Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

Dibutylbis(pentane-2,4-dionato-O,O')tin

EC Number: 245-152-0
CAS Number: 22673-19-4

CLH-O-0000001412-86-184/F

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

Adopted

5 December 2017

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification: Dibutylbis(pentane-2,4-dionato-O,O')tin

EC Number: **245-152-0**

CAS Number: **22673-19-4**

Index Number: **Not applicable**

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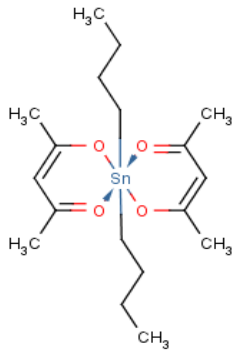
Version number: 2 **Date: November 22, 2016**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	Tin, dibutylbis(2,4-pentanedionato-kO2,kO4)-,
Other names (usual name, trade name, abbreviation)	Dibutyltin bis(2,4-pentanedionate) Dibutyltin bis(acetylacetonate) Dibutyltin ketonate Dibutyltin diketonate TIB KAT 226 NEOSTANN U-220-H
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	245-152-0
EC name (if available and appropriate)	Dibutylbis(pentane-2,4-dionato-O,O')tin
CAS number (if available)	22673-19-4
Other identity code (if available)	-
Molecular formula	$C_{18}H_{32}O_4Sn$
Structural formula	
SMILES notation (if available)	Resonance structure 1: CCCC[Sn](CCCC)(O\C(C)=C/C(C)=O)O\C(C)=C/C(C)=O Resonance structure 2: C(CCC)[CH2-].CC([CH-]C(=O)C)=O.CC([CH-]C(C)=O)=O.C(CCC)[CH2-].[Sn+4]
Molecular weight or molecular weight range	431.14
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-

Degree of purity (%) (if relevant for the entry in Annex VI)	-

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Dibutylbis(pentane-2,4-dionato-O,O')tin	No data	-	Acute Tox. 4, H302 Skin Corr. 1C, H314 Eye Dam. 1, H318 Skin Sens. 1, H317 Muta. 2, H341 Repr. 1B, H360FD STOT SE 1, H370 STOT RE 1, H372 Aquatic Chronic 1, H410 [REACH Registration Dossier (ECHA, 2015a)] **and** Flam. Liq. 3, H226 Acute Tox. 4, H312 Skin Irrit. 2, H315 Eye Irrit. 2, H319 Repr. 1A, H360FD STOT RE 2, H373 Aquatic Acute 1, H400 Aquatic Chronic 3, H412 Not classified [C&L Inventory (ECHA, 2015b)]

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
-	-	-	-	-	-

This CLH Report addresses the classification of dibutylbis(pentane-2,4-dionato)-O-O')tin for STOT-RE and reproductive toxicity. No studies with dibutylbis(pentane-2,4-dionato-O,O')tin are available for these endpoints; therefore reference is made to studies performed with the following read-across substances as part of a category approach:

Read-across substance	CLP Classification
Dibutyltin dichloride (DBTC)	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. **Harmonised classification, C&L Inventory (ECHA, 2015b)**
Dibutyltin dilaurate (DBTL)	Muta 2. H341; Repr. 1B, H360FD, STOT RE 1, H372. **Harmonised classification, RAC Opinion (2015)**
Dibutyltin maleate (DBTM)	Acute Tox. 4, H302, Acute Tox. 2, H330; Skin Corr. 1C, H314; Eye Dam. 1, H318; Skin Sens. 1, H317; Muta. 2, H341; Repr. 1B, H360FD; STOT SE 1, H370; STOT RE 1, H372; Aquatic Chronic 1, H410. **Self-classification, REACH registration (ECHA, 2015g)**
Dibutyltin (di)acetate (DBTA)	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, 2015f)**
Dibutyltin oxide (DBTO)	Acute Tox. 3, H301; Skin Irrit. 2, H315; Eye Dam. 1, H318; Skin Sens. 1, H317; Muta. 2, H341; Repr 1B, H360; STOT SE 1, H370; STOT RE 1, H372; Aquatic Chronic 1 H410. **Self-classification, REACH registration (ECHA, 2016d)**

The read-across substances used in the various studies referred to in this report and used to support the classification proposal are referred to in the table below; purity details are given where these are reported

Table 5: Test substances (non-confidential information) (this table is optional)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information
DBTC	98.57%	-	Waalkens-Berendsen (2003)
DBTC	>98%	-	REACH Registration Dossier (1994)
DBTC	Not reported	-	Ema *et al*., 1996b
DBTC	Not reported	-	Ema *et al*., 1995b Ema *et al*., 1995c
DBTC	Not reported	-	Ema *et al*., 1991
DBTC	Not reported	-	Ema *et al*., 1992b Ema *et al*.,1992a
DBTC	Not reported	-	Noda *et al*., 1993
DBTC	Not reported	-	Noda *et al*., 1992a
DBTC	Not reported	-	Farr *et al*., 2001
DBTC	Not reported	-	Noda *et al*., 1992c
DBTC	97%	-	Ema & Harazono, 2000a Ema *et al*., 2000 Ema & Harazono, 2000b
DBTC	99.5%	-	Ema *et al*., 2007a
DBTC	98%	-	Ema *et al*., 2009
DBTC	98%	-	Ema *et al*., 2007b Ema *et al*., 2006b Ema *et al*., 2006a
DBTC	98%	-	Ema *et al*., 2003a Ema *et al*., 2003b
DBTC	Not reported	-	Iwase *et al*., 1996
DBTC	Not reported	-	Iwase *et al*., 1997
DBTC	Not reported	-	Iwase *et al*., 1995
DBTC	Not	-	Harazono & Ema, 2003

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information
	reported		Harazono & Ema, 2001
DBTC	Not reported	-	Yonemoto *et al*., 1993
DBTC	Not reported	-	Ema *et al*., 1996a
DBTC	99.7%	-	Gaunt *et al*., (1968)
DBTC	96%	-	DeWitt *et al*., 2005b
DBTC	>98%	-	Seinen & Vos (1977) Penninks & Seinen, 1982
DBTC	Not reported	-	Barnes & Stoner, 1958
DBTC	Not reported	-	DeWitt *et al*., 2006b
DBTC	Not reported	-	Luebke *et al*., 2003
DBTC	Not reported	-	DeWitt *et al*., 2005a
DBTC	Not reported	-	DeWitt *et al*., 2006a
DBTC	Not reported	-	Ishizaka *et al*., 1989]
DBTA	Not reported	-	Kimmel *et al*., 1977
DBTA	Not reported	-	Noda *et al*., 1993
DBTA	Not reported	-	Noda *et al*., 1992b
DBTA	Not reported	-	Noda *et al*., 1992c
DBTA	Not reported	-	Noda *et al*., 2001
DBTL	Not reported	-	Schilt & Zondervan van der Beuken, 2004
DBTL	Not reported	-	Noda *et al*., 1993
DBTL	Not reported	-	Noda *et al*., 1992c
DBTM	Not reported	-	Schilt & Zondervan van der Beuken, 2004
DBTM	Not reported	-	Noda *et al*., 1993
DBTM	Not reported	-	Noda *et al*., 1992c
DBTO	Not	-	Schilt & Zondervan van der Beuken, 2004

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information
	reported		
DBTO	Not reported	-	Noda *et al*., 1993
DBTO	Not reported	-	Noda *et al*., 1992c

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	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	-	-	-	-	-	-	-	-	-	-	-
Dossier submitters proposal		Dibutylbis(pentane-2,4-dionato-O,O')tin	245-152-0	22673-19-4	Repr. 1B STOT RE 1	H360FD H372	 Danger	H360FD H372	-	-	-
Resulting Annex VI entry if agreed by RAC and COM		Dibutylbis(pentane-2,4-dionato-O,O')tin	245-152-0	22673-19-4	Repr. 1B STOT RE 1	H360FD H372	 Danger	H360FD H372	-	-	-

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	Hazard class not assessed in this dossier	No
Carcinogenicity	Hazard class not assessed in this dossier	No
Reproductive toxicity	-	Yes
Specific target organ toxicity-single exposure	Hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	-	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 is no harmonised classification and labelling for the substance.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

Dibutylbis(pentane-2,4-dionato-O,O')tin has CMR properties (reproductive toxicity). Harmonised classification and labelling for CMR and respiratory sensitisation is a community-wide action under article 36 of the CLP regulation.

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

Specific target organ toxicity – repeated exposure

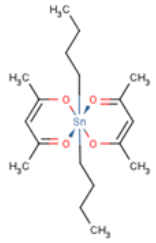
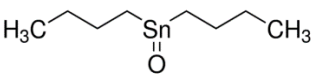
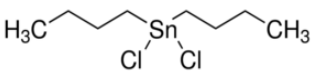
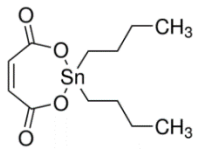
Reason for a need for action at Community level:

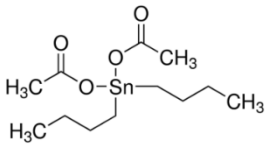
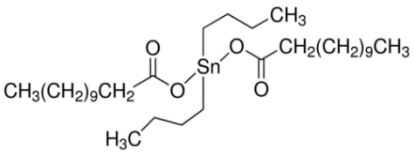
Differences in self-classification

RAC general comment

The DS proposed to classify Dibutylbis(pentane-2,4-dionato)-OO')tin (abbreviated throughout this document as DBTP) for STOT-RE and reproductive toxicity. Although no studies with DBTP are available for these endpoints, reference was made to studies performed with the following substances as part of a read-across category approach: DBTC, DBTM, DBTA, DBTL and DBTO (see Table below for the full substance names and structures).

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

Substance	EC # / CAS #	Structure	Purity (studies)	Purity / Impurity details (REACH Dossier)
Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	245-152-0 / 22673-19-4		>92%	>92% (TIB KAT 226) 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)

Dibutyltin (di)acetate (DBTA)	213-928-8 / 1067-33-0		Not reported	No further details (monoconstituent substance)
Dibutyltin dilaurate (DBTL)	201-039-8 / 77-58-7		Not reported	95-100% Monoconstituent substance; potential presence of tributyl(lauryloxy) stannane

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

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

In the initial hydrolytic studies an indirect detection method was used that could not determine the exact tin species that was formed, therefore it was thought that DBTP forms DBTC after hydrolysis.

However, in a recent *in vitro* hydrolysis study (Naßhan, 2015) a direct method of detection was used that allowed specific structural identification and demonstrated that both DBTP and DBTC form the same species, namely, the distannoxane $ClBu_2SnOSnBu_2Cl$ (Figure below).

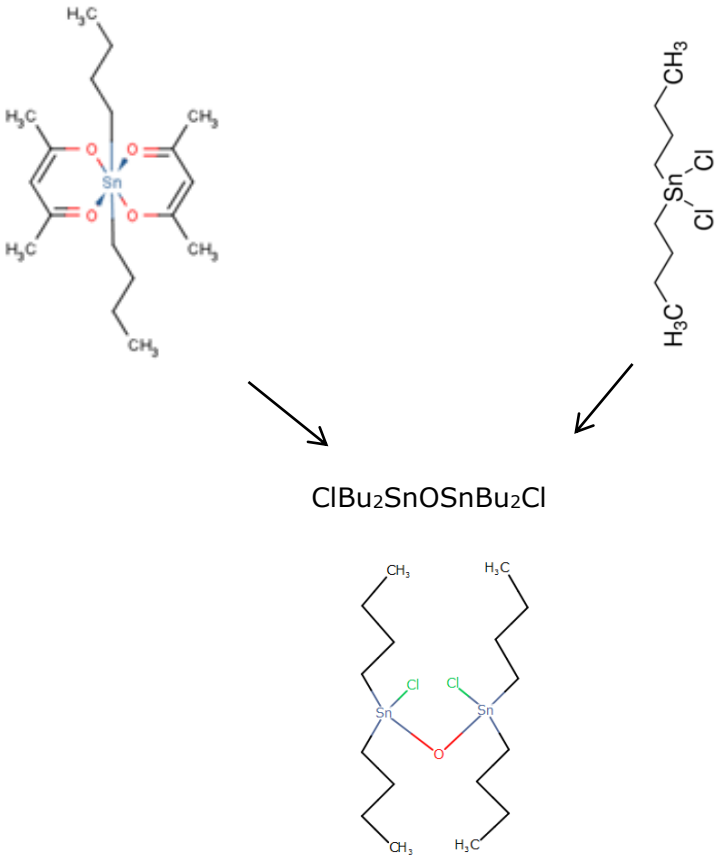


Figure: Overview of the hydrolysis of DBTP and DBTC as determined by Naßhan (2015)

In this study, DBTP was tested at a final concentration of 23.2 mM under low pH (~1-2) conditions (0.07 N HCl) at 37°C in order to simulate the hydrolytic action of mammalian gastric contents. DBTP rapidly formed the dimeric stannoxane $ClBu_2SnOSnBu_2Cl$ (119Sn-NMR: δ (ppm) -91, -144) in almost quantitative yield when exposed to conditions representative of the mammalian stomach. Minor amounts (~2 mol%) of non-hydrolysed DBTC were also detected.
Also DBTC formed the same bridged stannoxane (85% conversion after 1h and 90% conversion after 4 hours).

Complementary studies using indirect detection methods for other category members support the formation of common intermediate(s). This read-across approach is further supported by the available toxicological data that show similar toxicological profiles for the substances in this category.

Considering the available hydrolysis studies and similar toxicological profiles, the RAC agrees with the read-across approach proposed by the DS. In accordance with this approach, the classification proposal of DBTP is mainly supported by studies performed with DBTC and other related dibutyltin compounds. This is also consistent with the RAC opinion issued for dibutyltin dilaurate (DBTL) in 2015.

