

Committee for Risk Assessment
RAC

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

Dibutyltin maleate

EC Number: 201-077-5

CAS Number: 78-04-6

CLH-O-0000007032-86-01/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 maleate

EC Number: 201-077-5

CAS Number: 78-04-6

Index Number: -

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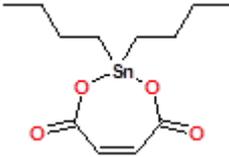
ABBREVIATIONS

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 development 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)	2,2- dibutyl-1,3,2-dioxastannepine-4,7-dione
Other names (usual name, trade name, abbreviation)	DBTM 1,3,2-Dioxastannepin-4,7-dione, 2,2-dibutyl-
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	201-077-5
EC name (if available and appropriate)	dibutyltin maleate
CAS number (if available)	78-04-6
Other identity code (if available)	-
Molecular formula	C ₁₂ H ₂₀ O ₄ Sn
Structural formula	
SMILES notation (if available)	-
Molecular weight or molecular weight range	346.99
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)	--

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 maleate EC 201-077-5	Conf	-	Acute Tox. 4, H302 Acute Tox. 2, H330 Skin Corr. 1B, H314 Skin Sens. 1, H317 Eye Dam. 1, H318 Muta. 2, H341 Repr. 1B, H360FD STOT SE 1, H370 (Thymus) STOT RE 1, H372 (Thymus) Aquatic Chronic 1, H410 Aquatic Acute 1, H400

Further information on the composition is not relevant for this dossier.

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
No information on impurities given in the registration dossier				

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					

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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 maleate	201-077-5	78-04-6	Add Muta. 2 Repr. 1B Acute Tox. 2 Acute Tox. 4 STOT RE 1 Skin Corr. 1 Eye Dam. 1	Add H341 H360FD H330 H302 H372 (immune system) H314 H318	Add GHS08 GHS06 GHS05 Dgr	Add H341 H360FD H330 H302 H372 (immune system) H314	-	Add inhalation: ATE = 0.317 mg/L (dusts and mists) oral: ATE = 510 mg/kg bw	-
Resulting Annex VI entry if agreed by RAC and COM	TBD	Dibutyltin maleate	201-077-5	78-04-6	Muta. 2 Repr. 1B Acute Tox. 2 Acute Tox. 4 STOT RE 1 Skin Corr. 1 Eye Dam. 1	H341 H360FD H330 H302 H372 (immune system) H314 H318	GHS08 GHS06 GHS05 Dgr	H341 H360FD H330 H302 H372 (immune system) H314	-	inhalation: ATE = 0.317 mg/L (dusts and mists) oral: ATE = 510 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	<i>hazard class not assessed in this dossier</i>	No
Flammable gases (including chemically unstable gases)	<i>hazard class not assessed in this dossier</i>	No
Oxidising gases	<i>hazard class not assessed in this dossier</i>	No
Gases under pressure	<i>hazard class not assessed in this dossier</i>	No
Flammable liquids	<i>hazard class not assessed in this dossier</i>	No
Flammable solids	<i>hazard class not assessed in this dossier</i>	No
Self-reactive substances	<i>hazard class not assessed in this dossier</i>	No
Pyrophoric liquids	<i>hazard class not assessed in this dossier</i>	No
Pyrophoric solids	<i>hazard class not assessed in this dossier</i>	No
Self-heating substances	<i>hazard class not assessed in this dossier</i>	No
Substances which in contact with water emit flammable gases	<i>hazard class not assessed in this dossier</i>	No
Oxidising liquids	<i>hazard class not assessed in this dossier</i>	No
Oxidising solids	<i>hazard class not assessed in this dossier</i>	No
Organic peroxides	<i>hazard class not assessed in this dossier</i>	No
Corrosive to metals	<i>hazard class not assessed in this dossier</i>	No
Acute toxicity via oral route	Acute Tox. 4, H302	Yes
Acute toxicity via dermal route	<i>data conclusive but not sufficient for classification</i>	Yes
Acute toxicity via inhalation route	Acute Tox. 2, H330	Yes
Skin corrosion/irritation	Skin Corr. 1, H314	Yes
Serious eye damage/eye irritation	Eye Dam. 1, H318	Yes
Respiratory sensitisation	<i>data lacking</i>	No
Skin sensitisation	<i>hazard class not assessed in this dossier</i>	No
Germ cell mutagenicity	Muta. 2, H341	Yes
Carcinogenicity	<i>hazard class not assessed in this dossier</i>	No
Reproductive toxicity	Repr. 1B, H360 FD	Yes
Specific target organ toxicity-single exposure	<i>data conclusive but not sufficient for classification</i>	Yes
Specific target organ toxicity-repeated exposure	STOT RE 1, H372 (immune system)	Yes
Aspiration hazard	<i>data lacking</i>	No
Hazardous to the aquatic environment	<i>hazard class not assessed in this dossier</i>	No
Hazardous to the ozone layer	<i>hazard class not assessed in this dossier</i>	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Dibutyltin maleate (DBTM) 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 DBTM for Repr. Cat. 2; R60-61, Muta Cat. 3; R68, T; R23-R48/25, Xi; R36, Xn; R22 and recommended its inclusion with the next ATP. However, this classification has not been included in the legislation.

The present C&L proposal to classify DBTM for Muta. 2; H341, Repr. 1B; H360FD and STOT RE 1; H372 (immune system) is based on a category approach which is in detailed described 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 derivatives 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 proposals also covers the following endpoints: acute toxicity (oral, dermal, inhalation route), skin corrosion/irritation, serious eye damage/eye irritation and STOT SE.

RAC general comment

The dossier submitter (DS) proposed to classify dibutyltin maleate (abbreviated throughout this document as DBTM) for acute oral toxicity, acute inhalation toxicity, skin corrosion, serious eye damage, mutagenicity, STOT RE and reproductive toxicity. In addition to studies performed with DBTM itself, reference was made to studies performed with the following substances as part of a read-across, category approach: DBTC, DBTO, 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 maleate (DBTM)	201-077-5 / 78-04-6		Not reported	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)

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Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	245-152-0 / 22673-19-4		>92%	>92% No further details (monoconstituent substance)
Dibutyltin oxide (DBTO)	212-449-1 / 818-08-6		Not reported	>97.5% No further details (monoconstituent substance)
Dibutyltin dilaurate (DBTL)	201-039-8 / 77-58-7		Not reported	95-100% Monoconstituent substance; potential 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 were 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 DBTM form the distannoxane ClBu₂SnOSnBu₂Cl.

The *in vitro* gastric simulation study with DBTM used an exposure time of 72 h at a pH of 1.2, after which DBTM was completely hydrolysed to the distannoxane ClBu₂SnOSnBu₂Cl (Umweltbundesamt, 2019).

In addition, an older simulated hydrolysis study which used GC-FPD detection and showed that the half-time of DBTM under gastric conditions was <0.5 hours (Schilt & Zondervan-van den Beuken, 2004).

Moreover, the available developmental toxicity studies with DBTM itself show 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 DBTM 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 oxide (DBTO), dilaurate (DBTL) and dibutylbis(pentane-2,4-dionato)-OO'tin (DBTP).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

DBTM 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. 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 December 2019):

	Use(s)	Technical function
Manufacture	Manufacture of the substance	-
Formulation	Manufacture of plastic products PC 32: Polymer preparations and compounds	-
Uses at industrial sites	Manufacture of plastic products PC 32: Polymer preparations and compounds SU 12: Manufacture of plastics products, including compounding and conversion	-
Uses by professional workers	Professional use of plastic products PC 32: Polymer preparations and compounds	-
Consumer Uses	-	-
Article service life	Professional use of plastic products AC 13: Plastic articles	-

6 DATA SOURCES

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

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

- Information from TSCA submissions (Acute Toxicity, Skin irritation, Eye Irritation)

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

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	white powder	REACH registration	
Melting/freezing point	ca. 107 - 139 °C	REACH registration	ASTM E537-86
Boiling point	-	REACH registration	ASTM E537-86; Decomposition from 260°C, no value for boiling point
Relative density	1 600 kg/m ³ (21.8°C)	REACH registration	OECD 109
Vapour pressure	2.6 x 10 ⁻⁴ Pa (25°C)	REACH registration	OECD 104
Surface tension	-	-	-
Water solubility	< 10 mg/L (20°C)	REACH registration	OECD 105
Partition coefficient n-octanol/water	1.27	REACH registration	OECD 107
Flash point	-	-	study technically not feasible
Flammability	non flammable	REACH registration	EU Method A.10
Explosive properties	-	-	-
Self-ignition temperature	-	-	-
Oxidising properties	-	-	
Granulometry	inhalable particle size <100 µm = 20.5% thoracic particle size <10.0 µm = 2.23 % respirable particle size <5.5 µm = 0.689 %	REACH registration	OECD 110
Stability in organic solvents and identity of relevant degradation products	-	-	-
Dissociation constant	-	-	-
Viscosity	-	-	-

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 detailed 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 gastric hydrolysis (¹¹⁹ Sn NMR detection), pH 1.2, 40°C Time points: 72 hrs	DBTM is hydrolysed to the dimer distannoxane ClBu ₂ SnOSnBu ₂ Cl 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 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	The formation of DBTM and DBTDL 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 DBTDL. The half-life of DBTM and DBTDL under simulated gastric hydrolysis conditions was < 0.5 hours. DBTO hydrolyzed to 87.3% after 4 hours, with a half-life at 3.5 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, 37°C	DBTP is rapidly hydrolyzed to the dimeric stannoxane ClBu ₂ SnOSnBu ₂ Cl under conditions representative for the mammalian stomach. After 2 hours almost all DBTP 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 ClBu ₂ SnOSnBu ₂ Cl under gastric conditions. The degree of hydrolysis was reported as approximately 70, 85 and 90 % after 30 seconds, 1 hour and 4 hours	Study to support read across Non-guideline study Test material:	Naßhan, 2016 [Annex I, 1.1.4]

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Method	Results	Remarks	Reference*
	respectively (not corrected for trace impurities of tributyltin chloride). The impurity tributyltin chloride remains unchanged during the hydrolysis.	DBTC Purity: >90 % (Tributyltin chloride (TBTC) was identified as impurity in small amounts)	
Combination <i>in vitro</i> and <i>in vivo</i> study			
<p>Microsomal metabolism <i>in vitro</i> and <i>in vivo</i> metabolism in swiss webster mice.</p> <p><i>In vitro</i>: 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</p> <p><i>In vivo</i>: 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.</p>	<p><i>In vitro</i>: DBTA was metabolised to dibutyl and monobutyl species.</p> <p><i>In vivo</i>: 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.</p> <p>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.</p>	<p>Reliability: 2 (reliable with restrictions) supporting study; non-guideline study published in a peer-reviewed journal</p> <p>Test material: DBTA Purity: >99%</p>	<p>Kimmel EC, Fish RH & Casida JE (1977)</p> <p>[Annex I, 1.1.5]</p>
<i>In vivo</i> study			
<p>Metabolism of DBTC in male Wistar rats <i>in vivo</i></p> <p>Intraperitoneal injection (4 mg/kg bw)</p> <p>Time points: 6-168 hours</p> <p>Samples: blood, urine, liver, kidney, spleen and brain</p> <p>Method: HPLC/MS</p>	<p>The half-life of DBTC in liver, kidney and blood was 3-5 days.</p> <p>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 the brain and blood).</p>	<p>Reliability: 2 (reliable with restrictions) supporting study; non-guideline study published in a peer-reviewed journal</p> <p>Test material: DBTC</p>	<p>Ishizaka et al., 1989</p> <p>[Annex I, 1.1.6]</p>

* 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(s)

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 DBTM only *in vitro* toxicokinetic data are available (Umweltbundesamt, 2019, Schilt & Zondervan-van den Beuken (2004)). Further studies were carried out with other category members.

In the *in vitro* gastric hydrolysis study carried out with DBTM (Umweltbundesamt, 2019) using the ^1H , ^{13}C and ^{119}Sn NMR analytical methods, it is demonstrated that DBTM forms identical DBTC derivatives as the category members DBTP and DBTC (Naßhan, 2015, Naßhan, 2016). Under gastric simulation conditions (pH 1.3, 37-40°C) the category members form dimer distannoxane ($(\text{ClBu}_2\text{SnOSnBu}_2\text{Cl})_2$) (see Figure 1).

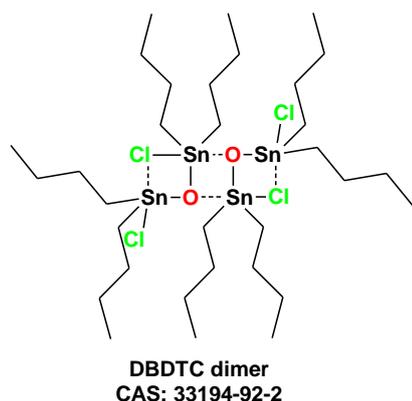


Figure 1: Dimer distannoxane ($(\text{ClBu}_2\text{SnOSnBu}_2\text{Cl})_2$)

In the study of Schilt & Zondervan van den Beuken it is demonstrated that DBTM and category members (DBTL, DBTO) formed 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. A faster hydrolysis (half-life < 0.5 hours) was reported for DBTM and DBTL to form DBTC (95% and 87% yields, after 4 hours). DBTO was reported to hydrolyse to DBTC with a half-life of 3.5 hours (87% yield 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 (DBTM, DBTO, DBTL) are converted to DBTC under simulated gastric conditions, however an unambiguous assignment of the structure of the common metabolite has not been made.

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 derivatives 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.

DBTM is a member of the category. For DBTO, 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

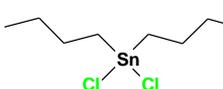
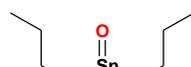
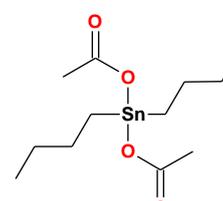
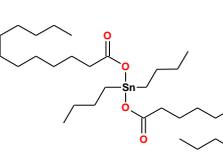
The read-across approach is limited to endpoints where toxicological data generated in experimental animal species *in vivo* by oral administration (e.g., *in vivo* mutagenicity, repeated dose toxicity, reproductive toxicity) are available. It is not applicable to studies using dermal or inhalation exposures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

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 toxicity - repeated exposure (Chapter 9.14).

