Committee for Risk Assessment

RAC

Annex 1

Background document

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

dichlorodioctylstannane

EC Number: 222-583-2
CAS Number: 3542-36-7

CLH-O-0000001412-86-230/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:

Dichlorodioctylstannane

EC Number: **222-583-2**

CAS Number: **3542-36-7**

Index Number: **050-021-00-4**

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

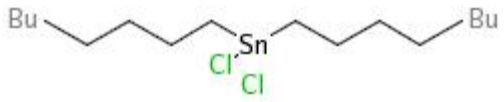
Version number: 2 **Date:** **July 7, 2017**

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

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

Name(s) in the IUPAC nomenclature or other international chemical name(s)	dichloro(dioctyl)stannane
Other names (usual name, trade name, abbreviation)	dichloro(dioctyl)stannane, DOTCl, SONGCAT DOTC
ISO common name (if available and appropriate)	Not available
EC number (if available and appropriate)	222-583-2
EC name (if available and appropriate)	Dichlorodioctylstannane
CAS number (if available)	3542-36-7
Other identity code (if available)	-
Molecular formula	C16H34Cl2Sn
Structural formula	
SMILES notation (if available)	CCCCCCCC[Sn](Cl)(Cl)CCCCCCCC
Molecular weight or molecular weight range	416.06
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	Not relevant

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)**
Dichlorodioctylstannane EC no. 222-583-2 CAS no. 3542-36-7	Typical conc: 96.04% Range: 94.5-100% [REACH Registration, 2016]	Acute Tox. 3 *, H331 STOT RE 1, H372** Aquatic Chronic 3, H412	Acute Tox. 2, H330 Acute Tox. 3, H331 STOT SE 2, H371 (thymus) STOT RE 1, H372 STOT RE 1, H372 (thymus) Aquatic Chronic 3, H412 Aquatic Chronic 4, H413 [C&L Inventory (ECHA,

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)
			2016b)]

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
Hexadecane EC no. 208-878-9 CAS no. 544-76-3	Typical conc: 0.23% (w/w) Range: 0-0.5% (w/w) [REACH Registration, 2016]	-	Skin Corr. 1B, H314 Skin Irrit. 2, H315 Eye Irrit. 2, H319 STOT SE 3, H335 (not specified) STOT SE 3, H335 (Respiratory tra...) STOT SE 3, H335 (eyes and skin) STOT SE 3, H335 (lung) (Inhalation) Asp. Tox. 1, H304 Not Classified	No, the impurity does not contribute to the classification and labelling in the current proposal.
Trichlorooctylstannane EC no. 221-435-4 CAS no. 3091-25-6	Typical conc: ca 3.05% (w/w) Range: > 0.0- ≤ 4.0% (w/w) [REACH Registration, 2016]	-	Acute Tox. 4, H332 Skin Irrit. 2, H315 Eye Dam. 1, H318 Repr. 2, H361 (Oral) Repr. 2, H361d (Oral) Repr. 2, H361 STOT RE 1, H372 (Oral) STOT RE 2, H373 (Thymus) (Oral) STOT RE 2, H373 Aquatic Acute 1, H400 Aquatic Chronic 1, H410 Not Classified	No, the impurity does not contribute to the classification and labelling in the current proposal. In the only available reproductive toxicity study (reproduction/ developmental screening study; OECD TG 421) of trichlorooctylstannane at ECHA dissemination site (2017) the purity of the test substance was stated to be 85.5%, with impurities of dioctyltin dichloride present at 11.07%. The LOAEL for reproductive effects was set to 22.3-33.0 mg/kg bw/day. In comparison with DOTC, trichlorooctylstannane is thus less potent (c.f. LOAEL 4.2-6.2 mg/kg bw/day). The contribution of this impurity in the

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**
				classification of DOTC as reproductive toxicant is considered not relevant. The LOAEL for effects on the thymus (STOT RE) was 31.5 mg/kg bw/day for trichlorooctylstannane. Effective dose levels of DOTC was 0.7 mg/kg bw/d. The contribution of this impurity in the classification of DOTC is considered not relevant for STOT RE.
Chlorotrioctylstannane EC no. 219-969-8 CAS no. 2587-76-0	Typical conc: 0.68% (w/w) Range: 0-1% (w/w) [REACH Registration, 2016]	-	Acute Tox. 2, H330 Skin Irrit. 2, H315 Eye Irrit. 2, H319 Skin Sens. 1, H317 Repr. 2, H361 STOT SE 3, H335 (No data) STOT SE 3, H335 (Lungs) STOT RE 1, H372 (Thymus) Aquatic Chronic 1, H410 Aquatic Chronic 4, H413 STOT SE 3; C ≥ 1 % Eye Irrit. 2; C ≥ 1 % Skin Irrit. 2; C ≥ 1 % M(Chronic)=100 Not Classified	No, the impurity does not contribute to the classification and labelling in the current proposal. In the only available reproductive toxicity study (combined repeated dose and reproduction / developmental screening study; OECD TG 422) of Chlorotrioctylstannane at ECHA dissemination site (2017) the purity of the test substance was not specified. The LOAEL for reproductive effects was 62 mg/kg bw/day. In comparison with DOTC, Chlorotrioctylstannane is thus less potent (c.f. LOAEL 4.2-6.2 mg/kg bw/day). The contribution of this impurity to the classification of DOTC as reproductive toxicant is considered not relevant. The LOAEL for

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
				effects on target organ (STOT RE) was based on the same study as mentioned above, and the LOAEL was also 62 mg/kg bw/day for effects on the thymus. Effective dose levels of DOTC was 0.7 mg/kg bw/d. The contribution of the impurity chlorotrioctylstannane to the classification of DOTC is considered not relevant for STOT RE.

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	-	-	-	-	-

Table 5: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
Dichlorodioctylstannane CAS no. 3542-36-7	92.1 % (as stated in original study report)	Identification of impurities of the test substance included: Monoctyltin trichloride, 2.25% Trioctyltin chloride, 0.70% Octylbutyltin dichloride, 0.16%	Supplier was ORTEP Association Stabilizer Task force	Appel, M.J. and D.H. Waalkens-Berendsen. (2004)
Dichlorodioctylstannane CAS no. 3542-36-7	97.7 %.(as stated in original study report)	Acetone was used as vehicle for formulation preparation.	Supplier was Galata Chemicals	Study report, 2014
Di-n-octyltin dichloride CAS no. 3542-36-7	No information available	No information available	Obtained from ABCR GmbH & Co.	Tonk et al., 2011

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	050-021-00-4	Dichlorodioctylstannane	222-583-2	3542-36-7	Acute Tox. 3 * STOT RE 1 Aquatic Chronic 3	H331 H372 H412	 Danger  Danger	H331 H372 ** H412	-	-	-
Dossier submitters proposal	-	Dichlorodioctylstannane	222-583-2	3542-36-7	Acute Tox. 2 Repr. 1B	H330 H360D	 Danger	H330 H360D	-	SCL ≥ 0.03%	-
Resulting Annex VI entry if agreed by RAC and COM	-	Dichlorodioctylstannane	222-583-2	3542-36-7	Acute Tox. 2 Repr 1B STOT RE 1 Aquatic	H330 H360D H372 H412	 Danger	H330 H360D H372 ** H412	-	-	-