5 IDENTIFIED USES

The REACH Registration Dossier (ECHA, 2015a) states that dibutylbis(pentane-2,4-dionato-O,O')tin is used as a catalyst in the following chemical product categories:

PC 1: Adhesives, sealants
PC 9a: Coatings and paints, thinners, paint removers
PC 26: Paper and board dye, finishing and impregnation products
PC 32: Polymer preparations and compounds
PC 34: Textile dyes, finishing and impregnating products

Dibutylbis(pentane-2,4-dionato-O,O')tin has the following sectors of end use:

SU 5: Manufacture of textiles, leather, fur
SU 6a: Manufacture of wood and wood products
SU 6b: Manufacture of pulp, paper and paper products
SU 11: Manufacture of rubber products
SU 16: Manufacture of computer, electronic and optical products, electrical equipment
SU 17: General manufacturing
SU 19: Building and construction work

Dibutylbis(pentane-2,4-dionato-O,O')tin has the following consumer use:

PC 1: Adhesives, sealants

6 DATA SOURCES

Data for dibutylbis(pentane-2,4-dionato-O,O')tin are limited to study summaries presented in the publically disseminated REACH Registration Dossier (ECHA, 2015a). Sources of information for the read-across substances are shown below:

ECHA: database of registered substances (ECHA, 2015c)

TOXLINE / MEDLINE: searches for CAS 22673-19-4, CAS 77-58-7, CAS 78-04-6, CAS, 683, 18-1, CAS 818-08-6, CAS 1067-33-0, 'dibutyltin'; October 2015

Published CLH Report for dibutyltin dilaurate (ECHA, 2014)

OECD SIDS Dossier for dibutyltin dichloride (2000)

OECD SIDS Dossier for dibutyltin dilaurate (2000)

OECD SIDS Dossier for dibutyltin maleate (2000)

OECD SIDS Initial Assessment Profile for dibutyltin dichloride and selected thioglycolate esters (2006)

REACH Registration Dossier (publically disseminated version) for dibutyltin dichloride (ECHA, 2015d)

REACH Registration Dossier (publically disseminated version) for dibutyltin dilaurate (ECHA, 2015e)

REACH Registration Dossier (publically disseminated version) for dibutyltin (di)acetate (ECHA, 2015f).

REACH Registration Dossier (publically disseminated version) for dibutyltin maleate (ECHA, 2015g).

REACH Registration Dossier (publically disseminated version) for dibutyltin oxide (ECHA, 2016d).

US Department of Health & Human Services ATSDR: Toxicological Profile for Tin and Tin Compounds (2005)

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

Full study reports are not available: data are taken from the publically disseminated REACH Registration Dossier for dibutylbis(pentane-2,4-dionato-O,O')tin (ECHA, 2015a).

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Liquid (yellow)	REACH Registration Dossier (ECHA, 2015a)	-
Melting/freezing point	25.1°C	REACH Registration Dossier (ECHA, 2015a)	Measured (OECD 102)
Boiling point	Not determined: substance decomposes at 142-158°C	REACH Registration Dossier (ECHA, 2015a)	-
Relative density	1.237 at 20°C	REACH Registration Dossier (ECHA,	Measured (OECD 109)

Property	Value	Reference	Comment (e.g. measured or estimated)
		2015a)	
Vapour pressure	3.8 x10^{-2} Pa at 25°C	REACH Registration Dossier (ECHA, 2015a)	Measured (OECD 104)
Surface tension	33.05mN/m	REACH Registration Dossier (ECHA, 2015a)	Measured (OECD 115)
Water solubility	Not determined	REACH Registration Dossier (ECHA, 2015a)	Not determined due to instant hydrolysis of the substance
Partition coefficient n-octanol/water	Not determined	REACH Registration Dossier (ECHA, 2015a)	Not determined due to instant hydrolysis of the substance
Flash point	87°C	REACH Registration Dossier (ECHA, 2015a)	Measured (Pensky-Martens method)
Flammability	Not determined	REACH Registration Dossier (ECHA, 2015a)	-
Explosive properties	Not determined	REACH Registration Dossier (ECHA, 2015a)	-
Self-ignition temperature	>400°C	REACH Registration Dossier (ECHA, 2015a)	Measured (EU A.15)
Oxidising properties	Not determined	REACH Registration Dossier (ECHA, 2015a)	-
Granulometry	Not applicable (liquid)	REACH Registration Dossier (ECHA, 2015a)	-
Stability in organic solvents and identity of relevant degradation products	Not determined	REACH Registration Dossier (ECHA, 2015a)	-
Dissociation constant	Not applicable	REACH Registration Dossier (ECHA, 2015a)	-
Viscosity	1.745 mPAs (dynamic) at 40°C	REACH Registration Dossier (ECHA, 2015a)	Measured (OECD 114)l rotational viscometer

8 EVALUATION OF PHYSICAL HAZARDS

Not considered in this CLH Report.

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
Dibutylbis(pentane-2,4-dionato)-O-O')tin: simulated gastric hydrolysis (^{119}Sn NMR detection)	Dibutylbis(pentane-2,4-dionato)-O-O')tin 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
Metabolism in the rat *in vivo*	DBTC administered to male rats by intraperitoneal 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.	Read-across substance: DBTC	Ishizaka *et al.*, 1989
Microsomal metabolism *in vitro* and in the mouse *in vivo*	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.	Read-across substance: DBTA	Kimmel *et al.*, 1977

Method	Results	Remarks	Reference
Simulated gastric hydrolysis (GC-FPD detection)	DBTL, DBTM and DBTO are rapidly hydrolysed to DBTC under conditions representative of the mammalian stomach. Within 30 minutes, the degree of hydrolysis was 82% (DBTL), 100% (DBTL) and 43%; within 1 hour the degree of hydrolysis was 78% (DBTL), 97% (DBTM) and 65% (DBTO); within 2 hours the degree of hydrolysis was 88% (DBTL), 98% (DBTM) and 80% (DBTO) and within 4 hours the degree of hydrolysis was 87%, (DBTL), 95% (DBTM) and 87% (DBTO).	Study used to support read-across. Read-across substances: DBTL, DBTM, DBTO	Schilt & Zondervan van der Beuken, 2004
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. Read-across substance: DBTC	Naßhan, 2016

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

Limited toxicity data are available for the substance dibutylbis(pentane-2,4-dionato-O,O')tin and no data are available for the endpoints considered in the CLH Report (reproductive toxicity and STOT RE). Classification for these endpoints following oral exposure is instead addressed using a read-across approach from DBTC and other dibutyltin substances (*i.e.* a category approach), justified on the basis of hydrolytic and toxicokinetic behaviour, and toxicological data (see section 9.2 below, read-across justification). A comparable approach which was accepted by RAC was used in the 2014 CLH report for DBTL to support the classification following oral exposure (ECHA, 2014).

In vitro simulated gastric hydrolysis studies for category members are discussed in detail in section 9.2. *In vivo* toxicokinetic data for category members are limited to data on DBTC and DBTA. DBTC is shown to be metabolised in the rat following intraperitoneal injection by hydroxylation of the butyl groups and by formation of monobutyltin trichloride (Ishizaka *et al*., 1989). Male Wistar rats were administered a single intraperitoneal administration of 4 mg/kg bw and terminated 6-168 hours following dosing. Blood, urine and selected tissues (liver, kidneys and brain) were collected. DBTC was detected in the liver and kidneys at the earliest time point, 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). The same authors also report the metabolism of DBTA following oral administration to the mouse. Findings show hydrolysis of DBTA to form an unidentified dibutyltin compound and liberation of the acetate moieties, which are further transformed and incorporated into normal cellular metabolism. The faeces also contained a proportion of non-metabolised DBTA and other dibutyltin derivatives. Extensive cleavage of the tin-carbon bond was also indicated, with further metabolism of the liberated butyl group to exhaled carbon dioxide and small quantities of butene.

9.2 Read-across

9.2.1 Background

A read-across approach for systemic endpoints after oral exposure has previously been used in the OECD HPV Chemicals Program for dimethyltin-, dibutyltin- and dioctyltin compounds on the basis of the common dialkyltin dichloride hydrolysis products. However, recent simulated gastric hydrolysis studies generated under REACH for specific dialkyltin compounds included in the OECD categories containing thioglycolate (EHMA) ligands, namely $DMT(EHMA)_2$ (EC: 260-829-0), $DBT(EHMA)_2$ (EC: 234-186-1) and $DOT(EHMA)_2$ (EC: 239-622-4), did not confirm the results of previous simulation experiments (ECHA, 2016a, b, c). Considering that distinct hydrolysis behaviour may be associated with the thioglycolate ligands under certain conditions, and additionally, that no toxicological data is available for $DBT(EHMA)_2$ for relevant endpoints, this substance is not included in the category below.

The proposed read-across approach is considered according to the 2008 ECHA Guidance Document for categories, *Guidance on information requirements and chemical safety assessment, Chapter R.6: QSARs and grouping of chemicals* (ECHA, 2008).

9.2.2 Category definition and its members

Category Hypothesis

The substances in the category are chemically comparable in that they contain a common functional dibutyltin (Bu_2Sn-) group, which is considered to be the toxic component. The hypothesis underpinning the category approach is that, following oral administration, all substances within the category behave in a predictable manner and will hydrolyse with the generation of DBTC (or derivatives thereof); systemic exposure will therefore be to the same substance regardless of the substance administered. The systemic toxic effects of the category members are due to common intermediates and will be comparable. Read-across is limited to those endpoints relying on toxicological data generated in experimental animal species *in vivo* by oral administration (*e.g.* reproductive toxicity, repeated dose toxicity) and is not applicable to *in vitro* studies or to studies using dermal or inhalation exposures. The category is established in order to address the mammalian toxicology of dibutyltin substances by reference to structurally-related substances, without the need to generate additional toxicological data *in vivo*.

Applicability domain of the category

The category includes substances with the generic formula Bu_2SnL_2 (L is a labile ligand) as well as DBTO (shown to form DBTC in gastric simulation studies). The category excludes dibutyltin substances with non-labile ligands which do not hydrolyse to form DBTC or derivatives thereof in gastric simulation studies, and where there is significant systemic exposure to the intact substance. Furthermore, the category is established with substances generating hydrolysis products (in addition to DBTC) which are of comparatively low toxicity. Substances generating additional hydrolysis products of toxicological significance would be excluded from the category.

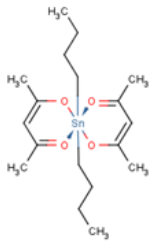
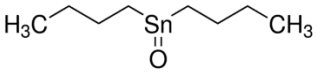
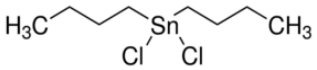
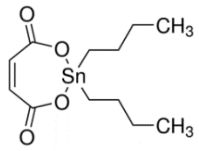
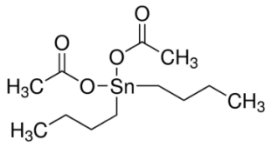
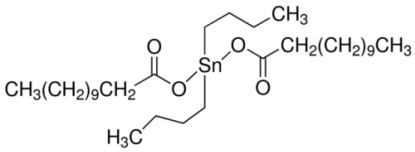
Category members have been chosen based on structural similarity and comparable hydrolytic behaviour. While additional substances could be included in the proposed category, these substances do not have any

toxicological data of relevance to the endpoints considered in the CLH proposal. These substances are excluded from the category based on the availability of data; however they could potentially form part of the category on the basis of their hydrolytic behaviour. The exclusion of these substances does not show any bias or decrease the level of confidence in the proposed approach.

Category Members

The chemical structures of the substances dibutylbis(pentane-2,4-dionato-O,O')tin, DBTC, DBTM, DBTA, DBTL and DBTO used to establish the proposed category for read-across are shown in Table 10 below.

Table 10 Substance characteristics*

Substance	EC # / CAS #	Structure	Purity (studies)	Purity / Impurity details (REACH Dossier)
Dibutylbis(pentane-2,4-dionato-O,O')tin	245-152-0 / 22673-19-4		>92%	>92% (TIB KAT 226) 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)
Dibutyltin (di)acetate (DBTA)	213-928-8 / 1067-33-0		Not reported	No further details (monoconstituent substance)
Dibutyltin dilaurate (DBTL)	201-039-8 / 77-58-7		Not reported	95-100% Monoconstituent substance; potenital presence of tributyl(lauryloxy) stannane

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

Purity / Impurities

Purity details for material tested in the toxicity studies are reported inconsistently for DBTC and not at all for other category members; however, where reported, the level of purity is relatively high. For studies where purity is not reported, the test materials used appear to have been obtained from reputable sources and can therefore be assumed to be of high purity. In general, dibutyltin compounds may contain small amounts of monobutyltin and tributyltin compounds as impurities. The potential presence of these impurities at variable levels does not affect the category approach. Tributyltin substances are metabolised to dibutyltin compounds (Appel, 2004); the dibutyltin metabolites are thought to be the more toxic moiety and are responsible for effects on the immune system and developing foetus. Studies with monobutyltin compounds show a lower level of toxicity; comparative developmental toxicity studies indicate that monobutyltin metabolites are detoxification products of dibutyltin compounds (Ema *et al*., 1995c; Ema *et al*., 1995b; Ema, 2001). All substances are notified under REACH as monoconstituent substances and, where impurities are also reported in the publically disseminated REACH Registration Dossiers, these are present at low levels and do not raise any specific toxicological concerns. It is unlikely, therefore, that variation in purity level or impurity profile will significantly affect the toxicity profile of the category substances for the endpoints of interest. In conclusion, the absence of purity details for a number of studies and the lack of impurity profiles for all studies is not considered to impact on the validity of the proposed category approach.

