Committee for Risk Assessment

RAC

Annex 1

Background document

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

2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate; [DOTE]

EC Number: 239-622-4
CAS Number: 15571-58-1

CLH-O-0000001412-86-257/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

30 November 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:

2-Ethylhexyl-10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate; DOTE

EC Number: **239-622-4**

CAS Number: **15571-58-1**

Index Number: **050-027-00-7**

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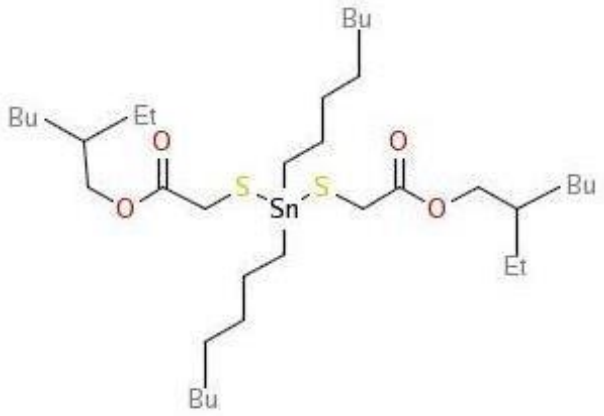
1 IDENTITY OF THE SUBSTANCE

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)	8-Oxa-3,5-dithia-4-stannatetradecanoic acid, 10-ethyl-4,4-dioctyl-7-oxo-, 2-ethylhexyl ester
Other names (usual name, trade name, abbreviation)	Dioctyltin bis(2-ethylhexyl)thioglycolate; Dioctyltin bis(2-ethylhexyl)mercaptoacetate; DOTE; $DOT(EHMA)_2$
EC number (if available and appropriate)	239-622-4
EC name (if available and appropriate)	2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate
CAS number (if available)	15571-58-1
Other identity code (if available)	
Molecular formula	$C_{36}H_{72}O_4S_2Sn$
Structural formula	
SMILES notation (if available)	O=C(CS[Sn](SCC(=O)OCC(CC)CCCC)(CCCCCCCC)CCCCCCCC)OCC(CC)CCCC
Molecular weight or molecular weight range	751.79 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	The molecule contains two chiral carbon atoms in the 2-ethylhexyl substituent of the acetic ester group. The substance is not known to show optical activity. It is unlikely that one of the possible enantiomers shows different reactivity.
Degree of purity (%) (if relevant for the entry in Annex VI)	> 90 % - < 99.9 %

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)
2-Ethylhexyl-10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate **EC no.: 239-622-4** **CAS No 15571-58-1**	> 90 % / <99.9 % (w/w)	Repr. 1B H360D GHS08 Dgr	Acute Tox. 4 (H302) Skin Sens. 1A (H317) Repr. 1B (H360d) STOT RE 1 (H372) Aquatic Acute 1 (H400) Aquatic Chronic 1 (H410)

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
see conf. Annex				

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

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
-					

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

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
-				

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: Proposed harmonised classification and labelling according to the CLP criteria

	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	050-027-00-7	2-ethylhexyl 10-ethyl-4,4- dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate	239-622-4	15571-58-1	Repr. 1B	H360D	GHS08 Dgr	H360D			
Dossier submitters proposal					**Modify** Repr. 2 **Add** STOT RE 1 Aquatic Chronic 2	**Modify** H361d **Add** H372 (thymus) H411	GHS08 GHS09 Dgr	H361d H372 (thymus) H411			
Resulting Annex VI entry if agreed by RAC and COM					Repr. 2 STOT RE 1 Aquatic Chronic 2	H361d H372 (thymus) H411	GHS08 GHS09 Dgr	H361d H372 (thymus) H411			

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

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

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

		CLP Regulation (EC) No1272/2008
2 October 2013	944/2013/EU, Amendment of Annex VI, Tables 3.1 and 3.2 CLP as follows	Repr 1B H360D GHS08 Dgr
8 June 2012	RAC adopted opinion that DOTE should be classified and labelled as follows	Repr 1B H360D GHS08 Dgr
25 March 2011	Arkema on behalf of ETINSA submitted CLH dossier with the following proposed classification.	Repr. 2 H361d

RAC general comment

Substance abbreviations used throughout the text:

DOTE: dioctyltin bis(2-ethylhexyl mercaptoacetate); 2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate.
MOTE: monooctyltin tris(2-ethylhexyl mercaptoacetate
DOTI: dioctyltin bis(isooctyl mercaptoacetate)
MOTI: monooctyltin tris(isooctyl mercaptoacetate)
DOTC: dioctyltin dichloride
DOTEC: dioctyltinchloro 2-ethylhexyl mercaptoacetate

DOTE contains two stable octyl groups and two labile 2-ethylhexyl-mercaptoacetate groups potentially available to hydrolysis. Commercially produced DOTE may contain varying concentrations of MOTE as an impurity (Costlow, 2017). Some toxicological tests have also been conducted using DOTE containing 20-30% MOTE (e.g., DOTE:MOTE, 80:20). MOTE differs from DOTE by containing one less octyl group and one extra 2-ethylhexyl-mercaptoacetate group.

DOTE is a large molecule, and the same applies to the read-across substance DOTI. DOTI and DOTE are isomers differing only slightly in the structure of the C-8 alcohol of the mercaptoester ligand (either iso-octanol or 2-ethylhexanol, respectively). Since these alcohols are so close in structure, their respective mercaptoacetate esters are expected to have very similar physicochemical and toxicological properties, including hydrolysis products.

It has previously been assumed that both DOTE and DOTI quickly hydrolyse in the gastrointestinal (GI) tract to the dichloride DOTC, and that DOTC is the active metabolite of both substances. DOTE has therefore previously been assessed based on read-across to studies conducted on DOTC and DOTI:MOTI (RAC, 2012). A new study was conducted in order to specifically examine the hydrolysis of DOTE. This study reported that the monochloride ester (DOTEC; still containing one 2-ethylhexylmercaptoacetate group) was the only identifiable hydrolysis product after several days in 0.1 M HCl. Costlow *et al.* (2017)

reported that DOTE hydrolysed to 70.8 Mol.% DOTEC, while 23 Mol.% remained unreacted and <2 Mol.% consisted of unidentified reaction products (Anonymous, 2015; later published as Costlow *et al*., 2017).

The dossier submitter (DS) stated that no DOTC is formed during *in vitro* hydrolysis of DOTE. It is noted that, the *in vivo* metabolism of DOTE and of its monochloride hydrolysis product (DOTEC) have not been studied, and the lack of information on further enzymatic metabolism, absorption, and potential toxicity, hamper the assessment of mode of action (MoA) and toxicity of DOTE. Likewise, the MoA for the toxicity of DOTC and DOTI are not fully known.

For these reasons, the new hydrolysis study describes the abiotic 'chemical' fate of DOTE at low pH, but does not inform about the *in vivo* fate of DOTE and its transformation products. Moreover, the results of the toxicity studies with DOTC, DOTI, and DOTE all show very similar adverse effects on the immune system.

RAC is of the view that studies on DOTE itself, DOTE:MOTE mixtures, and the structurally very similar analogues DOTI and DOTI:MOTI, should be considered in the hazard assessment of DOTE.

Studies cited in the CLH report are conducted using:

DOTE	90 days oral repeated dose toxicity study; similar to an OECD TG 408 study (Anonymous, 1970)
DOTE:MOTE	90 days oral repeated dose toxicity study; similar to an OECD TG 408 study (Anonymous, 1974)
DOTI:MOTI	OECD TG 416; Two-generation toxicity study (Anonymous, 1997)
DOTE	OECD TG 414; Developmental toxicity study in rabbits (Anonymous, 2014a)
DOTE	OECD TG 414; Developmental toxicity study in mice (Anonymous, 2014b)
DOTI:MOTI	Developmental toxicity study in rats; similar to an OECD TG 414 study (Battenfeld, 1991)
DOTI:MOTI	Developmental toxicity study in rabbits; similar to an OECD TG 414 study (Battenfeld, 1992)
DOTI:MOTI	Developmental toxicity study in mice; similar to an OECD TG 414 study (Faqi *et al*., 2001)
DOTC	OECD TG 421; Reproduction/developmental toxicity screening study (Appel & Waalkens-Berendsen, 2004)

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Reason for a need for action at Community level:

- New data
- Differences in self-classification (STOT RE, Aquatic Toxicity)

Further detail on need of action at Community level

According to Article 36(1), a substance that fulfils the criteria set out in Annex I of the CLP regulation for the following shall normally be subject to harmonized classification and labelling in accordance with Article 37:

(d) Reproductive toxicity, Category 1A, 1B or 2 (Annex I, section 3.7).

According to Article 37(6), manufacturers, importers and downstream users who have new information which may lead to a change of the harmonised classification and labelling elements of a substance in Part 3 of Annex VI shall submit a proposal in accordance with the second subparagraph of paragraph 2 to the competent authority in one of the Member States (MSCA) in which the substance is placed on the market.

CLH dossiers proposing a revision of a specific hazard class from an existing entry in Annex VI to the CLP Regulation can only be submitted directly to ECHA by an MSCA (Article 37(1), CLP). A revision of an existing entry can be justified in the event that new data has become available since the harmonised classification was agreed. The new data could, for example show that classification in a different category is justified.

Currently the harmonized classification of DOTE by the Commission Regulation (EU) No 944/2013 is Repr. 1B (H360D). The RAC assessment and the resulting opinion from 2012 are based solely on read across data.

Two new key studies are available which investigated the developmental toxicity with the DOTE substance itself. These new GLP studies complement earlier flawed studies with the most sensitive species, mice and rabbits. The new studies were conducted with 96 % purity DOTE and are fully complete guideline studies (OECD 414) following GLP which evaluated gestational integrity, external anomalies, soft tissue anomalies and skeletal anomalies.

Recent *in vitro* hydrolysis studies at gastric pH value of 1.2 and pH values of 9, 7 and 4, using 119-Sn-NMR to identify the reaction products, clearly show that Dioctyltinchloro 2-ethylhexylmercaptoacetate (DOTEC) is the only identifiable hydrolysis product of Dioctyltin bis(2-ethylhexylmercaptoacetate) (DOTE). No Dioctyltin dichloride (DOTC) could be detected under the conditions of the study.

Thus, industry found that the read across from DOTC to DOTE is no longer considered appropriate without restrictions. To their view the studies with DOTC (since DOTC is not formed during acidic hydrolysis) may have low significance for the hazard assessment and studies with dioctyltin bis(isooctyl mercaptoacetate)/Monooctyltin tris(isooctyl mercaptoacetate) 80:20 (DOTI/MOTI 80:20) on mice and rabbits and lower significance for the hazard assessment than studies with DOTE.

This CLH report was initially drafted by Industry (proposing no classification on reproductive toxicity) and modified by the German Competent Authority according to their evaluation of the data.

5 IDENTIFIED USES

DOTE is mostly used as a stabiliser in plastic. The identified uses of the substance cover uses at industrial sites.

6 DATA SOURCES

REACH dossiers (12/2016)

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	Liquid, clear colourless to slightly yellow	Baltussen E, 2010, Determination of physico-chemical properties of dioctyltin bis(2-ethylhexylmercaptoacetate), NOTOX B.V., Hambakenwetering 7, 5231 DD 'S-Hertogenbosch, The Netherlands	
Melting/freezing point	-39 °C	Elf Atochem NA. 1999. Material Safety Data Sheet. Thermolite (R) 890 Stabilizer. Revision 2. Issued 31 August 1999.	
Boiling point	275 °C	Baltussen E, 2010, determination of the boiling temperature of DOTE by differential scanning calorimetry, NOTOX BV, Hambakenwetering 7, 5231 DD Hertogenbosch, NL	The substance decomposes at T >275°C and normal pressure without boiling.
Relative density	1.07 g/cm^3 at 20 °C	Elf Atochem NA. 1999. Material Safety Data Sheet. Thermolite (R) 890 Stabilizer. Revision 2. Issued 31 August 1999.	
Vapour pressure	< 2.50 x 10^{-4} Pa	Baltussen E, 2010, Determination of physico-chemical properties of dioctyltin bis(2-ethylhexylmercaptoacetate), NOTOX B.V., Hambakenwetering 7, 5231 DD 'S-Hertogenbosch, The Netherlands	Due to the behaviour of the test material in the equipment, an exact value for the vapour pressure could not be calculated. Three tests were performed. Significant differences between the individual measurements were observed. The vapour pressure was therefore reported to be lower than the highest measured value at < 2.50 x 10^{-4} Pa
Surface tension			not technically feasible as the water solubility of the substance is less than 0.1 mg/l.
Water solubility	0.000000001 µg/l	EPI-QSAR	study technically not feasible
Partition coefficient n-octanol/water	Log Pow = 15.34	EPI-QSAR	study technically not feasible

Property	Value	Reference	Comment (e.g. measured or estimated)
Flash point	182 °C	Baltussen E, 2010, Determination of physico-chemical properties of dioctyltin bis(2-ethylhexylmercaptoacetate), NOTOX B.V., Hambakenwetering 7, 5231 DD 'S-Hertogenbosch, The Netherlands	Pensky-Martens closed cup method.
Flammability	Not flammable		
Explosive properties	Not explosive		Expert judgement based on physico-chemical properties and the substance's structure
Self-ignition temperature	390 °C at 989.6 - 999.2 hPa.	Baltussen E, 2010, Determination of physico-chemical properties of dioctyltin bis(2-ethylhexylmercaptoacetate), NOTOX B.V., Hambakenwetering 7, 5231 DD 'S-Hertogenbosch, The Netherlands	
Oxidising properties	No oxidising properties		Expert judgement based on physico-chemical properties and the substance's structure
Granulometry	Not relevant		Substance is liquid
Stability in organic solvents and identity of relevant degradation products	Substance is stable in apolar solvents		
Dissociation constant			Technically not feasible
Viscosity	57.9 mm2/s at 20 °C 24.0 mm2/s at 40 °C	Baltussen E, 2010, Determination of the kinematic viscosity of dioctyltin bis (2-ethylhexylmercaptoacetate), NOTOX B.V., Hambakenwetering 7, 5231 DD 'S-Hertogenbosch, The Netherlands	

The following QSAR estimations were done for water solubility and Log Pow:

Water Sol: 2.94e-011 mg/L
SMILES : O=C(CS[Sn](SCC(=O)OCC(CC)CCCC)(CCCCCCCC)CCCCCCCC)OCC(CC)CCCC
CHEM: MOL FOR: C36 H72 O4 S2 Sn1 MOL WT: 751.80
WSKOW v1.42 Results: Log Kow (estimated): 15.35 Log Kow (experimental): not available from database Log Kow used by Water solubility estimates: 15.35 Equation Used to Make Water Sol estimate: Log S (mol/L) = 0.693-0.96 log Kow-0.0092(Tm-25)-0.00314 MW + Correction Melting Pt (Tm) = -39.00 deg C (Use Tm = 25 for all liquids) Correction(s): Value
No Applicable Correction Factors Log Water Solubility (in moles/L): -16.408
Water Solubility at 25 deg C (mg/L): 2.94e-011

Log Kow(version 1.68 estimate): 15.35
SMILES : O=C(CS[Sn](SCC(=O)OCC(CC)CCCC)(CCCCCCCC)CCCCCCCC)OCC(CC)CCCC
CHEM: DOTE MOL FOR: C36 H72 O4 S2 Sn1 MOL WT: 751.80

TYPE	NUM	LOGKOW FRAGMENT DESCRIPTION	COEFF	VALUE
Frag	6	-CH3 [aliphatic carbon]	0.5473	3.2838
Frag	26	-CH2- [aliphatic carbon]	0.4911	12.7686
Frag	2	-CH [aliphatic carbon]	0.3614	0.7228
Frag	2	-C(=O)O [ester, aliphatic attach]	-0.9505	-1.9010
Frag	2	-S- [aliphatic attach]	-0.4045	-0.8090
Frag	1	Tin [Sn]	1.0600	1.0600
Const	-	Equation Constant	-	0.2290

Log Kow = 15.3542

8 EVALUATION OF PHYSICAL HAZARDS

Physical hazards are not evaluated in this dossier

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 9: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
in vitro study DOTE- Hydrolysis as a Function of pH (OECD TG 111)	The study showed that DOTE at pH 9, 7 and 4 can be considered hydrolytically stable. After 5 days at 50 °C less than 10 % DOTE was hydrolyzed (t 0.5 25 °C > 1 year). Under the simulated gastric conditions (0.1 M HCl / pH 1.2 / 37 °C, 5 days) DOTE was hydrolyzed to DOTEC, its monochloride ester.	2 (reliable with restrictions) key study experimental result Test material (EC name): 2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate	Anonymous (2015)
in vitro study rat and human epidermis dermal Exposure regime: 24 hour(s) Doses/conc.: 17,007 µg tin/cm^2 OECD Draft Guideline for Dermal Delivery and Percutaneous Absorption: In Vitro Method [OECD TG 428]	Main ADME results: Absorption: Absorption of tin from DOTE through rat epidermis significantly overestimates absorption through human epidermis. Evaluation of results: bioaccumulation potential cannot be judged based on study results	2 (reliable with restrictions) key study experimental result **Test material: DOTE** **(EC name): 2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate**	Ward, R.J. (2003)

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

Hydrolysis:

A new *in vitro* study simulating mammalian gastric conditions, using 119-Sn-NMR spectroscopy to identify the reaction products, clearly shows that Dioctyltin chloro 2-ethylhexyl mercaptoacetate (DOTEC) is the only identifiable hydrolysis product of DOTE (Dioctyltin bis(2-ethylhexyl mercaptoacetate)). No Dioctyltin dichloride (DOTC) could be detected under the conditions of the study.