					Chronic 3		 Danger				

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

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Hazard class not assessed in this dossier	No
Acute toxicity via dermal route	Hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	-	Yes
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	Hazard class not assessed in this dossier	No
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

Dichlorodioctylstannane has a harmonised classification Acute Tox. 3* H331, STOT RE 1 H372**, Aquatic Chronic 3 H412 included in CLP Annex VI via ATP01 and is a direct translation from the entry in Annex I of Directive 67/548/EEC (ATP 30, August 2008). The basis of the decision on classification and if reproductive toxicity was considered or not by the Technical Committee for Classification and Labelling is not known to the dossier submitter. However, at least two relevant new studies have become available after 2008: one prenatal developmental toxicity study in rat (Study report, 2014) and one extended one-generation reproductive toxicity study in rat similar to OECD TG 443 (Tonk et al., 2011).

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Dichlorodioctylstannane 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.

The justification for modification of the harmonised classification Acute Tox. 3* H331 is that this is a minimum classification and it is concluded that a refinement of the classification to a more severe category based on available data is warranted.

RAC general comment

Dichlorodioctylstannane, further referred to as DOTC in this document, is an organotin compound with two octyl chains and two chlorine groups. 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 were in favour of amongst others, STOT RE 1 (immune system) and Repr. 1B; H360FD. One other dioctyltin compound previously assessed by RAC, dioctyltin bis(2-ethylhexylmercaptoacetate) was classified as Repr. 1B; H360D, which is also proposed for DOTC.

Toxicokinetic studies performed with radioactively labelled DOTC (oral dosing 6.3 mg/kg bw) indicate a low to medium absorption of approximately 20 %, with the highest concentrations observed in the liver and kidney. The half-life was determined to be between 8 and 9 days. Hydrolysis testing under simulated gastric conditions indicates that 90 % of DOTC is transformed to the dimeric stannoxane ($ClOct_2SnOSnOct_2Cl$) at pH 1.2 within 4 h, while the remaining 10 % is unmetabolised DOTC.

5 IDENTIFIED USES

The publically disseminated REACH registration dossier (ECHA, 2016a) states that dichlorodioctylstannane is used in closed processes as an intermediate in synthesis or formulation and manufacture of other substances. It is also used as laboratory reagent.

6 DATA SOURCES

Data on dichlorodioctylstannane in the publically disseminated REACH registration dossier (ECHA, 2016a) 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 subchronic (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 8: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	The substance is a white/off white solid block	REACH registration (ECHA dissemination, 2016a)	No guideline followed. Visual inspection of the test material.
Melting/freezing point	The melting point of the substance is ca. 43 ºC	REACH registration (ECHA dissemination, 2016a)	No guideline available.
Boiling point	The test item has been determined to decompose from 230 ± 0.5 °C (503 ± 0.5 K) at 102.18 kPa. As the test item decomposed, no value for boiling temperature could be determined.	REACH registration (ECHA dissemination, 2016a)	Differential scanning calorimetry. GLP compliant with certificate.
Relative density	The specific gravity of the substance was found to be 1.361 at 60 ºC	REACH registration (ECHA dissemination, 2016a)	No guideline followed. Buoyancy method.
Vapour pressure	The vapour pressure of the substance at 25°C was extrapolated by linear regression and determined to be 5.16 x 10^-4 Pa at 25°C.	REACH registration (ECHA dissemination, 2016a)	OECD Guideline 104 (Vapour Pressure Curve) and EU Method A.4 (Vapour Pressure).
Surface tension	-	REACH registration (ECHA dissemination, 2016a)	Data waved: other justification.
Water solubility	An average value for the solubility of the substance of 1.6 ± 0.1 mg/l was obtained from the two independent runs.	REACH registration (ECHA dissemination, 2016a)	OECD Guideline 105 (Water Solubility).
Partition coefficient n-octanol/water	-	REACH registration (ECHA, 2016a)	Data waived: study technically not feasible.
Flash point	Flash point obtained using the Cleveland open cup method was 204 ºC.	REACH registration (ECHA dissemination, 2016a)	No guideline available. Cleveland open cup method.

Property	Value	Reference	Comment (e.g. measured or estimated)
Flammability	-	REACH registration (ECHA dissemination, 2016a)	Data waived: study scientifically unjustified.
Explosive properties	-	REACH registration (ECHA dissemination, 2016a)	Data waived: study scientifically unjustified.
Self-ignition temperature	-	REACH registration (ECHA dissemination, 2016a)	Data waived: other justification.
Oxidising properties	-	REACH registration (ECHA dissemination, 2016a)	Data waived: other justification.
Granulometry	Not determined.	REACH registration (ECHA dissemination, 2016a)	-
Stability in organic solvents and identity of relevant degradation products	Not determined.	REACH registration (ECHA dissemination, 2016a)	-
Dissociation constant	Not determined.	REACH registration (ECHA dissemination, 2016a)	-
Viscosity	Not determined.	REACH registration (ECHA dissemination, 2016a)	-

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this CLH proposal.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 9: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
The absorption, tissue distribution and excretion of radiolabelled DOTC in rats. No guideline GLP: not specified Wistar-derived rat, males 3 rats/group	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	[Reliability: 2 (reliable with restrictions)]	Penninks *et al.*, 1987

Method	Results	Remarks	Reference
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.	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.		
Distribution of 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	[Reliability: 2 (reliable with restrictions) according to the Registrant(s)]	Study report, 1987. Reach registration dossier, public version (ECHA dissemination, 2016a)
Simulated gastric hydrolysis (119Sn NMR (nuclear magnetic resonance) detection) in vitro. No guideline Purity of test substance >95 %	DOTC is rapidly hydrolysed at low pH to the distannoxane ClOct2SnOSnOct2Cl as the only detectable product under conditions representative of the mammalian stomach. More than 90% of ClOct2SnOSnOct2Cl is formed after 4 hours. Small amounts (<10%) of DOTC was also detected.	[Reliability: 2 (reliable with restrictions)]	Naβhan, 2016

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

DOTC is shown to be distributed to various tissues in Wistar rats 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) (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, 2016a).

No data are available about 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).

Recent simulated gastric hydrolysis studies demonstrate the rapid formation of intermediate(s) of DOTC (Naßhan, 2016). Using 119Sn NMR (nuclear magnetic resonance) spectroscopy, the distannoxane ClOct2SnOSnOct2Cl 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.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not evaluated in this CLH proposal.

10.2 Acute toxicity - dermal route

Not evaluated in this CLH proposal.

10.3 Acute toxicity - inhalation route

Not evaluated in this CLH proposal.

