

Study design Rat embryo limb bud cell cultures were used to assess the relative teratogenic potential of tributyltin oxide and its metabolites including dibutyltin chloride and monobutyltin chloride. Fifty percent inhibition concentrations for cell proliferation (IP_{50}) and cell differentiation (ID_{50}) and P/D ratio were calculated.

Findings With the exception of monobutyltin chloride, all of the organotin compounds investigated in this study showed very strong inhibition of cell differentiation (ID_{50} :0.13-1.71 μM) and cell proliferation (IP_{50}: 0.12-2.81 μM).

Conclusion The authors suggest that dibutyltin is directly teratogenic."

2.3 Specific target organ toxicity – single exposure/repeated exposure

Detailed summaries of studies relevant to classification for specific target organ toxicity – single exposure/repeated - are presented in this section.

The studies described below have been described in the CLH-dossier for DBTP (EC 245-152-0) and assessed by RAC in 2017. Where the description of the studies below are in quotes the text is a verbatim rendering of the text in the mentioned CLH-dossier.

Studies of reproductive or developmental toxicity are also reported in this section where relevant endpoints were assessed.

2.3.1 Animal data

2.3.1.1 Mechanistic investigation of thymic atrophy in the rat

Reference "Snoeij NJ, Penninks AH & Seinen W (1989). Thymus Atrophy and Immunosuppression Induced by Organotin Compounds. Archives of Toxicology S13: 171-174.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

>98% purity

Study design Male Wistar rats were gavaged with DBTC (in ethanol/corn oil) at dose levels of 0 (vehicle control) or 15 mg/kg bw; bodyweights and thymus weights (3 rats per group) were measured at 1, 2, 3, 4, 7 and 9 days after dosing. Suspensions of the thymus were prepared for the analysis of total cell count, cell sizing and the incorporation of radiolabelled DNA, RNA and protein precursors.

Findings A single oral dose of DBTC was associated with a decrease in absolute and relative thymus weights from the second day after dosing. Thymus weight reduction was maximal at Day 4, but was shown to recover by Day 9. 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. The incorporation of radioactivity was reduced on Days 1 and 2, but subsequently returned to control values

Conclusion Based on the reduction in thymus weight and loss of cellularity, the authors conclude that 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."

2.3.1.2 Sub-chronic dietary toxicity study in the rat

Reference "Gaunt IF, Colley J, Grasso P, Creasey M & Gangolli SD (1968) Acute and Short-term Toxicity Studies on Di-n-butyltin Dichloride in Rats. Food & Cosmetic Toxicology 6: 599-608.

Guideline None

Species / strain Rat (CFE)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

99.7% purity

Study design Groups of SPF-derived rats (16/sex) were fed diets containing DBTC at concentrations of 0 (control), 10, 20, 40 or 80 ppm for 90 days. Animals were observed daily for signs of toxicity. Bodyweights and food consumption were measured weekly. Blood samples were taken during Week 6 (control, 40 and 80 ppm groups) for the assessment of haematological parameters; haematological parameters were also assessed in terminal blood samples taken from rats of all groups. Terminal blood samples were also assessed for AST and ALT activity; serum amylase activity was additionally measured in the control and 80 ppm dose groups as a marker of pancreatic damage. Urinalysis was also performed. Renal function tests were performed during Week 6 and prior to termination. Investigations comprised assessment of the concentrating ability of the kidney by measuring the volume and specific gravity of urine produced under conditions of normal hydration, during a 6-hour period of water deprivation, during a 2-hour period following a water load of 25 mL/kg bw and during a 4-hour period commencing 16 hours after the water load.

Gross necropsy was performed on all rats; weights of the brain, pituitary, heart, thyroid, liver, spleen, kidneys, adrenals and gonads were recorded. These organs and additionally the salivary gland, trachea, lungs, diaphragm, lymph nodes, thymus, pancreas, stomach, ileum, colon, caecum, rectum, urinary bladder, sternum and uterus were investigated histopathologically. The duodenal loop with the pancreas and bile duct *in situ* were fixed flat so as to retain their anatomical relationship.

