CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

[1] Dioctyltin dilaurate, [2] Stannane, dioctyl-, bis(coco acyloxy) derivs.

EC Number: **222-883-3 [1], 293-901-5 [2]**

CAS Number: **3648-18-8 [1], 91648-39-4 [2]**

Index Number: **Not applicable**

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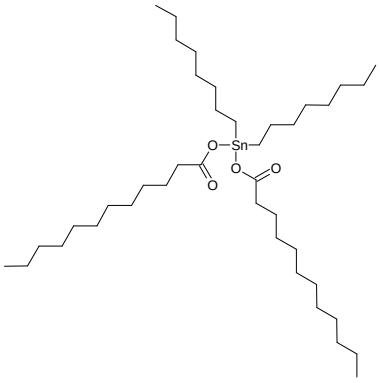
CONTENTS

1 IDENTITY OF THE SUBSTANCES

1.1 Name and other identifiers of the substance

Table 1: Substance identities and information related to molecular and structural formulas of the substances

[1] Dioctyltin dilaurate

Name(s) in the IUPAC nomenclature or other international chemical name(s)	IUPAC name: [dodecanoyloxy(dioctyl)stannyl] dodecanoate CAS name: stannane, dioctylbis[(1-oxododecyl)oxy]-
Other names (usual name, trade name, abbreviation)	bis(lauroyloxy)dioctylstannane stannane, bis(lauroyloxy)dioctyl- Mark DOTL Fomrez UL 59 E
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	222-883-3
EC name (if available and appropriate)	Dioctyltin dilaurate
CAS number (if available)	3648-18-8
Other identity code (if available)	-
Molecular formula	$C_{40}H_{80}O_4Sn$
Structural formula	
SMILES notation (if available)	CCCCCCCCCCCC(=O)[O-].CCCCCCCCCCCC(=O)[O-].CCCCCCCC[Sn+2]CCCCCCCC
Molecular weight or molecular weight range	743.7708
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)	n.a.
Degree of purity (%) (if relevant for the entry in Annex VI)	-

[2] Stannane, dioctyl-, bis(coco acyloxy) derivs.

Name(s) in the IUPAC nomenclature or other international chemical name(s)	-
Other names (usual name, trade name, abbreviation)	-
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	293-901-5
EC name (if available and appropriate)	Stannane, dioctyl-, bis(coco acyloxy) derivs.
CAS number (if available)	91648-39-4
Other identity code (if available)	-
Molecular formula	n.a. (UVCB)
Structural formula	n.a. (UVCB)
SMILES notation (if available)	n.a. (UVCB)
Molecular weight or molecular weight range	-
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)	n.a. (UVCB)
The substance *Stannane, dioctyl-, bis(coco acyloxy) derivs* is a complex mixture composed of dioctyltin units linked to fatty acids (ratio 1:2), the exact composition determined by the fatty acids distribution in coconut fatty acid. As an UVCB substance, the active substance is identified by its source, i.e. starting materials (a dioctyltin derivative and coconut fatty acid).	

The CLH-proposal embraces both the monoconstituent substance (EC no. 222-583-2) and the UVCB substance (EC no. 293-901-5). According to the REACH lead registrant, the substance currently on the European market is the UVCB substance although registered under EC no. 222-583-2 (October 2016). The name dioctyltin dilaurate or the abbreviation DOTL used in the current CLH-report refers to both substances unless otherwise noted. All data reported in the report refers to the UVCB substance unless otherwise noted.

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)
[1] Dioctyltin dilaurate CAS no. 3648-18-8 EC no. 222-583-2	degree of purity >90-100%	-	Acute Tox. 4, H302 Acute Tox. 4, H332 Skin Corr. 1C, H314 Eye Dam. 1, H318 Eye Irrit. 2, H319 Repr. 2, H361 Repr. 2, H361d STOT SE 2, H371 (Immune system) (Oral) STOT SE 2, H371 STOT RE 1, H372 (Eyes, Spleen) STOT RE 1, H372 (Thymus) STOT RE 1, H372 (Oral) STOT RE 1, H372 STOT RE 2, H373 (Organs) STOT RE 2, H373 (Oral) Aquatic Chronic 3, H412 Aquatic Chronic 4, H413
[2] Stannane, dioctyl-, bis(coco acyloxy) derivs CAS no. 91648-39-4 EC no. 293-901-5	degree of purity >90-100% Based on the fatty acid distribution of coconut fatty acid, the main constituent of this UVCB substance is dioctyltin dilaurate. The composition of the substance includes constituents characterised by a carbon chain distribution. The variability of the carbon chain lengths is related to the source used for manufacturing the substance. One example of carbon chain length distribution is (weight %): Caproic acid, C6: 0-0.8 Caprylic acid, C8: 5.0-9.0	-	Repr. 2, H361d STOT RE 1, H372 (Oral) Aquatic Chronic 4, H413

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)
	Capric acid, C10: 6.0-10.0 Lauric acid, C12: 44.0-52.0 Myristic, C14: 13.0-19.0 Palmitic acid, C16: 8.0-11.0 Stearic acid, C18: 1.0-3.0 Oleic acid, C18:1: 5.0-8.0 Linoleic acid, C18:2: 0.0-1.0 Arachidic acid, C20: 0.0-0.5		

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

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
Not relevant. According to various sources, dioctyltin substances may contain small amounts of monooctyltin and trioctyltin compounds as impurities. Although impurities are not defined for UVCB substances, the substances included in the current group entry are expected to have the same mono-/di-/tri-octyl ratios, determined by the dioctyltin source. The mono-/di-/tri-octyl ratios are not expected to affect the toxicity profile for the endpoints of interest and are not relevant for classification of the substances.				

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

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

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	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		Dioctyltin dilaurate; [1] Stannane, dioctyl-, bis(coco acyloxy) derivs. [2]	222-883-3 [1] 293-901-5 [2]	3648-18-8 [1] 91648-39-4 [2]	Repr. 1B STOT RE 1	H360D H372 (immune system)	 Danger	H360D H372 (immune system)	-	-	-
Resulting Annex VI entry if agreed by RAC and COM		Dioctyltin dilaurate; [1] Stannane, dioctyl-, bis(coco acyloxy) derivs. [2]	222-883-3 [1] 293-901-5 [2]	3648-18-8 [1] 91648-39-4 [2]	Repr. 1B STOT RE 1	H360D H372 (immune system)	 Danger	H360D H372 (immune system)	-	-	-

Table 6: 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

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

Dioctyltin dilaurate 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.

Justification that action is needed at Community level is required.

The need for a harmonised classification and labelling of Dioctyltin dilaurate for Specific target organ toxicity – repeated exposure is justified by *differences in self-classification.*

5 IDENTIFIED USES

- Tonnage band: 100-1000 (ECHA dissemination, 2016a)
- Used in production of products in the following product categories (ECHA dissemination, 2016a):
  - Adhesives, sealants
  - Coatings and paints, thinners, paint removes
  - Surface treatment products
  - Products such as ph-regulators, flocculants, precipitants, neutralisation agents
  - Leather tanning, dye, finishing, impregnation and care products
  - Paper and board dye, finishing and impregnation products: including bleaches and other processing aids
  - Polishes and wax blends
  - Polymer preparations and compounds

6 DATA SOURCES

Data on dioctyltin dilaurate and stannane, dioctyl-, bis(coco acyloxy) derivs in the publically disseminated REACH registration dossier (ECHA dissemination, 2016a) and the not publically available REACH registration dossier (24/07/2014) have been considered. In addition, dossier submitter have had full access to the original study reports of the simulated gastric hydrolysis (Naβhan, 2015 and 2016) as made available by the data owner/Registrant(s).

Data on the source substance dioctyltin dichloride (DOTC, EC name dichlorodioctylstannane) in the publically disseminated REACH registration dossier (ECHA dissemination, 2016b) and the not publically available updated joint submission of REACH registration dossier (08/09/2016) have been considered. Moreover, the dossier submitter have had full access to the original study reports of the sub chronic (13-weeks) oral toxicity study in rats (OECD 408) combined with a reproduction/developmental toxicity screening test (OECD 421) and the pre-natal developmental toxicity study (2004) as made available by the data owner/Registrant(s).

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101.3 kPa	The test material was described as a colorless liquid. Information regarding odor and form are not available.	REACH registration, publically disseminated version (ECHA dissemination, 2016a	No guideline followed. Observations on the physical state, and appearance, of the test material were performed.
Melting/freezing point	The freezing point of the test material was determined to be 9.5 ºC at 1018.2 hPa	REACH registration, publically disseminated version (ECHA dissemination, 2016a)	EU Method A.1 (Melting / Freezing Temperature) OECD Guideline 102 (Melting point / Melting range)
Boiling point	The boiling point of the test material was not determined as it started to decompose at 180 ºC. The test material was fully decomposed at 303 °C and the test was terminated.	REACH registration, publically disseminated version (ECHA dissemination, 2016a)	EU Method A.2 (Boiling Temperature) OECD Guideline 103 (Boiling point/boiling range)
Relative density	The density of the test material was determined to be 1.012 g/mL at 20 °C	REACH registration, publically disseminated version (ECHA dissemination, 2016a)	EU Method A.3. OECD Guideline 109 (Density of Liquids and Solids)
Vapour pressure	The vapour pressure of the test material at temperatures of 20, 25 and 50 °C were determined to be 0.0015, 0.0022 and 0.011 Pa, respectively.	REACH registration, publically disseminated version (ECHA dissemination, 2016a)	EU Method A.4 (Vapour Pressure) OECD Guideline 104 (Vapour Pressure Curve) EPA OPPTS 830.7950 (Vapour Pressure)
Surface tension	The surface tension of the test material was determined to be 33.96 mN/m at 20 °C	REACH registration, publically disseminated version (ECHA dissemination, 2016a)	EU Method A.5 (Surface Tension) OECD Guideline 115 (Surface Tension of Aqueous Solutions)
Water solubility	The water solubility of dioctyltin oxide was determined to be less than 1.52 x 10^{-5} g/L of solution at 20.0 ± 0.5 °C.	Data waived in REACH registration, publically disseminated version (ECHA dissemination, 2016a)	Water solubility testing on DOTL is not technically possible since the substance is hydrolytically unstable. On contact with water the substance hydrolyses to dioctyltin oxide and lauric acid. Instead, water solubility information on dioctyltin oxide is included to address this endpoint together with information on read-across substances to the other hydrolysis product, lauric acid.

Property	Value	Reference	Comment (e.g. measured or estimated)
			EU Method A.6 (Water Solubility) OECD Guideline 105 (Water Solubility)
Partition coefficient n-octanol/water	The calculated estimate of Log Pow was 9.26 for the read across substance dioctyltin oxide.	Data waived in REACH registration, publically disseminated version (ECHA dissemination, 2016a)	Partition coefficient n-octanol/water testing is not technically possible since DOTL is hydrolytically unstable at pH 4, 7 and 9, with a half-life of less than 4.5 hours. It was observed to immediately hydrolyse to dioctyltin oxide (insoluble) and lauric acid in the presence of water. The partition coefficient n-octanol/water was estimated by a computer estimate calculation using QSAR software KOWWIN (read-across from dioctyltin oxide).
Flash point	The flash point of the test material was determined to be 198 °C at 1015.8 hPa.	REACH registration, publically disseminated version (ECHA dissemination, 2016a)	EU Method A.9 (Flash-Point)
Flammability	Non-flammable.	Data waived in REACH registration, publically disseminated version (ECHA dissemination, 2016a)	The flammability of liquids is determined on the basis of their flash point (in combination with their boiling point), their ability to emit flammable gases upon contact with water and their pyrophoricity. The molecular structure of the substance does not contain groups that indicate potential reactivity with water or pyrophoric properties and handling of the substance indicates that this is the case. Furthermore the results of the submitted flash point study indicate that the substance is not a flammable liquid. On this basis, testing in accordance with EU Method A.10 (Flammability (Solids)), EU Method A.11 (Flammability (Gases)), EU Method A.12 (Flammability (contact with water)), and EU Method A.13 (Pyrophoric Properties of Solids and Liquids) are omitted.
Explosive properties	Non-explosive.	Data waived in REACH registration, publically disseminated version	From the structural formula it can be concluded that the substance is not explosive and

Property	Value	Reference	Comment (e.g. measured or estimated)
		(ECHA dissemination, 2016a)	testing is therefore not required.
Self-ignition temperature	Self-igniting: no	Data waived in REACH registration, publically disseminated version (ECHA dissemination, 2016a)	The flash point was determined to be 198 °C, which was found to be higher than the decomposition temperature 180 °C. Based on these results testing has been omitted.
Oxidising properties	Oxidising: no	Data waived in REACH registration, publically disseminated version (ECHA dissemination, 2016a)	Examination of the structure indicates that there are no groups associated with oxidising properties.
Granulometry	-	Data waived in REACH registration, publically disseminated version (ECHA dissemination, 2016a)	The substance is a liquid at room temperature, thus testing for this endpoint can be omitted.
Stability in organic solvents and identity of relevant degradation products	-	Data waived in REACH registration, publically disseminated version (ECHA dissemination, 2016a)	As the stability of the substance in organic solvents is not considered to be critical, testing has been omitted.
Dissociation constant	-	Data waived in REACH registration, publically disseminated version (ECHA dissemination, 2016a)	Since the substance is hydrolytically unstable and the half-life was determined to be less than 4.5 hours, testing has been omitted. Furthermore, it is scientifically not possible to perform the test, as the analytical method is not sensitive enough.
Viscosity	The viscosity of the test material was determined to be 27.74 m Pa s at 40 °C.	REACH registration, publically disseminated version (ECHA dissemination, 2016a)	OECD Test Guideline 114 (Viscosity of Liquids)

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Not evaluated in this CLH Report.