This *in vitro* study can be assumed to reasonably predict the behaviour of DOTE within the gastric contents, but it does not elucidate the full mode-of-action for oral toxicity studies because neither the full spectrum of possible *in vivo* metabolites, nor the toxicologically active species *in vivo*, are known. No definitive conclusion can be drawn for the mode-of-action from this study for repeated dose, *in vivo* genotoxicity, reproduction, and developmental effects, when they are assessed using oral administration.

The *in vitro* study leaves open the question whether any DOTE or DOTEC formed in the gastric environment might be taken up systemically and then be further metabolised into other dioctyltin species, monooctyltin species or non-organotin species. However, in the absence of any additional data read across from studies using DOTC as test material, must be considered carefully in the discussion of the reproductive toxicity endpoints.

The same study showed that DOTE at pH 9, 7 and 4 can be considered hydrolytically stable. After 5 days at 50 °C less than 10 % DOTE was hydrolyzed ($t_{0.5}$ 25°C > 1 year). However the study only considers abiotic conditions. The situation in an organism where metabolizing enzymes are present might be different.

Inhalation and dermal absorption:

With respect to inhalation and dermal mammalian toxicity, the thioesters have much higher molecular weights and considerably lower volatility than the chloride. The high molecular weights of the esters reduce their potential for absorption via the dermal route, and their volatility reduces their potential for absorption via the inhalation route relative to the chloride.

The absorption of DOTE was measured *in vitro* (Ward 2003) through both occluded and unoccluded human and rat epidermis. The absorption through rat epidermis was much faster than through human epidermis:

HUMAN EPIDERMIS: A dose of undiluted liquid DOTE, corresponding to 17,007 µg tin/cm² was determined to slightly reduce the measured electrical resistance across rat skin. Electrical resistance is one indicator of the integrity of the barrier function of the epidermis. The measured reduction was minimal [3.13 ohms versus $\leq$ 3.00 ohms indicative of undamaged skin]. Because human skin is typically more robust than rat skin, the authors chose to continue with the 17.007µg tin/cm^2 dose, which was the highest dermal dose achievable, and both the rat and the human skin samples were judged to be entirely adequate for the integrity of the dermal penetration test.

From the occluded and unoccluded applications, the rates of tin absorption over the 0-24 h exposure period were below the limit of quantification (0.001 µg/cm²/h). In terms of percent applied tin, 0.0001 % was absorbed from the occluded dose, and 0.0001 % was absorbed from the unoccluded dose after 24 hours of exposure.

RAT EPIDERMIS: Absorption of tin through rat epidermis was much faster than through human epidermis. From the occluded application, the maximum rate of tin absorption (0.035 µg/cm^2/h) occurred during 16-24 hours of exposure, and the mean rate of tin absorption over the whole 24-h exposure period was 0.021 µg/cm²/h. From the unoccluded application, the maximum rate of tin absorption occurred during 12-24 hours of exposure and was 0.033 µg/cm^2/h. The mean rate of tin absorption over the whole 24-h exposure period was 0.025 µg/cm^2/h. In terms of percent applied tin, 0.003 % was absorbed from the occluded dose, and 0.004 % was absorbed from the unoccluded dose after 24 hours of exposure. The overall recovery of tin from the test system after 24-h exposure was low and may be due to adsorption of the test substance to the glass equipment used. The recovery was 45.5 % (human) and 25.2 % (rat) of the applied occluded doses, and 29.6 % (human) and 30.5 % (rat) were recovered from the unoccluded test systems. Of the recovered tin, 2.1 % (human) and 5.5 % (rat) were obtained from the surface of the epidermis and donor chamber. The mean amounts of tin absorbed by 24 hours were 0.010 µg/cm² (unoccluded) and 0.011 µg/cm² (occluded) through human epidermis and 0.641 µg/cm² (unoccluded) and 0.547 µg/cm² (occluded) through rat epidermis.

These results show that the absorption of tin from DOTE through rat epidermis significantly overestimated the absorption from human epidermis. However, by 24 hours only a small amount of the applied tin (3 % in human and 1 % in the rat) is associated with the epidermis and is not regarded as systemically available. Thus, based on the low recovery the reliability of the study is highly questionable.

10 EVALUATION OF HEALTH HAZARDS

Read across approach for repeated exposure assessment:

The CLH dossier of 25 March 2011 and the RAC opinion of 8 June 2012 applied read across among three chemicals:

- DOTE
- DOTI
- DOTC

These substances are all members of the dioctyltin family of compounds, and the read across characteristics for this family were discussed in depth under the HPV program: SIDS Initial Assessment Reports “Dioctyltin dichloride and selected thioesters”. The dioctyltins (DOT) are tetravalent tin compounds comprised of two octyl groups bound to tin through tin-carbon bonds, and two other groups bound to tin.

The rationale for the read across was based on a simulated gastric hydrolysis study proposing that DOTE (dioctyltin bis(2-ethylhexylmercaptoacetate)) would readily hydrolyse under physiological conditions to

DOTC (dioctyltin dichloride). Thus DOTC was selected as an anchor compound and surrogate for the mammalian toxicology endpoints of repeated dose, *in vivo* genetic toxicity, reproduction, and developmental effects, when they are assessed using oral administration.

An inherent uncertainty exists in this approach due to the fact that the resulting tin species cannot be analysed and characterized as such by gas chromatography. The analytically necessary derivatisation modifies the chemical structure into tetraalkyltin derivatives, which are available to the gas phase. Thus a distinction between different ligands bound to the dioctyltin moiety during the simulated gastric hydrolysis is no longer possible.

Recent *in vitro* hydrolysis studies under simulated mammalian gastric conditions, using 119-Sn-NMR to identify the reaction products, clearly show that dioctyltinchloro 2-ethylhexylmercaptoacetate (DOTEC) is the only identifiable hydrolysis product. No dioctyltin dichloride (DOTC) could be detected under the conditions of the study.

Recent *in vitro* hydrolysis studies at gastric pH value of 1.2 and pH values of 9, 7 and 4, using 119-Sn-NMR to identify the reaction products, show that dioctyltinchloro 2-ethylhexylmercaptoacetate (DOTEC) is the only identifiable hydrolysis product. No dioctyltin dichloride (DOTC) could be detected under the conditions of the study (detection limit 0.5 % on pure DOTC).

However, the study does not allow to conclude, if and which other dioctyl species might be formed after systemic uptake of DOTE and/or DOTEC under *in vivo* conditions. Thus, read across to DOTC cannot be disregarded based on the results of this hydrolysis study. In addition to the read across to DOTC, the CLH dossier of 25 March 2011 and the RAC opinion of 8 June 2012 used a read across from the structural analogue substance DOTI, diisooctyl 2,2'-[(dioctylstannylene)bis(thio)]diacetate (CAS No. 26401-97-8).

Furthermore, read across at an “analogue level” as described in the SIDS report (SIDS Initial Assessment Report “Esters of Thioglycolic Acid” prepared for SIAM 23 (2006)) was applied to data on diisooctyl 2,2'-[(dioctylstannylene)bis(thio)]diacetate (CAS No. 26401-97-8, also named dioctyltin bis(isooctyl mercaptoacetate), dioctyltin bis(IOMA), DOTI). DOTI and DOTE are isomers differing only slightly in the structure of the C-8 alcohol of the mercaptoester ligand (either *iso*-octanol or 2-ethylhexanol, respectively). Since these alcohols are so close in structure, their respective mercaptoacetate esters are expected to have very similar physicochemical and toxicological properties, including hydrolysis products.

The recently conducted developmental toxicity studies with DOTE in two species may have higher significance than those with DOTI for reproduction hazard assessment. Therefore the significance of any effects read across from DOTI to DOTE is regarded in a weight-of-evidence assessment and the significance weighted accordingly (see Summary Tables of Dose-Response (Table 14 and Table 15) in Chapter 10.10.10, which suggest that DOTI might have a higher toxicological potency with respect to reproductive toxicity).

10.1 Acute toxicity - oral route

This point is not proposed for harmonization.

10.2 Acute toxicity - dermal route

This point is not proposed for harmonization.

10.3 Acute toxicity - inhalation route

Not evaluated in this dossier.

10.4 Skin corrosion/irritation

This point is not proposed for harmonisation.

10.5 Serious eye damage/eye irritation

This point is not proposed for harmonisation.

10.6 Respiratory sensitisation

Not evaluated in this dossier.

10.7 Skin sensitisation

This point is not proposed for harmonisation.

10.8 Germ cell mutagenicity

This point is not proposed for harmonisation.

10.9 Carcinogenicity

Not evaluated in this dossier

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 10: 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
Method: OECD Guideline 416 (Two-Generation Reproduction Toxicity Study) GLP key study 2 (reliable with restrictions) Rat (Sprague-Dawley) 25 male/25 female per group two-generation study oral: feed	**Test material: DOTI/MOTI 80:20** **In original study named: Dioctyltin bis(IOMA) [CAS no. 26401-97-8] : Octyltin tris(IOMA) [CAS no. 26401-86-5] (purity 78.8 : 16.9 %)** 0, 20, 60, and 200 ppm (nominal in diet) (P: ~1.5, 4.4, 15 mg test material/kg bw/d) (F1: ~1.6, 4.7, 16 mg test material/kg bw/d) Exposure: Duration of dosing of F0 generation males - 10 weeks prior to mating, during mating (3 weeks), and post mating until sacrifice; females - 10 weeks prior to mating and during mating. Mated females continued to receive test diets during gestation and lactation; unmated females received test diets until sacrifice. Test diets were prepared weekly and analysed for homogeneity and stability. Duration of dosing of F1 generation:	NOAEL (P): 20 ppm based on test material (~1.5 mg/kg bw/d male/female) (based on a reduction in the relative thymus weight of males) NOAEL (F1): 20 ppm based on test material (male/female) (The NOAEL for the F1 generation until weaning was 20 ppm (~1.6 mg/kg bw/d), based on a decrease in relative thymus weights in male and female pups at 60 ppm (~4.7 mg/kg bw/d). The NOAEL for the F1 generation post lactation was 20 ppm (~1.6 mg/kg bw/d, based on a slight decrease in the relative thymus weight of males and an increase in stillbirths at 60 ppm (~4.7 mg/kg bw/d). NOAEL (reproductive organs, fertility, gestational integrity) 200 ppm based on test material. No effect on reproductive organs or reproductive capacity was observed up to and including the highest dose tested.	Anonymous (1997)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	males - 14 weeks (starting at the end of lactation prior to mating), during mating (3 weeks), and post mating until sacrifice; females - 14 weeks (starting at the end of lactation prior to mating) and during mating (3 weeks). (continuously (in diet))	NOAEL (teratogenicity): 200 ppm based on test material (No teratogenic effect was observed within the limits of the experimental design up to and including the highest dose tested)	

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

10.10.2.1 Summary of effects on fertility

In the two generation study performed under GLP and according to OECD 416 (Anonymous, 1997), the test material consisting of 78.8 % DOTI (CAS No. 26401-97-8) and 16.9 % MOTI (Mono-n-octyltin tris(2-ethylhexylmercaptoacetate), CAS No. 26401-86-5, also named Octyltin tris(IOMA)) was administered to the F0 generation 10 weeks prior to mating, during mating (3 weeks) and post-mating. DOTI and DOTE are isomers, they are expected to have similar reactivity and to be toxicologically equivalent. However, the recently conducted developmental toxicity studies with DOTE in two species (see Table 11, Section 10.10.4) support that DOTI might have a higher toxicological potency with respect to reproductive toxicity (see Summary Tables of Dose-Response (Table 14 and Table 15) in Chapter 10.10.10).

The F_1 generation was treated 14 weeks during premating, 3 weeks during mating. Females continued to receive the test material during gestation and lactation.

The following treatment-related effects were observed:

P generation:

- Mortality: 1 male died at 200 ppm test material in the diet
- No substance-related mortality or changes in behaviour or external appearance
- Absolute food consumption reduced in females at 200 ppm (-6 % on lactation days 7-14, -9 % on lactation days 14-21)
- No substance-related changes in Mean pre-coital time, Mating Index, Pregnancy Rate, Fertility Index, Gestation Index, Mean pregnancy duration, live-born pups, or stillbirths
- Viability index slightly increased at 200 ppm (96.2 % in the controls vs. 98.6 % s).
- Lactation index significantly decreased at 200 ppm diet (88.6 % vs. 94.4 % in controls, $p < 0.05$) after 21 days lactation.
- Pup body weights not significantly decreased (approx. -3-4 % at 200 ppm) at birth, but significantly decreased at 200 ppm in both sexes after 14 and 21 days lactation (-19 to -21 %, $p < 0.01$; male: 34.58 g vs. 42.59 g for controls; female: 33.54 g vs. 42.27 g for controls).
- Slight delay in vaginal opening at 200 ppm (35.4 days vs. 33.2 days for controls).
- Slight decrease in relative thymus weight in males at 60 ppm (approx. 4.4 mg test material/kg bw/day); significant decrease in relative thymus weight in both sexes at 200 ppm.
- Increased incidence of thymic involution at 200 ppm (1/25 for control males; 9/25 for 200 ppm males; 0/25 for control females; 4/25 for 200 ppm females; significant for males only) at microscopic examination.
- Functional tests and examination of morphological landmarks revealed no substance-related findings at all dose-levels except for a slightly delayed in vaginal opening at 200 ppm.
- Microscopic examination of the other organs found no substance-related changes.

F_1 generation:

- No mortality.
- Body weight: significant reduction in F_1 males during their growth phase at 200 ppm [89 % of controls on test week 1 (TW1); 90 % of controls TW8; 91 % of controls TW22].
- Food consumption: generally within historical control values for both sexes with occasional statistical differences; not reduced in females during gestation, but reduced at 200 ppm and significant on lactation days 14-21 [-12 % for LD1-7, -15 % for LD7-14, -17 % for LD14-21].
- No substance-related changes in Mean pre-coital time, Mating Index, Pregnancy Rate, Fertility Index, Gestation Index, Mean pregnancy duration, or live-born pups
- Statistically significant increase in number of stillbirths at 200 ppm (26 vs. 5 in controls).
- Viability index: decreased at 200 ppm (82.0 % vs. 95.7 % in controls).
- Pup mortality: no significant differences from birth to PND4 prior to culling; significantly increased mortality at 200 ppm from PND4 after culling through PND21.
- Lactation index: decreased at 200 ppm (82.3 % vs. 94.4 %).
- Pup body weight for F_2 generation: reduced at 200 ppm; no significant differences at birth; significantly reduced at 200 ppm for males and females on PND 4, significantly reduced at 200 ppm for females on PND 7, 14, and 21 (male pups between approx. 3 % and 19 %; female pups between approx. 4 % and 21 %, $p < 0.01$).
- Morphological changes for F_2 generation: pinna unfolding, eye and ear opening were slightly, but not significantly, delayed (approx. 0.5 days) at 200 ppm, likely related to reduced post-natal body weight.
- Relative thymus weight of F_1 generation: showed a tendency towards a decrease [approx. 86 % of controls] in male and female rats at 60 ppm (approx. 4.7 mg test material/kg bw/day) and was significantly decreased in both sexes [males: 78 % of controls; females: 61 % of controls] at 200 ppm ($p < 0.01$).
- Relative thymus weight of F_2 generation: no significant differences in male or female rat pups at any dose on PND22.
- Relative spleen weight of F_1 generation: no significant differences for either sex at 60 or 200 ppm.
- Increased incidence of thymic involution for the F_1 generation at 200 ppm based on test material in feed (4/25 for control males; 13/25 for 200 ppm males; 1/25 for control females; 2/23 for 200 ppm females; significant for males) at microscopic examination.

The NOAEL for P males and females was 20 ppm (approx. 1.5 mg test material/kg bw/day) based on a slightly reduced relative thymus weight for males at 60 ppm (approx. 4.4 mg test material/kg bw/day).

The NOAEL for the F_1 generation was 20 ppm (approx. 1.6 mg test material/kg bw/day), based on a reduction in relative thymus weights for males and females at 60 ppm (approx. 4.7 mg test material/kg bw/day).

No teratogenic effects were observed in this study.