Findings There were no deaths and no signs of toxicity in any group. A slight reduction in weight gain was seen in both sexes at 80 ppm and was statistically significant in females. Some reduction in food intake was noted and was attributed to an effect of the test material on dietary palatability. Haematology revealed statistically significantly reduced haemoglobin concentrations at 80 ppm in females at Week 6 and in males at Week 13. Decreases were slight and were not associated with changes in other erythrocyte parameters or an indication of reticulocytosis. Clinical chemistry and urinalysis did not reveal any effects of treatment. Gross necropsy did not show any treatment-related findings; organ weights were comparable in all dose groups. Histopathology did not show any effects of treatment on any organ or tissue investigated (including the thymus).

Mean body weight values

Dietary level (ppm)	Body weight (g)			
	Week 0	Week 4	Week 8	Week 13
Males				
0	187	367	457	543
10	183	368	464	544
20	189	393	474	561
40	189	374	472	556
80	181	345	438	512
Females				
0	153	240	283	316

10	148	231	274	301
20	151	237	283	318
40	147	239	287	330
80	147	227	267*	299*

*Significantly different to controls, *P <0.05 Students t-test*

Haematology parameters at Week 6 and Week 13

Dietary level (ppm)	**Hb (g/dL)**	**Hct (%)**	**RBC (10^6/mm^3)**	**Retics (% of RBC)**	**Leucocytes**				
					Total (10^3/mm^3)	**Differential (%)**			
						N	**E**	**L**	**M**
Males – Week 6									
0	14.5	47	7.48	1.24	23.6	13	1	85	1
40	14.6	46	7.72	1.32	19.0	14	1	85	0
80	14.3	47	7.01	1.17	19.5	13	1	86	0
Females – Week 6									
0	14.7	45	7.44	1.66	23.2	9	0	91	0
40	14.1	44	7.22	1.38	18.8	12	2	85	1
80	13.7*	44	7.45	1.80	16.9	12	1	87	0
Males – Week 13									
0	14.6	45	7.42	1.30	7.4	16	2	79	3
10	14.3	46	7.61	1.14	6.0	14	1	81	4
20	13.9	40	7.41	1.21	5.9	13	1	82	4
40	13.9	44	7.49	1.47	6.0	16	3	78	3
80	13.2**	46	7.33	1.13	5.0	19	3	74	4
Females – Week 13									
0	14.2	43	6.52	1.32	4.1	13	1	83	3
10	13.6	44	6.46	1.29	3.2	18	3	76	3
20	14.0	43	6.76	1.36	3.1	19	2	75	4
40	13.5	43	6.27	1.42	3.5	14	1	82	3
80	14.0	45	6.61	0.99	3.2	15	1	81	3

*Significantly different to controls, *P <0.05, **P <0.01*

Conclusion Sub-chronic administration of DBTC to the rat resulted in a slight reduction in weight gain and a marginal effect on haemoglobin concentration at the highest dose level of 80 ppm (equivalent to approximately 4 mg/kg bw/d). A NOAEL of 40 ppm (equivalent to approximately 2 mg/kg bw/d) can therefore be determined for this study. No effects on the thymus were apparent at the highest dose level (4 mg/kg bw/d) in either sex."

2.3.1.3 Reproductive/developmental toxicity screening study in the rat

Reference "Unpublished report (2003).

[Full report not available: study details taken from the publically disseminated REACH Registration Dossier and the 2014 CLH report on dibutyltin dilaurate]