8.2 Flammable gases (including chemically unstable gases)

Not evaluated in this CLH Report.

8.3 Oxidising gases

Not evaluated in this CLH Report.

8.4 Gases under pressure

Not evaluated in this CLH Report.

8.5 Flammable liquids

Not evaluated in this CLH Report.

8.6 Flammable solids

Not evaluated in this CLH Report.

8.7 Self-reactive substances

Not evaluated in this CLH Report.

8.8 Pyrophoric liquids

Not evaluated in this CLH Report.

8.9 Pyrophoric solids

Not evaluated in this CLH Report.

8.10 Self-heating substances

Not evaluated in this CLH Report.

8.11 Substances which in contact with water emit flammable gases

Not evaluated in this CLH Report.

8.12 Oxidising liquids

Not evaluated in this CLH Report.

8.13 Oxidising solids

Not evaluated in this CLH Report.

8.14 Organic peroxides

Not evaluated in this CLH Report.

8.15 Corrosive to metals

Not evaluated in this CLH Report.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Dioctyltin dilaurate (DOTL): simulated gastric hydrolysis (^{119}Sn NMR (nuclear magnetic resonance) detection) in vitro. No guideline Purity of test substance >90 % (Test material as cited in the study report. The study owner confirmed that the test material corresponds to the industrially manufactured UVCB substance.)	DOTL is rapidly hydrolyzed at low pH under conditions representative of the mammalian stomach. Three species were detected by ^{119}Sn NMR: the distannoxane $ClOct_2SnOSnOct_2Cl$ (14-16%), DOTLC (43-47%) and a non-assigned tin-species (38-43%). No major change in product composition was observed from 0.5-4 hours.		Naβhan, 2015
Read-across data from source substance			
The absorption, tissue distribution and excretion of dioctyltin dichloride (DOTC) in rats. No guideline GLP: not specified Wistar-derived rat, males 3 rats/group Purity of test substance > 98% Vehicle: ethanol, tween 80 and phosphate buffered physiological saline (5 : 2 : 93, by volume) Oral gavage or i.v. Single exposure of 1.2 and 6.3 mg/kg bw Animals were killed at 1, 2, 4, and 7 days after administration. Following a single i.v. or oral dose of 1.2 and 2 mg [14C]DOTC/kg bw, respectively, the excretion of radioactivity in feces and urine was also determined.	Following a single intravenous (1.2 mg/kg bw) or oral (6.3 mg/kg bw) administration of [^{14}C]DOTC, highest concentrations of DOTC were found in liver and kidney. No selective accumulation was found in thymus. Following oral administration, absorption was calculated to be 20% of the dose. In separate excretion studies, the excretion half-life was determined to be 8.3 and 8.9 days for intravenous and oral administration, respectively.	Read-across substance: DOTC	Penninks *et al.*, 1987
Distribution of dioctyltin dichloride (DOTC) in rats. No guideline specified GLP: yes Wistar rat, females 5 rats/dose Purity of test substance unknown Vehicle: peanut oil Oral gavage	Following oral (25 mg/kg bw) administration of DOTC (^{113}Sn), highest proportions of DOTC at 24h post administration were found in liver and kidney.	Read-across substance: DOTC	Study report, 1987. [REACH registration dossier, publically disseminated version (ECHA dissemination 2016b)]

Method	Results	Remarks	Reference
Single exposure of 25 mg/kg bw 72h observation period following administration			
Dioctyltin dichloride (DOTC): simulated gastric hydrolysis (^{119}Sn NMR detection) in vitro. No guideline Purity of the test substance >95 %	DOTC is rapidly hydrolysed at low pH to the distannoxane $ClOct_2SnOSnOct_2Cl$ as the only detectable product under conditions representative of the mammalian stomach. More than 90% of $ClOct_2SnOSnOct_2Cl$ is formed after 4 hours.	Read-across substance: DOTC	Naβhan, 2016

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

Limited toxicity data are available for DOTL 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 dioctyltin dichloride (DOTC) (*i.e.* an analogue approach), justified on the basis of hydrolytic and toxicokinetic behaviour, and toxicological data (see section 9.2 below, read-across justification).

Toxicokinetic data are limited to the read-across substance dioctyltin dichloride (DOTC). Following intravenous (1.2 mg [^{14}C]DOTC/kg bw) and oral (6.3 mg [^{14}C]DOTC/kg bw) administration and subsequent termination (1-7 days), DOTC was shown to be distributed to various tissues in Wistar rats (Penninks *et al.*, 1987). Blood and selected tissues (e.g. liver, kidneys and brain) were collected. Radioactivity was detected in highest amount in the liver and kidney and to a lesser degree in adrenal, pituitary and thyroid glands. The lowest activity was recovered from blood and brain. No selective accumulation was observed in thymus, although thymus atrophy is the most sensitive parameter of dioctyltin toxicity in rats (Appel, K. E. 2004). The absorption following oral administration was calculated to be 20% of the dose. A similar distribution with highest concentration of radioactive [^{113}Sn]DOTC in liver (1.2% of the initial dose) and kidneys at 24h post administration (oral) was also reported in a separate study in the publicly disseminated REACH dossier (ECHA dissemination, 2016b).

No data are available on the metabolism of DOTC although it has been argued that dioctyltins are probably hardly metabolized (Penninks *et al.*, 1987, Appel, K. E. 2004). In excretion studies of DOTC, a single i.v. or a single oral (by gavage) dose of 1.2 mg and 2 mg [^{14}C]DOTC/kg bw respectively were given to rats, and urine and faeces were separately collected for 25 days. Similar half-life values were calculated for i.v. and oral administration, 8.3 and 8.9 days respectively, obtained from the faecal excretion of radioactivity (Penninks *et al.*, 1987).

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 common dialkyltin dichloride hydrolysis products at low pH. 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 no. 260-829-0), $DBT(EHMA)_2$ (EC no. 234-186-1) and $DOT(EHMA)_2$ (EC no. 239-622-4), did not confirm the results of previous simulation experiments (ECHA dissemination, 2016c, d, e). Instead, a monochloride species with one EHMA ligand still bonded to the Sn-centre was observed as the only hydrolysis product. Although the observations were not done at physiological representative conditions, the results indicate that the EHMA ligands may be strongly associated with the dialkyltin moieties (*e.g.* due to the soft character of the RS^- donor and/or

chelating ability of the EHMA ligand). In light of these results, a recent report concludes that grouping of organotins needs to consider in greater detail the nature of the labile ligands and the chemistry associated to the relevant organotin substances (Arcadis, 2016). On the basis of this knowledge, an analogue approach for read-across from the source substance dioctyltin dichloride (DOTC) to the target substance(s) DOTL was chosen in order to address the classification of DOTL for reproductive toxicity and STOT RE.

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).

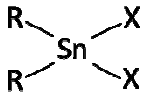
9.2.2 Hypothesis for the analogue approach

The read-across is based on the structural similarities between the source substance, DOTC, and the target substance(s) DOTL. The substances contain the common dioctyltin (Oct_2Sn-) group, considered to be the toxic component, as well as two labile ligands (X). The hypothesis for the analogue approach is that, following oral administration, both the source and target substances will hydrolyse with the generation of common intermediates; systemic exposure will therefore be to the same substance(s) regardless of the substance administered. 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 read-across approach is valid for both the monoconstituent substance (EC no 222-583-2) and the UVCB-substance (EC no 293-901-5) since they only differ in the structure of the labile carboxylate ligands. The hydrolysis product, the dioctyltin substance(s), will be the same/similar irrespective of what substance is administered and the carboxylates are considered to be of low toxicological relevance.

9.2.3 Source substance

Table 9: Substance characteristics

Substance	EC # / CAS #	Structure	Purity/Impurity details (REACH dossier)
Dioctyltin dichloride (DOTC) (EC name dichlorodi-octylstannane)	222-583-2 / 3542-36-7	 R = octyl X = Cl	94.5-100% (REACH registration, 2016) Typical impurities: octyltintrichloride and trioctyltinchloride

9.2.4 Purity / Impurities

In general, dioctyltin substances may contain small amounts of monooctyltin and trioctyltin substances as impurities. No purity details for DOTL are reported in the publically disseminated REACH dossier. The purity of DOTC in EU is 94.5-100 % and minor impurities typically include octyltintrichloride (~3%) and trioctyltinchloride (<1%) together with small amounts of hexadecane (<0.5%) (REACH registration, 2016). In the publically disseminated REACH registration dossier (ECHA dissemination, 2016b), DOTC is reported as a monoconstituent substance and no further purity details are reported.

The scope of the current CLH dossier for DOTL includes both monoconstituent and UVCB substances. The concept of impurities typically does not apply for UVCB substances but it may be anticipated that the current substances have similar mono-/di-/tri-octyl ratios as previously reported for other dioctyltin substances.

The potential presence of impurities at variable levels does not affect the analogue approach. The observed developmental toxicity of dioctyltin-/monooctyltin mixtures have been attributed to the dioctyltin substances as available data on monooctyltin substances indicate no adverse effects on the reproductive systems (Wiley-VCH, 2015). It is unlikely, therefore, that variation in purity level or impurity profile will significantly affect the toxicity profile of the source substance or target 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 analogue approach.

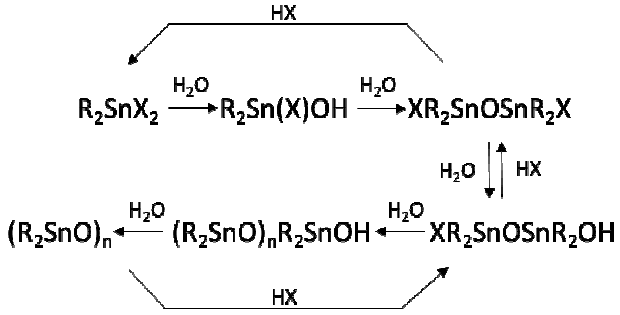
9.2.5 Analogue approach justification

Physicochemical properties

DOTL is liquid at room temperature (ECHA dissemination, 2016a) while DOTC is reported as an off-white solid (OECD, 2006; ECHA dissemination, 2016b). Due to variations in the carboxylate ligands for the UVCB substance, the molecular weight will differ which may be reflected in toxic potency, *i.e.* the mass proportion of dioctyltin moieties generated by hydrolysis will vary. Both the source substance and the target substance(s) are reported to be insoluble or of low water solubility.

Chemical similarities (hydrolytic behaviour)

Dialkyltin substances which contain labile ligands, *e.g.* chlorides or carboxylates, generally undergo hydrolysis in aqueous solution at room temperature with the ultimate 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, the labile ligand(s) X and the auxiliary ligands (R). Analogous equilibria in aqueous conditions for dioctyltin derivatives specifically was discussed in some detail in the PBT/vPvB evaluation of substituted mono- and dioctyltin substances (ECHA, 2011).



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

Based on general dialkyltin chemistry, the hydrolysis reactions for DOTC and DOTL at neutral pH to form dioctyltin oxide (DOTO) are expected to be facile. A reported water solubility study in the publically disseminated REACH dossier for DOTC states that hydrolysis occurs within 10 minutes and the study is therefore not technically feasible (ECHA dissemination, 2016b). The hydrolysis product is not identified. In a key study, a solubility of 1.6 ± 0.1 mg/l is reported for DOTC where it states that the result may be inaccurate due to possible unsuitability of the test method. In the OECD SIDS Initial Assessment Profile for dioctyltins (OECD, 2006), DOTC is reported to hydrolyze rapidly with the formation of insoluble DOTO and that the initial solubility of DOTC likely is very low. The insolubility of DOTO at neutral pH is reported in the publically disseminated REACH dossier (ECHA dissemination, 2016f). Where higher solubilities are reported it is generally assigned to various tin-based impurities.

The REACH dossier for DOTL supports the view that the carboxylates are rapidly liberated in water at neutral pH with the formation of insoluble DOTO and the corresponding carboxylates/carboxylic acids (ECHA dissemination, 2016a). In a key study, it is concluded that DOTL has a half-life below 4.5 hours (C_{12}) at pH 4, 7 and 9 and that the reaction rate constants could not be calculated due to fast hydrolysis. For the longer carboxylic acid homologues, it is concluded that the half-life is slightly longer than 4.5 hours which may reflect lower dissolution rates for more hydrophobic constituents of the UVCB substance. The carboxylates are not expected to hydrolyze further. Overall it can be concluded that the source substance DOTC and the target substance(s) behave similarly in water at neutral pH and hydrolyse to the same tin-species, DOTO, which precipitates out of solution. The similar behaviour supports the read-across approach for these dioctyltin substances.

The chemical behaviour at low pH is distinct from that at neutral pH in that the formation of insoluble DOTO is unfavored. Recent simulated gastric hydrolysis studies demonstrate the rapid formation of common intermediate(s) for the source substance DOTC and the target substance DOTL (Naßhan, 2015 and 2016). Using ^{119}Sn NMR (nuclear magnetic resonance) spectroscopy, the distannoxane $ClOct_2SnOSnOct_2Cl$ (*c.f.* Scheme 1) was observed from the hydrolysis of DOTC in >90% yield at pH 1.2 within 4 hours. The assignment was done based on reference NMR spectra and are in accordance to literature values for similar substances (Davies, 2004). Small amounts of DOTC (<10%) were also detected. The target substance (DOTL) also hydrolysed rapidly at pH 1.2. Three species were detected by ^{119}Sn NMR: $ClOct_2SnOSnOct_2Cl$ (~15%), DOTLC (a mono-chloride mono-carboxylate species, ~45%) and a non-assigned tin-species (~40%) which may be associated to polymeric structures different from DOTO. Only minor changes in product composition are observed from 30 min to 4 hours demonstrating the facile transformation of DOTL.