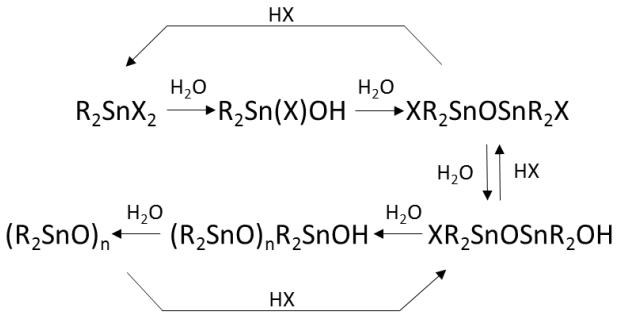
9.2.3 Category justification

Physicochemical properties

The category members are either solid or liquid at room temperature and pressure; (dibutylbis(pentane-2,4-dionato-O,O')tin is liquid at room temperatures, but is reported to have a melting point of 25.1°C (ECHA, 2015a) so will be liquid at mammalian body temperature. The category substances have molecular weights in the range 304-632 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 be reflected in toxic potency. 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 $XR_2SnOSnR_2X$ distannoxane 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 Scheme 1 where the composition at equilibrium will depend on factors such as the medium and the ionic strength.



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

A 2010 study of water solubility performed with dibutylbis(pentane-2,4-dionato-O,O')tin and reported in the publically disseminated REACH Registration Dossier states that the substance hydrolyses 'immediately' upon contact with water, with the formation of a hazy suspension of white particles (ECHA, 2015a). The hydrolysis products were not identified. Also for DBTC, the compound was reported to decompose in the publically disseminated REACH Registration Dossier but the product was not identified (ECHA, 2015d). 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 (ECHA, 2015e, f, g; ECHA, 2016d). In the OECD SIDS Initial Assessment Profile for dibutyltin dichloride and selected thioglycolate esters (OECD, 2006), it is stated however that DBTC, DBTL and DBTM all rapidly form oxides/hydroxides in contact with water in good agreement with the expected lability of the ligands.

The chemical behaviour at low pH is distinct from that at neutral pH. At pH 1-2 under simulated gastric conditions, the compounds in the category behave in a predictable manner and rapidly convert to common intermediates. Schilt & Zondervan van der Beuken (2004) reported the rapid (half-life < 0.5 hours) hydrolysis of DBTM and DBTL to form DBTC (95 % and 87 % yields respectively after 4 hours). Dibutyltin oxide (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. In contast to the simulated gastric conditions, DBTA has been shown to liberate free acetate following oral administration to the mouse (Kimmel *et al*., 1977) with the liberation of dibutyltin substances and free acetate.

Recent simulated gastric hydrolysis studies generated under REACH demonstrate the lability of the ligands also in dibutylbis(pentane-2,4-dionato-O,O')tin (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.

The hydrolytic behaviour of the substances in the category (dibutylbis(pentane-2,4-dionato-O,O')tin, DBTC, DBTM, DBTA, DBTL and DBTO) at neutral and low pH supports the read-across approach and demonstrate 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.

Toxicokinetic properties

No *in vivo* toxicokinetic data are available for dibutylbis(pentane-2,4-dionato-O,O')tin and only limited data are available for other dibutyltin substances, including DBTC. Nevertheless, the 'immediate' hydrolysis reported for the substance strongly suggests that, following oral administration, there will be no systemic exposure to the intact substance. Following absorption, therefore, there will be no relevant toxicokinetic differences for dibutylbis(pentane-2,4-dionato-O,O')tin or the other category members DBTC, DBTM, DBTA, DBTL and DBTO. The only toxicokinetic differences relate to the behaviour of the low toxicity ligands and are not considered to be of relevance.

Based on hydrolysis data for the category members and toxicokinetic data for DBTC, exposure of the biological targets (*i.e.* the thymus; the developing embryo/foetus; implantation, fertility) can be assumed.

Theoretically, a difference in response to the different category compounds may be caused by differences in the rate or extent of hydrolysis; however this is shown to be relatively rapid and extensive for all substances. 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. Of critical importance for this aspect of the assessment is the comparative developmental toxicity study of Noda *et al*., (1993), which clearly demonstrates exposure of the developing foetus following the administration of the category substances DBTC, DBTM, DBTA, DBTL and DBTO. The dose levels used in this study were equivalent (taking into account molecular weight). Toxicokinetic differences following the generation of DBTC in the gastrointestinal system are not relevant, as the toxicokinetics behaviour of DBTC will be the same, regardless of the substances dosed. Based on this evidence, exposure of the biological targets can be assumed.

Similar toxicological properties

Limited toxicological data are available for dibutylbis(pentane-2,4-dionato-O,O')tin. Where data are available (ECHA, 2015a) they are shown below and compared to equivalent data for the other category members (Table 11). Dibutylbis(pentane-2,4-dionato-O,O')tin is reported to be of moderate acute oral toxicity. The LD_{50} value (1864 mg/kg bw) is notably higher than the corresponding values for DBTC (219 mg/kg bw) and DBTO (172 mg/kg bw), but is comparable to data for the dibutyltin esters, most notably DBTL (2071 mg/kg bw).

The difference between the LD_{50} values could be taken as an indication that the formation of DBTC does not occur rapidly in the stomach. As stated however, the LD_{50} of dibutylbis(pentane-2,4-dionato-O,O')tin is comparable to that reported for DBTL (2071 mg/kg bw), which is shown to be rapidly hydrolysed to DBTC. Stoichiometric factors also need to be taken into consideration; however dibutylbis(pentane-2,4-dionato-O,O')tin would be expected to generate ~75% by mass of DBTC, which does not account for the large difference in oral LD_{50} values. The acute oral toxicity with dibutylbis(pentane-2,4-dionato-O,O')tin used a single gavage dose of 2000 mg/kg bw test material and no dosing vehicle. The use of a single high dose is likely to result in a reduced extent of hydrolysis, particularly (as in this case) when administered to fasted rats. The acute oral toxicity studies with DBTC and with dibutylbis(pentane-2,4-dionato-O,O')tin both report effects on the stomach (haemorrhage, loss of the gastric mucosa) which indicate that local toxicity may be an important cause of mortality. This is also reported for the other dibutyltin compounds. In this case, the slower generation of DBTC from the hydrolysis of dibutylbis(pentane-2,4-dionato-O,O')tin is likely to be an important factor. For other endpoints where toxicity data are available for dibutylbis(pentane-2,4-dionato-O,O')tin (acute dermal toxicity, skin and eye irritation, bacterial mutation), study outcomes are comparable with those of the other category members.

Comparative developmental toxicity data in the rat (Noda *et al*., 1993) show that DBTC, DBTM, DBTA, DBTL and DBTO result in a comparable spectrum of foetal malformations, further supporting read-across using a category approach. Based on this evidence, common biological targets can be assumed for the category members. Comparison of available toxicity data therefore supports the read-across approach within the category for reproductive toxicity and STOT RE.

The nature and potential toxicological significance of the liberated ligands meed to be considered. The dibutyltin esters in the category are linked to groups of relatively low systemic toxicity (i.e. lauric acid, maleic acid, acetic acid); the liberation of these hydrolysis products in the gastrointestinal tract does not affect the read-across argument. For dibutylbis(pentane-2,4-dionato-O,O')tin, hydrolysis will liberate acetylacetone which is similarly unlikely to have significant systemic toxicity.

Classification

The harmonised CLP classification for DBTC is shown in Table 11. The substance has a harmonised classification in Acute Tox. 3 (oral), Acute Tox. 4 (dermal), Acute Tox. 2 (inhalation), Skin Corr. 1B, Repr. 1B (fertility and developmental toxicity), Muta. 2, STOT RE 1 (thymus) and in Aquatic Acute 1 and Aquatic Chronic 1. DBTL has recently (ECHA, 2014) been considered by the RAC and a harmonised classification in Repr. 1B (fertility and developmental toxicity), Muta. 2 and STOT RE 1 (thymus) was agreed. Other

hazard classes were not considered by RAC. Other substances in the category do not have harmonised CLP classification, but the self-classification shown in the REACH Registration Dossiers for these substances is comparable to the harmonised classification for DBTC and for DBTL (for those hazard classes considered by RAC). The comparable classification of the substances in the category therefore indicates similar toxicological properties and further supports the read-across category justification. It is particularly notable that classification in reproductive toxicity (Category 1B: H360FD) and in organ toxicity after repeated exposure (Category 1; thymus) is the same for all members in the category and is also the same as that proposed for dibutylbis(pentane-2,4-dionato-O,O')tin.

The studies used to support the proposed classification of dibutylbis(pentane-2,4-dionato-O,O')tin for reproductive toxicity and for STOT RE are of variable quality. Few of the studies are OECD Testguideline- and GLP-compliant (and are therefore not classified in Category 1 according to Klimisch). The majority of the studies relied upon 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 consistent, providing a weight of evidence. Robust summaries are provided for those studies considered adequate (either individually or as part of a weight of evidence) to support the classification proposal.

9.2.4 Data matrix

Table 11. Data matrix for category members

Substance	***Dibutylbis(pentane-2,4-dionato-O,O')tin***	**Dibutyltin oxide (DBTO)**	**Dibutyltin dichloride (DBTC)**	**Dibutyltin maleate (DBTM)**	**Dibutyltin (di)acetate (DBTA)**	**Dibutyltin dilaurate (DBTL)**
CAS No	22673-19-4	818-08-6	683-18-1	78-04-6	1067-33-0	77-58-7
EC No	245-152-0	212-449-1	211-670-0	201-077-5	213-928-8	201-039-8
MW	431	249	304	347	351	632
PHYSICOCHEMICAL DATA						
Physical state	Liquid [REACH Registration Dossier (ECHA, 2015a)]	Solid [REACH Registration Dossier (ECHA, 2016d)]	Solid [REACH Registration Dossier (ECHA, 2015d)]	Solid [REACH Registration Dossier (ECHA, 2015g)]	Liquid [REACH Registration Dossier (ECHA, 2015f)]	Liquid [REACH Registration Dossier (ECHA, 2015e)]
Water solubility	Study technically not feasible. Hydrolysis on contact with water. [REACH Registration Dossier (ECHA, 2015a)]	2.55 mg/L [REACH Registration Dossier (ECHA, 2016d)]	Study technically not feasible. Hydrolysis on contact with water. [REACH Registration Dossier (ECHA, 2016d)]	Insoluble [REACH Registration Dossier (ECHA, 2015g)]	0.4 g/L [REACH Registration Dossier (ECHA, 2015f)]	Insoluble [REACH Registration Dossier (ECHA, 2015e)]
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 [Schilt & Zondervan-van den Beuken, 2004]	Not relevant	Formation of DBTC in gastric simulation studies: 100% in 0.5h, 97% in 1h, 98% in 2h, 95% in 4h [Schilt & Zondervan-van den Beuken, 2004]	No data	Formation of DBTC in gastric simulation studies: 82% in 0.5h, 78% in 1h, 88% in 2h, 87% in 4h [Schilt & Zondervan-van den Beuken, 2004]
Hydrolysis, low pH (^{119}Sn NMR detection)	Formation of $ClBu_2SnOSnBu_2Cl$ under gastric simulation studies: close to quantitative in 2 hours (2 mol% of DBTC also detected)	No data	Formation of $ClBu_2SnOSnBu_2Cl$ under gastric simulation studies: ~70% in 30s, ~85% in 1h, ~90% in 4h	No data	No data	No data

Substance	***Dibutylbis(pentane-2,4-dionato-O,O')tin***	**Dibutyltin oxide (DBTO)**	**Dibutyltin dichloride (DBTC)**	**Dibutyltin maleate (DBTM)**	**Dibutyltin (di)acetate (DBTA)**	**Dibutyltin dilaurate (DBTL)**
	[Naßhan, 2015]		[Naßhan, 2016]			
TOXICOLOGICAL DATA						
Oral LD50 (mg/kg bw)	1864 (1039-3344) [REACH Registration Dossier (ECHA, 2015a)]	172 (121-240) [REACH Registration Dossier (ECHA, 2016d)]	219 [REACH Registration Dossier (ECHA, 2016d)]	510 (263-777) [REACH Registration Dossier (ECHA, 2015g)]	1070 [REACH Registration Dossier (ECHA, 2015f)]	2071 (1207-5106) [REACH Registration Dossier (ECHA, 2015e)]
Dermal LD50 (mg/kg bw)	>2000 [REACH Registration Dossier (ECHA, 2015a)]	>2000 [REACH Registration Dossier (ECHA, 2016d)]	No Data	>2000 [REACH Registration Dossier (ECHA, 2015g)]	No Data	>2000 [REACH Registration Dossier (ECHA, 2015e)]
Skin irritation	Irritant but not corrosive *in vitro* [REACH Registration Dossier (ECHA, 2015a)] Corrosive *in vivo* [REACH Registration Dossier (ECHA, 2015a)]	Irritant *in vivo* [REACH Registration Dossier (ECHA, 2016d)]	Corrosive *in vivo* [REACH Registration Dossier (ECHA, 2016d)]	Corrosive *in vivo* [REACH Registration Dossier (ECHA, 2015g)]	Corrosive *in vitro* [REACH Registration Dossier (ECHA, 2015f)]	Corrosive *in vivo* [REACH Registration Dossier (ECHA, 2015e)]
Eye irritation	Serious eye damage *in vitro* [REACH Registration Dossier (ECHA, 2015a)]	Irritant *in vivo* [REACH Registration Dossier (ECHA, 2016d)]	Serious eye damage *in vivo* [REACH Registration Dossier (ECHA, 2016d)]	Serious eye damage *in vivo* [REACH Registration Dossier (ECHA, 2015g)]	No Data	Irritant *in vivo* [REACH Registration Dossier (ECHA, 2015e)]
Ames test	Negative [REACH Registration Dossier (ECHA, 2015a)]	Negative [REACH Registration Dossier (ECHA, 2016d)]	Negative [REACH Registration Dossier (ECHA, 2016d)]	Negative [REACH Registration Dossier (ECHA, 2015g)]	Negative [REACH Registration Dossier (ECHA, 2015f)]	Negative [REACH Registration Dossier (ECHA, 2015e)]
Reproductive toxicity – adverse effects on sexual function and	No data: read-across proposed	No data	Large increase in pre-implantation loss in studies in the rat, mouse & monkey [rat	No data	No data	No data