Guideline	OECD 421
Reliability	Klimisch 2: reliable with restrictions (guideline study, full report not available)
Species / strain	Rat (Wistar)
Test material	**Dibutyltin dichloride (DBTC)** CAS 683-18-1 EC 211-670-0 98.57% purity
Study design	Groups of 12 Wistar rats/sex were administered the test material in the diet at concentrations of 0, 5, 30 or 200 ppm for four weeks (males) or for two weeks prior to mating and to Day 4 or 6 *post partum* (females). Animals were observed daily for mortality and clinical signs. Bodyweights were measured pre-test and on Days 0, 7 and 14 of the pre-mating period. Bodyweights of males were measured on Days 21 and 28; bodyweights of females were measured during mating (Days 0, 7 and 14); on Days 0, 7, 14 and 21 of gestation; on Days 1 and 4 of lactation and at termination. Food consumption was measured for both sexes during the pre-mating period (Days 0-7, 7-13); for males during the post-mating period (Day 21-28); for females during the gestation period (Days 0-7, 7-14, 14-21) and during the lactation period (Days 1-4). At termination, weights of the kidney liver, spleen, testes, thymus and brain were recorded for parental animals. Gross necropsy was performed on all parental animals; the prostate, seminal vesicles and testes of male animals were investigated. The ovaries, uterus (including counting of implantation sites) and thymus were investigated in females. Pups were assessed for gross abnormalities.
Findings	Mean male bodyweights were significantly lower from Day 14-28 of the study at the highest dose level. Weight gains by males at the highest dose level were significantly lower over the study period; weight gain by males at 30 ppm was also reduced from Day 14-21. There was no effect of treatment on weight gain in males administered 5 ppm DBTC. Weight gain by females at the highest dose level was reduced over the premating, gestation and lactation periods; mean bodyweights of females in this group were significantly lower at Day 14 of the premating period, over the entire gestation period and during the lactation period. Food consumption by males at the highest dose level was reduced; significantly at Day 7-14. Food consumption by females in this group was significantly reduced during the pre-mating, gestation and lactation periods. Food consumption by females in the other treatment groups was comparable to controls. Gross necropsy and histopathology of males did not reveal any effects of treatment on the reproductive tract. Thymuses of males were not investigated histopathologically. Severe or very severe thymic lymphoid depletion was observed in females at 200 ppm; moderate to severe lymphoid depletion was seen in the majority of the pregnant (but not in the non-pregnant) females at 30 ppm. 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.
Conclusion	Administration of DBTC in the diet at concentrations of 30 and 200 ppm resulted in thymic lymphoid depletion in females. A NOAEL of 5 ppm can therefore be determined for this study."

2.3.1.4 Sub-acute toxicity study in the rat

Reference	"Seinen W & Vos JG (1977). Toxicity of Organotin. II. Comparative in Vivo and in Vitro Studies with Various Organotin and Organolead Compounds in Different Animal Species with special Emphasis on Lymphocyte Cytotoxicity. Toxicology & Applied Pharmacology 42:197-212.
Guideline	No guideline followed
Reliability	Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain Rat (Wistar)
Mouse (Swiss)

Test material **Dibutyltin dichloride (DBTC)**
CAS 683-18-1
EC 211-670-0
Purity >98%

Study design Groups of rats (10/sex) or 10 male mice were administered DBTC in the diet at concentrations of 0 (controls), 50 or 150 ppm for 4 weeks. Bodyweights were recorded weekly. Gross necropsy was performed on all animals; weights of the thymus, spleen, popliteal lymph node, liver, kidneys and adrenals were recorded; these tissues were also investigated histopathologically.

Findings Mortality occurred in rats administered 150 ppm DBTC (2 males, 4 females) in the second week of the study. Relative thymus weight was reduced at 50 ppm (by 53%) and at 150 ppm (by 68-72%); spleen weights (16% and 33%) and popliteal lymph node weights (16% and 28%) were also reduced at 50 ppm and 150 ppm, respectively. Gross necropsy revealed a marked reduction in the size of the thymus was found in all treated animals. Yellow discoloration of the liver, thickened and dilated bile ducts were also observed in a small number of rats at 150 ppm. Histopathology revealed severe proliferation of bile duct epithelial cells and bile ductules, associated with pericholangiolitis and periportal fibrosis in rats at 150 ppm.

The most prominent effect found was lymphocyte depletion in lymphoid organs; this was most pronounced in the thymic cortex. At 150 ppm, the cortex was almost completely depleted; however signs of cell destruction were not observed. Lymphocyte depletion was also observed in the thymus-dependent areas of the spleen (periarteriolar lymphocyte sheets) and popliteal lymph node (paracortex).