Table 10: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviation(s) if any	Species, strain, sex, no/group	Test substance, reference to table 5, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference

Inhalation (aerosol, nose/head only) According to Guideline: other: Sachsse et al. 1973 GLP compliance: no	Rat, Tif RAIf (SPF) male/female 9 animals per sex per dose (no control animals)	dioctyltin dichloride (composition not reported) Unknown purity MMAD: not reported Vehicle: ethanol	Dose levels: 221 ± 53, 443 ± 47, 696 ± 103 mg/m3 Duration of exposure: 4 h Duration of observation period following administration: 14 days	LC50 (4 h): 0.439 mg/l aerosol exposure for rats of both sexes	Study report, 1976 (as cited in the REACH registration public version, ECHA dissemination 2016a). [Reliability: 2 (reliable with restrictions) according to the Registrant(s)] Ciba-Geigy Ltd. 1976 (as cited in SIDS Initial Assessment Report for SIAM 23, 2006)
Inhalation (aerosol) According to guideline: other, not specified GLP compliance: no	Rat, Sprague-Dawley male 10 animals per dose	dioctyltin dichloride (composition not reported) Unknown purity MMAD: 2.5-3.5 µm Vehicle: polyethylene glycol 400	Dose levels: 0, 0.11, 0.29, 0.44, 0.83, and 0.91 mg/l Duration of exposure: 1 h Duration of observation period following administration: 14 days	LC50 (1 h): 0.39 mg/l aerosol exposure for male rats Converted[£] to LC50 (4 h): 0.0975 mg/l	Hazelton Laboratories America, Inc. 1976 (as cited in SIDS Initial Assessment Report for SIAM 23, 2006) [Reliability: 2 (reliable with restrictions) according to the Registrant(s)]

Inhalation (aerosol) According to guideline: other, not specified GLP compliance: no	Rat (strain not specified) male/female 5 animals per sex per dose	dioctyltin dichloride (composition not reported) Unknown purity MMAD: Droplets ranged from 3-10 µm Vehicle: sesame oil	Dose levels: 15, 25, 50, 90, and 120 mg/l Duration of exposure: 1 h Duration of observation period following administration: 14 days	LC50 (1 h): 37 mg/l aerosol exposure for rats of both sexes Converted[£] to LC50 (4 h): 9.25 mg/l	Wells Laboratories, Inc. 1976 (as cited in SIDS Initial Assessment Report for SIAM 23, 2006) [Reliability: 3 (not reliable) according to the Registrant(s). Rationale for reliability incl. deficiencies other: Method not reported, study pre-dates GLP, purity not reported. 1 h exposure]

(£) According to note (c) to CLP Annex I, Table 3.1.1 conversion of existing inhalation toxicity data which have been generated using a 1-hour exposure can be carried out by dividing by a factor of 4 for dusts and mists.

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

In the study (Study report, 1976) selected as key study in the REACH registration of DOTC, the acute inhalation toxicity of the test material was investigated according to a standard acute method (Sachsse et al., 1973) as stated by the Registrant(s). The test was not according to currently validated test guidelines and not according to GLP conditions (study was conducted prior to implementation of GLP). Groups of nine male and nine female Tif RAIf (SPF) rats were exposed by inhalation route to dioctyltin dichloride in ethanol for 4 hours at analytically determined concentrations of 221, 443 and 696 mg/m^3. Animals were then observed for 14 days. After the 4-hour exposure period the rats at all dose levels showed dyspnoea, tremor, lateral or ventral position and ruffled fur. These symptoms became more accentuated as the concentration was increased. Four out of nine males and two out of nine females were dead within 4 hours at 443 ± 47 mg/m^3, and at 696 ± 103 mg/m^3 all animals were dead within 4 hours (Table 11). At gross pathology of dead animals haemorrhages of the lungs and congested organs were seen. Animals killed at the termination of the study had no substance related gross organ changes.

The LC50 (95% confidence limits) of a 4 hour aerosol exposure for rats of both sexes was reported as 439 (394-489) mg/m^3, when evaluated for a 14 day post-treatment observation period.

Table 11: Rate of deaths

Concentration (mg/m^3)	Exposure Time (hrs)	No. of animals exposed		Died Within									
				0 – 4 hrs		24 hours		48 hours		7 days		14 days	
		M	F	M	F	M	F	M	F	M	F	M	F
221 ± 53	4	9	9	0	0	0	0	0	0	0	0	0	0
443 ± 47	4	9	9	4	2	5	3	5	3	5	4	5	4

696 ± 103	4	9	9	9	9	9	9	9	9	9	9	9	9

Two additional supporting studies were available in the DOTC REACH registration, but not in the public version of the registration at ECHA dissemination site. In addition, in the SIDS Initial Assessment Report for SIAM 23 (2006) DOTC was assessed in a chemical category consisting of DOTC and selected thioesters. In this report, the same two acute inhalation toxicity studies were briefly described (Wells Laboratories, Inc. 1976 and Hazelton Laboratories America, Inc. 1976) as well as the study referenced as Study report 1976 in the REACH registration. The latter study was given the reliability 4 (with the rationale that details of toxic effects were not reported other than lethal dose value) and the other studies were each given reliability 2 in the SIDS assessment. Several reporting deficiencies of the three available studies were noted by the dossier submitter, including lack of data on body weight, individual clinical signs and gross pathological findings, lack of data on the composition of the test substance and unknown purity, and no data on particle size distribution. Furthermore, all three studies predates GLP. These deficiencies are considered to impact the reliability of the interpretation of data and conclusion on acute toxicity via the inhalation route.

In the study by Hazelton Laboratories America, Inc. (1976) 10 male rats were exposed to aerosols of dioctyltin dichloride dissolved in 1.75-8.03 parts polyethylene glycol for 1 hour. Test solutions were placed in a nebulizer that delivered particles with an aerodynamic mass median diameter of 2.5-3.5 µm. Animals died between 4-48 hours of dosing. The number of deaths for each dose level/total animals exposed is indicated in table 12.

Table 12: Mortality of rats after exposure

Group no.	**DOTC concentration (mg/l)**	**Total no. dead in 72 h**	**No. exposed**	**No. dead at observation time (h)**				
				0	**4**	**24**	**48**	**72**
1	PEG400 control	0	10	0	0	0	0	0
2	0.11	0	10	0	0	0	0	0
3	0.29	4	10	0	0	4	0	0
4	0.44	6	10	0	0	5	1	0
5	0.83	9	10	0	6	3	0	0
6	0.91	7	10	0	1	6	0	0

All animals (including the control group) exhibited compound awareness, periodic restlessness alternating with inactivity, and preening. Deposition of a whitish material was noted in the nostrils of rats exposed to 0.29, 0.83, and 0.91 mg/l. Several animals in the 0.29 mg/l dose group and above exhibited erratic, rapid breathing. One animal in the 0.29 mg/l group had a reddish nasal discharge, several rats in the 0.44 mg/l group had a slight discharge around the eyes, and several animals in the 0.83 and 0.91 mg/l groups were prostrate near the end of the exposure period. LC50 (95% confidence limits) was reported as 0.39 (0.28-0.56) mg/l/hr. No data from gross pathology were included in the study summary of the REACH registration or in the SIDS report.