9.2.1.5 Category members

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

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

Substances	CAS	Structure	Purity/Impurity details (REACH dossier)
Dibutyltin maleate (DBTM)	78-04-6		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 oxide (DBTO)	818-08-6		>97.5% 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	22673-19-4		>92% No further details (mono-

Substances	CAS	Structure	Purity/Impurity details (REACH dossier)
(DBTP)			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 ($\text{XR}_2\text{SnOSnR}_2\text{X}$) 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).

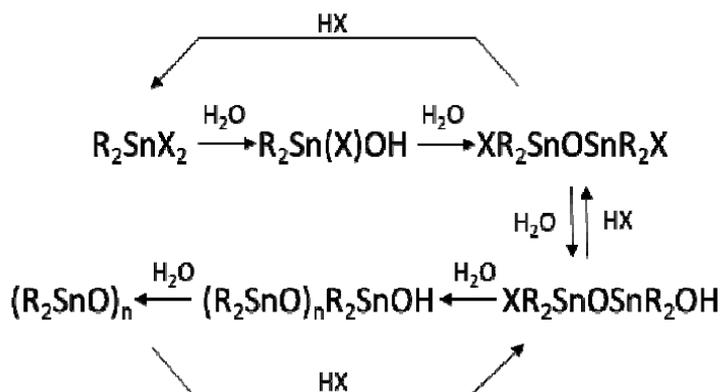


Figure 2: Simplified hydrolysis scheme for dialkyltins (Davies, 2004; Aylett et al., 1979) (reaction scheme as depicted in ECHA, 2016).

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 & Zondervan-van der Beuken (2004) reported that DBTM and DBTL forms DBTC under simulated stomach conditions very rapidly (half-life < 0.5 hours) (95% and 87%, respectively after 4 hours). DBTO forms DBTC with a half-life of 3,5 hours (87% after 4 hours). The category member 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 ^1H , ^{13}C and ^{119}Sn NMR spectroscopy demonstrate that DBTM (Umweltbundesamt, 2019), DBTC and the category member DBTP (Naßhan, 2015, 2016, cited in ECHA, 2017) form distannoxane (dimer) at pH 1.2.

No DBTC with the starting material DBTM were detected after 72 hours. Minor amounts of DBTC after the reaction were detected with the starting material DBTC and DBTP (10 mol% and 2 mol%, respectively, after 4 hours). The direct analytical method (with much higher substance concentrations) allow in contrast to gastric simulation studies of Schilt & Zondervan-van der Beuken (2004) a specific assignment of the formed substance.

These observations that distannoxane $\text{ClBu}_2\text{SnOSnBu}_2\text{Cl}$ 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).

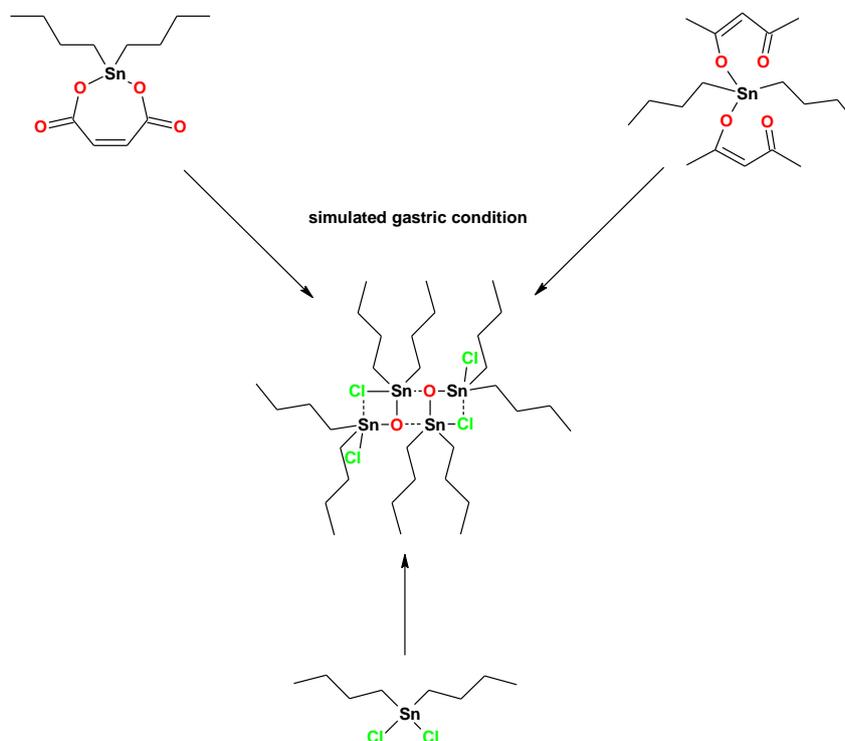


Figure 3: Overview of the hydrolysis of DBTM, DBTP and DBTC as determined in recent studies (Naßhan, 2015, 2016, Umweltbundesamt, 2019), which is in accordance with well established tin chemistry (Davies, 2004; Aylett et al., 1979).

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 DBTM has a water solubility $< 10 \text{ mg/L}$ (20°C). In the "OECD SIDS Initial Assessment Profile for dibutyltin dichloride and selected thioglycolate esters" (OECD, 2006), it is stated however that DBTM, DBTC and DBTL 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 (DBTM, DBTC, DBTO, 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 thereof. 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

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIBUTYLTIN MALEATE

A key study is the study of Schilt & Zoderan-van den Beuken (2004), in which DBTM and category members (DBTL, DBTO) 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 (DBTM, DBTC, DBTP). The used method (^{119}Sn NMR) allows to identify the structure of the DBTC derivatives. The studies demonstrate that after hydrolysis of the category members dimeric stannoxanes $\text{ClBu}_2\text{SnOSnBu}_2\text{Cl}$ are formed.

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

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

Based on the similar toxicokinetic behaviour it is plausible that upon exposure the same biological targets are affected 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 DBTM 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 DBTO a CLH proposal will be submitted by AT at the same time as for DBTM. 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 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.

DBTM is a white powder (at 20°C and 101,3 kPa) and is reported to have a melting point between 107-137°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.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIBUTYLTIN MALEATE

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 maleate (DBTM)	Dibutyltin dichloride (DBTC)	Dibutyltin oxide (DBTO)	Dibutyltin dilaurate (DBTL)	Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	Dibutyltin (di)acetate (DBTA)
CAS no	78-04-6	683-18-1	818-08-6	77-58-7	22673-19-4	1067-33-0
EC no	201-077-5	211-670-0	212-449-1	201-039-8	245-152-0	213-928-8
MW	347	304	249	632	431	351
Physical-chemical properties						
Physical state	Liquid	Solid	Solid	Liquid	Liquid	Liquid
Water solubility	Sparingly soluble	Study technically not feasible. Hydrolysis on contact with water.	2.55 mg/L	Insoluble	Study technically not feasible. Hydrolysis on contact with water.	Insoluble
Hydrolysis, low pH (GC-FPD detection)	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: 43% in 0.5h, 65% in 1h, 90% in 2h, 87% in 4h	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)	Formation of ClBu ₂ SnOSnBu ₂ Cl under gastric simulation studies: 100% in 72hrs	Formation of ClBu ₂ SnOSnBu ₂ Cl under gastric simulation studies: ~70% in 30s, ~85% in 1h, ~90% in 4hrs	No data	No data	Formation of ClBu ₂ SnOSnBu ₂ Cl 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)	510 (263-777)	219	172 (121-240)	2071 (1207-5106)	1864 (1039-3344)	1070
Dermal LD50 (mg/kg bw)	>2000	No data	>2000	>2000	>2000	No data

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIBUTYLTIN MALEATE

Substance	Dibutyltin maleate (DBTM)	Dibutyltin dichloride (DBTC)	Dibutyltin oxide (DBTO)	Dibutyltin dilaurate (DBTL)	Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	Dibutyltin (di)acetate (DBTA)
Skin corrosion/irritation	Corrosive <i>in vivo</i>	Corrosive <i>in vivo</i>	Corrosive <i>in vivo</i>	Corrosive <i>in vivo</i>	Irritant but not corrosive <i>in vitro</i> . Corrosive <i>in vivo</i>	Corrosive <i>in vitro</i>
Serious eye damage/eye irritation	Serious eye damage <i>in vivo</i>	Serious eye damage <i>in vivo</i>	Serious eye damage <i>in vivo</i>	Irritant <i>in vivo</i>	Serious eye damage <i>in vitro</i>	No data
Germ cell mutagenicity	Only Ames test, negative	Positive <i>in vivo</i> somatic cell mutagenicity test, as well as support from positive results from <i>in vitro</i> mutagenicity/genotoxicity tests.	Only Ames test, negative	Only Ames test, negative	Only Ames test, negative	Only Ames test, negative
Reproductive toxicity – adverse effects on sexual function and fertility	No data	Large increase in pre-implantation loss in studies in the rat, mouse & monkey	No data	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	Characteristic external and skeletal foetal malformations, largely affecting the jaw/skull	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	No data, read-across	Characteristic external and skeletal foetal malformations, largely affecting the jaw/skull
Repeated dose toxicity	No data, read across	Marked reduction in thymus size & cellularity; similar effects on the spleen and lymph nodes	No data, read across	No data, read-across	No data, proposed read-across	No data, proposed read-across
Harmonised	No harmonised	Acute Tox. 3*, H301	No harmonised	Muta. 2; H341	Adopted RAC opinion	No harmonised

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIBUTYLTIN MALEATE

Substance	Dibutyltin maleate (DBTM)	Dibutyltin dichloride (DBTC)	Dibutyltin oxide (DBTO)	Dibutyltin dilaurate (DBTL)	Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	Dibutyltin (di)acetate (DBTA)
classification	classification	Acute Tox. 4*, H312 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	classification	Repr. 1B; H360FD STOT RE 1; H372 (immune system) Based mainly on read-across from DBTC	(ECHA, 2017) Repr. 1B; H360FD STOT RE 1; H372 (immune system). Based mainly on read-across from DBTC	Classification; CLH proposal under evaluation: Repr. 1B, H360FD; STOT RE 1, H372 (immune system), Muta. 2 (H341); Based mainly on read-across from DBTC)

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

In the key study presented by the registrants (Anonymous, 1982a) rats (5/sex/group) were exposed to DBTM doses of 250, 500, 1000, 2500 mg/kg bw via gavage. Animals from all groups exhibited dyspnoea, curved position and ruffled fur within one hours after dosing. All surviving animals in the 250 and 500 mg/kg bw groups recovered till day nine. Decrease of body weight was noted in males at 500 mg/kg bw and above and in females at 1000 mg/kg bw and above. One female at 250 mg/kg bw died on day 8. Animals at 500 mg/kg and above died within 4-7 days following dosing. No compound-related gross organ changes were reported. DBTM was found to have a LD₅₀ of 510 mg/kg bw with confidence limits of 263 - 777 mg/kg bw in males/female. For males the LD₅₀ is 422 mg/kg bw and for females 647 mg/kg bw.

In the study by Schafer (1985) two exposure scenarios are described. For the first test, a range-finding method, 6 wild-trapped mice and a graduated dosage scaling was used. Each succeeding treatment was 50% higher than the preceding level and continued until mortality occurred using a single animal per level. DBTM was administered by gavage using water, corn oil, or 1.0% carbopol as carriers, followed by three days of observations for mortality. The approximate lethal dose was determined as 470 mg/kg. The second test is described as a feeding study. Mice were fed for 3 days with wheat seeds treated with 2.0% (wt/wt) of the test material. 2 to 4 animals were used per geometrically spaced dosage level. The ingested amount of chemical was calculated based on the average weight of wheat seeds and the weight of each individual mouse. The lethal dose reported for a 3-day feeding test was >250 mg/kg/day. No further details on study design are available.

In a third study only a dose of 50 mg/kg bw was tested in 10 rats. 4 rats died within 48h. No further information is available. The reliability of this old study is rather limited.

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 LD₅₀/ATE values are > 300 and ≤ 2000 mg/kg bw.
- Acute Tox 3 (oral) if the LD₅₀/ATE values are > 50 and ≤ 300 mg/kg bw.

For DBTM one well documented oral study (gavage) is available resulting in a LD₅₀ (m/f) of 510 mg/kg bw with a CI of 263 – 777 mg/kg bw. A second study with limited reliability (non standard test, wild trapped animals, limited reporting) a LD₅₀ of 470 mg/kg bw was found in mice, which is in the same order of magnitude.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on a LD₅₀ (m/f) of 510 mg DBTM/kg bw and the available criteria a classification as Acute Tox 4, H302 is proposed.

An ATE value of 510 mg/kg bw can be assigned.

RAC evaluation of oral acute toxicity

Summary of the Dossier Submitter's proposal

Three acute oral toxicity studies with DBTM are available, of which one is an OECD test guideline (TG) 401 study. This study was performed in rats by oral gavage and gave LD₅₀

values of 422 mg/g bw for males and 647 mg/kg bw for females. Animals from all groups exhibited dyspnoea, curved position, and ruffled fur within 1 h after dosing, but no gross organ changes were reported (Anonymous, 1982a).

The other two studies were non-guideline studies with limitations in reporting and large deviations from the guidelines, reducing their reliability. One used wild trapped mice exposed by gavage and through feed. The gavage LD₅₀ was 470 mg/kg bw (Schafer, 1985). For the other study, no more information was available other than that 10 rats were exposed to 50 mg/kg bw after which 4 died (Anonymous, 1975).

The DS based the classification proposal on the average LD₅₀ of 510 mg/kg bw from the OECD TG 401 study. This results in a classification as Acute Tox. 4 with and ATE value of 510 mg/kg bw.

Comments received during consultation

Three comments were received from Member State Competent Authorities (MSCAs), all in support of the proposed classification in Acute Tox. 4. Two MSCAs commented on the ATE, one to indicate a preference to use the lower male LD₅₀ of 422 mg/kg bw while the other supported the use of the mean value. The reasoning of the latter was the same as that of the DS, namely that there is no apparent difference in sensitivity between males and females for oral acute toxicity, and that there is a rather large range of confidence intervals for the LD₅₀ values.