The NMR experimental setup allows the determination of the formed product composition in the aqueous environment at certain time intervals. The determined species ratios correspond to a snapshot of the reaction taking place in the simulated gastric hydrolysis. On the basis of the known equilibria for dialkyltin substances in aqueous solution, one may argue that a change in species concentrations, *e.g.* depletion of a species A in the equilibrium mixture due to uptake, will drive the equilibrium to the formation of more species A. The amount of uptake of a specific species may therefore be different than the product ratios determined in the study.

The NMR studies are distinct from the previous simulated gastric hydrolysis studies for analogous tin substances (Schilt *et al.*, 2004 (mainly dibutyltin substances); ORTEP Association Stabilizer Task Force, 2000) in that a direct detection method is used (with much higher substance concentrations) which allow specific assignment of the product(s). The observation of the distannoxane $ClOct_2SnOSnOct_2Cl$ is in accordance with the well-established aqueous chemistry of dialkyltin substances where the product(s) may vary depending on the identity of the ligands and experimental conditions (*e.g.* solvent, pH, concentration) due to various equilibria.

The hydrolytic behaviour of the source and target substances at neutral and low pH supports the read-across approach. The target substance(s) display more complex chemistry during the specific experimental setup used in the simulated gastric hydrolysis study than the source substance which may be expected due to the coordinating carboxylate ligands which can bind to the tin moiety in various ways (monodentate, bidentate, bridging etc.). Also, the composition of DOTL (UVCB substance) with carboxylic acid homologues may affect the product composition. Altogether, it can be concluded however that both the source and target substances form the common intermediate $ClOct_2SnOSnOct_2Cl$ under simulated gastric conditions thus supporting the read-across approach.

Similar toxicological properties

In general, dioctyltin compounds are ascribed as having immunotoxic properties via the thymus gland. The use of dioctyltin compounds is therefore restricted according to REACH (EC) No 1907/2006 Annex XVII, entry 20 in a number of consumer articles (≥ 0.1 % by weight of tin).

Limited toxicological data are available for DOTL. Where data are available (ECHA dissemination, 2016a) they are shown in the matrix below and compared to equivalent data for the source substance (Table 11). Studies on repeated dose toxicity and reproductive toxicity are available for the source substance and used in the analogue approach for read-across to DOTL.

DOTL is reported to be of moderate acute oral toxicity. The LD_{50} value (>2000 mg/kg bw, rat) is similar to the corresponding values for DOTC (3300 and 4700 mg/kg bw for female and male rats, respectively).

For DOTC, increases in incidences of malformations (missing bones), post-implantation loss, stillborn pups and the number of runts were observed in rat in developmental and reproduction/developmental toxicity screening studies with effect dose levels from 0.8 mg/kg bw/day. A decrease in thymus weight and histopathological changes in the thymus were also observed in repeated dose toxicity studies with DOTC in rat, at dose levels from 0.7 mg/kg bw/day. It should be noted that DOTC has a harmonised classification in STOT RE 1 (thymus/immune system). DOTC have no previous harmonised classification for reproductive toxicity, however, the dossier submitter has simultaneously with the current CLH-proposal also submitted a CLH-proposal for DOTC in Repr. 1B, H360D.

9.2.6 Data matrix

Table 10: Data matrix for DOTL and DOTC

	DOTL **(target substance)**	**DOTC** **(source substance)**
Structure	$(Oct)_2Sn(laurate)_2$	$(Oct)_2SnCl_2$
CAS number	3648-18-8 / 91648-39-4	3542-36-7
EC number	222-883-3 / 293-901-5	222-583-2
Molecular weight	743.8 g/mol (monoconstituent substance)	416.1 g/mol
Current harmonized classification in CLP Annex VI	Not included in CLP Annex VI.	Acute Tox. 3 *, H331 STOT RE 1, H372** Aquatic Chronic 3, H412 Proposal submitted to ECHA: Acute Tox. 2, H330 Repr. 1B, H360D STOT RE 1, H372** Aquatic Chronic 3, H412
PHYSICOCHEMICAL DATA		
Physical state (20°C and 101,3 kPa)	Colorless liquid [REACH Registration, public version (ECHA dissemination, 2016a)]	Solid, white/off-white [REACH Registration, public version (ECHA dissemination, 2016b); OECD, 2006]
Water solubility	Study technically not feasible. Data waived in REACH Registration, public version (ECHA dissemination, 2016a)	1. Study technically not feasible. Rapid hydrolyses within 10 minutes to form a new solid distinct from DOTC. No further identification. [REACH Registration, public version (ECHA dissemination, 2016b)] 2. 1.6 ± 0.1 mg/l [REACH Registration, public version (ECHA dissemination, 2016b)]. The result may not be accurate

	DOTL **(target substance)**	**DOTC** **(source substance)**
		due to possible unsuitability of the test method.
Water stability	Hydrolysis rate could not be determined, half-life of hydrolysis determined to <4.5h at pH 4, 7 and 9. A white precipitate of DOTO was formed in all experiments. [REACH Registration, public version (ECHA dissemination, 2016a)]	Rapid hydrolysis in water to form DOTO (OECD, 2006).
Hydrolysis, low pH (^{119}Sn NMR detection)	Rapid hydrolysis at low pH under gastric simulation studies to form $ClOct_2SnOSnOct_2Cl$ (14-16%), DOTLC (43-47%) and a non-assigned tin-species (38-43%) (Naßhan, 2015)	Rapid formation of $ClOct_2SnOSnOct_2Cl$ at low pH under gastric simulation studies: more than 90% formed after 4 hours (Naßhan, 2016)
TOXICOLOGICAL DATA		
Acute oral toxicity	Key study [REACH registration, public version (ECHA dissemination, 2016a)], OECD 423. Rat LD50 > 2000 mg/kg bw	Key study [(REACH registration, public version (ECHA dissemination, 2016b)], OECD 401 (equivalent or similar to). Rat LD50 (males) = 4700 mg/kg bw LD50 (females) = 3300 mg/kg bw
Reproductive toxicity	No data: read-across proposed	Prenatal Developmental Toxicity Study (Study report, 2014), OECD TG 414 Rat 0, 10, 100 and 300 mg/kg (oral, feed), GD 5 to 19. Dose dependent increase in the incidence of total skeletal malformations (missing bones of the forepaw). NOAEL: <10 mg/kg diet, equivalent to <0.8 mg/kg bw/day (actual dose received). LOAEL: 10 mg/kg diet, equivalent to 0.8 mg/kg bw/day. Reproduction/developmental toxicity screening test (Appel and Waalkens-Berendsen 2004), OECD 421. Rat

	DOTL **(target substance)**	**DOTC** **(source substance)**
		Doses: 0, 10, 100, 300 mg/kg diet (oral, feed). Reproductive and developmental effects: animals showing only implantations at necropsy, animals delivering only dead pups, decreases in gestation, live birth and viability indices and increases in post-implantation loss and number of runts. NOAEL: 10 mg/kg diet, equivalent to 0.5-0.7 mg/kg bw/day. LOAEL: 100 mg/kg diet, equivalent to 4.2-6.2 mg/kg bw/day.
Specific target organ toxicity – repeated exposure	No data: read-across proposed	Repeated dose 90-day oral toxicity study (Appel and Waalkens-Berendsen 2004), OECD 408. Rat Doses: 0, 10, 100, 300 mg/kg diet (oral, feed). Effects: decreased thymus weight at 10 mg/kg diet (females) and at 100 and 300 mg/kg diet (males and females); histopathological changes in the thymus at 100 and 300 mg/kg diet (males and females). NOAEL: < 10 mg/kg diet, equivalent to <0.7 mg/kg bw/day. LOAEL: 10 mg/kg diet, equivalent to 0.7 mg/kg bw/day.

9.2.7 Conclusions

Overall, the data available are considered to justify the use of an analogue approach for read-across from DOTC in order to address the classification of DOTL for reproductive toxicity and for STOT RE, based on the following data:

- The hydrolytic behaviour at neutral and low pH supports the rapid formation of common intermediate(s). Due to rapid hydrolysis at low pH there will therefore be no systemic exposure to DOTL using oral dosing.

- Hydrolysis studies at low pH for DOTC and DOTL show partial formation of the *same species* which has been assigned based on a direct method allowing specific structural identification. The

chemistry at low pH for DOTL is more complex compared to DOTC due to the binding nature of the carboxylate ligands but the observed behavior are in accordance to established chemistry for dialkyltins under aqueous conditions.

- In general, dioctyltin compounds are considered to have adverse effects on the immune system after repeated exposure. Acute toxicity data demonstrate that both the target substance and the source substance are of moderate toxicity.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not evaluated in this CLH Report.

10.2 Acute toxicity - dermal route

Not evaluated in this CLH Report.

10.3 Acute toxicity - inhalation route

Not evaluated in this CLH Report.

10.4 Skin corrosion/irritation

Not evaluated in this CLH Report.

10.5 Serious eye damage/eye irritation

Not evaluated in this CLH Report.

10.6 Respiratory sensitisation

Not evaluated in this CLH Report.

10.7 Skin sensitisation

Not evaluated in this CLH Report.

10.8 Germ cell mutagenicity

Not evaluated in this CLH Report.

10.9 Carcinogenicity

Not evaluated in this CLH Report.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

There is no data on adverse effects on sexual function and fertility of DOTL. In this CLH-proposal read-across from the source substance DOTC have been utilized for the purpose of justifying harmonised classification.

Table 11: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Read-across data from source substance			
Repeated dose 90-day oral toxicity study (OECD TG 408) combined with a reproduction/ developmental screening test (OECD TG 421) (no significant deviations) GLP: yes Wistar rat 10 rats/sex/group in the main study (13-week study) 10 females/ group in the satellite study (reproduction/developmental screening) Male rats from the main study were mated after a premating period of 10 weeks with female rats of the satellite groups.	Dioctyltin dichloride, purity 92.1 % 0, 10, 100 and 300 mg DOTC/kg diet (nominal in diet) Actual dose: 0, 0.5-0.7, 4.2-6.2, and 8.4-17 mg/kg bw/day Animals in the main study were fed daily for 13 consecutive weeks. Female rats in the satellite study were fed daily during the 2 weeks of the premating period, and continued through mating, gestation and up to euthanasia at or shortly after PND 4.	**Parental generation** (i.e. males from main study and females from satellite group) <u>Mortalities and clinical observations</u> There were no mortalities in the study. *Males*: No clinical signs were observed *Females:* One or two females in the satellite study of the 100 and 300 mg/kg groups showed clinical effects during gestation and/ or lactation: thin, pale appearance, piloerection and/or blepharospasm (see table 1, Annex I). <u>Body weights</u> *Males:* ↓ body weight throughout the study at 300 mg DOTC/kg diet (approx. -9%, $p<0.05/0.01$, as compared to control). *Females:* Premating phase: ↓ mean body weight gain in the 100 and 300 mg DOTC/kg diet groups (0.28 g, $p<0.05$ and -4.03 g, $p<0.001$ respectively, compared to 4.8 g in control) during the first week of the premating period. As compared to controls, a slightly lower body weight was recorded for intermediate (-3.11%, not stat. sign.) and high dose (-4%, not stat. sign.) females at the end of premating period. Gestational phase: ↓ body weight gain in the 300 mg DOTC/kg diet	Appel and Waalkens-Berendsen. (2004)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		group (-34% to -60%, stat. sign. compared to control) during the entire gestation period. Consequently ↓ mean body weight from GD 7-21 in the 300 mg DOTC/kg diet group (-7% to -16%, stat. sign. compared to control). Lactational phase: ↓ mean body weight in the 300 mg DOTC/kg diet dose group (-18%/ -20%, stat. sign. compared to control) on PND 1 and 4. <u>Food consumption</u> *Males:* ↓ food intake at 300 mg DOTC/kg diet (approx. -8%) compared to control, however food efficiency values were similar compared to those of the control group. *Females:* ↓ food consumption at 300 mg DOTC/kg diet during the entire study (-18 to -68%, stat. sign. compared to control) and at 100 mg DOTC/kg diet during the premating period (-10 to -15%, $p<0.01$ compared to control) and from GD 7-14 (-11%, $p<0.05$ compared to control). **Organ weights and Histopathology** <u>Parental generation</u> *Males:* ↓ absolute and relative thymus weights in all treated groups in a dose-response manner, statistically significant at 100 mg DOTC/kg diet (-47/-48%) and 300 mg DOTC/kg diet (-75/-73%) compared to control. ↑ incidence of lymphoid depletion (in the 100 mg/kg group (5/10 males, severity score slight to moderate) and in the 300 mg/kg group (9/10 males, severity score, moderate to severe). Statistical significant changes in absolute or relative organ weights were reported for adrenals, spleen, kidney, liver and testes in the 300 mg DOTC/kg diet dose group compared to control, however, no adverse histopathological changes were noted. *Females:* ↓ absolute and relative thymus weight in all	

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
treated groups (but only stat. sign. at the 100 and 300 mg DOTC/kg diet) in a dose-dependent manner (-23/-24%, -38/-33%, -69/-62%, in the low intermediate and high dose groups, respectively)

↑ incidence of lymphoid depletion (severity score was severe to very severe) in all groups (1/10, 5/10, 10/10 and 10/10 at 0, 10, 100 and 300 mg DOTC/kg diet respectively).

Fertility, parturition and sexual function

No effects on fecundity or fertility indices, precoital time or gestational length were recorded.

