Committee for Risk Assessment

RAC

Annex 1

Background document

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

dioctyltin dilaurate; [1]
stannane, dioctyl-, bis(coco acyloxy) derivs. [2]

EC Number: 222-883-3 [1] 293-901-5 [2]
CAS Number: 3648-18-8 [1] 91648-39-4 [2]

CLH-O-0000001412-86-223/F

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

Adopted

14 September 2018

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**

Contact details for dossier submitter:

Swedish Chemicals Agency

Esplanaden 3a, P.O Box 2

SE-172 13 Sundbyberg, Sweden

kemi@kemi.se

+46 8 519 41 100

Version number: 2 **Date: July 7, 2017**

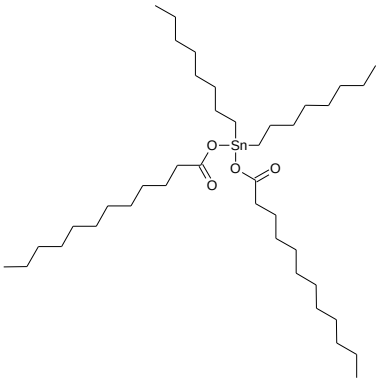
CONTENTS

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.*

RAC general comment
Dioctyltin dilaurate, further referred to as DOTL in this document, is an organotin compound with two octyl chains and two laurate (C12) groups. The CLH proposal for DOTL embraces both the mono constituent 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). 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. According to the dossier submitter (DS), 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. Other organotin compounds previously assessed by RAC include dibutyltin dilaurate and dibutylbis(pentane-2,4-dionate-O,O)tin that contain shorter alkyl side chains. The RAC opinions on these compounds concluded on classification as, amongst others, STOT RE 1 (immune system), Repr. 1B; H360FD and acute toxicity via inhalation. One other di-octyl tin compound previously assessed by RAC, dioctyltin bis(2-Ethylhexylmercaptoacetate), was classified as Repr. 1B; H360D, which was now also proposed for DOTL.

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	REACH registration, publically disseminated version (ECHA dissemination, 2016a)	EU Method A.2 (Boiling Temperature) OECD Guideline 103 (Boiling point/boiling range)

Property	Value	Reference	Comment (e.g. measured or estimated)
	terminated.		
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. 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	REACH registration,	EU Method A.9 (Flash-Point)

Property	Value	Reference	Comment (e.g. measured or estimated)
	test material was determined to be 198 °C at 1015.8 hPa.	publically disseminated version (ECHA dissemination, 2016a)	
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 (ECHA dissemination, 2016a)	From the structural formula it can be concluded that the substance is not explosive and 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	The substance is a liquid at room temperature, thus testing for this endpoint can be omitted.

Property	Value	Reference	Comment (e.g. measured or estimated)
		(ECHA dissemination, 2016a)	
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

Method	Results	Remarks	Reference
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 Single exposure of 25 mg/kg bw 72h observation period following administration	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)]
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).

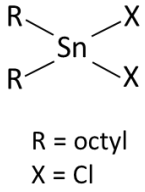
9.2.2 Hypothesis for the analogue approach

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

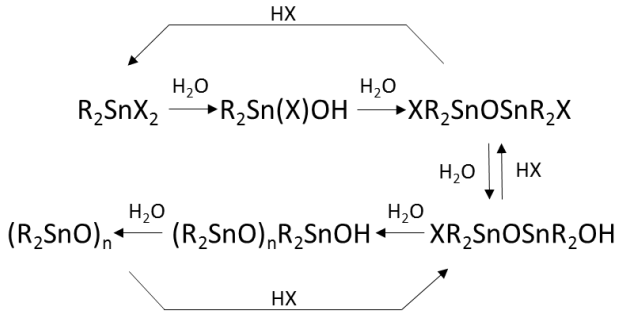
Physicochemical properties

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	$(Oct)_2Sn(laurate)_2$	$(Oct)_2SnCl_2$

	DOTL **(target substance)**	**DOTC** **(source substance)**
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 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)

	DOTL **(target substance)**	**DOTC** **(source substance)**
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 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 –	No data: read-across proposed	Repeated dose 90-day oral

	DOTL **(target substance)**	**DOTC** **(source substance)**
repeated exposure		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.

RAC evaluation on the proposed read across approach
Summary of the dossier submitter's proposal on the read across approach Because no data is available on DOTL for the endpoints of interest (STOT RE,

reproductive toxicity), a read across approach from dioctyltin dichloride (DOTC, EC 222-583-2) to DOTL was proposed based on hydrolytic and toxicokinetic behaviour, in accordance with the ECHA guidance document *Guidance on information requirements and chemical safety assessment, Chapter R.6: QSARs and grouping of chemicals (ECHA, 2008)*.

The read across approach is based on structural similarity between the source and target substance where the common dioctyltin group is considered the relevant toxic component. Both substances are hypothesised to hydrolyse with common intermediates to the same substances in the stomach. Therefore, the read across is limited to systemic endpoints by oral administration. The DS argued that the read across is applicable to both the UVCB and the mono constituent substance since they only differ in structure of the labile carboxylate ligands and therefore the hydrolysis of dioctyltin compounds will be similar. Two studies are available on *in vitro* simulated gastric hydrolysis of DOTL and DOTC by Naβhan (2015) and Naβhan (2016), respectively. 90% of DOTC was hydrolysed to the dimeric distannoxane ($ClOct_2SnOSnOct_2Cl$) within 4 h, while the remaining 10% was DOTC. DOTL is hydrolysed to several products including the aforementioned dimer $ClOct_2SnOSnOct_2Cl$ (14-16%), DOTLC (43-47%) and a non-assigned tin species (38-43%). This composition is reached after 4 h, but there are only minor differences compared to the composition after 30 min of incubation at acidic pH. Both DOTC and DOTL are transformed to DOTO (dioctyltin oxide) at neutral pH. At acidic pH, formation of DOTO is however not favoured, as is illustrated in the figure below.

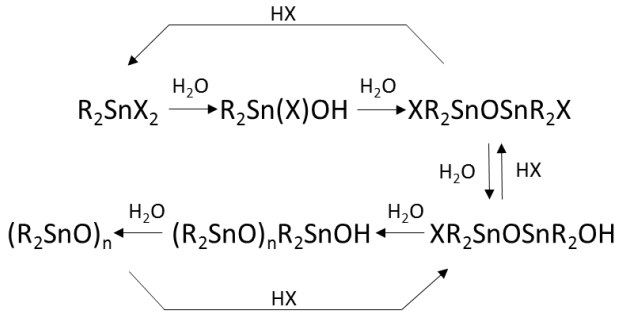


Figure: Hydrolysis scheme for dialkyltins (Davies, 2004; Aylett *et al.*, 1979)

Finally, the DS justified the read across approach with the following arguments:

- The hydrolytic behaviour at neutral and low pH supports the rapid formation of common intermediate(s). Subsequently, due to rapid hydrolysis at low pH there will be no systemic exposure to DOTL when administered orally.
- Hydrolysis studies at low pH for DOTC and DOTL show partial formation of the same species. 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 behaviour is in accordance with 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.

Comments received during public consultation

Three member state competent authorities (MSCAs) supported the proposed read across approach. However, two of the MSCAs addressed some uncertainties.

One of the MSCAs noted that the toxicological data in the data matrix were limited and the hydrolysis products of DOTC and DOTL are different because of the coordinating carboxylate ligands, while there is no demonstration of how this may impact the toxicological outcome. This MSCA suggested to also consider the tin compounds with shorter side groups, dibutyltin dichloride (DBTC) and dibutyltin dilaurate (DBTL), previously assessed by RAC, in the evaluation. Similar to the current proposal, classification for DBTL was proposed, predominantly based on read across from DBTC although some toxicological data was available based on DBTL itself. This MSCA ultimately considered the read across approach as plausible rather than giving full support.

The DS agreed that it is worth including DBTC and DBTL and to compare them with DOTC and DOTL. The DS noted that the results from a study by Milton *et al*. (2017) suggest a similar mode of action for both DBTC and DBTL and it is reasonable to assume that DOTC and DOTL may act via a similar mode of action as well. Further, DOTC and DBTC appear to have similar immunosuppressive properties, anti-proliferative effects and depletion of immature thymoblasts. DBTC is also hydrolysed to a dimer ClBu2SnOSnBu2Cl similar to DOTC and all of these (DOTC, DOTL, DBTC, DBTL) behave similarly in water at neutral pH. Data on DBTL itself indicates that it has similar toxic effects to DBTC. The information points to a common metabolite/intermediate *in vivo* and common biological targets for DBTL and DBTC. This also further supports the applicability of read across from DOTC to DOTL for reproductive toxicity and STOT RE since a similar relationship between DOTC and DOTL can be expected.

The other MSCA noted that transformation *in vivo* may be more complex due to (for example) the presence of enzymes. Further, if the common metabolite would be responsible for the observed effects, it may not fall within the criteria for STOT RE 1 as the potency is then expected to be lower for DOTL. This MSCA also suggested taking into account other dioctyltin compounds with organic/carboxylate ligands that show similar effects.

In response, the DS agreed that it is uncertain what the potency of DOTL could be after repeated exposure in comparison to DOTC. In MAK value documentation (2015) on n-octyltin compounds, studies are available, predominantly on DOTC, but also on Mono-n-octyltin trichloride (MOTC), Di-n-octyltin-bis(2-ethylhexylmercaptoacetate) (DOTE), Mono-n-octyltin tris (2-ethylhexyl mercaptoacetate) (MOTE), Di-n-octyltin-bis(isooctyl mercaptoacetate) (DOTI), and Mono-n-octyltin-tris(isooctyl-mercaptoacetate) (MOTI). DOTE/MOTE and DOTI/MOTI have thioester ligands and it is unclear if these can be compared to octyltin compounds with labile carboxylate ligands. The lowest effect levels were observed for DOTC and DOTI (thymus). Common reproductive toxicity findings were observed for DOTC/MOTC and MOTI/DOTI, but for prenatal development, only data for DOTI and MOTI were included in the MAK-document. The common findings include post-implantation loss, decreased gestation index, decreased litter size, increased number of stillbirths and increased postnatal mortality. More recent studies available for

DOTE and DOTC are however available and effects include cleft palate, reduced ossification and decreased foetal viability.

The lead registrant, supported by a single individual and three other companies, questioned the read across proposal. They mentioned that the hydrolysates of DOTC and DOTL have some commonalities but are also different. Further they believe that the adverse effects observed after exposure to DOTC may be attributed to the 10% leftover DOTC after hydrolysis rather than the dimeric distannoxane.

The DS disagreed that there is evidence that the effects observed after exposure to DOTC is attributed to DOTC itself rather than the dimeric distannoxane. In fact, there is evidence for dissociation in solution and the dimer with half the molecular weight can be present in significant concentrations in solution. Significant exposure to the intact substances in solution is unlikely as shown by the hydrolytic behaviour. Further, DBTC readily hydrolyses to the dimeric distannoxane ClBu2SnOSnBu2Cl, behaves similarly in water and has comparable developmental toxicity and immune toxicity data to DBTL and DOTC. This information points to a common metabolite/intermediate *in vivo*, and common biological targets, which support the applicability of a read across from DOTC to DOTL for reproductive toxicity and STOT RE.

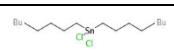
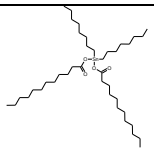
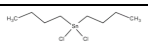
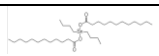
Assessment and comparison with the classification criteria

The DS hypothesised that DOTC and DOTL share structural similarity apart from two labile ligands and form similar hydrolysis and biologically active breakdown products. RAC considers that the source and target compound are structurally similar apart from the dichlorine (DOTC) compared to the dilaurate (DOTL) groups. Both these different structures are considered labile, di-substituted and readily hydrolysed. DOTC is up to 90% hydrolysed to a dimer ClOc2SnOSnOc2Cl at low pH. The same hydrolysis product is formed by DOTL, although to a lesser extent (approximately 15%).

It should be noted that only an *in vitro* gastric simulation study is available for both DOTC and DOTL. No information is provided on the *in vivo* metabolism of either substance, or on the toxicological properties of the other metabolites formed by DOTL. A study by Penninks *et al.* (1987) showed that after oral administration of DOTC to rats, 20% is absorbed and systemically bioavailable. The gastric simulation study indicates that 90% of DOTC is hydrolysed to the dimer within 4 h. This information suggests that the dimer is at least in part bioavailable and is likely to account for the effects observed after oral administration of DOTC.

During the public consultation, two MSCAs suggested to include additional information on these similar organotin compounds to further support the read across proposal in light of these uncertainties. RAC agreed that this helps to strengthen the read across proposal. To obtain a clearer view on the toxicity profiles of other organotin compounds relevant for DOTL, RAC has summarised the harmonised classifications and known toxicity of the alkyltin compounds that are closely related to DOTC and DOTL in the table below. The information presented in the table has been derived from the registration dossiers and the previous RAC opinion for DBTL.