Substance	*Dibutylbis(pentane-2,4-dionato-O,O')tin*	Dibutyltin oxide (DBTO)	Dibutyltin dichloride (DBTC)	Dibutyltin maleate (DBTM)	Dibutyltin (di)acetate (DBTA)	Dibutyltin dilaurate (DBTL)
fertility			[Ema & Harazono, 2000a; Ema & Harazono, 2000b; Ema *et al*., 2000; Waalkens-Berendsen, 2003], mouse [Ema *et al*., 2007a], monkey [Ema *et al*., 2006b; Ema *et al*., 2007a; Ema *et al*., 2009]]			
Reproductive toxicity – adverse effects ont the development of the offspring	No data: read-across proposed	Characteristic external and skeletal foetal malformations, largely affecting the jaw/skull [Noda *et al*., 1992c; Noda *et al*., 1993]	Characteristic external and skeletal foetal malformations, largely affecting the jaw/skull [Ema & Harazono, 2000a, b; Ema *et al*., 1991; Ema *et al*., 1992a, b; Ema *et al*., 1995b; Ema *et al*., 1996b; Ema, 2001; Ema *et al*., 2007b; Farr *et al*., 2001; Noda *et al*., 1992b; Noda *et al*., 1993; Study report, 1994; Waalkens-Berendsen, 2003]	Characteristic external and skeletal foetal malformations, largely affecting the jaw/skull [Noda *et al*., 1992c; Noda *et al*., 1993]	Characteristic external and skeletal foetal malformations, largely affecting the jaw/skull [Noda *et al*., 1992a, b, c; Noda *et al*., 1993; Noda *et al*., 2001]	Characteristic external and skeletal foetal malformations, largely affecting the jaw/skull [Noda *et al*., 1992c; Noda *et al*., 1993]
Repeated dose toxicity	No data: read-across proposed	No data	Marked reduction in thymus size & cellularity; similar effects on the spleen and lymph nodes [Barnes & Stoner, 1958; DeWitt *et al*., 2005b; Farr *et al*., 2001; Gaunt *et al*., 1968; Penninks &	No data	No data	No data

Substance	*Dibutylbis(pentane-2,4-dionato-O,O')tin*	Dibutyltin oxide (DBTO)	Dibutyltin dichloride (DBTC)	Dibutyltin maleate (DBTM)	Dibutyltin (di)acetate (DBTA)	Dibutyltin dilaurate (DBTL)
			Seinen, 1982; Seinen & Vos, 1977; Study report, 1994; Waalkens-Berendsen, 2003]			
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	Muta 2, H341 Repr 1B, H360FD STOT RE 1, H372 [Based mainly on read-across from DBTC]

9.2.5 Conclusions

Overall, the data available are considered to justify the use of a category approach for read-across from DBTC, DBTM, DBTA, DBTL and DBTO in order to address the classification of dibutylbis(pentane-2,4-dionato-O,O')tin for reproductive toxicity and for STOT RE, based on the following data:

- The hydrolytic behaviour at neutral and low pH support the rapid formation of common intermediate(s) across the category. Due to rapid hydrolysis at low pH there will therefore be no systemic exposure to dibutylbis(pentane-2,4-dionato-O,O')tin using oral dosing.

- Hydrolysis studies at low pH for dibutylbis(pentane-2,4-dionato-O,O')tin and DBTC show the formation of the *same species* which has been assigned based on a direct method allowing specific structural identification. Complementary studies using indirect detection methods for other category members support the formation of common intermediate(s).

- The available toxicological data for dibutylbis(pentane-2,4-dionato-O,O')tin and other category members support the category hypothesis.

- Toxicological data show comparable toxic effects for DBTC, DBTM, DBTA, DBTL and DBTO in a rat developmental study, further supporting the generation of common toxic hydrolysis products.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not considered in this CLH Report.

10.2 Acute toxicity - dermal route

Not considered in this CLH Report.

10.3 Acute toxicity - inhalation route

Not considered in this CLH Report.

10.4 Skin corrosion/irritation

Not considered in this CLH Report.

10.5 Serious eye damage/eye irritation

Not considered in this CLH Report.

10.6 Respiratory sensitisation

Not considered in this CLH Report.

10.7 Skin sensitisation

Not considered in this CLH Report.

10.8 Germ cell mutagenicity

Not considered in this CLH Report.

10.9 Carcinogenicity

Not considered in this CLH Report.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 12: Summary table of animal studies on adverse effects on sexual function and fertility

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. Developmental toxicity studies assessing the effects of treatment during very early gestation (prior to implantation) are also included in this section.

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
Studies in the rat						
OECD 421	No significant deviations	Wistar rat (12/sex)	DBTC: 98.57% purity	0, 5, 30, 200 ppm (diet); 2 weeks premating to PND 4	200 ppm diet: reduced maternal weight gain (values not reported). Reduced numbers of foetuses (10 compared to 101 in controls), reduced litter size (6.0 compared to 11.3). **NOAEL = 30 ppm (1.7-2.4 mg/kg bw/d)** **LOAEL = 200 ppm (12.0-15.4 mg/kg bw/d)**	Waalkens-Berendsen, 2003
None	-	Wistar rat (16-19/group)	DBTC: 97% purity	0, 3.8, 7.6, 15.2 mg/kg bw/d GD 0-3	Maternal toxicity at ≥3.8 mg/kg bw/d (clinical signs), weight loss during early gestation at 3.8 (-2 g), 7.6 (-14 g) and 15.2 mg/kg bw/d (-20 g); reduced food consumption (≥3.8 mg/kg bw/d) Increased pre-implantation loss at 7.6 (35.6%) and 15.2 mg/kg bw/d (87.9%), compared to 2.7% in controls. **LOAEL =3.8 mg/kg bw/d** **NOAEL <3.8 mg/kg bw/d**	Ema & Harazono, 2000a Ema *et al*., 2000 Ema & Harazono, 2000b

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
Study in the mouse						
None	-	CD1 mouse (12/group)	DBTC: 99.5% purity	0, 7.6, 15.2, 30.4 mg/kg bw/d GD 0-3	Maternal toxicity (mortality, clinical signs, reduced weight gain GD 0-4 (-82%) at 30.4 mg/kg bw/d reduced food consumption) at 7.6 (-18%), 15.2 (-8%) and 30.4 mg/kg bw/d (-19%). 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. **LOAEL =7.6 mg/kg bw/d** **NOAEL <7.6 mg/kg bw/d**	Ema *et al*., 2007a

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

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

Table 14: Summary table of other studies relevant for toxicity on sexual function and fertility

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.

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Mechanistic	DBTC: 98% purity	Investigation of the effects of progesterone on implantation failure.	Administration of progesterone on GD 0-8 offered some protection against implantation failure in Wistar rats treated with 7.6 or 15.2 mg/kg bw/d DBTC on GD 0-3. Pre-implantation losses were 8.6%, 62.8%, 81.3% at dose levels of 0, 7.6, 15.2 mg/kg bw without progesterone ; 10.5%, 25.9% and 60.0% with progesterone.	Ema *et al*., 2003a Ema *et al*., 2003b
Mechanistic	DBTC:	Investigation of the effects	DBTC administration (7.6 and	Harazono & Ema,

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	purity not reported	of DBTC on decidual cell response in pseudopregnant rats	15.2 mg/kg bw/d on GD 0-3 or GD 4-7) reduced uterus weight and serum progesterone levels. Oestradiol levels and corpora lutea numbers were unaffected by treatment. Administration of progesterone reversed the suppression of uterine decidualisation.	2003 Harazono & Ema, 2001

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

No data are available for dibutylbis(pentane-2,4-dionato-O-O'-tin; reference is made to studies performed with dibutyltin dichloride (DBTC) as part of the read-across (category) approach.

In a guideline compliant (OECD 421) screening study (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). Only 3 of the 7 pregnant females at the highest dose level delivered live offspring. The number of pregnant females in this group (7/12) is lower than controls (9/12); however the numbers of pregnant females in the other treated groups are also low without a dose-response relationship. Corpora lutea numbers were not measured in this study, therefore the extent of pre-implantation loss cannot be assessed. An effect of treatment on fertility at the highest dose level, however, cannot be totally excluded. The full study report is not available; summary data are taken from two sources, the publically disseminated REACH Registration Dossier for dibutylbis(pentane-2,4-dionato-O,O')tin (ECHA, 2015a) and the 2014 CLH report for dibutyltin dilaurate (ECHA, 2014). Complete details on the study methodology and findings are therefore not available. Notably, values for maternal bodyweight and bodyweight gain are absent from both sources; therefore the extent of maternal toxicity seen at the highest dietary concentration of 120 ppm cannot be fully assessed.

A number of developmental toxicity studies using administration of DBTC during early gestation (prior to implantation) are also available (see below) and report effects of treatment on implantation which, for the purposes of classification, is considered under reproductive toxicity (effects on sexual function and fertility). These published studies are of variable quality and do not fully comply with regulatory guidelines but are considered to be sufficiently robust to support the classification proposal as part of a weight of evidence.

A published study by Ema & Harazono (2000a) focussed on 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); findings were associated with maternal weight loss in all groups on GD 0-4. The same findings appear to be reported in abstracts published by Ema *et al*., (2000) and by Ema & Harazono (2000b).

A study 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; findings at this dose levels were associated with maternal toxicity including mortality (Ema *et al*., 2007a). A small number of deaths were seen in all treated groups in this study, but not in controls; however the absence of a dose-response relationship (mortality of 0/12, 2/12, 1/12 1/12 at 0, 7.6, 15.2 and 30.4 mg/kg bw/d, respectively) indicates that the deaths of dams may not be directly related to treatment with DBTC. Mortality in this study would appear more likely to be due to misdosing; the level of mortality seen is not

considered to invalidate the study. Other signs of maternal toxicity seen in this study were clinical signs, and moderate reductions in food consumption and weight gain.

Mechanistic data in the rat indicate that DBTC may result in the failure of implantation due to a suppression of the decidual cell response and reduction in circulating progesterone levels (Harazono & Ema, 2001; Harazono & Ema, 2003) and indicate that some protection against the failure of implantation is afforded by the administration of progesterone during early gestation (Ema *et al*., 2003a; Ema *et al*., 2003b). Administration of DBTC on GD 0-3 caused a marked increase in pre-implantation loss at 7.6 mg/kg bw/d (62.8%) and at 15.2 mg/kg bw/d (81.3%) compared to controls (8.6%); progesterone treatment reduced the level of pre-implantation loss to 25.9% and 60.0% at 7.6 and 15.2 mg/kg bw/d BTC, respectively. Mechanistic data therefore indicate that implantation failure caused by administration of DBTC is likely to be of relevance to humans.

10.10.3 Comparison with the CLP criteria

The definition of reproductive toxicity in the CLP Regulation (Annex I: 3.7.1.1) includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring.

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

For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. Within each category, effects on sexual function and fertility and on development are considered separately. Effects on lactation are allocated to a separate hazard category. Substances are allocated to Category 1 (substances that are a known or presumed human reproductive toxicant) or to Category 2 (substances that are a suspected human reproductive toxicant). Substances are classified in Category 1 when they are 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 is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B). 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.

No data are available for dibutylbis(pentane-2,4-dionato-O-O'-tin; however relevant data are available for DBTC for read-across (category approach). 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 less marked maternal toxicity is observed, however, marked effects increases in the level of pre-implantation loss are still apparent. The data suggest, therefore, that the adverse effect on reproduction is not considered to be a secondary non-specific consequence of other toxicity. Mechanistic data suggest that the increased level of pre-implantation loss may be to a reduction in circulating progesterone levels, which is also potentially of relevance to humans.

Classification of dibutylbis(pentane-2,4-dionato-O-O')tin for reproductive toxicity (adverse effects on sexual function and fertility) in Category 1B is therefore considered to be appropriate.

