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 bis(2-ethylhexanoate)

EC Numbers: **220-481-2**

CAS Numbers: **2781-10-4**

Index Numbers: **Not applicable**

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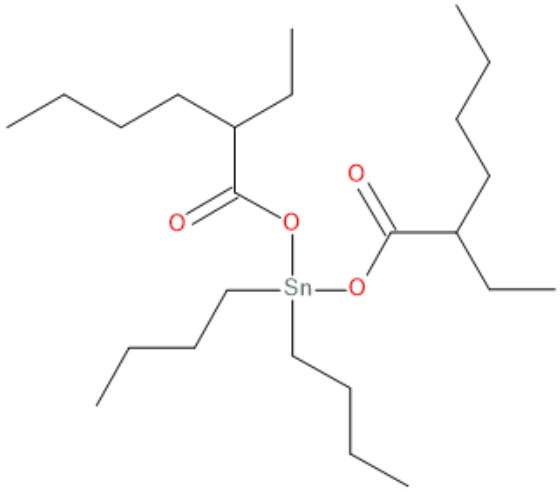
IDENTITY OF THE SUBSTANCES

1.1 Names and other identifiers of the substances

Table 1: Substance identity and information related to molecular and structural formula of dibutyltin bis(2-ethylhexanoate)

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Dibutylstannanebis(ylium) bis(2-ethylhexanoate)
Other names (usual name, trade name, abbreviation)	Dibutyltin bis(2-ethylhexanoate) (DBTE)
ISO common name (if available and appropriate)	Not available
EC number (if available and appropriate)	220-481-2
EC name (if available and appropriate)	Dibutyltin bis(2-ethylhexanoate)
CAS number (if available)	2781-10-4
Other identity code (if available)	Not available
Molecular formula	C24H48O4Sn
Structural formula	
SMILES notation (if available)	O=C(O[Sn](CCCC)(OC(=O)C(CC)CCCC)CCCC)C(CC)CCCC
Molecular weight or molecular weight range	519.3327 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	The substance shows stereochemistry (two stereocentres) and it is a multi-constituent substance including all possible stereoisomers as main constituents.
Description of the manufacturing process and identity of the source (for UVCB substances only)	The registrant describes this substance as a UVCB, possibly due to the manufacturing process. See annex III for confidential information.
Degree of purity (%) (if relevant for the entry in Annex VI)	Confidential. See annex III.

1.2 Composition of the substances

Table 2: Constituents (non-confidential information) of dibutyltin bis(2-ethylhexanoate) (CAS No: 2781-10-4)

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 bis(2-ethylhexanoate)		-	Acute Tox. 3 H301 Acute Tox. 4 H302 Skin Corr. 1B H314 Skin Corr. 1C H314 Skin Irrit. 2 H315 Skin Sens. 1 H317 Eye Dam. 1 H318 Eye Irrit. 2 H319 Muta. 2 H341 Repr. 1B H360 Repr. 2 H361 STOT SE 1 H370 STOT RE 1 H372 STOT RE 2 H373 Aquatic Acute 1 H400 Aquatic Chronic 1 H410 Aquatic Chronic 4 H413

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
-	-	-	-	-

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
-	-	-	-	-	-

Table 5: Test substances (non-confidential information)

None of the studies of this CLH-dossier are performed with dibutyltin bis(2-ethylhexanoate) (DBTE). Most of the studies are performed with the substances characterised as belonging to the category described in section 9.2, primarily dibutyltin dichloride (DBTC) and dibutyltin dilaurate (DBTDL) that both have harmonised classifications. Some studies are performed with dibutyltin di(acetate) (DBTA) which is also proposed to be classified in the same way as DBTC and DBTDL.

Identification of test substance	Purity	Classifications	The study(ies) in which the test substance is used, reference to studies in annex I
Dibutyltin dichloride (DBTC)	>90%-99.7% or not	Acute Tox. 3*, H301 Acute Tox. 4*, H312 Acute Tox. 2*, H330	2.1.2 – Toxicokinetics in the rat 2.1.8 – Simulated gastric hydrolysis 3.8.1.4 – *In vitro* cell gene mutation assay

Identification of test substance	Purity	Classifications	The study(ies) in which the test substance is used, reference to studies in annex I
	reported	Skin Corr. 1B, H314 Muta. 2, H341 Repr. 1B, H360FD STOT RE 1, H372 Aquatic Acute 1, H400 Aquatic Chronic 1, H410 **Harmonised classification, C&L Inventory**	3.8.1.7 – *In vitro* mam. chr. aberration test 3.8.1.5 – *In vitro* lymphocyte toxicity 3.8.1.6 – *In vitro* gene mutation, CHO 3.8.1.9 – Ames test 3.8.1.8 – Breakage of λ-DNA 3.8.1.10 – Bact SOS chromotest and rec-assay 3.8.1.11 – Condensate formation with DNA 3.8.1.12 – Effect on spindle structure, V79 3.8.1.13 – Aneuploidy in lymphocytes 3.8.1.14 – Effect on spindle-inhibition 3.8.2.1 – Micronucleus assay 3.8.2.2 – Micronucleus assay 3.10.1.1 – Repro/dvlp toxicity study, rat 3.10.1.2 – developmental toxicity study, rat 3.10.1.3 – developmental toxicity study, rat 3.10.1.4 – Developmental toxicity study, rat 3.10.1.5 – Developmental toxicity study, rat 3.10.1.6 – Developmental toxicity study, rat 3.10.1.7 – Developmental toxicity study, rat 3.10.1.8 – Developmental toxicity study, rat 3.10.1.12–Reproductive toxicity study, mouse 3.10.1.13 – Dvlp toxicity study, monkey 3.10.1.14 – Dvlp toxicity study, monkey 3.10.1.15 – Developmental toxicity study, rat 3.10.3.1 – Cultured rat embryo study 3.10.3.2 – Cultured rat embryo study 3.10.3.3 – Mechanistic study, rat 3.10.3.4 – Mechanistic study, rat 3.12.1.1 – Subchronic study, rat 3.12.1.3 – Subchronic study, rat 3.12.1.4 – Subacute study, rat 3.12.1.5 – Subchronic study, rat 3.12.1.6 – Subacute immunotox study, rat 3.12.1.7 – Dvlp immunotox, rat 3.12.1.8 - 3.12.1.9 – Mechanistic study 3.12.1.10 –Mechanistic study, thymic atrophy 3.12.1.14 – Mechanistic study
Dibutyltin dilaurate (DBTDL)	98.2-98.6%, or not reported	Muta. 2, H341 Repr. 1B, H360FD STOT RE 1, H372 **Harmonised classification, C&L Inventory**	2.1.1 – Simulated gastric hydrolysis 3.8.1.1 – Ames test 3.8.2.3 – DNA damage i rat cerebral cells 3.10.1.4 – Developmental toxicity study, rat 3.12.1.12 – Immunotoxicity study 3.12.1.13 – Neurotoxicity study
Dibutyltin (di)acetate (DBTA)	> 98% or not reported	Skin Corr. 1B, H314 Eye Dam. 1, H318 Skin Sens. 1B, H317 Muta. 2, H341 Repr. 1B, H360FD STOT SE 1, H370 STOT RE 1, H372 Aquatic Chronic 1, H410 **Self-classification, REACH registration (ECHA)**	2.1.3 – Metabolism *in vitro* and *in vivo* 3.8.1.3 – Ames test 3.10.1.4 – Developmental toxicity study, rat 3.10.1.9 - Developmental toxicity study, rat 3.10.1.10 - Developmental toxicity study, rat 3.10.1.11 - Developmental toxicity study, rat
Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	>90%	Repr. 1B, H360 STOT RE 1, H372 **RAC opinion, (ECHA 2017)**	2.1.7 – Simulated gastric hydrolysis

Identification of test substance	Purity	Classifications	The study(ies) in which the test substance is used, reference to studies in annex I
Dibutyltin maleate (DBTM)	99.2% or not reported	Acute Tox. 2, H330 Acute Tox. 4, H302 Repr. 1B, H360FD STOT RE 1, H372 Muta. 2, H341 Skin Sens. 1, H317 Aquatic Chronic 1, H410 Skin Corr. 1C, H314 STOT SE 1, H370 Eye Dam. 1, H318 **Self-classification, REACH registration (ECHA)**	2.1.1 – Simulated gastric hydrolysis 3.10.1.4 – Developmental toxicity study, rat
Dibutyltin oxide (DBTO)	99.2% or not reported	Acute Tox. 3, H301 Eye Dam. 1, H318 Muta. 2, H341 Aquatic Chronic 1, H410 Repr. 1B, H360 STOT RE 1, H372 Skin Irrit. 2, H315 STOT SE 1, H370 Skin Sens. 1, H317 **Self-classification, REACH registration (ECHA)**	2.1.1 – Simulated gastric hydrolysis 3.8.1.2 – Ames test 3.10.1.4 – Developmental toxicity study, rat
Dibutyltin bis EHMA (Dibutyltin bis(2-ethylhexyl mercaptoacetate))	Not reported	Acute Tox. 4, H302 Eye Irrit. 2, H319 STOT RE 1, H372 Skin Irrit. 2, H315 Muta. 2, H341 Skin Sens. 1, H317 Aquatic acute 1, H400 STOT SE1, H370 **Self-classification, REACH registration (ECHA)**	2.1.4 – Simulated gastric hydrolysis 2.1.5 – Simulated gastric hydrolysis 2.1.6 – Simulated gastric hydrolysis 2.1.9 – Dermal absorpsjon, *in vitro*

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: Dibutyltin bis(2-ethylhexanoate)

	Index No	International Chemical Identification	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	No current Annex VI entry										
Dossier submitters proposal		Dibutyltin bis(2-ethylhexanoate)	220-481-2	2781-10-4	Muta. 2 Repr. 1B STOT RE 1	H341 H360FD H372 (immune system)	GHS08 Dgr	H341 H360FD H372 (immune system)			
Resulting Annex VI entry if agreed by RAC and COM		Dibutyltin bis(2-ethylhexanoate)	220-481-2	2781-10-4	Muta. 2 Repr. 1B STOT RE 1	H341 H360FD H372 (immune system)	GHS08 Dgr	H341 H360FD H372 (immune system)			

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	Hazard class not assessed in this dossier	No
Skin corrosion/irritation	Hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Hazard class not assessed in this dossier	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	**Harmonised classification proposed**	**Yes**
Carcinogenicity	Hazard class not assessed in this dossier	No
Reproductive toxicity	**Harmonised classification proposed**	**Yes**
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	**Harmonised classification proposed**	**Yes**
Aspiration hazard	Hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	Hazard class not assessed in this dossier	No
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

There are no harmonised classifications and labellings for dibutyltin bis(2-ethylhexanoate) (DBTE).

This proposal is based on a category approach (see section 9.2). The substances in the category all have the same general formula and will hydrolyse to the same metabolite (DBTC). DBTC is classified as Muta. 2, Repr. 1B and STOT RE 1. This approach has been used for several other dibutyltin-substances (DBTDL, DBTP).

DBTE also hydrolyses to 2-ethylhexanoate (2-EHA) (EC no. 205-743-6). 2-EHA has a harmonised classification, as Repr. 2. In addition, a new classification proposal for 2-EHA is in process in order to include the salts of 2-EHA and to include more recent data generated after substance evaluation. The classification proposal is still Repr. 2. See annex II for the whole CLH proposal from Spain.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

Dibutyltin bis(2-ethylhexanoate) has CMR properties (mutagenic and reproductive toxicity). Harmonised classification and labelling for CMR and respiratory sensitisation is a community-wide action under article 36 of the CLP regulation.

Justification that action is needed at Community level is required.

The self-classifications notified by industry and published in the C&L Inventory shows a great degree of variety. STOT RE is closely related to the reproductive toxicity and it is therefore relevant to consider both of these hazard classes. This justifies a harmonized classification for dibutyltin bis(2-ethylhexanoate).

5 IDENTIFIED USES

Dibutyltin bis(2-ethylhexanoate) is manufactured and/or imported in the European Economic Area in 10 - 100 tonnes per year.

This substance is used in articles, formulation and in manufacturing. The technical function of the substance during formulation is as a stabiliser.

This substance is used in the following environmental release categories (ERC) and process categories (PROC):

Manufacture
ERC 1: Manufacture of substances
PROC 3: Use in closed batch process (synthesis or formulation)
PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities

Formulation
ERC 3: Formulation in materials

PROC 5: Mixing or blending in batch processes for formulation of preparations and articles (multistage and/or significant contact)
PROC 8b: Transfer of substance or preparation (charging/discharging) from/to vessels/large containers at dedicated facilities
PROC 14: Production of preparations or articles by tabletting, compression, extrusion, pelletisation
PROC 21: Low energy manipulation of substances bound in materials and/or articles

Article service life
ERC 10a: Wide dispersive outdoor use of long-life articles and materials with low release

6 DATA SOURCES

Experimental data and information on dibutyltin bis(2-ethylhexanoate) included in the present CLH report originates from the publically disseminated REACH Registration Dossiers (available at the ECHA website).

Sources of information for the read-across substances are shown below:

- ECHA: database of registered substances, publically disseminated version of REACH registration dossiers for
  - Dibutyltin bis(2-ethylhexanoate) DBTE
  - Dibutyltin dichloride (DBTC)
  - Dibutyltin dilaurate (DBTDL)
  - Dibutyltin di(acetate) (DBTA)
  - Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)
  - Dibutyltin maleate (DBTM)
  - Dibutyltin oxide (DBTO)
- TOXLINE / MEDLINE searches for:
  - CAS 2781-10-4 (DBTE)
  - CAS 683-18-1 (DBTC)
  - CAS 77-58-7 (DBTDL)
  - CAS 1067-33-0 (DBTA)
  - CAS 22673-19-4 (DBTP)
  - CAS 78-04-6 (DBTM)
  - CAS 818-08-6 (DBTO)
  - ‘dibutyltin’
- Published CLH Report for DBTDL (ECHA, 2014)
- Published CLH Report for DBTP (ECHA, 2016)
- RAC Opinion for DBTP (ECHA, 2017)
- OECD SIDS Initial Assessment Profile for dibutyltin dichloride and selected thioglycolate esters (2006)
- US Department of Health & Human Services ATSDR: Toxicological Profile for Tin and Tin Compounds (2005)

7 PHYSICOCHEMICAL PROPERTIES

The information on physiochemical properties originates from the publically disseminated REACH Registration Dossier. The values are taken from the key study or, in the absence of a key study, the study with the highest reliability score.