Table: Summary of closely related octyltin chemicals and their (harmonised) classifications

Chemical name & CAS number	DOTC 3542-36-7	DOTL (UVCB) 3648-18-8, 91648-39-4	DBTC 683-18-1	DBTL 77-58-7
Chemical Structure				
Harmonised classification (human health hazards)	STOT RE 1; H372 ** Acute Tox. 3; H331 Proposal: Modify/add: Acute Tox. 2; H330 Repr. 1B; H360D (SCL: 0.03%)	Current proposal (read across from DOTC): STOT RE 1; H372 (immune system) Repr. 1B; H360D	STOT RE 1; H372 ** Repr. 1B; H360FD Acute Tox. 2; H330 Acute Tox. 4; H312 Acute Tox. 3; H301 Muta. 2; H341 Skin Corr. 1B; H314	STOT RE 1; H372 (immune system) Repr. 1B; H360FD Muta. 2; H341
Simulated gastric hydrolysis pH = 1.2 <4 h	90% dimer, 10% DOTC.	14-16% dimer, 43-47% DOTLC and 38-43% non-assigned.	No information available.	88% DBTC in <2 h (basis for read across).
Repeated dose toxicity (thymus/immune toxicity)	**Yes,** based on substance specific information.	Current proposal: **Yes,** based on read across from DOTC.	**Yes,** based on substance specific information.	**Yes,** based on read across from DBTC and substance specific data
Reproductive toxicity	**Yes**, based on substance specific information.	Current proposal: **Yes,** based on read across from DOTC.	**Yes**, based on substance specific information.	**Yes,** based on read across from DBTC and supporting substance specific data.
Consulted information	CLH proposal on DOTL	CLH proposal on DOTL	RCOM for DOTL and RAC opinion on DBTL	RCOM for DOTL and RAC opinion on DBTL

DBTC was classified as STOT RE 1; H372 ** and Repr. 1B; H360FD based on similar effects to those induced by DOTC. DBTL was classified based on read across from DBTC. However, in addition to a hydrolysis study, there was also supportive evidence from studies with DBTL itself. These studies had limitations, but showed that DBTL has immunotoxic and developmental effects similar to those displayed by DBTC. This indicates that substitution of the chlorine groups by laurate groups is not the determinative factor for the toxicological properties of these organotin substances.

Additionally, effects on the immune system were also observed after exposure to other dioctyltin compounds with labile- or thioester ligands, as mentioned during the public consultation. All of these organotin compounds cause very similar (systemic) adverse effects. When there is substance specific information available and/or a harmonised classification, these compounds all adversely affect the immune system and most of them

also affect the reproductive system (similar effects include amongst others post-implantation loss, reduced postnatal viability, and skeletal effects).

The similarity in effects of these di-substituted organotin compounds strengthens the hypothesis that DOTL will have the same hazard properties. As DOTC is the most closely related di-substituted dioctyltin compound and has partially similar hydrolysis breakdown products under acidic conditions, this is the most appropriate available substance for read across. This is also consistent with previous RAC evaluations of di-substituted organotins, such as DBTL.

In light of these considerations, RAC agrees with the DS that the read across approach from DOTC to DOTL is appropriate for systemic endpoints after oral administration. Data on DOTC can therefore be used to assess reproductive toxicity or adverse effects after repeated exposure (STOT RE) to DOTL.

RAC acknowledged that there may be potency differences between DOTC and DOTL. The possible impact is discussed in the relevant sections.

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	Appel and Waalkens-Berendsen. (2004)

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

<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>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 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%</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, 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% 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	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	**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.	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.	and 0.27–0.55, 1.0–1.9, 2.9–5.2 mg/kg bw/day during lactation.	**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 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).

<u>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)</u>

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,	Study report, 2014

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		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 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.	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	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	Tonk et al., 2011

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
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.	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	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 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 group	25	25	25	25
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. (%)	 1 (0.8) 1 (4.5)	 8 (7.0) 6 (28.6)	 15 (13.3*) 9 (45.0)	 29 (27.1*) 16 (80.0)

Litter basis, no. (%)				
Split thoracic vertebrae centrum no. 12	0	1(1)	0	0
Missing caudal vertebral arch no 2	0	2(2)	0	3(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	22.33 ± 13.159	20.98 ± 7.114	49.23 ± 17.453	69.99 ± 14.713

Median value [N = number of females]	7 N=7	11 N=8	50 N=7	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.

RAC evaluation of reproductive toxicity

RAC accepted the read across approach from DOTC to DOTL. A proposal for classification of DOTC as Repr. 1B; H360D was simultaneously addressed together with this proposal for DOTL. The information in this section is hence from studies on DOTC. Comments from the public consultation for both DOTC and DOTL are included.

Summary of the Dossier Submitter's proposal

The DS proposed to classify DOTL, based on read across from DOTC, for effects on development, as Repr. 1B; H360D. To assess adverse effects on reproduction, three studies on DOTC were taken into account, a combined reproductive screening study according to OECD TG 421, an extended one generation reproduction toxicity study (EOGRTS) similar to OECD TG 443 and an additional prenatal developmental toxicity study performed according to OECD TG 414. All studies were carried out with the registered substance. An overview of the study designs and results submitted by the DS are presented in the table below. A more detailed summary, including tables with clear treatment-relationships on relevant adverse effects regarding parental and reproductive toxicity, is presented in the RAC assessment.

***Table**: Summary of setup and results of the reproductive toxicity studies*

Study	Dosing	Results
Appel and Waalkens-Berendsen, 2004 OECD TG 421 (Combined reproductive screening test) GLP Wistar rats 10/sex/dose in main 13-week sub-chronic toxicity study 10 females/dose in satellite reproductive screening study	DOTC, 92.1% pure 0, 10, 100, 300 mg/kg diet/d (corresponding to approx. 0, 0.5-0.7, 4.2-6.2 and 8.4-17 mg/kg bw/d respectively). Main study animals were fed for 13 weeks daily. Females from the satellite groups were fed for 2 weeks premating, and continued until shortly after PND4. Main study males were mated with female from the satellite groups after 10 weeks premating.	F0 at 300 mg/kg diet unless otherwise stated: Gestation: females: ↓ bw (not corrected, -16% on GD21). Lactation: females: ↓ bw (-20% on PND4). Food consumption: females: ↓ (-18 to -68% and -10 to -15% at 100 mg/kg diet; -11% during GD7-14). Organs: ↓ absolute relative thymus weight (males: -73 to -75% and -47 to -48% at 100 mg/kg diet, females: -62 to -69% and -33 to -38% at 100 mg/kg diet and non-stat sign -23 to -24% at 10 mg/kg diet). ↑ Lymphoid depl, (males: 9/9 (moderate-severe) and 5/10 at 100 mg/kg diet (slight-moderate), females: 10/10 (severe-very severe in all groups) and 10/10 at 100 mg/kg diet and 5/10 at 10 mg/kg diet). No effects on fertility indices. Males: Stat. sign. changes in absolute/relative weight of spleen, kidney, liver and testes at highest dose. **Reproductive toxicity:** Strongly decreased (but not stat. sign. at 100/300 mg/kg diet): ↓ gestation index (71%/50% vs 86% in control), ↑ mean post-implantation loss (of 49%/70% vs 22% in control). ↓ live birth index (53%/60% vs 99% in control). Stat. sign. effects: ↓viability index PND 0-4 (74/12% vs 94% in control). F1: Foetal weight at PND1, (3.9 at 300 mg/kg diet vs 4.76 g in control). ↑ no. of runts (weight below 2 std. deviation vs. mean weight, at 10, 100 and 300 mg/kg diet: 7, 10 and 6, respectively vs. 1 in control). ↑ no. of cold pups at 300 mg/kg diet.

Tonk *et al.*, 2011 OECD TG 443 – EOGRTS without cohorts 2/3 and extension of 1B. GLP unknown Wistar rats 24 females/group (20 in high dose group) Litters not standardised and pups weaned at PND21. Sexual maturation evaluated for 1 pup/litter, 8 F1 males/group for immune assessment	DOTC, purity unknown. 0, 3, 10, 30 mg/kg in diet (corresponding to F0 females: 0.17-0.21, 0.56-0.71 and 1.7-2.1 mg/kg bw/d during gestation and 0.27-0.55, 1.0-1.9, 2.9-5.2 mg/kg bw/d during lactation).	F0 females: ↓ bw (5%) during lactation at 10/30 mg/kg diet. No effects on fertility indices. No information on organ weights and histopathology of F0. **Development:** F1: At high dose only: ↓ mean no. of live pups/litter at PND4 (8.78 vs 10.48 in control). ↓absolute (-22%) & ↓ relative (-20%) thymus weight, ↓ thymus cellularity (-36% on PND42). Spleen at PND 42 (high dose only): ↓ absolute and relative No. of CD3+, CD3+CD4+ and CD3+CD8+ cells. ↓ T:B cell ratio. At PND70, CD3+CD4+ no longer stat. sign. reduced. Thymus at PND42 (high dose only): ↓ absolute no. CD4-CD8+, CD4+CD8+, immature (CD3low) and mature (CD3high) thymocytes. Not stat. sign. anymore at PND70. Delayed-type hypersensitivity (DTH): The DTH response at PND49 was stat. sign. ↑ at low/high dose (37% and 52%) and non-stat. sign. ↑ at mid dose. LOAEL: 30 mg/kg diet/d for developmental effects, NOAEL for F0 is 30 mg/kg diet/d in diet.
Study Report 2014 OECD TG 414 prenatal development toxicity study GLP Sprague Dawley rats 25 mated females/group	DOTC, purity 97.7% 0, 10,100, 300 mg/kg diet from GD5-GD19 Actual dose: 0, 0.8 ± 0.1, 7.2 ± 1.0, 22.4 ± 4.2 mg/kg bw/d	F0: ↓ bw on GD 20 (not corrected, -30% at high dose). ↓ bw gain on GD5-20 at mid- (-12%) & high dose (-31%). Organs: ↓ thymus size (7/25 mid dose, all at high dose), no details available. **Development (F1):** ↑ Pre-implantation loss at mid (7%) and high dose (10.4%) vs. control (1.5%). ↑ Post-implantation loss at low (6.8%), mid (4.9%) and high dose (6.9%) vs. control (0.8%). ↑ Skeletal malformations, predominantly missing bones in paws at mid (22) and high dose (47) vs. control (1). Increase also at low dose (11) but not stat. sign. ↑ Skeletal variations (predominantly poor ossification) at high dose (26 vs. 6 in control). Incidences at low/mid dose were 10/11 and not stat. sign. LOAEL for both maternal and developmental effects considered by the registrants to be 100 mg/kg diet or 7.2 mg/kg bw/d.

PND=postnatal day

According to the DS, the studies do not indicate adverse effects on fertility in either males or females up to dose levels of 300 mg/kg diet/d. However, the dose levels used were low, especially in the EOGRT study since it was mainly focused on assessing immunological effects. Therefore, the DS concludes that classification for effects on fertility is not warranted although adverse effects at higher concentrations cannot be excluded.

Adverse effects on development were observed in the prenatal developmental study and the combined reproductive screening study. Maternal toxicity in the form of lower body weight and effects on the immune system (thymus) were noted. However, the DS argued

that the lower maternal body weight is limited and there is no established link between the effects on the maternal thymus and developmental toxicity. Therefore, the developmental effects should be regarded as relevant.

Based on skeletal malformations (missing bones, considered rare) in the TG 414 study, decreased live birth index along with increased number of stillborn pups at the doses corresponding to 7.2 and 22.4 mg/kg bw/d and increased post-implantation loss seen in multiple studies, the DS concluded that classification as Repr. 1B; H360D was warranted. For DOTC, the DS proposed to add an SCL of 0.03 mg/kg bw/d since a 10% increased incidence (ED_{10}) of total skeletal malformations is caused by about 0.8 mg/kg bw/d, hence meeting the criteria for the high potency group ($ED_{10} \leq 4$ mg/kg bw/d) as outlined in the CLP guidance. For DOTL, however, considering that no direct estimate of the reproductive toxicity potency derived from an ED10 value is possible, no SCL was proposed.

Comments received during public consultation (DOTC)

Two member state authorities (MSCAs) responded and were in support of the proposed classification. One of them added that they agreed with the proposed SCL for DOTC of 0.03%.

Two industries commented, not agreeing with the proposed classification because they considered the developmental effects likely to be secondary to maternal toxicity. Additionally, they posed questions concerning whether the malformations are true malformations or a result of delayed ossification and whether the results were adequately reported and interpreted considering the staining techniques used for investigating ossification and missing bones.

In reply, the DS argued that the reported malformations cannot be interpreted in another way. The malformations are, according to the study authors, associated with delayed foetal ossification. The DS believed that this means that in addition to the missing bones, increased incidences of poor or incomplete ossification of sternum no. 5 and 6 (statistically significantly different compared to control in the high dose group) and metacarpal no. 5 in the low, intermediate and high dose groups were also evident. Furthermore, poor or incomplete ossification of proximal phalanx no. 3 and 4 were seen in all dose groups including the control group. However, there was no dose-dependent increase in incidences and no statistically significant differences between the groups; the study authors therefore considered that these effects were not treatment-related. The DS further clarified that based on the following text from the report, it is interpreted that double staining was used and malformations like missing bones or variations such as delayed ossifications should have been picked up and reported as such:

"*The live foetuses with odd numbers were skinned and eviscerated, fixed in 95% ethanol, subjected to preparation of Alcian blue staining for cartilage and Alizarin red S staining for bones and the specimens were examined under stereomicroscope for the presence or absence of skeletal malformation (variations)"*

The incomplete ossification of the same structures as the missing ones (proximal phalanx no. 3 and 4, metacarpal no. 5) were reported separately, therefore confirming that the staining technique distinguishes between incomplete ossification and missing bone correctly and the malformations should be interpreted accordingly.

Comments received during public consultation (DOTL)

Four MSCAs supported the read across proposal of DOTC to DOTL and specifically the proposed classification as Repr. 1B; H360D.

As mentioned under "RAC evaluation on the proposed read -across approach", the lead registrant supported by a single individual and three companies questioned the proposed read across and therefore also question the proposed classification as Repr. 1B (H360D).

Assessment and comparison with the classification criteria

Fertility

Two reproduction studies were available for DOTC, one reproduction screening study with doses up to 8.4-17 mg/kg bw/d DOTC) and an EOGRTS using very low doses (up to 1.7-2.1 mg/kg bw/d). In none of these studies were effects observed that would support classification for fertility. However, in the EOGRTS no effects were seen in parental animals and therefore, adverse effects on fertility at higher concentrations cannot be excluded. The EOGRTS was primarily conducted to assess developmental immunotoxicity. In addition, a reproduction screening study cannot be used to exclude effects on fertility, due to e.g. the limited endpoints and power. As a consequence, RAC proposed not to classify DOTC or DOTL for effects on fertility because there is a lack of relevant data.