Assessment and comparison with the classification criteria

Of the three studies available, only one was of sufficient quality as regards both performance and reporting to be used as a key study for classification. This study was performed in rats and showed LD₅₀ values of 422 and 647 mg/kg bw for males and females, respectively, with confidence limits of 263-777 mg/kg bw. Both LD₅₀ values fall within the classification range of Acute tox 4 of > 300 and ≤ 2000 mg/kg bw.

Hence, **classification of DBTM in Acute Tox. 4; H302 (Harmful if swallowed) is warranted.**

As also brought up during the consultation, the broad confidence interval makes it difficult to judge whether the difference in outcome between males and females is due to actual differences in sensitivity or a chance finding. For this reason, RAC supports the proposal of the DS to use the mean LD₅₀ value of **510 mg/kg bw** in the setting of the **ATE value**.

10.2 Acute toxicity - dermal route

Table 13: Summary table of animal studies on acute dermal toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels of duration exposure	Value LD ₅₀	Reference
OECD 402 GLP	Rat, Wistar	DBTM	2000 mg/kg bw Semiocclusive,	LD ₅₀ > 2000 mg/kg bw	Anonymous, 2010a

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels of duration exposure	Value LD ₅₀	Reference
	N=5/sex/dose		24h, 10% of the body surface, clipped moistened with arachis oil BP observation 14d	No mortalities observed Signs of dermal irritation	
-	Rabbit N=10	DBTM in propylene glycol	200 mg/kg bw Shaved, 24h	Mortalities: 48h: 4/10 72h: 5/10	Anonymous, 1975 (OTS0571954)

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

In the study by Anonymous (2010a) two animals (1m+1f) were given a single dermal application of DBTM (moistened with arachis oil BP) to intact skin (clipped, semi occluded, 24h) at a dose level of 2000 mg/kg bw. Based on the results of the initial test, a further group of eight animals (4m + 4f) was similarly treated. Animals were observed for 14 days and clinical signs and bodyweight development were monitored. All animals were subjected to gross necropsy. No deaths occurred and there were no signs of systemic toxicity. Signs of dermal irritation were very slight to well-defined erythema, haemorrhage of the dermal capillaries, small superficial scattered scabs, hardened light brown coloured scab, scab lifting to reveal bleeding, crust formation, glossy skin and scar tissue (see also Chapter 9.6). One female rat showed signs of dermal corrosion. Three males and one female showed bodyweight loss during the first week but expected gain in bodyweight during the second week. One other male showed no gain in bodyweight during the first week but expected gain in bodyweight during the second week. No abnormalities were noted at necropsy. The LD₅₀ of DBTM in Wistar rats was found to be greater than 2000 mg/kg bw.

In a TSCA submission document (Anonymous, 1950) test results of an application of DBTM (in propylene glycol) for 24h to shaved rabbits skin are reported. Four deaths occurred within 48h and five within 72h. No further information is available therefore only limited reliability was assigned.

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 LC₅₀/ATE values are > 1000 and ≤ 2000 mg/kg bw

Acute Tox 3 (dermal) if the LC₅₀/ATE values are > 200 ≤ 1000 mg/kg bw

The LD₅₀ of DBTM in Wistar rats was found to be greater than 2000 mg/kg bw in a GLP guideline study. Another study with limited reporting and reliability presents and LD₅₀ ≥ 200mg/kg bw.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the results (LD₅₀ > 2000 mg/kg) of one reliable, well documented guideline study DBTM does not meet the criteria for classification for this endpoint.

RAC evaluation of dermal acute toxicity

Summary of the Dossier Submitter's proposal

There are two acute dermal toxicity studies with DBTM, an OECD TG 402 study in rats and a non-guideline study in rabbits.

In the rat study semi-occlusive exposure to 2000 mg/kg bw DBTM in arachis oil for 24 h did not result in mortalities or systemic toxicity. There were signs of dermal irritation observed (Anonymous, 2010a).

The rabbit study was very old and only limited information is available. Shaved rabbits were exposed for 24 h to 200 mg/kg bw DBTM in propylene glycol. After 48 h 4/10 rabbits died, which increased to 5/10 after 72 h (Anonymous, 1950).

Due to the limitations in the rabbit study, the DS based the evaluation on the rat study. As the LD₅₀ was above 2000 mg/kg bw, no classification was proposed.

Comments received during consultation

Two comments from MSCAs specifically addressed acute dermal toxicity. One agreed with the proposal for no classification. The second suggested that the higher sensitivity of rabbits may have to be considered as well.

Assessment and comparison with the classification criteria

No mortalities were observed in an acute dermal toxicity study in rats with a top dose of 2000 mg/kg bw. On the other hand, mortality up to 50% occurred in a study in rabbits at 200 mg/kg bw. There are several possible reasons for this difference, including differences in sensitivity between species, higher absorption due to the solvent used (arachis oil vs. propylene glycol), but also potential flaws in animal husbandry in the rabbit study. As the rabbit study is very old and limited information is available, it is not possible to draw a conclusion on this, but RAC agrees with the DS that the level of confidence for this study is rather low. It should also be noted that all of the other category members for which acute dermal toxicity studies are available have LD₅₀ values > 2000 mg/kg bw (DBTO, DBTL, and DBTP). Considering the close similarities in toxicity between these substances, this increases the confidence for the use of the test guideline study in rats as the only key study.

In conclusion, RAC considers that **no classification is warranted for DBTM for Acute toxicity via the dermal route.**

10.3 Acute toxicity - inhalation route

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

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, , form and particle size (MMAD)	Dose levels, duration of exposure	Value LC ₅₀	Reference
Method of Sachsse (Sachsse, 1973)	Rat, Tif: RAIf (SPF) N=10/sex/group	DBTM inhalation: aerosol nose only vehicle: aerosol	0, 104, 212, 478 and 1004 mg/m ³ 4h Observation 14d	LC ₅₀ (m/f) = 317 mg/m ³ air (nominal) (CI: 240 – 416 mg/m ³) LC50 (m)= 313 (214-459) mg/m ³ LC50 (f) = 319 (198-504) mg/m ³	Anonymous, 1982b

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

In an acute inhalation toxicity study (Anonymous, 1982b) according to the method of Sachsse (1973) rats (10/sex/dose) were exposed nose-only to a DBTM aerosol in concentrations of 104, 212, 478 and 1004 mg/m³ (nominal). The aerosol was generated by injecting a 30 % (w/w) solution of DBTM in ethanol with a Perfusor IV Syringe System at a rate of 0.949, 3, 6.01, and 12 ml/h into a spray nozzle through which compressed filtered air was discharged into the inhalation chamber. Control exposure was done at the highest nominal solvent concentration used in the test material exposure. Particle size analysis was conducted twice during each exposure and the aerosol concentration was determined 5 times during exposure. Animals were exposed for 4h and observed for 14 days. Examination of clinical symptoms and mortalities were done during exposure (1,2,4h), 2h after exposure and daily for 14 days. Body weights were recorded prior to exposure and on day 7 and 14. Gross pathological examinations were done for all animals. Mortalities were seen at 212, 478 and 1004 mg/m³ in males and females. Control animals showed slight exophthalmus and ruffled fur. For animals exposed to 104 mg/m³ slight dyspnoea, exophthalmus, ruffled fur and curved body position are documented. Higher exposure concentrations resulted in slight sedation, ventral body position and moderate dyspnoea, exophthalmus, ruffled fur and curved body position. At the two higher concentrations a significant decrease in bodyweight was seen. Gross pathology showed mottled effects in the lungs in some animals at all exposure concentrations. At 207 mg/m³ and above odema of the lungs are documented. Other effects such as mottled liver, stomach expansion and small/large intestine expansion were seen in some animals at exposure concentrations of 474 mg/m³ and above. Based on these results an LC₅₀ (m/f) of 317 (240-416) mg/m³ was derived using the probit method. For males an LC₅₀ value of 313 (214-459) mg/m³ and females a value of 319 (198-504) mg/m³ has been derived.

10.3.2 Comparison with the CLP criteria

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

- Acute Tox 4 (inhal) if the LC₅₀ values are > 1.0 mg/L and ≤ 5.0 mg/L (4h exposure)
- Acute Tox 3 (inhal) if the LC₅₀ values are > 0.5 mg/L and ≤ 1.0 mg/L (4h exposure)
- Acute Tox 2 (inhal) if the LC₅₀ values are > 0.05 and ≤ 0.5 mg/L (4h exposure)

For the substance DBTM one acute inhalation toxicity study with an exposure duration of 4h is available resulting in a LC₅₀ value of 317 (240-416) mg/m³.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the classification criteria and a converted LC₅₀ of 0.317 mg/L a classification as Acute Tox, Cat 2, H330 for DBTM is indicated.

An ATE value of 0.317 mg/L (dusts and mists) can be assigned.

RAC evaluation of inhalation acute toxicity

Summary of the Dossier Submitter's proposal

An acute inhalation study was performed in rats with doses of 0, 104, 212, 478 and 1004 mg/m³ DBTM in ethanol administered by nose-only (Anonymous, 1982b). The exposure duration was 4 h with a 14-d observation period.

Control animals showed slight exophthalmus and ruffled fur. For animals exposed to 104 mg/m³ slight dyspnoea, exophthalmus, ruffled fur and curved body position were documented. Higher exposure concentrations resulted in slight sedation, ventral body position and moderate dyspnoea, exophthalmus, ruffled fur and curved body position. At the 478 and 1004 mg/m³ a significant decrease in bodyweight was seen. Gross pathology showed mottled effects in the lungs in some animals at all exposure concentrations. At 207 mg/m³ and above oedema of the lungs are documented. Other effects such as mottled liver, stomach expansion and small/large intestine expansion were seen in some animals at exposure concentrations of 474 mg/m³ and above. Based on these results an LC₅₀ (m/f) of 317 (240-416) mg/m³ was derived using the probit method. For males an LC₅₀ value of 313 (214-459) mg/m³ and females a value of 319 (198-504) mg/m³ was derived.

Based on the LC₅₀ value of 317 mg/m³ the DS proposed classification of DBTM as Acute Tox. 2 (inhalation). An ATE of 0.317 mg/L was proposed.

Comments received during consultation

Three MSCAs indicated their support for the proposed classification.

Assessment and comparison with the classification criteria

One acute inhalation study in the rat is available with DBTM. The outcome showed both local and systemic toxicity including mortality after 4 h exposure. Although the study did not follow an OECD test guideline, it did use a generally recognised method and both methodology and results are sufficiently well described.

The LD₅₀ values for males and females were very similar; hence, no difference in sensitivity between sexes is apparent and RAC agrees with the use of the mean LC₅₀ value of 0.317 mg/L. This value falls within the boundaries for Acute Tox. 2 of 0.05 and 0.5 mg/L (4-h exposure). Hence, RAC agrees that **classification of DBTM as Acute Tox. 2; H330 (Fatal if inhaled) is warranted**. An **ATE of 0.317 mg/L (dusts or mists)** is considered warranted for both sexes.

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 402 GLP	Rat, Wistar N=5/sex/dose	DBTM	2000 mg/kg bw Semioclusive, 24h, intact skin, 10% of the body surface, clipped moistened with arachis oil BP observation 14d	LD ₅₀ > 2000 mg/kg bw No mortalities observed Signs of dermal irritation (slight to well-defined erythema) and corrosion (haemorrhage, scab, scar formation)	Anonymous, 2010a
TSCA method (acute exposure, primary dermal irritation)	Rabbits, New Zealand white N=6 (2m + 4f)	DBTM (Thermolite 13)	500 mg, moistened with 0.5ml phys. saline 4h, clipped, 1x1cm Wiped after exposure Observation 3-14d	Mean scores (24, 48, 72h) for animals #1-#6: Erythema 1.67, 1.67, 1.67, 2, 1, 2 Oedema: 1.33, 0.33, 1.33, 1.67, 0, 1.67 Necrosis in 2/6	Anonymous, 1988b (OTS 0555419)

Table 16: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
OECD 431 (In Vitro Skin Corrosion) GLP	DBTM Purity >98% w/w	20 mg of the solid test material was applied Exposure: 3, 60 and 240 min	relative mean viability: 79.1% after 240 min exposure 90.6% after 60 min exposure 101.4% after 3 min exposure Positive control 10.1%	Anonymous, 2010b
EU Method B.46. (Reconstructed Human Epidermis Model Test) (Episkin) GLP	DBTM Purity >98%	10 mg of the test material was applied exposure period: 15 min incubation period: 42h	cell viability: 71.6% after 3 min exposure	Anonymous, 2010c

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

In the study by Anonymous (2010a) two animals (1m+1f) were given a single dermal application of DBTM (moistened with arachis oil BP) to intact skin at back and flanks (clipped, semi occlusive, 24h) at a dose level of 2000 mg/kg bw. A piece of surgical gauze was placed over the treatment area and semi-occluded with a piece of self adhesive bandage. To remove any residual test substance treated skin and surrounding hair was wiped with cotton wool moistened with arachis oil BP 24h after start of exposure. Based on the results of the initial test, a further group of eight animals (4m + 4f) was similarly treated. Animals were observed for 14 days and clinical signs and bodyweight development were monitored. All animals were subjected to gross necropsy. No deaths occurred and there were no signs of systemic toxicity. Three males and one female showed bodyweight loss during the first week but expected gain in bodyweight during the second week. One other male showed no gain in bodyweight during the first week but expected gain in bodyweight during the second week. No abnormalities were noted at necropsy. Signs of dermal irritation were very slight to well-defined erythema, haemorrhage of the dermal capillaries, small superficial scattered scabs, hardened light brown coloured scab, scab lifting to reveal bleeding, crust formation, glossy skin and scar tissue. One female rat showed signs of dermal corrosion. Treated skin sites of male animals appeared normal 6 - 13 days after dosing and the treated skin sites of three females appeared normal 6 or 12 days after dosing. Individual results are presented in Table 17. No details on the used scoring system are given.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIBUTYLTIN MALEATE

Table 17: Individual animal data after dermal exposure to 2000 mg DBTM/kg bw for 24h (Anonymous, 2010a).