Development
<table>
<tr><th>Dose level (mg/kg diet)</th><th>0</th><th>10</th><th>100</th><th>300</th></tr>
<tr><td># pregnant animals</td><td>7</td><td>8</td><td>7</td><td>8</td></tr>
<tr><td># dams with only implantation sites</td><td>1</td><td>0</td><td>0</td><td>3</td></tr>
<tr><td># dams with only stillborn pups</td><td>0</td><td>0</td><td>2</td><td>1</td></tr>
<tr><td># dams with live born pups</td><td>6</td><td>8</td><td>5</td><td>4</td></tr>
<tr><td># dams with live pups PND 4</td><td>6</td><td>7</td><td>3</td><td>1</td></tr>
</table>
↓ gestation index in the 100 and 300 mg DOTC/kg diet dose groups (71 and 50% respectively, not stat. sign. compared to 86% in control).

↑ mean post-implantation losses in the 100 and 300 mg DOTC/kg diet dose groups (49 and 70% respectively, not stat. sign. compared to 22% in control).

↓ live birth index in the 100 and 300 mg DOTC/kg diet dose groups (53 and 60%, respectively, not stat. sign. compared to 99% in control).

↓ viability index PND 0-4 in the 100 and 300 mg DOTC/kg diet dose groups (74 and 12%
</td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		respectively, compared to 94% in control). ↓ foetal weight at PND 1 at 300 mg DOTC/kg diet (3.9 g not stat. sign. compared to 4.76 g in control). ↑ number of runts‡ at 10, 100 and 300 mg DOTC/kg diet (7, 10 and 6 respectively, compared to 1 in control). ↑ number of cold pups at 300 mg DOTC/kg diet on PND 1. Macroscopic observations in stillborn pups and pups that died between PND 1 and 4 revealed no treatment related abnormalities in the pups. LOAEL for fertility and developmental effects was 100 mg DOTC/kg diet (equivalent to 6.5 mg/kg body weight/day in males and 4.2-6.2 mg/kg body weight for females) according to the Registrant(s). LOAEL for maternal toxicity was 10 mg DOTC/kg diet (equivalent to 0.5-0.7 mg/kg body weight/day) based on the observed histological changes in the thymus (lymphoid depletion) according to the Registrant(s).	
Similar to OECD TG 443 – Extended one-generation reproductive toxicity study (EOGRTS) without the Cohorts 2 and 3 and without the extension of Cohort 1B to mate the F1 animals to produce the F2 generation. GLP: not specified Wistar rats 24 females were mated per group, except in high dose group where 20 females were mated. Litters were not standardized and pups were weaned on PND 21. Evaluation of sexual maturation was performed using 1 pup/sex/litter. 8 F1 males per group were used for immune assessment, however, the	Di-n-octyltin dichloride, CAS no. 3542-36-7, was obtained from ABCR GmbH &Co. 0, 3, 10 or 30 mg/kg DOTC during the premating period, mating, gestation and lactation and subsequently F1 were exposed from weaning onwards. The substance intake for the treated F0 females was 0.17–0.21, 0.56–0.71, 1.7–2.1 mg/kg bw/day during gestation and 0.27–0.55, 1.0–1.9, 2.9–5.2 mg/kg bw/day during lactation.	**Parental generation** <u>Mortalities and clinical observations</u> No adverse behaviour or clinical signs. <u>Body weights</u> No statistically significant effects on body weights of F0 animals except for a statistically significant increased body weight (approximately 5%) of F0 females in mid and high dose groups compared to control during lactation. No effects on male body weights. **Organ weights and Histopathology** No information available on F0 animals. **Fertility, parturition and sexual function** Mating and fertility indices, precoital time, mean duration of pregnancy and gestation indices were similar among all groups. **Development** ↓ mean number of live pups per litter at PND 4 in	Tonk et al., 2011

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
design to assess the potential impact of chemical exposure on the developing immune system deviates substantially from that described for Cohort 3 in OECD TG 443.		high dose group (8.78, p<0.05 compared to 10.48). ↓ absolute (-22%, p<0.05) and relative (-20%, p<0.05) thymus weight and thymus cellularity (-36%, p <0.05) in high dose group on PND 42 compared to control. LOAEL for fertility and developmental effects is considered to be 30 mg DOTC/kg diet. No LOAEL identified for maternal toxicity. NOAEL for maternal toxicity is 30 mg DOTC/kg diet.	

(§) Main findings of the study are presented here, for further details see tables 18 and Annex I.

(ǂ) runts = pups with weight below 2 standard deviations as compared to mean pup weight of control group at PND 0

Table 12: Summary table of human data on adverse effects on sexual function and fertility

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

Table 13: Summary table of other studies relevant for toxicity on sexual function and fertility

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

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

There is no data available on adverse effects on sexual function and fertility of DOTL.

Read-across from the source substance to fill data gaps on adverse effects on sexual function and fertility of DOTL

To generate information on the potential reproductive toxicity of DOTL for the purpose of harmonized classification an analogue substance grouping approach was utilized. Read-across from data of DOTC was used for the purpose of hazard assessment and classification.

Justification

The read-across is based on the structural similarities between the source substance, DOTC, and the target substance(s). The substances contain the common dioctyltin (Oct2Sn-) group, considered to be

the toxic component, as well as two labile ligands (X). The hypothesis for the analogue approach is that following oral administration, both substances will hydrolyse with the generation of common intermediates; systemic exposure will therefore be to the same substance(s) regardless of the substance administered.

Adverse effects on sexual function and fertility of DOTL is therefore assumed to be predictable on the basis of existing data on DOTC in the current analogue approach for chemical grouping.

Source substance data

For examination of adverse effects on sexual function and fertility two studies are available, a sub-chronic (13 weeks) dietary toxicity study (OECD TG 408) in Wistar rats combined with a reproduction/developmental toxicity screening test (OECD TG 421) performed in female satellite groups (Appel and Waalkens-Berendsen, 2004) and a dietary extended one-generation reproductive toxicity study in Wistar rat, similar to OECD TG 443 (Tonk et al., 2011).

Repeated dose 90-day dietary toxicity study in rats (OECD TG 408) combined with a dietary reproduction/ developmental screening test (OECD TG 421) (Appel and Waalkens-Berendsen, 2004)

No effects on male or female fertility, mating indices, or gestational length were recorded in the available reproductive screening study at dose levels up to and including 300 mg DOTC/kg diet (Appel and Waalkens-Berendsen, 2004). Oestrus cycling and sperm parameters were not examined in the study.

No adverse histopathological findings or effects on organ weights (except for a slight statistically significant increased relative, but not absolute, weight of the testis) were recorded at the examination of the reproductive organs in males or in females dosed for 13 weeks (main study groups) and no effects were observed on reproductive organs (ovaries and uterus were examined grossly, but no histopathological examination performed) in the satellite females in the screening test. Moreover, no adverse effect were recorded at the histopathological examination of the reproductive organs in males that failed to produce pregnancy.

There were no adverse clinical findings in the males. Reduced body weight at 300 mg DOTC/kg diet were recorded at a similar level throughout the study, however food efficiency values were similar compared to those of the control group. Consequently the effects on body weight was at least partly related to low palatability of the test diet (the same phenomenon was also recorded for the females of the main study).

In females of the satellite study, there were no clinical findings recorded during the pre-mating period. Clinical findings during gestation and lactation is discussed in section 10.10.5.

The body weight of the females during pre-mating was not statistically significantly affected, however during the first week of pre-mating there was a statistically significant difference in body weight change in intermediate dose (0.28 g) and high dose animals (-4.03 g) compared to control (4.8 g). Food consumption of the female animals at 300 mg DOTC/kg diet in the satellite group was reduced during the entire study, a level of statistical significance was achieved during most periods. In the 100 mg/kg group food consumption was statistically significantly decreased during the premating period and during GD 7-14. Body weight and food consumption during gestation and lactation is discussed in section 10.10.5.

Relative thymus weights of dams in all treated groups were decreased in a dose-response manner (24% (not stat. sign.), -48%, $p<0.01$, -73%, $p<0.01$ at 10, 100 and 300 mg DOTC/kg diet respectively) compared to control with corresponding histopathological changes in the thymus manifested as lymphoid depletion, characterized by a decrease in the size of the thymic lobules. The lymphoid depletion was considered as severe to very severe in 5/10, 10/10 and 10/10 dams in the 10, 100 and in the 300 mg DOTC/kg diet groups. Similar effects on the thymus weight and histopathological changes, with less severe lymphoid depletion, were also observed in the males (as well as in female rats of the main study that had been dosed for 13 weeks).

Extended one-generation reproductive toxicity study similar to OECD TG 443 (Tonk et al., 2011)

In an extended one-generation reproductive toxicity study by Tonk et al. (2011) performed according to a protocol similar to OECD TG 443 DOTC was given orally via the diet to Wistar rats at dose levels up to and including 30 mg DOTC/kg diet (i.e. a dose level just above the lowest dose level used in the reproduction/developmental screening study).

All females of all dose groups were mated and precoital time, gestation time, and female fertility and fecundity indices were similar among all groups. The gestation index was 100 % in all groups. Post-implantation loss was increased in the high dose group (17.9 % compared to 8.8 % in control), however the difference compared to the control group was not statistically significant. Moreover, the mean number of pups delivered per litter was similar among the dose groups and the live birth index was 99-100% in all groups.

No adverse behaviour or clinical signs were reported and no statistically significant effects on body weights of F0 animals except for a statistically significant increased body weight (approximately 5%) of F0 females in intermediate and high dose groups compared to control during lactation was observed.

There was no information available on organ weights or histopathology for F0 animals. In F1 animals, it is stated in the publication (Tonk et al., 2011) that no treatment-related macroscopic changes were observed and that no treatment-related organ weight changes were observed in spleen, kidneys, adrenals, heart and testes. The absolute and relative thymus weight and thymus cellularity were decreased in the high dose group on PND 42 and there was a tendency to decreased cellularity in the spleen in the high dose group on PND 42.

Summary of available studies

The current data from the two available studies of adverse effects on sexual function and fertility of DOTC do not give a concern for effects on the integrity of the male and female reproductive organs and no adverse effects were recorded for female and male fertility or mating. However, it should be emphasised that the screening study covers a limited number of endpoints and has less statistical power than the more comprehensive reproductive toxicity studies (two-generation, one-generation or extended one-generation reproductive toxicity studies) and consequently an absence of signal should be interpreted with caution. Moreover, the focus of the available EOGRTS was to explore effects on the immune system of pups that had been exposed in utero/post-natally to DOTC (with the notion that organotin compounds are known to affect the immune system of adults) and therefore, far lower dose levels were used as compared to the dose levels administered in the reproduction/developmental toxicity screening study of DOTC. Hence, the lack of effects on reproductive parameters in the EOGRTS study at all dose levels (such as the gestation index) are in line with the observations at the lowest dose levels in the screening study. In addition, information on all relevant assessments (including histopathological examination, sperm parameters, oestrus cycling, and sexual maturation) was not included in the publication. It is therefore concluded that data may not be sufficiently detailed or complete for a comprehensive evaluation for adverse effects on sexual function and fertility, and that administered doses in the EOGRTS may be too low to detect reproductive potential of DOTC.

The available data indicate that all toxic effects of DOTC occur post implantation and does not seem to be related to adverse effect on parturition: decreased gestation indices, increased post-implantation loss and decreased live birth index. These effects are further described and discussed in section 10.10.4 Adverse effects on development.

10.10.3 Comparison with the CLP criteria

Based on the data from the presented reproductive/developmental toxicity screening study there is no indication for an effect on mating or fertility indices. No one- or two-generation study of DOTC is available and the design of the available reproductive/developmental toxicity screening study does not provide information on sexual maturation or information on sperm parameters. Moreover, the available study with EOGRTS design did not include information on sexual maturation or sperm parameters, and it is noted that no effect on mating or fertility indices were recorded in the study.

No adverse effects were observed at the histopathological examination of female and male reproductive organs that had been exposed for 13 weeks.

In conclusion, no adverse effects on fertility or sexual function were recorded in the available studies that fulfils the criteria for classification.

10.10.4 Adverse effects on development

There is no data on adverse effects on adverse effects on development of DOTL. In this CLH-proposal read-across from the source substance DOTC have been utilized for the purpose of justifying harmonised classification.