10.10.4 Adverse effects on development

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

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 Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
Studies in the rat						
OECD 421	No significant deviations	Wistar rat (12/sex)	DBTC: 98.57% purity	0, 5, 30, 200 ppm (diet); 2 weeks premating to Day 4 of lactation	200 ppm diet: reduced maternal weight gain (values not reported). Reduced numbers of foetuses (10 compared to 101 in controls), reduced litter size (6.0 compared to 11.3). **NOAEL = 30 ppm (1.7-2.4 mg/kg bw/d)** **LOAEL = 200 ppm (12.0-15.4 mg/kg bw/d)**	Waalkens-Berendsen, 2003
OECD 414	No significant deviations	Wistar rat (25/group)	DBTC: >98% purity	0, 1, 2.5, 5, 10 mg/kg bw/d GD 6-15	Maternal toxicity at 5 mg/kg bw/d (reduced weight gain) and 10 mg/kg bw/d (reduced weight gain and food consumption); values not reported. 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**	Study report, 1994
None	OECD 414	Wistar rat (25/group)	DBTC: purity not reported	0, 1, 2.5, 5, 10 mg/kg bw/d GD 6-15	Maternal toxicity (reduced weight gain (-17%) & reduced food consumption (-7%)) at 10 mg/kg bw/d Marginal increase in malformations (including single incidences of ankyloglossia, agnathia, mandibular defect at 10	Farr *et al.*, 2001

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
					mg/kg bw/d). **LOAEL = 10 mg/kg bw/d** **NOAEL =5 mg/kg bw/d**	
None	-	Wistar rat (10/group)	DBTC: purity not reported	0, 10, 15 mg/kg bw/d GD 7-8	Maternal toxicity (reduced weight gain) at 10 and 15 mg/kg bw/d (-29% and -51% respectively), with initial weight loss (-5 g, -8 g, respectively). 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. **LOAEL =10 mg/kg bw/d** **NOAEL <10 mg/kg bw/d**	Ema *et al*., 1995b Ema *et al*., 1995c
None	-	Wistar rat (10-12/group)	DBTC; purity not reported	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%). 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	Ema *et al*., 1991

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
					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 =5 mg/kg bw/d** **NOAEL =2.5 mg/kg bw/d**	
None	-	Wistar rat 11/group	DBTC; purity not reported	0, 20 mg/kg bw/d (GD 7-9, 10-12 or 13-15) 0, 20, 40 mg/kg bw/d (GD 6, 7, 8 or 9)	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%). Total resorption in 5/11 dams, resulting in low litter size (3.3 compared to 11.8 in controls). Mean foetal weight reduced (~40%). Increased malformations (largely omphalocoele and jaw defects) GD 10-12: reduced foetal weight (~15%); no malformations. GD 13-15: reduced foetal weight (~20%); no malformations. GD 6: increased post-implantation loss at 20 (18.9%) and 40 mg/kg bw/d (43.5%); total resorption at 20 (1/11) and 40 mg/kg bw/d (3/11). Marginal increase in malformations at 40 mg/kg bw/d. GD 7: increased post-implantation loss at 20 (24.6%) and 40 mg/kg bw/d (76.2%); total	Ema *et al*., 1992b Ema *et al*., 1992a

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
					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. **LOAEL =20 mg/kg bw/d** **NOAEL <20 mg/kg bw/d**	
None	-	Wistar rat (10/group)	DBTC: purity not reported	80 µmol/kg bw; (25 mg/kg bw), GD 8	Maternal toxicity details not reported. Foetal malformations; mainly affecting the jaw (cleft mandible, cleft lower lip, ankyloglossia or schistoglossia); exencephaly. **LOAEL = 25 mg/kg bw**	Noda *et al*., 1993 Noda *et al*., 1992c
			DBTL: purity not reported	80 µmol/kg bw (50 mg/kg bw) GD 8	Maternal toxicity details not reported. Foetal malformations; mainly affecting the jaw (cleft mandible, cleft lower lip, ankyloglossia or schistoglossia; exencephaly. **LOAEL = 50 mg/kg bw**	

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
			DBTA: purity not reported	80 µmol/kg bw (28 mg/kg bw), GD 8	Maternal toxicity details not reported. Foetal malformations; mainly affecting the jaw (cleft mandible, cleft lower lip, ankyloglossia or schistoglossia); exencephaly. **LOAEL = 28 mg/kg bw**	
			DBTM: purity not reported	80 µmol/kg bw (28 mg/kg bw), GD 8	Maternal toxicity details not reported. Foetal malformations; mainly affecting the jaw (cleft mandible, cleft lower lip, ankyloglossia or schistoglossia). **LOAEL = 28 mg/kg bw**	
			DBTO: purity not reported	80 µmol/kg bw (20 mg/kg bw) GD 8	Foetal malformations; mainly affecting the jaw (cleft mandible, cleft lower lip, ankyloglossia or schistoglossia); exencephaly. **LOAEL = 20 mg/kg bw**	
None	-	Wistar rat (9-10/group)	DBTA: purity not reported	0, 15, 30 mg/kg bw/d: GD 7-9	Details on maternal toxicity not reported. Foetal malformations (mainly affecting the jaw: cleft mandible, cleft lower lip, ankyloglossia or schistoglossia; exencephaly) **LOAEL =15 mg/kg bw** **NOAEL <15 mg/kg bw**	Noda *et al.*, 1992a
				0, 5.0, 7.2, 10.5, 15.2, 22 mg/kg bw/d: GD 8	Details on maternal toxicity not reported. Foetal malformations: (mainly affecting the jaw: cleft mandible, cleft lower lip, ankyloglossia or schistoglossia; exencephaly) **LOAEL =10.5 mg/kg bw** **NOAEL =15.2 mg/kg bw**	

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
None	-	Wistar rat 13-16/group	DBTA: purity not reported	0, 1.7, 5, 10, 15 mg/kg bw/d GD 7-17	Maternal toxicity (reduced weight gain) at 15 mg/kg bw/d. Reduced numbers of dams with viable foetuses at 15 mg/kg bw (7/16) due to foetal loss and total resorption (9/16). Reduced foetal weight at 10 mg/kg bw (~18%) and 15 mg/kg bw (~26%. Foetal malformations (mainly cleft mandible, cleft lower lip, ankyloglossia and schistoglossia) increased at ≥5 mg/kg bw/d. **LOAEL =5 mg/kg bw/d** **NOAEL =1.7 mg/kg bw/d**	Noda *et al.*, 1992b
None	-	Wistar rat (16-19/group)	DBTC: 97% purity	0, 3.8, 7.6, 15.2 mg/kg bw/d GD 4-7	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). 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. **LOAEL =3.8 mg/kg bw/d** **NOAEL <3.8 mg/kg bw/d**	Ema & Harazono, 2000a Ema *et al.*, 2000 Ema & Harazono, 2000b
None	-	Wistar rat (12-14/group)	DBTA; purity not reported	0, 7.5, 10, 15, 22 mg/kg bw/d GD 8	Reduced maternal weight gain at 22 mg/kg bw in 7.5 month-old dams (-33%). Reduced numbers of litters with	Noda *et al.*, 2001

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
					viable foetuses (6/13) due to total resorption (5/13) at 22 mg/kg bw (7.5 month-old dams). Implantation loss increased at 22 mg/kg bw in 3-month old (19.2%), 7.5 month-old (37.8%) and 12 month-old dams (95.2%). Foetal malformations (typically cleft mandible, cleft lower lip, ankyloglossia, schistoglossia, fused ribs, fused cervical and vertebral arches) at ≥7.5 mg/kg bw/d **NOAEL <7.5 mg/kg bw/d** **LOAEL =7.5 mg/kg bw/d**	
None	-	Wistar rat (11-13/group)	DBTC: purity not reported	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), reduced weight gain -70%, -88%). Reduced foetal weight at 50 (-29%) and 100 mg/kg bw/d (-34%). Increased post-implantation loss at 50 (22.0%) and 100 mg/kg bw/d (34.4%) compared to controls (9.8%). No clear increase in foetal malformations. **LOAEL =50 mg/kg bw/d** **NOAEL <50 mg/kg bw/d**	Ema *et al*., 1996b
None	-	Wistar rat (10/group)	DBTC: purity not reported	Limited details	Foetal malformations: limited detail provided	Ema, 2001
Studies in the mouse						
None	-	CD1 mouse (12/group)	DBTC: 99.5% purity	0, 7.6, 15.2, 30.4 mg/kg bw/d 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	Ema *et al*., 2007a

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
					(+1.9 g), 15.2 (+1.2 g) and 30.4 mg/kg bw (-0.3g) compared to +3.1 g in controls. Food consumption was reduced at 15.2 mg/kg bw (~25%) and 30.4 mg/kg bw (~28%). Total resorption at 7.6 (2/12), 15.2 (8/12) and 30.4 mg/kg bw (10/12); increased post-implantation loss at 7.6 (48.3%), 15.2 (94.4%) and 30.4 mg/kg bw (100%). Marginal increase in malformations at 7.6 mg/kg bw (omphalocoele, exencephaly) but not at 15.2 mg/kg bw. **NOAEL <7.6 mg/kg bw** **LOAEL =7.6 mg/kg bw**	
Studies in the monkey						
None	-	Cynomolgus monkey (5/group; 12 controls)	DBTC: 98% purity	0, 7.5 mg/kg bw/d: GD 19-21, 21-23, 24-26, 26-28, 29-31, 31-33, 34-36	Embryofoetal loss (GD 19-21 (1/5), 24-26 (2/5), 34-36 (1/5) compared to 1/12 controls. Findings associated with maternal toxicity (signs of toxicity, marginally reduced weight gain). **LOAEL =7.5 mg/kg bw/d** **NOAEL <7.5 mg/kg bw/d**	Ema *et al*., 2009
None	-	Cynomolgus monkey (10-12/group)	DBTC: 98% purity	2.5, 3.8 mg/kg bw/d: GD 20-50	Maternal toxicity (clinical signs, weight loss (-556 g) & reduced food consumption) at 3.8 mg/kg bw/d; clinical signs, weight loss (-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	Ema *et al*., 2007b Ema *et al*., 2006b Ema *et al*., 2006a

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
					at 3.8 mg/kg bw/d (4/10 females with embryofoetal loss) compared to 18/12 controls. **LOAEL =2.5 mg/kg bw/d** **NOAEL <2.5 mg/kg bw/d**	

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

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

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

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.

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
In vitro	DBTC: purity not reported	Cultured GD 9 rat embryos were exposed to DBTC at concentrations of 82, 165 or 330 nM (25, 50, 100 ng/mL) for 46 hours.	Exposure to 330 nM DBTC resulted in 100% lethality. A high incidence (87%) of craniofacial defects was seen at 165 nM. No effects were seen at 82 nM.	Iwase *et al*., 1996
In vitro	DBTC: purity not reported	Cultured GD 8 rat embryos were exposed for 68 hours; GD 9 rat embryos were exposed for 46 hours; GD 11 rat embryos were exposed for 48 hours to DBTC at concentrations of 3-1000 ng/mL.	GD 8 embryos exposed to 10 and 30 ng/mL showed incomplete turning of the body axis and craniofacial defects; GD 9 embryos showed similar effects following exposure to 50 and 100 ng/mL. No effects were seen in GD 11 embryos at 100 ng/mL; exposure to 300 ng/mL resulted in defects of the prosencephalon, forelimb bud and tail. Exposure to 1000 ng/mL resulted in 100% lethality.	Iwase *et al*., 1997
In vitro	DBTC: purity not reported	Cultured GD 8 rat embryos were exposed for 68 hours to DBTC at concentrations of 3, 10 or 30 ng/mL.	A reduction in vascularisation was seen in in the body and yolk sac at 30 ng/mL; yolk sac diameter, crown-rump length and number of somite pairs	Iwase *et al*., 1995

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			were also reduced. A concentration-dependent decrease in the overall morphological score and an increase in the incidence of embryos with anomalies were observed at all concentrations.	
In vitro	DBTC: purity not reported	Cultured rat embryo limb buds were used to assess the teratogenicity of DBTC.	DBTC showed very strong inhibition of cell differentiation (ID_{50} 0.13-1.71 µM and cell proliferation (IP_{50} 0.12-2.81 µM).	Yonemoto, 1992 Yonemoto *et al*., 1993
In vitro	DBTC: purity not reported	Cultured GD 8.5, GD 9.5 or GD 11.5 embryos were cultured for 68, 46 or 48 hours and were exposure to DBTC concentrations for 24, 46 or 46 hours respectively.	In GD 8.5 embryos DBTC caused decreases in placental diameter (≥10 ng/mL) and the number of somite pairs and the morphological score (30 ng/mL). In GD 9.5 embryos, significant decreases in yolk sac diameter and crown-rump length (100 ng/mL, reduction in the number of somite pairs (≥50 ng/mL) and a reduction in the morphological score (≥30 ng/mL). No adverse effects were seen in GD 11.5 embryos. Dysmorphogenesis was seen in embryos from GD 8.5 (≥10 ng/mL), GD 9.5 (≥50 ng/mL) and GD 11.5 (300 ng/mL). Incomplete turning and craniofacial defects in embryos cultured from GD 8.5 and GD 9.5 and defects of the forelimb buds and tail in embryos cultured from GD 11.5 were frequently observed.	Ema *et al*., 1996a

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

No data are available for dibutylbis(pentane-2,4-dionato-O-O')tin; reference is made to studies performed with dibutyltin dichloride (DBTC), dibutyltin dilaurate (DBTL), dibutyltin (di)acetate (DBTA), dibutyltin oxide (DBTO) and dibutyltin maleate (DBTM).

In a guideline compliant (OECD 421) screening study (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). Only 3 of the 7 pregnant females at the highest dose level

delivered live offspring; a high level of post-implantation loss (87.6% compared to 13.4% in controls) is reported.

In a guideline-compliant (OECD 414) study performed with DBTC at dose levels of 2.5, 5 or 10 mg/kg bw/d (Study report, 1994), no deaths occurred; evidence of maternal toxicity was seen at 5 mg/kg bw/d (reduced weight gain) and at 10 mg/kg bw/d (reduced weight gain and food consumption). The original study report is not available; therefore full methodological details and tabulated results (including details of maternal toxicity) are not available. The incidence of foetuses with malformations was increased 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 2.5 mg/kg bw/d can therefore be determined for this study, based on reduced weight gain at 5 and 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.

A number of published studies are also available, which do not fully comply with OECD 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 exencephaly, cleft jaw, cleft lip, ankyloglossia and club foot were apparent at 10 mg/kg bw/d; the incidences of exencephaly, cleft tongue and omphalocoele were additionally significantly increased at 15 mg/kg bw/d. Significantly increased incidences of vertebral column and rib deformities were observed in both treated groups; mandibular defects and fusion of the sternebrae were additionally observed at 15 mg/kg bw. Incidences of anophthalmia and microphthalmia were also increased. The same findings are also reported elsewhere (Ema *et al*., 1995c**)**. Although maternal toxicity was observed in this study (initial slight weight loss, overall reductions in weight gain) at dose levels of 10 and 15 mg/kg bw/d, the severity of maternal toxicity is not considered to be sufficient to account for the level of foetal malformations seen at these dose levels.

Ema *et al*. (1991) report increased 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); micrognathia, cleft palate, omphalocoele, exencephaly, anal atresia, club foot and anomalies of the tail (vestigial, kinky and short tail) were also frequently observed. A NOAEL for teratogenicity of 2.5 mg/kg bw/d can be determined for this study, based on increased malformation incidences at ≥5 mg/kg bw/d. Although malformations at 7.5 and 10 mg/kg bw/d were associated with marked maternal toxicity, it is notable that the increased incidence of foetal malformations at 5 mg/kg bw/d occurred in the absence of overt maternal toxicity.