Interpretation

The body weight of F1 animals showed no statistically significant differences from the controls in either sex at birth, PND4 (postnatal day 4 both before and after culling) or PND7. Beginning with PND14 there was a statistically significant decrease in both male and female pup body weights which persisted through PND21. This correlates exactly with the incidences of post-natal losses documented for these F1 animals. Up to PND4 the number of F1 pups was not different from controls for either sex. Pups were culled to 4/sex/litter on PND4 (a routine practice). After culling, the pup losses across the four dose groups between PND4 and PND21 were 0, 4, 11, and 20 for the control, 20, 60, and 200 ppm doses of DOTI/MOTI in feed, respectively. It may not be a reproductive effect as the effects may occur as a consequence of impaired nursing behaviour as a result of maternal toxicity and/or a consequence of a direct toxic effect of dioctyltin species on young animals who may well be receiving two sources of exposure, one from the maternal milk and a second from the diet as they begin to eat adult feed during the lactation period. The mg/kg bw/day dose to these juveniles is likely far higher than the dose to the lactating P females (the mothers) and the dose-response of the juvenile deaths supports this conclusion. There is no indication from clinical signs or body weights in females on a significant maternal toxicity that may have caused impaired nursing. Only food consumption was found to be lowered during the lactation period. In addition, the delay in vaginal opening of the high dose females is likely due to the non-

specific toxic effect and low weight gain resulting from this postnatal exposure. For the F1 generation, DOTI/MOTI did not produce an adverse effect on mating, fertility, pregnancy rates, gestation, litter size or neonatal body weight. There was an increase in stillbirths which was inexplicably marked both non-statistically significant and statistically significant at the high dose, however 12/26 still births were in a single litter which diminishes any statistical significance in the endpoint analysis. There was an effect on thymus weight of the F2 animals and the histopathology of the thymus was typical of the known target organ effect of dioctyltin substances on this gland.

The body weight of F2 animals at the mid and low doses showed no statistically significant differences from controls in either sex at birth, PND4 (postnatal day 4 both before and after culling) or PND7. The body weight of F2 animals at the high dose showed no statistically significant differences from controls in either sex at birth, however at PND4 (postnatal day 4 both before and after culling) and thereafter through PND21 body weights of both sexes were statistically decreased.

Just as in the F1 generation, the body weight outcome for the F2 animals correlated with the incidences of postnatal losses. Up to PND4 the number of F2 pups was not different from controls for either sex. Pups were again culled to 4/sex/litter on PND4. After culling, the pup losses across the four dose groups between PND4 and PND21 were 9, 26, 29, and 22 for the control, 20, 60, and 200 ppm doses of DOTI/MOTI in feed. This is not a reproductive effect for the F2 generation either. It is the second manifestation of a direct toxic effect of dioctyltin species on young animals who may well be receiving two sources of exposure, one from the maternal milk and a second from the diet as they begin to eat adult feed during the lactation period. As in the F1 pups, the mg/kg/day dose to these F2 juveniles is likely far higher than the dose to the lactating F1 females (the mothers) and the dose-response of the juvenile deaths (taking into account the slightly lower number of pups available in the F2 high dose group) supports this conclusion. The consequences of the significant maternal and paternal toxicity early in the F1 generation at the high dose can be seen to be played out in the manifestation of the reproductive results in the F2 generation. No assessment of vaginal opening or testicular descent was done in the F2 animals because the experiment was terminated at PND21, prior to the occurrence of these developmental landmarks. As with the F1 generation, the effects on the offspring are not reproductive effects as envisioned by the classification criteria.

There are no adverse reproductive effects at any dose of DOTI/MOTI in the F1 generation. The postnatal losses are most probably the consequence of dose-related direct toxic effects of dioctyltin species on the F1 animals beginning after PND4 possibly coupled with impaired nursing behaviour, which is causally related to maternal toxicity. The effects on body weight and survival of the F1 animals at the high dose support a conclusion that there is carry-over toxicity, both maternal and paternal. This is manifested again during the physiological stress of producing the F2 generation pups, which are similarly adversely affected, but only after PND4.

The reproductive NOEL is the high dose; the maternal and paternal NOEL is 20 ppm (the low dose) which is driven by the effect of the DOTI/MOTI test material on the thymus gland. The effect on the PND4-PND21 pups of the F1 and F2 generations should not be used in setting the NOEL because the delivered dose to these animals via lactation is unknown, and likely higher than that of the adults because of dual exposure via milk and diet.

Conclusion for effects on fertility

Under the experimental conditions of the two generation study on DOTI/MOTI 80:20, the NOAEL for the F0 parental generation was 20 ppm (~1.5 mg test material/kg bw/day), based on a reduction in the relative thymus weight of males at 60 ppm (~4.7 mg test material/kg bw/day). The NOAEL for the F1 generation until weaning was 20 ppm (~1.6 mg test material/kg bw/day), based on a decrease in relative thymus weight in male and female pups at 60 ppm. The NOAEL for the F1 generation post-lactation was 20 ppm, based on a slight decrease in the relative thymus weight of males and an increase in stillbirth at 60 ppm.

Indices of mating, fertility, gestation and the pregnancy rates were within the range of the control group at 20 and 60 ppm. The mean pre-coital time, duration of pregnancy in days and duration in hours did not show any substance related effects at all dose-levels. The fertility index was slightly decreased at 200 ppm but was within the range of historical control data. The viability and lactation indices were decreased at 200 ppm in both the

F0 and F1 generation, however this was associated with a decrease in pup body weight (by 3 to 4 %) in the F0 generation and a significant decrease in pups weight in the F1 generation (males pups between approx. 3 % and 19 %; female pups between approx. 4 % and 21 %, at $p < 0.01$) during the lactation period that may be unrelated to in utero exposure.

It was concluded by RAC in its 2012 assessment, that the data may not be sufficiently detailed or complete for a comprehensive evaluation for the endpoint fertility.

No effects on the male or female reproductive organs or on fertility, or on reproductive capacity, or gestational integrity were observed in this study. Further, there was no evidence of a teratogenic effect observed at any dose within the limits of the design of this study.

There is a GLP-conform reproductive toxicity screening study according to OECD guideline 421 (Appel and Waalkens, 2004) performed with dioctyltin dichloride (CAS 3542-36-7) and described in detail in section 0. In this GLP study, comparable effects to the two generation study were obtained; indeed thymus effects were also recorded. Dose-related effects were seen at 10, 100 and 300 ppm (corresponding to 0.5-0.7, 4.2-6.2 and 8.4-17.0 mg DOTC/kg bw/d), with post-implantation losses in the top two dose groups. The maternal LOAEL was set at 10 ppm diet (equivalent to 0.7 mg DOTC/kg bw/day for males and 0.5-0.7 mg DOTC/kg bw/day for females) for treatment related effects to dams including moderate to very severe lymphoid depletion in the thymus, which was considered related to treatment.

Lymphoid depletion was characterized by a decrease in the size of the thymic lobules which can be ascribed to extensive loss of cortical and medullary small lymphocytes. Consequently, the distinction between the cortical and medullary area was blurred. Lymphoid depletion was observed in 5/10 of the 10 ppm group and in all animals of the 100 and 300 ppm groups.

One control animal also had very severe lymphoid depletion in the thymus. However, this was most probably associated with the fact this animal was physiologically disturbed, as was demonstrated by 12 resorptions in the uterus and an abnormal kidney (gross change: flabby and yellow patches).

Some animals of the 10 ppm group showed thymic involution as a result of pregnancy/lactation. This appearance was similar to the thymic pregnancy/lactation involution in control animals and was characterized by a decreased size of thymic lobules exhibiting normal architecture. This phenomenon is a common observation in pregnant or lactating animals. However, the lymphoid depletion in the animals of the 10 ppm group was similar to the thymic change in the animals of the 100 and 300 ppm groups. Therefore, lymphoid depletion in animals of the 10, 100 and 300 ppm groups was considered related to treatment with DOTC.

In the screening reproductive toxicity study performed with DOTC, no effects were observed on the mating index, the pre-coital time was comparable for the control and the treated groups, the female fecundity index, female fertility index and male fertility index were not affected while the gestation index was 86, 100, 71 and 50 % in the control, 10, 100 and 300 ppm groups, respectively. The livebirth index was 99, 95, 53 and 60 % in the control, 10, 100 and 300 ppm groups, respectively. Post-implantation loss was 22.3, 21.0, 49.2 and 70 % for the control, 10, 100 and 300 ppm groups, respectively.

It was concluded by RAC in its 2012 assessment that the data do not allow a comprehensive evaluation for the endpoint fertility.

10.10.3 Comparison with the CLP criteria

The discussion and comparison with the CLP Criteria for adverse effects on fertility is done in Section 10.10.6 together with the discussion and comparison of CLP criteria for developmental effects and effects via lactation

10.10.4 Adverse effects on development

Table 11: 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
OECD TG 414 (Prenatal Developmental Toxicity Study) GLP key study 1 (reliable without restriction) rabbit (New Zealand White) 24 presumed-pregnant females per dose	**Test material: DOTE** **In original Study (EC name): 2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate (96.1 % purity)** 0 (vehicle control), 4, 20, 80 mg/kg bw/day (nominal conc.) oral: gavage Vehicle: peanut oil Exposure: Daily from gestation day 6 to gestation day 28 (inclusive). (Daily throughout treatment period.)	NOAEL (maternal toxicity): 20 mg/kg bw/day (actual dose received) based on test material (biologically relevant depression [> 10 %] in thymus weight at 80 mg/kg bw/day) LO(A)EL (developmental toxicity): 80 mg/kg bw/day (actual dose received) based on test material (Biologically relevant effect on foetal weight (-11.9 %) and foetal crown-rump length (-10.7 %) relative to controls, statistically significant negative trend on foetal weight and foetal crown-rump length/1 high-dose litter with foetal loss [within historical controls] excluded) LO(A)EL (maternal toxicity): 80 mg/kg bw/day (actual dose received) based on test material (Biologically relevant depression (-12.8 %) in thymus weight, dose-dependent: -9.6 % at mid and -5.1 % at low dose group)	Anonymous (2014a)
Method: OECD Guideline 414 (Prenatal Developmental Toxicity Study) GLP key study 1 (reliable without restriction) mouse (Swiss) 25 pregnant females per dose	**Test material: DOTE** **In original Study (EC name): 2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate (96.1 % purity)** 0 (vehicle control), 15, 30, 60 mg/kg bw/day (nominal conc.) oral: gavage Vehicle: peanut oil Exposure: Females received test material daily from gestation day 5 to gestation day 17 (inclusive). (Daily throughout treatment period.)	NOAEL (maternal toxicity): 15 mg/kg bw/day (actual dose received) based on test material (Statistically significant decrease in thymus size at 30 mg/kg bw/day (2 animals) and 60 mg/kg bw/day (8 animals), statistically significant decrease in thymus weight from 30 mg/kg bw/day) Developmental toxicity: statistically significant positive trend on percentage of post-implantation loss: 0.9 ± 2.8 at low, 1.5 ± 4.9 at mid, and 2.6 ± 5.6 at high dose, respectively) LOAEL (maternal toxicity): 30 mg/kg bw/day (actual dose received) based on test material (Statistically significant depression in thymus size, stat. sign. depression (23 %) in thymus weight, 35 % at 60 mg/kg bw/day, treatment-related statistically not significant reduction in corrected maternal body weight gain -26.5% (2.34±2.41 g) in the high dose mice relative to controls, mid and low dose -16.7% and -17.7%, respectively)	Anonymous (2014b)
similar to OECD TG 414 (Prenatal Developmental Toxicity Study) supporting study GLP	**Test material: DOTI/MOTI 80:20.** In original study named: **Dioctyltin bis(IOMA) [CAS No. 26401-97-8] : Octyltin tris(IOMA) [CAS No. 26401-86-5] (80:20 %)** (See endpoint	NOAEL (maternal toxicity): 5 mg/kg bw/day (slight but non-significant decrease in corrected body weight and corrected body weight gain of the dams indicating a marginal maternal toxic effect of the test material) NOAEL (developmental toxicity): 5 mg test material/kg bw/day (significant increase in the percentage of dead foetuses)	Battenfeld, R. (1991)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
2 (reliable with restrictions) rat (Han-Wistar SPF) 25 per treatment group	summary for justification of read-across) 1, 5, and 25 mg/kg/day (actual ingested) oral: gavage Vehicle: peanut oil Exposure: days 6-15 of gestation (once/day x 10 days)		
similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) GLP 3 (not reliable) Rabbit (New Zealand White) 23-24 females/group	**Test material: DOTI/MOTI 80:20.** In original study named: **Dioctyltin bis(IOMA) [CAS No. 26401-97-8] : Octyltin tris(IOMA) [CAS No. 26401-86-5] (80:20 %)** (See endpoint summary for justification of read-across) 1.0, 10, and 100 mg/kg bw/day (actual ingested) oral: gavage Vehicle: peanut oil Exposure: days 6-18 of gestation (once/day x 13 days)	NOAEL (maternal toxicity): 10 mg/kg bw/day (based on increased incidence of abortions at 100 mg/kg bw/day) NOAEL (developmental toxicity): 10 mg test material/kg bw/day: Slight non-significant increase in minor skeletal head anomalies (incompletely ossified bones in the skull). 100 mg test material/kg bw/day: Significantly increased incidence of abortions, post implantation loss, minor visceral anomalies (severely dilated renal pelves and additional small vessels originating from the aortic arch), minor skeletal head anomalies (incompletely ossified bones in the skull), and skeletal variations of the sternum and feet bones (not or incompletely ossified sternebrae and feet bones); and a significant reduction in foetal body weight.)	Battenfeld, R. (1992)
Method equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study) 3 (not reliable) Mouse (NMRI) 22 to 25 females/group	**Test material: DOTI/MOTI 80:20.** In original study named: **Dioctyltin bis(IOMA) [CAS No. 26401-97-8] : Octyltin tris(IOMA) [CAS No. 26401-86-5] (80:20 %)** (See endpoint summary for justification of read-across) 20, 30, or 45 mg/kg bw/day (group 1); 67 or 100 mg/kg bw/day (group 2) (actual ingested) oral: gavage Vehicle: peanut oil Exposure: days 6-17 of gestation (once/day x 12 days)	NOAEL (maternal toxicity): 30 mg test material/kg bw/day (Based on a significant decrease in thymus weight [-15 %] at 45 mg/kg bw/day.) NOAEL (developmental toxicity): 45 mg test material/kg bw/day (based on an increased incidence of cleft palate [+5.5 %] in foetuses from dams exposed to 67 mg test material/kg bw/day.)	Faqi, A.S., H. Schweinfurth, and I. Chahoud (2001)

Table 12: Summary table of human data on adverse effects on development

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

Table 13: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance, dose levels, duration of exposure	Relevant information about the study (as applicable)	Observations	Reference
OECD Guideline 421- reproduction/ developmental screening study: sub-chronic (13 week) oral toxicity study in rats (OECD Test guideline 408), including a satellite group for a reproduction/ developmental screening study (OECD Test guideline 421) GLP Rat (Wistar) male, female 44 females (four dose groups of 10 rats/sex) Oral: feed	Test material: **Dichlorodioctylstannane (CAS no 3542-36-7) (purity 94 %)** 10, 100, 300 ppm (nominal in diet) The test substance intake of the female animals of the 10, 100 and 300 ppm groups were, respectively: Premating period days 0-7: 0.6, 5.8 and 13.5 mg/kg bw/day days 7-14: 0.7, 5.9 and 16.4 mg/kg bw/day; Gestation period GD 0-7: 0.7, 6.2 and 16.6 mg/kg bw/d, GD 7-14: 0.7, 6.2 and 17.0 mg/kg bw/day, GD 14-21: 0.5, 4.2 and 11.0 mg/kg bw/day; Lactation period: PND 1-4: 0.7, 5.0 and 8.4 mg/kg bw/day. Duration of exposure: females: daily for 2 consecutive weeks during the premating period, daily during gestation (up to 26 days after study initiation) and up to euthanasia at or shortly after postnatal day (PND) 4. (daily); males: daily for 13 weeks prior to mating	Read-across from supporting substance (structural analogue or surrogate) (See endpoint summary for justification of read-across) Reliability: 2 (reliable with restrictions)	NOAEL (reproduction toxicity): 10 ppm (0.5 — 0.7 mg/kg bw/day (female)) (Based on reproductive and developmental effects: animals showing only implantations at necropsy, animals delivering only dead pups, decreases in gestation, live birth and viability indices and increases in post-implantation loss and number of runts) LOAEL (general toxicity): 10 ppm (0.5 — 0.7 mg/kg bw/day (female)) (decreases in absolute and relative thymus weights associated with treatment related lymphoid depletion at 10, 100 and 300 ppm groups)	Appel, M.J. and D.H. Waalkens-Berendsen. (2004)

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

Two new key studies, investigating the developmental toxicity of DOTE itself, are available; one study was conducted in mice and the other in rabbits. Both studies were performed under GLP conditions and in accordance with the standardised guideline OECD 414.

1/ In the new mice developmental toxicity study (Anonymous, 2014b), dams were given DOTE 96.1 % purity at 0, 15, 30, and 60 mg test material/kg bw/day (corresponding to 14.4, 28.8, and 57.7 mg DOTE/kg bw/day) during day 5 to 17 of pregnancy.