Development

In the single prenatal developmental study available on DOTC (Study report 2014) performed with SD rats, no significant maternal toxicity was observed. The maternal body weight gain and body weight was significantly lower at the highest dose at GD20. However, the corrected body weight was not significantly lower at GD20 (-6.8%) than the controls. Lower thymus weight than the control animals was reported in maternal animals at an incidence of 7/25 in the mid dose and all animals in the high dose. No data on the level of thymus weight was available to the DS or RAC. In addition, thymus effects were absent/limited at the low/mid dose while increased incidences of malformations were already seen in those treatment groups. These data indicate that developmental effects do occur in the absence of measured maternal thymus toxicity. RAC concluded that based on the information available, no direct relationship between the effects on the maternal thymus and effects on development can be established.

Skeletal malformations were seen in the form of missing bones predominantly at metacarpal no. 5 and proximal phalange no. 3, in the forepaws of foetuses. The most important adverse effects are summarised in the table below. The malformations at metacarpal no. 5, proximal phalange no. 3 and no. 4 were all statistically significantly increased at the mid and high doses in a dose-dependent manner. Skeletal variations in the form of poor or incomplete ossification of sternum no. 5, 6 and metacarpal no. 5 were significantly increased in the high dose group. Additionally, poor and incomplete ossification was also observed in the proximal phalange no. 3 and no. 4 (not shown in the table below), although not in a dose-dependent manner. As suggested by the DS, RAC considered it possible that these skeletal variations may be a milder form of the malformations (missing bones) in the same position.

Table: *Summary of the OECD TG 414 (prenatal development toxicity study; study report 2014)*

Test substance intake	**0 ± 0.0 mg/kg bw/d**	**0.8 ± 0.1 mg/kg bw/d**	**7.2 ± 1.0 mg/kg bw/d**	**22.4 ± 4.2 mg/kg bw/d**
Foetal data				
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)
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)

The lead registrant questioned if the malformations reported were true malformations or limited/absent ossification, which should be considered reversible. The DS explained that based on the staining techniques, no other interpretation is possible. RAC considered the clarification by the DS plausible and therefore interpreted the malformations and skeletal variations as described in the study report and by the DS.

In the combined reproductive screening test by Appel and Waalkens-Berendsen (2004), a non-statistically significant, but high incidence of post-implantation loss was observed (~50% and 70% in the mid and high dose groups, respectively; results summarised in the table below). The lack of statistical significance is likely due to high variation in some animals and a single dam in the control group with only implantation sites, resulting in a high control incidence of post-implantation loss (23%). As noted by the DS, the median values rather than the mean reflect the actual data better because of the high variation in some animals. The median post-implantation loss was 7%, 11%, 50% and 95% in the control, low, mid and high doses, respectively, and thus indicates a dose-response relationship. The post-implantation loss was accompanied by a statistically significant

decrease in live birth index (53% and 60% in mid and high dose groups compared to 99% in the control), followed by a reduction in postnatal viability (PND1-PND4) in the mid- and high dose groups of -22% and -87%, respectively. The pup weight was statistically significantly lower at PND1 in the high dose group (3.9 g vs 4.76 g in control), the number of runts was increased in a non-dose-dependent manner in all dose groups and the number of cold pups was increased in the high dose group (amount not mentioned in the CLH report).

***Table**: Results summary of the Combined reproductive screening test (Appel and Waalkens-Berendsen, 2004)*

Dose level	**Control**	**10 mg/kg diet**	**100 mg/kg diet**	**300 mg/kg diet**
Test substance intake	0 mg/kg bw/d	0.5-0.7 mg/kg bw/d	4.2-6.2 mg/kg bw/d	8.4-17 mg/kg bw/d
Number of pregnant females	7	8	7	8
Mean number of implantations	12.6	13.4	11.3	10.3
Number of dams with only implantation sites observed at necropsy	1	0	0	3
Post-implantation loss (%) Mean value Median value	 22.33 ± 13.16 7	 20.98 ± 7.11 11	 49.23 ± 17.45 50	 69.99 ± 14.71 95[£]
Pups delivered (total) (N)	70	88	72	43
Pups delivered (live + dead mean) [N= number of litters]	11.67 ± 0.80 N=6	11.00 ± 0.71 N=8	10.29 ± 052 N=7	8.60 ± 1.21 N=5
Mean viable litter size PND 1 [N= number of litters]	11.50 ± 0.72 N=6	10.50 ± 0.95 N=8	7.60 ± 1.63 N=5	6.50 ± 2.22 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 ± 0.60 N=6	11.00 ± 0.79 N=7	9.33 ± 0.67 N=3	3.00 ± 0.00 N=1
Pup weight (g) PND 1 (all viable pups)	4.76 ± 0.23	4.74 ± 0.23	4.19 ± 0.35 (-12%)	3.90 ± 0.09 (-18%)
Pup weight gain (g) PND 1 to PND 4	2.17 ± 0.26	1.86 ± 0.38	1.41 ± 0.58	-0.57 ± 0.00
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 was observed in the form of lower body and thymus weight compared to the controls. The maternal body weight was 16% lower at GD21 and 20% lower at PND4 in the high dose group compared to the control. No corrected body weights were provided in the report. However, RAC notes that the lower body weights in the high dose groups were at least in part due to the high post-implantation losses and the reduced pups/foetal weights. Moreover, no significant lower maternal body weight was observed at the mid dose group while the median post-implantation loss and decreased live birth index were already statistically significantly increased at this dose level. RAC concludes that the effects seen in the mid and high doses are not secondary to effects on maternal body weight or body weight gain.

Thymus weights of parental animals were significantly lower compared to the control animals and were accompanied by significant lymphoid depletion in both sexes. 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 other treatment related clinical effects: thin, pale appearance, piloerection and/or blepharospasm. For the majority of these dams, there was no correlation between onset of clinical signs and intrauterine or postnatal death of pups.

Based on the information available, no link between thymus toxicity and reproductive effects can be established. As mentioned, the developmental effects were not secondary to effects on maternal body weight and weight gain. Therefore, RAC concluded that the adverse effects on development in the combined reproductive screening test are relevant

for classification.

The third study summarised by the DS was an EOGRTS similar to OECD TG 443 (Tonk *et al*., 2011). However, the animals were dosed at low concentrations that resulted in limited post-implantation loss (non-significant increase) and postnatal viability (small but significant increase). It is to be noted that the highest dose level in the EOGRTS (1.7-2.1 mg/kg bw/d) was lower than the mid dose group in the reproduction screening study, where also an increase in post-implantation loss was seen. The EOGRTS focused on developmental immunotoxicity and no maternal toxicity was measured up to the highest dose group (1.7- 2.1 mg DOTC kg bw/d). Notably, maternal toxicity was not observed other than adverse behaviour. In addition, the dose spacing was rather narrow, which might have affected the detection of a dose response relationship. In view of the low dose levels, no meaningful conclusions on fertility or development can be drawn. Effects on the developing immune system observed included changes of thymus weight and immunologic cell populations in the pups. Significant changes in immunologic cell populations and thymus weight were observed at the highest dose only, which corresponds to 1.7-2.1 mg/kg bw/d during gestation and 2.9-5.2 mg/kg bw/d during lactation. The delayed type hypersensitivity (DTH) response, evaluated at PND 49, was increased in all dose groups with statistical significance in the low- and high dose groups. The increased DTH response and lower thymus weight in the pups at dose levels up to 5.2 mg/kg bw/d confirm adverse effects on the immune system also in developing animals. At slightly higher dose levels (4.2-6.2 and 7.2 mg/kg bw/d), effects on thymus weights were also observed in some maternal animals of the reproductive screening study and the prenatal developmental study. Based on the available information, RAC agrees with the DS that the pups may be more sensitive compared to parental animals, but the available study is not robust enough for definite conclusions. Thus, the effects on the developing immune system in this study are supportive, but not clear evidence for effects on development.

Comparison with the criteria

Clear adverse effects on development were observed in the prenatal development study and combined reproductive screening study on DOTC.

These adverse effects are:

- Skeletal malformations (missing bones, dose-dependent) at the mid- and high dose groups in the absence of significant maternal toxicity (mid dose group)
- Statistically significantly reduced pup viability and increased post-implantation loss in the mid- and high dose groups following a dose-dependent manner with significant maternal toxicity (reduction of body weight) only at the highest dose tested.

Further effects observed that can be considered supportive include reduced ossification in partially the same position as the missing bones (at lower concentrations), limited increased post-implantation loss and postnatal viability and increased DTH response in the EOGRTS, as well as reduced pup weight, increased runts (not dose-dependent) and cold pups in the combined reproductive screening test. RAC was of the opinion that these effects warrant classification as Repr. 1B; H360D.

Lactation

RAC agreed with the DS that no effects were observed that can be solely attributed to

exposure via lactation. Therefore, no classification for reproductive effects via lactation is warranted.

Conclusion

RAC is of the opinion that DOTL should be classified as Repr. 1B; H360D based on read across from DOTC, where clear adverse effects on development were seen in the prenatal development toxicity study (Study report, 2014) and combined reproductive screening study (Appel and Waalkens-Berendsen, 2004).

Information on other related dioctyltin chemicals also support classification for development. The potency of DOTL may be different from DOTC, e.g. because of differences in the amount of dimer formed and difference in molecular weight. Therefore, RAC considered that an SCL is not justified.

In conclusion, RAC is of the opinion that **classification of Repr. 1B; H360D for dioctyltin dilaurate is warranted, without a specific concentration limit.**

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 ≤ 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.

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

Summary of the Dossier Submitter's proposal

The DS proposed to classify DOTL as STOT RE 1; H373 (immune system) based on read across from DOTC since no substance specific information on adverse effects after repeated dosing is available. DOTC has a harmonised classification as STOT RE 1 translated from the R48 classification in Directive 67/548/EEC (ATP 30, August 2008). To support the proposal, the DS summarised the same three studies that were also used as evidence for classification of reproductive toxicity. In addition, a few older repeated dose studies were summarised in the CLH proposal for effects after repeated exposure. All of these studies were carried out with DOTC.

In the combined developmental and sub-chronic toxicity study by Appel and Waalkens-Berendsen (2004), treatment related changes were seen including decreased

haemoglobin, packed cell volume, total white blood cells, absolute number of lymphocytes and an increase in prothrombin time that was considered biologically relevant at the highest dose (8.4-17 mg/kg bw/d). A dose-dependent decrease in absolute and relative thymus weights (-14% to -73%) were observed at all dose levels, being statistically significant in the mid- and high-dose levels. These effects were correlated with histopathological findings, including a high incidence of lymphoid depletion (mid/high dose groups) and decreased size of thymic lobules resembling thymus atrophy as described in the literature for organotin compounds. A non-statistically significant lymphoid depletion was also observed at the low dose in females, indicating a toxicologically relevant treatment related effect. Similarly, 7/25 dams from the developmental toxicity study in the 7.2 and 22 mg/kg bw/d dose groups had reduced thymus sizes.

In the EOGRTS, F1 animals had reduced absolute and relative thymus weights. In addition, thymus cellularity was significantly reduced at around 3 mg/kg bw/d on PND42. However, no difference was seen at PND70. Statistically significant effects on lymphocyte subpopulations were seen in the thymus on PND42, and on PND70 but these were not statistically significant, and in the spleen on PND42 and PND70. Adverse effects were further investigated by assessing the T-cell dependent antibody response to Keyhole Limpet haemocyanin (KLH) following subcutaneous immunizations with KLH on PND21 and PND35. The DTH was evaluated on PND49. The DTH response was increased in all dose groups, with statistically significant differences in the low and high dose groups.

A few older studies were also mentioned, that indicate effects on the immune system in rats, including lymphocyte depletion and thymus atrophy after exposure to DOTC. A 14-d repeated dosing study (Penninks and Seinen, 1982) caused reduced thymus and spleen weights with reductions up to -70% in thymus weights. Lymphocyte depletion was also observed in the thymic cortex and splenic periarteriolar lymphocyte sheets.

Upon a request during public consultation, the DS provided an overview with the adverse effects considered to fall within the criteria for STOT RE 1 **in bold**:

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

- Decreased absolute and relative thymus weights at 0.5-0.7, **4.2-6.8 and 8.4-17 mg/kg bw/d** in males **(-47/-48% and -75/-73%**), in pregnant females (-23/-24%, **-38/-33% and -69/-62%)** compared to control.
- Decreased relative thymus weight in females in all treated groups in a dose-dependent manner: at 0.7 (-14%), 6.5-6.8 (-69%) and 19.3-19.8 mg/kg bw/d (-70%) compared to control.
- Increased incidence of lymphoid depletion at **0.5-0.7, 4.2-6.8 and 8.4-17 mg/kg bw/d** in males (mid dose **5/10** slight-moderate **and 9/10** moderate-severe**)** in pregnant females **(5/10**, **10/10,** slight-very severe **and 9/10**, slight-very severe, respectively) compared to control.
- Increased incidence of lymphoid depletion in females: at **6.5-6.8 (10/10** slight-very severe**)** and 19.3-19.8 mg/kg bw/d (9/10, slight-very severe) compared to control.

Repeated dose 14-d oral toxicity study in young male rats (Penninks & Seinen, 1982): (note that concentrations were recalculated to 90-d equivalent doses)

- The relative weights of lymphoid organs (thymus and spleen) were decreased in a dose-related manner at 50 and 150 ppm DOTC in the diet (estimated to be **6 and 18 mg/kg bw/d** using a default subacute conversion factor). The decrease in thymus weight was the more pronounced and amounted to more than 70% in the 150 ppm group.
- Lymphocyte depletion was the most prominent histopathological feature seen in all treated animals, particularly in the thymic cortex, but also in the splenic periarteriolar lymphocyte sheets.