Further work by the same authors (Ema *et al*., 1992b**;** Ema, 2001; Ema *et al*., 1992a) using higher and maternally toxicity dose levels of 20 or 40 mg/kg bw/d, identify the sensitive period for DBTC teratogenicity to be GD 7 or 8, with a higher incidence of foetuses affected by administration on GD 8. Exposure at later time points resulted in increased post-implantation loss, reduced litter size and reduced foetal weight. A comparative study with DBTC, DBTA, DBTM, DBTL and DBTO (Noda *et al*., 1993) using a single gavage

administration on GD 8, shows a comparable spectrum of effects for all substances, in the absence of maternal toxicity. The study used dose levels of 80 µmol/kg bw, equivalent to dose levels of 25 mg/kg bw (DBTC), 50 mg/kg bw (DBTL), 28 mg/kg bw (DBTA), 28 mg/kg bw (DBTA) and 20 mg/kg bw (DBTO). Treatment showed a comparable incidence of foetal malformations for DBTC (17.3%), DBTA (28.3%), DBTL (30.6%), DBTM (12.5%) and DBTO (20.7%). 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

Further investigations using DBTA confirm that administration on GD 7 resulted in foetal malformations including cleft mandible, cleft lower lip, ankyloglossia, schistoglossia and exencephaly (Noda *et al*., 1992a). 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 in this study at 15 mg/kg bw/d (reduced weight gain) but not at 10 mg/kg bw/d. A conference abstract by Noda *et al*., (1992c) appears to report findings reported elsewhere in more detail in a full paper (Noda *et al*., 1993).

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

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

A study by Noda *et al*, (2001) investigated 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 and in foetuses from 7.5 month-old dams at ≥10 mg/kg bw. Malformations (cleft mandible, cleft lower lip, ankyloglossia and/or schistoglossia) were comparable in both groups.

Ema *et al*. (1996b) showed a reduction in foetal weight but no evidence of embryofoetal mortality or 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 to 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.

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

A study with DBTC in cynomolgus monkeys (Ema *et al*., 2009), reports embryofoetal loss but no foetal malformations following treatment with 7.5 mg/kg bw/d between GD 19-36. Findings were associated with maternal toxicity (signs of toxicity and slightly reduced weight gain). A further study in monkeys (Ema *et al*., 2007b, also reported by Ema *et al*., 2006b and Ema *et al*., 2006a) reports embryofoetal loss but no foetal malformations following treatment with dose levels of 2.5 and 3.8 mg/kg bw/d on GD 20-50. Findings were associated with signs of toxicity and weight loss. The dosing periods in these studies were designed to cover organogenesis (GD 20-50).

In vitro, studies in cultured rat limb bud cells clearly demonstrate the potential of DBTC to inhibit cell differentiation and cell proliferation (Yonemoto *et al*., 1993; Yonemoto, 1992). Studies in cultured explanted rat embryos (Ema *et al*., 1995a; Iwase *et al*., 1995; Iwase *et al*., 1996; Iwase *et al*., 1997) 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

The definition of reproductive toxicity in the CLP Regulation (Annex I: 3.7.1.1) includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring.

Adverse effects on development of the offspring (Annex I: 3.7.1.4) includes 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 post-natally, to the time of sexual maturation. As classification for developmental toxicity is primarily intended to provide a hazard warning for pregnant women and for men and women of reproductive capacity, for pragmatic purposes, classification for developmental toxicity is essentially intended to encompass adverse effects induced during pregnancy, or as a result of parental exposure.

For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. Within each category, effects on sexual function and fertility and on development are considered separately. Effects on lactation are allocated to a separate hazard category. Substances are allocated to Category 1 (substances that are a known or presumed human reproductive toxicant) or to Category 2 (substances that are a suspected human reproductive toxicant). Substances are classified in Category 1 when they are 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 is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B). 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.

No data are available for dibutylbis(pentane-2,4-dionato-O-O'-tin; however relevant data are available for DBTC and related substances as part of a read-across (category) approach.

Data show consistently that DBTC and related compounds have the potential to cause foetal malformations (a characteristic pattern of external and skeletal malformations, predominantly affecting the skull and jaw) in studies in the rat, and that the sensitive period of exposure is Gestation Day 8. Exposure is also shown to cause post-implantation loss (and subsequently a reduced litter size), as well as a reduction in foetal weight. Many studies used relatively high dose levels sufficient to cause marked maternal toxicity; however effects

are also apparent at lower dose levels not causing marked maternal toxicity and also (albeit inconsistently) at dose levels not causing overt maternal toxicity. Studies in the mouse and cynomolgus monkey show foetotoxicity and increased post-implantation loss but do not replicate the characteristic pattern of malformations seen consistently in studies in the rat. Although no evidence of teratogenicity is seen in these species, it cannot be excluded that effects are masked by the relatively high level of post-implantation loss. In vitro and mechanistic data confirm the sensitivity of the rat foetus to malformations induced by DBTC, but do not further elucidate the potential species-specificity of this effect.

Based on the clear and consistent evidence of effects on the developing foetus (post-implantation loss, skeletal and external malformations) in rat studies with only mild or no overt maternal toxicity and in the absence of data indicating that effects are not relevant to humans, classification of dibutylbis(pentane-2,4-dionato-O-O')tin for reproductive toxicity (adverse effects on development) in Category 1B is therefore considered to be appropriate.

10.10.7 Adverse effects on or via lactation

No relevant studies are available.

Table 18: Summary table of animal studies on effects on or via lactation

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

Table 19: Summary table of human data on effects on or via lactation

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

Table 20: Summary table of other studies relevant for effects on or via lactation

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

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

No relevant data are available. The only study including maternal exposure during lactation is an OECD 421 screening study with DBTC (Waalkens-Berendsen, 2003), which reports a high level of early post-natal

mortality (50%) at the highest dietary concentration of 200 ppm. It is unclear if this effect is due to exposure of offspring via lactation.

10.10.9 Comparison with the CLP criteria

The definition of reproductive toxicity in the CLP Regulation (Annex I: 3.7.1.1) includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring.

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

Adverse effects on or via lactation are included under reproductive toxicity, but for classification purposes such effects are treated separately (Annex I: 3.7.1.5).

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Classification of dibutylbis(pentane-2,4-dionato-O,O')tin for reproductive toxicity in Category 1B (H360FD: May damage fertility. May damage the unborn child) is considered to be appropriate.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

A category approach, supported on the basis of hydrolytic and toxicokinetic behaviour, and toxicological data, was used by the DS to justify that studies on DBTC can be taken into consideration when classifying DBTP for the hazard class reproductive toxicity.

Fertility

No data are available for DBTP, thus the evaluation was based on studies with DBTC and related substances.

The fertility effects of DBTC observed in the OECD TG 421 reproduction/developmental toxicity screening study (Waalkens-Berendsen, 2003) did not include noticeable effects on males. However, in females rats reduced weight gain was observed over the pre-mating, gestation, and lactation periods at the higher dose level (200 ppm). The corpora lutea numbers were not measured in this study and the full study report was also not available.

A fertility study with DBTC (Ema & Harazono, 2000a; but also reported in Ema *et al.*, 2000 and Ema & Harazono, 2000b), which was used as a key study for the classification of DBTC, is included in the CLH report. Observed effects included an increase in the number of non-pregnant females, a reduced number of implantations and an increased incidence of pre-implantation loss upon exposure to DBTC on GD 0-3, and an increased incidence of early total resorption upon exposure to DBTC on GD 4-7.

In a developmental toxicity study in the mouse with DBTC (Ema *et al*., 2007a) a lower level of pregnancies was associated with pre-implantation losses and it followed a clear dose-response

relationship. Post-implantation loss increase was also associated with an increase in dosing. In addition, there was a small number of deaths of dams observed across all dosing groups, however they were not considered to be treatment related due to the absence of a dose-response relationship.

Two supportive mechanistic studies explored the effect of progesterone on implantation failure induced by dibutyltin dichloride in rats (Ema *et al.*, 2003a,b). Other two supportive studies (Harazono & Ema, 2003; Harazono & Ema, 2001) also focused on the effects of DBTC in decidual cell response and progesterone levels during pseudo-pregnancy.

Based on 1) the increased number of non-pregnant individuals among successfully mated females, the reduced number of implantations, the increased pre-implantation losses and increased early total resorptions in the key fertility study with DBTC; 2) the harmonised classification of DBTC as Repr. 1B for effects on fertility and sexual function; and 3) given the hydrolytic behaviour of the substances in the category at neutral and low pH, the DS considered that DBTP should have the same classification as DBTC. The DS therefore proposed Repr. 1B for effects on fertility and sexual function for DBTP.

Development

No data are available for DBTP, thus the evaluation was based on studies with DBTC and related substances.

Two guideline compliant studies are presented in the CLH report: a screening study (OECD TG 421) and a prenatal developmental toxicity study (PNDT, OECD TG 414). At the high dose of the screening study (12.0-15.4 mg/kg bw/d), the number of foetuses was strongly reduced (10 compared to 101 in the controls). The PNDT reported severe malformations in 4 foetuses from 3 high dose litters (10 mg/kg bw/d). Both studies reported maternal toxicity at these doses, in particular reduced body weight gain, however, the values were not reported.

In addition, several non-compliant PNDT studies were presented as supportive evidence.

Five studies by Ema *et al.* (2003a,b; 2007a,f,g) identified that the sensitive period for DBTC teratogenicity is GD 7 or 8. Exposure in this period resulted in a characteristic pattern of external and skeletal malformations, predominantly affecting the skull and jaw. Other reported effects included increased post-implantation loss, reduced litter size and reduced foetal weight. Although also maternal toxicity was observed at doses of 7.5 mg/kg bw/d and higher, foetal malformations occurred at 5 mg/kg bw/d in absence of significant maternal toxicity (Ema *et al.*, 1991).

Studies by Noda *et al.* (1992 a,c, 1993) showed that similar foetal malformations are induced by other di-n-butyltin moieties after administration on GD 8, including DBTA, DBTO, DBTL, and DBTM.

Foetotoxicity and increased post-implantation loss was also observed in mice and cynomolgus monkeys, but these did not show the malformations observed in rats, although the effects may have been masked by the high level of post-implantation loss.

Based on the clear and consistent evidence of effects on the developing foetus (post-implantation loss, skeletal and external malformations) in rat studies and in the absence of severe maternal toxicity, the DS proposed to classify DBTP for reproductive toxicity (adverse effects on development) in Category 1B.

Comments received during public consultation

Two MSCAs agreed with the proposed classification as Repr. 1B (development). One asked for additional clarification regarding the read-across approach and purity of the compound.

The DS noted there are no indications that DBTC is an impurity of DBTP, and even if it would be, this would not change the classification as the read-across is based on DBTC.

It is also clarified that the hydrolysis studies of Schilt (2004) and Naβhan (2015, 2016) used different concentrations and analytical techniques. As a result, it is not certain that DBTL, DBTO, and DBTM also form the distannoxane, although they seem to form identical tin moieties.

One Company/Manufacturer submitted comments expressing their different interpretation of the hydrolysis data. Their main argument is that, since the hydrolysis of DBTP is faster than DBTC it will result in a different bioavailability of the tin compound. The company also considers that the other tin compounds that are part of the category (DBTA, DBTM) have different hydrolysis rates and initiate different products therefore making them not suitable for read across.

In their response, the DS considered that DBTC and DBTP have hydrolysis rates of equivalent magnitude and result in the same bridged stannoxane, thus supporting the category read-across approach based on hydrolysis and toxicokinetics. The DS also replied that they were not in the possession of gastric hydrolysis studies for the other dibutyltin compounds referred in the comment from industry (DBTL, DBTA and DBTM).

Furthermore, to further justify the inclusion of the category members, the DS indicated a comparative rat developmental study that included all category members. The findings of the study by Noda *et al*. (1992c and 1993) demonstrate that all category members cause similar characteristic external and skeletal foetal malformations (largely affecting the jaw/skull).

Assessment and comparison with the classification criteria

Fertility

The reprotoxic effects of DBTC observed in the OECD TG 421 reproduction/developmental toxicity screening (Waalkens-Berendsen, 2003) were a significant increase in the incidence of ovarian cysts in nine of the twelve high-dosed females (200 mg/kg diet; corresponding to 6.2-15.4 mg/kg bw/d), an increase in the number of dams with post implantation loss, a reduction in the number of live pups and a reduction in the gestation index. In line with the conclusion of the dossier submitter, RAC considers these effects as relevant for classification for fertility of DBTC, although it is recognized that some of these effects (e.g. reduced number of live pups) could also be due to developmental toxicity.

Another rat fertility study (non-guideline, non-GLP) with DBTC is presented (Ema & Harazono, 2000a). Successfully mated female rats were exposed via gastric intubation to DBTC in olive oil (0-3.8-7.6-15.2 mg/kg bw/d) on GD 0-3 or GD 4-7. In addition to a control group (olive oil), also a pair-fed group (feed restricted to same amounts as high dose DBTC-group) was included. A significantly higher number of non-pregnant dams was observed after being exposed to the mid and high dose of DBTC on GD 0-3 (high dose: 87%, mid dose: 31.3%, low dose: 0%, control: 0%, pair-fed: 5.9%). In addition, a reduced number of implantations (high dose: 1.8±4.8, mid dose: 10.1±7.1, low dose: 15±1.5, control: 15±1.4, pair-fed: 13.4±4.3)

and increased incidences of preimplantation loss (high dose: 87.9%, mid dose: 35.6%, low dose: 4.1%, control: 2.7%, pair-fed: 16.4%) were observed in these DBTC-exposed groups as evidence for effects on fertility. The fertility effects for the high dose group were statistically significantly different from the control group as well as from the pair-fed group, whereas effects for the mid dose groups were statistically significantly different from the control group. Slight general toxicity was observed and included reduced body weight and feed consumption in the high dose group (for details on body weight (BW) changes please refer to Annex I of the background document (BD))). Upon DBTC-exposure during GD 4-7 increased early total resorptions were observed in the high dose group (87.5%; statistically significantly different from the control (0%) and from the pair-fed groups (11.8%)). Also in these animals, some slight general toxicity was observed and included reduced adjusted bw gain (i.e. excl. the uterus) and reduced feed consumption (for details on BW changes please refer to Annex I of the BD). RAC evaluated the general toxicity effects and concluded that the reproductive effects are not due to a secondary non-specific consequence of parental toxicity.