Table 14: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
Read-across data from source substance			
Prenatal Developmental Toxicity Study OECD TG 414 (no significant deviations) GLP: yes Sprague Dawley rat 25 mated females/group	Dioctyltin dichloride, purity 97.7 %. 0, 10, 100 and 300 mg/kg in the diet from GD 5 to 19. Actual dose: 0 ± 0.0, 0.8 ± 0.1, 7.2 ± 1.0, 22.4 ± 4.2 mg/kg bw/day	**Maternal toxicity** ↓ body weight on GD 20 (-30%, p<0.001 as compared to control) at high dose level. ↓ body weight change GD 5-20 at intermediate and high dose level (-12%, p<0.05 and -31%, p<0.001 respectively compared to control). ↓ thymus size at intermediate (7 of 25 females) and high (all females) dose levels. No data on weight available and only gross necropsy performed. **Developmental effects** ↑ pre-implantation loss at the intermediate dose (7.0%) and high dose (10.4%, p<0.05) levels as compared to control (1.5%). ↑ post-implantation loss in all treated groups (6.9, 4.9, 6.9% in 10, 100 and 300 mg DOTC/kg diet groups respectively), not statistically significantly different from control (0.8%) and no dose-response relationship. ↑ no. fetuses with skeletal malformations (mainly missing bones in the paws) at the intermediate dose (22, p<0.01) and high dose (47, p<0.001) levels as compared to controls (1). Incidence at	Study report, 2014

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		low dose level was 11 (not stat. sign.). ↑ no. of fetuses with skeletal variants (mainly poor ossification) at the high dose level (26, $p<0.01$) as compared to controls (6). Incidences at low and intermediate dose levels were 10 and 11, respectively. LOAEL for both maternal toxicity and developmental toxicity was set to 100 mg DOTC/kg diet (7.2 mg/kg bw/day) by the the Registrant(s).	
Repeated dose 90-day oral toxicity study (OECD TG 408) combined with a reproduction/ developmental screening test (OECD TG 421) (no significant deviations) GLP: yes Wistar rat 10 rats/sex/group in the main study (13-week study) 10 females/ group in the satellite study (reproduction/developmental screening) Male rats from the main study were mated after a premating period of 10 weeks with female rats of the satellite groups.	Dioctyltin dichloride, purity 92.1 % 0, 10, 100 and 300 mg DOTC/kg diet (nominal in diet) Actual dose: 0, 0.5-0.7, 4.2-6.2, and 8.4-17 mg/kg bw/day Animals in the main study were fed daily for 13 consecutive weeks Female rats in the satellite study were fed daily during the 2 weeks of the premating period, and continued through mating, gestation and up to euthanasia at or shortly after PND 4.	See Table 11 for a summary of adverse findings. Main finding was a marked and dose-related increase in post-implantation loss (at the intermediate and high dose levels). Maternal toxicity during gestation and lactation was reported at the highest dose as decreased body weight (down to -16% at GD 21 and -20% at PND 4) and body weight gain (down to -60% during GD 14-21) compared to control. LOAEL for fertility and developmental effects was 100 mg DOTC/kg diet (equivalent to 6.5 mg/ kg body weight/day in males and 4.2-6.2 mg/kg body weight for females) according to the Registrant(s). LOAEL for maternal toxicity was 10 mg DOTC/ kg diet (equivalent to 0.5-0.7 mg/kg body weight/day) based on the observed histological changes in the thymus (lymphoid depletion) according to the Registrant(s).	Appel and Waalkens-Berendsen. (2004)
Similar to OECD TG 443 – Extended one-generation reproductive toxicity study (EOGRTS) without the Cohorts 2 and 3 and without the extension of Cohort 1B to mate the F1 animals to produce the F2 generation. GLP: not specified Wistar rats 24 females were mated per group, except in high dose group where 20 females were mated. Litters were not standardized and pups were	Di-n-octyltin dichloride, CAS no. 3542-36-7, was obtained from ABCR GmbH &Co. 0, 3, 10 or 30 mg/kg DOTC during the premating period, mating, gestation and lactation and subsequently F1 were exposed from weaning onwards. The substance intake for the treated F0 females was 0.17–0.21, 0.56–	See Table 11 for a summary of adverse findings. Main finding was a statistically significant decrease in the mean number of live pups per litter at PND 4 in high dose group, and decreased absolute and relative thymus weight and thymus cellularity in F1 high dose animals on PND 42 compared to control. LOAEL for fertility and developmental effects is considered to be 30 mg DOTC/kg diet. No LOAEL identified for maternal toxicity. NOAEL for maternal toxicity is 30 mg DOTC/kg diet. **Immunotoxicological assessment of F1** <u>Lymphocyte subpopulations – spleen</u> On PND 42 the absolute and relative number of	Tonk et al., 2011

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
weaned on PND 21. Evaluation of sexual maturation was performed using 1 pup/sex/litter. 8 F1 males per group were used for immune assessment, however, the design to assess the potential impact of chemical exposure on the developing immune system deviates substantially from that described for Cohort 3 in OECD TG 443.	0.71, 1.7–2.1 mg/kg bw/day during gestation and 0.27–0.55, 1.0–1.9, 2.9–5.2 mg/kg bw/day during lactation	CD3+, CD3+CD4+ and CD3+CD8+ cells showed statistically significant decrease in the high dose group together with a decreased T:B cell ratio. The decrease in CD3+CD4+ splenocytes was no longer statistically significant at PND 70. <u>Lymphocyte subpopulations – thymus</u> On PND 42 the absolute number of CD4-CD8+, CD4+CD8+, immature (CD3low) and mature (CD3high) thymocytes were statistically significantly decreased in the high dose group compared to the control group. Same trend at PND 70, however, not statistically significant. <u>Delayed-type hypersensitivity (DTH)</u> The DTH response to KeyHole Limpet Hemocyanin (KLH) was evaluated at PND 49. There was an increased DTH response in all dose groups compared to the control, reaching statistical significance in the low and high dose groups (37% and 52% increase in thickening of the ear compared to control).	

(§) Main findings of the study are presented here, for further details see tables 17, 18 and Annex I.

Table 15: Summary table of human data on adverse effects on development

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

Table 16: Summary table of other studies relevant for developmental toxicity

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

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

There is no data available on adverse effects on development of DOTL.

Read-across from the source substance to fill data gaps on adverse effects on the development of the offspring of DOTL

To generate information on the potential reproductive toxicity of DOTL for the purpose of harmonized classification an analogue substance grouping approach was utilized. Read-across from data of DOTC was used for the purpose of hazard assessment and classification.

Justification

The read-across is based on the structural similarities between the source substance, DOTC, and the target substance(s). The substances contain the common dioctyltin (Oct2Sn-) group, considered to be the toxic component, as well as two labile ligands (X). The hypothesis for the analogue approach is that following oral administration, both substances will hydrolyse with the generation of common intermediates; systemic exposure will therefore be to the same substance(s) regardless of the substance administered.

Adverse effects on the development of the offspring of DOTL is therefore assumed to be predictable on the basis of existing data on DOTC in the current analogue approach for chemical grouping.

Source substance data

For examination of developmental effects three studies of the source substance DOTC are available, a dietary prenatal developmental toxicity test in Sprague Dawley rats with dosing of females from gestation day 5 to 19, a dietary reproduction/developmental toxicity screening test in Wistar rats according to OECD 421 and a dietary extended one-generation reproductive toxicity study in Wistar rat, similar to OECD TG 443 with focus on immunotoxicological assessment.

The source substance DOTC have no previous harmonised classification for reproductive toxicity, however, the dossier submitter has simultaneously with the current CLH-proposal also submitted a CLH-proposal for DOTC in Repr. 1B, H360D.

Pre-natal developmental toxicity study, OECD TG 414 (Study report 2014)

In an GLP compliant OECD TG 414 Prenatal Developmental Toxicity Study in rats the main developmental effect was a dose dependent increase ($p < 0.5$ at intermediate, and $p < 0.01$ at high dose compared to control) in the incidence of total skeletal malformations, where missing bones (metacarpal no 5 and proximal phalange no. 3, bilateral) of the forepaws was the predominant malformation (Table 17).

The incidences of total skeletal variations were not dose-dependently increased, and only statistically significantly increased in the 300 mg DOTC/kg diet dose group on a foetal basis (Table 17). The predominant finding was poor ossification of sternum no. 5 or of sternum no. 6. In addition, a dose dependent and treatment related increase in the incidence of poor ossification of metacarpal no. 5 was observed (1.0 and 3.7 % at 100 and 300 mg DOTC/kg diet, respectively, as compared to 0 % in the control).

Table 17: Main maternal and developmental effects

Dose level	**0**	**10 mg/kg diet**	**100 mg/kg diet**	**300 mg/kg diet**
Test substance intake	0 ± 0.0 mg/kg bw/day	0.8 ± 0.1 mg/kg bw/day	7.2 ± 1.0 mg/kg bw/day	22.4 ± 4.2 mg/kg bw/day
Pregnancy data				
Initial animals per	25	25	25	25

group				
Mortalities	0	0	0	0
Confirmed pregnancy at necropsy	22	21	20	20
Maternal data				
Initial body weight (g) at GD 0	195.62 ± 12.45	197.88 ± 11.99	197.79 ± 9.62	198.01 ± 9.52
Body weight (g) at GD 5	211.44 ± 11.70	212.10 ± 11.95	213.88 ± 12.32	213.59 ± 9.70
Final body weight (g) at GD 20	305.34 ±18.98	300.90 ±18.42	296.62 ±18.08	278.54 ± 25.85*** (-8.8 %)
Body weight gain (g) from GD 5-20	93.9 ± 11.96	88.80 ± 12.92	82.74 ± 12.43* (-12%)	64.95 ± 20.95 *** (-31.2 %)
Corrected body weight (g)	235.38	238.67	233.36	219.44
Corrected body weight change (g) GD 5-20	23.94 ± 15.48	26.57 ± 10.57	19.47 ± 11.98	5.85 ± 18.22***
Foetal data				
Malformations				
Malformations (total) Foetal basis, no. (%) Litter basis, no. (%)	 1 (0.8) 1 (4.5)	 11 (9.6) 8 (38.0)	 22** (21.0) 11 (55.0)	 47*** (43.9) 19 (95.0)
Metacarpal no. 5 bilateral Foetal basis, no. (%) Litter basis, no. (%)	 1 (0.8) 1 (4.5)	 3 (2.6) 3 (14.3)	 12 (11.4*) 6 (30.0)	 37 (34.6*) 18 (90.0)
Proximal phalanx no. 3 bilateral Foetal basis, no. (%) Litter basis, no. (%)	 1 (0.8) 1 (4.5)	 9 (7.8) 7 (35.0)	 15 (14.3 *) 10 (50.0)	 29 (28.0*) 16 (80.0)
Proximal phalanx no.4 bilateral Foetal basis, no. (%) Litter basis, no. (%)	1 (0.8) 1 (4.5)	8 (7.0) 6 (28.6)	15 (13.3*) 9 (45.0)	29 (27.1*) 16 (80.0)
Split thoracic vertebrae centrum no. 12	0	1(1)	0	0
Missing caudal	0	2(2)	0	3(2)

vertebral arch no 2				
Variations				
Variations (total) Foetal basis, no. (%) Litter basis, no. (%)	 6 (4.5) 5 (22.7)	 11 (9.6) 7 (33.3)	 10 (9.5) 4 (20.0)	 26* (24.3) 12 (60.0)
Poor or incomplete ossification of sternum no. 5 Foetal basis, no (%) Litter basis, no. (%)	 0 0	 1 (0.9) 1 (4.8)	 0 0	 7 (6.5*) 4 (20.0)
Poor or incomplete ossification of sternum no. 6 Foetal basis, no (%) Litter basis, no. (%)	 0 0	 0 0	 2 (1.9) 1 (5.0)	 16 (14.0*) 8 (40.0)
Poor or incomplete ossification of metacarpal no. 5 Foetal basis, no (%) Litter basis, no. (%)	 0 0	 0 0	 1 (1.0) 1 (5.0)	 4 (3.7) 3 (15.0)

* p<0.05

** p<0.01

*** p<0.001

A statistically significant increase in pre-implantation loss was observed in the high dose group compared to control (10.4% compared to 1.5%, p<0.05), however it is noted that the incidence in the control group is unusually low. No clinical signs of toxicity or mortality of the dams were noted at any dose. A statistically significant decrease (6.5-8.8%) in body weight (without a concurrent effect on food consumption) was reported towards the end of the gestation in the high dose group compared to control and consequently a decreased body weight gain (28-48 % decrease as compared to control) during gestation (GD 0-20) was recorded. The corrected body weight change GD 5-20 was also statistically significantly reduced in the 300 mg DOTC/kg diet dose group compared to control (5.85 g versus 23.94 g in control, p<0.001) but the corrected body weight was only slightly reduced in high dose group compared to the control group (-6.8%). The weight of uteri in high dose dams (59.1 g) was 10.86 g (16%) lower compared to control (69.96 g), however, since the difference cannot be accounted for by differences in foetal weight (approx.. 4 g in all groups) and the slight difference in mean litter size (10.1 compared to 11.4 foetuses in control), there appears to be some toxicity to the uterus.

In conclusion, malformations (mainly missing bones in the forepaws) was seen at all dose levels with incidences increased in a dose response manner (and the dossier submitter considers that no NOAEL can be identified in the study) with or without maternal toxicity in the form of effects on body weight. In addition, effects on the degree of ossification (without a concurrent effect on foetal weight) were also recorded at these dose levels. The maternal effects on the thymus is not considered to cause the observed malformations.

Reproduction/developmental toxicity screening test, OECD TG 421 (Appel and Waalkens-Berendsen, 2004)

In the OECD TG 421 Reproduction/Developmental Toxicity Screening Test an increase of post-implantation losses in the 100 and 300 mg DOTC/kg diet dose groups (50% and 70%, respectively compared to 22% in control) was reported. The mean values were not statistically significantly different from control and there was no dose-response. However, a 70% increase in post-implantation loss is considered as a biological concern, despite the relatively high incidence of post-implantation loss in control animals. The post-implantation loss in the control group was due to one animal with implants at necropsy, but no pups delivered (Table 18). Three pregnant females with implants but no pups delivered was also seen in the high dose group. Total number of lost implantations were 19, 23, 41 and 56 in control, low dose, intermediate dose and high dose respectively. The median value (instead of mean value) better reflects the actual data of post-implantation losses due to the great variations in one or a few animals. The median values are 7, 11, 50 and 95% in control, 10, 100 and 300 mg DOTC/kg diet dose groups, respectively. Hence, the median values of incidences of post-implantation loss give a dose-response relationship and trend-analysis of the median values demonstrates a statistical significant difference between groups ($p = 0.003$).