Repeated dose 6-week oral toxicity study in male and female rats, 4-week study in male rats, and a time-response study up to 28 days in female rats (Seinen and Willems, 1976): (note that concentrations were recalculated to 90-d equivalent doses)

- Thymic atrophy and lymphocyte depletion at **6 mg/kg bw/d** and at 18 mg/kg bw/d. All DOTC-fed animals showed atrophy of the thymus.
- At 18 mg/kg bw/d, the cortex was almost completely depleted of lymphocytes. At **6 mg/kg bw/d**, lymphocytes depletion of the thymus was less pronounced.
- Decreased thymus weight: -51/-67% and -73%/-75% at **6** and 18 **mg/kg bw/d** respectively, in males/females.
- Total thymocyte counts diminished to 33% and 6% of the control value at week 4 in animals dosed with **6** and 18 **mg/kg bw/d** DOTC, respectively.
- Thymus cell viability was significantly decreased at day 14 in the 18 mg/kg bw/d group ($p < 0.05$) and at day 28 at both **6** and 18 **mg/kg bw/d** ($p < 0.001$).

OECD TG 414 Developmental toxicity study in rats (Study report, 2014):

- Decreased thymus size at 7.2 mg/kg bw/d (7 of 25 females) and at 22.4 mg/kg bw/d (all females).

Similar to OECD TG 443 – Extended one-generation reproductive toxicity study in rats (Tonk *et al*., 2011):

- Decreased absolute (-22%, $p<0.05$) and relative (-20%, $p<0.05$) thymus weight and thymus cellularity (-36%, $p <0.05$) in F1 (**1.7-5.2 mg/kg bw/d**) on PND 42 compared to control.

The DS concluded that adverse effects on the immune system, predominantly lymphoid depletion and reduced thymus sizes, were seen with DOTC at dose levels well below the guidance values for STOT RE 1, supporting classification in STOT RE 1 for DOTL. Regarding possible potency differences, the DS noted that adverse effects are already observed at dose levels around 0.5-0.7 mg/kg bw/d of DOTC in the 90-d sub-chronic (combined) toxicity study. Based on 20% absorption after oral administration, it may be assumed that almost 20% of the distannoxane dimer is absorbed since this is the dominant (90%) hydrolysis product indicated by gastric simulation. This same dimer is formed at a smaller fraction of around 15% (and thus ~6x lower) after hydrolysis at acidic pH of DOTL. If, hypothetically, this would be the only toxic tin moiety that is

bioavailable for DOTL, an almost 10-fold higher dose (corrected for differences in molecular weight, 743 g/mol for DOTL and 416 g/mol for DOTC) is required to obtain the same adverse effects as for DOTC. This would still be below the cut-off criteria for STOT RE 1 (<10 mg/kg bw/d).

No specific route is proposed, since effects cannot be excluded after exposure via other routes. In consideration of the effects on the immune system and potential potency differences, the DS proposes to classify DOTL as STOT RE 1; H372.

Comments received during public consultation

Four MSCAs supported the read across proposal of DOTC to DOTL and three of them supported the proposed classification as STOT RE 1, H372 based on the data with DOTC. The fourth MSCA also supported classification for STOT RE but noted there may be a potency difference between DOTL and DOTC since the hydrolysis products are predominantly different

One MSCA noted that the LOAEL of 0.7 mg/kg bw/d was based on a limited effect (reduced thymus weight of 14%) and therefore may not be enough for classification as STOT RE 1 when DOTL is considered to have a < 10-fold lower bioavailability. In response, the DS provided an overview with the key and supportive effects including the dose levels.

As mentioned in the general section, the lead registrant, supported by a single individual and three companies, questioned the proposed read across and therefore disagreed with the proposed classification for STOT RE.

Assessment and comparison with the classification criteria

RAC accepted the read across proposal for systemic effects after oral administration and therefore relevant information with DOTC can be used to classify DOTL.

The effects observed on the immune system are considered sufficiently severe to fulfil the classification criteria for STOT RE. These effects (thymus atrophy and lymphoid depletion) occur at concentrations below 10 mg/kg bw/d and some effects considered as sufficiently severe were observed already at concentrations <1 mg/kg bw/d.

Overall, RAC agreed with the DS arguments regarding the potency of DOTC and DOTL. As noted by the DS, in the event that the dimer is the only toxic component of DOTL, this will result in an about 10-fold decrease in potency as compared to DOTC, which still falls within the guidance values for STOT RE 1. Further, RAC acknowledged there are some uncertainties about the impact of the other hydrolysis products on the toxicity, as well as on the bioavailability of the dimer and other toxic products. Considering all available information, RAC is of the opinion that the effects seen on the immune system for closely related compounds justify classification of DOTL as STOT RE. RAC considers Category 1 most appropriate in view of the high potency of DOTC and other organotin compounds and the fact that even a 10-fold lower potency for DOTL would still result in the same classification. RAC considers the information as insufficient for derivation of an SCL for DOTL.

Therefore, RAC concludes that **classification of dioctyltin dilaurate as STOT RE 1; H372 (immune system) is warranted.**

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.

Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

[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**

Contact details for dossier submitter:

Swedish Chemicals Agency

Esplanaden 3a, P.O Box 2

SE-172 13 Sundbyberg, Sweden

kemi@kemi.se

+46 8 519 41 100

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1 PHYSICAL HAZARDS

1.1 Explosives

Not evaluated in this CLH Report.

1.2 Flammable gases (including chemically unstable gases)

Not evaluated in this CLH Report.

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Not evaluated in this CLH Report.

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Not evaluated in this CLH Report.

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Not evaluated in this CLH Report.

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Not evaluated in this CLH Report.

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Not evaluated in this CLH Report.

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Not evaluated in this CLH Report.

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Not evaluated in this CLH Report.

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Not evaluated in this CLH Report.

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Not evaluated in this CLH Report.

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Not evaluated in this CLH Report.

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Not evaluated in this CLH Report.

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Not evaluated in this CLH Report.

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Not evaluated in this CLH Report.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1 Toxicokinetics in rats

Reference	Penninks, A. H.; Hilgers, L.; Seinen, W. (1987). The absorption, tissue distribution and excretion of di-n-octyltin dichloride in rats. Toxicology **44**, 107-120.
Guideline	No guideline followed
Reliability	Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)
Species / strain	Rat (Wistar)
Test material	**Dioctyltin dichloride (DOTC)** CAS 3542-36-7 EC 222-583-2 Purity > 98%
Study design	The absorption, tissue distribution and excretion of DOTC were investigated in Wistar rats after oral and intravenous administration of [^{14}C]DOTC. Following a single i.v. administration with 1,2 mg [^{14}C]DOTC/kg bw or after oral administration with 6,3 mg [^{14}C]DOTC/kg bw, rats were terminated at time points of 1-7 days and blood, organs and tissues were analysed for radioactivity. Following a single i.v. or oral dose of 1,2 and 2 mg [^{14}C]DOTC/kg bw, respectively, the excretion of radioactivity in feces and urine was also determined.
Findings	The highest amount of radioactivity was found in 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 DOT toxicity in rats. The tissue radioactivity was 3-4 times higher after i.v. administration with 1,2 mg [^{14}C]DOTC/kg bw than after oral administration with 6,3 mg [^{14}C]DOTC/kg bw, but the relative accumulation of radioactivity between organs/tissues was indenpendant of administration route. The radioactivity declined time-dependantly in all organs and tissues except in kidney where the activity remained constant during the 7 days experimental period. Absorption was calculated to be approximately 20% of the oral dose. <u>Tissue (radioactivity, dpm/mg tissue, oral administration) day 1:</u> Liver (679), kidneys (144), adrenals (103), pituitary gland (95), thyroid gland (86), spleen (75), peripheral lymph nodes (64), lungs (50), pancreas (41), heart (38), submaxillary glands (31), epididymal adipose (31), parotid gland (30), perirenal adipose (27), inguinal adipose (25), thymus (23), skeletal muscle (20), testis (14), blood (13), brain (6). <u>Tissue (radioactivity, dpm/mg tissue, i.v. administration) day 1:</u>

Liver (2598), kidneys (831), adrenals (510), spleen (300), thyroid gland (243), pituitary gland (232), submaxillary glands (221), peripheral lymph nodes (217), heart (213), lungs (182), pancreas (156), parotid gland (106), perirenal adipose (94), epidydimal adipose (93), skeletal muscle (86), inguinal adipose (83), thymus (66), testis (48), blood (34), brain (26).

In the excretion studies, 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 feces were separately collected for 25 days. Following i.v. administration, most of the radioactivity was excreted in feces and characterized by a biphasic excretion pattern. The first 4 days showed an increase in radioactivity excretion, while from day 5 the excretion gradually declined with a halflife of 8.3 days. The urinary excretion of radioactivity appeared to be independent of the body burden since the daily excretion was nearly constant during 25 days. From the orally administered dose, more than 80% was excreted in the feces the first 2 days which is in accordance to the higher tissue radioactivity after i.v. administration. From day 3, the excretion in feces followed first order kinetics with a halflife of 8,9 days. Also for oral administration, the urinary excretion of radioactivity appeared to be independent of the body burden.

Conclusion The results of this study show that DOTC distributes to various tissues after intravenous or oral administration, with an absorption of approximately 20% of the oral dose. The highest concentrations are found in liver and kidney, while DOTC concentrations in thymus are much lower. It was concluded that the selective thymus effects of DOTC can not be correlated with a specific distribution to this organ.

2.1.2 Toxicokinetics in rats

Reference Study report, Anonymous (1987). Summary included in the publically disseminated REACH Registration Dossier for dichlorodioctylstannane (ECHA, 2016a).

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions according to Registrant(s).

Species / strain Rat (Wistar), female

Test material Dichlorodioctylstannane (dioctyltin dichloride, DOTC)

CAS 3542-36-7

EC 222-583-2

Purity not specified

Study design The distribution of DOTC were investigated in Wistar rats after oral administration of radiolabelled DOTC (^{113}Sn). Following a single administration with 25 mg DOTC/kg bw in peanut oil, the proportions in blood and organs were measured up to 72h after exposure.

Findings The proportions of concentrations (in ng DOTC-equivalents/g tissue) at 1 h post administration were as follows:

blood (1) < kidneys (1.7) < brain (2.6) < thymus (5.3) < liver (21.1)

and at 24 h post administration:

brain (1) < blood (2.9) < thymus (4.5) < kidneys (32.5) < liver (131.3).

Most of the ^{113}Sn_DOTC was found in the liver, where at 1 h post administration 0.2 % and after 24 h 1.2 % of the initial dose was measured. The activity in liver and all other organs except for brain did not decrease within 72 h. The blood levels were comparatively low but still increasing up to the last measurement in blood at 24 h. An evaluation of the time dependencies as e.g. DOTC showed a slight affinity to the corpuscular compartments of blood: the solid/liquid partition factor, calculated for three points of measure, was about 2 and seemed to decrease slowly.

Half-life of the substance was calculated to be 67 h.

Conclusion The results of this study show that DOTC distributes to various tissues after oral administration. The highest concentrations (at 24 h post administration) are found in liver and kidney, while DOTC

concentrations in thymus are lower.

2.1.3 Simulated gastric hydrolysis

Reference Naßhan H (2015).
Dioctyltin dilaurate [DOTL] CAS number: 3648-18-8. In-vitro Metabolism Study
Galata Chemicals GmbH, Chemiestrasse 22, 68623 Lampertheim, Germany

Guideline None followed

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

Species / strain Not relevant: *in vitro* study

Test material Dioctyltin dilaurate
CAS 3648-18-8
EC 222-883-3
Purity >90 %
(Test material as cited in the study report. The study owner confirmed that the test material corresponds to the industrially manufactured UVCB substance.)

Study design Simulated gastric hydrolysis studies were performed using dioctyltin dilaurate.
The extent of hydrolysis was assessed under low pH (1.2) conditions at 37°C at a concentration of 13.4 mM in aqueous HCl. The degree of hydrolysis was measured after 0.5h and 4h respectively, after extraction in hexane and subsequent ^{119}Sn NMR analysis in toluene-d^8 which allowed positive identification of the hydrolysis product(s). Any remaining tin-residues in the aqueous phase (decomposition products and/or water soluble substances) was analysed by atomic absorption spectrometry (AAS).

Findings Simulated gastric hydrolysis studies demonstrate that dioctyltin dilaurate rapidly hydrolyses at low pH. Three major products were observed after 0.5h and assigned to $ClOct_2SnOSnOct_2Cl$ (14%, ^{119}Sn-NMR: δ (ppm) -92, -145), DOTLC - a mono-chloride mono-carboxylate species (43%, ^{119}Sn-NMR: δ (ppm) -35), and a non-assigned tin-species (43%, ^{119}Sn-NMR: δ (ppm) -150, broad peak). Only minor changes in product composition were observed from 0.5h to 4h.
The non-assigned tin-species can be associated to some polymeric structure distinct from DOTO (dioctyltinoxide). The recativity of the non-assigned tin-species was further analysed by addition of excess DOTC to the organic solvent, the non-assigned tin-species instantly formed DOTLC.
Only trace amounts of tin-residues were observed in the remaining water fractions.

Hydrolysis of DOTL

Time	DOTLC (mol %)	$ClOct_2SnOSnOct_2Cl$ (mol %)	Non-assigned tin-species (mol %)
0.5 h	43 %	14 %	43 %
4 h	47 %	16 %	38 %

Conclusion Dioctyltin dilaurate is shown to be rapidly hydrolysed under conditions representative of the mammalian stomach to form three major products. The complex chemistry observed may be expected due the coordinating carboxylate ligands which can bind to the tin moiety in various ways (e.g. monodentate, bidentate, bridging). The generation of a common intermediate, identical to the major product observed upon hydrolysis of dioctyltin dichloride (see 2.1.4) supports the analogue

approach for read-across from dioctyltin dichloride to dioctyltin dilaurate.