Table 8: Summary of physicochemical properties

Property	Value	Reference [1]	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid	Study report 2013	Visual observation
Melting/freezing point	>54.2 - <57.9 °C at 101.3 kPa	Study report 2012	Measured
Boiling point	>400 °C at 96.8 kPa	Study report 2013	Experimental value
Relative density	Data waived	-	
Vapour pressure	0.1 Pa at 293 K	Registration, ECHA website	The study report included was found to be unreliable and so the value was estimated. "From the molweight, the structure, interaction with other molecules and experience with similar substances the vapour pressure of the substance is approx. 10E-02 Pa. In due of safety a vapour pressure of 0.1 Pa is set at this point."
Surface tension	Data waived	-	-
Water solubility	4 mg/L +/- 0.15 at pH 6.1, 20 °C, not DBTE, see remark	Study report 1989	Study performed with DBTO, not quite according to OECD guideline
Partition coefficient n-octanol/water	2.64 at 25 °C, not DBTE, see remark	Study report 2013	Study performed with 2-ethylhexanoid acid
Flash point	118 °C at atmospheric pressure 1 013 hPa, not DBTE, see remark	Fire protection guide to hazardous materials 13 ed, National Fire Protection Association.	The substance is the condensation product of dibutyltin oxide and 2-ethylhexanoic acid. In contact with humidity decomposition occurs. From experience the falsh point of the ethylhaxanoic acid is lower than of dibutyltin oxide, therefore ethylhexanoic acid is in the worst case responsible for the flash point. Equivalent or similar to EU Method A.9
Flammability	Data waived	-	-
Explosive properties	Based on the information and review of the substance, it is deemed not to be potentially explosive, based on the chemical structure and oxygen balance values.	Study report 2011	Estimated. The oxygen balance is calculated from the empirical formula of a compound in percentage of oxygen required for complete conversion of carbon to carbon dioxide, hydrogen to water, and metal to metal oxide.
Self-ignition temperature	365°C at atmospheric pressure 1 013 hPa	Study report 2013	Observed
Oxidising properties	Based on the information and review of the substance, it is deemed	Study report 2011	Recently conducted calculation undertaken by an ISO9001:2008 accredited facility, using the

Property	Value	Reference [1]	Comment (e.g. measured or estimated)
	not to be potentially oxidising, based on the chemical structure and oxygen balance		latest methods in accordance with the general fundamental principles of recognised guidelines and deemed scientifically acceptable based on the results
Granulometry	Data waived	-	-
Stability in organic solvents and identity of relevant degradation products	Data waived	-	-
Dissociation constant	Data waived	-	-
Viscosity	Data waived	-	not required, substance is solid at 40°C

[1] As cited in the publically disseminated REACH registration dossier

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 9: Summary table of toxicokinetic studies. An overview of the studies is presented in the table below and in the following text; more detailed study summaries are presented in Annex I to this CLH report.

Method	Results	Remarks	Reference
In three separate experiments, each of the test substances: **- dibutyltin dilaurate (DBTDL)** **- dibutyltin maleate (DBTM)** **- dibutyltin oxide (DBTO)** were individually tested under low pH (1-2) conditions (0.07 N HC1) at 37 °C in order to simulate the hydrolytic action by mammalian gastric contents. 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. The degree of hydrolysis for the test substances DBTM, DBTDL and DBTO was studied by determination of the amount of DBTC formed after 0.5, 1.0, 2.0 and 4.0 hours, using GC-FPD. Where possible the ligand was also analyzed. The analytical approach to the individual ligands, maleate, laurate, and oxide, was different due to the unique chemical properties of each.	The hydrolysis 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. Evaluation of results: DBT may be hydrolyzed to a great extent in simulated mammalian gastric contents.	Reliability 2 (reliable with restrictions) Key study read-across from supporting substance (structural analogue or surrogate)	Schilt & Zondervan-van den Beuken (2004)
DBTC	DBTC administered to rats		Ishizaka *et*

Method	Results	Remarks	Reference
Metabolism in the male rat *in vivo.* Intraperitoneal injection (4 mg/kg bw).	by IP injection (4 mg/kg bw) 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). The half-life of DBTC in liver, kidney and blood was 3-5 days.		*al*., (1989)
Test material (EC name): **Dibutyltin di(acetate)** (DBTA) Microsomal metabolism *in vitro* and in the mouse (swiss webster) *in vivo.* *In vitro:* The metabolic fate of dibutyltin acetate was examined in a microsomal monooxygenase metabolism system (MO) derived from either rat or rabbit livers. Comparative data was also provided on other alkyltins in the MO system. Doses/conc.: 0.003 µmol of [14C]butyltin derivative, 0.5 µmol of unlabeled compound *In vivo*: Oral, gavage study, with male mice. One single dose: 1.1 mg/kg bw. The urine and faeces of the animals were examined for metabolites of the parent compound. Tissues were also examined for uptake at 138 hours post dosing.	In the mouse *in vivo*, following oral administration hydrolysis for DBTA to form dibutyltin and liberation of acetate (incorporated into normal cellular metabolism). Faeces contained a proportion of non-metabolised DBTA and other 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. *In vitro*, DBTA was metabolised to dibutyl and monobutyl species.	Reliability: 2 (reliable with restrictions) supporting study experimental result	Kimmel EC, Fish RH & Casida JE (1977)
Simulated gastric hydrolysis of **dibutyltin bisEHMA** (2-ethylhexyl 4,4-dibutyl-10-ethyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate, CAS nr 10584-98-2)	Under acidic conditions, mono- or di- alkyltin mercaptides undergo a tin-EHMA bond break releasing EHMA. The free EHMA undergoes additional hydrolysis with ethyl hexanol and thioglycolic acid as products. EHMA and ethyl hexanol are easily quantified at low ppm level by GC-AED. The water soluble thioglycolic acid could be determined indirectly by total sulfur analysis-ICP emission spectroscopy.	Reliability 2 (reliable with restrictions) key study read-across from supporting substance (structural analogue or surrogate)	Unnamed(2000). ORTEP summary report (2000) (Study 005 in DBTE registration)
Simulated gastric hydrolysis of **Bu2Sn(EHMA)2** (2-ethylhexyl 4,4-dibutyl-10-ethyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate, CAS no: 10584-98-2).	Results indicate that the hydrolysis of Bu2Sn(EHMA)2 is very rapid at pH = 1.2.	Reliability: 2 (reliable with restrictions) weight of evidence read-across from supporting substance (structural analogue or surrogate)	Unnamed (2000) ORTEP summary report (2000). (Study 006 in DBTE registration)
Simulated gastric hydrolysis of **Bu2Sn(EHMA)2** (2-ethylhexyl 4,4-dibutyl-10-ethyl-7-	In conclusion, these data show that the hydrolysis of organotins such as Bu2Sn(EHMA)2 depends of the	Reliability: 2 (reliable with restrictions)	Unnamed (2000) ORTEP

Method	Results	Remarks	Reference
oxo-8-oxa-3,5-dithia-4-stannatetradecanoate, CAS no: 10584-98-2).	amount of acid HCl added to the solution, and hydrolysis is very fast at pH = 1. The results indicate that the hydrolysis occurs more completely at pH = 1 than at pH = 4.	weight of evidence read-across from supporting substance (structural analogue or surrogate)	summary report. (Study 007 in DBTE registration)
DBTP Simulated gastric hydrolysis (^{119}Sn NMR detection)	DBTP is rapidly hydrolyzed to the distannoxane $ClBu_2SnOSnBu_2Cl$ under conditions representative of the mammalian stomach. After 2 hours the degree of hydrolysis was almost quantitative, approximately 2 mol% of DBTC was also detected.		Naßhan, 2015
DBTC Simulated gastric hydrolysis (^{119}Sn NMR detection)	DBTC is rapidly hydrolysed to the distannoxane $ClBu_2SnOSnBu_2Cl$ under conditions representative of the mammalian stomach. The degree of hydrolysis was reported as approximately 70, 85 and 90 % after 30 seconds, 1 hour and 4 hours respectively (not corrected for trace impurities of tributyltinchloride).	Study used to support read-across.	Naßhan, 2016
Dibutyltin bis(2-ethylhexyl mercaptoacetate (same substance as **Bu2Sn(EHMA)2** used in studies above) Dermal absorption. *In vitro* study, human and Wistar rat epidermis. The absorption of dibutyltin bis(2-ethylhexyl-mercaptoacetate), containing 18.5 % w/w tin, was measured *in vitro* through human and rat epidermis. The first phase of the study was to identify the highest dose that could practically be applied, which was also regarded as likely to be non-damaging to human epiderms, using rat epidermis as a model. During the second phase of the study, the absorption of tin was determined through human and rat epidermis from both occluded and unoccluded applications of this non-damaging dose (100 µL/cm^2 eq. 21120 µg tin/cm^2).	100 µL/cm^2 (= 21120 µg tin/cm^2) was found to alter the barrier function of the rat epidermis. At 100 µL/cm^2, approximately up to 18-45 % of the tin dose was unaccounted for, possibly due to adherence of the test material to the glass apparatus. The absorption of tin through human epiderims was very slow, when compared with the absorption rates of other penetrants. The proportions of dibutyltin bis(2-ethylhexylmercaptoacetate) absorbed through human epidermis were 0.0004% and 0.0010% (occluded and unoccluded respectively) after 24 hours exposure, compared to 0.261% and 0.189% through rat epidermis. The majority of the applied tin dose was washed from the surface of the epidermis during decontamination, only a relatively small proportion of the dose (human up to 1%; rat up to 10%) remained associated with the epidermis and therefore was not regarded as systemically available.	Reliability: 2 (reliable with restrictions) Key study. Read-across from supporting substance (structural analogue or surrogate).	Unnamed (2003)

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

No toxicokinetic or other toxicological data are available for dibutyltin bis(2-ethylhexanoate) (DBTE). In this CLH report a category approach is therefore used when considering toxicokinetic data and other endpoints relevant for this classification proposal, and is based on data available for other substances in the same category. This category approach is justified on the basis of hydrolytic and toxicokinetic behaviour, and toxicological data from dibutyltin dichloride (DBTC) and other dibutyltin compounds (see section 9.2 below). The same approach was accepted by RAC for dibutyltin dilaurate (DBTDL) in 2014 (ECHA, 2014) and for dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP) in 2017 (ECHA, 2017). It is also the same approach used in the CLH proposal for dibutyltin di(acetate) (DBTA), which will be on public consultation at the same time as DBTE.

An *in vivo* toxicokinetic study was performed with DBTA, reporting the metabolism of DBTA following oral administration to the mouse (Kimmel *et al.*, 1977). The study showed that there is hydrolysis of DBTA, forming an unidentified dibutyltin compound and liberation of the acetate moieties, which are further transformed and incorporated into normal cellular metabolism. In the faeces there was also a proportion of non-metabolised DBTA and other dibutyltin derivatives. There was also extensive cleavage of the tin-carbon bond, with further metabolism of the liberated butyl group to exhaled carbon dioxide and small quantities of butene.

Another *in vivo* study was performed with DBTC (Ishizaka *et al.*, 1989). Male Wistar rats received an intraperitoneal injection of 4 mg/kg bw and DBTC was shown to be metabolised by hydroxylation of the butyl groups and by formation of monobutyltin trichloride. DBTC was detected in the liver and kidneys at the earliest time point (6 hours), 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. Butyl(3-hydroxybutyl)tin dichloride, butyl(4-hydroxybutyl)tin dichloride and butyltin trichloride were detected as metabolites by HPLC and MS. 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 the biliary and hepatic lesions. The generation of monobutyltin derivatives from DBTC is also shown in microsomal preparations *in vitro* (Kimmel *et al*., 1977).

In vitro performed simulated gastric hydrolysis studies for category members are discussed in detail in section 9.2.

9.2 Category approach

9.2.1 Background and definition of category

Dibutyltin compounds have been seen as belonging to one same category for mammalian toxicology studies by the oral route based on the common DBTC hydrolysis products. This has been done by the OECD (OECD 2006), by the European Chemicals Bureau's Technical Committee on Classification and Labelling in 2006 and more recently in the classification proposals for DBTDL (ECHA, 2014), for DBTP (ECHA, 2017) and for DBTA (in process, 2019).

Although DBTE does not fulfill all the criteria to be a member of this category (see definition below), due to the toxicity of its' hydrolysis product, its' other inherent properties are the same as

the other members of the category and for the purposes of this CLH proposal the same principles apply for DBTE as for the other category members and DBTE will be considered as part of the same category.

Rationale for the category, specific considerations for DBTE

The substances in the category are chemically comparable in that they contain a common functional dibutyltin (Bu2Sn-) group. Following oral administration, the substances belonging to this category will hydrolyse and generate DBTC (or derivatives thereof). Systemic exposure to any of the substances in the category will therefore result in exposure to the same substance, ie DBTC, and will therefore cause the same toxic effects to the exposed individuals. Since the hydrolysis products are thought to be generated by gastric hydrolysis, i.e. by the oral route, a category approach is not possible by other routes of administration.

For the other category members the common functional group (Bu2Sn-group) is considered to be the only toxic component. For DBTE the Bu2Sn-group is the most toxic group, but in addition the other hydrolysis product (2-EHA) is also classified as reprotoxic. The classification of 2-EHA is however Repr. 2, whereas the proposal for the DBTE is Repr. 1B. The reproductive toxicity of 2-EHA is therefore considered not relevant in the context of this category approach, as it will not weaken the Repr. 1B proposal for DBTE. For more details on 2-EHA's toxicity see the CLH proposal from Spain, in Annex II. Apart from the classification of the hydrolysis product, DBTE's properties fit with the rest of the category members as it has the same generic formula and is therefore considered to have the same behaviour in gastric conditions. i.e. expected to form DBTC at low pH.

Definition of the category

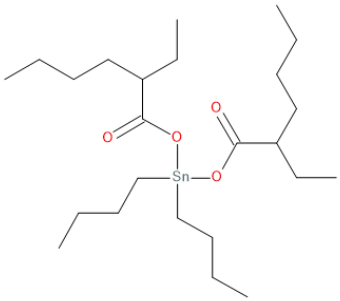
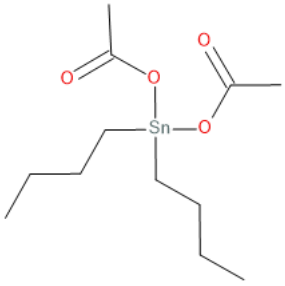
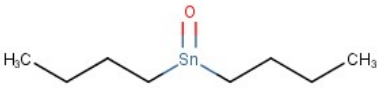
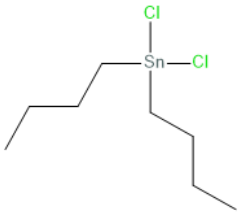
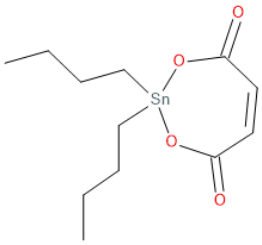
The category includes substances (as shown in Table 10 Substance characteristicsbelow) with the generic formula Bu2SnL2, where L is a labile ligand. It also includes DBTO since it has been shown to form DBTC in gastric simulation studies. Dibutyltin substances with nonlabile ligands, and where there is significant systemic exposure to the intact substance, are not included in the category. Furthermore, the category includes substances generating hydrolysis products (in addition to DBTC) which are of comparatively low toxicity. Substances generating additional hydrolysis products of toxicological significance are therefore not considered to be a part of this category. As explained above, although DBTE generates 2-EHA, which has a Repr. 2 classification, this classification is not considered to impact on the classifications proposed in this CLH dossier as Repr. 2 is a weaker classfication than the Repr. 1B proposed. 2-EHA is in this context therefore not considered as toxicologically significant and DBTE can therefore be considered as being a part of this category.