In the study by Wells Laboratories, Inc. (1976) groups of 10 rats (5 males/5 females) were exposed to the test material dissolved in sesame oil (pH was 1.25-1.5) for 1 hour. Solution was sprayed into chamber by means of an atomizer and the size of the droplets ranged from 3-10 µm. Animals that were administered the test substance exhibited heart failure, very bloody lungs, fluid in the chest cavity, and hemorrhagic stomach and intestines at gross necropsy. There were no deaths in control rats which inhaled sesame oil and there were no findings at gross pathology. The number of deaths for each dose level/total animals exposed is given below:

15 mg/l: 3/10
25 mg/l: 6/10

50 mg/l: 7/10
90 mg/l: 8/10
120 mg/l: 9/10

LC50 (95% confidence limits) was reported as 37 (22.0-62.16) mg/l/hr.

10.3.2 Comparison with the CLP criteria

Currently, DOTC is classified according to Regulation (EC) No. 1272/2008 as Acute Tox 3* via the inhalation route. In the REACH registration of DOTC (2016) the test substance is self-classified as Acute Tox. 2, H330. This is based on the LC50 0.439 mg/l of a 4 hour aerosol exposure of rats of both sexes (Study report, 1976). Classification in Acute Tox 2 is warranted since the LC50 value is within $0.05 < \text{ATE} \leq 0.5$ (dusts and mists) meeting the criteria for category 2. The Registrant(s) states that this study is the only four hour exposure study available and therefore considered to provide a reliable result for hazard assessment. The two additional available studies were considered as supporting studies only.

The dossier submitter notes that the LC50 from the Study report, 1976 is close to the upper limit for category 2 ($\text{ATE} \leq 0.5$) and the lower limit of category 3 ($\text{ATE} > 0.5$) and that there are reporting deficiencies bringing some uncertainty in the interpretation of the results. Due to the lack of data on particle size distribution and MMAD of the particles it is unclear how large fraction of the particles of the administered aerosol that was respirable and actually reached all regions of the respiratory tract and thus the systemic circulation. However, considering the clear effects reported: a high rate of mortality observed already within 4 hours after dosing and clinical signs reported to occur at all dose levels with more emphasized effects at higher doses, the test substance could potentially have a higher acute inhalation toxicity than observed in the current study.

In support of a potentially higher acute toxicity is the 4-hour LC50 value of 0.0975 mg/l (males and females), derived from a 1-hour LC50 based on aerosol exposure with particles of known MMAD (but unknown particle size distribution) within the respirable range of 1-4 µm in rat (Hazelton Laboratories America, Inc., 1976). This is the lowest 4 h LC50 of the three available studies and the value is well within $0.05 < \text{ATE} \leq 0.5$ and thus also meets the criteria of category 2 for acute inhalation toxicity of dusts and mists.

In contrast, in the study from Wells Laboratories (1976) the 4 hour LC50 derived from a 1-hour LC50 based on aerosol exposure with droplets in the size range 3-10 µm was 9.25 mg/l. In this case the LC50 does not meet the criteria for classification. However, in the absence of information on the particle size distribution it is not possible to conclude that a sufficient fraction was of respirable size and consequently the acute toxicity may be higher than what was recorded in the current study.

In summary, two out of three available acute inhalation toxicity studies meet the criteria for classification in Acute Tox. 2, and the lowest 4 h LC50 (derived from 1 h exposure) is 0.0975 mg/l (males and females) in rat exposed to aerosol of particles with MMAD 2.5-3.5 µm. The differences in LC50 values between the three available studies could be explained by differences in experimental conditions. Three different rat strains and three different vehicles have been used in the studies. In two of the studies the duration of exposure was 1 hour, and in the third study exposure continued for 4 hours. Moreover, the size distribution of the particles and the purity of the test substance is unknown in all three studies and these factors could also potentially influence the resulting acute toxicity.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Based on the criteria for the inhalation route of exposure classification of DOTC as **Acute Tox. 2, H330** is warranted.

Currently DOTC has a harmonised classification as Acute Tox. 3* (H331) for the inhalatory route of exposure. A removal of the asterisk (*) and a more stringent classification in category 2 is proposed.

RAC evaluation of acute inhalation toxicity

Summary of the Dossier Submitter's proposal

The current classification of dichlorodioctylstannane (DOTC) as Acute Tox. 3; H331 was transposed from the previous Dangerous Substances Directive (DSD) and considered a minimum classification as depicted by the asterisk. Based on the available information, the Dossier Submitter (DS) proposed to update the classification to Acute Tox. 2; H330 via the inhalation route. Notably, the registrants have self-classified DOTC as Acute Tox. 2; H330 in the REACH registration dossier instead of Acute Tox. 3; H331. In total, three studies were summarized in the classification proposal, all dating from 1976.

Study 1 (Ciba-Geigy Ltd., 1976)

Tif RAIf (SPF) rats (9/sex/dose) were exposed to DOTC in ethanol aerosols of unknown sizes at concentrations of 221 ± 53, 443 ± 47 and 696 ± 103 mg/m^3 for 4 h. The rats showed dyspnoea, tremors and ruffled fur, all becoming more accentuated at higher doses. Within 4 hours, 4/9 males and 2/9 females died at the mid-dose while all animals died at the high-dose. After 24 h, 5 males and 3 females were found dead and one additional female died within 7 days in the mid-dose group. Gross pathology of dead animals revealed haemorrhages of the lungs and congested organs. Animals killed at termination of the study after the 14-day observational period had no substance related gross organ changes. The LC_{50} was reported as 439 or 394-489 (95 % confidence interval) mg/m^3 or 0.439 mg/L for both sexes. The study was considered reliable with restriction (Klimisch score 2) by the registrants and SIDS from 2006.

Study 2 (Hazelton Laboratories America, Inc. 1976)

Ten male SD rats/dose were exposed to DOTC in polyethylene glycol as aerosols at dose levels of 0, 0.11, 0.29, 0.44, 0.83 and 0.91 mg/L for 1 h. The nebulizer used was considered to deliver particles with an aerodynamic mass median diameter of 2.5-3.5 µm. At 0.29 mg/L, 4/10 animals died within 24 h. The mortality incidences increased at higher dose levels to 6, 9 and 7 at 0.44, 0.83 and 0.91 mg/L, respectively. One of the animals at 0.44 mg/L died after 24 h and before 72 h. All animals exhibited compound awareness and associated stress symptoms including periodic restlessness alternating with inactivity and preening. The LC_{50} reported was 0.39 (0.28-0.56) mg/L/h corresponding to 0.0975 mg/L for a 4 h period.