In a developmental toxicity study with the CD1 mice, groups of mated female were administered DBTC (in olive oil) via gavage at dose levels of 0 (vehicle control), 7.6, 15.2 or 30.4 mg/kg bw/d on GD 0-3 or GD 4-7. Dam mortality occurred in all treated groups but without a dose-response relationship. Other signs of toxicity (vaginal discharge, hypo activity, hypothermia) were also observed at all dose levels and jaundice in the medium and high doses. Body weight and food consumption were also affected negatively. Regarding the number of pregnant females, there was a dose related increase in the pre-implantation loss with the dose administered (29.7% - 7,6 mg/kg bw/d, 34.0% - 15.2 mg/kg bw/d, 58.3% - 30.4 mg/kg bw/d at low, mid and high dose respectively) that was statistically significant in the high dose. As for the post implantation losses, they also were increased in a dose-dependent manner and the effect was statistically significant from the mid dose (15.2 mg/kg bw/d).

Four additional studies on the mechanism of toxicity of DBTC seem to support that DBTC negatively influences the levels of progesterone, hence causing the observed implantation losses (Ema *et al.*, 2003a,b; Harazono & Ema, 2003; Harazono & Ema, 2001).

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

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

Altogether, RAC supports the conclusion of the DS that DBTP should be classified as toxic to reproduction for effects on sexual function and fertility as Repr. 1B (H360F: May damage fertility).

Development

The reprotoxic effects of DBTC observed in the OECD TG 421 reproduction/developmental toxicity screening study (Waalkens-Berendsen, 2003) included an increase in the number of dams with post implantation loss, a reduction in the number of live pups and a reduction in the gestation index.

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

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

Three additional studies on developmental toxicity conducted to assess the most sensitive gestation window for exposure to DBTC indicated that induced teratogenic effects were observed following exposure on GD 7-8 and were most pronounced when dams were exposed on GD 8 (Ema *et al.*, 1992, 1995, 1996). Embryo-lethality was observed at all tested time-points for exposure during gestation (GD 6-15).

A comparative study with DBTC, DBTA, DBTM, DBTL and DBTO (Noda *et al.*, 1993) using a single gavage administration of 80 µmol/kg bw on GD 8, shows a comparable spectrum of effects (incidence and type of foetal malformations) for all substances, in the absence of maternal toxicity.

In addition, the sensitivity of the rat foetus to DBTC was confirmed by several *in vitro* studies.

There is some uncertainty whether the typical pattern of malformations observed is rat specific; however, as there is insufficient information on other species, RAC agrees with the DS that these findings should be considered relevant.

Overall, several studies consistently showed foetal effects (malformations, post-implantation loss and weight reduction) at doses with limited or no maternal toxicity, and there is no basis to question the human relevance of these effects. Thus, RAC considers that there is clear evidence of an adverse effect on development upon exposure to DBTC.

Given that both DBTP and DBTC are hydrolysed to the same distannoxane, RAC is of the opinion that data on DBTC can be used for classification of DBTP for effects on development (see also under *RAC general comment*).

RAC supports the conclusion of the DS that DBTP should be classified as toxic to reproduction for developmental toxicity in Category 1B, leading to the overall classification of **Repr. 1B** (**H360D; May damage the unborn child**).

Specific Concentration Limits (SCL)

Setting of Specific Concentration Limits is considered not necessary for reproduction toxicity (effects on sexual function and fertility and on development), given that ED_{10}-values for DBTDL fall within the ranges of a medium potency group (i.e. 4 mg/kg bw/d < ED_{10} < 400 mg/kg bw/d) and modifying factors which might changing the potency group are considered not needed, resulting in the GCL of 0.3% (see ECHA Guidance on the Application of the CLP Criteria v. 5.0, section 3.7.2.5).

10.11 Specific target organ toxicity-single exposure

Not considered in this CLH Report.

10.12 Specific target organ toxicity-repeated exposure

Table 21: Summary table of animal studies on STOT RE

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
Comparable to OECD 407	DBTC (>98% purity)	Wistar (WU-CPB) rat (M, F); 10/sex/ group	Oral (diet)	50, 150 ppm (28 days)	Deaths 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%). Liver/bile duct pathology at 150 ppm. Lymphocyte depletion in the thymic cortex and PALS at 50 and 150 ppm **LOAEL = 50 ppm (~2.5 mg/kg bw/d)** **NOAEL <50 ppm (~2.5 mg/kg bw/d)**	Seinen & Vos, 1977 Penninks & Seinen, 1982
		Swiss mouse (M)		50, 150 ppm (28 days)	No effects of treatment	
Comparable to OECD 408	DBTC (99.7% purity)	Rat (M, F)	Oral (dietary)	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)**	Gaunt *et al*., 1968
OECD 421	DBTC	Wistar rat	Oral (dietary)	5, 30, 200	Reduced weight gain, food	Waalkens-

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
	(98.75% purity)	(M, F)		ppm: 2 (F) or 4 weeks (M) pre-mating to PND 4	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 (F). The thymus was not investigated in males. **LOAEL =30 ppm** **NOAEL =5 ppm (0.4 mg/kg bw/d)**	Berendsen, 2003
OECD 414	DBTC (>98% purity)	Wistar rat (F)	Oral (gavage)	1, 2.5, 5, 10 mg/kg bw/d (GD 6-15)	Reduced weight gain & food consumption at 10 mg/kg bw/d; slightly reduced weight gain at 5 mg/kg bw/d. Thymic atrophy at 10 mg/kg bw/d and (to a lesser extent) at 2.5 and 5 mg/kg bw/d. **LOAEL = 2.5 mg/kg bw/d** **NOAEL <2.5 mg/kg bw**/d	Study report, 1994
OECD 414	DBTC: purity not reported	Wistar rat (F)	Oral (gavage)	1, 2.5, 5, 10 mg/kg bw/d (GD 7-17)	Reduced maternal weight gain (~17%) & food consumption (~7%) at 10 mg/kg bw/d. Reduced thymus weight (-23%) at 10 mg/kg bw/d. **LOAEL = 10 mg/kg bw/d** **NOAEL =5 mg/kg bw/d**	Farr *et al*., 2001
None	DBTC: 96% purity	CD rat (M, F)	Oral (drinking water)	10, 25 mg/L equivalent to 0.9, 1.9 mg/kg bw/d	No bodyweight effects. Reduced water consumption at 25 mg/L (M, F). No effects on thymus weight, antibody production, DTH response or NK cell activity. **NOAEL >2.5 mg/kg bw/d** **NOAEL =2.5 mg/kg bw/d**	DeWitt *et al*., 2005b
None	DBTC (purity unknown)	Rat (strain not reported)	Oral (diet)	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	Barnes & Stoner, 1958

Method Guideline, Deviation(s) from the guideline (if any)	Test substance, reference to table 5	Species Strain Sex no/group	Route of exposure	Dose levels duration of exposure	Results	Reference
					treatment for 54-55 days. Treatment for 6 months resulted in mortality (75 and 100 ppm), reduced weight gain and food consumption (≥50 ppm), bile duct and pancreas pathology (≥50 ppm). **LOAEL =50 ppm (2.5 mg/kg bw/d)** **NOAEL = 20 ppm (1 mg/kg bw/d)**	

Table 22: Summary table of human data on STOT RE

Type of data/report	Test substance, reference to table 5	Route of exposure Relevant information about the study (as applicable)	Observations	Reference
No human data are available				

Table 23: Summary table of other studies relevant for STOT RE

Type of study/data	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
Mechanistic study	DBTC: purity not reported	SCID mice engrafted with human foetal thymus and liver tissue fragments (SCD-hu mice) were exposed to a single intraperitoneal dose of DBTC (0, 0.03, 1.0 mg/kg bw).	No bodyweight effects were observed. Histopathology showed reduced thymus size and a reduction in the size of the thymic cortex following DBTC exposure.	de Heer *et al*., 1995
None	DBTC >98% purity	Wistar rat; gavage dose levels of 0 or 15 mg/kg bw (single dose). Measurement of thymus weight, histopathology and incorporation of radiolabelled precursors into DNA, RNA and protein.	Rapid (from Day 2, maximal at Day 4) but reversible (by Day 9) reduction in thymus weight. Reduced thymus cellularity, cell populations were normal at Day 9. **NOAEL <15 mg/kg bw**	Snoeij *et al*., 1989
None	DBTC: >98% purity	WU rat WAG rat Swiss mouse Dietary concentrations of 0 or 150 ppm; treatment three weeks. Weights of the thymus, spleen, adrenals and lymph	Allograft rejection was significantly delayed; other measures of immune function were unaffected by treatment.	Seinen *et al*., 1977

Type of study/data	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
		node were recorded; allograft rejection response measured in rats.		
None	DBTC: purity not reported	SD rat (F, maternal); pregnant rats administered DBTC in drinking water at 0, 10 or 25 mg/L on GD 6-PND 21. DTH and NK response assessed in offspring at PND 42. SD rat (M, F; pups) gavaged with DBTC at 0, 1.0 or 2.5 mg/kg bw from PND 3 (3/week). DTH and NK response assessed in offspring at PND 42.	No effects of treatment Reduced weight gain (2.5 mg/kg bw/d) No clear effects on immune parameters **NOAEL = 2.5 mg/kg bw/d**	DeWitt *et al*., 2006b Luebke *et al*., 2003 DeWitt *et al*., 2006a
None	DBTC: purity not reported	SD rat (M, F) administered DBTC in drinking water (0, 10, 25 mg/L) for 28 days; immune responses (DTH, primary and secondary antibody response to SRBC, NK cell activity) measured on Day 29.	No effects on bodyweight; decreased water consumption (25 mg/L) No effects on immune parameters. **NOAEL =25 mg/L**	DeWitt *et al*., 2005a

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

No studies are available for dibutylbis(pentane-2,4-dionato-O-O')tin; reference is instead made to studies performed using dibutyltin dichloride (DBTC) as part of a read-across (category) approach.

With regard to classification for repeated dose toxicity effects on the immune system (notably the thymus) are critical and have been assessed in a number of studies intended to address repeated dose toxicity. Additional relevant information is also provided by studies primarily intended to assess developmental and/or reproductive toxicity, but which include measurement of thymus weight or assessment of thymus histopathology.

In a 90-day sub-chronic toxicity study performed at dietary concentrations of 0, 10, 20, 40 and 80 ppm DBTC (Gaunt *et al*., 1968), reduced weight gain and food consumption and a marginal effect on haemoglobin concentration were seen at the highest dietary concentration. No effects on the thymus were reported in this study at the highest dietary concentration of 80 ppm (equivalent to approximately 4 mg/kg bw/d). A 28-day study in rats and mice (Seinen & Vos., 1977) performed with DBTC at dietary concentrations of 0, 50 and 150 ppm did not identify any effects of treatment in mice. Mortality occurred in rats at 150 ppm. Thymus size, 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 NOAEL for immune system effects of <50 ppm (equivalent to approximately 2.5 mg/kg bw/d) can be determined for this study. A 14-day study in rats (Penninks & Seinen, 1982) performed using DBTC at dietary concentrations of 0, 50 and 150 ppm reports mortality and signs of toxicity (severe jaundice) at 150 ppm; weight gain was reduced in both treated groups. Increased liver weight and histopathology were noted at 150 ppm. Marked lymphoid depletion of the thymus and spleen is reported in both treated groups. NOAEL for immune system effects of <50 ppm (equivalent to approximately 2.5 mg/kg bw/d) can also be determined

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

Thymus histopathology was investigated in female (but not male) rats in an OECD 421 screening study (Waalkens-Berendsen, 2003) using dietary concentrations of 0, 5, 30 or 200 ppm DBTC. Female rats were exposed of two weeks prior to mating, throughout mating, gestation and to PND 4. Weight gain and food consumption by females at 200 ppm were reduced over the pre-mating, gestation and lactation periods. Histopathology revealed thymic depletion at 200 ppm (graded as severe to very severe) and 30 ppm (moderate to severe); findings at 30 ppm were apparent in the majority of pregnant females but were not observed in non-pregnant rats. Lymphoid depletion was characterised by decreased size of the thymic lobules due to extensive loss of cortical and medullary lymphocytes. The border between the thymus cortical and medullary areas was therefore indistinct. In the most severe cases, the cortex was very small or partially absent. The remaining cells visible in the cortical areas were mainly lymphoblasts and reticular epithelial cells. A NOAEL of 5 ppm (0.4 mg/kg bw/d) can be determined for thymus histopathology in this study. Thymus 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; 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.

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 parked at four days but was reversible by nine days. The numbers of large cells were reduced from one day after treatment; whereas small and intermediate cells were reduced from three days following treatment. Cell populations had recovered by nine days. Incorporation of radioactivity into DNA, RNA and protein precursors was reduced on Days 1 and 2 only. 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. A number of published studies (DeWitt *et al*., 2006b; Luebke *et al*., 2003; DeWitt *et al*., 2005a; DeWitt *et al*., 2006a) investigate the potential developmental immunotoxicity of DBTC following *in utero* and/or early post-natal exposure. Studies involved exposure of maternal rats to DBTC in the drinking water (from GD 6) and direct exposure of offspring (to PND 21) by gavage. The dose levels used in these studies were relatively low (up to 5 mg/kg bw/d for direct exposure of offspring) but do 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) shows a reduction in thymus cortex size following treatment with DBTC.