Associated with the post-implantation losses was a decrease in live birth index (99, 95, 53 and 60% in control, 10, 100, 300 mg/kg groups respectively) with a concomitant statistically significant increase in number of stillborn pups in the 100 and 300 mg/kg dose groups compared to control. The number of dams that delivered only stillborn pups were 2 and 1 respectively, in intermediate and high dose groups (see Table 11 and Annex I, Table 2) and 4 litters in total were entirely stillborn or lost up to PND 4 in both these dose groups.

Thus, DOTC appears to have adverse effects on the pregnancy outcome and the available data indicate that the toxic effects occur post implantation. The gestation index was 71% and 50% at 300 and 100 mg DOTC/kg diet, respectively compared to 86% in the control group (no statistically significant difference). At 10 mg DOTC/kg diet the gestation index was 100%.

Furthermore, the survival of the pups was poor up to PND 4 notably in the high dose group but also in the intermediate dose group. Viability index between PND 1-4 was decreased at intermediate (-21%) and high (-87%) dose (not statistically significant compared to control).

Runts, indicative for developmental retardation, were observed in the 100 and 300 mg DOTC/kg diet dose groups and the mean pup body weight was decreased at PND 1 – 4 in the 300 mg DOTC/kg diet dose group (note that there was only one pup at PND 4). An increased number of cold pups was also recorded in the 300 mg DOTC/kg diet group.

Table 18: Summary of pup data

Dose level	Control	10 mg/kg diet	100 mg/kg diet	300 mg/kg diet
Test substance intake	0 mg/kg bw/day	0.5-0.7 mg/kg bw/day	4.2-6.2 mg/kg bw/day	8.4-17 mg/kg bw/day
Number of pregnant females	7	8	7	8
Mean number of implantations	12.6	13.4	11.3	10.3
Number of dams with total intrauterine death (only implantation sites observed at necropsy)	1	0	0	3
Post implantation loss (%) Mean value Median value [N = number of females]	 22.33 ± 13.159 7 N=7	 20.98 ± 7.114 11 N=8	 49.23 ± 17.453 50 N=7	 69.99 ± 14.713 95[£] N=8
Pups delivered (total) (N)	70	88	72	43
Pups delivered (live + dead; mean) [N= number of litters]	11.67 ± 0.803 N=6	11.00 ± 0.707 N=8	10.29 ± 0522 N=7	8.60 ± 1.208 N=5

Mean viable litter size PND 1 [N= number of litters]	11.50 ± 0.719 N=6	10.50 ± 0.945 N=8	7.60 ± 1.631 N=5	6.50 ± 2.217 N=4
Total no. of live born pups[f] (Live birth index)	69 99	84 95	38[#] 53	26[#] 60
Total no. of stillborn pups[f] (% stillborn)	1 1.4	4 4.5	34[#] 47	17[#] 40
Total number of dead pups PND 0 to PND 4[f]	4	7	10[**]	23[#]
Total number of pups dying perinatally	5	11	44	40
Mean viability index PND 1-4	94	92	74	12
Mean viable litter size PND 4 [N= number of litters]	10.83 ± 0.601 N=6	11.00 ± 0.787 N=7	9.33 ± 0.667 N=3	3.00 ± 0.000 N=1
Pup weight (g) PND 1 (all viable pups) [N= number of litters]	4.76 ± 0.229 N=6	4.74 ± 0.229 N=8	4.19 ± 0.346 (-12%) N=5	3.90 ± 0.088 (-18%) N=4
Pup weight gain (g) PND 1 to PND 4	2.17 ± 0.257	1.86 ± 0.382	1.41 ± 0.584	-0.57 ± 0.000
Pup weight (g) PND 4 (all viable pups) [N= number of litters]	6.93 ± 0.447 N=6	6.69 ± 0.743 N=7	6.10 ± 0.719 N=3	3.10 ± 0.000 N=1
Total number of runts [ǂ] [N= number of litters]	1 N=1	7 N=3	10 N=3	6 N=1

(ǂ) runts = pups with weight below 2 standard deviations as compared to mean pup weight of control group at PND 0
(f) Fishers exact test
* $p<0.05$, ** $p<0.01$, # $p<0.001$
(£) Statistical significant trend, $p<0.01$

Maternal toxicity in the 300 mg DOTC/kg diet dose group during gestation was observed as a statistically significantly decreased mean body weight (from GD 7 and onwards) and at GD 14 and GD 21 the decreases were 12% and 16% respectively compared to the control group. No weight loss was reported in the high dose animals during the gestation period. The decrease in body weight persisted during lactation day 1 (-18% compared to control) and at lactation day 4 (-20% compared to control). Consequently, the body weight gain was also statistically significantly reduced during most of the study period (except for week two of the pre-mating period and lactation day 1-4) and during GD 14-21 the body weight gain was 60% less than control. The total body weight gain from GD 0 to 21 was 65.8, 69.6, 53.4 and 34.4 g in control, 10, 100 and 300 mg DOTC/kg diet, respectively. Excluding the 3 females with intrauterine loss does not affect the mean body weight in the high dose group. Moreover, the lower number of pups (viable + dead) in the high dose group does not account for the difference in maternal body weight compared to control. At 100 mg DOTC/kg diet, the body weight was not significantly affected as compared to control throughout the entire study period. However, during the first week of the premating period, the body weight gain was statistically significantly reduced in the 100 mg DOTC/kg diet dose group as compared to control.

Food consumption was statistically significantly decreased (23-25%) in the high dose group during the whole gestation period compared to control group and also during lactation day 1-4 (-68%). In the 100 mg DOTC/kg diet group food consumption was statistically significantly reduced (-11%) during GD 7-14 compared to control, but not at any other time point. No food conversion efficiency values were available for the dams.

The study report of the combined repeated dose 90-day dietary toxicity study with reproduction/ developmental toxicity screening test does not discuss the palatability of the test diet in the screening study, however, it is noted that the reduced food intake was concluded to be related to reduced palatability of the test diet in the 90-day repeated dose toxicity study. Thus, one can assume that the decrease in food consumption in the screening study also is, at least partly, related to the palatability of the food.

In a study by Carney et al (2004), determining the effects of feed restriction in rat during in utero and postnatal life on standard reproductive toxicity and developmental immunotoxicity end points, reductions in maternal body weights down to 32% were not considered to cause any significant effects on offspring viability, or litter size at birth or at PND 4. Thus, the decrease (-12 to -20% compared to control) in maternal body weight during gestation at 300 mg DOTC/kg diet is not considered to influence the observed post-implantation losses and pup mortality and there are no conclusive evidence to prove that the observed developmental effects are being secondary to the maternal toxicity. Furthermore, increase in incidence of post-implantation losses, statistically significant decrease in live born pups and statistically significant increase in number of stillborn pups were also evident at 100 mg DOTC/kg diet where marked maternal toxicity was absent (3-7% decrease in body weight compared to control and 23-28% decrease in body weight gain, not statistically significant compared to control). The mean viability index PND 1-4 was also decreased (but not statistically significant) at this dose.

One female in the high dose group showed indications of treatment related clinical effects at the end of the gestation (piloerection and blepharospasm). During the lactation period one female in the control group, three females in the intermediate dose group and two females in the high dose group also displayed treatment related clinical effects: thin, pale appearance, piloerection and/or blepharospasm (Table 1 in Appendix 1). For the majority of these dams there was no correlation between onset of clinical signs and intrauterine death or postnatal death of pups. All of these animals with clinical observations showed implants at necropsy but had no viable pups, except for one female in high dose group that delivered one viable pup and nine dead pups.

There were no consistent effects recorded for haematological or clinical chemistry parameters in any of dams in the three dose groups. Histopathological examination revealed severe lymphoid depletion in the thymus in 10 out of 10 animals at 100 and 300 mg DOTC/kg diet. This correlated with statistically significantly decreased relative thymus weight in the same dose groups (-33% and -62%, respectively compared to control). The lymphoid depletion in thymus is not considered to impact on post-implantation loss or perinatal death.

Extended one-generation reproductive toxicity study similar to OECD TG 443 (Tonk et al., 2011)

In the extended one-generation reproductive toxicity study by Tonk et al. (2011) performed according to a protocol similar to OECD TG 443 a minor increase in post-implantation loss was reported in all treated groups, however not statistically significant different from control and only a weak dose-response was noted. In the high dose group the post-implantation loss was 17.9 % compared to 8.8 % in control, which is not considered as biologically relevant increase. Moreover, there were no stillborn pups in treated groups, the live birth index was 99-100% in all groups and the mean number of pups delivered per litter was similar among the dose groups.

Postnatal viability was affected at PND 4 with statistically significantly decreased viable litter size in the 30 mg DOTC/kg diet dose group (8.78 live pups compared to 10.48 in control group).

Male pup weight in the 30 mg DOTC/kg diet dose group was statistically significantly increased on PNDs 8, 10, and 13 when compared to the pup weight in the control group (data only presented graphically in the publication). After weaning, no effects of DOTC on body weight, food consumption and sexual maturation were observed according to study authors (no data available).

No adverse behaviour or clinical signs of F0 animals were reported and no statistically significant effects on body weights except for a statistically significant increased body weight (approximately 5%) of F0 females in intermediate and high dose groups compared to control during lactation was observed. There was no information available on organ weights or histopathology for F0 animals.

The apparent absence of maternal toxicity at the highest dose tested does not make it possible to convincingly conclude on the potential developmental toxicity of DOTC in this study. The highest dose selected in this study is not near the maximum recommended dose for oral repeated toxicity testing (1000 mg/kg bw/day) according to OECD test guidelines, and is lower than the dose levels used in the reproductive/developmental toxicity screening test, and there is no relevant toxicokinetics data to demonstrate that higher doses are not appropriate, or no limitations by physical/chemical nature of the

test substance. Consequently, higher doses should have been tested to explore the full reproductive toxicity potential of DOTC.

Developmental immunotoxicity

The present study focused on immunotoxicological assessment of the F1 generation after pre- and postnatal exposure of DOTC in rats. Responses were measured on PNDs 21, 42 and 70 and effects on thymus weight, and on lymphocyte subpopulations of both the thymus and the spleen were reported.

Both absolute and relative thymus weight and thymus cellularity were decreased in the highest dose group on PND 42, however, no effects were observed on absolute and relative spleen weights, although there was a tendency at PNDs 42 and 70 to a decreased cellularity at the high dose groups. Relative liver weight showed a statistically significant increase in the low and mid dose groups on PND 70 (4.12 g in the control versus 4.45 g in the low and 4.53 g in the mid dose group). These minor changes were not dose related. At necropsy no treatment-related macroscopic changes were observed in F1 animals

Changes in lymphocyte subpopulations in the spleen were noted on PND 42 as a statistically significant decrease in the absolute and relative number of CD3+, CD3+CD4+ and CD3+CD8+ cells in the high dose group together with a decreased T:B cell ratio. The decrease in CD3+CD4+ splenocytes was no longer statistically significant at PND 70.

Changes in lymphocyte subpopulations in the thymus were also noted on PND 42 with a statistically significantly decrease in the absolute number of CD4-CD8+, CD4+CD8+, immature ($CD3^{low}$) and mature ($CD3^{high}$) thymocytes in the high dose group compared to the control group. Same trend was observed at PND 70, however, the difference was no not statistically significant compared to control.

The DTH response to KLH was evaluated at PND 49 to aid in the evaluation of cell-mediated immunity. There was an increased DTH response in all dose groups compared to the control, reaching statistical significance in the low and high dose groups.

The recorded decrease in thymus weight and decrease in lymphocyte subpopulations of both spleen and thymus confirms the adverse effects on the immune system that is known for dioctyltin compounds in adult animals. It is, however, unclear how the increased DTH response correlates with the findings in spleen and thymus and the Th2-skewing. The study authors suggest that the findings in the present study may indicate a disturbed immune balance.

The thymus is a target organ of organotin compounds in the developing animals, as well as in adults, and there is some evidence to suggest that young animals are more sensitive than adults (Seinen et al., 1977; Smialowicz et al., 1988). However, the dossier submitter considers that there is not enough evidence to suggest that young animals are more sensitive than adults to effects of DOTC on the immune system.

Summary of available studies

The main adverse effect of developmental toxicity in the pre-natal developmental toxicity study was skeletal malformations of the fore limb, where missing bones of the forepaws was the predominant malformation Malformations was observed starting at 0.8 mg/kg bw/day (10 mg DOTC/kg diet) and at 7.2 mg/kg bw/day and 22.4 mg/kg bw/day (100 and 300 mg DOTC/kg diet) the increased incidence on a foetal basis was statistically significantly increased compared to control. The dose-dependent increase in incidences supports a treatment related effect. Moreover, the malformations are considered as rare and occur at high incidences with only one foetus affected in the concurrent control. No historical control data was available to the dossier submitter.

Pups were only examined externally for gross abnormalities in the reproduction/developmental toxicity screening test, and therefore no corresponding findings were recorded in that study. The main effects found in the reproduction/developmental toxicity screening test, with similar dose levels as the PNDT study, were increased post implantation loss, decreased live birth index and increased number of stillborn pups at intermediate and high dose compared to control, and an increased number of runts in

all treated groups. Moreover, a marked (but not statistically significant different from control) decrease in mean viability index PND 1-4 at intermediate and high dose and consequently also a substantially decreased (but not statistically significant) viable litter size at PND 4 at high dose. Similar to the screening study, a decreased pup viability (statistically significantly different from control) at PND 4 was also observed at the highest dose level in the Tonk (2011) study. No clear pre-natal effects were recorded in the Tonk study as seen at intermediate and high dose levels in the screening test, however, it is noted that the highest dose level (30 mg DOTC/kg diet, equivalent to 1.7-2.1 mg/kg bw/day) in the Tonk study is just above the lowest dose level (10 mg DOTC/kg diet, equivalent to 0.5-0.7 mg/kg bw/day) used in the reproductive/developmental screening test. In the PNDT study, no statistical significant or biologically relevant increase in incidences of pre-natal death was recorded at any dose, in contrast to the screening test. This could at least partly be explained by the difference in length of treatment between the two study designs. In the screening study exposure to the test substance starts already prior to implantation and lasts past GD 19, whereas in the PNDT study administration of the test substance starts at GD 5 and ends at GD 19. The actual internal dose in the animals in the screening study is probably higher at the time after implantation since administration starts two week prior to mating and considering the relatively long half-life (approx. 8 days) of the test substance. From the available information it is not possible to decide if the observed post-implantation losses in the screening study occurs early or late during the gestation.