2.1.4 Simulated gastric hydrolysis

Reference Naßhan H (2016).
Dioctyltin dichloride [DOTC] CAS number: 3542-36-7. In-vitro Metabolism Study
Galata Chemicals GmbH, Chemiestrasse 22, 68623 Lampertheim, Germany

Guideline None followed

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

Species / strain Not relevant: *in vitro* study

Test material Dioctyltin dichloride
CAS 3542-36-7
EC 222-583-2
Purity >95 %

Study design Simulated gastric hydrolysis studies were performed using dioctyltin dichloride.
The extent of hydrolysis was assessed under low pH (1.2) conditions at 37°C at a concentration of 24.0 mM in aqueous HCl. The degree of hydrolysis was measured after 30 s, 1 h, and 4 h respectively, after extraction in hexane and subsequent ^{119}Sn NMR analysis in toluene-d^8 which allowed positive identification of the hydrolysis product.

Findings Simulated gastric hydrolysis studies demonstrate that dioctyltin dichloride rapidly forms the dimeric stannoxane $ClOct_2SnOSnOct_2Cl$ (^{119}Sn-NMR: δ (ppm) -92, -145) as the only observed hydrolysis product when exposed to conditions representative of the mammalian stomach. Small amounts (~10 mol%) of non-hydrolyzed DOTC remains after 4 hours. The recovery rate of organotins, defined by the isolated mass after extraction vs. the mass of the test sample was determined to 91-101%.

Conversion of DOTC to $ClOct_2SnOSnOct_2Cl$

Time	DOTC (mol %)	$ClOct_2SnOSnOct_2Cl$ (mol %)
30 s	46	54
1 h	23	77
4 h	10	90

Conclusion Dioctyltin dichloride is shown to be rapidly converted to $ClOct_2SnOSnOct_2Cl$ under conditions representative of the mammalian stomach. The generation of a common intermediate, identical to one of the hydrolysis products of dioctyltin dilaurate (see 2.1.3), supports the analogue approach for read-across from DOTC to dioctyltin dilaurate.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

Not evaluated in this CLH Report.

3.2 Acute toxicity - dermal route

Not evaluated in this CLH Report.

3.3 Acute toxicity - inhalation route

Not evaluated in this CLH Report.

3.4 Skin corrosion/irritation

Not evaluated in this CLH Report.

3.5 Serious eye damage/eye irritation

Not evaluated in this CLH Report.

3.6 Respiratory sensitisation

Not evaluated in this CLH Report.

3.7 Skin sensitisation

Not evaluated in this CLH Report.

3.8 Germ cell mutagenicity

Not evaluated in this CLH Report.

3.9 Carcinogenicity

Not evaluated in this CLH Report.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Reproduction/developmental toxicity screening study in the rat

Reference Appel MJ and Waalkens-Berendsen DH. (2004). Dichlorodioctylstannane [CASRN # 3542-36-7]: Sub-chronic (13 week) oral toxicity study in rats, including a reproduction/developmental screening study. Testing laboratory: TNO Nutrition and Food Research. Report no.: V3964. Owner company: ORTEP. Report date: 2004-04-01.

Guideline OECD 421 (Reproduction/Developmental Toxicity Screeining Test)

Reliability Klimisch 1: reliable without restriction (guideline-compliant study with no or minor deviations not affecting the quality of the results, GLP-compliant study with certificate), according to Registrant(s).

Species / strain Rat (Wistar)

Test material Dichlorodioctylstannane (dioctyltin dichloride, DOTC)

CAS 3542-36-7

EC 222-583-2

Purity 92.1 %

Study design The repeated dose toxicity of the test material was studied using continuous administration via the diet for 13 consecutive weeks according to OECD 408 (study summarised in section 3.12.1.1). In satellite groups of female rats a reproduction/developmental screening test was performed according to OECD 421. The main 13-week study used four groups of 10 rats/sex and the satellite reproduction/developmental screening study used four groups of 10 female rats. For both studies the control group was kept on untreated diet and the three test groups received diets containing 10, 100 and 300 mg/kg of the test material.

In the satellite study administration of female rats started two weeks prior to the mating period and continued through mating, gestation, and up to PN 4 or shortly thereafter. After a premating period of 10 weeks, male rats from the main study were mated with female rats of the satellite groups, which were administered the same dose of test diet.

The study summary continued below refers to the satellite study, i.e. OECD 421 (Reproduction/Developmental Toxicity Screeining Test).

Animals were observed daily for mortality and, if necessary, clinical signs. Body weights were measured pre-test (day -5), on gestation day 0, 7, 14 and 21 and on postnatal day 1 and 4. All animals were weighed on the day of necropsy in order to determine their correct organ to body weight ratios. This was in addition to a weekly measurement being taken throughout the study.

The numbers of females placed with males, males mated with females, successful copulations, males that became sire and pregnant females were noted, as was pre-coital time. At the end of the gestation period, females were examined twice daily for signs of parturition. Any difficulties occurring during parturition were recorded.

At necropsy, ovaries, uterus (after counting of the implantation sites), thymus and gross lesions from all females were weighed, and samples of the organs were preserved. Microscopic examination of the thymus was performed. The ovaries and uterus of the females of the control and 300 mg/kg were microscopically examined. Furthermore, the reproductive organs of the males of the 10 and 100 mg/kg groups that failed to sire (did not mate or female was not pregnant) and the reproductive organs of females of the 10 and 100 mg/kg groups that were non-mated or non-pregnant were microscopically examined.

The total litter size and numbers of each sex as well as the number of stillbirths, live and dead pups and grossly malformed pups were evaluated on days 1 and 4 of lactation. The pups were weighed individually and litter weight was calculated for days 1 and 4 of lactation. Mean pup weight was calculated as litter weight/number pups. The number of runts (defined as pup weight less than 2 standard deviations from the litter mean) were noted and reported. A necropsy was performed on stillborn pups and pups dying during the study and macroscopic abnormalities were recorded. Pups were examined externally for gross abnormalities.

Findings **Parental animals *(Note: only maternal data is presented here. Please refer to section 3.12.1.1 for data on males)***

CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS)

No clinical signs were observed during the premating period. During the gestation period piloerection

was observed in animal A115 (GD 21 -24) of the control group and animal D161 (GD 23-24) of the 300 mg/kg group. In addition, blepharospasm was observed in animal D161 (GD 23-24). During lactation piloerection was observed in animal C157 (PND 2-4) of the 100 mg/kg group and D163 (PND 2-4) and D167 (PN 1) of the 300 mg/kg group. Blepharospasm was observed in animal D167 of the 300 mg/kg group. Animals C149 (PND 4-5) and C157 (PND 4) of the 100 mg/kg group and animal D163 (PND 4) of the 300 mg/kg group were considered to be thin. In addition animals C149 and C159 showed a pale appearance. Some animals were sparsely haired during gestation and/or lactation; this finding is normal for this strain.

BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS)

During the premating period no significant differences in mean body weight were observed. Mean body weight change was statistically significantly reduced in the 100 and 300 mg/kg groups during the first week of the premating period. During the gestation period, mean body weight was statistically significantly reduced from GD 7-21 in the 300 mg/kg group. Body weight change was statistically significantly reduced during the entire gestation period. During the lactation period, the mean body weight was statistically significantly reduced in the 300 mg/kg group.

During the premating period, mean food consumption (expressed as g/animal/day and as g/kg body weight/day) of the female animals of the 100 and 300 mg/kg groups was statistically significantly decreased. During the gestation period, food consumption (g/animal/day) of the females of the 100 mg/kg group was statistically significantly decreased from GD 7-14. Mean food consumption of the 300 mg/kg group (expressed as g/animal/day) was statistically significantly decreased during the entire gestation period and as g/kg body weight/day from GD 0-14. During the lactation period food consumption of the female animals of the 300 mg/kg group was statistically significantly decreased.

TEST SUBSTANCE INTAKE (PARENTAL ANIMALS)

The test substance intake of the female animals of the 10, 100, and 300 mg DOTC/kg diet was respectively:

Pre-mating period days 0-7: 0.6, 5.8 and 13.5 mg/kg bw/day

Pre-mating period days 7-14: 0.7, 5.9 and 16.4 mg/kg bw/day

Gestation period days 0-7: 0.7, 6.2 and 17.0 mg/kg bw/day

Gestation period days 7-14: 0.7, 6.2 and 17.0 mg/kg bw/day

Gestation period days 14-21: 0.5, 4.2 and 11.0 mg/kg bw/day

Lactation period days 1-4: 0.7, 5.0 and 8.4 mg/kg bw/day

The overall intake of the test substance for the 10, 100, and 300 mg DOTC/kg diet, respectively was approximately 0.7, 6.5 and 19.3 mg/kg bw/day in males.

FERTILITY, PARTURITION AND SEXUAL FUNCTION (PARENTAL ANIMALS)

Effects on sperm parameters and oestrous cycling were not investigated.

The pre-coital time was not statistically significantly different between groups (2.33, 2.40, 1.50 and 2.80 days in control, 10, 100 and 300 mg DOTC/kg diet respectively).

No effects on gestational length.

Female fecundity index, female fertility index and male fertility index were comparable for the control group and treated groups and ranged between 70-80%. The female mating index was 90% in control and 100% in the treated groups.

The gestation index was 86, 100, 71 and 50% in the control, 10, 100 and 300 mg/kg groups, respectively. The number of females with liveborn pups was 6, 8, 5 and 4 for the control, 10, 100 and 300 mg/kg groups, respectively. The number of females with stillborn pups amounted to 1, 1, 4 and 3 for the control, 10, 100 and 300 mg/kg groups, respectively. The number of females with all stillborn pups was 0, 0, 2 and 1 in the control, 10, 100 and 300 mg/kg groups, respectively.

ORGAN WEIGHTS (PARENTAL ANIMALS)

Absolute and relative uterus and ovary weight were similar in all groups.

The absolute and relative thymus weights were decreased in all treated groups (but only stat. sign. at the

100 and 300 mg DOTC/kg diet) in a dose-dependent manner (-23/-24% (not stat. sign.), -38/-33%, $p<0.05$ and -69/-62%, $p<0.001$ in the low intermediate and high dose groups, respectively).

GROSS PATHOLOGY (PARENTAL ANIMALS)

No effects

HISTOPATHOLOGY (PARENTAL ANIMALS)

Microscopic examination revealed moderate to very severe lymphoid depletion in the thymus, which was considered related to treatment. Lymphoid depletion was characterised by a decrease in the size of the thymic lobules which can be ascribed to extensive loss of cortical en medullary small lymphocytes. Consequently, the distinction between the cortical and medullary areas was blurred. Lymphoid depletion was observed in 5/10 animals of the 10 mg/kg group and in all animals of the 100 and 300 mg/kg groups. Severity score was severe to very severe in all groups. One control animal (A115) also had very severe lymphoid depletion in the thymus. However, this was most probably associated with the fact that this animal was physiologically disturbed, as was demonstrated by 12 resorptions in the uterus and an abnormal kidney (gross changes: flabby and yellow patches). Some 10 mg/kg animals showed thymic involution as a result of pregnancy/lactation. This picture was similar to the thymic pregnancy/lactation involution in control animals and was characterised by a decreased size of thymic lobules exhibiting normal architecture. This phenomenon is a common observation in pregnant or lactating animals. However, the lymphoid depletion in the 10 mg/kg animals was similar to the thymic change in the 100 and 300 mg/kg animals. Therefore, lymphoid depletion in the 10, 100 and 300 mg/kg animals was considered related to treatment with the test substance. The other histopathological changes observed are common findings in rats of this strain and age or occurred in a single animal only.