Key studies are the guideline-comparable 28-day study (Seinen & Vos (1977; Peninks & Seinen (1982)) and the 90-day study (Gaunt *et al*., 1968). The 28-day study identifies a LOAEL of 2.5 mg/kg bw/d which is extrapolated to a NOAEL of 0.8 mg/kg bw/d. The 90-day study identifies a LOAEL of 4 mg/kg bw/d. Both of these studies therefore support classification for STOT (RE) in Category 1. Findings are consistent with those of the OECD 421 screening study (Waalkens-Berendsen, 2003), which reports a LOAEL of 30 ppm (1.7-2.4 mg/kg bw/d) for reduced thymus weight in females (total exposure approximately 56 days). Studies were performed with DBTC, whereas dibutylbis(pentane-2,4-dionato-O,O')tin is hydrolysed to form DBTC in the mammalian stomach; the quantity of DBTC resulting from the total hydrolysis of dibutylbis(pentane-2,4-dionato-O,O')tin is approximately 70% based on molecular weight. Classification as STOT RE1 is therefore supported for dibutylbis(pentane-2,4-dionato-O,O')tin.

Table 24: Extrapolation of equivalent effective dose for toxicity studies of greater or lesser duration than 90 days

Study reference	Effective dose (mg/kg/d)	Length of exposure	Extrapolated effective dose when extrapolated to 90-day exposure	Classification supported by the study
Seinen & Vos (1977 Penninks & Seinen, 1982	2.5 mg/kg bw/d (LOAEL)	28 days	0.8 mg/kg bw/d	STOT RE1
Gaunt *et al*., (1968)	4 mg/kg bw/d (LOAEL)	90 days	-	STOT RE1
Waalkens-Berendsen (2003)	1.7-2.4 mg/kg bw/d	~56 days	-	STOT RE1

10.12.2 Comparison with the CLP criteria

Specific target organ toxicity (repeated exposure) is defined in the CLP Regulation (Annex I, 3.9.1.1) as specific, target organ toxicity arising from repeated exposure to a substance. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included in this definition. The adverse health effects relevant for STOT RE classification include consistent and identifiable toxic effects in humans, or, in experimental animals, toxicologically significant changes which have affected the function or morphology of a tissue/organ, or have produced serious changes to the biochemistry or haematology of the organism and these changes are relevant for human health. With respect to animal data, Annex 1, Section 3.9.2.5 of the CLP Regulation notes that the standard animal studies in rats or mice that provide this information are 28-day, 90-day or lifetime studies that include haematological, clinical chemistry and detailed macroscopic and microscopic examination to enable the toxic effects on target tissues/organs to be identified. Data from repeat dose studies performed in other species may also be used, if available and other long-term exposure studies such as carcinogenicity, neurotoxicity or reproductive toxicity may also provide evidence of specific target organ toxicity that could be used in the assessment of STOT RE classification.

Substances are classified in STOT RE Category 1 based on evidence of significant toxicity in humans or where there is evidence from studies in experimental animals that they can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. For classification in Category 1, either reliable good quality human data (evidence from human cases or epidemiological studies) or animal data (observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were observed at generally low exposure concentrations) is required. 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 are classified in STOT RE Category 2 based on evidence from studies in experimental animals that they can be presumed to have the potential to be harmful to human health following repeated exposure. For classification in Category 2, animal data (observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were observed at generally moderate exposure concentrations) is required. 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 clear from the available data that dibutyltin dichloride has the potential to cause effects on the thymus (lymphoid depletion) following single or repeated administration and is therefore classified for STOT SE 1 and STOT RE 1. Effects on other organs including the liver, pancreas and bile duct are reported at higher dose levels and are therefore less critical with regard to classification.

Effects on the thymus are shown 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. Studies consistently report 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. Following stoichiometric correction, the LOAEL for dibutylbis(pentane-2,4-dionato-O-O')tin is calculated to be approximately 4.2 mg/kg bw/d. Following correction for study duration, the corrected dose for a 90-day study is estimated to be approximately 1-2 mg/kg bw/d. A mechanistic study in SCID mice grafted with human thymus fragments also report effects, indicating that DBTC is also likely to have similar effects in humans.

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 STOT RE in Category 1 is considered to be appropriate.

Classification of dibutylbis(pentane-2,4-dionato-O,O')tin for STOT RE in Category 1 (H372: Causes damage to the immune system) is considered to be appropriate.

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

Summary of the Dossier Submitter's proposal

Although there are no studies with DBTP for STOT RE, a category approach, based on the hydrolytic and toxicokinetic behaviour, and on the toxicological data, was used by the DS to justify that studies on DBTC can be considered when classifying DBTP for this hazard class. The CLH report does not contain any relevant studies with DBTP but it includes several animal studies with repeated oral exposure to DBTC. No studies are available for the dermal and inhalation route.

DBTC

The following studies were included in the CLH report:

- OECD TG 407 (comparable study) repeated dose 28-day oral toxicity in rat (Wister) (Seinen & Vos, 1977)
- OECD TG 407 (comparable study) repeated dose 28-day oral toxicity with Swiss mouse (Penninks & Seinen, 1982)
- OECD TG 408 (comparable study) in rat (CFE - Carworth Farms Elia) 90-d oral feeding (Gaunt *et al.*, 1968)
- OECD TG 421 reproductive/developmental toxicity screening test (diet) in rat (Wistar) (Waalkens-Berendsen, 2003)
- OECD TG 414 prenatal developmental toxicity study (gavage) in rat (Wistar) (Study Report, 1994)
- OECD TG 414 prenatal development toxicity studies (gavage) in rat (Wistar) (Farr *et al.*, 2001).
- 28 day drinking water sub-acute toxicity study of immune function in the rat (DeWitt *et al.*, 2005b)
- sub-chronic oral feeding study (dietary concentrations of max. 100 ppm) for up to 6 months (Barnes & Stoner, 1958).

A number of non-standard and mechanistic studies are also available for DBTC.

- Single oral gavage rat study (Snoeij *et al.*, 1988)

- Mechanistic investigation of thymic atrophy in the rat, the mouse and the Guinea pig (Seinen *et al.*, 1977)
- 28 day drinking water sub-acute toxicity study of immune function in the rat (DeWitt *et al.*, 2005a)
- Sub-acute toxicity study of immune function in rats exposed during development (drinking water) (DeWitt *et al.*, 2006a)
- Developmental immunotoxicity study in the rat (dams: drinking water; pups: gavage or untreated) (DeWitt *et al.*, 2006b) (also reported by Luebke *et al.*, 2003; in conference abstract)
- Effect of single dose intraperitoneal injection on SCID mice engrafted with human foetal thymus and liver tissue fragments (de Heer *et al.*, 1995)

The immune system was clearly the target organ, as observed after oral exposure to DBTC. Effects included reduced thymus weight, thymus atrophy, immune system depression (e.g. inhibitory effect on T-lymphocyte activity), reduced weight gain in pups and reduced water and food consumption in some cases. The DS considered the two OECD TG 407 comparable 28-d repeated dose studies as key studies (Seinen & Vos, 1977 and Penninks & Seinen, 1982). In these studies a LOAEL of 2.5 mg/kg bw/d was derived based on reduced thymus, spleen and lymph node weight (M/F) and lymphocyte depletion in the thymic cortex and PALS. The DS concluded that the available data supported classification for specific target organ toxicity following repeated exposure as STOT RE 1 with the immune system as target organ.

Comments received during public consultation

Three MSCAs commented and agreed with the proposed classification for STOT RE during the public consultation. One of the MSCAs expressed their doubts regarding the study of Gaunt *et al.* (1968) being considered a key study. They proposed that it should be considered as supportive due a lack of identified effects on thymus. The DS agreed to this proposal.

Assessment and comparison with the classification criteria

Summary of the most relevant studies

An OECD TG 421 reproductive/developmental toxicity screening test (diet) in rats (Waalkens-Berendsen, 2003) showed, in addition to reduced body weight gain and food consumption in male and female animals, a reduced relative thymus weight and moderate to severe lymphoid depletion in dams exposed to 1.7-2.4 mg/kg bw/d (exposure 41 days for females and 28 days for males). A dose of 6.2- to 15.4 mg/kg bw/d induced a reduced absolute and relative thymus weight and a severe to very severe lymphoid depletion in dams. Lymphoid depletion was characterized by a decrease in size of thymic lobules due to an extensive loss of cortical and medullary small lymphocytes. The distinction between cortical and medullary areas was blurred. In the severe cases, the cortex was very small or partially absent. The effects on fertility and development, as observed in this study, are described and evaluated in the section RAC evaluation of Reproductive Toxicity.

An OECD TG 414 prenatal developmental toxicity study in rats (oral gavage; 0, 1, 2.5, 5, 10 mg/kg bw/d on GD 6-15) showed clear maternal toxicity (Study Report, 1994). Effects included reduced bw gain (≥ 5 mg/kg bw/d), reduced food consumption (≥ 10 mg/kg bw/d) and significantly increased number of animals with thymus atrophy (≥ 10 mg/kg bw/d). Maternal

toxicity was not observed at a dose of 1 mg/kg bw/d. The effects on development, as observed in this study, are described and evaluated in the section "RAC evaluation of Reproductive Toxicity".

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

A 28-d rat drinking water study (0, 0.9, 1.9 mg DBTC/kg bw/d in an initial experiment; 0, 1.0, 2.8 mg DBTC/kg bw/d in the replicate experiment), which focussed on immunotoxic effects, did not reveal any treatment-related effect on organ weight (including the thymus and spleen), antibody production, delayed type hypersensitivity response or natural killer cell activity. A slight reduction in water consumption was observed in the high dose group (DeWitt *et al.*, 2005b). Another study (DeWitt *et al.*, 2006b) also did not provide evidence that DBTC affected the rat immune system at low concentrations (1.0 to 4.4 mg/kg bw/d).

A 28d rat/mouse immunotoxicity study (rat: 2 weeks, mouse: 4 weeks) with doses of DBTC of 0, 50 and 150 ppm in the diet (corresponding to 0, 2.5, 7.5 mg/kg bw/d for rats and 0, 7.1, 21.4 mg/kg bw/d for mice) was included in the CLH report (Seinen & Vos, 1977). No treatment-related effects were observed in mice. In rats, mortality was observed in the 7.5 mg/kg bw/d group (4/10 females and 2/10 males). Further, clear dose-dependent effects on the thymus were observed. Reductions in relative organ weights were noticed for the thymus (2.5 mg/kg bw/d: 53%, 7.5 mg/kg bw/d: 68-72%), but also the spleen (2.5 mg/kg bw/d: 16%, 7.5 mg/kg bw/d: 33%) and popliteal lymph nodes (2.5 mg/kg bw/d: 16%, 7.5 mg/kg bw/d: 28%). A pronounced reduction in size of the thymus was found in all DBTC-treated animals. The most important effect observed was lymphocyte depletion in lymphoid organs, which was most pronounced in the thymic cortex of DBTC-treated animals. At the 7.5 mg/kg bw/d level, the thymic cortex was almost completely depleted, although no signs of cell destruction were observed. Lymphocyte depletion was also present in the thymus-dependent areas of the spleen and popliteal lymph nodes. In addition, effects on liver were observed and included thickened and dilated bile ducts accompanied by irregularly yellowish discoloured livers. These effects were found in the animals that died and in 2 male and 2 female survivors of the high dose group. Microscopic analysis revealed severe proliferation of bile duct epithelial cells and bile ducts which was associated with pericholangiolitis and periportal fibrosis in livers of 4 male and 6 female rats of the high dose group. Other treatment-related histopathological changes were not observed.

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

In another study, dose- and time-effects of DBTC administration were studied (Snoeij *et al.*, 1988). Rats were given DBTC via oral gavage in a single dose of 15 mg/kg bw and killed after 1, 2, 3, 4, 5, 7 and 9 days. A second group of rats received doses varying between 5 and 35 mg/kg bw and were killed 4 days post-exposure. A dose-dependent reduction in thymus weight was observed, and further, thymus weights returned to normal at day 9 post-exposure.

Assessment and comparison with the criteria

The available data indicates that the immune system is clearly affected after oral exposure to DBTC.

Given that both DBTP and DBTC are hydrolysed to the same distannoxane, RAC is of the opinion that data on DBTC can be used on this basis for the STOT-RE classification of DBTP (see also the RAC general comment).

Studies on DBTC revealed effects on the immune system. The effects included thymus atrophy with lymphoid depletion, loss of organ-structure and reduced immune response. The effective observed dose levels for DBTC are:

- ≥ 1.7-2.4 mg/kg bw/d in a reproductive/developmental toxicity screening test (exposure period of 41 days for adult animals in this study) (Waalkens-Berendsen, 2003),
- ≥ 5 mg/kg bw/d in a rat prenatal developmental toxicity study (dams were exposed during 10 days, GD 6-15) (Study Report, 1994),
- ≥ 2.5 mg/kg bw/d in combined rat subacute/developmental toxicity studies (Seinen & Vos, 1977)

When considering differences in molecular weight between DBTC and DBTP (DBTP: 431.14 g/mol, DBTC: 303.84 g/mol), these effective dose levels would correspond to effective dose levels expressed as DBTP as:

- ≥ 2.4-3.4 mg/kg bw/d (in a reproductive/developmental toxicity screening test exposure period of 41 days for adult animals in this study),
- ≥ 7.1 mg/kg bw/d (in a rat prenatal developmental toxicity study, dams were exposed during 10 days, GD 6-15),
- ≥ 3.6 mg/kg bw/d (combined rat subacute/developmental), respectively.

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

Effective dose-levels for DBTP are below the extrapolated guidance values for classification as STOT RE 1 (i.e. 10, 30 and 60 mg/kg bw/d for a 90 day, 28 day and 14 day study, respectively).

RAC therefore supports the conclusion of the dossier submitter that DBTP should be classified as **STOT RE 1 (H372: Causes damage to the immune system through prolonged or repeated exposure).**

Setting of Specific Concentration Limits is not considered necessary, given the small margin between the effective dose levels and the guidance values for STOT RE.

10.13 Aspiration hazard

Not considered in this CLH Report.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not considered in this CLH Report.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not considered in this CLH Report.

13 ADDITIONAL LABELLING

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15 ANNEXES

Annex I to the CLH report.