Sex	No	Observation	1day	2 day	3 day	4 day	5 day	6 day	7 day	8 day	9 day	10 day	11 day	12 day	13 day	14 day	
Male rats	1	Erythema	0	1	1	1	1	0	0	0	0	0	0	0	0	0	
		Oedema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		Other	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2	Erythema	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
		Oedema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Other	0	0	Hd	HdCf	Cf	Cf	0	0	0	0	0	0	0	0	0
	3	Erythema	1	1	1	2	1	1	1	1	1	1	0	0	0	0	0
		Oedema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Other	0	0	Hd	HdCf	HdCf	Cf	Cf	Cf	Cf	Cf	Cf	0	0	0	0
	4	Erythema	2	2	2	2	2	2	1	1	1	0	0	0	0	0	0
		Oedema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Other	0	0	Hd	HdCf	HdCf	CfSp	CfSsG	CfSsG	SsG	SsG	SsG	G	0	0	
	5	Erythema	2	2	2	2	2	2	1	1	0	0	0	0	0	0	0
		Oedema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Other	0	0	Hd	HdCf	HdCfSs	CfSs	CfSs	CfSs	Cf	Cf	Cf	Cf	Cf	0	0
Female rats	1	Erythema	1	2	2	2	1	1	1	1	0	0	0	0	0	0	
		Oedema	0	0	1	0	0	0	0	0	0	0	0	0	0	0	
		Other	0	Ss	Hd	HdCf	HdCf	HdCf	HdCf	HdCf	HdCf	CfSs	CfSc	Ss	Ss	Ss	
	2	Erythema	1	1	1	1	1	1	1	1	0	0	0	0	0	0	
		Oedema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
		Other	0	0	Hd	HdCf	CfSb	CfSbSs	CfSbSs	CfSbSs	SbSs	Ss	Ss	0	0	0	
	3	Erythema	2	2	2	1	1	1	1	1	0	0	0	0	0	0	
		Oedema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIBUTYLTIN MALEATE

		Other	0	Ss	SsHd	HdCfSs	CfSs	Ss	Ss	Ss	G	SsG	SsG	0	0	0
4	Erythema	1	2	1	0	0	0	0	0	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Other	0	Ss	Ss	Cf	Cf	0	0	0	0	0	0	0	0	0	0
5	Erythema	2	2	2	2	1	1	1	1	0	0	0	0	0	0	0
	Oedema	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Other	0	0	0	HdCf	CfSp	CfSp	CfSpSs	CfSpSs	SsSpG	SsG	SsG	SsG	SsSc	SsSc	

0,1,2...scoring (no detailed information in registration information; due to description of effects a Draize score can be assumed)

Hd...Haemorrhage of dermal capillaries

Ss...Small superficial scattered scabs

Sb...Scab lifting to reveal bleeding

Cf...Crust formation

Sp...hardened light brown coloured scab

G...Glossy skin

Sc...Scar Tissue

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIBUTYLTIN MALEATE

In a well documented irritation study submitted for TSCA (Anonymous, 1988b) clipped backs of six rabbits were exposed to moistened DBTM (500 mg). After 4h of exposure the test sites were wiped with castile soap. Skin irritation was evaluated for up to 14d. Individual scoring data is presented in Table 18. Mean scores for erythema were between 1 and 2 and for oedema between 0 and 1.67. Erythema persist till end of the study (14d) and oedema were irreversible for three rabbits. Necrosis was documented for one animal and superficial necrosis for another.

Table 18: Dermal irritation study – individual irritation Draize-scores (Anonymous, 1988b)

	Animal No. (sex)	#1 (f)	#2 (f)	#3 (f)	#4 (m)	#5 (f)	#6 (m)
30min	Erythema	1	2	2	1	1	1
	Oedema	0	0	1	0	0	0
	Other	-	-	-	-	-	-
24h	Erythema	2	2	2	2	2	2
	Oedema	2	1	2	2	0	2
	Other	-	-	-	-	-	-
48h	Erythema	2	2	2	2	0	2
	Oedema	1	0	2	2	0	2
	Other	-	-	-	-	-	-
72h	Erythema	1	1	1	2	1	2
	Oedema	1	0	0	1	0	1
	Other	-	-	-	-	-	-
7d	Erythema	1	2	2	4	2	2
	Oedema	0	0	0	1	0	0
	Other	-	-	D	N	-	-
10d	Erythema	2	2	4	4	2	3
	Oedema	0	0	1	1	0	1
	Other	-	-	D, S	N	D	-
14d	Erythema	2	2	4	4	2	3
	Oedema	0	0	1	2	0	2
	Other	-	D	D, S	N	D	D
Mean score (24, 48, 72h)							
Erythema		1.67	1.67	1.67	2	1	2
Oedema		1.33	0.33	1.33	1.67	0	1.67

D...desquamation, S...superficial necrosis, N...necrosis

In an *in vitro* Human Skin Model Test (EPISKIN) (OECD 431) reconstituted human epidermis was topically exposed to 20 mg of DBTM (uniform coverage of the tissue) to assess a possible corrosivity of the substance. Duplicate tissues were treated with the test material for exposure periods of 3, 60 and 240 minutes. The toxicity is determined by the metabolic conversion of the vital dye MTT to formazan by viable cells in the treated cultures relative to the negative control. Formazan then was extracted and measured at 540nm. The relative viability (MTT reduction on treated tissue relative to control tissue) was 79.1%, 90.6%

and 101.4% after exposure duration of 240 min, 60 min and 3 min respectively. The test material was considered as non-corrosive to skin (Anonymous, 2010b).

To evaluate the skin irritation potential of DBTM using the EPISKINTM reconstituted human epidermis model cells were exposed for 15min, followed by a post exposure incubation period of 42 hours. The measurement of cytotoxicity was done by the MTT reduction assay. The concentration of the inflammatory mediator IL-1 α in the culture medium retained following the 42 h post exposure incubation period is also determined for test materials which are found to be borderline. The assay was done in triplicates. The relative mean viability of the DBMT treated tissues was 71.6% after the 15 min exposure period. The test material was considered to be non irritating (Anonymous. 2010c).

10.4.2 Comparison with the CLP criteria

A corrosive substance is a substance that produced destruction of skin tissue in at least one tested animal after exposure up to 4h duration. Corrosive reaction are typified by ulcers, bleeding, bloody scabs and by end of observation at 14 days, by discoloration due to bleaching, complete areas of alopecia and scars. Three subcategories are provided:

		Corrosive in > 1 of 3 animals	
	Corrosive subcategories	Exposure	Observation
Category 1: Corrosive	1A	≤ 3 min	≤ 1h
	1B	> 3min - ≤ 1h	≤ 14 d
	1C	> 1 h - ≤ 4h	≤ 14 d
Skin Irritation Category 2	In the case of testing six animals: (1) Mean score of $\geq 2,3$ - $\leq 4,0$ 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.		

Guidance on use of dermal acute toxicity animal data according to the CLP guidance (ECHA, 2017): If the substance is proven to be either an irritant or a corrosive in an acute dermal toxicity test carried out with rabbits with the undiluted test substance (liquids) or with a suitable suspension (solids), the following applies: In case of signs of skin corrosion, classify as Skin Corrosive (subcategorisation as 1A, 1B or 1C, where possible). In case the test was performed in other species, which may be less sensitive (e.g. rat), evaluation must be made with caution. [...] Only in case of evidence of skin corrosivity in the rat dermal toxicity test can the test substance be classified as Skin Corrosive Category 1. All other data should be used in a weight of evidence.

Available *in vitro* studies on reconstituted human epidermis gave negative results.

In a primary dermal irritation test (Anonymous, 1988b) in rabbits, with an exposure periode of 4h, mean scores for erythema were between 1 and 2 and for oedema between 0 and 1.67. Effects were irreversible for 6/6 animals and two showed necrosis after 14 days.

However, in an acute toxicity study (Anonymous, 2010a) with dermal exposure of rats to 2000 mg/kg bw signs of skin irritation and corrosion were documented. The body weight of rats was at least 200 g and the

area of exposure was 10% of the body surface (clipped, intact skin) resulting in a maximum total amount of 400 mg/animal. 10 animals were tested under semi-occlusive conditions with an exposure duration of 24h. Animals were observed for 14 days. Erythema with scores up to 2 were seen in all treated rats. Small superficial scattered scabs were seen in three females on day 2 and Haemorrhage of dermal capillaries were documented for 7 animals on day 3. Scabs and/or crust formation were seen in 9/10 animals. One female rat showed scar tissue at the end of the observation period. Effects in other animals were reversible (Table 17).

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

In the dermal irritation study scores below the classification criteria are reported, however, irreversibility at study termination on day 14 is documented for erythema and oedema becoming even worse with study duration.

Based on the ECHA guidance (ECHA, 2017) on the use of acute (rat) toxicity data for classification the seen corrosive effects (haemorrhage, scabs, scar tissue) in rats are relevant for classification. 8/10 animals showed haemorrhage of dermal capillaries from day 3 upwards. Scabs and crust formation is documented for 9/10 rats. One female rat had scar tissue at end of observation periode. Irreversible effect were seen in one rat (f) and sever but reversible skin damage in 8/10 rats.

Based on the severity of effects seen in two species and irreversibility at study termination a classification as Skin Corr. 1 is proposed.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The dossier contains one dermal irritation study in rabbits, an acute dermal toxicity study in rats, and two *in vitro* skin corrosion assays. All studies were performed with DBTM.

The dermal irritation study exposed six rabbits to doses of 500 mg for four hours (Anonymous, 1988b). Mean scores for erythema were between 1 and 2 and for oedema between 0 and 1.67. The effects were irreversible in all animals in 14 days and in fact progressed to scores for erythema between 2 and 4 and for oedema between 0 and 2. Necrosis was documented for one animal and superficial necrosis for another animal.

Dermal irritation was also recorded in the acute dermal toxicity study in rats (Anonymous, 2010a). The dose was 2000 mg/kg bw, which is equivalent to about 400 mg/animal and animals were exposed semi-occlusive for 24 h. Erythema with scores up to 2 were seen in all treated rats. Small superficial scattered scabs were seen in three females on day 2 and haemorrhage of dermal capillaries were documented for 7 animals on day 3. Scabs and/or crust formation were seen in 9/10 animals. One female rat showed scar tissue at the end of the observation period. Effects in other animals were reversible.

The *in vitro* assays were both the human skin model EPISKIN (OECD TG 431), using respectively 10 and 20 mg test material (Anonymous, 2010b and c). Exposure periods were 15 min in one study and 3, 60, and 240 min in the other. Although both studies found a reduction in cell viability (at most 71.6% and 79.1%, respectively), both were above the threshold value of 50% for this test.

Based on the outcome of the studies in rats and rabbits, which both showed severe, irreversible effects after 4 h exposure, the DS proposed to classify DBTM as Skin Corr. 1.

Comments received during consultation

Two MSCAs indicated their support for classification as Skin Corr. 1. However, one added that sub-category 1C could also be considered as the exposure inducing the effects was 4 h. This was agreed on by the DS based on irreversibility.

Assessment and comparison with the classification criteria

There is one dermal irritation study performed with DBTM in six rabbits. Animals were exposed for 4 h to 500 mg and test sites were wiped at the end of this period. The individual scores are given in the CLH report, table 18. Notable is that, while all animals have comparable scores 30 minutes after exposure (1/2 for erythema, 0/1 for oedema), in three animals there is a pronounced increase in severity in the 14-d observation period (3/4 for erythema, 1/2 for oedema), while the scores stayed practically the same in the other three. At the end of the observation period, desquamation (4), superficial necrosis (1), or necrosis (1) were observed.

Dermal irritation was also observed in the acute dermal toxicity study in rats. This study used a slightly lower dose of 400 mg, but an exposure period of 24 h. Erythema scores ranged from 0 to 2 and no oedema was observed. Also scabbing, crust formation and in one animal scar tissue were observed. Most effects were reversible in 14 days.

Two *in vitro* Human Skin Model Tests were negative with all exposure periods tested.

According to the CLP Regulation "A substance is corrosive to skin when it produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis in at least one tested animal after exposure for up to 4 hours". Visible necrosis was observed in two rabbits after 4 h exposure. Effects were also irreversible in all animals in this study. No information is available on the effects after shorter durations of exposure, which would allow division into a subcategory. Hence, RAC considers that no sub-categorisation can be done.