Maternal toxic effects

Reduction in thymus weight from 30 mg/kg bw/day

Embryotoxic / teratogenic effects

None

MATERNAL DATA

- Clinical Signs of Toxicity and Morbidity/Mortality
The animals did not reveal any clinical signs of toxicity and mortality at any of the tested doses throughout the experiment.

- Maternal Body Weight
There were no statistically significant differences in maternal body weights across the dose groups on any single gestation day. However, there was a clear dose-related pattern of reduced body weights beginning after GD6, the first day of dosing, and continuing for the duration of the study. The high dose was the most severely affected, though a dose-related decrement relative to the control body weights can be seen across all doses particularly from GD16 to GD18. At 30 mg/kg bw/day the maternal weight effect was marginal, but maternal body weight gain in the 60 mg/kg bw/day high dose group was 11.5 % [uncorrected] and 26.6 % [corrected] less (2.34 ± 2.41 g) than the vehicle control (3.19 ± 2.23 g). The corrected body weight gain in the low (15 mg/kg bw/day) and mid (30 mg/kg bw/day) dose groups were -16.7 % (2.62 ± 2.03 g) and -17.7 % (2.66 ± 2.46 g) when compared to the controls.

- Feed Consumption
No treatment related differences in average feed consumption were observed at any dose.

- Gross Pathology [Maternal]
There was a treatment-related macroscopic finding of reduced maternal thymus weight.
The mean maternal thymus weight was statistically significantly reduced (-23 %) in the 30 mg/kg bw/day [mid] and 35 % in the 60 mg/kg bw/day [high] dose groups. The mean maternal thymus weight in the low dose mice was reduced relative to controls, but was not statistically significant. These observations are indicative of a treatment-related specific target organ toxicity resulting from exposure to the test material. No other gross pathological findings were noted in any dose group.

PREGNANCY DATA

A total number of 21 (84 %), 21 (84 %), 20 (80 %) and 20 (80 %) mated females were confirmed pregnant at the time of caesarean section for groups G1, G2, G3 and G4, respectively.

UTERINE OBSERVATIONS

There were no statistically significant differences in these gravid uterus weights, number of implantation sites, pre- and post-implantation loss or early or late resorptions across dose groups when compared to the vehicle control.

REPRODUCTION DATA

No treatment related effects were noted in mean gravid uterus weight, no. of corpora lutea, no. of implantations in all the groups, and no. of early or late resorptions. There was a statistically significant positive trend on percentage of post implantation loss of 0.9 ± 2.8 at the low, 1.5 ± 4.9 at the mid and 2.6 ± 5.6 at the high dose group, respectively.

There were no statistically significant differences in the sex ratio, mean litter size or the number of live foetuses per dam across dose groups when compared to the vehicle control.

FOETAL DATA

- Foetal Weight
The mean foetal weights [combined sexes] were 1.35, 1.37, 1.30 and 1.31 grams for groups G1, G2, G3 and G4, respectively. Mean foetal weights were not statistically significantly different across the dose groups when compared to controls.

- External Examination
No external abnormalities were noted during gross examinations of foetuses at any dose.

- Visceral Examination
No treatment-related abnormalities were observed during visceral examinations of foetuses at any dose. The noted findings [pale coloured kidneys and dilated renal pelvis] are common findings for foetuses of this species and strain. The observations were not dose dependent, nor was the severity of the anomaly increased with dose. This result supports the conclusion that the findings are incidental and that the test material did not produce an adverse effect during foetal development of the soft tissues.

- Skeletal Examination
There was a single incidence in one litter in the high dose group of fused sternum and a single incidence in a different high dose litter of short rib. Single incidences, even of rare malformations (which these are not), cannot be reliably attributed to treatment. The other noted anomalies (poorly ossified frontal, parietal and inter-parietal bones; ossification site at first lumbar vertebrae; supplementary ribs) are common findings for foetuses of this species and strain. These morphologic observations did not occur in a dose-dependent pattern, nor was the severity of the anomaly increased with dose. The findings were therefore considered to be incidental, and not indicative of a teratogenic effect.

- Crown-rump length
The average crown-rump lengths were 23.2, 24.0, 23.3 and 22.9 mm for groups, G1 through G4, respectively. There were no statistically significant differences in length across all dose groups when compared to the control.

2/ In the new rabbit developmental toxicity study (Anonymous, 2014a), dams were given DOTE 96.1 % purity during day 6-28 of pregnancy at 0, 4, 20 and 80 mg test material/kg bw/day (corresponding to 3.8, 14.4, and 76.9 mg DOTE/kg bw/day).

Maternal toxic effects

Biologically relevant depression [> 10 %] in thymus weight at 80 mg/kg bw/day

Embryotoxic / teratogenic effects

None

PREGNANCY DATA

A total number of 19, 21, 19 and 20 mated females were confirmed pregnant in groups G1, G2, G3 and G4, respectively (0, 4, 20 and 80 mg/kg bw/day, respectively). This is a pregnancy rate of 79, 88, 79 and 83 % at the time of caesarean section for groups G1, G2, G3 and G4, respectively.

MATERNAL DATA

- General Tolerability: No deaths or abortions were observed during the experimental period and there were no clinical signs recorded which were indicative of overt toxicity.

- Maternal Body weight, Body Weight Gain, and Corrected Body Weight Gain: There were no statistically significant differences in maternal body weights across the dose groups on any single gestation day, nor was there a dose-related pattern of reduced body weights beginning after GD6, the first day of dosing. There were no statistically significant differences in uncorrected maternal body weight gain across the dose groups for any single gestational period. Treated does had higher weights than controls on GD29; this was considered to be a random occurrence and not related to test material administration.

- Food Consumption: No treatment related differences in average feed consumption were observed at any dose.

- Gross Pathology: There was a treatment-related macroscopic finding of reduced maternal thymus weight. The mean maternal thymus weights were -5.1, -9.6, and -12.8 % in the 4 mg/kg bw/day [low], 20 mg/kg bw/day [mid], and 80 mg/kg bw/day [high] dose groups, respectively when compared to controls. While these decreases were not statistically significant, the results were dose-dependent relative to controls and are consistent with data from other species [rat and mouse] which demonstrate the thymus is a target organ for octyltin compounds. These observations are indicative of a treatment-related specific target organ toxicity resulting from exposure to the test material. No other gross pathological findings were noted in any dose group.

REPRODUCTION DATA

No treatment related effects were noted in the number of corpora lutea, number of implantation sites, number of early or late resorptions, or percentage of post implantation loss across all the groups.

The mean gravid uterus weights were 230.1, 309.5, 253.7 and 196.9 g for groups G1 through G4, respectively. A statistically significant increase in the mean gravid uterus weight was noted at the low dose when compared to the vehicle control. This difference was attributed to the increase in the number of foetuses compared to the vehicle control group and other dose groups.

The mean pre-implantation losses were 0.9, 0.8, 2.3, and 4.9 % for groups G1, G2, G3 and G4, respectively. Mean post-implantation losses for these treatment groups were 3.1, 3.5, 6.4 and 5.7, respectively. These losses are within the historical control range and there is no clear evidence of a dose-response across dose groups.

There were no statistically significant differences in either the mean litter size or the number of live foetuses per dose across dose groups when compared to the vehicle control.

No treatment-related effect on the sex ratio of foetuses was noted at any dose compared to the vehicle controls.

FOETAL DATA

- Foetal Weight

The mean foetal weights [combined sexes] were 36.6, 37.3, 35.5, and 32.3 g for groups G1, G2, G3 and G4, respectively. Mean foetal weights were not statistically significantly different across the dose groups when compared to controls. At the high dose the mean foetal body weight was -11.9 % relative to controls which suggests a biologically-relevant, but marginal effect on foetal maturation. However, the mean weight for this group was disproportionately affected by low foetal body weights in a single litter in this group. There was a statistically significant negative trend on foetal weight (mean foetal weight 33.5 g for group G4 with single litter excluded).

- External Examination

No external abnormalities were noted during gross examinations of foetuses at any dose.

- Visceral Examination

No treatment-related abnormalities were observed during visceral examinations of foetuses at any dose. The noted findings [pale coloured kidneys and dilated renal pelvis] are common findings for foetuses of this species and strain. The observations were not dose dependent, nor was the severity of the anomaly increased with dose. This result supports the conclusion that the findings are incidental and that the test material did not produce an adverse effect during foetal development of the soft tissues.

- Crown rump length:

The mean foetal crown-rump length for both sexes was 92.1, 91.1, 89.3 and 82.3 mm for groups G1, G2, G3 and G4, respectively. A statistically significant reduction in the mean foetal crown-rump length was noted in the high dose group when compared to controls. This was -10.7 % relative to the vehicle control suggesting a marginal but biologically relevant effect on foetal maturation which correlated to the degree of skeletal ossification. One litter in this group had low crown-rump lengths which were found to disproportionately affect the mean. There was a statistically significant negative trend when not considering all the litters of this group (mean foetal crown-rump length 83.8 mm for group G4 with single litter excluded). There were no correlating statistically significant differences for any other parameter.

- Skeletal Examination:

The noted skeletal anomalies in the study are common findings for foetuses for this species and strain. The morphologic observations did not occur in a dose-dependent pattern, nor was the severity linked to an increase in dose. The incidences of absent sternum No. 5, a measure of delayed ossification were found to be higher in controls compared to any other treatment group. Other indicators of delayed ossification such as the absence of proximal phalanges were found to be single incidences within a litter both in the control group and treatment groups. Poor ossification in sternum No. 5 and No. 6, an indicator of delayed ossification, was the only variation to be found to occur as multiple incidences within a litter. This variation however is considered to be less significant than the absence of sternum No. 5. The conflicting results indicate that these findings are incidental.

Supporting information is available in the form of an embryotoxicity and teratogenicity study which was conducted with a read across material, consisting of DOTI [CAS No. 26401-97-8] : MOTI [CAS No. 26401-86-5] (80:20 %). Read across is considered justified as DOTE and DOTI are isomers of the same compound and are structural analogues of each other. Based on the recently conducted developmental toxicity studies in two species with DOTE it is considered that the use of data on DOTI would be considered in a weight-of-evidence assessment of the registered substance.

3/ In the developmental toxicity study in rats (Battenfeld, 1991), dams were treated with DOTI/MOTI (80:20 %) at 1, 5 and 25 mg/kg bw/day during day 6-15 of gestation.

Maternal effects:

Alopecia was observed in single animals of all four groups and was not attributed to treatment. There was a slight (non-significant) decrease in corrected body weight and corrected body weight gain from day 6 to day 21 at 25 mg/kg bw/day dose. This reduction was attributed largely to one single dam (dam No.97).

Foetal observations:

No embryofoetal effects were reliably attributed to treatment as the observed embryolethality was marginal (observed in only one dam) and is therefore considered to have occurred by chance

It should be noted that for rats exposed to DOTI/MOTI [GD6-15] the foetuses did not show the malformations or variations of bone formation as observed in mice and rabbits. For this test material the rat was the least sensitive species.

At 1 and 5 mg/kg/day dose-levels no treatment-related effects were observed. At the 25 mg/kg/day dose level there was a slight, but not statistically significant decrease in corrected body weight and corrected body weight gain of the dams. This reduction was to a great extent due to the loss of corrected body weight in one single dam (-58g in dam No. 97) Seven dead foetuses were observed, all in one litter in group 4. This led to an artefact in the statistics of the percentage of dead foetuses. All dead foetuses in the group were from a single dam (No. 97), and in addition to the high maternal weight loss, four early resorptions and an extremely low mean weight (2.2 g) of the two living foetuses were found in this animal. There were no treatment-related effects (external, visceral or skeletal malformations) at any dose-level. The frequency of all external, visceral and skeletal variations was within historical control limits and there was no dose-response.

For DOTI/MOTI, no adverse effects were observed at the low [1 mg/kg/day] or intermediate [5 mg/kg/day] doses. The high dose 25 mg/kg/day] produced marginal maternal toxicity and no embryofoetal effects were reliably attributed to treatment. The effects noted in a single high dose dam were considered to have occurred by chance.

RAC position (2011):
"Indeed there is only weak evidence on developmental effects from this rat study since dead foetuses were seen only from one dam"

4/ Two generation study performed under GLP and according to OECD 416 with Dioctyltin bis(IOMA)/Octyltin tris(IOMA) (Anonymous, 1997)

Please refer to section 'effects on fertility' for details**.**

The NOAEL for P males and females was 20 ppm diet (approx. 1.5 mg test material/kg bw/day) based on a slightly reduced relative thymus weight for males at 60 ppm (approx. 4.4 m test materialg/kg bw/day).

The NOAEL for the F1 generation was 20 ppm (approx. 1.6 mg test material /kg bw/day), based on a reduction in relative thymus weights for males and females at 60 ppm (approx. 4.7 mg test material/kg bw/day).

No teratogenic effects were observed in this study.

Three additional studies had been considered by RAC in its scientific opinion reached on 8 June 2012. They have been regarded for hazard classification in a weight-of-evidence assessment and the significance weighted accordingly.

5/ In the mice developmental toxicity study (Faqi, 2001), dams were given DOTI/MOTI (80:20 %) at 20, 30, 45, 67 and 100 mg test material/kg bw/day (corresponding to 16, 24, 36, 53.6, and 80 mg DOTI/kg bw/day) during day 6 to 17 of pregnancy.

Maternal effects:

There was a dose dependent decrease (-43 % at the high dose and -10 % at 67 mg test material/kg bw/day, respectively) in maternal body weight gain (mean (control): 5.1 ± 7.0 g, mean ± SD: 2.9 ± 4.8 g at 100 mg test material/kg bw/day), but differences were not significant in mice exposed to the test substance. No signs of toxicity were observed with the exception of one dam in the 100 mg/kg bw/day dose group that died. Pregnancy rates were comparable between treated groups and the control groups.

The mean maternal thymus weights in the 45 and 100 mg test material/kg bw/day dose groups were significantly lower than the control groups (-27 %, $p < 0.05$ at 100 mg test material/kg bw/day). At 67 mg/kg bw/day, the mean maternal thymus weight was slightly (-13 %) but not significantly decreased. Maternal liver weights were significantly lower in the 100 mg/kg bw/day dose group (-23 %, $p<0.05$ at 100 mg test material/kg bw/day). The number of implantations per litter was comparable between treated groups and the control groups. Resorption rates were significantly increased in mice treated with 67 (13 %) or 100 mg test material/kg bw/day (16 %).

Foetal observations:

Foetal weights were significantly decreased in the 67 and 100 mg test material/kg bw/day groups (-9 and -18 %, respectively). There were no dead foetuses in any of the treated groups. There were no external malformations reported in the foetuses exposed to 20, 30, or 45 mg test material/kg bw/day, however a significantly increased incidence of cleft palate in the foetuses exposed to 67 or 100 mg test material/kg bw/day were observed (5.5 and 9.3 %, respectively), and incidences of bent forelimbs (18.5 %) and exencephaly (7.1 %) were significant in the foetuses exposed to 100 mg test material/kg bw/day. Skeletal variations reported in the low dose groups included unossified digit (4.4 % at 45 mg test material/kg bw/day) and supernumerary cervical ribs (significantly increased at 20 and 45 mg test material/kg bw/day, but not at 30 mg test material/kg bw/day); hind paw incompletely ossified (9.3 %), Os frontale misshapen (25.2 %), and interparietale incompletely ossified (24.4 %) (Significantly increased at 45 mg test material/kg bw/day); and supernumerary lumbar or cervical ribs (significantly increased at 20, 30, and/or 45 mg test material/kg bw/day). There was a significant increase in skeletal abnormalities in the foetuses of dams exposed to 67 or 100 mg test material/kg bw/day. Skeletal abnormalities reported in these dose groups included bent forelimbs, bent hindlimbs, dislocated sternum, fused or bent ribs (6.4 and 35.5 % at the 67 or 100 mg test material/kg bw/day groups, repectively), or bent vertebral column. Skeletal variations were observed in the low dose groups (20, 30, or 45 mg test material/kg bw/day).

The authors defined malformations as a permanent or irreversible structural change that is likely to adversely affect survival or health. The authors reported a no-observed-adverse-effect-level (NOAEL) for each endpoint examined, i. e., malformations, variations, organ toxicity.

- The embryo-foetal NOAEL for malformations was reported as 45 mg test material/kg bw/day, based on an increased incidence of cleft palate (5.5 %) in foetuses from dams exposed to 67 mg test material/kg bw/day.
- A NOAEL for skeletal variations could not be determined, but would be expected to be < 20 mg/kg bw/day, based on an increased incidence of supernumerary lumbar ribs (68.5 %) observed at 20 mg test material/kg bw/day.
- The authors reported that the NOAEL for maternal organ toxicity was 30 mg test material/kg bw/day, based on a significant decrease in thymus weight (15 %) at 45 mg test material/kg bw/day.