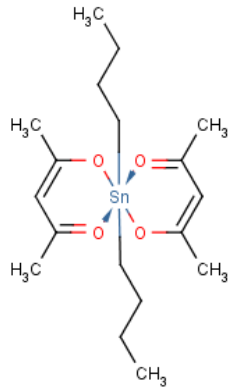
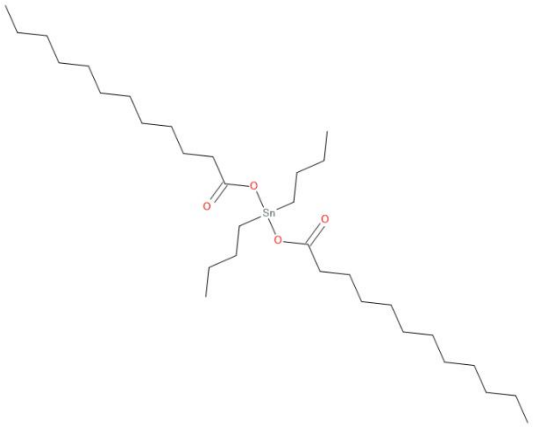
Category Members

The table below shows the proposed category members: DBTA, DBTC, DBTM, DBTDL, DBTO and DBTP, in addition to DBTE.

Table 10 Substance characteristics

Substance	**EC # / CAS #**	**Structure***	**Purity (studies)**	**Purity / Impurity details (REACH Dossier)**

Dibutyltin bis(2-ethylhexanoate) (DBTE)	220-481-2 / 2781-10-4		Confidential	The registrant calls this a UVCB substance. It is not clear why they categorise it as such, and it seems to be a mono-consitituent, just like the members of the category.
Dibutyltin (di)acetate (DBTA)	213-928-8 / 1067-33-0		Not reported	No further details (monoconstituent substance)
Dibutyltin oxide (DBTO)	212-449-1 / 818-08-6		Not reported	>97.5% No further details (monoconstituent substance)
Dibutyltin dichloride (DBTC)	211-670-0 / 683-18-1		96-99.7% where reported for studies	93-100% Monoconstituent substance; tributyltin chloride (0.25-1%) in some sources
Dibutyltin maleate (DBTM)	201-077-5 / 78-04-6		Not reported	No further details (monoconstituent substance)

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

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

9.2.2 Category justification

Physicochemical properties

The category members are either solid or liquid at room temperature and pressure. DBTE is solid at room temperature, and is reported to have a melting point of 54°C. The category substances have molecular weights in the range 304-632 g/mol due to differences in the groups linked to the dibutyltin moiety. The difference in molecular weight means that the mass proportion of DBTC generated by hydrolysis will vary, this may in some degree influence the toxic potency. The quantity of DBTC resulting from the total hydrolysis of DBTE is approximately 59% based on molecular weight. Category members are all reported to be insoluble or of low water solubility. Physicochemical properties are not critical to the inclusion of substances in the category, but relevant properties are comparable.

Chemical similarities (hydrolytic behaviour)

Dialkyltin compounds which contain labile ligands, *e.g.* chlorides or carboxylates, generally undergo hydrolysis in aqueous solution at room temperature with the formation of various oxide/hydroxide species. The hydrolysis reactions have been thoroughly studied and depending on the reaction conditions various products may be isolated (Beckmann *et al.*, 2002; Davies, 2004), where the partly hydrolysed distannoxane ($XR_2SnOSnR_2X$) is frequently encountered. Further hydrolysis in an aqueous environment of this compound eventually forms the insoluble polymeric $(R_2SnO)n$. Importantly, 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). A general mechanistic pathway is presented in Figure 1 where the composition at equilibrium will depend on factors such as the medium and the ionic strength.

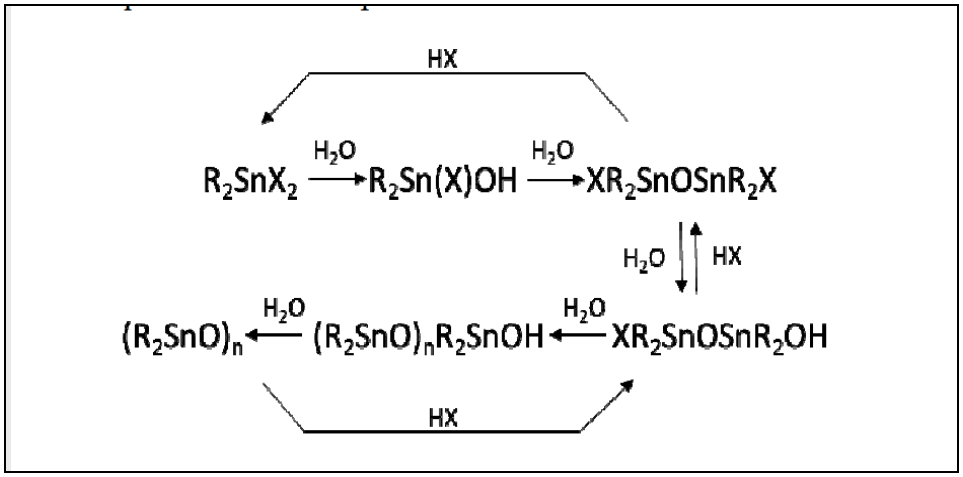


Figure 1. Simplified hydrolysis scheme for dialkyltins (Davies, 2004; Aylett *et al.*, 1979)

The water solubility of DBTE has not been tested. The registrant has included a study for the water solubility of DBTO. Based on the DBTO study the registrant claims that DBTE is either insoluble or has very low water solubility. The study suggests a water solubility of 4 mg/L, although it was not performed quite according to guidelines. The registrant regards the substance as slightly soluble. Information on DBTC in the publically disseminated REACH Registration Dossier indicates that DBTC decomposes, but the product was not identified. For the other compounds in the category, the water solubilities were reported as low or as insoluble in the REACH registration dossiers and the possible formation of decomposition products were not discussed. In the "OECD SIDS Initial Assessment Profile for dibutyltin dichloride and selected thioglycolate esters" (OECD, 2006), it is stated however that DBTC, DBTDL and DBTM all rapidly form oxides/hydroxides in contact with water, as expected due to the lability of the ligands.

An important common property for these substances is the chemical behaviour at low pH, as opposed to that at neutral pH. At pH 1-2, simulating gastric conditions, all the compounds in the category behave in the same way and rapidly hydrolyse to form the same product. Schilt & Zondervan van der Beuken (2004) reported the rapid hydrolysis (half-life < 0.5 hours) of DBTM and DBTDL to form DBTC (95% and 87% yields respectively after 4 hours). DBTO was also reported to form DBTC with a half-life of 3,5 hours (87% yield after 4 hours). DBTC was detected and quantified with GC-FPD using prepared stock solutions of DBTC while the liberated ligands (maleic acid and lauric acid) were analysed using HPLC-UV and GC-MS respectively. The results demonstrate that the substances are hydrolysed and converted to DBTC under conditions representative of the stomach although an unambiguous assignment of the structure of the common intermediate has not been made. The hydrolysis of DBTA has not been studied *in vitro*, however, in a study from 1977 (Kimmel *et al.*, 1977) DBTA was shown to hydrolyse to dibutyltin and free acetate following oral administration to mice, as would be expected for a substance in this category.

Recent simulated gastric hydrolysis studies generated under REACH demonstrate the lability of the ligands also in DBTP (Naßhan, 2015). Using ^{119}Sn NMR spectroscopy, it was shown that the acetylacetone ligands are liberated almost quantitatively at pH 1.2 within 2 hours with the formation of the distannoxane $ClBu_2SnOSnBu_2Cl$ (c.f. Scheme 1), assigned based on reference NMR spectra and in accordance to literature values (Davies, 2004). Minor amounts of DBTC (2 mol%) were also

detected. The NMR studies are distinct from the previous simulated gastric hydrolysis studies (Schilt *et al.*, 2004) in that a direct method is used (with much higher substance concentrations) which allow a specific assignment of the formed substance. An analogous behaviour was observed for the reference compound DBTC which formed the identical distannoxane $ClBu_2SnOSnBu_2Cl$ as the only hydrolysis product (Naßhan, 2016). Small amounts of unreacted DBTC (<10 mol%) were also detected (after 4 hours). The formation of the distannoxane $ClBu_2SnOSnBu_2Cl$ is in accordance with the well established chemistry of dialkyltin substances, some of which indicates that DBTC and the distanoxane is in a pH dependant equilibrium (figure 2) (Davies, 2004; Aylett *et al.*, 1979).

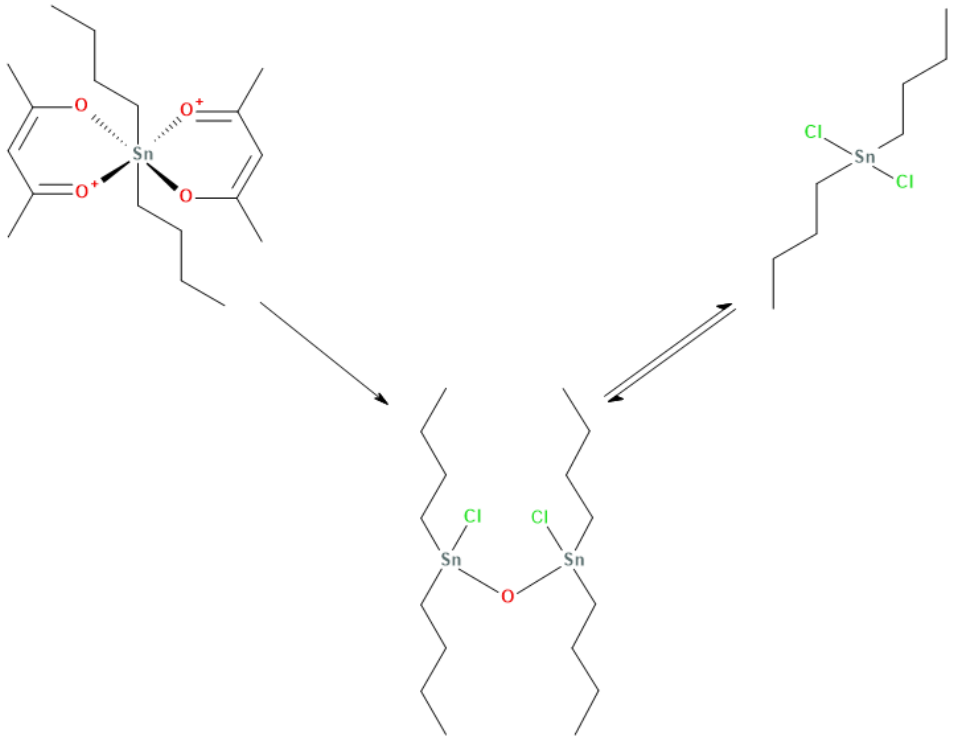


Figure 2. Overview of the hydrolysis of DBTP and DBTC as determined in a recent study (Naßhan, 2015), which is in accordance with well established tin chemistry (Davies, 2004; Aylett *et al.*, 1979).

The hydrolytic behaviour of the substances in the category (DBTA, DBTC, DBTM, DBTDL, DBTO and DBTP) at neutral and low pH supports the category approach and demonstrates that systemic exposure to the intact substances following oral administration is unlikely. All substances behave in a predictable manner and will hydrolyse with the generation of DBTC (or derivatives there of) as common intermediates at low pH. The observed intermediate(s) may vary depending on experimental conditions (e.g. solvent, temperature, pH, concentration) due to various equilibria in aqueous conditions. Although there are no studies performed that show the hydrolysis of DBTE, DBTE's structure has the same general formula as the other category members. The 2-ethylhexanoate does not introduce any strong electron donating or electron withdrawing moieties, nor does it contain any additional polar moieties or steric hindrance. Hence, the tin-oxygen bond in DBTE should be of similar properties as those bonds in the other members in the group. It is therefore considered that the same hydrolysis will happen with DBTE as with the other substances in the category.

Toxicokinetic and toxicological properties

Very limited data are available for DBTE, and no data is available for the toxicokinetic and toxicological properties. Some of the available data is shown below and compared with data for the other category members in a data matrix (Table 11).

As shown above, gastric hydrolysis is expected to be rapid and extensive for all substances in the category, and therefore, following absorption there will be no toxicokinetic differences between DBTE or the other substances in the category. The only toxicokinetic differences relate to the behaviour of the low toxicity ligands and are not considered to be of relevance.

Based on similar toxicokinetic properties it can also be assumed that all members of the category will give rise to the same exposure of the biological targets (i.e. the thymus; the developing embryo/foetus; implantation, fertility; genetic material). Small differences in toxic potency may also be seen and are attributable to differences in the molecular weight of the category substances, however any differences in potency are consistent with the proposed category approach. The comparative developmental study by Noda *et al.* (1993) is a good illustration of the category substances' similar toxicokinetic behaviour, since it shows that all five of the tested substances (DBTA, DBTC, DBTM, DBTDL and DBTO) have the same toxic effect on the developing foetus. The dose levels used in this study were equivalent taking into account molecular weight.

Based on this evidence, common biological targets can be assumed for the category members. Comparison of available toxicity data therefore supports the category approach for both reproductive toxicity, mutagenicity and and STOT RE.

Classification
As shown in Table 11, two of the category substances (DBTC and DBTDL) have harmonised classifications and a CLH for the third one (DBTP) has been adopted by the RAC. All three are classified Repr. 1B (fertility and developmental toxicity), and STOT RE 1 (thymus). DBTC and DBTDL are also classified Muta. 2. Mutagenicity was not assessed in the CLH proposal for DBTP due to shortage of resources in the evaluating MSCA, but the substance is self-classified as Muta. 2.

Other substances in the category do not have harmonised CLP classification, but the self-classifications shown in the REACH Registration Dossiers for these substances is comparable to the harmonised classification for DBTC and for DBTDL (for those hazard classes considered by RAC). The comparable classifications of the substances in the category therefore indicate similar toxicological properties and further support the category justification. It is particularly notable that classification in mutagenicity (Category 2; H341), reproductive toxicity (Category 1B; H360FD) and in STOT RE (Category 1; immune system (thymus)) is the same for all members in the category.