No effects of treatment were observed in mice.

Body weight and relative organ weights (means ± SD)

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

Conclusion A NOAEL of <50 ppm (equivalent to approximately 2.5 mg/kg bw/d) can be determined for this study, based on effects on the thymus, spleen and lymph nodes (lymphoid depletion) in both groups of treated rats."

2.3.1.5 Sub-acute toxicity study in the rat

Reference "Penninks AH & Seinen W (1982). Comparative toxicity of alkyltin and estertin stabilisers
Food & Chemical Toxicology 20:909-916.

Guideline None followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**
CAS 683-18-1
EC 211-670-0
Purity unknown

Study design Groups of ten male Wistar (WU-CPB) rats (bodyweight 40-45 g) were administered DBTC in the diet at concentrations of 0 (controls), 50 or 150 ppm for 14 days. Bodyweights were measured weekly. Gross necropsy was performed and the weights of the thymus, spleen, liver, kidneys and adrenals were recorded. These organs were investigated histopathologically.

Findings Two rats in the 150 ppm group died during Week 2 of the study and are reported to have showed signs of severe jaundice. A dose-related reduction in bodyweight gain was seen in the treated groups. Relative weights of the thymus and spleen were reduced in both treated groups; the decrease in thymus weight was pronounced and was equivalent to a reduction of greater than 70% at 150 ppm.

Gross necropsy showed yellow liver discolouration in some rats at 150 ppm; relative liver weight was increased in this group. Microscopically, rats administered 150 ppm showed hepatotoxicity (severe proliferation of the bile duct epithelium, associated with pericholangitis, periportal fibrosis and accumulation of bile pigment in hepatocytes). The most prominent histopathological feature in all treated animals was lymphocyte depletion; this findings was noted particularly in the thymic cortex, but was also apparent in the splenic periarteriolar lymphocyte sheets.

Summary of findings

Concentration (ppm)	**0**	**50**	**150**
Terminal bodyweight (g)	115.3	107.7**	92.1**
Liver weight (%)	4.25	4.29	4.93**
Thymus weight (%)	0.38	0.17**	0.10**
Spleen weight (%)	0.36	0.30**	0.24**
Kidney weight (%)	1.07	1.04	1.06
Adrenal weight (%)	0.025	0.021	0.022

***significantly different to controls (P<0.001)*

Conclusion A NOAEL of <50 ppm can be determined for this study based on reduced thymus and spleen weights and associated histopathology (lymphocyte depletion) in both treated groups."

2.3.1.6 Sub-chronic dietary toxicity study in the rat

Reference "Barnes JM & Stoner HB (1958). Toxic properties of some dialkyl and trialkyl tin salts
British Journal of Industrial Medicine 15:15-22.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain Rat (unspecified)

Test material **Dibutyltin dichloride (DBTC)**
CAS 683-18-1
EC 211-670-0
Purity unknown

Study design Groups of 12 rats were administered DBTC in the diet at concentrations of 0 (controls), 20, 50, 75 or 100 ppm for periods of up to six months.

Findings In groups of rats administered DBTC for 54 or 55 days, a dose-related reduction in weight gain and food consumption was apparent in all groups; weight gain was significantly reduced at dietary concentrations of 50 ppm and above.

Bodyweight and food consumption effects

Dietary concentration	54 days		55 days	
	Weight gain (g)	Food consumption (g)	Weight gain (g)	Food consumption (g)
20 ppm	-11%	-2%		
50 ppm	-19%*	-21%	-22%	-23%
75 ppm			-35%*	-26%
100 ppm	-42%**	-29%	-30%*	-19%

Rats administered 20 ppm DBTC for 6 months grew normally and showed no lesions at gross necropsy. At 50 ppm, growth and food intake were reduced; gross necropsy showed thickening and dilatation of the bile duct and fibrosis of the pancreas. At 75 and 100 ppm, rats showed some mortality and a greater depression of growth. Gross necropsy of animals surviving to termination showed variable levels of bile duct damage.