Study 3 (Wells Laboratories, Inc. 1976)

Five rats/sex/dose were exposed to the test compound in sesame oil sprayed into air by an atomizer producing droplet sizes between 3-10 µm for 1 h. The concentrations were 0, 15, 25, 50, 90 and 120 mg/L resulting in 0, 3, 6, 7, 8 and 10 deaths, respectively. No details about deaths/sex were given. The LC_{50} reported was 37 (22-62.16) mg/L/h, corresponding to 9.25 mg/L for a 4 h period.

The first of these three studies were reported by the registrants and SIDS to have a Klimisch reliability score of 2, while the third study had a lower score with the rationale that details of toxic effects were not reported other than lethal dose values in the SIDS Initial Assessment Report for SIAM 23 (2006). Several reporting deficiencies in the three available studies were noted by the DS, including lack of data on body weight, individual

clinical signs, gross pathological findings, composition of the test substance and purity, and limited data on particle size distribution. Furthermore, all three studies predated GLP.

The two studies with a reliability score of 2 indicated median lethal concentrations (LC_{50}) that met the criteria for classification as Acute Tox. 2; H330 since their LC_{50} were within the range of 0.05 < ATE ≤ 0.5 mg/L (dust and mists). The third study, with the lowest reliability score, indicated an LC_{50} in the range for Acute Tox. 3; H330. Based on all studies, the DS concluded that DOTC met the criteria for classification as Acute Tox. 2 rather than Acute Tox. 3.

Comments received during public consultation

One comment was received from a member state competent authority (MSCA) and one from industry. Both supported the proposed classification as Acute Tox. 2; H330.

Assessment and comparison with the classification criteria

RAC evaluated the information in the CLH report on the three studies. RAC notes that in the first study, males seem more sensitive compared to female rats shortly after exposure, but after longer observation periods (> 7 days) there is no relevant difference between males and females. Therefore, RAC agrees with the LC_{50} of 439 mg/m^3 calculated for both sexes.

RAC acknowledges that two out of three acute inhalation toxicity studies indicate an LC_{50} between 0.05 and 0.5 mg/L, meeting the criteria for Acute Tox. 2; H330. The third study indicates an LC_{50} in the range of Acute Tox. 3; H330, but has a particle size range between 3-10 µm which is mostly outside the range of 1-4 µm considered to penetrate deep into the lungs (CLP 3.1.2.3.2). Although RAC acknowledges limitations in all studies, it agrees with the former evaluations and the DS that the third study may be considered of more limited value (lower Klimisch score, sizes of most particles likely > 4 µm), and this study is therefore not considered further.

The LC_{50} values from the second study were extrapolated from 1 h to 4 h exposure duration, while the LC_{50} reported in the first study was based on an actual 4 h exposure period resulting in an LC_{50} (0.439 mg/L) just below the criteria cut-off for category 2. The general ATE for Acute Tox. 2 is the same as the lower bound of the criteria (0.05 mg/L) according to Table 3.1.2 of the CLP regulation. The lowest LC_{50} reported was 0.0975 mg/L in the second study with a particle size distribution (MMAD of 2.5-3.5 µm) that is within the recommended range of 1-4 µm for acute inhalation studies. Therefore, RAC considers this LC_{50} appropriate for ATE derivation. RAC notes that only male rats were used in this study, but the two other studies do indicate that male rats are equally or more sensitive as compared to females. The use of the LC_{50} calculated from a study with only males is therefore justified for derivation of an ATE.

In conclusion, RAC supports the proposal of the DS to classify dichlorodioctylstannane as **Acute Tox. 2; H330 (fatal if inhaled)**. In addition, RAC proposes an **ATE value of 0.098 mg/L (dust and mist)**.

10.4 Skin corrosion/irritation

Not evaluated in this CLH proposal.

10.5 Serious eye damage/eye irritation

Not evaluated in this CLH proposal.

10.6 Respiratory sensitisation

Not evaluated in this CLH proposal.

10.7 Skin sensitisation

Not evaluated in this CLH proposal.

10.8 Germ cell mutagenicity

Not evaluated in this CLH proposal.

10.9 Carcinogenicity

Not evaluated in this CLH proposal.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 13: 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**
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.	Dichlorodioctylstannane, 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 3, 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).	Study report, 2004 Appel, M.J. and D.H. Waalkens-Berendsen. (2004)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results [§]	Reference
		Females: Premating phase: ↓ mean body weight gain in the 100 and 300 mg DOTC/kg diet groups (0.28 g, p<0.05 and -4.03 g, p<0.001 respectively, compared to 4.8 g in control) during the first week of the premating period. As compared to controls, a slightly lower body weight was recorded for intermediate (-3.11%, not stat. sign.) and high dose (-4%, not stat. sign.) females at the end of premating period. Gestational phase: ↓ body weight gain in the 300 mg DOTC/kg diet 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/-	

<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>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 (9/9 males, severity score, moderate to severe).

Statistical significant changes in absolute or relative organ weights were reported for adrenals, spleen, kidney, liver and testes in the 300 mg DOTC/kg diet dose group compared to control, however, no adverse histopathological changes were noted.

Females:

↓ absolute and relative thymus weight in all 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></td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results** [§]	**Reference**
		↓ gestation index in the 100 and 300 mg DOTC/kg diet dose groups (71 and 50% respectively, not stat. sign. compared to 86% in control). ↑ mean post-implantation losses in the 100 and 300 mg DOTC/kg diet dose groups (49 and 70% respectively, not stat. sign. compared to 22% in control). ↓ live birth index in the 100 and 300 mg DOTC/kg diet dose groups (53 and 60%, respectively, not stat. sign. compared to 99% in control). ↓ viability index PND 0-4 in the 100 and 300 mg DOTC/kg diet dose groups (74 and 12% 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[ǂ] 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).	
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	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	**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.	Tonk et al., 2011

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results §	Reference
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.	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.	No effects on male body weights. **Organ weights and Histopathology** No information available on F0 animals. **Fertility, parturition and sexual function** Mating and fertility indices, precoital time, mean duration of pregnancy and gestation indices were similar among all groups. **Development** ↓ mean number of live pups per litter at PND 4 in 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 table 20 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 14: 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 15: 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

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

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

No effects on male or female fertility, mating indices, or gestational length were recorded in the available reproductive screening study at dose levels up to and including 300 mg DOTC/kg diet (Appel and Waalkens, 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

Table 16: 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
Prenatal Developmental Toxicity Study OECD TG 414 (no significant deviations) GLP: yes Sprague Dawley rat 25 mated females/group	Dichlorodioctylstannane, 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 (7.0%) and high (10.4%, p<0.05) dose levels as compared to control (1.5%). ↑ post-implantation loss in all treated groups (6.9, 4.9, 6.9% in 10, 100 and 300 mg DOTC/kg diet groups respectively), not statistically significantly different from control (0.8%) and no dose-response relationship. ↑ no. fetuses with skeletal malformations (mainly missing bones in the paws) at the intermediate (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 Registrant(s).	Study report, 2014
Repeated dose 90-day oral	Dichlorodioctylstannane,	See Table 13 for a summary of adverse	Study report,

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
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.	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.	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).	2004 Appel, M.J. and D.H. Waalkens-Berendsen. (2004)
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.	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	See Table 13 for a summary of adverse findings. Main finding was a statistically significant decrease in the mean number of live pups per litter at PND 4 in high dose group, and decreased absolute and relative thymus weight and thymus cellularity in F1 high dose animals on PND 42 compared to control. LOAEL for fertility and developmental effects is considered to be 30 mg DOTC/kg diet. No LOAEL identified for maternal toxicity. NOAEL for maternal toxicity is 30 mg DOTC/kg diet. **Immunotoxicological assessment of F1** <u>Lymphocyte subpopulations – spleen</u> On PND 42 the absolute and relative number of 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-	Tonk et al., 2011

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		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 19, 20 and Annex I.