The studies used to support the proposed classifications are of variable quality. Few of the studies are OECD test guideline- and GLP-compliant (and are therefore not scored in Category 1 according to Klimisch). The majority of the studies are not fully compliant with the relevant guidelines but do, however, include reliable assessment of appropriate endpoints and key parameters of relevance to classification. Furthermore, the majority of studies are reported in sufficient detail to support the classification proposal and are also providing consistent evidence for the hazard assessment.

9.2.4 Data matrix

Table 11 Data matrix for category members

Substance	Dibutyltin bis(2-ethylhexanoate) (DBTE)	Dibutyltin oxide (DBTO)	Dibutyltin dichloride (DBTC)	Dibutyltin maleate (DBTM)	Dibutyltin (di)acetate (DBTA)	Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	Dibutyltin dilaurate (DBTL)
Cas no	**2781-10-4**	818-08-6	683-18-1	78-04-6	1067-33-0	22673-19-4	77-58-7
EC no	**220-481-2**	212-449-1	211-670-0	201-077-5	213-928-8	245-152-0	201-039-8
MW	**519**	249	304	347	351	431	632
Physical-chemical properties							
Physical state	Solid	Solid	Solid	Liquid	Liquid	Liquid	Liquid
Water solubility	No data, value in the registration is for DBTO	2.55 mg/L	Study technically not feasible. Hydrolysis on contact with water.	Insoluble	Insoluble	Study technically not feasible. Hydrolysis on contact with water.	Insoluble
Hydrolysis, low pH (GC-FPD detection)	No data	Formation of DBTC in gastric simulation studies: 43% in 0.5h, 65% in 1h, 90% in 2h, 87% in 4h	Not relevant	Formation of DBTC in gastric simulationst udies: 100% in 0.5h, 97% in 1h, 98% in 2h, 95% in 4h	No data	No data	Formation of DBTC in gastric simulation studies: 82% in 0.5h, 78% in 1h, 88% in 2h, 87% in 4h
Hydrolysis, low pH (119Sn NMR detection)	No data	No data	Formation of $ClBu_2SnOSnBu_2Cl$ under gastric simulation studies: ~70% in 30s, ~85% in 1h, ~90% in 4h	No data	No data	Formation of $ClBu_2SnOSnBu_2Cl$ under gastric simulation studies: close to quantitative in 2 hours (2 mol% of DBTC also detected)	No data
Toxicological data							

[04.01-MF-003.01]

Substance	Dibutyltin bis(2-ethylhexanoate) (DBTE)	Dibutyltin oxide (DBTO)	Dibutyltin dichloride (DBTC)	Dibutyltin maleate (DBTM)	Dibutyltin (di)acetate (DBTA)	Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	Dibutyltin dilaurate (DBTL)
Oral LD50 (mg/kg bw)	No data, value in the registration is for DBTDL	172 (121-240)	219	510 (263-777)	1070	1864 (1039-3344)	2071 (1207-5106)
Dermal LD50 (mg/kg bw)	No data, value in the registration is for DBTDL	>2000	No data	>2000	No data	>2000	>2000
Skin irritation	No data, value in registration is for DBTDL	Irritant *in vivo*	Corrosive *in vivo*	Corrosive *in vivo*	Corrosive *in vitro*	Irritant but not corrosive *in vitro.* Corrosive *in vivo*	Corrosive *in vivo*
Eye irritation	No data, value in registration is for DBTDL	Irritant *in vivo*	Serious eye damage *in vivo*	Serious eye damage *in vivo*	No data	Serious eye damage *in vitro*	Irritant *in vivo*
Mutagenic toxicity	No data, values from other category members	Only Ames test, negative	Positive *in vivo* somatic cell mutagenicity test, as well as support from positive results from *in vitro* mutagenicity/ genotoxicity tests.	Only Ames test, negative	Only Ames test, negative	Only Ames test, negative	Only Ames test, negative
Reproductive toxicity – adverse effects on sexual function and fertility	No data, proposed read-across	No data	Large increase in preimplantation loss in studies in the rat, mouse & monkey rat	No data	No data	No data, read-across	No data, read across
Reproductive toxicity – adverse effects ont the development of the offspring	No data, proposed read-across	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	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, proposed read-across	No data	Marked reduction in thymus size & cellularity; similar effects on the spleen and lymph nodes	No data	No data, proposed read-across	No data, proposed read-across	No data, read-across

Substance	Dibutyltin bis(2-ethylhexanoate) (DBTE)	Dibutyltin oxide (DBTO)	Dibutyltin dichloride (DBTC)	Dibutyltin maleate (DBTM)	Dibutyltin (di)acetate (DBTA)	Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	Dibutyltin dilaurate (DBTL)
CLP Classification	No harmonised classification	No harmonised classification	Acute Tox. 3*, H301 Acute Tox. 4*, H312 Acute Tox. 2*, H330 Skin Corr. 1B, H314 Muta. 2, H341 Repr. 1B, H360FD STOT RE 1, H372 Aquatic Acute 1, H400 Aquatic Chronic 1, H410	No harmonised classification	No harmonised classification	No harmonised classification (Adopted RAC opinion 5 Dec, 2017: Repr. 1B, H360FD STOT RE 1, H372. Based mainly on read-across from DBTC).	Muta. 2, H341 Repr. 1B, H360FD STOT RE 1, H372 (Based mainly on read-across from DBTC)

10 EVALUATION OF HEALTH HAZARDS

10.1 Acute toxicity – oral route

Not evaluated in this dossier.

10.2 Acute toxicity – dermal route

Not evaluated in this dossier

10.3 Acute toxicity – inhalation route

Not evaluated in this dossier

10.4 Skin corrosion/irritation

Not evaluated in this dossier.

10.5 Serious eye damage/eye irritation

Not evaluated in this dossier.

10.6 Respiratory sensitisation

Not evaluated in this dossier.

10.7 Skin sensitisation

Not evaluated in this dossier.

10.8 Germ cell mutagenicity

Table 12: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	**Test substance**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
In vitro gene mutation study in bacteria	DBTDL	Dose selection was based on a pre-test. Doses ranged between 0.005 and 5000 µg/plate	**Negative**, the test material was considered to be non-mutagenic under the conditions of this test.	Annex I 3.8.1.1 Anonymous, 2010 (DBTE registration dossier) Key study in the registration dossier
In vitro gene mutation study in bacteria	DBTO	Dose selection was based on a pre-test. Doses ranged between 1.25 and 20 µg/plate	**Negative**, the test material was considered to be non-mutagenic under the conditions of this test.	Annex I 3.8.1.2 Anonymous, 2002 (DBTE registration dossier) Key study in

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
				the registration dossier
In vitro gene mutation study in bacteria	DBTA	Dose selection was based on a pre-test. Doses ranged between 0.15 and 500 µg/plate	**Negative**, the test material was considered to be non-mutagenic under the conditions of this test.	Annex I 3.8.1.3 Anonymous, 2010. Not in the DBTE registration, taken from DBTA registration.
In vitro mammalian cell gene mutation assay – lung fibroblast	DBTC	Chinese hamster lung fibroblasts (V79) ± S9 metabolic activation. Test concentrations: -S9: 0.000001 to 0.000060 µl/ml; +S9: 0.00020 to 0.00050 µl/ml.	**Negative**, the test material did not show a mutagenic potential in the HGPRT/V79 mammalian cell gene mutation test neither - nor + S9 mix in two independently performed experiments.	Annex I 3.8.1.4 Anonymous, 1989 (DBTE registration dossier) Key study in the registration dossier
In vitro lymphocyte toxicity	DBTC	Lymphocytes isolated from Fischer 344 rats were tested without metabolic activation. Test concentrations: 9 - 75 µg/mL.	**Positive** without metabolic activation.	Annex I 3.8.1.5 Li *et al.*, 1982.
In vitro mammalian cell gene mutation assay	DBTC	Chinese hamster ovary (CHO) cells. Graded concentrations of DBTC dissolved in DMSO were added. The final concentration of DBTC was 0.05 - 0.3 µg/ml.	**Positive** without metabolic activation. DBTC induced mutations at the HGPRT gene locus in CHO cells.	Annex I 3.8.1.6 Li *et al.*, 1982
In vitro mammalian chromosome aberration test	DBTC	Human peripheral blood lymphocytes ± S9 metabolic activation. Test concentrations: -S9: 0.001 - 0.4 µl/ml; +S9: 0.050 - 3.0 µl/ml	**Positive**, the study indicates a clastogenic potential of the test material in the human lymphocyte test *in vitro* at cytotoxic concentrations. In the registration it is written that it was difficult to reproduce the result due to the substance's very steep toxicity curve. In spite of the cytotoxicity, the result is still considered solid enough since it is supported by other positive results and borderline negative results.	Annex I 3.8.1.7 Anonymous, 1990a (DBTE registration dossier) Key study in the registration dossier
Breakage of naked λ-DNA (±H_2O_2)	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 H_2O_2.	Annex I 3.8.1.8 Hamasaki *et al.*, 1995
Bacterial reverse mutation	DBTC	Doses ranged between 0.1 and 10 µg/tube.	**Positive** without metabolic activation.	Annex I 3.8.1.9 Hamasaki *et al.*, 1993

Method, guideline, deviations if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
assay				
Bacterial SOS chromotest and rec-assay	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.	Annex I 3.8.1.10 Hamasaki *et al.*, 1992
Condensate formation with DNA	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.	Annex I 3.8.1.11 Piro *et al.*, 1992
Effect on spindle structure in V79 Chinese hamster cells	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).	Annex I 3.8.1.12 Jensen *et al.*, 1991a
Aneuploidy in human peripheral lymphocytes	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	Annex I 3.8.1.13 Jensen *et al.*, 1991b
Effect on spindle-inhibition as chromosomal contractions in human lymphocytes	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.	Annex I 3.8.1.14 Jensen *et al.*, 1989

Table 12: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Micronucleus assay	DBTC	ICR Mice were given a single oral dose of DBTC at 2, 10 or 50 mg/kg bw. Five mice/sex/group were terminated 24, 48 and 72 hours after treatment. Dose selection was based on a preliminary toxicity test. A total of at least 2000 bone marrow erythrocytes per animal were examined.	**Positive**, a biologically and 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 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	Annex I 3.8.2.1 Anonymous, 1991 (DBTE registration dossier) Key study in the registration dossier

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
			control group animals given chlorambucil at 30 mg/kg bw ($p<0.01$).	
Micronucleus assay	DBTC	NMRI mice were given a single oral dose of DBTC at 0, 50, 100 and 200 mg/kg bw. Five mice/sex/group were terminated 24, 48 and 72 hours after treatment. Dose selection was based on a preliminary toxicity test. The slides were examined for the incidence of micro-nucleated cells per 2000 polychromatic (PCE) and 1000 normo-chromatic (NCE) erythrocytes per animal.	**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 bw. Triaziquone, the positive reference, gave the expected mutagenic response.	Annex I 3.8.2.2 Anonymous, 1990b (DBTE registration dossier)
DNA damage in rat cerebral cortical cells	DBTDL	10 rats/dose group were gavaged with DBTDL in corn oil at doses 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.	**Positive**, a significant dose-dependent increase in DNA damage was seen in rat cerebral cortical cells.	Annex I 3.8.2.3 Jin *et al.*, 2012.

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

No mutagenicity study have been performed with DBTE. However, using the rationale of the category approach explained above (9.2.2), and based on the knowledge of the toxicokinetic and hydrolytic behaviour of the substances in the category, it is justified to use data on the other substances in the category to assess the mutagenic potential of DBTE. We have therefore included studies performed with category substance, especially DBTDL and DBTC, which both are classified as mutagenic in category 2.

Repeated DBTDL gavage administration in rats increases *in vivo* DNA damage in isolated cerebral cortical cells (Jin, 2012). Studies performed with DBTC show a variable outcome both for *in vitro* and *in vivo* studies, but overall most studies are positive. Positive *in vitro* mutagenicity (Li, 1982; Study report 1990; Hamasaki, 1993) and genotoxicity tests exist where the latter indicate clastogenicity (Study report 1990; Anonymous, 1991) and effects on spindle formation during mitosis (Jensen, 1991a). 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). One *in vivo* GLP study (OECD TG 474 (Mammalian Erythrocyte Micronucleus Test)) assessing mutagenicity in mice is positive, at the highest dose (50 mg/kg bw) (Anonymous, 1991). A similar GLP *in vivo* study (a Mammalian Erythrocyte Micronucleus Test in mice) observed no

mutagenicity up to 200 mg/kg bw (Anonymous, 1990b). 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 study of Dance (1991) is taken forward for the evaluation. Overall, the studies show a mixed outcome both for *in vitro* and *in vivo* studies, but in general most studies are positive.

10.8.2 Comparison with the CLP criteria

The CLP Regulation states:

> **Annex I:** ***3.5.2.1*** *This hazard class is primarily concerned with substances that may cause mutations in the germ cells of humans that can be transmitted to the progeny. However, the results from mutagenicity or genotoxicity tests in vitro and in mammalian somatic and germ cells in vivo are also considered in classifying substances and mixtures within this hazard class.*

Subtances can be classified in category 1A, 1B or 2. Substances in category 1 are:

> *"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."*

Substances in category 2 are:

> *"Substances which cause concern for humans owing to the possibility that they may induce heritable mutations in the germ cells of humans."*

Classification in Category 1A

Classification in Category 1A demands evidence for classification primarily from human data. There is no available human data. Muta. 1A is therefore not relevant.

Classification in Category 1B

In Category 1B evidence is primarily from animal data, either positive *in vivo* heritable germ cell mutagenicity, or *in vivo* somatic cell mutagenicity tests in combination with some evidence that the substance has potential to cause mutations to germ cells.

There are no germ cell mutagenicity studies available, neither for DBTE nor for any of the category substance. There is not sufficient evidence either for a potential to cause mutations to germ cells.

Classification in Category 2

Substances are classified in Category 2 when there is positive evidence from studies in mammals and/or in some cases from *in vitro* tests, obtained from: *in vivo* somatic cell studies in mammals, or other *in vivo* somatic cell tests which are supported by positive results from *in vitro* studies.

For DBTC there is one positive well conducted (GLP-compliant) *in vivo* somatic cell mutagenicity test (Anonymous, 1991) as well as supporting evidence from positive results from *in vitro* mutagenicity/genotoxicity tests. Thus, DBTC is a suspected germ cell mutagen. DBTC has a harmonised classification with Muta. 2; H341. There is also one positive study with DBTDL where there is evidence of genotoxicity in isolated brain cells (Jin, 2012). DBTDL also has a harmonised classification as Muta. 2; H341. Based on the category approach it is justified to classify DBTE in the same way as DBTC and DBTDL.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

Classification of DBTE for germ cell mutagenicity in Category 2; H341 "Suspected of causing genetic defects", is considered warranted.