The mouse study reported by **Faqi (2001)** with DOTI/MOTI (80:20 %) failed to fulfil the requirements of a GLP OECD 414 guideline study since detailed information on the following were not included: maternal body weight, food consumption, housing, dosing, analysis of the dosing formulations or individual animals data. Furthermore, no information was given on foetal sex ratio, foetal weight per sex or internal malformations and

no comparison to historical control data was carried out. The study was therefore considered to be deficient and the results are taken forward for hazard classification with lower significance than the new key study, investigating the developmental toxicity with the substance itself on mice.

6/ In the rabbit developmental toxicity study (Battenfeld, 1992), dams were given DOTI/MOTI (80:20 %) during day 6-18 of pregnancy at 1, 10 and 100 mg test material/kg bw/day (corresponding to 1, 8, and 80 mg DOTI/kg bw/day).

Maternal effects:

Except for a nasal haemorrhage in one dam of Group 2 (1 mg/kg bw/day), slight torticollis in one dam of Group 3 (10 mg/kg bw/day), and bloody outflow in 3 dams of Group 4 (100 mg/kg bw/day), no clinical observations were made. In total, 18 of 24 dams in Group 1, 23/23 in Group 2, 18/22 in Group 3, and 17/24 in Group 4 survived until day 28. Two dams in Group 1 and 3 dams in Group 3 died after treatment had commenced. Death resulted from infectious diseases (pneumonia or enteritis), and there was no dose-related increase. Therefore, these deaths were not attributed to the test substance. In Group 1, 3 dams were eliminated because of normal deliveries before day 28. Before start of treatment, one dam in Group 1 and one dam in Group 2 were found dead. Maternal body weight data did not reveal differences between treatment groups. Abortion was diagnosed in one dam of Group 1 and 4 dams of Group 4. All abortions occurred after termination of treatment. The high incidence of abortion in Group 4 was considered to result "at least partly from a slight maternal toxic effect of the test compound."

Foetal observation:

Total foetal death was found only in Groups 1 and 4. In both groups, total post-implantational loss occurred in 3 dams. Percentages of post-implantation losses per group were 17.7 % (control), 10.5 % (1 mg/kg bw/day), 5.7 % (10 mg/kg bw/day), and 28.4 %, $p < 0.05$ (100 mg/kg bw/day). The significant increase in post-implantation loss at the high dose-levels was explained by a significant increase of total resorptions (28.4 %, $p < 0.05$ vs. 17.1 % in controls).

External examination revealed two nasal clefts and an encephalocele in one foetus of Group 2. Umbilical hernia was found in one foetus of the control group and in one foetus each in Groups 3 and 4. These were not associated with treatment. Other findings, such as malformations of the vertebral column (one animal in Group 4) and absence of the right kidney and adrenal gland (one animal in Group 4) were regarded as chance findings and not attributed to treatment due to their single occurrence and because they represented totally different types of malformations. The lack of a statistically significant difference to the control group and inconsistency regarding the type of anomaly found did not "point towards a compound-related effect." Foetuses with minor external anomalies (flexion of digits and limbs, open eyelids, shortened tail) were observed in all four groups, and not attributed to the test substance. Minor visceral anomalies found included severely dilated renal pelves and additional small vessels originating from the aortic arch. The statistically significant increase in the incidence of visceral anomalies of foetuses in Group 4 is an indication of retardation in foetal development. Individual body weights of the foetuses in Group 4 with minor visceral anomalies were approximately 40 % lower than the mean weight of control foetuses.

Suspected or definite compound-related changes noted included:
-1 mg/kg bw/day: No substance-related effects.
-10 mg/kg bw/day: Slight non-significant increase in minor skeletal head anomalies (incompletely ossified bones in the skull).
-100 mg/kg bw/day: clear substance-related embryotoxic effects were noted i. e. significantly increased incidence of abortions, post-implantation loss, minor visceral anomalies (severely dilated renal pelves and additional small vessels originating from the aortic arch), minor skeletal head anomalies (incompletely ossified bones in the skull), and skeletal variations of the sternum and feet bones (not or incompletely ossified sternebrae and feet bones); and a significant reduction in foetal body weight.

In conclusion, the author of the rabbit developmental toxicity study reported that the evaluation of reproduction data and foetal weights indicated a slight embyrolethal and moderate retarded effect (with regard to foetal development) at the high dose level (100 mg/kg bw/day) associated with maternal toxicity (abortions).

In the rabbit study reported by **Battenfeld (1992)** with DOTI/MOTI (80:20 %) it was not possible to interpret the results of the study based on the information presented in the study report. During the study maternal disease was reported; the disease that is described in the report is common in rabbits of the age and strain used in the study. It was not possible to ascertain whether maternal effects which were observed resulted from the disease or from treatment with the test material since detailed examination of the immune system are not included in developmental toxicity studies and were not included as part of this study. There was one case of torticollis, a symptom of encephalitozoonosis, a parasitic disease occurring in immune deficient animals, which was reported. Since no effects were seen in the rangefinder study, at doses up to 30 mg test material/kg bw/day, the maternal findings observed in the main study were considered spurious. The exposure time was rather short (13 days) as also noted by RAC in its 2012 assessment. Overall the study was considered to be deficient and the results are taken forward for hazard classification with lower significance than the new key study, investigating the developmental toxicity with the DOTE substance itself on rabbits.

7/ In the Reproduction / Developmental Toxicity Screening Test (according to OECD 421) (Appel and Waalkens, 2004) rats were given DOTC > 94 % purity at 10, 100, 300 mg/kg in the diet:

At 10 ppm (equivalent 0.7 mg/kg bw/day for males and 0.5-0.7 mg/kg bw/day for females), treatment-related effects to dams included lymphoid depletion were observed in dams.

At 100 ppm (equivalent to 6.5 mg/kg bw/day for males -6.8 mg/kg bw/day for females, treatment-related effects included increased post-implantation loss (49 %), decreased gestation index (71 %) decreased live birth index (53 %), decreased viability index (74 %), increased number of runts, increased pup mortality (PND 1 and 4), and decreased absolute and relative thymus weights and lymphoid depletion in the dams.

At 300 ppm (equivalent to 19.3 mg/kg bw/day for males -19.8 mg/kg bw/day for females), treatment-related effects included increased in post-implantation loss (70 %), decreased gestation index (50 %), decreased live birth index (60 %) decreased viability index (12 %), increased number of runts, decreased pups weights (PND 1 and 4), increased pup mortality (PND 1 and 4), and decreased absolute and relative thymus weights and lymphoid depletion (dams).

Summary of litter data
- **Litter size**: The mean number of pups delivered per litter amounted to 11.7, 11.0, 10.3 and 8.6 for the control, 10, 100 and 300 ppm groups, respectively.
- **Litter weight**: Mean pup weights and pup weight changes were similar in the 10 and 100 ppm groups when compared to the control group. Pup weight of the 300 ppm group (PND 1, 3 litters and PND 4, 1 litter) was reduced.
- **Pup mortality**: 1.4, 4.5, 47 and 40 % in the control, 10, 100 and 300 ppm groups, respectively (PND 1); 5.8, 8.3, 26 and 88 % in the control, 10, 100 and 300 ppm, respectively (PND 4).
- **Number viable**: The viability index (PND 1-4) was 94, 92, 74 and 12 % in the control, 10, 100 and 300 ppm groups, respectively.
- **Number live pups per litter**: 11.5, 10.5, 7.6 and 6.5 for the control, 10, 100 and 300 ppm groups, respectively (PND 1); 10.8, 11.0, 9.3 and 3.0 for the control, 10, 100 and 300 ppm groups, respectively (PND 4).
- **Sex ratio**: No difference was observed in the sex ratio between the groups.

The above developmental effects were associated with maternal toxicity substantiated by a statistically significant decrease in absolute and relative thymus weight in the 100 (c. 62 and 67 % in male and females,) and 300 ppm group (31 and 38 % in males and females) and a moderate to very severe lymphoid depletion in dams (5/10 animals at 10 ppm and in all animals of the 100 and 300 ppm groups).

Based on reproductive and developmental effects in the screening reproductive toxicity assay (particularly severe post-implantation losses and foetal losses) observed after mating of 100 and 300 ppm female of the satellite groups with male animals of the main study, the low dose level of 10 ppm in diet (equivalent to 0.7 mg/ kg bw/day in males and 0.5-0.7 mg/kg bw/day for females) can be considered as a NOAEL for fertility and developmental effects.

Based on the treatment related histological changes in the thymus (lymphoid depletion) of the 10 mg/kg female animals of the satellite groups, 10 ppm in diet (equivalent to 0.5-0.7 mg/kg bw/day) was considered to be a LOAEL for maternal toxicity.
To assess teratogenic effects was not subject of this study. Thus, the animals were not in depth examined regarding external, soft tissue or skeletal abnormalities. However, grossly visible abnormalities were recorded.

This study reported by **Appel, M.J. and D.H. Waalkens-Berendsen. (2004)** was regarded in a weight-of-evidence assessment and the significance weighted accordingly based on the results of the recent hydrolysis study (Anonymous, 2015, see section 9.1), which indicate the substance DOTEC is the only hydrolysis product of DOTE which was formed at pH 1.2 and which, however, does not exclude that no dioctyltin dichloride can be formed in vivo.

A direct comparison of the studies using DOTI/MOTI (80:20 %) with the newer studies on the substance itself is given at the end of section 10.10.10 within the conclusion on classification and labelling of DOTE.

10.10.6 Comparison with the CLP criteria

The classification criteria for reproductive toxicity for the ECHA are documented in Section 3.7.2 of the Guidance on the Application of the CLP Criteria, Guidance to Regulation (EC) No. 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Version 4.1, June 2015.

For the purpose of classification the hazard class Reproductive Toxicity is differentiated into:

- adverse effects
    - on sexual function and fertility, or
    - on development;
- effects on or via lactation.

Annex I: 3.7.1.4. defines adverse effects on development of the offspring as follows:

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

The hazard categories for the reproductive endpoint are presented in Table 3.7.1 (a) of the referenced document.

Rationale for classification

There are no human reproductive data on DOTE, therefore DOTE is not a candidate for Category 1A.

There is no animal evidence that DOTE interferes with sexual function or fertility, therefore DOTE should not be classified for these endpoints.

Based on recently conducted developmental toxicity studies according to OECD TG 414 (Anonymous, 2014a, b) there is animal evidence in the most sensitive species, rabbit and mouse, that DOTE interferes with gestational integrity in mice and the embryological development in rabbits. In mice, study results show a statistically significant positive trend on percentages of post implantation loss. In rabbits, at the high dose (80 mg/kg bw/day) the mean foetal body weight decreased about 12 % relative to controls, suggesting a marginal but biologically relevant effect on foetal maturation. Furthermore, a statistically significant reduction in the

mean foetal crown-rump length was noted in this study (-10.7 % relative to the controls), again suggesting a marginal but biologically relevant effect on foetal maturation which moreover correlated to the degree of skeletal ossification. DOTE, however, produced no statistically significant adverse effects on foetal morphology of skeletal elements or soft tissues even in the presence of slight maternal toxicity. Observed effects were only statistically significant in trend tests, supporting the assumption that the effects were treatment- and dose-related. Furthermore, in comparison to other studies on analogues the highest dose tested using DOTE was notably lower. According to the OECD TG 414 the highest dose used in the experiment should be chosen with the aim to induce some developmental and/or maternal toxicity (clinical signs or a significant decrease in body weight) but not death or severe suffering. The descending sequence of dose levels should further be selected with a view to demonstrating any dosage-related response and NOAEL. Hence, the highest dose tested (Anonymous 2014a, b) might have been too low be able to detect such a dose-response relationship and thus might only reflect the starting point of a potential dosage-related response.

There is evidence of postnatal toxicity from a two generation study with a related substance, DOTI/MOTI 80:20. DOTI and DOTE are isomers differing only slightly in the structure of the C-8 alcohol of the mercaptoester ligand (either *iso*-octanol or 2-ethylhexanol, respectively). Since these alcohols are so close in structure, their respective mercaptoacetate esters are expected to have very similar physicochemical and toxicological properties, including hydrolysis products. In the two generation study with DOTI/MOTI, postnatal deaths in the F1 and F2 generations occurred, however, the offspring did not die during the perinatal period. Deaths occurred after PND4 and hence might rather be attributed to postnatal exposure of lactating pups than to in utero exposure. In this case, these findings do not meet the criteria noted in bold text of the definition above.

There were relevant developmental effects in the two generation study and in the developmental toxicity studies performed with DOTI/MOTI, particularly the effects on pups such as the increased incidence of abortions, marked retardations of foetal development, increased number of runts, decreased foetal weight, decreased number of pups per litter, increased post-implantation loss, and decreased thymus weight for the F0 parent and F1 progeny.

In addition, the findings of the screening reproductive toxicity feeding study with DOTC were consistent with a part of these particular findings (increase in post-implantation loss, decreased viability index, increased number of runts, decreased pups weights). These effects were observed at dose levels inducing some maternal toxicity (statistically significant decrease in absolute and relative thymus weight and a moderate to very severe lymphoid depletion in dams). A recent *in vitro* hydrolysis study under simulated mammalian gastric conditions, admittedly only identified DOTEC as a hydrolysis product and no DOTC could be detected under the particular study conditions, however this study does not allow to conclude, if and which other dioctyl species might be formed after systemic uptake of DOTE under *in vivo* conditions. Hence, read-across from studies using DOTC as test material have to be included when discussing potential reproductive effects of DOTE and cannot be disregarded.

The above reported effects (increased post-implantation loss, increase incidence of resorption, increase pups mortality, depressed foetal weight) are indicative of developmental effects. These effects observed in all the above reported studies were associated with no or only slight maternal (thymo-)toxicity that has no bearing on the developmental toxicity. In the absence of significant/systemic maternal toxicity, DOTE should be classified for developmental toxicity.

In summary, the weight-of-evidence analysis for DOTE for toxicity studies in experimental animals there is some evidence which is not sufficiently convincing to place the substance in Category 1. The effects recorded with DOTI/MOTI are considered of toxicological significance. For DOTE per se there is some evidence of developmental toxicity occurring in two different species with low toxicological significance (trend only). Repr. 1B should only be chosen if there is clear evidence. Therefore, in accordance with the criteria for classification as defined in Annex I, Regulation (EC) No. 1272/2008 (CLP), classification of the substance with respect to developmental toxicity as Repr. 2 (H361d) is appropriate.

10.10.7 Adverse effects on or via lactation

Not evaluated in this dossier

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

The postnatal deaths in the F1 and F2 generations of the 2G study with DOTI/MOTI occurred during the lactation period. The increase in pup mortality, respectively decrease in viability at birth and probably growth retardation during the lactational period has been discussed in Section 10.10.6 and considered as indicative of developmental effects. However, the low weight gain and deaths occurring after PND4 were explained as a consequence of repeated-dose exposure from the maternal milk and/or from the diet.
As a contribution of test substance in the milk cannot be estimated, and the (assumed) presence in the milk only is not sufficient to justify a classification for effects on/via lactation, no classification for effects on or via lactation is proposed.

10.10.9 Comparison with the CLP criteria

The discussion and comparison with the CLP Criteria for adverse effects via lactation is done in Section 10.10.6 together with the discussion and comparison of CLP criteria for developmental effects and effects on fertility.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

A two generation study in rats with DOTI/MOTI (78.8:16.9, 80:20 ratio) showed maternal effects on the thymus and this result was the critical toxic effect for the adult NOAEL.

The body weight of F1 animals showed no statistically significant differences from controls in either sex at birth, PND 4 or PND 7, but showed a statistically significant decrease in both male and female pup body weights at PND 14 which persisted through PND 21. This correlates exactly with the incidences of postnatal losses documented for these F1 animals. Up to PND 4 the number of F1 pups was not different from controls for either sex. Pups were culled to 4/sex/litter on PND 4 (a routine practice). After culling, the pup losses across the four dose groups between PND4 and PND21 were 0, 4, 11, and 20 for the control, 20, 60, and 200 ppm doses. This is most probably not a reproductive effect; it can be best explained as a consequence of a direct toxic effect of dioctyltin species on these young animals who may well be receiving two sources of exposure, one from the maternal milk and a second from the diet as they begin to eat adult feed during the lactation period. It may (in theory) also be a consequence of impaired nursing behaviour as a result of maternal toxicity for which no clear indication was given. The mg/kg/day dose to these juveniles is likely higher than the dose to the lactating F0 females (the mothers) and the dose-response of the juvenile deaths supports this conclusion. In addition, the delay in vaginal opening of the high dose females is likely due to the non-specific toxic effect and low weight gain resulting from this postnatal exposure.

For the reproductive testing of the F1 generation, DOTI/MOTI did not produce an adverse effect on mating, fertility, pregnancy rates, gestation, litter size or neonatal body weight. There was an increase in stillbirths which was inexplicably marked both non-statistically significant and statistically significant at the high dose; however 12 of the 26 still births for the group were in a single litter. This diminishes any statistical significance in the endpoint analysis. There was an effect on thymus weight of the F2 animals and the histopathology of the thymus was typical of the known target organ effect of multiple dioctyltin substances on this gland.

The body weight of F2 animals at the mid and low doses showed no statistically significant differences from controls in either sex at any time. The body weight of F2 animals at the high dose showed no statistically significant differences from controls in either sex at birth, however at PND4 (postnatal day 4 and thereafter through PND21 body weights of both sexes were statistically decreased.