In conclusion, RAC considers that **DBTM warrants classification as Skin Corr. 1; H314 (Causes severe skin burns and eye damage).**

10.5 Serious eye damage/eye irritation

Table 19: Summary table of animal studies on serious eye damage/eye irritation

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose duration exposure	levels of	Results - Observations and time point of onset - Mean scores/animal - Reversibility	Reference
OECD 405 GLP	Rabbit, New Zealand White	DBTM (>98% w/w) Impurities:	85 mg Exposure 72h (no washing)		Cornea mean scores (24,48,72h): #1 = 1; #2 = 1; #3 = 1.33 Scattered/diffuse/ translucent corneal	Anonymous, 2010d

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	N=3	Dibutyltin oxide (818-08-6) <1%, Maleic anhydride <1% No vehicle	Observation 14d	opacity Iris mean scores (24,48,72h): #1 = 1; #2 = 0.67 #3 = 1.33 Conjunctivae redness mean scores (24,48,72h): #1 = 2; #2 = 1,67; #3 = 2 Conjunctivae chemosis mean scores (24,48,72h): #1 = 2.33; #2 = 1.67; #3 = 2.33 Individual observations: #3: Blepharitis, blood stained discharge, petechial haemorrhage covering whole of the nictitating membrane #3 killed for humane reasons	
TSCA method (Acute exposure, eye irrit)	Rabbits, New Zealand white N=6/dose (unwashed) N=3/dose (washed)	DBTM (Thermolite 13), fine powder	42.2 mg Eyes of 3 rabbits were washed after 30 seconds Observation up to 21 days	<u>Unwashed eyes:</u> Mean scores for animals #1 to #6, respectively: Cornea mean scores (24,48,72h): 1.67, -, 2, -, 3.67, 3 Iris mean scores (24,48,72h): 3, -, -, -, 1.33, - Conjunctivae redness mean scores (24,48,72h): 3, 3, 3, 3, 3, 3 Conjunctivae chemosis mean scores (24,48,72h): 3, 3.67, 3.67, 4, 3.33, 3.67 Necrosis, ulceration, sacrificed after 72h due to severe response <u>Washed eyes:</u> Mean scores for animals #7 to #9, respectively: Cornea mean scores (24,48,72h): 3, 2.67, 2.67 Iris mean scores (24,48,72h): - Conjunctivae redness mean scores (24,48,72h): 2.33, 2.67, 3 Conjunctivae chemosis mean scores (24,48,72h): 2, 1, 2 Necrosis, ulceration; irreversibility of	Anonymous, 1988 (OTS 0555428)

				redness and cornea opacity at 21d.	
-	Russian bred rabbits N=6 (3m+3f)	DBTM	0.1 mg 3/6 animals rinsed after 30 seconds 3/6 unrinsed	Draize MAS scores: Rinsed eyes: cornea 1.7, iris 0 and conjunctiva 7.9. unrinsed eyes: cornea 74.7, iris 10, conjunctivae 19.2	OECD (2008)

Table 20: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
SkinEthic Reconstituted Human Corneal model (pre-validated method, not in line with OECD 492, but similar to SkinEthic™ HCE EIT*) GLP	DBTM >98% w/w (due to same batch number as for the <i>in vivo</i> study above the same impurities can be assumed)	transformed human keratinocytes of the cell line HCE 30mg DBTM applied Duration of treatment: 10min, washing cell viability testing after treatment with MTT	Not irritating Cell viability (%): DBTM: 93.6% Pos control: 40.7% Neg control: 100%	Anonymous, 2010e

*Based on the limited information available

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

In an OECD 405 study the eyes of 3 rabbits were exposed to a single application of DBTM (85mg, 98% purity). The test substance produced scattered or diffuse or translucent corneal opacity at 24 and 48h, iridial inflammation was noted in all treated eyes as well as moderate to severe conjunctival irritation. Other ocular effects noted were a pale area on the conjunctival membrane and/or petechial haemorrhage of the nictitating membrane. Other ocular effects noted in one treated eye were blepharitis and blood stained discharge. Individual animal data are shown in Table 22. Bodyweight loss was noted in animal No 3. Due to worsening reactions and signs of pain and discomfort, one animal was killed for humane reasons immediately after the 72 h observation. The pH of a 10% w/w aqueous preparation of the test item was 3.2 immediately after preparation and 2.4 after 10 min.

In another well documented study submitted for TSCA (Anonymous, 1988) DBTM has been tested for its corrosivity in rabbit eyes. A single dose of 42.2 mg (0.1cc) DBTM has been administered to the lower conjunctival sac of the right eyes of 9 New Zealand White rabbits. The left eye served as control. Eyes of three rabbit were washed after 30 seconds with lukewarm water. Ocular irritation was evaluated 1h, 24h, 48h, 72h, 7d, 10d, 14d and 21d after treatment according to the Draize scale. Fluorescein dye was used to identify corneal ulceration. Individual ocular scores and observations are presented in Table 23. DBTM produced severe ocular irritation (washed and unwashed). For the unwashed eyes all mean scores (24, 48, 72h) for conjunctivae redness and chemosis were ≥ 3 . Iris scores could not be assigned to all timepoints due to severity of response. Cornea opacity mean scores from 1.67 to 3.67 were found. Conjunctivae necrosis was documented at 24h, 48h, 72h and ulceration at 72h for all six rabbits. Also cornea ulceration is documented for all animals. Because of the severity of reactions all animals with unwashed eyes were sacrificed after 72h. Washed eyes of three rabbits showed similar effects maybe because the removal of the test material was not complete (residuals were documented in the test protokoll after 1h and 24h for two animals). Necrosis was still present at the end of the observation periode (21d). Individual data are presented in Table 24.

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In a briefly described study (taken from OECD, 2008) six rabbits were exposed to 0.1mg DBTM in the conjunctival sac of the left eye. For 3/6 animals the eyes were flushed with warm water after 30 seconds. Eye irritation score in rinsed eyes were cornea 1.7, iris 0 and conjunctiva 7.9. In unrinsed eyes the scores were cornea 74.7, Iris 10, Conjunctivae 19.2 (Draize MAS scores). Due to limited information this study was not used for classification.

An *in vitro* test (SkinEthic Reconstituted Human Corneal model, prevalidated method) was used to determine the eye irritation potential of DBTM after a treatment period of 10 minutes (Anonymous, 2010e). 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. Triplicate tissues (transformed human keratinocytes of the cell line HCE) were treated with 30 mg of DBTM for 10 min. As negative control served triplicate tissues treated with 30 µl of solution A and triplicate tissues treated with 30 µl of 1% w/v SDS serve as positive controls. Two tissues per group were taken for MTT loading. The remaining tissues were retained for possible histopathology. Tissue viability (in duplicate) was quantified by MTT uptake measuring the optical density at 540 nm. Relative mean tissue viability (%) = (mean OD₅₄₀ of test material / mean OD₅₄₀ of negative control) x 100. The test material itself was not able to directly reduce MTT. The relative mean viability of the test material treated tissues after a 10 minute exposure was 93.6%. The positive control showed a viability of 40.7% (see Table 22) . According to the study author the quality criteria required for acceptance of results in the test were satisfied. According to the test criteria a substance has to be considered as irritant if the tissue viability is <60%. Therefore DBTM was considered to be a non-irritant according to this *in vitro* test. 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.

Table 21: Reconstituted Human Corneal model – viability of tissue after treatment (Anonymous, 20103)

	Mean tissue viability	Mean OD ₅₄₀	Viability (%)
Neg. control	0.995	0.974	100
	0.953		
Pos Control	0.342	0.396	40.7
	0.449		
DBTM, 30 mg	0.882	0.912	93.6
	0.942		

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Table 22: Eye Irritation, individual animal data (Anonymous, 2010d).

	Animal #1								Animal #2						Animal #3 (a)				
	1h	24h	48h	72h	7d	14d	21d	Mean (24,48,7 2h)	1h	24h	48h	72h	7d	Mean (24,48,7 2h)	1h	24h	48h	72h	Mean (24,48, 72h)
Cornea																			
Degree of opacity	0	1	1	1	2	1	0	1	0	1	1	1	0	1	0	1	1	2	1.33
Area of cornea involved	0	4	3	3	2	1	0	-	0	3	2	1	0	-	0	4	4	4	-
Iris																			
Iris	0	1	1	1	1	0	0	1	0	1	1	0	0	0.67	0	1	1	1	1
Conjunctivae																			
Redness	2	2	2	2	2	1	0	2	2	2	2	1	0	1.67	2	2	2	2	2
Chemosis	2	3	2	2	2	1	0	2.33	2	2	2	1	0	1.67	2	2	2	3	2.33
Discharge	2	3	3	3	1	0	0	-	2	2	2	1	0	-	2	2	2	2	-
others	-	Pt	Pt	Pt	Pt	Pt	-	-	-	Pt	Pt	-	-	-	-	Pt	Pt	(b)	-

Pt ... Petechial haemorrhage covering whole of the nictitating membrane

(a) Animal killed for humane reasons

(b) Blepharitis, Blood stained discharge, Petechial haemorrhage covering whole of the nictitating membrane

Table 23: Eye irritation – individual scores for six rabbits with unwashed eyes (Anonymous, 1988).

	Time after administration	Conjunctiva redness	Conjunctiva Chemosis	Iris	Cornea opacity	comment
Animal 1 (m) unwashed	1	2	2	0	0	Conjunctivae necrosis at 24h, 48h, 72h Conjunctivae ulceration at 72h Cornea ulceration at 48h, 72h (scores 2, 1)
	24	3	3	1	0	
	48	3	3	1	1	
	72	3	3	1	4	
	Mean score (24, 48,72h)	3	3	3	1.67	Brown conjunctival discharge at 24h, 48h Residual test material at 1h, 24h Only portion of the cornea visible at 48h, 72h
Animal 2 (f) unwashed	1	3	3	1	0	Conjunctivae necrosis at 24h, 48h, 72h Conjunctivae ulceration at 72h Cornea ulceration at 24h, 48h, 72h (scores 2, 3, 4)
	24	3	4	#	#	
	48	3	4	1	3	
	72	3	3	#	3	
	Mean score (24, 48,72h)	3	3.67	-	-	Brown conjunctival discharge at 24h, 48h Residual test material at 1h, 24h, 48h Only portion of the cornea visible at 24h
Animal 3 (m) unwashed	1	3	3	0	0	Conjunctivae necrosis at 24h, 48h, 72h Conjunctivae ulceration at 72h Cornea ulceration at 24h, 48h, 72h (scores 2, 3, 3)
	24	3	4	1	0	
	48	3	4	#	3	
	72	3	3	1	3	
	Mean score (24, 48,72h)	3	3.67	-	2	Brown conjunctival discharge at 24h, 48h Red conjunctival discharge at 72h Residual test material at 1h, 24h Only portion of the cornea visible at 24h, 48h, 72h
Animal 4 (f) unwashed	1	2	3	1	0	Conjunctivae necrosis at 24h, 48h, 72h Conjunctivae ulceration at 72h Cornea ulceration at 24h, 48h, 72h (scores 2, 1, #)
	24	3	4	1	3	
	48	3	4	#	#	
	72	3	4	#	#	
	Mean score (24, 48,72h)	3	4	-	-	Brown conjunctival discharge at 24h, 48h Residual test material at 1h, 24h Only portion of the cornea visible at 24h, 48h

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Animal 5 (f) unwashed	1	2	3	1	0	Conjunctivae necrosis at 24h, 48h, 72h Conjunctivae ulceration at 72h Cornea ulceration at 24h, 48h, 72h (scores 3, 4, 4) Brown conjunctival discharge at 24h, 48h Residual test material at 1h, 24h Only portion of the cornea visible at 24h
	24	3	4	1	3	
	48	3	3	2	4	
	72	3	3	1	4	
	Mean score (24, 48,72h)	3	3.33	1.33	3.67	
Animal 6 (m) unwashed	1	2	4	+	0	Conjunctivae necrosis at 24h, 48h, 72h Conjunctivae ulceration at 72h Cornea ulceration at 24h, 48h, 72h (scores 3, 4, 2) Brown conjunctival discharge at 24h Residual test material at 1h, 24h Only portion of the cornea visible at 24h, 72h
	24	3	4	1	2	
	48	3	3	#	4	
	72	3	4	#	3	
	Mean score (24, 48,72h)	3	3.67	-	3	

not evaluated due to severity of response

Table 24: Eye irritation – individual scores for six rabbits with washed eyes (Anonymous, 1988).

	Time after administration	Conjunctiva redness	Conjunctiva Chemosis	Iris	Cornea opacity	comment
Animal 7 (f) washed	1	2	1	0	0	Conjunctivae necrosis at 24h, 48h, 72h, 10d, 14d, 21d Conjunctivae ulceration at 72h Cornea ulceration at 24h, 48h, 72h, 7d, 14d (scores 1, 1, 1, 1, 1) Residual test material at 1h, 24h, 48h Pannus at 7d, 10d, 14d, 21d
	24	2	2	0	1	
	48	2	2	+	4	
	72	3	2	1	4	
	7d	3	0	+	0	
	10d	2	1	0	4	
	14d	1	1	0	4	
	21d	1	0	0	3	
Mean score (24, 48,72h)	2.33	2	-	3		
Animal 8 (f) washed	1	1	1	+	0	Conjunctivae necrosis at 24h, 48h, 72h, 7d, 10d, 14d, 21d Cornea ulceration at 48h, 72h (scores 1, 1)
	24	3	1	0	0	
	48	3	1	+	4	
	72	2	1	+	4	
	7d	1	1	0	4	
	10d	1	1	0	3	

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	14d	1	1	0	3	
	21d	0	0	0	0	
	Mean score (24, 48,72h)	2.67	1	-	2.67	
Animal 9 (m) washed	1	2	2	1	0	Conjunctivae necrosis at 24h, 48h, 72h, 7d, 10d, 14d, 21d Conjunctivae ulceration at 72h Cornea ulceration at 24h, 48h, 72h (scores 1, 1, 1) Residual test material at 1h, 24h Pannus at 7d, 10d, 14d
	24	3	2	0	0	
	48	3	2	+	4	
	72	3	2	1	4	
	7d	1	1	0	4	
	10d	1	1	0	4	
	14d	1	1	0	4	
	21d	1	0	0	2	
	Mean score (24, 48,72h)	3	2	-	2.67	

not evaluated due to severity of response

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 (case for 6 rabbits according ECHA, 2017):

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 4 of 6 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.
Category 2:	Substances that produce in at least in 4 of 6 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

In the available *in vitro* test with a short exposure periode of 10 min no effects on cell viability have been seen.

In an *in vivo* study (Anonymous, 2010d) eyes of three rabbits were exposed to DBTM showing scattered or diffuse or translucent corneal opacity at 24 and 48h (mean scores: 1, 1, 1.33), iridial inflammation in all treated eyes (mean scores: 1, 0.67, 1) as well as moderate to severe conjunctival irritation (mean score redness: 2, 1.67, 2; chemosis: 2.33, 1.67, 2.33). In addition pale area on the conjunctival membrane and/or petechial haemorrhage of the nictitating membrane were seen. One treated eye showed blepharitis and blood stained discharge; this animal was killed for human reasons (Anonymous, 2010d).

In a second *in vivo* study (Anonymous, 1988) severe effects on rabbit eyes (n=6) were described with mean scores (24, 48, 72h) for conjunctivae redness and chemosis ≥ 3 . Opacity was ≥ 3 in 2 animals, however two animals could not be scored due to severity of response. Iris score was 3 for one animals and four animals could not be fully evaluated due to severity of response. Necrosis and ulceration are described for all animals. All animals were killed for human reasons after 72h observation. Rabbits with washed eyes showed similar severe reactions and observation till day 21 showed that redness and opacity did not recover for 2/3 animals. Necrosis was seen at all timepoints.