10.9 Carcinogenicity

Not evaluated in this dossier.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 26: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Reproductive/ developmental toxicity screening study in the rat. OECD 421 Rats, Wistar (12/sex/group)	DBTC Dose levels: 0, 5, 30 or 200 ppm for 4 weeks (males) or for 2 weeks prior to mating and to day 4 or 6 post partum (females).	200 ppm diet: reduced maternal weight gain (values not reported). Increase in number of ovarian cysts in the high-dose females. Reduced number of foetuses (10 compared to 101 in controls), reduced litter size (6.0 compared to 11.3). The number of corpora lutea were not measured and so the extent of pre-implantation loss is not known. Although the reduced number of foetuses/litters may be due to developmental toxicity, it is included here because these effects are also considered as relevant for classification for fertility, in line with the RAC opinion for DBTP (RAC opinion, adopted 5 dec 2017). **NOAEL = 30 ppm (1.7-2.4 mg/kg bw/d)** **LOAEL = 200 ppm (12.0-15.4 mg/kg bw/d)**	Annex I 3.10.1.1 Waalkens-Berendsen 2003 (study summary in DBTC registration dossier) Key study in the registration
Adverse effects of DBTC on initiation and maintenance of rat pregnancy. Non-guideline study. Rats, Wistar (16-19/group)	DBTC Dose levels: 0, 3.8, 7.6 or 15.2 mg/kg bw/d on GD 0-3 or GD 4-7. Rats terminated at GD 20	Maternal toxicity at ≥3.8 mg/kg bw/d (clinical signs), weight loss during early gestation (D0-4) 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 for groups exposed on GD 0-3. **LOAEL =3.8 mg/kg bw/d** **NOAEL =7.6 mg/kg bw/d**	Annex I 3.10.1.5 Ema & Harazono, 2000
Early pregnancy failure induced by DBTC in mice. Non-guideline study. Mice, (CRlj:CD1(ICR) (12/group)	DBTC Dose levels: 0, 7.6, 15.2 or 30.4 mg/kg bw on GD 0-3 or GD 4-7	Maternal toxicity (mortality not dose-related (0/12, 2/12, 1/12, 1/12 at 0, 7.6, 15.2 and 30.4 mg/kg bw/d, respectively), clinical signs, reduced weight gain GD 0-4 (-82%; 0.3 g vs 1.7 g in control) 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%). 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 for groups exposed on GD 0-3. Serum progesterone levels were significantly lower in mice administered DBTC at the high dose on both GD 0-3 and GD 4-7 of pregnancy. **LOAEL =7.6 mg/kg bw/d** **NOAEL <7.6 mg/kg bw/d**	Annex I 3.10.1.12 Ema *et al.*, 2007a

Table 13: Summary table of human data on adverse effects on sexual function and fertility

No human data

Table 14: 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
Investigation of the effects of progester-one on implantation failure. Non-guideline	DBTC	Rats, wistar, 14-15/group Dose levels: 0, 7.6, 15.2 mg/kg bw/day on GD 0-3, with or without progesterone supplementation (subcutaneous injection of 2 mg progesterone GD 0-8).	Administration of progesterone on GD 0-8 offered some protection against implantation failure. 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 60.0% with progesterone. Marked weight loss and reduced food consumption were observed at both dose levels of DBTC. These effects were reduced slightly by the administration of progesterone at 7.6 mg/kg bw/d, however progesterone administration had little effect at 15.2 mg/kg bw/d.	Annex I 3.10.3.3 Ema *et al.*, 2003
Investigation of the effects on decidual cell response in pseudo-pregnant rats Non-guideline	DBTC	Rats, wistar Dose levels: 0, 3.8, 7.6, 15.2 mg/kg bw/day 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.	DBTC 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.	Annex I 3.10.3.4 Harazono & Ema, 2003

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

No data is available for DBTE. However, using the rationale of the category approach explained above (9.2.2), and based on knowledge of the toxicokinetic and hydrolytic behaviour of the substances in the category, it is justified to use data for the other substances in the category to assess the reproductive toxicity potential of DBTE. We have therefore included studies performed with DBTC, a substance that is classified as a reproductive toxicant in category 1B; H360F.

In a guideline (OECD TG 421) screening study with rats (Waalkens-Berendsen, 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 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). Body weight effects were also seen in high-dose males (significantly lower mean body weight from Day 14-28 and significantly lower weight gain over the study period). Significantly lower body weight gain in males from Day 14-21 in the mid-dose group was also seen. There was also a significant increase in the incidence of ovarian cysts in the high-dose females. Gross necropsy and histopathology of males did not reveal any effects of treatment on the reproductive tract. Only 3 of the 7 pregnant females at the highest dose level delivered live offspring. The number of pregnant females in the high and mid-dose groups (both 7/12) was slightly lower than in the control group (9/12). Although the reduction in number of pregnant females is not significant and without a dose-

respons, it supports the other positive findings on pre-implantation loss. Corpora lutea numbers were not measured in this study, therefore the extent of pre-implantation loss cannot be assessed. Although the reduced number of foetuses/litters may be due to developmental toxicity, it is included here because these effects are also considered as relevant for classification for fertility, in line with the RAC opinion for DBTP (RAC opinion, adopted 5 Dec. 2017). An effect of treatment on fertility at the highest dose level, cannot be excluded. The full study report is not available and summary data are taken from the publically disseminated REACH Registration Dossier for DBTP, DBTA, the CLH report for DBTDL (ECHA, 2014) and the CLH report for DBTP (ECHA, 2016). Complete details on the study methodology and findings, such as values for maternal bodyweight and bodyweight gain are therefore not available, therefore the extent of maternal toxicity seen at the highest dietary concentration of 200 ppm cannot be fully assessed. We do not have details about the effects seen at the mid-dose group (30 mg/kg bw/day).

A number of developmental toxicity studies performed with DBTC report effects of treatment on implantation which, for the purposes of classification, is considered relevant to assess effects on sexual function and fertility. Although these published studies are non-guideline and may be of variable quality, they are considered to be sufficiently robust to support the classification proposal as part of a weight of evidence.

A published study by Ema & Harazono (2000) studied the effects of DBTC administration during early gestation in the rat. Treatment on GD 0-3 with DBTC at dose levels of 7.6 and 15.2 mg/kg bw/d 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 at 7.6 mg/kg bw/d (11/16) and 15.2 mg/kg bw/d (2/16). There was maternal weight loss in all treated groups on GD 0-4. In assessing this study in 2017 (RAC opinion for DBTP, ECHA 2017), RAC concluded that the reproductive effects seen in this study are not due to a secondary non-specific consequence of parental toxicity.

A study by Ema *et al.* (2007a) with DBTC in CD1 mice 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. A small number of maternal 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 related to treatment with DBTC. Other signs of maternal toxicity seen in this study were clinical signs, jaundice (at 15.2 and 30.4 mg/kg bw/d), and moderate reductions in food consumption and weight gain, however adjusted body weight gain was not statistically significantly reduced in any of the exposed groups. The effect on pre-implantation loss is considered a direct effect of DBTC and not a secondary consequence of maternal toxicity.

Mechanistic studies in the rat, performed with DBTC, may indicate that the failure of implantation could be due to a suppression of the decidual cell response and reduction in circulating progesterone levels (Harazono & Ema, 2001; Harazono & Ema, 2003). They also show that administration of progesterone may give some protection against pre-implantation loss (Ema *et al.*, 2003). 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%). In groups of rats receiving DBTC + progesterone, 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. In Ema *et al.* 2003, maternal toxicity was evident by a marked body weight loss in both treated groups (both +/- progesterone) but it was still possible to see an effect of progesterone on the pre-implantation loss. Body weight gain days 0-4 in grams (-/+progesterone respectively) was 8/7 (control group), -24/-24 (low dose) and -31/-28 (high dose) and for days 4-9: 12/14 (control group), -11/-22 (low dose), and -35/-31g in the high dose group. Food consumption days 0-4 in grams (-/+ progesterone respectively) was 48/46 (control group), 10/9 (low dose) and 4/3 (high dose); and for days 4-9: 80/78 (control group),

25/15 (low dose), and 2/4 (high dose). The mechanistic studies do not raise a doubt about the relevance of the observed implantation failure for humans.

10.10.3 Comparison with the CLP criteria

According to the CLP Regulation adverse effects on sexual function and fertility is defined as follows:

> **Annex I*: 3.7.1.3.*** *Adverse effects on sexual function and fertility*
> *Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is 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 systems.*

Substances can be classified in category 1A, 1B or 2. Substances in category 1 are:

" *Known or presumed human reproductive toxicant"*

Substances in category 2 are:

"Suspected human reproductive toxicant"

To be classified in category 1 a substance is known to have produced an adverse effect on sexual function and fertility or development in humans, or when there is evidence from animal studies providing a strong presumption that the substance has the capacity to cause effects in humans.

Classification in Category 1A
Classification in category 1A demands evidence for classification primarily from human data.
There is no available human data. Repr. 1A is therefore not relevant.

Classification in Category 1B:
In category 1B evidence is primarily from animal data. 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.

Classification in Category 2
Substances are classified in Category 2 when there is some evidence from experimental animals 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. Effects are relevant for classification where these have been observed in the absence of other toxicity, if the adverse effect on reproduction is considered not to be a secondary non-specific consequence other toxicity.

There are no studies on sexual function and fertility performed with DBTE. There are however relevant studies performed with the dibutyltin-category member DBTC. Data clearly show that DBTC causes marked effects on fertility in studies in the rat and mouse, through a reduction in implantations. Effects are seen at maternally toxic dose levels, including relatively high dose levels causing marked bodyweight effects, reduced food consumption, signs of toxicity and possible mortality. At lower dose levels, where there is less maternal toxicity however, marked increases in the level of pre-implantation loss are still apparent (Ema and Harazano, 2000; Ema et al, 2007a).

Even at maternally toxic levels the effects on pre-implantation could not be ascribed to maternal toxicity, as seen in the study with pair-fed rats (Ema and Harazano, 2000) where the high-dose group had significant higher pre-implantation losses compared to the rats that experienced the same weight loss due to feed deprivation to mimick the effects in the high dose group. The data suggest therefore, that the adverse effect on reproduction is not a secondary non-specific consequence of other toxicity. In addition, mechanistic data suggest that the increased level of pre-implantation loss may be due to a reduction in circulating progesterone levels, and the mechanistic studies do not raise a doubt about the relevance of the observed implantation failure for humans.

DBTC has a harmonised classification with Repr. 1B; H360F. DBTDL also has a harmonised classification with Repr. 1B; H360F, largely based on data from DBTC. Also for DBTP RAC adopted a harmonised classification with Repr. 1B; H360F in 2017.

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

10.10.4 Adverse effects on development

Table 15: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Reproductive/ developmental toxicity screening study in the rat. OECD TG 421 Wistar rat, 12/sex/group	DBTC Dose levels: 0, 5, 30 or 200 ppm for 4 weeks (males) or for 2 weeks before mating and to Day 4/6 post partum (females).	200 ppm diet: reduced maternal weight gain (values not reported). A marked increase in post-implantation loss was seen at 200 ppm; only three females in this group had live offspring. Reduced numbers of foetuses (10 compared to 101 in controls), reduced litter size (6.0 compared to 11.3). 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%). **NOAEL = 30 ppm (1.7-2.4 mg/kg bw/d)** **LOAEL = 200 ppm (12.0-15.4 mg/kg bw/d)**	Annex I 3.10.1.1 Waalkens-Berendsen 2003 (study summary in DBTC registration dossier)
Developmental toxicity study in the rat OECD TG 414 Wistar rat, 25/group	DBTC Dose levels: 0, 1, 2.5, 5, 10 mg/kg bw/d GD 6-15	Maternal toxicity at 5 mg/kg bw/d (slightly reduced weight gain) and 10 mg/kg bw/d (reduced weight gain and food consumption); values not reported. Marginally increased foetal malformations 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. **LOAEL =10 mg/kg bw/d** **NOAEL =5 mg/kg bw/d**	Annex I 3.10.1.2 Study report 1994 (DBTE registration dossier) Key study in the registration
Developmental toxicity in the rat – susceptible period for teratogenicity	DBTC Dose levels: 0 and 20 mg/kg bw/d on GD:	Details on maternal toxicity not reported. GD 7-9: increased resorption (9.9) compared to controls (1.3) and increased post-implantation loss (75.1% compared to 10.2% in control group). 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	Annex I 3.10.1.3 Ema *et al.*, 1992

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Non-guidline Wistar rat, 11/group	7-9, 10-12 or 13-15. 0, 20, 40 mg/kg bw/d on GD: 6, 7, 8 or 9	(largely omphalocoele and jaw defects) GD 10-12: reduced foetal weight (~15%); no malformations. GD 13-15: reduced foetal weight (~20%); no malformations. GD 6: increased post-implantation loss at 20 (18.9%) and 40 mg/kg bw/d (43.5%); total resorption at 20 (1/11) and 40 mg/kg bw/d (3/11). Marginal increase in malformations at 40 mg/kg bw/d. GD 7: increased post-implantation loss at 20 (24.6%) and 40 mg/kg bw/d (76.2%); total resorption at 20 (1/11) and 40 mg/kg bw/d (7/11). Increase in malformations at 20 and 40 mg/kg bw/d. GD 8: increased post-implantation loss at 20 (42.8%) and 40 mg/kg bw/d (79.7%); total resorption at 20 (3/11) and 40 mg/kg bw/d (7/11). Increase in malformations at 20 and 40 mg/kg bw/d. GD 9: increased post-implantation loss at 40 mg/kg bw/d (31.7%); total resorption at 40 mg/kg bw/d (3/11). Marginal increase in malformations at 20 mg/kg bw/d. The results of this study identity Gestation Day 7-8 as the critical period for DBTC-mediated teratogenicity in the rat; the most sensitive period was shown to be GD 8. Malformations were not induced following exposure on GD 6 or on GD 9 or later. Exposure at later time points resulted in post-implantation loss, reduced litter size and reduced foetal weight. **LOAEL =20 mg/kg bw/d** **NOAEL <20 mg/kg bw/d**	
Developmental toxicity study in the rat – various di-n-butyltins with different anions Wistar rat, 10/group	DBTA and various di-n-butyltins at GD 8. Dose levels: 80 µmol/kg bw corres-ponding to: DBTA: 28 mg/kg bw DBTC: 25 mg/kg bw DBTDL: 50 mg/kg bw DBTM: 28 mg/kg bw DBTO: 20 mg/kg bw	For all substances: no maternal mortality or signs of maternal toxicity. A significantly higher incidence of external and skeletal foetal malformations/variations was observed in all the treated groups; the nature of malformations was similar in all groups. External malformations: exencephaly and mandible findings (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 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. **DBTA**: **LOAEL = 28 mg/kg bw** **DBTC**: **LOAEL = 25 mg/kg bw** **DBTDL: LOAEL = 50 mg/kg bw** **DBTM**: **LOAEL = 28 mg/kg bw** **DBTO**: **LOAEL = 20 mg/kg bw**	Annex I 3.10.1.4 Noda *et al.*, 1993
Adverse effects of DBTC on	DBTC	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).	Annex I