Table 17: 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 18: 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

For examination of developmental effects three studies 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.

<u>Pre-natal developmental toxicity study, OECD TG 414 (Study report 2014)</u>

In an GLP compliant OECD TG 414 Prenatal Developmental Toxicity Study in rats the main developmental effect was a dose dependent increase ($p < 0.05$ 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 19).

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 19). 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/kg, respectively, as compared to 0 % in the control).

Table 19: 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. (%) Litter basis, no. (%)	1 (0.8) 1 (4.5)	8 (7.0) 6 (28.6)	15 (13.3*) 9 (45.0)	29 (27.1*) 16 (80.0)
Split thoracic vertebrae centrum no. 12	0	1(1)	0	0
Missing caudal 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/kg 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 fetal weight (approx. 4 g in all groups) and the slight difference in mean litter size (10.1 compared to 11.4 fetuses 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 fetal 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/kg 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 20). 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/kg 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 13 and Annex I, Table 4) 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/kg dose groups and the mean pup body weight was decreased at PND 1 – 4 in the 300 mg/kg 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/kg group.

Table 20: Summary of pup data

Dose level	Control	10 mg/kg diet	100 mg/kg diet	300 mg/kg diet
Test substance intake	0 mg/kg bw/day	0.5-0.7 mg/kg bw/day	4.2-6.2 mg/kg bw/day	8.4-17 mg/kg bw/day
Number of pregnant females	7	8	7	8
Mean number of implantations	12.6	13.4	11.3	10.3
Number of dams with total intrauterine death (only implantation sites observed at necropsy)	1	0	0	3
Post implantation loss (%) Mean value Median value [N = number of females]	 22.33 ± 13.159 7 N=7	 20.98 ± 7.114 11 N=8	 49.23 ± 17.453 50 N=7	 69.99 ± 14.713 95[£] N=8
Pups delivered (total) (N)	70	88	72	43
Pups delivered (live + dead; mean) [N= number of litters]	11.67 ± 0.803 N=6	11.00 ± 0.707 N=8	10.29 ± 0522 N=7	8.60 ± 1.208 N=5
Mean viable litter size PND 1 [N= number of litters]	11.50 ± 0.719 N=6	10.50 ± 0.945 N=8	7.60 ± 1.631 N=5	6.50 ± 2.217 N=4
Total no. of live born pups[f] (Live birth index)	69 99	84 95	38[#] 53	26[#] 60
Total no. of stillborn pups[f] (% stillborn)	1 1.4	4 4.5	34[#] 47	17[#] 40
Total number of dead pups PND 0 to PND 4[f]	4	7	10[**]	23[#]
Total number of pups dying perinatally	5	11	44	40
Mean viability index PND 1-4	94	92	74	12
Mean viable litter size PND 4 [N= number of litters]	10.83 ± 0.601 N=6	11.00 ± 0.787 N=7	9.33 ± 0.667 N=3	3.00 ± 0.000 N=1
Pup weight (g) PND 1 (all viable pups) [N= number of litters]	4.76 ± 0.229 N=6	4.74 ± 0.229 N=8	4.19 ± 0.346 (-12%) N=5	3.90 ± 0.088 (-18%) N=4
Pup weight gain (g) PND 1 to PND 4	2.17 ± 0.257	1.86 ± 0.382	1.41 ± 0.584	-0.57 ± 0.000
Pup weight (g) PND 4 (all viable pups) [N= number of litters]	6.93 ± 0.447 N=6	6.69 ± 0.743 N=7	6.10 ± 0.719 N=3	3.10 ± 0.000 N=1
Total number of runts [ǂ] [N= number of litters]	1 N=1	7 N=3	10 N=3	6 N=1

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

Maternal toxicity in the 300 mg DOTC/kg diet dose group during gestation was observed as a statistically significantly decreased mean body weight (from GD 7 and onwards) and at GD 14 and GD 21 the decreases were 12% and 16% respectively compared to the control group. No weight loss was reported in the high dose animals during the gestation period. The decrease in body weight persisted during lactation day 1 (-18% compared to control) and at lactation day 4 (-20% compared to control). Consequently, the body weight gain was also statistically significantly reduced during most of the study period (except for week two of the pre-mating period and lactation day 1-4) and during GD 14-21 the body weight gain was 60% less than control. The total body weight gain from GD 0 to 21 was 65.8, 69.6, 53.4 and 34.4 g in control, 10, 100 and 300 mg DOTC/kg diet, respectively. Excluding the 3

females with intrauterine loss does not affect the mean body weight in the high dose group. Moreover, the lower number of pups (viable + dead) in the high dose group does not account for the difference in maternal body weight compared to control. At 100 mg DOTC/kg diet, the body weight was not significantly affected as compared to control throughout the entire study period. However, during the first week of the premating period, the body weight gain was statistically significantly reduced in the 100 mg DOTC/kg diet dose group as compared to control.

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

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

In a study by Carney et al (2004), determining the effects of feed restriction in rat during in utero and postnatal life on standard reproductive toxicity and developmental immunotoxicity end points, reductions in maternal body weights down to 32% were not considered to cause any significant effects on offspring viability, or litter size at birth or at PND 4. Thus, the decrease (-12 to -20% compared to control) in maternal body weight during gestation at 300 mg DOTC/kg diet is not considered to influence the observed post-implantation losses and pup mortality and are there 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 3 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 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 also in the developing animals 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 10 mg DOTC/kg diet and at 100 and 300 mg DOTC/kg diet the increased incidence on a fetal 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 the same dose levels as the PNDT study, were increased postimplantation 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), 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 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 DOTC have adverse effect on human fetal development.

Classification in Repr. 1B, H360D is warranted since the evidence for developmental toxicity is considered to be *clear*. Based on a dose dependent statistically significant increase in incidence of skeletal malformations (missing bones) in a prenatal developmental toxicity study in rat from 0.8 mg/kg bw/day, a marked decrease in live birth index and increase in number of stillborn pups at 7.2 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 21: 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 22: 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 23: 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 are no relevant studies on toxicokinetics of 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

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 a specific concentration limit for adverse effects on development is considered justified based on the increased incidence of total skeletal malformations (on fetal basis) observed at the ED10 (0.8 mg/kg bw/day). The substance is shown to be of high potency (ED10 $\leq$ 4 mg/kg bw/day) and should therefore be allocated to the high potency group with a SCL of 0.03%.