This result for the F2 pups is parallel to the outcome from the F1 pups. The effect on weight correlated exactly with the incidences of postnatal losses documented for the F2 animals. Up to PND 4 the number of F2 pups

was not different from controls for either sex. After culling, the pup losses across the four dose groups between PND 4 and PND 21 were 9, 26, 29, and 22 for the control, 20, 60, and 200 ppm doses. This is not a reproductive effect for the F2 generation either. It is the second manifestation of a direct toxic effect of dioctyltin species on young animals who may well be receiving two sources of exposure, one from the maternal milk and a second from the diet as they begin to eat adult feed during the lactation period. The dose-response of the juvenile deaths supports this conclusion. No assessment of vaginal opening or testicular descent was done in the F2 animals because the experiment was terminated at PND 21, prior to the occurrence of these developmental landmarks.

The reproductive NOEL is the high dose, 200 ppm. The maternal and paternal NOEL is 20 ppm (the low dose) which is driven by the effect of DOTI/MOTI test material on the thymus gland.

The outcomes for the PND4-PND21 pups of the F1 and F2 generations, however, should not be used in setting the reproductive NOEL, because the effect does not appear to be a reproductive effect, rather a direct toxic effect of repeated doses delivered dose to these animals. The actual dose is unknown, but likely higher than the adults because of the probable dual exposure routes. Taken together, this study does not meet the classification criteria of clear evidence for reproductive effects of DOTI, which suggests the study has diminished significance when extrapolating to the named substance in the classification proposal, DOTE.

Two new key studies, investigating the developmental toxicity with the DOTE substance itself focused on the most sensitive species, mice and rabbits. These new GLP studies were conducted with 96 % purity DOTE and are compliant guideline studies (OECD 414) following GLP which evaluated gestational integrity, external anomalies, soft tissue anomalies and skeletal anomalies. However, in comparison to other studies on analogues the highest dose tested using DOTE was notably lower (Table 14), maybe too low to detect a dose-response relationship and thus the highest dose tested might rather reflect the starting point of a potential dosage-related response.

A maternal and/or a developmental/reproductive LOAEL specifically for the DOTE substance can be derived from the data above. Earlier estimates of the NOAEL should be disregarded in light of the new data set for DOTE.

As noted in the text above (Chapter 10.10.5), pregnant rats exposed orally to DOTI/MOTI did not show any variations of bone formation as seen in mice and rabbits. Rat dams had no treatment-related adverse effects on their foetuses. At a high dose of 25 mg/kg/day there was marginal maternal toxicity and the adverse outcomes noted in a single high dose dam were considered to have occurred by chance.

Quantitative comparison of new data on DOTE with data on DOTI / MOTI 80:20

Table 14: Summary of Dose-Response: DOTI/MOTI 80:20 vs DOTE in Developmental Toxicity Study with Mice. This is a dose-to-dose comparison of the DOTI and DOTE content in the test material stepping down from highest to lowest dose.

DOTI/MOTI 80:20 Effects Observed at dose levels of 100, 67, 45, 30, and 20 mg test material/kg bw/d corresponding to the amounts of the dioctyl species given below) **Mouse** (Faqi et al. 2001)	**DOTE** (purity 96.1 %) Effects Observed at dose levels of 60, 30, and 15 mg test material/kg bw/d corresponding to the amounts of the dioctyl species given below) **Mouse** (Anonymous 2014b)
80 mg DOTI/kg bw/day for 12 days **Maternal Toxicity** 1 death; resorption rate ↑ [16 %] thymus gland weight ↓ [27 %], liver weight ↓ [23 %]; corrected maternal body weight gain ↓ [43 %, mean (control): 5.1 ± 7.0 g, mean ± SD: 2.9 ± 4.8 g] **Developmental Toxicity** foetal body wt. ↓ [18 %]; litter size ↓ ; foetal loss ↑; major malformations related to treatment: ↑ incidence of cleft palate [9.3 %], exencephaly [7.1 %], bent ribs [35.5 %] and forelimbs [18.5 %]	No data for this dose level.
53.6 mg DOTI/kg bw/day for 12 days **Maternal Toxicity** resorption rate ↑ [13 %] thymus gland weight ↓ [13 % statistically not significant]; corrected body weight ↓ [10%] **Developmental Toxicity**– THRESHOLD* foetal body wt. ↓ [9 %]; litter size ↓; foetal loss ↑ ↑ incidence of cleft palate [5.5 %], bent ribs [6.4 %]	**57.7 mg DOTE/kg bw/day** for 13 days [higher dose of dioctyltin species, longer duration] **Maternal Toxicity** thymus gland weight ↓ [35 %]; corrected body weight ↓ [26.6 %] **Developmental Toxicity** statistically significant positive trend on percentage of post implantation loss: mean ± SD: 2.6 ± 5.6
36 mg DOTI/kg bw/day for 12 days **Maternal Toxicity** – THRESHOLD* thymus gland weight ↓ [15 %]; NO effect on body weight **Developmental Toxicity** – NOAEL (malformations) ↓ ossification of skull bones [25.2 %] and digits, [4.4 %] supernumerary ribs ↑	No data for this dose level
No data for this dose level	**28.8 mg DOTE/kg bw/day** for 13 days **Maternal Toxicity** – THRESHOLD thymus gland weight ↓ [23 %] ; marginal effect on body weight ↓ **Developmental Toxicity** statistically significant positive trend on percentage of post implantation loss: mean ± SD: 1.5 ± 4.9
24 mg DOTI/kg bw/day for 12 days **Maternal Toxicity** - NONE Maternal - NOAEL **Developmental Toxicity** supernumerary lumbar ribs ↑ [67.8 %]	No data for this dose level
16 mg DOTI/kg bw/day for 12 days **Maternal Toxicity** - NONE **Developmental Toxicity** skeletal variations: supernumerary ribs ↑	**14.4 mg DOTE/kg bw/day** for 13 days [~equal dose of dioctyltin species, longer duration] **Maternal Toxicity**- NONE Maternal NOAEL **Developmental Toxicity** statistically significant positive trend on percentage of post implantation loss: mean ± SD: 0.9 ± 2.8

* In the Table the word THRESHOLD appears in capital letters, always to the right of bold text indicating either Maternal Toxicity or Developmental toxicity, e.g. in this cell of the Table 36 mg DOTI/kg bw/day is the threshold for maternal toxicity. The word THRESHOLD highlights the threshold dose for Maternal Toxicity or Developmental toxicity.

Table 15: Summary of Dose-Response: DOTI/MOTI 80:20 vs DOTE in Developmental Toxicity Study with Rabbits. This is a dose-to-dose comparison of the DOTI and DOTE content in the test material stepping down from highest to lowest dose.

DOTI/MOTI 80:20 Effects Observed at dose levels of 100, 10, and 1 mg test material/kg bw/d corresponding to the amounts of the dioctyl species given below) **Rabbit** (Battenfeld 1992)	**DOTE** (purity 96.1 %) Effects Observed at dose levels of 80, 20, and 4 mg test material/kg bw/d corresponding to the amounts of the dioctyl species given below) **Rabbit** (Anonymous 2014a)
80 mg DOTI/kg bw/day for 13 days [GD6-18] **Maternal Toxicity**– THRESHOLD* incidence of abortion ↑ [4 dams], total resorptions [28.4 %]; body weight unaffected **Developmental Toxicity** mean foetal body wt. per litter ↓ [~20 %]; foetolethality [3 dams]: incidence of post implantation loss ↑ [28.4 % per group] incidence of skeletal/visceral abnormities ↑ [~60 %]: ossification of skull bones, sternebrae and feet bones ↓; dilated renal pelvis and additional small vessels originating from the aortic arch	**76.9 mg DOTE/kg bw/day** for 23 days [GD6-28] [~equal dose of dioctyltin species, longer duration] **Maternal Toxicity**– THRESHOLD NO abortion, or resorption thymus gland weight ↓ [12.8 %]; body weight unaffected **Developmental Toxicit**y – biologically relevant effect on foetal body wt. (mean -11.9 % relative to controls) and foetal crown-rump length (mean -10.7 % relative to controls) statistically significant negative trend on foetal weight and on foetal crown-rump length / 1 litter with foetal loss [within historical controls] excluded
8 mg DOTI/kg bw/day for 13 days [GD6-18] **Maternal Toxicity** - NOAEL **Developmental Toxicity** – THRESHOLD marginal retardative effect (non-significant increase): ossification of skull bones and digits ↓;	**14.4 mg DOTE/kg bw/day** for 23 days [GD6-28] [higher dose of dioctyltin species and longer duration] **Maternal Toxicity**- NOAEL **Developmental Toxicity** statistically significant negative trend on foetal weight and on foetal crown-rump length
1 mg DOTI/kg bw/day for 13 days [GD6-18] **Maternal Toxicity** - NONE **Developmental Toxicity**– NOEL	**3.8 mg DOTE/kg bw/day** for 23 days [GD6-28] [higher dose of dioctyltin species and longer duration] **Maternal Toxicity**- NONE **Developmental Toxicity** statistically significant negative trend on foetal weight and on foetal crown-rump length

* In the Table the word THRESHOLD appears in capital letters, always to the right of bold text indicating either Maternal Toxicity or Developmental toxicity, e.g. in this cell of the Table 76.9 mg DOTE/kg bw/day is the threshold for maternal toxicity. The word THRESHOLD highlights the threshold dose for Maternal Toxicity or Developmental toxicity.

The NOAEL for maternal toxicity in mice is 14.4 mg DOTE/kg bw/day [GD5-17]. The NOAEL for developmental/reproductive toxicity cannot be determined as there is statistically significant positive trend on percentage of post implantation loss [GD5-17]. The NOAEL for maternal toxicity in rabbits is 76.9 mg DOTE/kg bw/day [GD6-28] and the LOAEL for developmental/reproductive toxicity is 76.9 mg DOTE/kg bw/day [GD6-28]. Therefore the overall oral NOAEL for maternal toxicity is 14.4 mg DOTE/kg/day, based on slight maternal thymus toxicity. The overall developmental NOAEL [which is defined as the highest dose producing no adverse effect] cannot be determined, but is based on increasing percentages of post implantation loss in mice and effect on foetal weight and foetal crown-rump length in rabbits.

These data support the conclusion that there are adverse developmental effects from DOTE in mice and rabbits, the most sensitive species. The developmental effects in rats with DOTI/MOTI were related to the observation that this species is less sensitive and that further testing of DOTE in rats is (a) unlikely to alter the conclusion, and (b) unnecessary for classification, since DOTI is structurally very similar to DOTE differing only slightly at the C-8 alcohol of the mercaptoester ligand and their respective mercaptoacetate esters are expected to have very similar physicochemical and toxicological properties, including hydrolysis products. However, the recently conducted developmental toxicity studies with DOTE in two species (see Table 11, Section 10.10.4) suggest that DOTI might have a higher toxicological potency with respect to developmental toxicity as supported by the Summary Tables Table 14 and Table 15 of Dose-Response.

There are no data to suggest that classification of the substance for reproductive toxicity is required, however, not all doubts could be dismissed regarding developmental toxicity of DOTE, and thus the following classification is appropriate:

CLP
Classification as Repr. 2 (H361d)

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

There was one oral two-generation reproduction toxicity study (OECD TG 416; Anonymous, 1997) performed with the read-across substance DOTI:MOTI (80:20) in rats. The dose levels in this study were 0, 20, 60, and 200 ppm (nominal in diet) (approximately 1.5, 4.4, and 15 mg test material/kg bw/d in P0 animals and 1.6, 4.7, 16 mg test material/kg bw/d in F1 animals).

The effects observed included slight decreases in maternal food consumption during lactation, slightly decreased pup body weights and lower thymus weight in both pups (F1 but not in F2) and parental animals. In the F1 generation at the high dose there was a statistically significant increase in stillbirths (26 vs. 5 in controls), and increased pup mortality after PND4, resulting in a lower viability and lactation indexes. No teratogenic effects were observed in this study.

In addition, there was a reproductive toxicity screening study (OECD TG 421; Appel & Waalkens-Berendsen, 2004) with DOTC. The dose levels in this study were 0, 10, 100 and 300 ppm (corresponding to 0.5-0.7, 4.2-6.2 and 8.4-17.0 mg DOTC/kg bw/d). The main effect in the dams was lymphoid depletion in the thymus, which occurred at all doses with a dose-dependent increase in severity.

No effects were observed on the mating index, pre-coital time, female fecundity index, female fertility index or male fertility index. The gestation index was 86, 100, 71 and 50 % in the control, 10, 100 and 300 ppm groups, respectively. The live birth index was 99, 95, 53 and 60% in the control, 10, 100 and 300 ppm groups, respectively. Post-implantation loss was 22.3, 21.0, 49.2 and 70% for the control, 10, 100 and 300 ppm groups, respectively.

As no effects were observed on fertility, no classification for fertility was proposed by the DS.

Development

Five prenatal developmental toxicity studies (PNDT, OECD TG 414) were presented in the CLH report, two performed with DOTE (Anonymous, 2014a (rabbit) and 2014b (mouse)) and three with DOTI/MOTI 80:20 (called DOTI in the rest of the opinion; Battenfeld, 1991 (rat) and 1992 (rabbit); Faqi *et al*., 2001 (mouse)).

The first study with DOTE was performed in rabbits with doses of 0, 4, 20, 80 mg/kg bw/d. The LOAEL was 80 mg/kg bw/d for both maternal and developmental toxicity. Maternal effects consisted of a dose-dependent depression in maternal thymus weight compared to

controls (5.1, 9.6 and 12.8% in the low-, mid-, and high dose group, respectively), which was considered biologically relevant at the high dose. Foetal effects at the high dose included decreased body weight (-11.9%) and crown-rump length (-10.7%) compared to controls, and one high dose litter with total foetal loss.

The second PNDT with DOTE was performed in mice with doses of 0, 15, 30, 60 mg/kg bw/d. Maternal effects consisted of a depression in thymus weight at the mid- and high dose groups (23 and 35%, respectively). Also, a statiscally non-significant decrease in maternal body weight gain was observed at the high dose. The only developmental effect was a statistically significant trend in the percentage of post-implantation loss (0.0 ± 0.0 in controls, 0.9 ± 2.8 at low-, 1.5 ± 4.9 at mid-, and 2.6 ± 5.6 at high dose).

The first PNDT with DOTI was performed in rats with doses of 1, 5, and 25 mg/kg bw/d. Both maternal and developmental effects occurred only at the top dose of 25 mg/kg bw/d. Maternal toxicity consisted of a slight, non-significant decrease in corrected body weight and body weight gain. There was a significant increase in the percentage of dead foetuses, however, all dead foetuses were from a single dam.

The second study with DOTI was performed in rabbits at 1, 10, and 100 mg/kg bw/d. Effects at the high dose consisted of an increased incidence of abortions, post-implantation loss, minor visceral anomalies, minor skeletal head anomalies, skeleletal variations of the sternum and feet bones, and a significant reduction in foetal body weight. As maternal disease was noted in some animals, the DS considered that this study was less significant in the assessment compared to the new DOTE study.

The third study with DOTI was performed in mice at 20, 30, or 45 mg/kg bw/d (group 1); and at 67 or 100 mg/kg bw/d (group 2). Maternal toxicity consisting of a significant decrease in thymus weight occurred from 45 mg/kg bw/d. At 67 mg/kg bw/d an increase in foetal incidence of cleft palates (+5.5%) was observed. The DS considered that this study was less significant than the new DOTE study as detailed information on several endpoints was not reported.

The studies with DOTE itself in mice and rabbits showed some developmental effects, as well as slight maternal toxicity. The DS noted that the highest dose tested might have been too low to enable the detection of a dose-response relationship and thus it might only reflect the starting point of a potential dose-related response. The studies with the read-across substance DOTI as well as with DOTC consistently showed developmental effects, including post-implantation loss, retardations of foetal development and decreased foetal weight.

In the oral two-generation reproduction toxicity study in rats (OECD TG 416; Anonymous, 1997) performed with the read-across substance DOTI:MOTI (80:20), slightly decreased pup body weights and lower thymus weight in F1 pups (but not in F2) were seen. In the F1 generation at the high dose there was a statistically significant increase in stillbirths (26 vs. 5 in controls), resulting in a lower viability index. No teratogenic effects were observed in this study.

As the studies with DOTE itself showed only some evidence of developmental toxicity and its potency seemed to be lower than that of DOTI, the DS proposed classification as Repr. 2; H361d.

Lactation

In the two-generation study with DOTI/MOTI, postnatal deaths occurred during the lactation period. However, the DS concluded that a contribution of the test substance in milk for the observed effects could not be estimated, and only the (assumed) presence of substance in milk was not sufficient justification for classification for effects on/via lactation. No classification for effects on or via lactation was proposed by the DS.

Comments received during public consultation

Five comments that addressed toxicity to reproduction were received during the public consultation.