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
initiation and maintenance of rat pregnancy. Non-guideline study. Rats, Wistar (16-19/group)	Dose levels: 0, 3.8, 7.6 or 15.2 mg/kg bw/d on GD 0-3 or GD 4-7. Rats terminated at GD 20	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. Litter size and mean foetal weights were significantly reduced at ≥7.6 mg/kg bw/d. **LOAEL =3.8 mg/kg bw/d** **NOAEL <3.8 mg/kg bw/d**	3.10.1.5 Ema & Harazano, 2000
Developmental toxicity study in the rat Non-guideline Wistar rat, 10-12/group	DBTC Dose levels: 0, 2.5, 5.0, 7.5, 10 mg/kg bw/d GD 7-15	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 at 5 mg/kg bw/d. 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. **LOAEL $_{teratogenicity}$ =5 mg/kg bw/d** **NOAEL $_{teratogenicity}$ =2.5 mg/kg bw/d**	Annex I 3.10.1.6 Ema *et al.*, 1991
Developmental toxicity study in the rat Non-guideline Wistar rat, 10/group	DBTC Dose levels: 0, 10, 15 mg/kg bw/d GD 7-8	Maternal toxicity: reduced adjusted weight gain at 15 mg/kg bw/d (-25% as compared to controls, but not statistically significant), with weight loss on GD 7-9 (8g, -5 g, -8 g at 0, 10 and 15 mg/kg bw/d respectively). 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. Administration of DBTC to at dose levels of 10 and 15 mg/kg bw on Days 7-8 of gestation results in embryolethality and teratogenicity. Findings were associated with minor maternal toxicity (not statistically significant reduced adjusted weight gain in the high-dose group). Teratogenicity was characterised by increased incidences of external, skeletal and visceral malformations. **LOAEL =10 mg/kg bw/d** **NOAEL <10 mg/kg bw/d**	Annex I 3.10.1.7 Ema *et al.*, 1995b
Developmental toxicity study in the rat – late organogenesis Non-guideline Wistar rat,11-	DBTC Dose levels: 0, 50, 100 mg/kg bw/d GD 13-15	Maternal toxicity at 50 and 100 mg/kg bw/d: mortality at 50 (1/11) and 100 mg/kg bw/d (3/13), there was weight loss at both dose groups from GD 13 giving a reduced weight gain of -70%, -88%. Also, the adjusted weight gain was negative in both dose groups (-13 and -26 g in 50 and 100 mg/kg bw/d) (statistically significant) whereas the control animals gained 38g. Reduced foetal weight at 50 (-29%) and 100 mg/kg bw/d (-34%). Increased post-implantation loss at 50 (22.0%) and 100 mg/kg bw/d (34.4%) compared to	Annex I 3.10.1.8 Ema *et al.*, 1996b

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
13/group		controls (9.8%). No clear increase in foetal malformations. 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. **LOAEL =50 mg/kg bw/d** **NOAEL <50 mg/kg bw/d**	
Critical gestational day of teratogenesis by DBTA in rats Non-guideline study Wistar rat, 9-10/group	DBTA Dose levels: 15 mg/kg bw/d: on GD 7-9 or 10-12, or 13-15 or 16-17. 0, 15, 30 mg/kg bw/d: GD 7-9 or 0, 5.0, 7.2, 10.5, 15.2, 22 mg/kg bw/d: GD 8	Details on maternal toxicity not reported. Rats treated with DBTA at 15 mg/kg bw/d for 2 or 3 consecutive days were most susceptible to teratogenesis on GD 7-9: higher number of resorptions (ca 40% resorbed/dead fetuses on GD 7-9, compared to 10-20% in animals treated on later GD); and higher number of malformed foetuses were observed (4 out of 5 litters, and 31.5% of fetuses, compared to no fetuses with external or skeletal malformations in the other 3 groups treated with DBTA on days 10-12, 13-15 and 16-17). Rats administered single doses of DBTA on GD 8 had the highest proportion of foetal malformations (28/33 malformed fetuses); treatment on GD 7 to 9 resulted in a lower frequency of malformations (5/33 malformed fetuses). The incidence of foetal malformations was significantly increased at 30 mg/kg bw/day of DBTA. External malformations observed in the DBTA treated rats included cleft mandible, cleft lower lip, ankyloglossia, schistoglossia and exencephaly. 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. **NOAEL =10.5 mg/kg bw** **LOAEL =15.2 mg/kg bw**	Annex I 3.10.1.9 Noda *et al.*, 1992a
Developmental toxicity study in the rat. Comparable to OECD TG 414 Wistar rat, 13-16/group	DBTA Dose levels: 0, 1.7, 5, 10, 15 mg/kg bw/d GD 7-17	Maternal toxicity (reduced weight gain) at 15 mg/kg bw/d (weight at GD 20 254 g compared to 314 g in control rats), however dams with living fetuses at 15 mg/kg bw/day did not have reduced weight gain (corrected body weight gain is not specified in the study). Reduced numbers of dams with viable foetuses at 15 mg/kg bw/d (7/16) due to foetal loss and total resorption (9/16). Reduced foetal weight at 10 mg/kg bw/d (~18%) and 15 mg/kg bw/d (~26%). External and skeletal malformations increased at ≥5 mg/kg bw/d (mainly cleft mandible, cleft lower lip, ankyloglossia and schistoglossia). The results of this study demonstrate that DBTA is teratogenic in the rat **LOAEL =5 mg/kg bw/d** **NOAEL =1.7 mg/kg bw/d**	Annex I 3.10.1.10 Noda *et al.* 1992b
Developmental toxicity study in the rat. Non-guideline study Wistar rat, 12-	DBTA Dose levels: 0, 7.5, 10, 15, 22 mg/kg bw/d GD 8	Reduced maternal weight gain at 22 mg/kg bw/d in 7.5 month-old dams (-33%), but the adjusted body weight gain was not statistically significantly reduced compared to the control group. Reduced number of litters with viable foetuses (6/13) due to total resorption (5/13) at 22 mg/kg bw/d (7.5 month-old dams). Implantation loss increased at 22 mg/kg bw/d in 3-month old (19.2%), 7.5 month-old (37.8%) and 12 month-old dams (95.2%). Foetal malformations at ≥7.5 mg/kg bw/d (typically cleft mandible, cleft lower lip, ankyloglossia, schistoglossia, fused ribs, fused cervical and	Annex I 3.10.1.11 Noda *et al.*, 2001

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
14/groups of different ages: 3, 7.5 or 12 months at mating		vertebral arches). The study confirms that GD 8 is the susceptible period for teratogenesis caused by DBTA. The results of this study also indicate an influence of maternal age on the susceptibility of the rat to the developmental toxicity of DBTA. **NOAEL <7.5 mg/kg bw/d** **LOAEL =7.5 mg/kg bw/d**	
Early pregnancy failure induced by DBTC in mice. Non-guideline study. Mice, (CRlj:CD1(ICR) (12/group)	DBTC Dose levels: 0, 7.6, 15.2 or 30.4 mg/kg bw on GD 0-3 or GD 4-7	In mice exposed G 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 (+0.5 g) and 30.4 mg/kg bw (-0.3 g) compared to +3.1 g in controls. Adjusted weight gain was also significantly reduced for the mid- and high dose group (8.3 g, 8.1 g, 3.2 g and 3.8 g in control, low, mid and high dose groups respectively). Food consumption was reduced at 15.2 mg/kg bw (~25%) and 30.4 mg/kg bw (~28%). Total resorption at 7.6 (2/12), 15.2 (8/12) and 30.4 mg/kg bw (10/12). Increased post-implantation loss at all dose levels (7.6: 48.3%; 15.2: 94.4% and 30.4: 100%) for groups exposed on GD 4-7. Marginal increase in malformations at 7.6 mg/kg bw (omphalocoele, exencephaly). This was not seen at 15.2 mg/kg bw, however the number of pups examined was very low. Serum progesterone levels were significantly lower in mice administered DBTC at the high dose on both GD 0-3 and GD 4-7 of pregnancy. **LOAEL =7.6 mg/kg bw/d** **NOAEL <7.6 mg/kg bw/d**	Annex I 3.10.1.12 Ema *et al.*, 2007a
Developmental toxicity study in the monkey Non-guideline study Cynomolgus monkey, 10-12/group	DBTC Dose levels: 0, 2.5, 3.8 mg/kg bw/d on GD 20-50	Maternal toxicity (clinical signs, weight loss on GD 20-51 (-556 g) and reduced food consumption) at 3.8 mg/kg bw/d; clinical signs, weight loss on GD 20-51 (-242 g) and reduced food consumption at 2.5 mg/kg bw/d. 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 1/12 controls. No external, visceral or skeletal malformations/variations were observed in any group. **LOAEL =2.5 mg/kg bw/d** **NOAEL <2.5 mg/kg bw/d**	Annex I 3.10.1.13 Ema *et al.* 2007b
Developmental toxicity study in the monkey Non-guideline study Cynomolgus monkey, 5/group; 12 controls	DBTC Dose levels: 0, 7.5 mg/kg bw/d: GD 19-21, 21-23, 24-26, 26-28, 29-31, 31-33, or 34-36	Maternal signs of toxicity were marginally reduced weight gain and some vomiting/diarrhea. Embryofoetal loss (GD 19-21 (1/5), 24-26 (2/5), 34-36 (1/5) compared to 1/12 controls. There were no effects 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. **LOAEL =7.5 mg/kg bw/d** **NOAEL <7.5 mg/kg bw/d**	Annex I 3.10.1.14 Ema *et al.*, 2009
Developmental toxicity study in	DBTC Dose levels:	Maternal toxicity (reduced weight gain on GD 6-16 (-17%) & reduced food consumption (-7%)) at 10 mg/kg bw/d	Annex I 3.10.1.15

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
the rat. OECD TG 414 Wistar rat, 25/group	0, 1, 2.5, 5, 10 mg/kg bw/d GD 6-15	Marginal increase in malformations (including single incidences of ankyloglossia, agnathia, mandibular defect at 10 mg/kg bw/d). **LOAEL = 10 mg/kg bw/d** **NOAEL =5 mg/kg bw/d**	Farr *et al.*, 2001

Table 16: Summary table of human data on adverse effects on development

No human data

Table 17: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Dysmorphogenic effects of di-n-butyltin dichloride in cultured rat embryos. Non-guideline Cultured Wistar rat embryo	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. 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. The study indicates that exposure of explanted GD 8 rat embryos to DBTC *in vitro* at concentrations of ≥3 ng/mL causes dysmorphogenesis.	Annex I 3.10.3.1 Ema *et al.*, 1995a
Change of embryotoxic susceptibility to di-n-butyltin dichloride in cultured rat embryos. Non-guideline Cultured rat embryo	DBTC	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.	GD 8.5-embryos: decreases in placental diameter (≥10 ng/mL) and number of somite pairs and the morphological score (30 ng/mL). 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). GD 11.5-embryos: no adverse effects were seen in. 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. The study shows that exposure to DBTC interferes with normal embryonic development during three different stages	Annex I 3.10.3.2 Ema *et al.*, 1996a

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			of organogenesis, and that susceptibility to the embryotoxicity and dysmorphogenic potential of DBTC varies with developmental stage	

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

No developmental toxicity studies are performed with DBTE. However, using the rationale of the category approach explained above (9.2.2), and based on knowledge of the toxicokinetic and hydrolytic behaviour of the substances in the category, it is justified to use data for the other substances in the category to assess the reproductive toxicity potential of DBTE. We have therefore included studies performed with DBTC, a substance that is classified as a reproductive toxicant in category 1B; H360D, and four studies with DBTA which is proposed classified as a reproductive toxicant 1B; H360D. One study is also performed with DBTDL, DBTM, DBTO, and DBTA in addition to DBTC and compares the toxicity of these category substances.

Four developmental studies by Noda and co-workers are performed with DBTA. Three of the studies (1992a, 1992b and 2001) investigate DBTA's teratogenic potential in general, and two of these (1992a and 2001) more particularily which days of gestation are most sensitive to DBTA's teratogenic effect. The investigations showed that administration of DBTA on GD 7 resulted in foetal malformations including cleft mandible, cleft lower lip, ankyloglossia, schistoglossia and exencephaly (Noda *et al.*, 1992a). No information was included in the study regarding maternal toxicity. 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). Maternal toxicity was observed at 15 mg/kg bw/d (reduced weight gain) but not at 10 mg/kg bw/d. Since maternal toxicity was not seen at 10 mg/kg bw/day in the presence of developmental toxicity the effect of DBTA on the fetuses is not considered secondary to maternal toxicity. The 2001 study by Noda *et al.*, investigated also the effects of maternal age on the teratogenicity of DBTA administered on GD 8. Malformations were seen in foetuses from 3 month old dams at dose levels of ≥15 mg/kg bw/d and in foetuses from 7.5 month-old dams at ≥10 mg/kg bw/d (significantly different from control). Malformations (cleft mandible, cleft lower lip, ankyloglossia and/or schistoglossia) were comparable in both groups. In the 12-month old dams the number of living foetuses was very low in all dose groups (including control) so no relevant findings were seen. Adjusted body weight gain was not statistically reduced in any dose group, or age group. No other signs of maternal toxicity was reported.

The fourth study by Noda *et al.* (1993) is a comparative study with DBTA, DBTC, DBTM, DBTDL and DBTO. After a single gavage administration on GD 8, a comparable spectrum of effects for all substances were seen, in the absence of maternal toxicity. The study used dose levels of 80 µmol/kg bw, equivalent to dose levels of 25 mg/kg bw (DBTC), 50 mg/kg bw (DBTDL), 28 mg/kg bw (DBTA), 28 mg/kg bw (DBTM) and 20 mg/kg bw (DBTO). Treatment showed a comparable incidence of foetal malformations for DBTC (17.3%), DBTA (28.3%), DBTDL (30.6%), DBTM (12.5%) and DBTO (20.7%) (0% in controls). The nature of foetal malformations (predominantly jaw defects (cleft mandible, cleft lower lip, ankyloglossia or schistoglossia) and exencephaly) was also broadly comparable with the exception of no exencephaly noted for DBTM. The results of this 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.

Two guideline studies are performed with DBTC. A screening study (OECD TG 421) with rats (Waalkens-Berendsen, 2003) and 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) showed 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 high dose level produced live offspring due to a very high level of post-implantation loss (87.6% compared to 13.4% in controls). In addition a significantly lower pup weight and increased pup mortality was seen.

The other guideline study (OECD TG 414) was performed with DBTC at dose levels of 2.5, 5 or 10 mg/kg bw/d (Study report, 1994). The main effect seen in dams was reduced weight gain and food consumption at 10 mg/kg bw/d. There was also a slightly reduced weight gain in dams at 5 mg/kg bw/d. The original study report is not available; therefore full methodological details and tabulated results are not available. There seems however to be a clear increase in the incidence of foetuses with malformations at 10 mg/kg bw/d (four foetuses from three litters). Oedema (anasarca) was observed in one foetus; the remaining three foetuses showed more severe malformations. One foetus 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. A maternal NOAEL of 5 mg/kg bw/d can therefore be determined for this study, based on reduced weight gain at 10 mg/kg bw/d. A NOAEL for teratogenicity of 5 mg/kg bw/d can be determined, based on the increased incidence of foetal malformations at the highest dose level of 10 mg/kg bw/d.