Conclusion A NOAEL of 20 ppm (equivalent to approximately 1 mg/kg bw/d) can be determined for this study based on reduced weight gain at 50 ppm (equivalent to approximately 2.5 mg/kg bw/d)."

2.3.1.7 Developmental toxicity study in the rat

Reference "Study report (1994) included in the publically disseminated REACH Registration Dossier for dibutyltin dichloride.

Guideline OECD 414; no deviations

Reliability Klimisch 2: reliable with restrictions (guideline study, full report not available)

Species / strain Rat (Wistar) Crl:CD(Wi)BR

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

>98% purity

Study design Groups of 25 mated Wistar female rats were gavaged with the test material (in corn oil) at dose levels of 0, 1, 2.5, 5 or 10 mg/kg bw/d on Days 6-15 of gestation. Dams were investigated for mortality and clinical signs. Bodyweights and food consumption were recorded at regular intervals.

Dams were subject to gross necropsy; the thymus, liver, bile duct and mesenteric lymph nodes were investigated histopathologically.

Findings No deaths occurred and no signs of maternal toxicity were observed during the study period. Reduced weight gain and food consumption were seen at 10 mg/kg bw/d; weight gain was also slightly reduced at 5 mg/kg bw/d. Gross necropsy of dams did not reveal any treatment-related findings. Mean thymus weight was significantly lower in dams at 10 mg/kg bw/d. Histopathology of the thymus showed atrophy at 10 mg/kg bw/d and to a lesser extent at 5 and 2.5 mg/kg bw/d.

Conclusion A NOAEL for maternal toxicity of 1 mg/kg bw/d can be determined for this study based on thymic atrophy at dose levels of ≥2.5 mg/kg bw/d; reduced weight gain was seen at ≥5 mg/kg bw/d."

2.3.1.8 Developmental toxicity study in the rat

Reference "Farr CH, Reinisch K, Holson JF & Neubert D (2001). Potential teratogenicity of di-n-butyltin dichloride and other dibutyltin compounds. Teratogenesis, Carcinogenesis & Mutagenesis 21(6):405-15.

Guideline OECD 414

Reliability Klimisch 2: reliable with restrictions (guideline study summary, published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design A developmental toxicity study was conducted in the rat according to OECD guidelines and GLP. Groups of 25 mated female Wistar rats were gavaged with DBTC (in olive oil) at dose levels of 0, 1, 2.5, 5 or 10 mg/kg bw on GD 6-15. Evaluation of pregnancy outcome was performed on day 20 of pregnancy.

Findings Maternal toxicity (reduced food consumption, bodyweight gain and reduced thymus weight) were seen at 10 mg/kg bw. No evidence of embryotoxicity as assessed by numbers of total resorptions, viable foetuses or foetal weight was noted in any treated group. A slightly increased frequency of total malformations was seen at 10 mg/kg bw (4/262 foetuses) compared to the control group (1/269 foetuses). The authors consider that the nature and pattern of malformations does not suggest any effect of treatment; however the nature of findings (including single incidences of ankyloglossia, agnathia, mandibular defect) are consistent with the results of other studies and therefore indicate a relationship to treatment with DBTC

Maternal findings

Dose level (mg/kg bw)	**0**	**1.0**	**2.5**	**5.0**	**10.0**
Inseminated females (#)	25	25	25	25	25
Pregnant females (#)	20	25	23	19	20
100% intrauterine deaths (#)	0	1	0	1	0
Females with viable foetuses (#)	20	24	23	18	20
Malformed foetuses (#)	1/269	0-343	0-292	1/224	4/262
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**
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

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

Conclusion A NOAEL of 5 mg/kg bw can be determined for teratogenicity and developmental toxicity, based on the slightly elevated incidence of characteristic foetal malformations at 10 mg/kg bw/d. A NOAEL of 5 mg/kg bw/d can be determined for maternal toxicity, based on reduced bodyweight gain, food

consumption and reduced thymus weight at the highest dose level."