One was from the Organotin REACH Consortium, which argued that only the two PNDT studies with DOTE itself, and the two-generation study with DOTI should be considered for classification for reproductive toxicity of DOTE, as these were the most relevant studies. According to the registrants, these studies did not warrant classification for reproductive toxicity.

The other four comments were from MSCAs, which all opposed the proposed revision of the current harmonised classification from Repr. 1B to Repr. 2 for development. The main arguments provided were:

- The effects observed in the studies with DOTE are consistent with those induced by DOTI and DOTC;
- As maternal toxicity was very limited, the developmental effects could not be considered secondary to maternal toxicity;
- The new studies had too low dose levels, but the effects observed at the high doses supported classification;
- Differences in the potency between DOTE and DOTI did not result in different hazard categories for reproductive toxicity.

Regarding the two PNDT studies with DOTI, it was also stated that the previous RAC opinion for DOTE considered these studies sufficiently reliable for classification.

An additional notion was that a comparison of ED10 between DOTE and its analogues could be useful for a comparison of their potency.

Assessment and comparison with the classification criteria

Fertility

There is no reproductive toxicity study available with DOTE itself that includes information on effects on sexual function and fertility. However, a two-generation study (OECD TG 416) with DOTI/MOTI (80:20) and a reproductive toxicity screening study (OECD TG 421) with DOTC are presented in the CLH dossier. The DOTI/MOTI study is relevant, but it is not clear to what extent the DOTC data can be read across to DOTE considering the new *in vitro* data showing no transformation of DOTE to DOTC *in vitro*.

No effects were observed on the reproductive organs or fertility indices in these studies. In the two-generation study, a significant increase in stillbirths occurred in the F1-generation. In the screening study on DOTC, the mid- and high dose induced an increase in post-

implantation loss. However, as all effects occurred post-implantation, RAC agrees with the DS that they should be considered as developmental effects.

As there were no effects on sexual function and fertility observed in the two-generation study with the read-across substance DOTI/MOTI (80:20), **RAC concludes that no classification is warranted for effects on sexual function and fertility**.

Development

In 2012, DOTE was classified by RAC as Repr. 1B; H360D, based on read-across from DOTI and DOTC. The dossier included the same five read-across studies as the current dossier, but lacked the PNDT studies with DOTE itself.

The classification at that time was based on the following effects, exerted by DOTI:

- reduction in foetal body weight in rabbits and mice;
- increased post-implantation loss in rabbits;
- abortions in rabbits;
- increased number of stillbirths in rats;
- increased rate of pup mortality in rats (PND 4 > PND 1, reduced lactation index
- (PND 21));
- increased incidences of minor visceral anomalies, skeletal head anomalies, and skeletal variations in rabbits;
- increased incidences of skeletal variations, skeletal abnormalities, cleft palate, and exencephaly in mice;
- reduced thymus weights in F1 pups (indicative for developmental immunotoxicity ≥60 ppm (≥4.4 mg/kg bw/d, two-generation study, rat));

In addition, the classification opinion referred to these effects seen after exposure to DOTC:

- increased post-implantation loss in rats and mice;
- reduced T-cell mitogen response (indicative for an immunosupressive effect) in directly dosed weanlings (rats) on PND 3-24, indicating that weanlings are more sensitive than young adults.

RAC considered these effects as clear evidence of developmental toxicity in three species, while there were no or only slight signs of maternal (thymo-)toxicity (RAC, 2012). Regarding the limited reporting of the PNDT study with DOTI in mice, RAC stated: "*Details may be lacking since data requirements for a full study report to achieve compliance to testing guidelines are higher. Nevertheless, there is no obvious reason to question the results of this published study. Dose dependency of effects and consistency with other studies support the reliability of the study.*"

As to the incidence of infectious disease in the PNDT in rabbits with DOTI, RAC noted: "*Industry concluded that robustness of this study was compromised by infections. RAC did not share this view: The original study did not report other animals to be affected by infectious diseases.*" Also Industry's view is not in agreement with the overall conclusion of the study author in the original study report: "*At the high dose level of 100 mg/kg/d, clear-cut embryotoxic effects, i.e. an increased rate of abortions and embryolethal effects as well as marked retardations of fetal development, were induced by the test substance.*" and

"*marginal retardation effects on fetal development could be attributed to treatment with the intermediate dose of 10 mg/kg/day*".

RAC also concluded that the observed developmental toxicity was not considered to be a secondary non-specific consequence of the (thymo-)toxicity.

In the two-generation study (OECD TG 416; Anonymous, 1997) performed with the read-across substance DOTI:MOTI, a statistically significant increase in stillbirths occurred in the F1-generation, leading to a lower viabilitiy index. In the screening study on DOTC, the mid- and high dose induced a reduced live birth index and an increase in post-implantation loss.

Considering the previous RAC opinion, the main question is whether the two new PNDT studies with DOTE show a qualitative difference in the developmental toxicity of DOTE compared to DOTI and DOTC, which would invalidate the previous conclusion.

As described previously in this opinion (see RAC general comment), new *in vitro* hydrolysis data indicate that DOTC is not formed from DOTE, although the exact *in vivo* metabolism of both DOTE and DOTC is still unclear. DOTC and DOTE induce similar thymotoxicity, which indicates they share similarities in their toxicity profiles. There is no data on the MoAs of these organotins for either reproductive toxicity or thymotoxicity, i.e. it is not known if it is the parent substance or active metabolites that exert the toxicity. For these reasons, RAC considers that the DOTC data cannot be ignored and should be used in a weight of evidence (WoE), but not necessarily in a strict read across approach. In fact, it is noted that the effects seen in the studies with the close analogue DOTI are already sufficient to support for classification and labelling, whitout the need for considering DOTC data.

It is difficult to assess whether there are qualitative differences between these substances based on the available database, but small differences would not affect the classification.

The PNDT study with DOTE in rabbits showed a significant negative trend on foetal weight and crown-rump length (see table below, and also table 3 in the response to comments document). In the rabbit PNDT study with DOTI there was also a reduction in foetal weight at the high dose (100 mg DOTI/kg bw/d). The crown-rump length was not mentioned, but there were multiple skeletal/visceral abnormities that indicate a disturbance/delay in foetal development. The study with DOTI also showed an increase in post-implantation loss at 100 mg/kg bw/d. This effect was not seen in the study with DOTE, however, there was one litter with only dead foetuses.

It should be noted though that the control group in the rabbit PNDT with DOTE had relatively small litters (mean size 4.9±1.4 in controls, no comparison with historical control data (HCD) was available) and high incidence of skeletal malformations/variations, which decreases the chances of finding a statistically significant effect. Moreover, only slight maternal toxicity was observed at the top dose (thymus weight decreased by 13% as compared to controls), which indicates that the dose levels were too low to really study developmental toxicity and to determine a dose-response relationship in this study.

Nevertheless, the effects on foetal weight and crown-rump length confirm that DOTE interferes with foetal development in rabbits.

***Table**: Summary of results in the rabbit PNDT study with DOTE*

	0 mg/kg bw/d	4 mg/kg bw/d	20 mg/kg bw/d	80 mg/kg bw/d[1]
Maternal thymus weight (g)	2.24 (100%)	2.12 (94.9%)	2.02 (90.4%)	1.95 (87.2%)
Implantation sites	5.1	6.0	5.5	5.0
Live foetuses	4.9	5.7	5.0	4.6
Dead foetuses	0	2	2	3
Gravid uterus w	230.1	309.5	253.7	196.9
Pre-implantation loss (%)	0.9	0.8	2.3	4.9
Post-implantation loss (%)	3.1	3.5	6.4	5.7
Foetal weight (g)	36.6	37.3	35.5	32.3
Crown rump length (mm)	92.1	91.1	89.3	82.3*
Sternum No 5 absent	7 (7.6%)	4 (3.4%)	4 (4.2%)	5 (4.3%)
Sternum No 5 poor ossification	4 (4.4%)	1 (0.8%)	4 (4.2%)	10 (8.7%)
Sternum No 6 poor ossification	4 (5.3%)	1 (0.8/%)	3 (3.2%)	10 (8.7%)

[1]One litter with foetal loss excluded

*P<0.05

In the PNDT with DOTE in mice, the only developmental effect was a significant positive trend in the percentage of post-implantation loss (0.0±0.0, 0.9±2.8, 1.5±4.9, 2.6±5.6% in control, low-, mid- and high dose, respectively). However, as noted by the DS and several member states, the highest dose in this study (60 mg/kg bw/d) was notably lower compared to the highest doses in the studies with the analogues. The maternal effects observed at 60 mg DOTE/kg bw/d were restricted to a numerically decreased corrected body weight gain, but the decrease was not statistically significant.

According to the registration dossier of DOTE, the dose selection rationale of this study was based on the PNDT study with DOTI. The choice of 60 mg/kg bw/d was based on the maternal and developmental effects observed with DOTI at 67 and 100 mg/kg bw/d:

"*Therefore, the high dose chosen for this study is 60 mg/kg, to reflect a dioctyltin dose with minimal maternal and foetal toxicity as the upper bound. It is anticipated that this dose will meet the developmental toxicity test guideline criteria of producing some maternal toxicity without compromising the survival of the pregnant dam, the integrity of pregnancies to Day 18, or the survival of the developing foetuses*."

The use of such dose levels might have been justified if there would have been overt maternal toxicity in the PNDT study with DOTI. However, as stated by RAC in 2012, maternal toxicity caused by the dose levels used (67 and 100 mg/kg bw/d) can be characterised as slight (mainly decreased thymus weight). Moreover, if the goal is to determine whether a substance is toxic for development, dose levels minimising the chances to observe adverse effects on the integrity of pregnancies or the survival of the developing foetuses may not be appropriate.

Thus, RAC notes that the highest dose of DOTE was similar to the dose of DOTI where the dose-response for reproductive toxicity started.

The trend to an increase in post-implantation loss does indicates that DOTE induces foetal mortality in mice, similar to DOTI.

Considering the outcome of the PNDT studies with DOTE, RAC considers it justified to assume that DOTE induces developmental effects similar to DOTI and DOTC. The low dose levels in the studies with DOTE limits the opportunity to perform a direct comparison of the quantitative differences in the potency. There is no ground to derive a specific concentration limit for DOTE.

As there are no data in humans, classification in Category 1A is not warranted.

The differentiation between Category 1B and Category 2 depends on the strength of the evidence, including the nature of the effects observed, the quality of the data, and the relevance of the effect for humans.

The DS proposed Category 2 for development, based on the two PNDT studies with DOTE that show only slight adverse effects on development. However, considering the marginal maternal toxicity at the highest dose levels (60-80 mg/kg bw/d) and the significant trends in developmental toxicity seen, it is highly probable that DOTE would show clear evidence of adverse effects on development at higher dose levels. This is also supported by the results of the studies with the closely related substance DOTI, which included increased post-implantation loss, increase incidence of resorption, increase pup mortality. Given the close structural similarity between DOTE and DOTI, the clear evidence of developmental toxicity in the studies with DOTI and the outcome of the PNDT studies with DOTE which indicate that DOTE has comparable effects, RAC considers that there is sufficient evidence to **retain the current harmonised classification of DOTE as Repr. 1B (H360D)**.

Lactation

In the two-generation study with DOTI, there was an increase in growth retardation and pup losses between PND4 and PND21 in all dose groups in both F1 and F2 generations (dead pups: 0, 4, 11, and 20 in the F1 and 9, 26, 29, and 22 in the F2 at 0, 1.6, 4.7, 16 mg/kg bw/d). The explanation presented by the DS was that pups experience a higher exposure as they are exposed through both maternal milk and from the diet, although exposure via milk was only assumed and not measured. This explanation seems plausible, however RAC notes that although pup body weight was not significantly decreased compared to controls at birth (3-4%), whereas it was significantly decreased (19-21%) by PND14, no further decrease in pup body weight was noted from PND14 to 21 when additional exposure via food becomes more important. Although the lactational period seems the most sensitive for pup toxicity, the concentration of DOTI in maternal milk and the (relative) exposure of the pups through milk were not determined. Also, it is possible that the growth retardation and pup mortality were late effects of the developmental

toxicity experienced during gestation, which RAC already concluded that DOTE warrants classification for.

Hence, RAC agrees with the DS that there is at present **insufficient evidence to justify classification for effects on or via lactation**.

10.11 Specific target organ toxicity-single exposure

This point is not proposed for harmonization.

10.12 Specific target organ toxicity-repeated exposure

Table 16: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
equivalent or similar to OECD TG 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) supporting study 2 (reliable with restrictions) Rat (Sprague-Dawley) 4 groups of 40 rats 20 male/20 female subchronic	**Test material: DOTE/MOTE** **Dioctyltin bis(2-EHMA) [CAS No. 15571-58-1] : Octyltin tris(2-EHMA) [CAS No. 27107-89-7] (purity 70:30 %)** oral: feed 25, 50, and 100 ppm (nominal in diet) (0, 1.6, 3.3, and 6.6 mg/kg bw/day) Exposure: 90 days (continuously) Post-exposure period: 30 days	NOAEL: 25 ppm (male/female) based on: test material (At 50 and 100 ppm: significant dose-related reduction in absolute and relative thymus gland weights [~25-35 %].) 25 ppm calculated as 1.25 mg/kg bw/day, based on a food factor of 0.05.	Anonymous (1974)
equivalent or similar to OECD TG 408 (Repeated Dose 90-Day Oral Toxicity in Rodents) key study 2 (reliable with restrictions) rat (Wistar) male/female (15 rats/sex/group) subchronic	**Test material: DOTE (97 %)** **Dioctyltin bis(2-EHMA) [CAS No. 15571-58-1] : [CAS No. 27107-89-7] Octyltin tris(2-EHMA) : Trioctyltin (2-EHMA) [CAS No. 61912-55-8] (purity 97: 0.3 : 2.17 %)** oral: feed 100, 500, and 1000 ppm (experiment 1) (nominal in diet) 50 and 250 ppm (experiment 2) (nominal in diet) 10 and 25 ppm (experiment 3) (nominal in diet) Exposure: 90 days (continuously)	NOAEL: 10 ppm (male/female) based on: test material (reduced thymus weight [~20 %]) 10 ppm is equivalent to 0.5 mg/kg bw/day	Anonymous (1970)

Table 17: Summary table of human data on STOT RE

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

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

The two subchronic studies (Anonymous, 1974 and 1970) with DOTE (CAS No. 15571-58-1) and MOTE (CAS No. 27107-89-7) at 70/30 % Dioctyltin (2-EHMA)/Monooctyltin (2-EHMA) and 97:2.17 % Dioctyltin (2-EHMA) and Monooctyltin (2-EHMA) demonstrated that the substance causes clear target effects substantiated by thymus lymphocyte depletion. A GLP deficiency for these two tests exists in that the reports do not contain information on the test substance homogeneity and stability; however other data for the octyltin family and more broadly for organotin thioesters indicate that the substances are stable in animal feed. Therefore, the studies can be considered "valid with deficiencies" and used for classification of the substance. In the summaries below, the high mortality in the 500 and 1000 ppm groups indicated these doses were too high to accurately and meaningfully assess the non-lethal adverse effects. For this reason, calculations of % change in measured parameters were done only for doses below 500 ppm.

1/ In the first subchronic diet non GLP study (Anonymous, 1970), rats were given 100, 500 and 1000 ppm (test 1), 50, 250 ppm (test 2), 10, 25 ppm (test 3) Dioctyltin (2-EHMA) during 90 days. The following effects were observed:

- Mortality: 9/15 males and 4/15 females died in the 500 ppm diet group; 15/15 males and 14/15 females died in the 1000 ppm diet group;
- Food consumption and food efficiency: slightly, but not significantly reduced at 500 and 1000 ppm.
- Haematology:
  - Significant decrease of RBC at 100 ppm diet for males (92 % of controls), and at 500 ppm diet for females (week 6).
  - Significant decrease in percentage of lymphocytes and neutrophils at 500 ppm diet (both sexes) (weeks 6 and 12).
  - Significant decrease in haemoglobin content at 100 ppm diet for males (week 12) [97 % of controls], and at 500 ppm diet for females (weeks 6 and 12).
  - Significant decrease in percentage of packed cell volume at 100 ppm diet for males [96 % of controls] and a significant increase for females [105 % of controls] at week 12.
  - Significant decrease in percentage of packed cell volume at 500 ppm diet for females but not for males at week 12.

  Because these haematological findings show small percentage differences, are not consistent across sexes, nor do they follow a clear dose-response, it is uncertain if the findings are treatment-related.
- Urinalysis: Specific gravity of the urine was significantly decreased and UGOT levels were significantly increased at 500 ppm diet (both sexes). Specific gravity of the urine of females at 100 ppm diet was also significantly decreased.
- Biochemical: The sugar content of the blood was significantly decreased in males and females at 500 ppm diet. SGOT levels were significantly increased in females at 10 ppm diet. SGPT levels were significantly increased in females at 10 ppm diet and in males at 500 ppm diet. SAP levels were significantly increased at 100 and 500 ppm diet for both sexes.
- The water content of the brain was significantly decreased at 500 ppm diet.