Several published studies are also available, which do not fully comply with OECD TG 414 but which are considered to be sufficiently robust to support the classification proposal as part of a weight of evidence.

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 external, skeletal and visceral malformations were seen. These malformations (predominantly exencephaly and mandibular defects) are characteristic of those induced by dibutyltin compounds in other studies. Although maternal toxicity was observed in this study on GD 7-9 (slight weight loss, however adjusted weight gain was not significantly reduced) 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.

Another study by Ema *et al.* (1991) reported an increase in foetal 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. 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), but not at 2.5 or 5 mg/kg bw/d. 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) however other malformations were also frequently observed. Increased malformation incidences were observed at 5 mg/kg bw/d in the absence of overt maternal toxicity.

Further work by the same authors (Ema *et al.*, 1992) with higher dose levels of 20 or 40 mg/kg bw/d, given at different GD intervals identified 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 only information on maternal toxicity in the study was that there were no maternal deaths.

Farr *et al.* (2001) administered DBTC to rats on GD 6-15. The highest dose level, 10 mg/kg bw/d, resulted in a slightly increased frequency of foetal malformations (1.5% compared to 0.4% in controls). The only data on maternal toxicity was reduced maternal weight gain (55.7 g in highdose group, compared to 67.2 g in control group) and food consumption (23.7 g in highdose group, compared to 25.5 g in control group). The reductions were not very big, but were statistically significant. Adjusted body weight gain was not reported. 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 and should therefore be considered to be potentially related to treatment, in the absence of marked maternal toxicity.

Ema & Harazono (2000) focused on the effects of DBTC administration during early gestation in the rat. Treatment on GD 4-7 with DBTC at dose levels of 3.8, 7.6 and 15.2 mg/kg bw/d resulted in statistically siginificantly increased post-implantation loss (13.9%, 39.9% and 91.5% compared to 7% in the control group). No increase in foetal malformations was seen. There were some signs of maternal toxicity (initial weight loss on GD 4-8 and 14 and 86% decrease in adjusted weight gain as compared to controls at 7.6 and 15.2 mg/kg bw/d). However, a group of pair-fed rats were provided with the same amount of diet as consumed by rats administered the test material at 15.2 mg/kg bw/d. This group of rats experienced the same weight loss, and the adjusted weight gain was even lower than in the high dose group, and yet post-implantation loss was much lower (18.3%) and not significant, showing that the effects on post-implantation seen in the treated groups were not secondary effects to maternal toxicity.

Ema *et al*. (1996b) showed a reduction in foetal weight, and increased post-implantation loss in both dose groups (the latter finding not statistically significant) but there was no evidence of malformations following the gavage administration of DBTC at dose levels of 50 or 100 mg/kg bw/d on GD 13-15. These dose levels were sufficient to cause significant maternal toxicity, including mortality, thereby limiting the relevance of the study for classification purposes. Although the dose levels used in this study are significantly higher than those shown to result in teratogenicity in other studies, the dosing period used in this study is shown by other authors not to cause teratogenicity. The absence of foetal malformations is therefore consistent with other data.

In a study with DBTC in CD1 mice (Ema *et al*., 2007a), treatment on GD 4-7 resulted in a marked increase in post-implantation loss at all dose groups (7.6, 15.2 and 30.4 mg/kg bw/day), which reached 100% at 30.4 mg/kg bw/d. No foetuses were examined at 30.4 mg/kg bw/d and the numbers of foetuses examined at 15.2 was very low so it is difficult to conclude on foetal effects, however 2/76 foetuses showed malformations at 7.6 mg/kg bw/day, compared to 0/144 in the control group. Signs of maternal toxicity were reduced food consumption and weight gain, including adjusted weight gain in the two highest dose groups. Reduced weight gain in the lowest dose group was also seen, but adjusted weight gain was not statistically significant. There was a marked increase in post-implantation group in the lowest dose group which cannot be attributed to a secondary effect of maternal toxicity.

Two studies with DBTC in cynomolgus monkeys are reported. Ema *et al*., 2009, reports embryofoetal loss but no foetal malformations following treatment with 7.5 mg/kg bw/d between GD 19-36. 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. Findings were associated with maternal toxicity (vomiting and diarrhoea and slightly reduced weight gain, not statistically significant). The other 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 increased incidences of vomiting and diarrhoea and marked

weight loss on GD 20-51. The dosing periods in these studies were designed to cover organogenesis (GD 20-50). Due to marked maternal toxicity and no clear dose-response effects no clear conclusion can be drawn from these two studies.

A study in cultured explanted rat embryos (Ema *et al*., 1995a;) show that DBTC causes craniofacial defects (as seen in studies *in vivo*), and also that the period of sensitivity was comparable to that seen in studies in the rat *in vivo*.

10.10.6 Comparison with the CLP criteria

According to the CLP Regulation adverse effects on development is defined as follows:

> **Annex I*: 3.7.1.4.*** *Adverse effects on development of the offspring*
> *Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.*

Substances can be classified in category 1A, 1B or 2. Substances in category 1 are:

" *Known or presumed human reproductive toxicant"*

Substances in category 2 are:

"Suspected human reproductive toxicant"

To be classified in category 1 a substance is known to have produced an adverse effect on sexual function and fertility or development in humans; or when there is evidence from animal studies providing a strong presumption that the substance has the capacity to cause effects in humans.

Classification in Category 1A
Classification in category 1A demands evidence for classification primarily from human data.
There is no available human data. Repr. 1A is therefore not relevant.

Classification in Category 1B
In category 1B evidence is primarily from animal data. 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.

Classification in Category 2
Substances are classified in Category 2 when there is some evidence from experimental animals 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. Effects are relevant for classification

where these have been observed in the absence of other toxicity, if the adverse effect on reproduction is considered not to be a secondary non-specific consequence other toxicity.

Although there are no studies on development performed with DBTE there are quite a few studies with category subtances that show teratogenic effects in the offspring of the rat. Very similar effects are seen in several studies with substances in the dibutyltin-category, mostly DBTC but also DBTA, which consistently show that these substances have the potential to cause foetal malformations. A characteristic pattern of external and skeletal malformations, predominantly affecting the skull and jaw, are seen in studies in the rat, and the studies show that the sensitive period of exposure is Gestation Day 8. Post-implantation loss, with a subsequent reduction in litter size, as well as a reduction in foetal weight, is also a consistent finding in developmental toxicity studies. Some of the studies are performed with relatively high dose levels, sufficient to cause maternal effects. However, effects are also apparent at lower dose levels not causing marked maternal toxicity.

A few studies are also performed in the mouse and cynomolgus monkey. These show foetotoxicity and increased post-implantation loss, but the characteristic pattern of malformations seen in the rat studies is not seen in the mouse and monkey. This may however be due to study design (lack of examination of fetuses in the mouse study for instance) or that the effects are masked by the increase in post-implantation loss. *In vitro* and mechanistic data confirm the sensitivity of the rat foetus to malformations induced by DBTC.

Based on the category approach and 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 DBTE for reproductive toxicity (adverse effects on development) in Category 1B; H360D is therefore considered to be appropriate.

This is also supported by the classification of category substances. DBTC has a harmonised classification with Repr. 1B; H360D. DBTDL also has a harmonised classification with Repr. 1B; H360D, largely based on data from DBTC. For DBTP RAC adopted a harmonised classification with Repr. 1B; H360D in 2017. DBTA is proposed classified Repr. 1B; H360D.

10.10.7 Adverse effects on or via lactation

Not evaluated in this dossier.

10.10.8 Conclusion on classification and labelling for reproductive toxicity

When considering both adverse effects on sexual function and fertility and adverse effects on development, classification of DBTE for reproductive toxicity in Category 1B; H360FD is therefore considered to be appropriate.

10.11 Specific target organ toxicity-single exposure

Not evaluated in this dossier.

10.12 Specific target organ toxicity-repeated exposure

Table 18: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Sub-chronic dietary toxicity study. Comparable to OECD 408 Rat, M + F (16/sex/group)	DBTC Dose levels 0, 10, 20, 40, 80 ppm (90 days)	Reduced weight gain (~5%) at 80 ppm (significant in females). Marginally reduced Hb concentration at 80 ppm. No effects on the thymus. **LOAEL > 80 ppm (~4 mg/kg bw/d)** **NOAEL = 80 ppm (~4 mg/kg bw/d)**	Annex I 3.12.1.1 Gaunt *et al.*, 1968 Key study in the registration
Reproductive/developmental toxicity screening study OECD 421 Rats, Wistar (12/group)	DBTC 5, 30, 200 ppm: 2 (F) or 4 weeks (M) pre-mating to PND 4	Reduced weight gain, food consumption and mean bodyweight at 200 ppm (M, F); reduced weight gain at 30 ppm (M). Severe/very severe lymphoid depletion of the thymus at 200 ppm (F); moderate/severe lymphoid depletion at 30 ppm in the pregnant females (not in non-pregnant). The thymus was not investigated in males. **LOAEL = 30 ppm (1.7-2.4 mg/kg bw/d)** **NOAEL = 5 ppm (0.4 mg/kg bw/d)**	Annex I 3.12.1.2 Waalkens-Berendsen 2003 Key study in the registration
Short term repeated dose oral toxicity study Rats, white Simonsen (5/sex/group in 1. trial; 6/sex/group in 2. study; 10/sex/group in 3. study)	DBTDL 3 trials: 1. 4 weeks; 25, 50, 100, 200 ppm 2. 3 months; 25, 50, 100, 200 and 400 ppm 3. 13 weeks; 500, 1000, 1500 and 2000 ppm.	Clinical signs and mortality: 1/20 rat, 5/20 rats and 7/20 rats died in the 1000, 1500 and 2000 ppm dose groups, respectively - significance not reported. Few tissue changes which were observed appeared physiological or infectious and no evidence of toxic changes was observed upon histopathological examination. At 1000 ppm, weight gain and feed intake were significantly affected. An enlarged bile duct was the most common gross necropsy observation attributable to dose. No effects on thymus reported. **NOAEL > 400 ppm or 26.6 mg/kg bw/day**	Annex I 3.12.1.3 Anonymous, 1961 (DBTE registration dossier)

Comparative toxicity of alkyltin and estertin stabilisers Non-guideline (comparable to 407) Rats, Wistar, (10/group)	DBTC Dose levels: 0, 50, 150 ppm (14 days)	Deaths (2/10) at 150 ppm. 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%). Reduced lymph node weights in males and females at 50 ppm (-22%, -19%) and at 150 ppm (-29%, -16%). The most prominent histopathological feature in all treated animals was lymphocyte depletion; this finding was noted particularly in the thymic cortex, but was also apparent in the splenic periarteriolar lymphocyte sheets. There was also liver/bile duct pathology in the highdose group only (severe proliferation of the bile duct epithelium, associated with pericholangitis, periportal fibrosis and accumulation of bile pigment in hepatocytes). **LOAEL = 50 ppm (~2.5 mg/kg bw/d)** **NOAEL < 50 ppm (~2.5 mg/kg bw/d)**	Annex I 3.12.1.4 Penninks & Seinen 1982
Sub-chronic dietary toxicity study Non-guideline Rats, 12/group	DBTC Dose levels: 0, 20, 50, 75, 100 ppm (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. "At 50 ppm growth and food intake were usually reduced and seven out of 12 rats killed after six months had visible bile duct damage shown by thickening and dilatation of the duct and fibrosis of the pancreas. At 75 and 100 ppm the rats showed a greater depression of growth than those on 50 ppm. There were deaths in each group on the higher level during the first four weeks but those surviving this period remained active and well. All survivors killed at six months showed some bile duct damage and this varied considerably in extent". This study is quite old and the quality of reporting is very poor. The results reported here is only a small part of a large study widely diverging in substances, doses, exposure time, species and administration pathway. Due to the uncertainties of the study design, no clear conclusions can be drawn from this study. **LOAEL = 50 ppm (2.5 mg/kg bw/d)** **NOAEL = 20 ppm (1 mg/kg bw/d)**	Annex I 3.12.1.5 Barnes & Stoner 1958
Immune responses in rats exposed to DBTC in drinking water Non-guideline Sprague-Dawley CD rat (M, F) (8/sex/group)	DBTC emulsified in ethoxylated castor oil, and diluted in drinking water. Dose levels: 0, 10, 25 mg/L equivalent to 0, 0.9, 1.9 mg/kg bw/d (28 days)	No bodyweight effects. Reduced water consumption at 25 mg/L (M, F). No effects on thymus weight, antibody production, Delayed-Type Hypersensitivity (DTH) Response or NK cell activity. **LOAEL > 1.9 mg/kg bw/d** **NOAEL = 1.9 mg/kg bw/d**	Annex I 3.12.1.6 DeWitt *et al.*, 2005

Developmental study, sub-acute. Non-guideline Sprague-Dawley rats,	DBTC, emulsified in ethoxylated castor oil, and diluted in drinking water. Mothers: GD 6-PND 21. Pups: gavage from PND3 (3/week) Dose levels: 0, 1.0, and 2.5 mg/kg bw/day	Mothers: No effects of treatment Pups: DTH and NK response assessed in offspring at PND 42. Reduced weight gain (2.5 mg/kg bw/d) No clear effects on immune parameters **NOAEL = 2.5 mg/kg bw/d**	Annex I 3.12.1.7 DeWitt *et al.*, 2006
Sub-acute toxicity in the rat and mouse. Comparable to OECD TG 407 Rat (Wistar); and Swiss mice (10/group)	DBTC 0, 50, 150 ppm (28 days)	Rats: Deaths (2 males, 4 females) at 150 ppm. 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%). Reduced lymph node weights in males and females at 50 ppm (-22%, -19%) and at 150 ppm (-29%, -16%). 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). There was also some liver/bile duct pathology in a few rats at 150 ppm. **LOAEL = 50 ppm (~2.5 mg/kg bw/d)** **NOAEL < 50 ppm (~2.5 mg/kg bw/d)** Mice: No measurements were affected by DBTC.	Annex I 3.12.1.8 Seinen & Vos 1977
Mechanistic investigation of thymic atrophy in the rat, mouse and the guinea pig Non-guideline Rat (Wistar), mouse (Swiss), guinea pig (Hartley)	DBTC Dose levels: 0, 50, 150 ppm. 3 weeks of treatment, after which rats were sensitised of subcutaneous injection of complete adjuvant	Allograft rejection was significantly delayed; other measures of immune function were unaffected by treatment. 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 in rats. 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). Altered immune functions were not found in mice or guinea pigs exposed to DBTC.	Annex I 3.12.1.9 Seinen *et al.*, 1977