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

The study by Anonymous (1988) shows severe effects of DBTM on rabbit eyes. Mean scores (24, 48, 72h) for opacity were ≥ 3 in two animals, however two animals could not be scored due to severity of response. Iris score was 3 for one animals and four animals could not be fully evaluated due to severity of response. Necrosis and ulceration are described for all animals. The longer observation periode for three animals (with washed eyes after 30 sec treatment but still residual test substance) showed that redness and opacity were not reversible after a observation periode of 21 days. Necrosis were documented for all timepoints ≥ 24 h. Based on this study a classification as Eye Dam. 1, H318 is proposed.

DBTM 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

Two *in vivo* eye irritation studies were performed with DBTM in rabbits.

In the first study in three rabbits DBTM showed scattered or diffuse or translucent corneal opacity at 24 and 48 h (mean scores: 1, 1, 1.33), iridial inflammation in all treated eyes (mean scores: 1, 0.67, 1) as well as moderate to severe conjunctival irritation (mean score redness: 2, 1.67, 2; chemosis: 2.33, 1.67, 2.33). In addition, pale area on the conjunctival membrane and/or petechial haemorrhage of the nictitating membrane were seen. One treated eye showed blepharitis and blood-stained discharge; this animal was killed for humane reasons (Anonymous, 2010d).

The second study with six animals found mean scores of ≥ 3 for conjunctivae redness and chemosis. Opacity score was ≥ 3 in 2 animals, however, two animals could not be scored due to severity of response. Iritis score was 3 for one animal and four animals could not be fully evaluated due to severity of response. Necrosis and ulceration were described for all animals. All animals were killed for humane reasons after 72 h observation period. Rabbits with washed eyes showed similar severe reactions, and observation until day 21 showed that redness and opacity did not recover for 2/3 animals. Necrosis was seen at all timepoints (Anonymous, 1988).

An available *in vitro* test (SkinEthic Reconstituted Human Corneal model, pre-validated method) found no irritative properties, but used only 10 min exposure (Anonymous, 2010e).

Based on the severe, irreversible effects observed in the study by Anonymous (1988), including redness, opacity, ulceration, and necrosis in eyes of all animals, the DS proposed classification as Eye Dam. 1.

Comments received during consultation

Two comments were received by MSCAs, both agreed with the proposal.

Assessment and comparison with the classification criteria

There are two *in vivo* eye irritation studies available in rabbits. Both showed consistent eye damage induced by DBTM, which was particularly severe in the Anonymous (1988) study, but also in the Anonymous (2010d) study one animal had to be killed for humane reasons (one treated eye showed blepharitis and blood-stained discharge).

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 hours after installation of the test material.

In Anonymous (1988) two out of three animals with washed eyes still showed conjunctival redness and cornea opacity after 21 days. Moreover, all six animals with unwashed eyes had such severe reactions that they had to be killed after 72 h.

As the criteria are met, RAC concludes that **DBTM warrants classification as Eye Dam. 1; H318 (Causes serious eye damage)**. As also classification for skin corrosion is proposed, classification for eye damage is the default classification.

10.6 Respiratory sensitisation

No data available.

10.7 Skin sensitisation

Not evaluated.

10.8 Germ cell mutagenicity

Information on germ cell mutagenicity of DBTM and category member DBTC has been retrieved from REACH registration dossier of DBTM 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 25, 26) *in vitro* data of DBTM 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 25: Summary table of mutagenicity/genotoxicity tests *in vitro* with DBTM and DBTC (DBTO registration and 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 OECD Guideline 471 GLP	DBTM Purity: not indicated on dissemination site	0.0, 0.3, 0.8, 2.3, 7.0, 21, 62 µg/plate Cytotoxic concentration. 7-62 µg/plate DBTM was toxic to all strains at highest concentration (+/- S9) and also at 21 µg/plate (except for TA 98 and TA 1537 without S9). Test substance was toxic at 7 µg/plate to TA 1535 (without S9 and to TA 1537 with S9).	Negative in absence and presence of S9-mix and with all strains tested - TA 1535, TA 1537, TA 98 and TA 100.	Krul (2002) (ECHA dissemination site, REACH registration dossier DBTM and OECD SIDS (2006))

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Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
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 0.00050 µl/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)
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	Positive. 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 < 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).	Reimann R & Gramlich U (1990)
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-	Li AP et al., (1982)

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Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
			forming cells (AFC) was reduced to approximately 10 % of the control.	
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)

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 26: 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-GLP	DBTC	Purchased λ-DNA (0.5 µg, double-stranded) was incubated with DBTC at 37°C for 2 h.	Negative. DBTC did not induce dsDNA breaks in the presence or absence of H2O2.	Hamasaki et al., 1995
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

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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 27: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo* with DBTC and DBTL (DBTO registration and 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 (Mammalian Erythrocyte Micronucleus Test)	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 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 effect was apparent for any group killed 24 hours after DBTC treatment.	Anonymous (1991) [Annex I, 2.1.1]
Micronucleus assay (chromosome aberration) mouse (NMRI) male/female oral: gavage, Non OECD	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)	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	Anonymous (1990) [Annex I, 2.1.2]

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
guideline, GLP not stated		tests)	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).	
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 was observed.	Jin et al., 2012 [Annex I, 2.1.3]

Table 28: 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 DBTM 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, DBTM 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 thus 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 ($p < 0.05$) 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. 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).

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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 (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 cells of humans
Subcategory 1A	The classification in Cat. 1A is based on positive evidence from 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 <i>in vivo</i> heritable germ cell mutagenicity tests in mammals; or — positive result(s) from <i>in vivo</i> 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 <i>in vivo</i> , 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

	<p>from <i>in vitro</i> experiments, obtained from:</p> <ul style="list-style-type: none"> — somatic cell mutagenicity tests <i>in vivo</i>, in mammals; or — other <i>in vivo</i> somatic cell genotoxicity tests which are supported by positive results from <i>in vitro</i> mutagenicity assays.
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No epidemiological studies are available for DBTM 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 DBTM read across to category members (DBTC and DBTL) is applied, for which (despite information from *in vitro* cell mammalian studies) *in vivo* studies with laboratory rodents are available.

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) and there is evidence from *in vitro* studies that DBTC interacts with the gene material.

DBTC and category member DBTL are harmonised classified for Muta. 2; H341. Currently there is a CLH proposal under evaluation to classify 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 (Krul, 2002) has been performed with DBTM 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. 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 (\pm S9-mix) was reported with positive results (Reimann & Gramlich, 1990). 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 (\pm 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 (\pm 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 it did not affect chromosomal length in human peripheral lymphocytes (Jensen *et al.*, 1989), or 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 exposure. 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 females compared to males. 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 24h, 48h and 72h 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 DBTM also as Muta. 2 based on the category approach.

Comments received during consultation

Comments were received from three MSCAs, indicating support for the proposed classification based on the category approach.

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 DBTM 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. As DBTM forms at least in part the same metabolite as DBTC, RAC considers the proposed read-across valid for germ cell mutagenicity (see also 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, 1990).

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 DBTM 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.

RAC concludes that **DBTM warrants classification for germ cell mutagenicity as Muta. 2; H341 (Suspected of causing genetic defects).**

10.9 Carcinogenicity

Not evaluated in the present 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 DBTM and/or in frame of harmonised classification proposal of category members (e.g. ECHA, 2016, ECHA 2019A and B). All of 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 29: Summary table of animal studies on adverse effects on sexual function and fertility (adopted from ECHA, 2017)

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

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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 fetuses (10 compared to 101 in controls). Gestation index: 43% vs 100% (high dose vs control) Post-implantation loss: 87.6% vs 13.4% (high dose vs control). Gross necropsy and histopathology in males did not reveal any effects of treatment on the reproductive tract. NOAEL (for general toxicity) = 5 ppm (0.3-0.4 mg/kg bw/day) (thymus effects) NOAEL (reproduction) = 30 ppm (1.7-2.4 mg/kg bw/d) LOAEL (reproduction) = 200 ppm, (12.0-15.4 mg/kg bw/d)	Unpublished report, 2003 [Annex I, 2.2.1.1]
Wistar rat (16-19 female/group) non-guideline study	DBTC Purity: 97% 0, 3.8, 7.6, 15.2 mg/kg bw/d, GD 0-3 (and GD 4-7) Termination: GD 20	Maternal toxicity at ≥ 3.8 mg/kg bw/d (clinical signs), weight loss during early gestation at 3.8 (-2 g), 7.6 (-14 g) and 15.2 mg/kg bw/d (-20 g); reduced food consumption (≥ 3.8 mg/kg bw/d) Increased pre-implantation loss at 7.6 (35.6%) and 15.2 mg/kg bw/d (87.9%), compared to 2.7% in controls. LOAEL =3.8 mg/kg bw/d NOAEL <3.8 mg/kg bw/d	Ema & Harazono, 2000 [Annex I, 2.2.1.2]
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 30: 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 31: 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	DBTC	Investigation of the effects of progesterone on	Administration of progesterone on GD 0-8	Ema et al., 2003

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Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Wistar rats (14-15 female/group) 0, 7.6, 15.2 mg/kg bw/day (with and without progesterone) subcutaneous injection of 2 mg progesterone GD 0-8	Purity: 98%	implantation failure.	offered some protection against implantation failure in Wistar rats treated with 7.6 or 15.2 mg/kg bw/d DBTC on GD 0-3. Pre-implantation losses were 8.6%, 62.8%, 81.3% at dose levels of 0, 7.6, 15.2 mg/kg bw without progesterone ; 10.5%, 25.9% and 60.6% with application of progesterone	[Annex I, 2.2.1.4]
Mechanistic 0, 3.8, 7.6, 15.2 mg/kg bw Pseudopregnant 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 the category member 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

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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) group). 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

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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	<p>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.</p> <p>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.</p>

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 nonspecific consequence of other toxicity.

Classification of DBTM 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

With DBTM itself a non-guideline conform comparative study is available, which investigates the effects of various category members (DBTC, DBTL, DBTA, DBTO) on reproductive parameters (Noda et al., 1993). All other available studies have been carried out with the category members DBTC, DBTA or DBTO.

The studies listed below, except the recent prenatal developmental toxicity study (OECD 414) with DBTO, have been considered in the registration of DBTM and/or in the frame of harmonised classification proposals of category members (e.g. ECHA, 2017, ECHA 2019A and B). All of the studies described below have already been 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.

Table 32: 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
Comparative study with different di-n-btyltin compounds			
Wistar rat (10 females /group) Single dose, gavage, 80 µmol/kg bw, GD 8 Non-guideline conform study	DBTM: purity not reported 80 µmol/kg bw (28 mg/kg bw), GD 8 DBTC: purity not reported 80 µmol/kg bw; (25 mg/kg bw), GD 8 DBTO: purity not reported 80 µmol/kg bw (20 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	The nature of malformations was similar in all treatment groups. The di-n-butylin 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. DBTM LOAEL = 28 mg/kg bw DBTC LOAEL = 25 mg/kg bw DBTO LOAEL = 20 mg/kg bw DBTL LOAEL = 50 mg/kg bw DBTA LOAEL = 28 mg/kg bw A NOAEL cannot be determined for this study.	Noda et al., 1993 [Annex I, 2.2.1.6]
DBTO			
OECD 414 (Prenatal development toxicity)	DBTO	At 6 mg/kg bw dams with clinical signs, lower body weights, lower food consumption. Lower	Unpublished report, 2017

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Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
study) Sprague Dawley (25 females/group)	Purity: > 97% 0, 0.75, 3 and 6 mg/kg bw/d GD 0-19, gavage	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	[Annex I, 2.2.1.18]
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); 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]
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). No effects were reported on post-implantation loss. 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]
OECD 414 (Prenatal developmental toxicity	DBTC	Marginal increase in malformations (including single incidences of ankyloglossia, agnathia,	Farr et al.,

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Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
study) Wistar rat (25 females/group) Oral gavage	purity not reported 0, 1, 2.5, 5, 10 mg/kg bw/d GD 6-15	mandibular defect at 10 mg/kg bw/d. Maternal toxicity (reduced weight gain (-17%) & reduced food consumption (-7%)) at 10 mg/kg bw/d. LOAEL = 10 mg/kg bw/d (reproductive toxicity) NOAEL = 5 mg/kg bw/d (reproductive toxicity)	2001 [Annex I, 2.2.1.8]
Wistar rat (10-12 females/group) Non-guideline conform study Oral gavage	DBTC purity not reported 0, 2.5, 5.0, 7.5, 10 mg/kg bw/d GD 7-15	Increased resorptions at 7.5 (10.0%) and 10 mg/kg bw/d (5.3%) compared to controls (1.3%); increased post-implantation loss at 7.5 (77.0%) and 10 mg/kg bw/d (37.9%) compared to controls (10.2%). Reduced number of live foetuses at 7.5 mg/kg bw/d (3.6, compared to 11.8 in controls). Reduced foetal weight at 5 (~15%), 7.5 (~38%) and 10 mg/kg bw/d (~30%). Foetal malformations at ≥5 mg/kg bw/d, typically cleft jaw and related mandibular defects. Maternal toxicity: mortality at 7.5 (5/12) and 10 mg/kg bw/d (9/12), clinical signs, weight loss or reduced weight gain during the dosing period at 7.5 and 10 mg/kg bw/d (-9 g, 6 g 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]
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 omphalocele 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	Ema et al., 1992 [Annex I, 2.2.1.10]