2.3.1.9 Sub-acute study of immunotoxicity in the rat

Reference "DeWitt JC, Copeland CB & Luebke RW (2005). Immune responses in Sprague-Dawley rats exposed to dibutyltin dichloride in drinking water as adults. Journal of Immunotoxicology 2(3):151–60.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain Rat (Sprague-Dawley CD)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

96% purity

Study design Groups of 60-day old Sprague-Dawley (CD) rats (8/sex) were administered DBTC in drinking water containing 0.5% Alkamuls at concentrations of 0 (controls), 0 or 25 mg/L for 28 days. Achieved dose levels were equivalent to 0, 0.9 and 1.9 mg/kg bw/d for the initial study; 0, 1.0 and 2.5 mg/kg bw/d for the confirmatory study. Water bottles were changed and water consumption monitored twice weekly; body weights were recorded weekly. Delayed-type hypersensitivity (DTH), primary and secondary antibody responses to sheep red blood cells (SRBCs), and natural killer (NK) cell activity were evaluated in groups of treated and control animals on Day 29 of the study.

Primary (IgM) and secondary (IgG) T-cell-dependent antibody responses against SRBCs were assessed in animals were immunized on Study Day 24 (intravenous injection of 2×10^8 SRBCs in 0.5 mL sterile saline); blood samples were taken on Study Day 29. The same animals were administered a booster immunization (intravenous injection of 2×10^8 SRBCs in 0.5 mL sterile saline) on study Day 39. Blood samples collected on study Day 44 were analysed for SRBC-specific IgG. The relative serum titre of SRBC-specific IgM and IgG antibodies were measured by ELISA.

Delayed-Type Hypersensitivity Response (DTH): Sensitized with purified bovine serum albumin (BSA; Sigma) in Freund's complete adjuvant subcutaneously into the caudal tail fold. Seven days later, animals were challenged by 0.1 mL BSA into the right rear footpad. The left rear footpad was the injection control. After 24 h, footpad thickness (triplicate measurements) was determined. Swelling was calculated by subtracting the mean saline-injected, left footpad thickness from the mean BSA-injected right footpad thickness.

Natural killer (NK) cell activity was measured in splenocyte single cell suspensions prepared and cultured with ^{51}Cr-labeled murine YAC-1 lymphoma target cells. ^{51}Cr release was determined using liquid scintillation counting.

Findings No statistically significant effects were seen on bodyweight. Water consumption by males (-17%) and females (-21%) was significantly decreased at the highest concentration. Absolute and relative thymus and spleen weights were unaffected by treatment. No clear effects of treatment were seen on antibody production, DTH 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.

Conclusion A NOAEL of 2.5 mg/kg bw/d can be determined for this study, in the absence of any effects of treatment."

2.3.1.10 Sub-acute toxicity study of immune function in the rat

Reference "DeWitt J, Copeland C & Luebke R (2006). Immune Function In Rats Developmentally Exposed To Dibutyltin Dichloride. Toxicological Sciences 90(1-S):388.

The study is reported as a conference abstract only, and appears to report findings by the same

authors reported elsewhere [DeWitt et al., 2005b]

Guideline No guideline followed

Reliability Klimisch 4: not assignable (insufficient experimental detail)

Species / strain Rat (Sprague-Dawley)

Test material **Dibutyltin dichloride (DBTC)**
CAS 683-18-1
EC 211-670-0

Study design Individually housed pregnant female SD rats were given administered DBTC (in 0.35% Alkamuls) in the drinking water at concentrations of 0, 10 or 25 mg/L from GD 6 to PND 21. Litters were sexed, weighed and culled to 8 pups (4/sex) on PND 2. From PND 3, the litters from half of the dams of each group were gavaged with DBTC (in 0.5% Alkamuls) at dose levels of 0, 1.0, or 2.5 mg bw, three times a week for a total of ten doses. Delayed-type hypersensitivity (DTH), primary and secondary antibody responses and natural killer (NK) cell activity were evaluated in offspring (6/sex/group) after PND 42.