Effects on thymus size, weight and/or lymphoid depletion in the thymus were seen in the dams in the treated groups in both the pre-natal developmental toxicity study and the reproduction/developmental studies, however, the recorded serious developmental effects, i.e. rare skeletal malformations and increased foetal/pup mortality, are not considered as being secondary to the maternal thymus effects. No specific mode of action has been identified to show that developmental effects can be caused by a specific thymus (-lymphocyte)-related mechanism. Moreover, it needs to be demonstrated that the specific mode of action for developmental effects would not be relevant for humans. In absence of such evidence, downgrading of the classification category is not justified.

According to Registrant(s) of DOTC, all noted effects in the available reproductive and developmental toxicity studies conducted with the registered substance were observed at maternally toxic doses only. They consider that it is generally accepted that such developmental effects are produced by a non-specific secondary consequence of general toxicity. Therefore, the Registrant(s) classifies the registered substance DOTC as a Reproductive Toxicant Category 2 (H361).

10.10.6 Comparison with the CLP criteria

Classification in Repr. 1A, H360D is not justified since there is no human data that indicates that the source substance DOTC have adverse effect on human foetal development.

Classification in Repr. 1B, H360D is warranted since the evidence for developmental toxicity of the source substance is considered to be *clear*. Based on a dose dependent statistically significant increase in incidence of skeletal malformations (missing bones) starting from 0.8 mg/kg bw/day in a prenatal developmental toxicity study in rat, a marked decrease in live birth index and increase in number of stillborn pups at 7.2 mg/kg bw/day and 22.4 mg/kg bw/day, and a dose dependent (median values) statistically significant increase in incidences of post implantation losses in treated groups compared to control in a reproductive/developmental toxicity study in rat, available data fulfils the criteria for adverse effects on the development of the offspring and a classification in Repr. 1B is warranted. Thus, there is *clear* evidence of both death of the organism and structural abnormalities. Moreover, the recorded effects are relevant for humans, and are not considered to be secondary to maternal toxicity.

Classification in Repr. 2 is not justified since the evidence for developmental toxicity is considered to be *clear* and not *some evidence* of developmental toxicity.

10.10.7 Adverse effects on or via lactation

Table 19: 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 20: 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 21: 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

There is no data available on adverse effects on or via lactation of DOTL.

Read-across from the source substance to fill data gaps on adverse effects on or via lactation of DOTL

To generate information on the potential reproductive toxicity of DOTL for the purpose of harmonized classification an analogue substance grouping approach was utilized. Read-across from data of DOTC was used for the purpose of hazard assessment and classification.

Justification

The read-across is based on the structural similarities between the source substance, DOTC, and the target substance(s). The substances contain the common dioctyltin (Oct2Sn-) group, considered to be the toxic component, as well as two labile ligands (X). The hypothesis for the analogue approach is that following oral administration, both substances will hydrolyse with the generation of common intermediates; systemic exposure will therefore be to the same substance(s) regardless of the substance administered.

Adverse effects on or via lactation of DOTL is therefore assumed to be predictable on the basis of existing data on DOTC in the current analogue approach for chemical grouping.

Source substance data

There are no relevant studies on toxicokinetics of the source substance DOTC demonstrating the presence of the substance in breast milk and there are no studies available that demonstrate that DOTC interferes with lactation or cause adverse effects to offspring via lactation. There are two studies available with maternal exposure of DOTC during lactation in rats: an OECD TG 421 reproductive toxicity screening study (Apple and Waalkens-Berendsen, 2004) and a study similar to an OECD TG 443 EOGRTS (Tonk et al., 2011). Both studies report early post-natal mortality after dietary administration of the dams during pre-mating, mating, gestation and lactation. However, it is unclear if the observed losses of pups are due to exposure of the offspring via lactation.

10.10.9 Comparison with the CLP criteria

Since no conclusive data are available, comparison with the CLP criteria is inapplicable.

According to CLP Annex I classification of substances for effects on or via lactation can be assigned on the:

(a) human evidence indicating a hazard to babies during the lactation period; and/or

(b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or

(c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Based on read-across data from the source substance DOTC the dossier submitter concludes on the following classification of DOTL:

No classification for adverse effects on fertility and sexual function is warranted.

Classification as **Repr. 1B (H360D)** according to the CLP criteria is considered justified.

Setting of specific concentration limit is not considered appropriate. Based on read-across from the source substance (DOTC) to the target substance (DOTL) no direct estimate of the reproductive toxicity potency derived from an ED10 value is possible. The expected potency between the target substance and the source substance may vary and for that reason a SCL for DOTL is not proposed. This is in line with the Guidance on the Application of the CLP Criteria (ECHA, 2017), section 3.7.2.6.2. regarding substances causing reproductive toxicity.

No classification for effects on or via lactation is warranted.

10.11 Specific target organ toxicity-single exposure

Not evaluated in this CLH Report.

10.12 Specific target organ toxicity-repeated exposure

There is no data on adverse effects on specific organ toxicity after repeated exposure of DOTL. In this CLH-proposal read-across from the source substance DOTC have been utilized for the purpose of justifying harmonised classification.

Table 22: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
Read-across data from source substance			
Repeated dose 90-day oral toxicity study (OECD TG 408)§ combined with a reproduction/ developmental screening test (OECD TG 421) (no significant deviations) GLP: yes Wistar rat 10 rats/sex/group in the main study (13-week study)	Dioctyltin dichloride, purity 92.1 % 0, 10, 100 and 300 mg DOTC/kg diet (nominal in diet) Actual dose: 0, 0.7, 6.5-6.8, and 19.3-19.8 mg/kg bw/day Animals were fed daily for 13 consecutive weeks.	**Organ weights and Histopathology** Males: ↓ absolute and relative thymus weights in all treated groups in a dose-response manner, statistically significant ($p<0.01$) at 100 mg DOTC/kg diet (-47/-48%) and 300 mg DOTC/kg diet (-75/-73%) compared to control. ↑ incidence of lymphoid depletion (in the 100 mg/kg group (5/10 males, severity score slight to moderate) and in the 300 mg/kg group (9/10 males, severity score, moderate to severe). Statistical significant changes in absolute or relative organ weights were reported for adrenals, spleen, kidney, liver and testes in the 300 mg DOTC/kg diet dose group compared to control, however, no corresponding adverse histopathological changes were noted. Females: ↓ absolute thymus weight in all treated groups in a dose-dependent manner (-14%, $p<0.05$, -68%, $p<0.01$, -73%, $p<0.01$ in 10, 100 and 300 mg DOTC/kg diet groups compared to control). ↓ relative thymus weight in all treated groups in a dose-dependent manner (-14%, $p<0.05$, -69%, $p<0.01$, -70%, $p<0.01$ in 10, 100 and 300 mg DOTC/kg diet groups compared to control). ↑ incidence of lymphoid depletion (severity score was slight to very severe) in the 100 mg/kg group (10/10 females) and in the 300 mg/kg group (9/10 males). LOAEL: 0.7 mg/kg bw/day (female) (10 mg DOTC/ kg diet) based on decreases in absolute and relative thymus weights associated with treatment related lymphoid depletion at 10, 100 and 300 mg/kg/day groups). BMDL05: 0.45 mg/kg bw/day (nominal) (female) based on: test mat. (The BMDL of mg/kg/day is recommended as a surrogate for a NOAEL for the effect of dioctyltin dichloride on absolute and relative thymus weight) BMD: 0.5 mg/kg bw/day (nominal) (female) based on: test mat. (for decreased absolute and relative thymus weights.)	Appel and Waalkens-Berendsen (2004) Kim J (2004)

Similar to OECD TG 443 – Extended one-generation reproductive toxicity study (EOGRTS) GLP: not specified Wistar rats 24 females were mated per group, except in high dose group where 20 females were mated. Litters were not standardized and pups were weaned on PND 21. Evaluation of sexual maturation was performed using 1 pup/sex/litter. 8 F1 males per group were used for immune assessment, however, the design to assess the potential impact of chemical exposure on the developing immune system deviates substantially from that described for Cohort 3 in OECD TG 443.	Di-n-octyltin dichloride, CAS no. 3542-36-7, was obtained from ABCR GmbH &Co. 0, 3, 10 or 30 mg/kg DOTC during the premating period, mating, gestation and lactation and subsequently F1 were exposed from weaning onwards. The substance intake for the treated F0 females was 0.17–0.21, 0.56–0.71, 1.7–2.1 mg/kg bw/day during gestation and 0.27–0.55, 1.0–1.9, 2.9–5.2 mg/kg bw/day during lactation.	<u>Organ weights and Histopathology</u> No information available on F0 animals. <u>Organ weights and Histopathology</u> No treatment-related macroscopic changes were observed and no treatment-related organ weight changes in kidneys, adrenals, heart and testes of F1 animals were reported. Absolute and relative thymus weight were decreased in high dose group on PND 42. Relative weights were 0.28 g/ 100 g bw compared to 0.35 g/100 g bw, $p<0.05$. Thymus cellularity were decreased in high dose group on PND 42 ($98.40 \cdot 10^7$ compared to $153.79 \cdot 10^7$ in control, $p<0.05$). **Immunotoxicological assessment** <u>Lymphocyte subpopulations – spleen</u> On PND 42 the absolute and relative number of CD3+, CD3+CD4+ and CD3+CD8+ cells showed statistically significant decrease in the high dose group together with a decreased T:B cell ratio. The decrease in CD3+CD4+ splenocytes was no longer statistically significant at PND 70. <u>Lymphocyte subpopulations – thymus</u> On PND 42 the absolute number of CD4-CD8+, CD4+CD8+, immature ($CD3^{low}$) and mature ($CD3^{high}$) thymocytes were statistically significantly decreased in the high dose group compared to the control group. Same trend at PND 70, however, not statistically significant. <u>Delayed-type hypersensitivity (DTH)</u> The DTH response to KeyHole Limpet Hemocyanin (KLH) was evaluated at PND 49. There was an increased DTH response in all dose groups compared to the control, reaching statistical significance in the low and high dose groups (37% and 52% increase in thickening of the ear compared to control). LOAEL is considered to be 30 mg DOTC/kg diet based on decreases in absolute and relative thymus weights associated with decreases in lymphocyte subpopulations in F1 males.	

OECD 414 (no significant deviations) Sprague Dawley rat 25 mated females/group	Dichlotodioctylstannane, purity 97.7 %. 0, 10, 100 and 300 mg/kg in the diet from GD 5 to 19. Actual dose: 0 ± 0.0, 0.8 ± 0.1, 7.2 ± 1.0, 22.4 ± 4.2 mg/kg bw/day	Reduced body weight at 300 mg DOTC/kg diet and reduced body weight gain at 100 and 300 mg DOTC/kg diet. No statistical significant difference in food consumption was observed at any time point in any dose group administered DOTC in the diet compared to control. Reduced thymus size at 100 mg DOTC/kg diet (7 of 25 females) and 300 mg DOTC/kg diet (all females). LOAEL for effects on thymus is considered to be 100 mg DOTC/kg diet.	Study report, 2014

(§) Only information on the 90-day study is presented in this table. For information on the OECD TG 422-study, see table 11 and Annex I.

Table 23: Summary table of human data on STOT RE

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

Table 24: Summary table of other studies relevant for STOT RE

Type of study/data	**Test substance**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No relevant studies.				

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

There is no data available on specific target organ toxicity after repeated exposure of DOTL. In general, dioctyltin compounds are ascribed as having immunotoxic properties via the thymus gland. The use of dioctyltin compounds is therefore restricted according to REACH (EC) No 1907/2006 Annex XVII, entry 20 in a number of consumer articles (≥ 0.1 % by weight of tin).

Read-across from the source substance to fill data gaps on specific target organ toxicity – repeated exposure of DOTL

To generate information on the specific organ toxicity of DOTL after repeated exposure for the purpose of harmonized classification an analogue chemical grouping with read-across from data of DOTC was used.

Justification

The read-across is based on the structural similarities between the source substance, DOTC, and the target substance(s). The substances contain the common dioctyltin (Oct2Sn-) group, considered to be the toxic component, as well as two labile ligands (X). The hypothesis for the analogue approach is that following oral administration, both substances will hydrolyse with the generation of common intermediates; systemic exposure will therefore be to the same substance(s) regardless of the substance administered.

The specific organ toxicity after repeated exposure of DOTL is therefore assumed to be predictable on the basis of existing data on DOTC in the current analogue approach for chemical grouping.

Source substance data

The source substance DOTC has a harmonised classification in STOT RE 1 for effects on the thymus/immune system. The classification R48 was formerly concluded by Technical Committee for Classification and Labelling and hence included in Annex I of Directive 67/548/EEC (ATP 30, August 2008) and later translated and included in CLP Annex VI.