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Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		<p>40 mg/kg bw/d.</p> <p>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 mal-formations at 20 and 40 mg/kg bw/d.</p> <p>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.</p> <p>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.</p> <p>Details on maternal toxicity not reported.</p> <p>The study demonstrates that the most sensitive period is GD 8.</p> <p>LOAEL =20 mg/kg bw/d (reproductive toxicity) NOAEL <20 mg/kg bw/d (reproductive toxicity)</p>	
<p>Wistar rat (10 females/group) Non-guideline conform study Oral gavage</p>	<p>DBTC purity not reported 0, 10, 15 mg/kg bw/d GD 7-8</p>	<p>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.</p> <p>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).</p> <p>LOAEL =10 mg/kg bw/d (reproductive toxicity) NOAEL <10 mg/kg bw/d (reproductive toxicit<)</p>	<p>Ema et al., 1995b [Annex I, 2.2.1.11]</p>
<p>Wistar rat (11-13 females/group) Non-guideline conform study Oral gavage</p>	<p>DBTC purity not reported 0, 50, 100 mg/kg bw/d GD 13-15</p>	<p>Reduced foetal weight at 50 (-29%) and 100 mg/kg bw/d (-34%).</p> <p>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.</p> <p>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%).</p> <p>LOAEL =50 mg/kg bw/d (reproductive toxicity) NOAEL <50 mg/kg bw/d (reproductive</p>	<p>Ema et al., 1996b [Annex I, 2.2.1.12]</p>

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Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		toxicity)	
Wistar rat (16-19 females/group) Non-guideline conform study Oral gavage	DBTC 97% purity 0, 3.8, 7.6, 15.2 mg/kg bw/d GD 4-7	Total resorption was seen at 7.6 mg/kg bw (3/16) and 15.2 mg/kg bw (14/16); post-implantation loss was increased at 3.8 (13.9%), 7.6 (39.9%) and 15.2 mg/kg bw (91.5%) compared to controls (7.0%). Foetal weight was decreased at 7.6 (~13%) and 15.2 mg/kg bw (~24%). No malformations were observed. Maternal toxicity: Exposure on GD 4-7 resulted in signs of maternal toxicity and weight loss during the dosing period at 7.6 mg/kg bw (-2 g) and 15.2 mg/kg bw (-14 g) DBTC casues pre- and post-implantation embryonic loss when adminisiter to maternal rats during early pregnancy. LOAEL =3.8 mg/kg bw/d (reproductive toxicity) NOAEL <3.8 mg/kg bw/d (reproductive toxicity)	Ema & Harazono, 2000 [Annex I, 2.2.1.2]
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 between 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	DBTA;	Implantation loss increased at 22 mg/kg bw in 3-month old (19.2%), 7.5 month-old (37.8%)	Noda et al., 2001

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Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
(12-14 females/group) No guideline study Oral gavage	purity not reported 0, 7.5, 10, 15, 22 mg/kg bw/d GD 8	and 12 month old dams (95.2%). Foetal malformations (typically cleft mandible, cleft lower lip, ankyloglossia, schistoglossia, fused ribs, fused cervical and vertebral arches) at ≥ 7.5 mg/kg bw/d Reduced numbers of litters with viable foetuses (6/13) due to total resorption (5/13) at 22 mg/kg bw (7.5 month-old dams). <u>Maternal toxicity</u> : 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)	[Annex I, 2.2.1.15]

Table 33: 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 34: 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

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Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Cynomolgus monkey (10-12 females/group) Non-guideline study nasogastric intubation	DBTC: 98% purity 2.5, 3.8 mg/kg bw/d GD 20-50 (period of organogenesis) Pregnancy out come was determined on GD 100	Reduced foetal survival at 2.5 mg/kg bw/d (8/12 females with embryofoetal loss) and at 3.8 mg/kg bw/d (4/10 females with embryofoetal loss) compared to 18/12 controls. <u>Maternal toxicity:</u> clinical signs, reduced weight gain (-242±423g and -556 ± 526 g) on GD 20-51 compared to control (+ 57±237g) accompanied with reduced food consumption at 2.5 and 3.8 mg/kg bw/d; DBTC causes embryolethal effects, but no malformations were observed; LOAEL =2.5 mg/kg bw/d (reproductive toxicity) NOAEL <2.5 mg/kg bw/d (reproductive toxicity)	Ema et al., 2007b [Annex I, 2.2.1.16]
Cynomolgus monkey (5/group; 12 controls) Non-guideline study nasogastric intubation	DBTC: 98% purity 0, 7.5 mg/kg bw/d: GD 19-21, 21-23, 24-26, 26-28, 29-31, 31-33, 34-36	Embryofoetal loss (GD 19-21 (1/5), 24-26 (2/5), 34-36 (1/5) compared to 1/12 controls. Findings associated with maternal toxicity <u>Maternal toxicity:</u> 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 35: 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 36: 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
<i>In vitro</i> 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.	Ema et al., 1995a [Annex I, 2.2.3.1]

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Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			Reduced yolk sac diameter, crown-rump length and number of somite pairs at 30 ng/ml; decrease in the overall morphological score; increase in the incidence of embryos with anomalies (all concentration, statistically significant for embryos exposed to 10 and 30 ng/mL)	
<i>In vitro</i> <i>Cultured rat embryo study</i>	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.	<p>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).</p> <p>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.</p> <p>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.</p>	Ema et al., 1996a [Annex I, 2.2.3.2]
<i>In vitro</i> <i>Cultured rat embryo study</i>	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 μ M and cell proliferation (IP50 0.12-2.81 μ M).	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 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 μ mol/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,

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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 37: 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	-	-	-	-	-	-
<i>Pes varus</i>	-	-	1 (1)	-	-	-
<i>Pes valgus</i>	-	-	-	-	-	-
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 38: 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)

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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 39: 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 13 th rib	-	-	5 (2)*	-	3 (1)	-

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

Category member DBTO (OECD TG 414)

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.

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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 40: 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	92.0	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

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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.

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 41: 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,

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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 42: 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, omphalocele, 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 43: 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 44: 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)
Omphalocele (#)	-	-	2 (2)	5 (1)	6 (2)*
Exencephaly (#)	-	-	1 (1)	3 (1)	1 (1)
Ecephalocele (#)	-	-	-	5 (1)	2 (1)
Open eye (#)	-	-	-	1 (1)	-
Anal atresia (#)	-	-	4 (2)	1 (1)	1 (1)
Anasarca (#)	-	-	-	1 (1)	-
<i>Ectopia cordis</i> (#)	-	-	-	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 45: 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 omphalocele 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 sternbrae 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 46: 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 embryofetal 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.

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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).

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, omphalocele, 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 47: 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 48: 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)

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Omphalocele (#)	-	-	2 (2)	5 (1)	6 (2)*
Exencephaly (#)	-	-	1 (1)	3 (1)	1 (1)
Ecephalocele (#)	-	-	-	5 (1)	2 (1)
Open eye (#)	-	-	-	1 (1)	-
Anal atresia (#)	-	-	4 (2)	1 (1)	1 (1)
Anasarca (#)	-	-	-	1 (1)	-
<i>Ectopia cordis</i> (#)	-	-	-	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 49: 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)

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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 omphalocele 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 sternbrae 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 50: 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)**
Encephalocele	-	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)	-
Omphalocele	-	2 (1)	3 (3)*
Kinked tail	-	1 (1)	-
Club foot	-	10 (5)**	3 (3*)
Hind limb deformity	-	1 (1)	1(1)
Anasarca	-	-	3 (2)

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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 embryofetal 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 51.

Table 51: 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)

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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 52: 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	3.2/1.9	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 ($p < 0.01$)

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON DIBUTYLTIN MALEATE

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 53: 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 embryolethal 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.

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 were 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.

In the recently conducted PNDT study with DBTO (according to OECD 414) pregnancy outcome parameters were adversely affected 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 appearance 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 PNDD 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 DBTC 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.

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 category members.

Based on the clear and consistent evidence of effects on the developing foetus (post-implantation loss, skeletal and external malformations) in rat studies with only mild or no overt maternal toxicity and in the absence of data indicating that effects are not relevant to humans, classification of DBTM 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 DBTM for reproductive toxicity in Category 1B (H360 FD: 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 DBTM; thus, the evaluation was based on studies with DBTC. This evaluation has been made previously for several category members, including DBTA, DBTP and DBTDL.

The OECD TG 421 study (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 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 gastric intubation to DBTC in olive oil (0-3.8-7.6-15.2 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 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 (Ema *et al.*, 2003). The other two supportive studies (Harazono & Ema, 2003; Harazono & Ema, 2001) also focused on the effects of DBTC in decidual cell response and progesterone levels during pseudopregnancy.

Based on the increased number of non-pregnant females among successfully mated females, the reduced number of implantations, 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 DBTM should have the same classification as DBTC. The DS therefore proposed Repr. 1B for adverse effects sexual function and fertility for DBTM.

Adverse effects on development

There is one comparative study performed with DBTM in addition to DBTDL, DBTA, DBTO, and DBTC (Noda *et al.*, 1993). All studies except one performed with DBTO have been evaluated previously for category members. The CLH dossier divides the studies into four groups: the Noda *et al.* (1993) , the study with DBTO, studies with DBTC, and studies with DBTA.

The comparative study with DBTM, DBTC, DBTA, DBTO, and DBTL (Noda *et al.*, 1993) using a single gavage administration of 80 µmol/kg bw on GD 8 (28 mg/kg bw DBTM), showed a comparable spectrum of developmental effects for all substances, in the absence of maternal toxicity. For DBTM external malformations were observed in 12.5% of the pups (n=16), consisting of cleft mandible, cleft lower lip, ankyloglossia, schistoglossia or cleft upper lip. Skeletal malformations had an incidence of 9.3% (n=12), consisting of mandibular fixation, and cranial hypoplasia. Treatment showed a comparable incidence and type of foetal malformations for all organotin substances.

Study 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 6 mg/kg bw/d. 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 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.

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 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 fetuses 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 fetuses 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 fetuses, 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) with category members and evidence that DBTM induces similar effects and in the absence of data indicating that effects are not relevant to humans, the DS proposed classification of DBTM as Repr. 1B; H360D.

Comments received during consultation

Comments were received from three MSCAs, indicating support for the proposed classification based on the category approach.

Assessment and comparison with the classification criteria

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 DBTM and DBTC are hydrolysed to the same distannoxane, RAC is of the opinion that data on DBTC can be used for classification of DBTM for adverse effects on sexual function and fertility (see also under 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 Guidance on the application of the CLP

criteria; CLP guidance, 2017) the ED₁₀-values for DBTO fall within the ranges of a 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%

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

Development

There is only one developmental toxicity study with DBTM itself, which was the study by Noda *et al.*, 1993, in which single doses of several dibutyltins, including DBTM, were given on the critical day for organotin toxicity, namely GD 8. DBTM showed an induction in foetal malformations similar to those induced by other category members. There was no maternal mortality or signs of general toxicity.

In addition, there are numerous studies with DBTC, DBTO, 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 is 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 DBTM.

Altogether, RAC supports the conclusion of the DS that **classification of DBTM for developmental toxicity as Repr. 1B; H360D (May damage the unborn child) is warranted.**

Specific concentration limit

Setting of an SCL is not considered necessary for effects on development, given that the ED₁₀-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).

10.11 Specific target organ toxicity-single exposure

Table 54: 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

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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 55: 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 DBTM 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 µm³), intermediate (volume between 130 and 225 µm³) and large cells (volume > 225 µm³) 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 µm³) was decreased at days 1 and 2, the numbers of small (volume <130 µm³) 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 56: Influence of a single oral dose of DBTC on small, intermediate, large and total cell count (x10⁷) per thymus at various days after administration (Snoeij et al., 1989)

mg/kg bw	Small		Intermediate		Large		Total cells	
	0	15	0	15	0	15	0	15

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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 DBTM 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 DBTM 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 (Snoeijs 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 DBTM 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 and SCID-hu mice (SCID mice engrafted with human foetal thymus and liver tissue fragments)

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 (Snoeijs *et al.*, 1989).

Lower doses of 0.03 and 1.0 mg/kg bw were given intraperitoneally to SCID-humice engrafted with human thymus and liver tissue fragments. DBTC induced a reduction in thymus cortex size (de Heer *et al.*, 1995).

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

One MSCA commented indicating their disagreement with the proposal for no classification, stating that reversibility and a classification proposal for STOT RE 1 are not valid arguments to not classify for STOT SE. Instead, they preferred classification as STOT SE 1 for immune system effects.

The DS responded that the main reason to not propose a classification was related to the limitations of the two studies available. The rat study used only one, relatively high, dose and

only three animals per group. The mouse study had an unusual design by using engrafted mice with human thymus and liver tissue, as well as intraperitoneal exposure. It was also noted that both studies have been included in previous discussions for category members, which were not classified for STOT SE.

Assessment and comparison with the classification criteria

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. In the rat study, significant reductions of cell numbers in the thymus were observed after exposure to 15 mg/kg bw, with the lowest number at day 4 (-70%). At day 9 cell numbers had reversed back to control levels.

In the mouse study a reduction in cortex size of the human thymus graft was observed. This i.p. study is considered of limited relevance for STOT SE classification due to, amongst other reasons, the route of administration.

Although effects were seen in a single dose study in rats, RAC notes that the effects were reversible, the study does not allow to assess whether there is: "clear evidence of marked disfunction" and the endpoint, immune system, is already taken into consideration in the STOT RE classification, for which there is a much stronger burden of proof.

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

10.12 Specific target organ toxicity-repeated exposure

No studies have been performed with DBTM thus reference is made to studies with DBTC (read across substance) as part of the category (see details Chapter 9.2). Further information is retrieved from a PNDT study carried out with category member DBTO. The studies listed in the table below (except unpublished study, 2017) have been either included in the registration of DBTM and/or have been considered in the frame of harmonised classification proposals of category members (e.g. ECHA, 2016, ECHA 2019A and B). All of 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 CLH report.