Mechanistic investigation of thymic atrophy in the rat Non-guideline Rat (Wistar)	DBTC Dose levels: 0 or 15 mg/kg bw (single dose). Measurement of thymus weight, histopathology and incorporation of radiolabelled precursors into DNA, RNA and protein.	The number of cells isolated from the thymus was significantly reduced at Days 3, 4 and 7, with recovery by Day 9. Thymus weight was reduced from Day 2. Maximal weight reduction was seen on Day 4 and was shown to recover by Day 9. Recovery was shown by a rise in the number of large cells and an increase in macromolecular synthesis **NOAEL < 15 mg/kg bw**	Annex I 3.12.1.10 Snoeij *et al.*, 1989
Developmental toxicity study. OECD 414 Rat (Wistar)	DBTC Dose level: 0, 1, 2.5, 5 or 10 mg/kg bw/d on Days 6-15 of gestation, gavage	Reduced weight gain & food consumption at 10 mg/kg bw/d; slightly reduced weight gain at 5 mg/kg bw/d. 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 (non-significant). **LOAEL ≥ 2.5 mg/kg bw/d**	Annex I 3.12.1.11 Study summary, 1994 (DBTC registration dossier)
Immuno-toxicity study Non-guideline Rats (male, albino). 5-6/group	DBTDL Dose levels: 0, 2, 4, 8 or 16 mg/kg bw/day for 5 days per week for 2 weeks.	A marked dose dependent reduction in thymus weight and its nucleated cell counts with histological alterations were observed at 4, 8 and 16 mg/kg bw/day. Thymus relative organ weight was also significantly reduced at 2 mg/kg bw/day. **LOAEL = 4 mg/kg bw/day** based on significant organ weight reduction and reduced cell counts in the thymus.	Annex I 3.12.1.12 Subramoniam *et al.*, 1994
Neurotoxicity study Non-guideline Rat (Wistar) (10 rats/group)	DBTDL Dose levels: 0, 5, 10 and 20 mg/kg bw/day for 5 days/week for 7 weeks. Gavage.	DBTDL reduced superoxide dismutase and glutathione peroxidase activities, and increased the malondialdehyde content in rat brain tissue. DBTDL increased nitric oxide content and nitric oxide synthase activity in rat brain tissue. DNA damage and apoptosis was seen with increasing frequency and intensity with increased DBTDL dose. 20 mg/kg bw/day resulted in apparent neuropil cavitation in the brains, as well as other ultrastructural changes, with glial filaments dissolving within the axon. **LOAEL = 20 mg/kg bw/day** based on ultrastructural changes in brain and glial filaments dissolving within the axon.	Annex I 3.12.1.13 Jin *et al.*, 2012
Immuno-toxicity, mechanistic study Non-guideline Mouse (SCID-hu), 36 females were engrafted with human foetal thymus and liver tissue fragments.	DBTC Single dose by i.p. injection at dose levels of 0, 0.3 or 1.0 mg/kg bw and sacrificed five days later. The human thymus transplants were removed and assessed morpho-metrically and histo-pathologically.	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 at both dose levels. **LOAEL < 0.3 mg/kg bw**	Annex I 3.12.1.14 de Heer *et al.*, 1995

Table 19: Summary table of human data on STOT RE

No human data.

Table 20: Summary table of other studies relevant for STOT RE

All studies are reported above

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

No studies are avaible for DBTE. However, using the rationale of the category approach explained above (9.2.2), and based on knowledge of the toxicokinetic and hydrolytic behaviour of the substances in the category, it is justified to use data for the other substances in the category to assess the repeated dose toxicity potential of DBTE. We have therefore included studies performed with DBTDL and DBTC, both substances that are classified as STOT RE in category 1.

A number of studies have been performed to study the effect of DBTC and DBTDL on the immune system, particularly the thymus. Only two are 90-day studies, and they are both non-guideline and quite old. However, a few 28-day studies, some developmental studies and other non-guideline studies that looked particularly at the effect on the thymus after exposure to category-substances are also available.

In the 90-day sub-chronic toxicity study at dose levels of 0, 10, 20, 40 and 80 ppm DBTC (Gaunt *et al*., 1968), only 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.

In another rat study carried out with DBTDL in several trials (4 weeks, 3 months and 13 weeks) at dose levels of 25-2000 ppm mortality was seen in the 1000 (1/20), 1500 (5/20) and 2000 (7/20) ppm groups respectively (Anonymous, 1961). Body weight and feed intake was affected in the 1000 ppm group. No mention is given on these effects in the 1500 and 2000 ppm group. The only other effect mentioned is enlarged bile duct, but it is not clear at what dose this occurs.

A 28-day study in rats and mice (Seinen & Vos., 1977) performed with DBTC (dose levels of 0, 50 and 150 ppm) did not identify any effects of treatment in mice. Mortality occurred in rats at 150 ppm and thymus size and thymus and spleen weights were markedly reduced in rats at 50 and 150 ppm. Histopathology revealed effects on the liver and bile duct at 150 ppm and on the thymus (cortex), spleen (PALS) and popliteal lymph node (paracortex) 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 LOAEL for immune system effects of 50 ppm (equivalent to approximately 2.5 mg/kg bw/d) can be determined for this study. A NOAEL could not be determined.

In a 14-day study in rats by Penninks & Seinen (1982) with dose levels of DBTC of 0, 50 and 150 ppm marked lymphoid depletion of the thymus and spleen was reported in both treated groups. In addition there was reduced weight gain, severe jaundice and two deaths in the high dose group. LOAEL for immune system effects of 50 ppm (equivalent to approximately 2.5 mg/kg bw/d) can also be determined for this study. A NOAEL could not be determined.

In an old study, reported with very few details (Barnes & Stoner, 1958), rats were exposed to DBTC for periods of up to 6 months at dietary concentrations of up to 100 ppm. Mortality was reported at 75 and 100 ppm during the first weeks. Reduced weight gain and food consumption were reported at all dietary concentrations (≥20 ppm). All survivors killed at six months showed som bile duct damage and this varied considerably in extent. The thymus or other immune tissues seem not to have been investigated in this study.

Thymus histopathology was investigated in female (but not male) rats in a reproductive/ developmental toxicity screening study (OECD 421) (Waalkens-Berendsen, 2003) using dose levels of 0, 5, 30 or 200 ppm DBTC. Female rats were exposed from two weeks prior to mating until PND 4 (~41days). Weight gain and food consumption by females at 200 ppm were reduced over the whole dosing periods. Histopathology revealed thymic depletion graded as severe to very severe at 200 ppm and moderate to severe at 30 ppm (in pregnant, but not 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 weight and histopathology were investigated in a guideline-compliant rat developmental toxicity study (Study report, 1994) using DBTC dose levels of 0, 1, 2.5, 5 and 10 mg/kg bw/d. Reduced weight gain and food consumption were observed at 10 mg/kg bw/d. Thymus weight was reduced at 10 mg/kg bw/d and 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.

Maternal thymus weight was investigated in an additional developmental toxicity study in the rat (Farr *et al*., 2001) 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.

In a non-guideline neurotoxicity study (Jin et al., 2012) rats were exposed to DBTDL in dose levels of 0, 5, 10 and 20 mg/kg bw/day for 5 days/week for 7 weeks (10 rats/dose group). DBTDL reduced superoxide dismutase and glutathione peroxidase activities, and increased the malondialdehyde content in rat brain tissue. DBTDL increased nitric oxide content and nitric oxide synthase activity in rat brain tissue. DNA damage and apoptosis was seen with increasing frequency and intensity with increased DBTDL dose. In the high dose group apparent neuropil cavitation was seen in the brains, as well as other ultrastructural changes, with glial filaments dissolving within the axon. However, considering the low quality of the reporting of the results, the study alone is not sufficient for a classification for STOT RE neurotoxicity.

A number of non-standard and mechanistic studies are also available for DBTC. Snoeij *et al*. (1989) demonstrates that a single gavage exposure of rats to DBTC (15 mg/kg bw) is sufficient to result in a marked and rapid, but reversible reduction in thymus weight and cellularity. Thymus weight reduction was apparent from two days following treatment, was most marked at four days, but was reversible by nine days.

Seinen *et al*. (1977) report a significant delay in allograft rejection caused by administration of DBTC. No other measures of immune function were affected, leading the authors to conclude that DBTC has a selective inhibitory effect on T-lymphocyte activity. The authors also conclude that effects are most marked in animals exposed during the developmental phase of the lymphoid system.

DeWitt *et al.* (2006) investigated the potential developmental immunotoxicity of DBTC following *in utero* and/or early post-natal exposure. Maternal rats were exposed to DBTC in the drinking water from GD 6 and the pups were directly exposed by gavage until PND 21. The dose level used was relatively low (up to 5 mg/kg bw/d for direct exposure of offspring) and did not identify any effects of treatment on immune system function. A study in SCID mice engrafted with human thymus fragments (de Heer *et al*., 1995) showed a reduction in thymus cortex size following treatment with DBTC.

Effects on the thymus were seen in several studies that taken together give rise for concern. The LOAEL ranged from 1.7-2.5 mg/kg bw/day, which supports classification in category 1.

Table 21: Extrapolation of equivalent effective dose for toxicity studies of lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Adjusted effective dose for DBTE (M=519 g/mol)	Length of exposure	Extrapolated guidance value for STOT RE 1	Classification supported by the study
Seinen & Vos (1977)	≥2.5 mg/kg bw/d of DBTC (MW: 304) (LOAEL: reduced thymus and spleen and lymph node weights and associated lymphocyte depletion)	≥4.6 mg/kg bw/d	28 days	30 mg/kg bw/d	STOT RE1 immune system
Penninks & Seinen (1982)	2.5 mg DBTC/kg bw/d (LOAEL) based on reduced thymus and spleen weights and associated histopathology (lymphocyte depletion)	4.6 mg/kg bw/d	14 days	60 mg/kg bw/d	STOT RE1 immune system
Study summary (1994)	≥2.5 mg/kg bw/d of DBTC (LOAEL: thymic atrophy)	≥4.6 mg/kg bw/d	9 days	~100 mg/kg bw/day	STOT RE1 immune system
Subramoniam *et al.* (1994)	4 mg/kg bw/day of DBTDL (MW 632) (LOAEL: reduced cell counts in thymus)	~3.3 mg/kg bw/day	10 days	~90 mg/kg bw/day	STOT RE1 immune system
Waalkens-Berendsen (2003)	≥1.7-2.4 mg/kg bw/d (30 ppm) of DBTC (lymphoid depletion in the pregnant females)	≥2.9-4 mg/kg bw/d	~41 days	~20 mg/kg bw/d	STOT RE1 immune system

10.12.2 Comparison with the CLP criteria

According to the CLP regulation specific target organ toxicity- repeated exposure is defined as follows:

> **Annex I: *3.9.1.1.*** *Specific target organ toxicity (repeated exposure) means specific, target organ toxicity arising from a repeated exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included. However, other specific toxic effects that are specifically addressed in Chapters 3.1 to 3.8 and Chapter 3.10 are not included here.*

Substances can be classified in Category 1 or 2:

> **Annex 1: *3.9.2.1.*** *Substances are classified as specific target organ toxicants following repeated exposure by the use of expert judgement (see 1.1.1), on the basis of the weight of all evidence available, including the use of recommended guidance values which take into account the duration of exposure and the dose/concentration which produced the effect(s), (see 3.9.2.9), and are placed in one of two categories, depending upon the nature and severity of the effect(s) observed (Table 3.9.1).*

Substance in Category 1 are (Table 3.9.1 from CLP regulation):

> *Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below (see 3.9.2.9), to be used as part of a weight-of- evidence evaluation.*

Annex I, Section 3.9.2.9.6 of the CLP Regulation provides a 'guidance value' of ≤10 mg/kg bw/d from a 90-day rat study to assist in Category 1 classification.

Substances in Category 2 are (Table 3.9.1 from CLP regulation):

> *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. Guidance dose/concentration values are provided below (see 3.9.2.9) in order to help in classification.*
> *In exceptional cases human evidence can also be used to place a substance in Category 2 (see 3.9.2.6).*

Annex I, Section 3.9.2.9.7 of the CLP Regulation provides a 'guidance value' of 10-100 mg/kg bw/d from a 90-day rat study to assist in Category 2 classification.

It is a clear and consistent finding from the available data that DBTC has the potential to cause effects on the lymphoid organs, especially the thymus (severe lymphoid depletion, organ weight reduction) following repeated administration and is therefore classified for STOT RE 1, immune system. Effects on liver, bile duct and pancreas, have also been reported. These are reported at higher dose levels and not as consistently as the thymus-effects and are therefore not considered as enough evidence to justify a classification for these organs. A neurotoxicity study of newer date (Jin *et al.*, 2012), but of low quality, is also described in a study with DBTDL. The effects in this study were also seen at higher dose levels than the thymus effects seen in other studies. The Jin *et al.* study is not considered on its own to be sufficient to justify a classification for STOT RE neurotoxicity. A mechanistic study in SCID mice grafted with human thymus fragments also report effects, indicating that the effects caused by DBTC seem to be relevant to humans.

Effects on the thymus are shown in one study with single dose exposure (Snoeij *et al.*, 1989) to be reversible and the functional consequences are unclear. Nevertheless, the effects observed on the thymus are considered to represent a significant health effect as defined in the CLP Regulation, and the fact that an effect seems reversible does not exclude it from being classified as STOT RE.

As seen in table 33, studies consistently report thymus effects at <10 mg/kg bw/d. 14- and 28-day studies in the rat report LOAEL values for DBTC of approximately 2.5 mg/kg bw/d. Another study, reproductive/developmental toxicity screening test (exposure 41 days of adult animals) (Waalkens-Berendsen, 2003) had effects at 1.7-2.4 mg/kg bw/d. Two studies of shorter duration (9-10 days) support these findings. When adjusting for differences in molecular weight, the LOAELs for DBTE in these studies are calculated to be approximately 3-5 mg/kg bw/d. Although these figures are approximate they are clearly below their extrapolated guidance value for STOT RE 1, justifying a category 1 classification.

10.12.3 Conclusion on classification and labelling for STOT RE

Based on the thymus effects seen in studies with DBTC and following correction for stoichiometry and study duration, classification for DBTE for STOT RE in Category 1 (H372: Causes damage to the immune system) is considered to be appropriate.

10.13 Aspiration hazard

Not evaluated in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this dossier.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this dossier.

13 ADDITIONAL LABELLING

Not relevant.

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15 ANNEXES

15.1 Annex I: Detailed information on the studies in the CLH report for dibutyltin bis(2-ethylhexanoate)

15.2 Annex II: CLH report for 2-ethylhexanoate, proposal sent in by Spain (2019)

15.3 Annex III: Confidential information from the CSR/IUCLID for dibutyltin bis(2-ethylhexanoate)