Indeed, clear and critical effects of DOTC on the thymus were observed in the repeated dose toxicity study from 2004 and also in the two studies primarily intended to assess developmental and/or reproductive toxicity (which include measurement of thymus weight or assessment of thymus histopathology). In the key study of DOTC (Apple and Waalkens-Berendsen, 2004) the test substance was administered in the diet to Wistar rats at 10, 100, 300 mg DOTC/kg diet (0.7, 6.5-6.8, and 19.3-19.8 mg DOTC/kg bw/day) for 90 days. No treatment-related changes were observed in clinical signs, food conversion, neurobehavioral testing, ophthalmoscopy and urinary volume and density. Reduced body weights and body weight changes were observed at 300 mg DOTC/kg diet in males and females. In addition, reduced food intake was recorded in males and females of the main study at 300 mg DOTC/kg diet, however food efficiency values were similar compared to those of the control groups. The decreased body weight associated with reduced food consumption in males and females of the 300 mg/kg/day group was most probably due to reduced palatability of the test item, according to the study report.

A number of treatment related changes were observed (decreased in haemoglobin, packed cell volume, mean corpuscular haemoglobin, total white blood cells, absolute numbers of lymphocytes and an increase in prothrombin time). These changes involved the 300 mg/kg/day group and were considered toxicologically relevant. Furthermore, a number of treatment-related clinical chemistry changes were observed (decreases in total protein and calcium and increases in alkaline phosphatase, albumin to globulin ratio, bilirubin and bile acids). These changes were observed in the 100 and 300 mg/kg/day groups and were considered toxicologically relevant.

A number of treatment related changes in organ weights were observed, notably a decrease in thymus weights and increases in kidney and liver weights. However, treatment related histopathological changes were only observed in the thymus.

A dose-dependent decrease in absolute and relative thymus weights were observed at all dose-levels in females (-14%/-14%, $p<0.05$, -68%/-69%, $p<0.01$, -73%/-70%, $p<0.01$ in 10, 100 and 300 mg DOTC/kg diet groups compared to control) and males (statistically significant ($p<0.01$) at 100 mg DOTC/kg diet (-47/-48%) and 300 mg DOTC/kg diet (-75/-73%) compared to control).

Changes in thymus weight were correlated with histopathological effects in the 100 mg/kg group (5/10 males, 10/10 females) and in the 300 mg/kg group (9/9 males, 9/9 females) and were manifested as lymphoid depletion, characterized by a decrease in the size of the thymic lobules. The microscopic appearance of the affected thymus resembled thymus atrophy as described in the literature for organotin compounds, according to the study authors.

The decreased absolute and relative thymus weights in females of the 10 mg/kg group, although not accompanied by histopathological changes, were also considered to reflect a toxicologically-relevant change in the thymus, which was in accordance with the shown toxicity profile of the test substance (i.e. thymotoxicity).

In females of the satellite study, absolute and relative thymus weight were statistically significantly decreased in the 100 and 300 mg/kg groups (-38 and -33%, respectively at 100 mg/kg; -69 and -62%, respectively at 300 mg/kg). Moderate to severe lymphoid depletion was observed in all treated groups (5/10, 10/10 and 10/10 at 10, 100 and 300 mg DOTC/kg diet respectively). One animal in control group also had very severe lymphoid depletion. This was considered to be because the animal was physiologically disturbed according to the study report (12 resorptions and an abnormal kidney). The

reported lymphoid depletion in treated groups was characterised by a decrease in size of the thymic lobules which can be ascribed to extensive loss of cortical en medullary small lymphocytes.

A NOAEL for sub chronic toxicity was not established in the repeated dose 90-day oral toxicity study (OECD TG 408). The LOAEL was determined to be 0.7 mg/kg bw/day.

In line with the above findings, seven out of 25 dams in the 7.2 mg/kg bw/day dose group and all dams in the 22.4 mg/kg bw/day dose group had reduced thymus size at necropsy in the pre-natal developmental toxicity study of DOTC in rat (Study report, 2014), where dams were dosed GD 5-19. However, no data on thymus weight or histopathology were available to the dossier submitter.

In a study similar to EOGRTS in rat the F1 animals were evaluated for changes in immune function. Absolute (-22%) and relative (-20%) thymus weight and thymus cellularity (-36%) were statistically significantly decreased in the highest dose group (30 mg DOTC/kg diet, approx. 3 mg/kg bw/day) on PND 42 compared to control, but no difference was noted at PND 70. No effects were observed on absolute and relative spleen weights, although there was a tendency at PNDs 42 and 70 to a decreased cellularity at the high dose groups. Relative liver weight was statistically significantly increased in the low and mid dose groups on PND 70 (4.12 g in the control versus 4.45 g in the low and 4.53 g in the mid dose group). These minor changes were not dose related and considered to be of no toxicological significance. No treatment-related organ weight changes were observed in kidneys, adrenals, heart, and testes (no information on organ weights or histopathology for F0 animals was available to the dossier submitter).

There were no treatment-related macroscopic changes observed in F1 animals at necropsy and there was no difference in general condition or behaviour among groups of F1 pups. Male pup mean body weights were statistically significantly increased on PNDs 8, 10 and 13 in the high dose group compared to control. However, after weaning, no effects on body weight or food consumption were observed (according to study authors, no data available).

Immune assessments by several immune parameters were performed at PNDs 21, 42 and 70. Effects on lymphocyte subpopulations in the thymus of F1 animals were observed in the 30 mg DOTC/kg diet group on PND 42, whereas effects on lymphocyte subpopulations in the spleen were found in the 30 mg DOTC/kg diet group on both PNDs 42 and 70.

Changes in lymphocyte subpopulations in the spleen were noted on PND 42 as a statistically significant decrease in the absolute and relative number of CD3+, CD3+CD4+ and CD3+CD8+ cells in the high dose group together with a decreased T:B cell ratio. The decrease in CD3+CD4+ splenocytes was no longer statistically significant at PND 70.

Changes in lymphocyte subpopulations in the thymus were also noted on PND 42 with a statistically significantly decrease in the absolute number of CD4-CD8+, CD4+CD8+, immature ($CD3^{low}$) and mature ($CD3^{high}$) thymocytes in the high dose group compared to the control group. Same trend was observed at PND 70, however, the difference was no not statistically significant compared to control.

To aid in the evaluation of cell-mediated immunity the T cell-dependent antibody response to Keyhole Limpet hemocyanin (KLH) was assessed following subcutaneous immunizations with KLH on PNDs 21 and 35 and the delayed-type hypersensitivity response (DTH) against KLH was evaluated at PND 49. There was an increased DTH response in all dose groups compared to the control, reaching statistical significance in the low and high dose groups.

The decreases in thymus weight and in lymphocyte subpopulations of both spleen and thymus confirms the adverse effects on the immune system that is known for dioctyltin compounds in adult animals. It is however unclear how the increased DTH response correlates with the findings of lymphoid depletion in the spleen and the thymus and the Th2-skewing. Moreover, it is unclear if this is a specific developmental immunotoxicological effect. The study authors suggest that the findings in the present study may indicate a disturbed immune balance.

There are a number of older repeat dose toxicity studies in the open literature that have demonstrated that DOTC induce thymus atrophy in rats at dietary levels from 50 ppm (including Seinen and Willems, 1976; Seinen et al., 1977; Seinen and Penninks, 1979; Miller, Scott, and Foster 1984), and there are data

to suggest that the T cell may be a primary target for dialkyltin compounds like DOTC. Dialkyltin compounds induce lymphocyte depletion in the thymus and in the thymus dependent areas of the peripheral organs, and as a consequence they cause immunosuppression, especially of the T-lymphocyte–dependent immunity (Penninks and Seinen, 1984).

As a supporting study in the REACH registration of DOTC, an oral 14-days repeated dose toxicity in young male rats by Penninks & Seinen (1982) was included. DOTC was administered via the diet at levels of 50 and 150 ppm, since 450 ppm killed the animals within the two weeks of the test period. DOTC caused growth retardation at 150 ppm. The relative weights of lymphoid organs (thymus and spleen) were decreased in a dose-related manner. The decrease in thymus weight was the more pronounced and amounted to more than 70% in rats fed 150 ppm. The most prominent histopathological feature in all treated animals was lymphocyte depletion. This was seen particularly in the thymic cortex, but also in the splenic periarteriolar lymphocyte sheets.

In conclusion, the effects observed on the immune system including thymus atrophy with lymphoid depletion were clearly dose related and were observed at dose levels starting from 0.5-0.7 mg/kg bw/day. These findings provide an important basis for classification for specific target organ toxicity after repeated exposure. It is noted that females appears to be the more sensitive sex, and there are indications that developing animals may be more sensitive to effects of DOTC on the immune system.

10.12.2 Comparison with the CLP criteria

The available data of the source substance DOTC point towards the immune system as a clear target organ after oral exposure, and accordingly DOTC already has a harmonised classification in STOT RE 1 for effects on the thymus/immune system. Consequently, based on read-across from DOTC classification of DOTL in STOT RE 1 is also warranted.

Overall, the dossier submitter considers that the effects on the thymus/immune system as demonstrated in available studies of the source substance DOTC are sufficiently significant to fulfil the classification criteria for STOT RE 1. The effects on the immune system include morphological changes in the thymus that provide clear evidence of marked organ dysfunction and are considered as significant organ damage noted at necropsy and subsequently confirmed at microscopic examination. Thus, the effects observed on the thymus are considered to represent a significant health effect as defined in the CLP Regulation.

Repeated exposure to DOTC during 90 days revealed reduced thymus size and lymphoid depletion with effective dose levels of 0.7 mg/kg bw/d (Apple and Waalkens-Berendsen, 2004). This is below the guidance value C $\leq$ 10 mg/kg bw/day (90-day repeated-dose study, oral, rat) for category 1 classification in STOT RE.

The effect level of DOTC in the reproduction/developmental toxicity screening test (approx. 54 days of exposure) was 0.5-0.7 mg/kg bw/day for females. Taking the shorter study duration into consideration the effect level is well below the guidance value for category 1. Less significant findings supporting the classification in category 1 were decreased thymus weight and effects on lymphocyte subpopulations in the thymus and the spleen in the F1 generation of the 30 mg DOTC/kg diet (approx. 3 mg/kg bw/day) dose group on PND 42 in the Tonk study (2011). Moreover, reduced thymus size at necropsy were noted in the PNDT study (15 days of exposure) from 7.2 mg/kg bw/day (Study report 2014) which is in line with the significant findings in previous studies and also below the guidance value for category 1.

The read-across from the source substance DOTC to the target substance DOTL for classification in STOT RE 1 may require some consideration with regards to stoichiometry and potency of the observed organ toxicity. At pH 1.2 the distannoxane ClOct2SnOSnOct2Cl was observed from the in vitro hydrolysis of DOTC in >90% yield within 4 hours making it likely to be responsible for the toxicological effects of DOTC observed after oral administration. In comparison, an approximately 15% yield of DOTL within 30 minutes to 4 hours was reported under the same conditions. Available

toxicokinetic studies of DOTC indicate that the absorption following oral administration was 20% of the dose. Since the distannoxane was the main hydrolysis product formed in the gastric hydrolysis study of DOTC, one can assume that the absorption of distannoxane is 20%. Moreover, the absorption from the gastrointestinal tract of the hydrolysis product distannoxane is assumed to be similar for DOTC and DOTL. However, based on differences in hydrolysis yields (at least 6 times higher for DOTC compared to DOTL) and based on differences in the mass proportion of dioctyltin moieties generated by hydrolysis (due to different molecular weight) an approximately ten times higher dose on a mass basis of DOTL compared to DOTC is required to achieve the same internal dose, on a molar basis. Considering that adverse effects of DOTC (on thymus/immune system) are evident at fairly low doses (0.5-0.7 mg/kg bw/day), and that DOTL have been demonstrated to have moderate to low acute toxicity, an approximately ten times higher dosing appears to be achievable. Thus, the read-across from DOTC to DOTL for systemic effects is relevant and justified. A 10 times higher dose of DOTL (10 x 0.7 mg/kg bw/day) would still be below the guidance value for category 1.

10.12.3 Conclusion on classification and labelling for STOT RE

Based on read-across data from the source substance DOTC on effects on the thymus/immune system and following correction for stoichiometry, classification of DOTL (CAS numbers 3648-18-8 [1], 91648-39-4 [2]) in **STOT RE 1, H372: Causes damage to the immune system** is considered to be appropriate.

No specific route of exposure should be indicated in the hazard statement, since no such data are available to conclusively prove that no other route of exposure can cause the hazard. It is noted that the source substance DOTC has a harmonised classification in STOT RE 1, H372 with the reference (**) stating that route of exposure cannot be excluded. The classification is derived from the translation of the classification (R48) listed in Annex I to directive 67/548/EEC. Under Directive 67/548/EEC the route of exposure is indicated for classifications with R48 when there was data justifying the classification for this route of exposure. The classification under 67/548/EEC indicating the route of exposure has been translated into the corresponding class and category according to this Regulation, but with a general hazard statement not specifying the route of exposure as the necessary information is not available.

10.13 Aspiration hazard

Not evaluated in this CLH Report.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Not evaluated in this CLH Report.

11.2 Environmental transformation of metals or inorganic metals compounds

Not evaluated in this CLH Report.

11.3 Environmental fate and other relevant information

Not evaluated in this CLH Report.

11.4 Bioaccumulation

Not evaluated in this CLH Report.

11.5 Acute aquatic hazard

Not evaluated in this CLH Report.

11.6 Long-term aquatic hazard

Not evaluated in this CLH Report.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not evaluated in this CLH Report.

13 ADDITIONAL LABELLING

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15 ANNEXES

Annex I to the CLH report.