No classification for effects on or via lactation is warranted.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed to classify DOTC as Repr. 1B; H360D. To assess adverse effects on reproduction, three studies were summarized, a repeated dose 90-day oral toxicity study (OECD TG 408) combined with a reproduction/ developmental screening test according to OECD TG 421, an extended one generation reproduction toxicity study (EOGRTS) similar to OECD TG 443 and an additional pre-natal development study performed according to OECD TG 414. All studies were carried out with the registered substance. An overview of the study designs and results are presented in the table below. A more detailed summary on adverse effects regarding parental and reproductive toxicity is presented in the RAC assessment section.

***Table**. Summary of 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	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.

maturation evaluated for 1 pup/litter, 8 F1 males/group for immune assessment		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.

According to the DS, the studies did not indicate adverse effects on fertility in both males and females up to dose levels of 300 mg/kg diet/day. However, the dose levels used were low, especially in the EOGRT study since it was mainly focused on assessing immunological effects. Therefore, the DS concluded that classification for effects on fertility was not warranted although adverse effects at higher concentrations could not be excluded.

Adverse effects on development were observed in the pre-natal development study and in 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 was limited and that there was no established link between the effects on the thymus and developmental toxicity. Therefore, the DS regarded the developmental effects as relevant.

Based on skeletal malformations (missing bones, considered as rare findings) in the OECD TG 414 study, decreased live birth index along with increased number of stillborn pups at 7.2 and 22.4 mg/kg bw/day and increased post-implantation loss seen in multiple studies, the DS concluded that classification as Repr. 1B; H360D was warranted.

The DS further proposed to add an SCL of 0.03 mg/kg bw/day since a 10 % increase in the incidence (ED_{10}) of total skeletal malformations was caused by about 0.8 mg/kg bw/day of test substance meeting the criteria for the high potency group (ED_{10} ≤ 4 mg/kg bw/day) as outlined in the CLP guidance.

Comments received during public consultation

Two MSCAs commented and supported the proposed classification. One of them added that they agreed with the proposed SCL of 0.03 %.

Two industry representatives provided comments and expressed their disagreement with the proposed classification, because they considered the developmental effects likely to be secondary to maternal toxicity. Additionally, they questioned whether the malformations were true malformations or the result of delayed ossification and whether the results were adequately reported and interpreted considering the staining techniques used for ossification and missing bones.

The DS replied that the authors and registrant(s) had categorized the findings as "malformations" and these could not be interpreted in another way as they did not have the raw data for review. According to the study authors, the malformations were associated with delayed foetal ossification. The DS interpreted this statement as that in addition to the missing bones, increased incidences of poor or incomplete ossification of sternum no. 5 and 6 (statistically significantly different in high-dose group compared to control) and metacarpal no. 5 in 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 but they were not dose-dependent or statistically significant and the study authors therefore considered that these effects were not treatment-related. The DS further clarified that based on the cited text below from the report, it was interpreted that double staining was used and malformations like missing bones or variations such as delayed ossifications should have been picked up and reported:

"*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 [a] 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.

RAC considers the clarification by the DS plausible and therefore interprets the malformations and skeletal variations as described in the study report and by the DS.

Assessment and comparison with the classification criteria

Fertility

Two reproduction studies were available, one reproduction screening study with doses up to 8.4-17 mg/kg bw/day and an EOGRT study using very low doses (up to 1.7-2.1 mg/kg bw/day). In neither of these studies, were effects observed that would support

classification for fertility. However, in the EOGRT study no effects were seen in parental animals and therefore, adverse effects on fertility at higher concentrations cannot be excluded. The EOGRT study was primarily conducted to assess developmental immunotoxicity. In addition, a reproduction screening study cannot be used to exclude effects on fertility, amongst others due to the limited endpoints and power of the experimental design. **As a consequence, RAC proposes not to classify DOTC for adverse effects on sexual function and fertility because there is a lack of relevant data.**

Development

In the single pre-natal development study available (2014) performed with SD rats, 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 summarized 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 table below), although not in a dose-dependent way. As suggested by the DS, RAC considers it possible that these skeletal variations may be milder forms of the malformations (missing bones) in the same position.

Table. Results summary of the OECD TG 414 Pre-natal development toxicity study (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. (%)	1 (0.8)	11 (9.6)	22** (21.0)	47*** (43.9)
Litter basis, no. (%)	1 (4.5)	8 (38.0)	11 (55.0)	19 (95.0)
Metacarpal no. 5 bilateral				
Foetal basis, no. (%)	1 (0.8)	3 (2.6)	12 (11.4*)	37 (34.6*)
Litter basis, no. (%)	1 (4.5)	3 (14.3)	6 (30.0)	18 (90.0)
Proximal phalanx no. 3 bilateral				
Foetal basis, no. (%)	1 (0.8)	9 (7.8)	15 (14.3*)	29 (28.0*)
Litter basis, no. (%)	1 (4.5)	7 (35.0)	10 (50.0)	16 (80.0)
Proximal phalanx no.4 bilateral				
Foetal basis, no. (%)	1 (0.8)	8 (7.0)	15 (13.3*)	29 (27.1*)
Litter basis, no. (%)	1 (4.5)	6 (28.6)	9 (45.0)	16 (80.0)
Variations (total)				
Foetal basis, no. (%)	6 (4.5)	11 (9.6)	10 (9.5)	26* (24.3)
Litter basis, no. (%)	5 (22.7)	7 (33.3)	4 (20.0)	12 (60.0)

No significant maternal toxicity was observed in this study. When compared to controls, the maternal body weight gain and body weight were significantly lower at the highest dose at GD20. However, the corrected body weight was not significantly lower at GD20 (-6.8 %) than that of the controls. Lower thymus weight compared to the controls was reported in maternal animals at an incidence of 7/25 in the mid dose and all animals in the high dose. No raw data on thymus weight was available to the DS and RAC. In addition, thymus effects were absent/limited at the low/mid dose while increased incidences of malformations were already seen in those groups. These data indicate that developmental effects do occur in the absence of measured thymus toxicity. Furthermore, RAC concludes that based on the information available, no direct relationship between the effects on the thymus and effects on development can be established.

In the repeated dose 90-day oral toxicity study (OECD TG 408) combined with a reproduction/developmental screening test (OECD TG 421) (2004), a statistically non-significant, but high incidence of post-implantation loss was observed (50 % and 70 % in the mid and high dose groups, respectively; results summarized in 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 dose, 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 22 and 87 % reduction in postnatal viability (PND1-4) in the mid and high dose groups, 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 (incidence not provided in the CLH report).