Table 1: Maternal effects

Dose level	**0 mg/kg diet (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	4.2-5.9 mg/kg bw/day	8.4-17 mg/kg bw/day
Number of pregnant animals	7	8	7	8
Mortalitites	0	0	0	0
Clinical observation during pre-mating	0	0	0	0
Clinical observation during gestation	1/10 piloerection (GD 21-24)	0	0	1/10 piloerection and blepharospasm (GD 23-24)
Clinical observation during lactation	0	0	1/10 piloerection (PND 2-4), thin (PND 4) 1/10 thin and pale appearance (PND 4-5) 1/10 pale appearance (PND 4-5)	1/10 piloerection (PND 2-4) and thin (PND 4) 1/10 piloerection and blepharospasm (PND 1)
Food consumption [g/rat/day] during pre-mating (week 0 - 1)[d]	13.05 ± 0.230	12.79 ± 0.317	11.05 ± 0.134** (-15%)	8.81 ± 0.173** (-33%)
Food consumption [g/rat/day] during pre-mating (week	13.05 ± 0.255	13.20 ± 0.146	11.78 ± 0.177** (-10%)	10.73 ± 0.019** (-18%)

1 - 2) [d]				
Food consumption [g/rat/day] during gestation (d 0-7)[t]	14.37 ± 0.265 (N=6)	15.37 ± 0.431 (N=8)	13.04 ± 0.215 (N=7)	11.09 ± 0.482[#] (-23%) (N=7)
Food consumption [g/rat/day] during gestation (d 7-14)[t]	16.00 ± 0.356 (N=7)	15.82 ± 0.383 (N=8)	14.17 ± 0.418* (-11%) (N=7)	12.05 ± 0.453** (-25%) (N=8)
Food consumption [g/rat/day] during gestation (d 14-21)[t]	10.72 ± 0.802 (N=7)	12.02 ± 0.649 (N=8)	10.27 ± 0.439 (N=7)	8.19 ± 0.535* (-24%) (N=8)
Food consumption [g/rat/day] during lactation (d 1-4) [t]	15.25 ± 1.555 (N=6)	14.63 ± 1.791 (N=8)	9.71 ± 3.059 (-34%) (N=5)	4.92 ± 2.686* (-68%) (N=4)
Body weight [g] pre-mating day 0	197.88 ± 2.036	195.65 ± 2.240	197.91 ± 1.989	198.07 ± 1.953
Body weight [g] pre-mating day 7	202.68 ± 2.414	199.05 ± 2.660	198.19 ± 2.612	194.04 ± 1.794 (-4.4%)
Body weight [g] pre-mating day 14	206.29 ± 2.222	202.99 ± 2.670	201.30 ± 2.455	198.28 ± 2.772 (-3.9%)
Body weight gain [g] pre-mating day 0-7[d]	4.80 ± 1.077	3.40 ± 0.953	0.28 ± 1.399*	-4.03 ± 0.699**
Body weight gain [g] pre-mating day 7-14[d]	3.61 ± 1.019	3.94 ± 1.088	3.11 ± 0.787	4.24 ± 1.417
Body weight [g] GD 0	207.49 ± 3.083	205.95 ± 4.331	201.53 ± 2.823	195.01 ± 2.955
Body weight [g] GD 7	223.31 ±3.352	224.94 ± 4.136	217.77 ± 2.963	207.96 ± 4.490* (-6.9%)
Body weight [g] GD 14	248.07 ± 4.309	245.84 ± 4.234	236.74 ± 3.308	219.35 ± 4.555[#] (-11.6%)
Body weight [g] GD 21[d]	273.30 ± 8.546	275.58 ± 5.872	254.90 ± 4.975	229.39 ± 4.463[#] (-16%)
Body weight gain [g] GD 0-7[d]	15.83 ± 1.113	18.99 ± 1.065	16.24 ± 0.655	10.41 ± 2.149* (-34%)
Body weight gain [g] GD 7-14[d]	24.76 ± 1.532	20.90 ± 0.910	18.97 ± 1.464	11.39 ± 2.418[#] (-54%)
Body weight gain [g] GD 14-21[d]	25.23 ± 6.137	29.74 ± 2.453	18.16 ± 3.196	10.04 ± 3.239[#] (-60%)
Body weight [g] at lactation day 1[d]	200.13 ± 4.043	198.19 ± 5.017	189.18 ± 4.166	164.98 ± 6.147[#] (-18%)
Body weight [g] at lactation day 4	211.65 ± 2.814	212.93 ± 5.479	194.26 ± 6.470	168.85 ±12.768** (-20%)
Body weight gain [g] lactation day 1-4[d]	11.52 ± 3.198	14.74 ± 2.278	5.08 ± 6.072	3.88 ± 11.062
Relative thymus weight [%][d]	0.612 ± 0.0612 (N=6)	0.466 ± 0.0441 (-24%) (N=8)	0.408 ± 0.0511* (-33%) (N=5)	0.231 ± 0.0298[#] (-62%) (N=4)
Lymphoid depletion in thymus (revealed at histopathlogical examination)[f]	1/10 (1 very severe)	5/10 (4 severe, 1 very severe)	10/10*** (5 severe, 5 very severe)	10/10*** (3 severe, 7 very severe)

# p<0.001
* p<0.05
** p<0.01

*** p<0.001
(d) Anova & Dunnett test
(f) Fishers exact test
(t) t-test with Bonferroni correction

Tabel 2: Summary of reproductive data

Dose level	0 mg/kg diet (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	4.2-5.9 mg/kg bw/day	8.4-17 mg/kg bw/day
Number of animals in the study (females + males)	10 + 10	10 + 10	10 + 10	10 +10
Number of mated females	9	10	10	10
Number of pregnant females -Thereof no. with only implantation sites at necropsy -Thereof no. with only stillborn pups	7 1 0	8 0 0	7 0 2	8 3 1
Number of females with liveborn pups	6	8	5	4
Female mating index[1]	90	100	100	100
Female fecundity index[2]	78	80	70	80
Female fertility index[3]	70	80	70	80
Male fertility index[4]	70	80	70	80
Gestation index[5]	86	100	71	50

1) female mating index = number of mated females/number of females placed w males
2) female fecundity index = number of pregnant females/number of females with confirmed mating
3) female fertility index = number of pregnant females/number of females placed with males
4) male fertility index = number of males that become sire/number of males placed with females
5) gestation index = number of females with live born/number of females with evidence of pregnancy

UTERINE OBSERVATIONS

The mean number of implantation sites were reduced in the 100 and 300 mg DOTC/kg diet dose groups (11.3 and 10.3 respectively, not stat. sign. compared to 12.6 in control).

Animal 115 of the control group and animals D161, D171 and D173 of the 300 mg/kg group showed only implantation sites at necropsy.

Mean percentage of incidences of post-implantation loss was 22.3, 21.0, 49.2 and 70.0% for the control, 10, 100 and 300 mg/kg groups, respectively.

VIABILITY (OFFSPRING)

The mean number of pups (live + dead) delivered per litter amounted to 11.7, 11.0, 10.3 and 8.6 or the control, 10, 100 and 300 mg/kg groups, respectively. There was an increased number of stillborn pups in the 100 and 300 mg DOTC/kg diet dose groups (34 and 17, respectively, $p<0.001$, compared to 1 in control) and the live birth index was reduced in the 100 and 300 mg DOTC/kg diet dose groups (99, 95, 53 and 60% in the control, 10, 100 and 300 mg/kg groups, respectively). Pup mortality on PND 1 was 1.4, 4.5, 47, and 40% in the control, 10, 100 and 300 mg/kg groups, respectively. All pups of the following animals died between PND 1-4: B137 of the 10 mg/kg group, C145 and C159 of the 100 mg/kg group and D165, D175 and D177 of the 300 mg/kg group. Pup mortality on PND 4 was 5.8, 8.3, 26 and 88 %. Viability index (PND 1 -4) was 94, 92, 74 and 12% in the control, 10, 100 and 300 mg/kg groups, respectively. The number of live pups per litter on PND 1 amounted to 11.5, 10.5, 7.6, 6.5 for the control, 10, 100 and 300 mg/kg groups, respectively and on PND 4 the number of live pups per litter amounted to 10.8, 11.0, 9.3 and 3.0 for the control, 10, 100 and 300 mg/kg groups, respectively.

No difference was observed in the sex ratio between the groups.

CLINICAL SIGNS (OFFSPRING)

On PND 1 and 4, the number of runts was statistically significantly increased in the 100 and 300 mg/kg groups (1, 7, 10 and 6 respectively in control, 10, 100 and 300 mg DOTC/kg diet). In addition the number of cold pups was increased in the 300 mg/kg group on PND 1.

BODY WEIGHT (OFFSPRING)

Mean pup weight and pup weight change were similar in the 10 and 100 mg/kg groups when compared to the control group. Pup weight of the 300 mg/kg group at PND 1 (3 litters, 3.9 g not stat. sign. compared to 4.76 g in control) and PND 4 (1 litter) was reduced.

SEXUAL MATURATION (OFFSPRING)

Not examined

ORGAN WEIGHTS (OFFSPRING)

Not examined

GROSS PATHOLOGY (OFFSPRING)

Macroscopic observations in stillborn pups and pups that died between PND 1 and 4 revealed no treatment related abnormalities in the pups.

HISTOPATHOLOGY (OFFSPRING)

Not examined

Table 3: Summary of pup data

Dose level	**Control**	**10 mg/kg diet**	**100 mg/kg diet**	**300 mg/kg diet**
Number of pregnant females	7	8	7	8
Mean number of implantations	12.6	13.4	11.3	10.3
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 liveborn pups[f] (Live birth index)	69 99	84 95	38[#] 53	26[#] 60
Total no. of stillborn pups[f] (% stillborn) [N = number of litters]	1 1.4 N=1	4 4.5 N=1	34[#] 47 N=4	17[#] 40 N=3
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, $p<0.01$

Conclusion LOAEL for fertility and developmental effects was 100 mg DOTC/kg diet (equivalent to 6.5 mg/ kg body weight/day in males and 4.2-5.9 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).

3.10.1.2 Prenatal developmental toxicity study in rats

Reference Study report (2014) Prenatal developmental toxicity study of DOTC administered orally in diet to Sprague Dawley rats.

Guideline	OECD 414 (Prenatal Developmental Toxicity Study)
Reliability	Klimisch 1: reliable without restriction (guideline-compliant study, GLP-compliant study with certificate), according to the Registrant(s).
Species / strain	Rat (Sprague Dawley)
Test material	Dichlorodioctylstannane (dioctyltin dichloride, DOTC) CAS 3542-36-7 EC 222-583-2 Purity 97.7 %
Study design	Groups of 25 mated females were administered the test material in the diet at concentrations of 0, 10, 100 or 300 mg/kg from gestation day (GD) 5 to 19. Animals were observed once daily for clinical signs of toxicity and twice daily for mortality/morbidity. Individual animal body weight was taken on GD 0, 3, 5, 8, 11, 14, 17, 19 and 20 (day of caesarean section). All surviving animals were subjected to detailed necropsy on the day of caesarean section (GD 20). The ovaries, uterus, thymus, spleen and liver were collected and preserved. and gross lesions from all females were weighed, and samples of the organs were preserved. The weight of the gravid uterus including cervix was recorded for each pregnant female. The foetuses were taken out and femals were sublected to macroscopic examination including numbers of corpora lutea, implantations, live and dead foetuses, and early and late resorptions. All foetuses were examined for sex, weight, external appearance (including oral cavity), and external anomalies. Approximately one half of live foetuses from each litter were examined for skeletal alterations, and the other half for visceral alterations.
Findings	PREGNANCY DATA A total number of 22, 21, 20 and 20 mated females were confirmed with pregnancy at a pregnancy rate of 88%, 84%, 80% and 80% at the time of caesarean section at 0, 10, 100 and 300 ppm respectively. MATERNAL DATA <u>General Tolerability</u> No deaths and abortions were noted during the experimental period. <u>Body weight, Body Weight Change and Corrected body weight</u> There was a statistically significant decrease in maternal body weight for the period of gestation days (GD) 17-20 and maternal body weight change for the periods GD 5-8, GD 11-14, GD 14-17 and GD 17-19 for dams in the 300 ppm group was noted. There were no statistically significant decreases in maternal body weight and maternal body weight change for dams in the 10 or 100 ppm dose groups on any gestation day when compared to control dams. No statistically significant decrease in body weight change GD 5-20 for dams in the 10 ppm and 100 ppm groups compared to controls was noted. A statistically significant decrease in body weight change GD 5-20 for dams in the 100 ppm (11.9%) and in 300 ppm (30.8%) groups as compared with controls was noted. The corrected body weight change and the percent change were similar to controls in the 10 and 100 ppm groups. In the 300 ppm group the corrected body weight change and the percent change (75.6%) were both statistically significantly decreased compared to controls. Gravid uterine weight was similar to controls at all doses. <u>Feed Consumption and Test Article Consumption</u> There were no treatment related differences in average feed consumption at any of the tested dose. Test Article consumption for each dose was calculated as 0.8, 7.2 and 22.4 mg/kg/day for the low, mid and high dose groups, respectively. <u>Gross Pathology</u> Macroscopic observations of reduced size of thymus in 7 of 25 females at 100 ppm and in all females (25 of 25) at 300 ppm were observed. These observations were judged to be treatment-related. No gross pathological observations were noted in 10 ppm animals.

MATERNAL DATA (UTERINE OBSERVATIONS)

No treatment related differences in mean gravid uterus weight, number of corpora lutea per dam, number of implantation sites per dam, incidence of early and late resorptions, number of dead and live fetuses, pre and post-implantation losses and male/female sex ratio were noted at all the doses.

The occurrence of early resorptions at 100 ppm, late resorptions at 10 and 300 ppm, pre-implantation loss at 300 ppm and post-implantation loss at 10, 100 and 300 ppm were judged as incidental and not treatment related.

Number of abortions

No effects observed

Pre-Implantation Loss

No treatment-related pre-implantation loss was noted across all doses when compared to the vehicle control. The statistically significant difference in this parameter for the 300 ppm group was attributed to two dams [Ra6262 and Ra6270] which had pre-implantation losses of 44.4% and 60.4%, respectively. In the 100 ppm group two dams [Ra6248 and Ra6249] were noted with a pre-implantation loss of 45.5% and 62.5% respectively. These occurrences were judged as incidental and not treatment related.

Post-Implantation Loss

No statistically significant difference in the percentage of post-implantation loss was observed for any dose group when compared to vehicle controls.

The observed post-implantation losses were 6.9%, 4.9% and 6.9% in the 10, 100, and 300 ppm groups, respectively versus 0.8% in the vehicle control.

Total litter losses by resorption

No effects observed

Effects on pregnancy duration

No effects observed

Early Resorptions

The incidence of early resorptions was statistically significantly increased at the mid dose compared to the vehicle control. This was judged to be an incidental occurrence. One dam (Animal No. Ra6232) in this group had 4 early resorptions compared to a maximum of 2 early resorptions in any dam. In addition the incidence of early resorptions did not demonstrate a dose-response.

Late Resorptions

No statistically significant differences in the number of late resorptions per dam were observed across groups.

Dead fetuses

No dead fetuses were observed in the 100 or 300 ppm groups or in the controls. In the 10 ppm group, 2 dead fetuses were observed in a single litter.

Changes in pregnancy duration

No effects observed

Changes in number of pregnant

No effects observed

Other effects

No effects observed

FETAL DATA

Fetal Sex Ratio, Average Fetal Weight and Average Crown Rump Length

No treatment related effects on the fetal sex ratio, average fetal weight and average crown-rump length were noted at any of the dose.

External Examination

No gross external abnormalities were noted within any group after external examination of fetuses.

Visceral Examination

Malformations: Lateral ventricular dilation of the 3rd ventricle of the brain. No dose-response.