Table 57: 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

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<p>OECD 414 (Prenatal development toxicity study) Sprague Dawley (25 females/group)</p>	<p>DBTO Purity: > 97% 0, 0.75, 3 and 6 mg/kg bw/d GD 0-19, gavage</p>	<p>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</p>	<p>Unpublished report, 2017 [Annex I, 2.2.1.18]</p>
<p>comparable to OECD 408 (Repeated dose 90 day oral toxicity study in rodents) Rat CFE (m, f) 16/sex</p>	<p>DBTC Purity: 99.7% Oral (dietary) 10, 20, 40, 80 ppm (approximately, 0.5, 1, 2 and 4 mg/kg bw) (for 90 days)</p>	<p>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)</p>	<p>Gaunt et al., 1968 (REACH registration, DBTO) [Annex I, 2.3.1.2]</p>
<p>OECD 421 Reproduction/ Developmental toxicity screening test Wistar rat 25 females/group</p>	<p>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</p>	<p>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) (thymus effect) NOAEL =5 ppm (0.3-0.4 mg/kg bw/d) (thymus effect)</p>	<p>Unpublished report, 2003 (REACH registration, DBTO) [Annex I, 2.3.1.3]</p>
<p>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</p>	<p>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)</p>	<p>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</p>	<p>Seinen & Vos, 1977 Penninks & Seinen, 1982 (REACH registration, DBTO) [Annex I, 2.3.1.4, 2.3.1.5]</p>

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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	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	No effects of treatment	DeWitt et al., 2006 [Annex I, 2.3.1.10]

<p>SD rat (m, f; pups)</p> <p>No guideline study</p>	<p>DBTC/kg bw while nursing)</p> <p>DTH and NK response assessed in offspring at PND 42.</p> <p>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.</p>	<p>Reduced weight gain (2.5 mg/kg bw/d)</p> <p>No clear effects on immune parameters</p> <p>NOAEL = 2.5 mg/kg bw/d</p>	
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Table 58: 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 59: 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	<p>WU rat</p> <p>WAG rat</p> <p>Swiss mouse</p> <p>Dietary concentrations of 0 or 150 ppm; ; rats were sensitised after three weeks, hypersensitive 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.</p>	<p>Allograft rejection was significantly delayed; other measures of immune function were unaffected by treatment.</p>	<p>Seinen et al., 1977 [Annex I, 2.3.1.11]</p>

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

No studies are available for DBTM; studies are available with category members DBTO and DBTC which are considered as part of the category approach. The studies have been submitted in the REACH registration of DBTM and/or have been described previously (e.g. ECHA, 2016) and have been already considered by RAC in 2017 (except unpublished report, 2017).

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The most critical effect is the thymus toxicity 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 60: 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 61: 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*

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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 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 62: Maternal weight gain, food consumption and maternal thymus 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 63.

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

Table 63: 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
Seinen & Vos (1977) Penninks & Seinen (1982)	2.5 mg/kg bw/d (LOAEL)	28 days	0.8 mg/kg bw/d	STOT RE 1
Unpublished report, (2003)	1.7-2.4 mg/kg bw/d (LOAEL)	~56 days	1.25 mg/kg bw/d	STOT RE 1

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	<p>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:</p> <ul style="list-style-type: none"> — 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	<p>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.</p> <p>In exceptional cases human evidence can also be used to place a substance in Category</p>

2.

The repeated dose and other relevant rodent studies clearly demonstrate that category members and therefore also DBTM 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).

Therefore, DBTM 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 studies with category members a classification for STOT RE in Category 1 (H372: causes damage to the immune system) is considered to be appropriate for DBTM.

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

Summary of the Dossier Submitter's proposal

There are no repeated dose studies available with DBTM itself. The classification proposal is based on read-across with DBTC and a PNDD study with DBTO and supported by the toxicokinetic and hydrolytic behaviour of the substances in the category.

In the developmental toxicity study with DBTO 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. 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 a reduced absolute and relative thymus weight and a severe to very severe lymphoid depletion in dams.

A 28-d rat/mouse immunotoxicity study with doses of 0, 50 and 150 ppm of 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 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 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 reproductive/developmental toxicity screening study (Unpublished report, 2003) to be key studies. All three studies showed thymus toxicity at low dose levels. As DBTM 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 (see RAC note on the 56-d study further down) studies 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 consultation

Comments were received from three MSCAs, indicating support for the proposed

classification based on the category approach.

Assessment and comparison with the classification criteria

Given that both DBTM and DBTC are hydrolysed to the same distannoxane, RAC is of the opinion that data on DBTC can be used for classification of DBTM for STOT RE (see also under 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.

Seinen & Vos (1977) noted reductions in thymus weight 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 of exposure (it is unclear how the 56 days in the CLH dossier was calculated).

In addition, an OECD TG 414 study with DBTO was included for the assessment of STOT RE (Unpublished report, 2017). Mean absolute thymus weights were 19%, 34%, and 44% lower than the mean control values in the 0.75, 3.0, and 6.0 mg/kg bw/d dose groups, respectively, and relative to the adjusted GD 20 body weights, they were 20%, 35%, and 37% lower, respectively.

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 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 DBTM (346.99 g/M), this applies to the equivalent values of DBTM 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 **DBTM 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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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 maleate

(DBTM)

EC Number: 201-077-5
CAS Number: 78-04-6
Index Number: Not applicable

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In the following a detailed study description is provided for studies cited and referred in the CLH report for dibutyltin maleate (DBTM).

The studies described in the section Toxicokinetic (Chapter 1) and Reproductive Toxicity (Chapter 2.2) and Specific Target Organ Toxicity (Chapter 2.3) (except unpublished report, 2017) below 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 described 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 prenatal developmental toxicity study with DBTO (unpublished report, 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: <i>in vitro</i> 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

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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 aqueous HCL, 72h, 40°C). The degree of hydrolysis was measured after workup with dichloromethane-D₂ by ¹¹⁹Sn NMR.

Findings

Hydrolysis studies demonstrate that DBTM is hydrolysed to the dimeric stannoxane ClBu₂SnOSnBu₂Cl.

Conclusion

Dibutyltin maleate is shown to be converted to ClBu₂SnOSnBu₂Cl 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: <i>in vitro</i> 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 ₂ SnOSnBu ₂ Cl (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 ₂ SnOSnBu ₂ Cl 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: <i>in vitro</i> 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 ₂ SnOSnBu ₂ Cl (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 ClBu₂SnOSnBu₂Cl

Time	DBTC	ClBu ₂ SnOSnBu ₂ Cl	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 ClBu₂SnOSnBu₂Cl 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 ¹⁴C-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 *in vitro* phase, the metabolites of ¹⁴C 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 ¹⁴C butyl labelled dibutyltin (di)acetate to dibutyl and monobutyl species by both nonenzymatic destannylation and by α- 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.”

1.5.1 Toxicokinetics in the rat

- Reference** “Ishizaka T, Suzuki T & Saito Y (1989)
 Metabolism of Dibutyltin Dichloride in Male Rats

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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-life 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)

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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 preceding 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 regarding 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 pre-mating, gestation and lactation periods; mean bodyweights of females in this group were significantly lower at Day 14 of the pre-mating 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.

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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,

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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

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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. <i>Environmental Toxicology</i> 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 17 β -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 all 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

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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 (omphalocele, 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

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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	
	-P	+P	-P	+P	-P	+P
Progesterone +/-						
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	<p>Dibutyltin dichloride (DBTC)</p> <p>CAS 683-18-1</p> <p>EC 211-670-0</p> <p>Purity not reported</p> <p>Dibutyltin di(acetate) (DBTA)</p> <p>CAS 1067-33-0</p> <p>EC 211-670-0</p> <p>Purity not reported</p> <p>Dibutyltin maleate (DBTM)</p> <p>CAS 78-04-6</p> <p>EC 201-077-5</p> <p>Purity not reported</p> <p>Dibutyltin dilaurate (DBTL)</p> <p>CAS 77-58-7</p> <p>EC 201-039-8</p> <p>Purity not reported</p> <p>Dibutyltin oxide (DBTO)</p> <p>CAS 818-08-6</p> <p>EC 212-449-1</p> <p>Purity not reported</p>
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	<p>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.</p> <p>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).</p>

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)**

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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	-	-	-	-	-	-
<i>Pes varus</i>	-	-	1 (1)	-	-	-
<i>Pes valgus</i>	-	-	-	-	-	-
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

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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 13 th 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) CrI: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

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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*

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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

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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 severe cleft jaw was associated with ankyloglossia and/or cleft tongue. Micrognathia, cleft palate, omphalocele, 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 10 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 omphalocele and exencephaly; 59% of the malformed foetuses had cleft jaw and ankyloglossia. The majority of affected foetuses in this group

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had a relatively slight cleft jaw. At 7.5 mg/kg bw/d, 12% of the malformed fetuses had micrognathia only. 61% of the malformed fetuses had cleft jaw, ankyloglossia and/or cleft tongue. At 10 mg/kg bw/d, all malformed fetuses showed multiple findings; 88% of the malformed fetuses 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)
Omphalocele (#)	-	-	2 (2)	5 (1)	6 (2)*
Exencephaly (#)	-	-	1 (1)	3 (1)	1 (1)
Ecephalocele (#)	-	-	-	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 fetuses 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 fetuses 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)	-

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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

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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, omphalocele, open eye, tail anomalies and club foot was seen, compared to controls. Of the 26 affected foetuses, one had a single malformation (omphalocele), while the remainder had multiple findings. 54% of the malformed foetuses had omphalocele 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

	Controls	Days of treatment		
		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

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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 fetuses at 20 mg/kg bw 23/34 fetuses 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 fetuses had a single malformation such as exencephaly, omphalocele and encephalocele following treatment with DBTC on GD 7. 50% (at 20 mg/kg bw) and 13% (at 40 mg/kg bw) of the malformed fetuses had a single malformation such as omphalocele, 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 fetuses with skeletal anomalies. The highest increase in the incidence of skeletal malformations resulted treatment with DBTC on GD8; 21 of the 63 fetuses at 20 mg/kg bw and 22 of 23 fetuses at 40 mg/kg bw showed malformations. Cleft sternum was the predominant finding in fetuses 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 sternbrae 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 fetuses (#)	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*
<i>External malformations</i>				
No. examined (#)	127 (10)	89 (8)	116 (10)	41 (4)
Total malformations (#)	0	2 (2)	14 (6)*	5 (4)*
<i>Skeletal malformations</i>				
No. examined (#)	85 (10)	59 (8)	78 (10)	27 (3)
Total malformations (#)	0	1 (1)	13 (6)*	1 (1)
<i>Internal malformations</i>				
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

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	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*
<i>External malformations</i>				
No. examined (#)	95 (8)	34 (4)	141 (11)	112 (8)
Total malformations (#)	14 (6)*	23 (4)*	3 (2)	0
<i>Skeletal malformations</i>				
No. examined (#)	63 (8)	23 (4)	93 (11)	75 (8)
Total malformations (#)	21 (6)*	22 (4)*	3 (2)	5 (3)
<i>Internal malformations</i>				
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 identify 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

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numbers of live and dead fetuses and resorptions were counted. Fetuses were sexed, weighed and inspected for external malformations and malformations of the oral cavity. Approximately two thirds of the fetuses in each litter stained with Alizarin Red S and examined for skeletal malformations. The remaining fetuses 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 fetuses 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 fetuses (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 omphalocele 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 fetuses (51%) at 10 mg/kg bw and in 15/29 fetuses 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 sternbrae 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)**
Encephalocele	-	8 (3)	4 (3)*
Spina bifida	-	1 (1)	-
Cleft jaw	-	14 (6)**	11 (4)**
Micrognathia	-	6 (3)	2 (1)

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Cleft lip	-	11 (4)*	10 (5)**
Ankyloglossia	-	18 (5)**	7 (4)**
Cleft tongue	-	5 (3)	3 (3)*
Cleft palate	-	2 (2)	-
Omphalocele	-	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 embryoletality 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 $\mu\text{mol/kg}$ 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

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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

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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
<i>External observations</i>						
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)
<i>Skeletal observations</i>						
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 ($p < 0.05$); ** ($p < 0.01$)

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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	3.2/2.9	3.0/2.8	2.6**/2.5**	2.3**/2.3**
External malformations (#)	-	-	2 (2)	43 (10)**	19 (7)**

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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 rate 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

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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	3M	-	-	-	30.2*	62.6*

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malformations (%)	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 17 β -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*

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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 embryofetal 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)

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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). Dibutyltin oxide: 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 0, 0,75, 3 und 6 mg/kg bw/day

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design 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 sacrificed 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		Individual gestation body

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	body weight values		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 adjusted body weight/body weight change values is provided in the following table:

Endpoint	0 mg/kg bw/d (Mean \pm SD) n=24	0.75 mg/kg/bw/d (Mean \pm SD) n=24	3.0mg/kg bw/d (Mean \pm SD) n=23	6 mg/kg bw/d (Mean \pm 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 \pm SD)	0.75 mg/kg bw/day (Mean \pm SD)	3 mg/kg bw/day (Mean \pm SD)	6 mg/kg bw/day (Mean \pm SD)
Thymus, g	0.239 ± 0.062	$0.193^x \pm 0.042$	$0.158^x \pm 0.043$	$0.134^x \pm 0.046$
Thymus/adjusted GD 20 BWT, %	0.0891 ± 0.0192	$0.0716^x \pm 0.0123$	$0.0581^x \pm 0.0143$	$0.0558^x \pm 0.0108$

^xstatistically 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

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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	92.0	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

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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 fetuses evaluated	150	169	154	106
Head - Retina folded				
No. of litters (%)	0 (0)	0 (0)	1 (4.3)	0 (0.0)
No. of fetuses (%)	0 (0)	0 (0)	1 (0.6)	0 (0.0)
Mouth – Palate, rugae irregular				
No. of litters (%)	1 (4.2)	3 (12.5)	6 (26.1)	0 (0)
No. of fetuses (%)	3 (2.0)	3 (1.8)	6 (3.9)	0 (0.0)
Thyroid, smaller than normal				
No. litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.6)
No. fetuses (%)	0 (0.0)	0 (0.0)	0 (0.0)	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 fetuses (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). Dymorphogenic 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)

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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 <i>in vitro</i> 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	[<i>In vitro</i> 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). <i>In vitro</i> 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	[<i>In vitro</i> 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 (IP50) and cell differentiation (ID50) 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 (ID50 :0.13-1.71 µM) and cell proliferation (IP50: 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

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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				

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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 ($10^6/\text{mm}^3$)	Retics (% of RBC)	Leucocytes				
					Total ($10^3/\text{mm}^3$)	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 pre-mating, gestation and lactation periods; mean bodyweights of females in this group were significantly lower at Day 14 of the pre-mating 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.”
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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

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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).”
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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) CrI: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

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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	-	-	-	-

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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.

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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 F1 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.”