Findings Weight gain by litters gavaged with 2.5 mg/kg bw DBTC was decreased, but recovery was seen and bodyweights reached control levels by PND 50. DTH response and NK cell activities were unaffected by treatment. In female offspring, IgM was lower in some treated groups relative to control groups. In male offspring, IgG was elevated in the 25 mg/L group relative to controls. Findings were, however, not replicated in a second study assessing antibody production.

Conclusion No clear effects of DBTC treatment were seen under the conditions of this study."

2.3.1.11 Mechanistic investigation of thymic atrophy in the rat, the mouse and the Guinea pig

Reference "Seinen W, Vos JG, van Krieken R, Penninks A, Brands R & Hooykaas H (1977). Toxicity of organotin compounds. III. Suppression of thymus-dependent immunity in rats by di-n-butyltindichloride and di-n-octyltindichloride. Toxicology and Applied Pharmacology 42(1):213-24.

Guideline No guideline followed

Species / strain Rat (Wistar WU, WAG inbred)
Mouse (Swiss)
Guinea pig (Hartley)

Test material **Dibutyltin dichloride (DBTC)**
CAS 683-18-1
EC 211-670-0
>98% purity

Study design Groups of rats and mice were administered diet containing DBTC at concentrations of 0 (control), 50 or 150 ppm.

After three weeks of treatment, male WU rats were sensitised by subcutaneous injection of complete adjuvant; delayed hypersensitive response was tested by intradermal tuberculin injection after 5 or six weeks. At termination, weights of the thymus, spleen, adrenals and popliteal lymph node were recorded.

Tail skin grafts from WAG x B F_1 hybrid rats were performed on WAG rats; allograft rejection was assessed microscopically.

Immune response in rats was also assessed using plaque forming cell, haemagglutination, haemolysis and *in vitro* phagocytosis (carbon clearance) assays.

Findings Allograft rejection was significantly delayed by DBTC at 150 ppm (11.9 days) compared to controls (9.4 days), but not at 50 ppm (10.1 days). The antibody response against *E. coli* LPS, was unaffected by DBTC. The humoral immune response against sheep red blood cells (SRBC) was depressed by DBTC. Haemagglutination and haemolysin titres and the number of direct plaque-forming cells

against SRBC were decreased in a dose-related manner by DBTC. Altered immune functions were not found in mice or guinea pigs exposed to DBTC.

Conclusion The authors conclude that DBTC causes immunotoxicity in rats by a selective inhibition of T-lymphocyte activity. Effects were most pronounced in animals exposed to the chemicals during the developmental phase of the lymphoid system."

2.3.2 Human data

No human data are available.

2.3.3 Other data

2.3.3.1 Mechanistic study

Reference "de Heer C, Schuurman HJ, Houben GF, Pieters RH, Penninks AH & van Loveren H (1995). The SCID-hu mouse as a tool in immunotoxicological risk assessment: effects of 2-acetyl-4(5)-tetrahydroxybutyl-imidazole (THI) and di-n-butyltin dichloride (DBTC) on the human thymus in SCID-hu mice. Toxicology 100(1-3):203-11.

Guideline No guideline followed

Species / strain Mouse (SCID-hu)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design 36 female SPF-derived homozygous C.B-17 *scid*/*scid* (SCID) mice aged 4-5 weeks were engrafted with human foetal thymus and liver tissue fragments. Mice were exposed to a single dose of DBTC by intraperitoneal injection at dose levels of 0 (vehicle), 0.3 or 1.0 mg/kg bw and sacrificed five days later. The human thymus transplants were removed and assessed morphometrically and histopathologically.

Findings Bodyweights were unaffected by treatment with DBTC. Relative spleen weight was increased in the treated groups, a finding attributed to increased extramedullary haematopoiesis. DBTC treatment resulted in reduced cortical size of the human thymus graft. Histopathological examination of the human thymus grafts of SCID-hu mice exposed to DBTC showed a reduction in the relative size of the thymus cortex.

Conclusion The results of this study indicate that the human thymus is a target for DBTC."