Variations: Abnormal liver lobation and renal pelvis dilation. No dose-response.

The noted observations, abnormal liver lobation and dilation of the renal pelvis, are common findings for rat fetuses and were judged as incidental occurrences.

Skeletal Examination

Malformations:

Statistically significant and treatment related increases were observed in the percentage of malformations of missing metacarpal No. 5 (11.4 and 34.6 % at 100 and 300 mg/kg, respectively, as compared to 0.8 % in the control), proximal phalanx No. 3 bilateral (14.3 and 28.0 % at 100 and 300 mg/kg, respectively, as compared to 0.8 % in the control) and proximal phalanx No. 4 (13.3 and 27.1 % at 100 and 300 mg/kg, respectively, as compared to 0.8 % in the control).

Observations of skeletal malformations were:

- 1 incidence in 132 foetuses (1 of 22 litters affected) at 0 mg DOTC/kg diet
- 11 incidences in 115 foetuses (8 of 21 litters affected) at 10 mg DOTC/kg diet
- 22 incidences in 105 foetuses (11 of 20 litters affected) at 100 mg DOTC/kg diet
- 47 incidences in 107 foetuses (19 of 20 litters affected) at 300 mg DOTC/kg diet

Split thoracic vertebra centrum no. 12 was noted as a single occurrence in a single litter at 10 ppm.

Missing caudal vertebral arch no. 2 on both sides was noted in 2 litters (2 fetuses) at 10 ppm and 2 litters (3 fetuses) at 300 ppm. Both these observations were judged to be incidental occurrence.

Variations:

Statistically significant and treatment related increases were observed in the percentage variations of poor ossification of sternum No. 5 (6.5 % at 300 mg/kg as compared to 0 % in the control) and sternum No. 6 (14.0 % at 300 mg/kg as compared to 0 % in the control). A dose dependent and treatment related increase in poor ossification of metacarpal No. 5 was observed (1.0 and 3.7 % at 100 and 300 mg/kg, respectively, as compared to 0 % in the control).

Observations of skeletal variations were:

- 6 incidences in 132 foetuses (5 of 22 litters affected) at 0 DOTC/kg diet
- 11 incidences in 115 foetuses (7 of 21 litters affected) at 10 DOTC/kg diet
- 10 incidences in 105 foetuses (4 of 20 litters affected) at 100 DOTC/kg diet
- 26 incidences in 107 foetuses (12 of 20 litters affected) at 300 DOTC/kg diet

Table 4: Main maternal and developmental effects

Nominal dose in the diet (Actual test substance intake)	**0 ppm** **(0 mg/kg/d)**	**10 ppm** **(0.8 mg/kg/d)**	**100 ppm** **(7.2 mg/kg/d)**	**300 ppm** **(22.4 mg/kg/d)**
Pregnancy data				
Initial animals per group	25	25	25	25
Mortalities	0	0	0	0
Confirmed pregnancy at necropsy	22	21	20	20
Pregnancy rate (%)	88	84	80	80

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*	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***
Uterine observation				
Gravid uterus weight (g)	69.96 ± 15.06	62.23 ± 14.46	63.26 ± 16.20	59.10 ± 19.67
Corpora lutea (no.)	11.7 ± 2.1	11.2 ± 1.9	11.4 ± 1.8	11.6 ± 2.5
Total implantation per female (no.)	11.5 ± 2.1	11.1 ± 1.9	10.7 ± 2.6	10.7 ± 3.3
Live foetuses (no.)	11.4 ± 2.2	10.1 ± 2.8	10.1 ± 2.7	10.1 ± 3.8
Dead foetuses (no.)	0.0 ± 0.0	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
Early resorptions (no.)	0.0 ± 0.2	0.4 ± 0.6	0.6 ± 1.1*	0.4 ± 0.6
Late resorptions (no.)	0.0 ± 0.2	0.1 ± 0.4	0.0 ± 0.0	0.2 ± 0.5
Pre-implantation loss (%) 4/22	1.5 ± 3.3 2/21	0.8 ± 2.4	7.0 ± 16.8 5/20 animals with pre-implantation loss (8.3, 8.3, 45.5, 62.5, 15.4%)	10.4 ± 17.1* 8/20 animals with pre-implantation loss (6.3, 44.4, 60.0, 20.0, 21.4, 27.3, 20.0, 8.3%)
Post-implantation loss (%) 1/22	0.8 ± 3.6 10/21	6.9 ± 10.2	4.9 ± 10.0 6/20 animals with post-implantation loss (40, 15.4, 9.1, 7.1, 9.1, 18.2)	6.9 ± 13.8 7/20 animals with post-implantation loss (40, 8.3, 50.0, 8.3, 9.1, 12.5, 9.19
Litter data	**0 ppm** **(0 mg/kg/d)**	**10 ppm** **(0.8 mg/kg/d)**	**100 ppm** **(7.2 mg/kg/d)**	**300 ppm** **(22.4 mg/kg/d)**

Litter size (no.)	11.4 ± 2.2	10.2 ± 2.8	10.1 ± 2.7	10.1 ± 3.8
Total (male + female) live foetuses (no.)	11.4 ± 2.2	10.1 ± 2.8	10.1 ± 2.7	10.1 ± 3.8
Live male foetuses (no.)	5.1 ± 1.6	4.7 ± 1.7	4.7 ± 1.8	5.4 ± 2.6
Live female foetuses (no.)	6.3 ± 2.0	5.8 ± 1.8	5.5 ± 2.3	4.8 ± 2.1*
Average fetal weight (g)	4.0 ± 0.3	4.0 ± 0.5	4.2 ± 0.2	4.0 ± 0.2
Fetal data				
Total no. of live fetuses	251	220	202	202
Total no. of foetuses examined for visceral examination	119	105	97	95
Total no. of foetuses examined for skeletal examination	132	115	105	107
Total foetuses available for gross external evaluation (no.)	11.4 ± 2.2	10.5 ± 2.2	10.1 ± 2.7	10.1 ± 3.8
Foetuses available for visceral examination (no.)	5.4 ± 1.2	5.0 ± 1.2	4.8 ± 1.4	4.8 ± 1.8
Foetuses available for skeletal examination (no.)	6.0 ± 1.0	5.5 ± 1.1	5.3 ± 1.3	5.4 ± 2.0
External Examination				
Malformations, no. of foetuses %	 0 0.0	 0 0.0	 0 0.0	 0 0.0
Variations no. of foetuses %	 0 0.0	 0 0.0	 0 0.0	 0 0.0
Visceral Examination				

Malformations no. of foetuses %	 0 0.0	 3 2.9	 1 1.0	 1 1.1
Variations no. of foetuses %	 0 0.0	 3 2.9	 1 1.0	 1 1.1
Skeletal examinations				
No. foetuses examined No. litters examined	132 22	115 21	105 20	107 20
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)
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)
Split Thoracic vetrtebra centrum no. 12	0	1(1)	0	0
Mising caudal vertebral arch no 2	0	2(2)	0	3(2)
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

Conclusion The main developmental effect was a dose dependent increase, starting at low dose ($p < 0.5$ 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 phalang no. 3, bilateral) of the forepaws was the predominant malformation.

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).

3.10.1.3 Extended one-generation reproductive toxicity study in rats

Reference Tonk *et al*. (2011) Developmental immunotoxicity of di-*n*-octyltin dichloride (DOTC) in an extended one-generation reproductive toxicity study. Toxicol. Lett. 204: 156-163.

Guideline 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.

(*) note that 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.

Reliability Klimisch 1: reliable without restriction, according to Registrant(s), however, it is noted that GLP-compliance and purity of test substance are unknown.

Species / strain Rat (Wistar)

Test material di-*n*-octyltin dichloride (DOTC)

CAS 3542-36-7

EC 222-583-2

Purity not reported.

Study design Rats were randomly assigned to the treatment groups and received the test diets with 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 dose levels were selected based on in house dose range finding studies (data not shown). At the end of the two-week pre-mating period, rats were mated at a ratio of 2 females:1 male. The day of sperm detection in the vaginal smear was considered day 0 of gestation and the mated F0 females were housed individually.

The morning after birth was considered postnatal day (PND) 1. Litters were not standardised and pups were weaned on PND 21. Evaluation of sexual maturation was performed using 1 pup/sex/litter.

Throughout the study, all animals were checked daily for clinical signs and abnormal behavior. The body weights of all males and females were recorded weekly during the premating period, and the body weights of the males weekly thereafter. Mated females were weighed on gestational days (GD) 0, 6, 14, and 21 and during lactation on days 1, 4, 8, 10, 13, 17, and 21. Pup body weights were recorded at PNDs 1, 4, 8, 10, 13, 17, and 21 and weekly from weaning.

During the premating period, food consumption was measured weekly for each cage by weighing the feeders. Individual food consumption of all mated females was recorded from GD 0–6, 6–14, and 14–21 and for all females with live pups from postpartum days 1–4, 4–8, 8–10, and 10–13. F1 food consumption was recorded weekly from weaning.

Subsets of F_1 male rats (n = 8/dose) originating from different litters, were evaluated at PNDs 21, 42, and 70 for changes in immune function. Terminal body weights were recorded, and the following organs were weighed: liver, thymus, spleen, kidneys, adrenals, heart, and testes.

The following immunotoxicological assessment was peformed:

- Subpopulations of spleenocytes and thymocytes were analysed using flow cytometric analysis of cell surface markers
- NK cell activity using in vitro ^{51}Cr-release assay
- NO/TNF-α production by adherent splenocytes stimulated with LPS
- Lymphoprolifertive responses assessed in splenocytes using ConA and LPS and in thymocytes using ConA.
- Cytokine production of splenocytes after stimulation with ConA
- T-cell antibody response to Keyhole Limpet hemocyanin (KLH) was assessed following subcutaneous immunizations with KLH on PNDs 21 and 35.
- Delayed type hypersensitivity response (DTH) against KLH was evaluated at PND 49.
- KLH-specific lymphoproliferative response and cytokine production of splenocyte cultures of KLH-immunized rats.

Findings

Parental generation

Parental animals showed no adverse behavior or clinical signs. No statistically significant effects of DOTC on the body weights of the F_0 rats were observed, except for the F_0 females during lactation. On lactation days 4, 8, 10, 17, and 21 the F_0 females in the mid and high dose groups (on day 8 only in the high dose) showed a slight (approximately 5%), but statistically significantly increased body weight when compared to controls. There were no effects of DOTC on the food consumption of the F_0 females during gestation or lactation. The substance intake for the treated F_0 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 for the 3, 10, and 30 mg DOTC/kg diet dose groups, respectively.

Organ weights and Histopathology

No information available on F0 animals.

Fertility, parturition and sexual function

Mating and fertility indices were similar among all groups.

Precoital time similar among all groups (1.8 days in all groups except in the 10 mg DOTC/ kg diet group where the precoital time was 2.5 days, not statistically significantly different from control).

The gestation index was 100% at all dose levels

Mean duration of pregnancy was comparable between all test substance-treated groups and the controls (21.3-21.5 days).

Development

↑ post-implantation loss (12.2, 13.7 and 17.9 % in 3, 10 and 30 mg/kg dose groups, repectively, not stat. sign. compared to 8.8% in control)

Mean number of pups (live + dead) delivered per dam was not different among groups and there was no difference in live birth index or number of stillborn pups among groups.

↓ mean number of live pups per litter at PND 4 in high dose group (8.78, $p<0.05$ compared to 10.48).

Male pup mean body weights were statistically significantly increased on PNDs 8, 10 and 13 in the high dose group compared to control.

After weaning, no effects on body weight, food consumption and sexual maturation were observed (according to study authors, no data available).

F_1 animals showed no differences in the animals' appearance, general condition or behavior among treatment and control groups.

At necropsy 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 (-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.

Relative liver weigth was statistically significantly increased in low and mid dose groups at PND 70.

Table 5: Delivery and offspring data

DOTC (mg/kg diet)	0	3	10	30
Test substance intake (F0 females; mg/kg bw/day)	0	0.17–0.21 (gestation) 0.27–0.55 (lactation)	0.56–0.71 (gestation) 1.0–1.9 (lactation)	1.7–2.1 (gestation) 2.9–5.2 (lactation)
Parameters of reproductive performance				
Females mated	24	24	24	20
Mating index (%)[a]	100	100	100	100
Fertility[b]/fecundity[c] index (%)	88	100	96	90
Gestation index (%)[d]	100	100	100	100
Precoital time (days)[e]	1.8 ± 1.1	1.8 ± 0.9	2.5 ± 1.2	1.8 ± 0.9
Females pregnant	21	24	23	18
Gestation time (days)[e]	21.3 ± 0.46	21.4 ± 0.52	21.5 ± 0.52	21.5 ± 0.53
Number of females with implanation loss	8(12)[f]	11(19)	13(18)	7(13)
Implantation loss per animal (%)[e,g]	8.8 ± 8.6	12.2 ± 17.3	13.7 ± 13.6	17.9 ± 25.1
Females with liveborn pups	21	24	23	18
Females with still born pups	3	0	0	0
Offspring data				
Pup delivered	10.76 ± 2.09	10.5 ± 3.09	10.39 ± 2.08	9.78 ± 2.24

(mean)[e]				
Live birth index (%)[h]	99	100	100	100
Sex ratio day 1 (%)i	50	54	53	47
Live pups/litter				
Day 1[e]	10.62 ± 2.01	10.50 ± 3.09	10.39 ± 2.08	9.72 ± 2.24
Day 4[e]	10.48 ± 2.02	10.13 ± 3.13	10.00 ± 1.95	8.78* ± 12.60
Day 21[e]	10.48 ± 2.02	9.88 ± 3.01	9.78 ± 2.13	8.78 ± 2.60
Viability index day 1–4 (%)[j]	98.7	96.6	96.6	91.0
Vialbility index day 4–21 (%)[k]	99.6	97.8	97.6	100

a (No. of females mated/no. of females placed with males) ×100.
b (No. of females pregnant/no. of females placed with males) × 100.
c (No. of females pregnant/no. of females mated) × 100.
d (No. of females with live pups/no. of females pregnant) × 100.
e Values are means ± SD.
f No. of females evaluated.
g (No. of implantation sites–number of live pups/no. of implantation sites) × 100).
h (No. pup born alive/no. of pups born) × 100.
i (No. live male pups/no. of live pups) × 100.
j (No. pups surviving 4 days/no. of liveborn pups at day 1) × 100.
k (No. pups surviving 21 days/no. of liveborn pups at day 4) ×100.
* $p < 0.05$.