***Table**. Results summary of the Combined reproductive screening test (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	11.67 ± 0.80	11.00 ± 0.71	10.29 ± 052	8.60 ± 1.21

litters]	N=6	N=8	N=7	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 CLH report. However, RAC notes that the lower body weights in the high dose group were at least in part due to the high incidence in post implantation losses and to the reduced pup/foetal weights. Moreover, maternal body weight was not significantly lower in the mid dose group as compared to the controls while the increase in post-implantation loss and the decrease in live birth index were already statistically significant at this dose level. RAC concludes that the effects seen in the mid and high dose groups are not secondary to effects on maternal body weight or weight gain. Thymus weight of parental animals was statistically significantly lower in high and mid dose groups compared to the control animals and accompanied by significant lymphoid depletion in both sexes (see the table under the heading "Summary of the Dossier Submitter proposal"). 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 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 concluded to

be not secondary to effects on maternal body weight and weight gain. Therefore, RAC concludes that the adverse effects on development in the combined reproductive screening test are relevant for classification.

The third study (Tonk *et al.*, 2011) summarized by the DS was an EOGRT study similar to OECD TG 443. This EOGRT study focused specifically on developmental immune system toxicity and no maternal toxicity was reported up to the highest dose (1.7-2.1 mg/kg bw/day). These doses resulted in a non-significant increase in post-implantation loss and small but significant increase in postnatal viability. It is to be noted that the highest dose level (1.7-2.1 mg/kg bw/day) in the EOGRT study was lower than the mid dose group in the reproduction screening study, in which also an increase in post-implantation loss was seen. Apart from behavioural changes, maternal toxicity was not assessed. In addition, the dose spacing was rather narrow, which might have affected the derivation of a dose response. In view of the low dose levels, no conclusions on fertility and development can be derived.

Effects on the developing immune system observed included changes in thymus weight and in immunologic cell populations in the pups (see the table under the heading "Summary of the DS's proposal"). 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/day during gestation and to 2.9-5.2 mg/kg bw/day during lactation. The delayed type hypersensitivity (DTH) response, evaluated at PND49, 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/day 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/day), effects on thymus weights were also observed in some maternal animals of the reproductive screening study and of the pre-natal development 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. In conclusion, the effects on the developing immune system are supportive, but not clear evidence for effects on development.

Comparison with the criteria

Clear adverse effects on development were observed in the pre-natal developmental study and combined reproductive screening study.

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 are considered as supportive evidence include: reduced ossification partially in the same position as the missing bones (at lower concentrations),

small increase in post implantation loss and postnatal viability and an increase in DTH response in the EOGRT study. Reduced pup weight, increased number of runts (not dose-dependent) and cold pups in the combined reproductive screening test.

RAC concludes that these effects warrant classification as **Repr. 1B; H360D (May damage the unborn child)**.

The DS proposed to add an SCL of 0.03 % based on an ED_{10} of 0.8 mg/kg bw/day for total skeletal malformations. The DS did not explicitly explain how the ED_{10} was calculated. RAC notes that the lowest concentration in the pre-natal developmental study was 0.8 mg/kg bw/day and that the incidence of total skeletal malformations observed at that dose was 9.6 %. The control incidence was 0.8 % and therefore the corrected ED_{10} should be higher than 0.8 mg/kg bw/day. However, since the cut-off criteria for the high potency group according to the CLP guidance is 4 mg/kg bw/day, RAC concludes that the ED_{10} for skeletal malformations is below 4 mg/kg bw/day and that **a SCL of C ≥ 0.03 % is therefore justified.**

Lactation

RAC agrees with the DS that no effects were observed that can be solely attributed to exposure via lactation. Therefore, **no classification for effects on or via lactation is warranted**.

Conclusion

In conclusion, RAC concurs with the DS that **dichlorodioctylstannane should be classified as Repr. 1B; H360D with a SCL of 0.03 %**.

10.11 Specific target organ toxicity-single exposure

Not evaluated in this CLH proposal.

10.12 Specific target organ toxicity-repeated exposure

Not evaluated in this CLH proposal.

10.13 Aspiration hazard

Not evaluated in this CLH proposal.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not evaluated in this CLH proposal.

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Not evaluated in this CLH proposal.

13 ADDITIONAL LABELLING

Not applicable.

14 REFERENCES

Appel, K.E. (2004). Organotin compounds: Toxicokinetic aspects. Drug Metab. Rev., 36 (3&4), 763-786.

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.

Carney EW, Zablotny CL, Marty MS, Crissman JW, Anderson P, Woolhiser M, Holsapple M. (2004) The effects of feed restriction during in utero and postnatal development in rats. Toxicol Sci., 82(1):237-49.

ECHA (2011). PBT/vPvB evaluation of substituted mono- and dioctyltin compounds http://echa.europa.eu/documents/10162/8a50d5ca-d720-4fd3-a0a1-06522b92bea4

ECHA dissemination, 2016a. http://echa.europa.eu/sv/registration-dossier/-/registered-dossier/14165

ECHA dissemination, 2016b: https://echa.europa.eu/sv/information-on-chemicals/cl-inventory-database/-/discli/details/66503

Naßhan, H. (2016). Dioctyltin dichloride [DOTC], CAS number: 3542-36-7. In-vitro metabolism study. Galata Chemicals GmbH, Lampertheim, Germany.

OECD (2006) SIDS Initial Assessment Report on the chemical category of Dioctyltin dichloride and selected thioesters, for SIAM 23, Jeju, South Korea, 17–20 October 2006.

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.

Sachsse K, Ullmann L, Voss G & Hess R (1973) Measurement of inhalation toxicity of aerosols in small laboratory animals. in: Experimental model systems in toxicology and their significance to man. Proc. Eur. Soc. Study of Drug Toxicity Vol XV. Excerpta Medica Intern. Congress Series No 311, Zurich [Sh sub no 3308 A3162/11 Box 82 Vol 21]

Seinen, W., Vos, J.G., Van Krieken, R., Penninks, A., Brands, R., Hooykaas, H. (1977) Toxicity of organotin compounds III. Suppression of thymus-dependent immunity in rats by di-n-butyltindichloride and di-n-octyltindichloride. Toxicol. Appl. Pharmacol. 42, 213–224.

Smialowicz, R.J., Riddle, M.M., Rogers, R.R., Rowe, D.G., Luebke, R.W., Fogelson, L.D., Copeland, C.B. (1988) Immunologic effects of perinatal exposure of rats to dioctyltin dichloride. J. Toxicol. Environ. Health 25, 403–422.

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

Tonk EC, de Groot DM, Penninks AH, Waalkens-Berendsen ID, Wolterbeek AP, Piersma AH, van Loveren H. (2011) Developmental immunotoxicity of di-n-octyltin dichloride (DOTC) in an extended one-generation reproductive toxicity study. Toxicol Lett., Jul 28;204(2-3):156-63.

15 ANNEXES

Annex I to the CLH-report