Immunotoxicological assessment of F1

Lymphocyte subpopulations – spleen

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 (see table 6)

Lymphocyte subpopulations – thymus

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 (see table 6).

NK cell activity

No effect.

NO/TNF-α production by adherent splenocytes

No effect.

Lymphoproliferative responses

No effect.

Cytokine production

See table 6.

T-dependent antibody response

No statistically significant effects.

Delayed-type hypersensitivity (DTH)

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).

KLH-specific lymphoproliferative response and cytokine production

No effect.

Table 6: Immunotoxicological assessment

	PND 21				PND 42				PND 70			
DOTC (mg/kg diet)	0	3	10	30	0	3	10	30	0	3	10	30
Hematology												
MPV								↓				
RDW								↑			↑	
Cytometric analysis of splenocytes in F1 males												
Spleen weight												
cellularity spleen												↓
T Cell (CD3+)								↓				↓
CD4+CD8-								↓				↓
CD4-CD8+								↓				↓
NK cell								↓				↓
B Cell								↓				↓
Cytometric analysis of thymocytes in F1 males												
Thymus weight								↓				↓
cellularity thymus								↓				↓
CD3 high								↓				↓
CD3 low								↓				↓
CD4-CD8+								↓			↓	↓
CD4+CD8+								↓			↓	↓
CD4-CD8-								↑			↓	↑
CD4+CD8-								↓			↓	↓
ConA stimulated cytokine production by splenocytes in F1 males												
IL-4						↑	↑	↑			↑	↑
IL-13		↓					↓	↑			↑	↑
IL-10								↑				
IFN-γ				↑								

Conclusion 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. There was also an increased DTH response in all dose groups compared to the control, reaching statistical significance in the low and high dose groups.

3.11 Specific target organ toxicity – single exposure

Not evaluated in this CLH Report.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Sub-chronic oral toxicity study in the rat

Reference Appel MJ and Waalkens-Berendsen DH. (2004). Dichlorodioctylstannane [CASRN # 3542-36-7]: Sub-chronic (13 week) oral toxicity study in rats, including a reproduction/developmental screening study. Testing laboratory: TNO Nutrition and Food Research. Report no.: V3964. Owner company: ORTEP. Report date: 2004-04-01.

Guideline OECD 408 (Repeated Dose 90-day Oral Toxicity Study in Rodents)

Reliability Klimisch 1: reliable without restriction (guideline-compliant study with no or minor deviations not affecting the quality of the results, GLP-compliant study with certificate), according to Registrant(s).

Species / strain Rat (Wistar)

Test material Dichlorodioctylstannane (dioctyltin dichloride, DOTC)

CAS 3542-36-7

EC 222-583-2

Purity 92.1 %

Study design The repeated dose toxicity of the test material was studied using continuous administration via the diet for 13 consecutive weeks according to OECD 408. In satellite groups of female rats a reproduction/developmental screening test was performed according to OECD 421 (study summarised in section 3.10.1.1). The main 13-week study used four groups of 10 rats/sex and the satellite reproduction/developmental screening study used four groups of 10 female rats. For both studies the control group was kept on untreated diet and the three test groups received diets containing 10, 100 and 300 mg/kg of the test material.

In the satellite study administration of female rats started two weeks prior to the mating period and continued through mating, gestation, and up to PN 4 or shortly thereafter. After a premating period of 10 weeks, male rats from the main study were mated with female rats of the satellite groups, which were administered the same dose of test diet.

The study summary continued below refers to the main study, i.e. OECD 408 (Repeated Dose 90-day Oral Toxicity Study in Rodents).

Clinical observations (daily), growth (body weight recorded once weekly), food consumption (measured weekly), food conversion efficiency, neurobehavioural testing, ophthalmoscopy (prior to the start of treatment in all animals and towards the end of the treatment period in all surviving animals of the control group and the 300 mg/kg group), haematology (at necropsy at the end of treatment), clinical chemistry (at necropsy at the end of treatment), renal concentration test (shortly before the end of treatment), urinalysis (shortly before the end of treatment), organ weights and gross examination at necropsy (in the week after the end of study), and microscopic examination of various organs and tissues (samples preserved during gross examination) were used as criteria for detecting effects of the treatment.

At final necropsy in the 13-week study, the following organs were weighed (paired organs together) as soon as possible after dissection to avoid drying:

- adrenals
- ovaries
- brain
- spleen
- epididymides
- testes
- heart
- thymus
- kidneys
- thyroid (with parathyroids)
- liver

Histopathological examination was performed on all tissues and organs listed above - except those marked with an asterisk - of all animals of the control group (group A) and of the 300 mg/kg group (group D). In addition, lungs, liver, kidneys and gross lesions were examined microscopically in all rats of the intermediate dosegroups. Since treatment-related changes were found in the thymus of males and females of the 300 mg/kg group, histopathology on this organ was extended to males and females of the intermediate-dose groups.

adrenals
parathyroid
aorta
* parotid salivary glands
*axillary lymph nodes pituitary
brain (brain stem, cerebrum, cerebellum)
prostate
caecum
rectum
colon
* seminal vesicles with coagulating glands
epididymides
* skeletal muscle (thigh)
* exorbital lachrymal glands
skin (flank)
eyes
small intestine (duodenum, ileum, jejunum)
*femur with joint spinal cord (at three levels)
GALT (gut associated lymphoid tissue, including Peyer's patches)
spleen
sternum with bone marrow
* Harderian gland stomach (glandular and non-glandular)
heart
sublingual salivary glands
kidneys
submaxillary salivary glands
liver
testes
lungs
thymus
mammary gland (females)

thyroid
* mandibular (cervical) lymph nodes
* tongue
mesenteric lymph nodes
trachea/bronchi
* nasal cavity
urinary bladder
nerve-peripheral (sciatic)
uterus (with cervix)
oesophagus
* vagina
ovaries
* Zymbals gland
pancreas
all gross lesions.

(*) The tissues marked with an asterisk were preserved but not processed for histopathological examination, since histopathological examination was not considered necessary on the basis of the results of gross observations.

Findings

CLINICAL SIGNS AND MORTALITY

No treatment-related clinical signs or mortalities were observed.

BODY WEIGHT AND WEIGHT GAIN

Body weights were statistically significantly decreased by about 9% in males and females of the 300 mg/kg group and females throughout the study. The decrease in body weight accompanied by reduced food intake in males and females of the 300 mg/kg group was in the study report considered to be due to reduced palatability of the test diet.

FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption was slightly decreased in males and females of the 300 mg/kg group (by about 8 and 11%, respectively). On a number of days the difference reached the level of statistical significance. Food consumption was generally similar among the control, 10 and 100 mg/kg groups in males and females. An occasional statistically significant difference was seen among these groups.

The overall intake of the test substance for the 10, 100, and 300 mg DOTC/kg diet, respectively was approximately 0.7, 6.5 and 19.3 mg/kg bw/day in males and 0.7, 6.8 and 19.8 mg/kg bw/day in females.

FOOD EFFICIENCY

Food conversion efficiency was similar among the groups in males and females throughout the study. An occasional statistically significant difference was seen.

OPHTHALMOSCOPIC EXAMINATION

No treatment-related ocular changes were observed.

HAEMATOLOGY

In the 300 mg/kg group decreases in haemoglobin, packed cell volume, mean corpuscular haemoglobin, total white blood cells, absolute numbers of lymphocytes and an increase in

prothrombin time were observed.

The following statistically significant changes in haematology parameters were observed:

- Hb was decreased in females of the 300 mg/kg group;
- PCV was decreased in females of the 300 mg/kg group;
- MCV was decreased in males of the 100 mg/kg group;
- MCH was decreased in the 100 (males) and 300 mg/kg groups (males and females);
- Reticulocytes were decreased in males of the 100 mg/kg group;
- Prothrombin time was increased in females of the 300 mg/kg group;
- Total WBC was decreased in males of the 300 mg/kg group. Atlthough not statistically significant, a similar decrease was also seen in emales of the 100 and 300 mg/kg groups;
- The absolute number of lymphocytes was decreased in males of the 300 mg/kg group;
- The absolute numbers of monocytes were decreased in females of all treated groups.

CLINICAL CHEMISTRY

In the 100 and 300 mg/kg groups increases in alkaline phosphatase and bilirubin were observed.

The following statistically significant changes in clinical chemistry parameters were observed:

- ALP was increased in males and females of the 100 and 300 mg/kg groups;
- TP was decreased in females of the 300 mg/kg group;
- The A/G ratio was increased in the 10 (females) and 300 mg/kg groups (males and females);
- Total bilirubin was increased in females of the 100 and 300 mg/kg groups;
- Direct bilirubin was increased in females of the 300 mg/kg group;
- Cholesterol was decreased in females of the 300 mg/kg group;
- Bile acids were increased in males of the 300 mg/kg group. Although not statistically significant, a similar increase was also seen in females of the 300 mg/kg group;
- Phospholipids was increased in males of the 10 mg/kg group and decreased in females of the 300 mg/kg group;
- Calcium was decreased in females of the 300 mg/kg group;
- Sodium was decreased in males of the 100 and 300 mg/kg groups.

URINALYSIS

Urinary volume and density were similar among the groups. Urinary crystals were statistically significantly increased in females of the 300 mg/kg group. Further semi-quantitative and microscopic urinary observations were similar among the groups.

NEUROBEHAVIOUR

No neurotoxic effects of treatment were observed from neurobehavioural measures and motor activity assessment in any of the groups at any time point during the 13-week treatment period. Some abnormalities were observed in individual animals that were not considered to be related to treatment. On one occasion during arena testing, a tilted head was observed in one female. This single observation was not considered to be related to treatment.

Tiptoe walking was observed in some females of different groups in various weeks of the study. Tiptoe walking was not considered to be related to treatment, for it was observed in females only, but in all groups, including the control group. Further, it was not consistently observed in the concerned animals from first occurrence towards the end of the test period and the severity of this gait abnormality did not increase over time.

The results of the neurobehavioural observations and motor activity assessment did not indicate any neurotoxic potential of the test substance.

ORGAN WEIGHTS

Statistically significant changes in organ weights were observed in the 300 mg/kg group for relative kidney weight (8.4 % increase in females), relative liver weight (6.2 and 16.4 % increase in males

and females, respectively), relative testis weight (9.7 % increase), absolute spleen weight (11.6 % decrease in females) and absolute adrenal weight (16.4 % decrease in females). A statistically significant decrease of 12.2 % was observed for relative adrenal weight in females of the 100 mg/kg group.

A marked and dose-related decrease in absolute and relative thymus weight was observed. In males the absolute and relative thymus weights in all treated groups were decreased 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.

In females, the absolute thymus weight in all treated groups was decreased 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) as well as the 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).

GROSS PATHOLOGY

At necropsy, treatment-related gross changes were not observed.

HISTOPATHOLOGY: NON-NEOPLASTIC

At microscopical examination, treatment-related histopathological changes were observed in the thymus. The histopathological changes comprised lymphoid depletion, characterised by a decrease in the size of the thymic lobules which can be ascribed to extensive loss of cortical en medullary small lymphocytes. Consequently, the distinction between the cortical and medullary areas was blurred. The microscopic appearance of the affected thymus resembled thymus atrophy described in the literature for organotin compounds.

Lymphoid depletion was observed in the thymus in 5/10 (severity score slight to moderate) and 9/9 (severity score, moderate to severe) males of the 100 and 300 mg/kg group, respectively, and in 10/10 and 9/9 females (severity score was slight to very severe) of the 100 and 300 mg/kg group, respectively. Lymphoid depletion was not observed in any of the animals of the control group or the 10 mg/kg group.

All other histopathological changes were common findings in rats of this strain and age. They were about equally distributed amongst the different treatment groups or occurred in one animal only. Therefore, they were not considered to be related to treatment.

Table 7: Incidence of lesions in the thymus

	Males				Females			
	0 mg/kg diet	10 mg/kg diet	100 mg/kg diet	300 mg/kg diet	0 mg/kg diet	10 mg/kg diet	100 mg/kg diet	300 mg/kg diet
Lymphoid depletion								
Number of animals examined	10	10	10	9	10	10	10	9
Slight	0	0	3	0	0	0	1	0
Moderate	0	0	2	4	0	0	4	2
Severe	0	0	0	5	0	0	5	5
Very severe	0	0	0	0	0	0	0	2
Total score	0	0	5*	9***	0	0	10***	9***

* $p<0.05$

** p<0.01

*** p< 0.001

Conclusion Administration of dioctyltin chloride in the diet at the concentrations of 10, 100 and 300 mg/kg caused a decrease in thymic weight which was correlated with histopathological effects (lymphoid depletion) observed in the 100 and 300 mg DOTC/kg diet dose groups and were considered as adverse effects. 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). Thus, no NOAEL could be set in the present study.

3.13 Aspiration hazard

Not evaluated in this CLH Report.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

Not evaluated in this CLH Report.

4.2 Bioaccumulation

Not evaluated in this CLH Report.

4.3 Acute toxicity

Not evaluated in this CLH Report.

4.4 Chronic toxicity

Not evaluated in this CLH Report.

4.5 Acute and/or chronic toxicity to other aquatic organisms

Not evaluated in this CLH Report.