- Organ weights: The following statistically significant changes were observed:
    - Terminal body weight: decreased in females at 100 ppm diet, and in males and females at 500 ppm diet;
    - Relative heart weight: increased in females at 500 ppm diet;
    - Relative kidney weight: increased in males and females at 500 ppm diet;
    - Relative liver weight: increased in males at 10 ppm diet and in females at 500 ppm diet;
    - Relative spleen weight: increased in females at 500 pm diet;
    - Relative brain weight: increased in males and females at 500 ppm diet;
    - Relative gonads weight: increased in males at 500 ppm diet;
    - Relative thymus weight: decreased in males and females at 100 and 500 ppm diet
- Histopathology: 2/5 females at 100 ppm diet, and 5/5 males and 5/5 females at 500 ppm diet had almost complete depletion of lymphocytes resulting in a very small thymus with a uniform picture of the remaining reticula parenchyma, which hardly permitted a distinction between cortex and medulla. This damage of the thymus was occasionally accompanied with little active lymph nodes and a slight reduction of splenic lymphoid cells. In the kidney, 3/5 males and 2/5 females exhibited swollen tubular epithelial cells containing a granular or finely vacuolated cytoplasm.

The NOAEL was determined to be 10 ppm diet (equivalent to 0.5 mg/kg bw/day), on the basis of reduced thymus weight at 25 ppm diet. The LOAEL was determined to be 25 ppm diet (calculated as 1.07-1.24 mg/kg bw/day in males and 1.46-1.51 mg/kg bw/day in females). Calculation of dosage was performed using body weights of 340 g (males) and 200 g (females), and average food consumption of 14.6-16.8 g/rat/day (males) and 11.7-12.1 g/rat/day (females).

2/ In the second subchronic non GLP study (Anonymous, 1974), rats were given Dioctyltin (2-EHMA)/Monooctyltin (2-EHMA) (70/30 %) at 25, 50 and 100 ppm in diet (equivalent to an average daily intake of 0, 1.6, 3.3 and 6.6 mg/kg bw/day) during 90 days. The following relevant effects were observed:

Significant dose-related reduction in absolute and relative thymus weights in the 50 ppm (3.3 mg/kg bw/day) and 100 ppm (6.6 mg/kg bw/day) dose groups. It was considered a toxicologically-relevant change in the thymus (i.e. thymotoxicity) which was in accordance with the known toxicity profile of the test substance and related octyltin substances.

The overall NOAEL was determined to be 25 ppm in the diet (calculated as 1.25 mg/kg bw/day and a food factor of 0.05) and was based on the 50 ppm threshold for thymus toxicity.

There were two recovery assessments associated with this study, a 15-day and a 30-day recovery. The specific target organ, the thymus, recovered completely within 15 days of the cessation of treatment for both the 50 and 100 ppm doses. There were no gross findings related to treatment or adverse histopathological findings in the thymus for either sex at either 15 or 30 days post-exposure.

The two new developmental toxicity studies with DOTE have been regarded for hazard classification as supporting evidence taking into account shorter exposure periods and pregnancy of the animals.

3/ In the new mice developmental toxicity study (Anonymous, 2014b), dams were given DOTE 96.1 % purity at 0, 15, 30, and 60 mg test material/kg bw/day (corresponding to 14.4, 28.8, and 57.7 mg DOTE/kg bw/day) during day 5 to 17 of pregnancy.

Please refer to section 'effects on development' for details**.**

Maternal toxic effects

Reduction in thymus weight from 30 mg/kg bw/day

MATERNAL DATA

- Gross Pathology [Maternal]

There was a treatment-related macroscopic finding of reduced maternal thymus weight.

The mean maternal thymus weight was statistically significantly reduced (-23 %) in the 30 mg/kg bw/day [mid] and 35 % at the 60 mg/kg bw/day [high] dose groups. The mean maternal thymus weight in the low dose mice was reduced relative to controls, but was not statistically significant. These observations are indicative of a treatment-related specific target organ toxicity resulting from exposure to the test material. No other gross pathological findings were noted in any dose group.

4/ In the new rabbit developmental toxicity study (Anonymous, 2014a), dams were given DOTE 96.1 % purity during day 6-28 of pregnancy at 0, 4, 20 and 80 mg/kg bw/day (corresponding to 3.8, 14.4, and 76.9 mg DOTE/kg bw/day).

Please refer to section 'effects on development' for details**.**

Maternal toxic effects

Biologically relevant depression [> 10 %] in thymus weight at 80 mg/kg bw/day

MATERNAL DATA

- Gross Pathology: There was a treatment-related macroscopic finding of reduced maternal thymus weight. The mean maternal thymus weights were -5.1, -9.6, and -12.8 % in the 4 mg/kg bw/day [low], 20 mg/kg bw/day [mid], and 80 mg/kg bw/day [high] dose groups, respectively when compared to controls. While these decreases were not statistically significant, the results were dose-dependent relative to controls and are consistent with data from other species [rat and mouse] which demonstrate the thymus is a target organ for octyltin compounds. These observations are indicative of a treatment-related specific target organ toxicity resulting from exposure to the test material. No other gross pathological findings were noted in any dose group.

10.12.2 Comparison with the CLP criteria

Category 1: Substances that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of:

- Observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally low exposure concentrations.

Classification in Category 1 is applicable, when significant toxic effects observed in a 90-day repeated-dose study conducted in experimental animals are seen to occur at or below the guidance values: oral (rat) C ≤ 10 mg/kg body weight/day.

Significant toxic effects on thymus are observed in both oral 90-day studies in rats well below the guidance value for Category 1 classification.

These observations are supported by significant toxic effects on thymus in mice and treatment-related as well as dose-dependent effects on thymus in rabbits demonstrating specific target organ toxicity on thymus in several species.

10.12.3 Conclusion on classification and labelling for STOT RE

The evaluation of the repeated dose toxicity was based on four studies:

- Two subchronic oral toxicity tests (rat) with DOTE (70 and 97 % purity) - no guideline studies;

No data on repeated dose toxicity via the dermal or inhalation route are available.

- Two developmental toxicity tests (mouse and rabbit) with DOTE (96 % purity) – OECD TG 414

Based on the four studies, DOTE is proposed to be classified as STOT RE 1, H372: Causes damage to thymus through prolonged or repeated exposure. **Furthermore, the tests illustrate that no adverse effects on the reproductive organs were recorded in either of these studies. These observations remain relevant for the broader assessment on the reproductive toxicity endpoint.**

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

Summary of the Dossier Submitter's proposal

Repeated dose toxicity was assessed based on two 90-d oral studies in rats, and two developmental toxicity studies in mice and rabbits, respectively. The relative thymus weight was reduced by 20-30% after 90 days exposure to 2-3 mg/kg bw/d in rats, and by 10-20% in the dams of the developmental toxicity studies in mice and rabbits after exposure to 20-30 mg/kg bw/d for 12 and 22 days, respectively.

Classification in STOT RE Category 1 was considered applicable as significant toxic effects were observed in the 90-day oral studies in rats at or below the guidance value of 10 mg/kg bw/d for STOT RE 1. These observations were supported by significant toxic effects on thymus in mice and treatment-related as well as dose-dependent effects on thymus in rabbits, demonstrating specific target organ toxicity on thymus in several species. There were no studies using inhalation or dermal exposure. DOTE was thus proposed by the DS to be classified as STOT RE 1, H372: Causes damage to thymus through prolonged or repeated exposure.

Comments received during public consultation

Comments were recieved from three Member State Competent Authorities (MSCAs) and one industry organisation. All four comments supported classification with STOT RE 1, but the industry organisation proposed to postpone the decision on classification until the results are available from ongoing (single dose) studies aiming to determine whether STOT RE or STOT SE would be a more appropriate classification for the observed effects.

Assessment and comparison with the classification criteria

Both 90-d studies in rats are old (from 1970 and 1974), pre-dating OECD test guidelines and GLP, but they are similar to OECD TG 408 studies. Both were considered by the DS as reliable with restrictions. They are very briefly described in the CLH report, but they show consistent results with regard to thymus toxicity.

In one 90-d oral study, DOTE (97%) was administered via the diet at concentrations of 10, 25, 50, 100, 250, 500 and 1000 ppm to groups of male and female rats (Anonymous, 1970). The exposure roughly corresponded to 0.55, 1.3, 2.6, 5.3, 13, 26 and 53 mg/kg bw/d. Substantial mortality was observed from 500 ppm and the terminal body weight

(magnitude not given) was decreased from 100 ppm in females and from 500 ppm in males, as compared to controls. At 100 ppm (approx. 5.3 mg/kg bw/d), almost complete depletion of lymphocytes and small thymus was reported in 2/5 females, effects that were seen in all males and females at 500 ppm. Thymus weight (absolute and/or relative weight not stated) was reduced by 20% also at 25 ppm (1-2 mg/kg bw/d).

In the other oral 90-d study (Anonymous, 1974), rats were given DOTE:MOTE 70:30% in the diet at concentrations of 25, 50, and 100 ppm (0, 1.6, 3.3 and 6.6 mg/kg bw/d). Effects on body weight gain were not given, but dose-dependent reduction of absolute and relative thymus weight was observed from 50 ppm. Thymus weight was completely recovered 15 days after cessation of exposure.

In addition, decreased thymus weights were observed in the two new developmental toxicity studies (with DOTE; Anonymous 2014a and 2014b) in mice and rabbits after 12 and 22 days exposure, respectively. The weight reduction was in the order of 10-20% at exposure levels of 30 and 20 mg/kg bw/d in mice and rabbits, respectively. As noted above, the exposure durations were very short and a comparison with the data from the 90-d studies or with the guidance value for a 90-d study is not appropriate. Significantly decreased relative thymus weights were also observed in the rat two-generation study (DOTI:MOTI; Anonymous, 1997), e.g., at 15-16 mg/kg bw/d in both sexes of the parental animals in the P0- and F1-generation.

RAC concludes, based on the above data on three species, and supported by the general knowledge of thymotoxicity being a characteristics of organotin compounds, that the thymus is a target organ after repeated exposure to DOTE, likely leading to an impaired function of the immune system. RAC suggests to specify the immune system (rather than the thymus) as the target organ in the hazard statement to be consistent with the target organ specification in the hazard statement for other organotins. Adverse effects occured below the guidance value for STOT RE of 10 mg/kg bw/d in the oral 90-d study on DOTE (Anonymous, 1970), and classification as STOT RE 1 is therefore supported by RAC. Thymus toxicity is also evident at low oral exposure levels in the 90-day study on DOTE:MOTE and in the two-generation study on DOTI:MOTI. No studies by the dermal or inhalation route exist, and although toxicity through these routes are likely to be lower than via the oral route, the lack of data precludes specifying the route in the hazard statement. Thus, RAC concludes in line with the DS proposal that **classification of DOTE as STOT RE 1; H372 (Causes damage to the immune system through prolonged or repeated exposure) is warranted**.

10.13 Aspiration hazard

Not evaluated in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 18: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
OECD Guideline 111 (Hydrolysis as a function of pH)	25°C pH 4: half-life > 1 year pH 7: half-life > 1 year pH 9: half-life > 1 year 37°C, 0.1 N HCl pH 1.2: half-life < 1 min degradation product: DOTEC (monochloride ester)	Rel. 2 no GLP-study	Anonymous (2015)
Electrospray ionization mass spectrometry	Rapid hydrolysis within minutes to hours	Rel. 3 (major methodological deficiencies)	Anonymous (2003)
OECD Guideline 301 F (Manometric Respirometry Test)	22±2°C 29-43% degradation (BOD) after 28 days Abiotic control: 0 – 10 % degradation (BOD) after 74 days Reference substance: 87 % degradation (BOD) after 13 days Inhibition control: 40-50 % degradation (BOD) after 14 days	Rel. 1 GLP-study	Anonymous (2000)
OECD Guideline 301B (CO_2 Evolution)	22±2°C 23 % ThCO2 evolution after 28 days Reference substance: 75 % ThCO2 evolution after 13 days	Rel. 2 (inoculum concentration not mentioned) GLP-study	Anonymous (1992d)
OECD Guideline 301B (CO_2 Evolution)	22±2°C 19 % $ThCO_2$ evolution after 28 days (10.2 mg/L initial concentration) 11 % $ThCO_2$ evolution after 28 days (21.4 mg/L initial concentration) Reference substance: 75 % ThCO2 evolution after 13 days	Rel. 2 (inoculum concentration not mentioned) GLP-study	Anonymous (1992e)

11.1.1 Ready biodegradability

Ready biodegradation of DOTE was investigated in a study conducted according to OECD Guideline 301 F using 30 mg solids/L inoculum (activated sludge, domestic, non-adapted) and 50 mg/L test substance. After 28 days 29-43 % degradation was observed. As the biodegradation of DOTE was shown to be slow, the test was prolonged to 74 days (36-68% degradation, validity criteria not fulfilled as difference of replicate values is > 20 %). Degradation of the reference substance (aniline) was 87 % after 13 days. 40-50 % degradation at day 14 was shown in the toxicity control (Anonymous, 2000)).

In addition, two ready biodegradation tests according to OECD Guideline 301 B are available. In the first study inoculum from activated sludge of a wastewater treatment plant was used (adaption and concentration not specified). The initial concentration of the test substance was 11.9 mg/L and 23.8 mg/L. For both concentration

23 % $ThCO_2$ evolution was observed after 28 days. $ThCO_2$ evolution of the reference substance (aniline) was 75 % after 13 days (Anonymous, 1992d). The second OECD Guideline 301 B study was carried out with 10.2 mg/L and 21.4 mg/L test substance. The concentration of the inoculum (activated sludge, adaption not specified) was also not mentioned. After 28 days 19 % $ThCO_2$ was observed for the lower initial concentration and evolution and 11% ThCO2 for the higher initial concentration. The reference substance was degraded to 75 % after 13 days (Anonymous, 1992e)).

In conclusion, DOTE is not readily biodegradable.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

The hydrolysis of DOTE was tested according to OECD Guideline 111. After 5 days less than 10 % DOTE was hydrolyzed at 50°C (pH 4, 7, 9). The half-life for 25°C was calculated by the registrant to be > 1 year (Anonymous, 2015)). At simulated gastric conditions (0.1 M HCl, pH 1.2, 37°C) the half-life was < 1 min and 75% DOTE was hydrolysed to dioctyltin chloro 2-ethylhexylmecaptoacetate (DOTEC).

11.1.4 Other convincing scientific evidence

No data available.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Not relevant for this dossier.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

No data available.

11.1.4.4 Photochemical degradation

Not relevant for this dossier.

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for this dossier.

11.2.1 Summary of data/information on environmental transformation

Not relevant for this dossier.

11.3 Environmental fate and other relevant information

Based on the estimated high log Kow value (15.35) adsorption to sediment and soil is expected.

No further data available.

11.4 Bioaccumulation

Table 19: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
OECD Guideline 305	0.25 µg/L: BCF = 1294 L/kg (steady state) BCF = 1078.3 L/kg (steady state, lipid normalized) 2.5 µg/L BCF = 99 L/kg (steady state) BCF = 82.5 L/kg (steady state, lipid normalized)	Rel. 3 GLP-study	Anonymous (2010)

11.4.1 Estimated bioaccumulation

A Log Kow value of 15.35 was estimated (KOWWIN v 1.68, see chapter 7).

11.4.2 Measured partition coefficient and bioaccumulation test data

The bioconcentration factor (BCF) of DOTE was measured for *Onchorhynchus mykiss* using OECD Guideline 305. The study was carried out in a flow through system using the solvent acetone (100 mg/L) and two exposure concentrations (0.25 and 2.5 µg/L). After an uptake phase of 30 days the BCF values were assumed to be 1294 L/kg for the lower exposure level and 99 L/kg for the higher exposure level. The BCF values are based on the limit of quantification, as DOTE was not detected above the limit of quantification in the fish tissues. The used treatment concentrations are far above the calculated solubility of DOTE and therefore the study is regarded as invalid according to the validity criteria defined in Guideline OECD 305. Further shortcomings of the study are the high limit of quantification, no proof of a steady state concentration in fish and the use of a solvent, which should generally be avoided. It should be noted that DOTE is technically difficult to test in a BCF study based on the high calculated K_{ow}, the low calculated water solubility and the analytical challenges. However, based on the conducted study, neither a reliable BCF value, nor an estimation regarding the bioaccumulation criterion can be derived.

11.5 Acute aquatic hazard

Table 20: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
OECD 203/ EU C.1 Limit-test	*Danio rerio*	CAS 15571-58-1	96h-LC_{50} > 24.8 mg/L (measured)	Rel. 1 GLP-study	Anonymous (2004a)
EU Method C.1 GLP study	*Danio rerio*	CAS 15571-58-1	96h-LC_{50} > 5.8 mg/L (measured end conc.)	Rel. 3 (purity TS only 70%; analytical approach not specified, vehicle used, results based on end conc.)	Anonymous (1993c)
OECD 202 (1984) No vehicle used	*Daphnia magna*	D 27-231 TK 10974 "DOT bis thioglykester"	24h-EC_{50} > 0.11 mg/L (measured)	Rel. 3 No details on analytical method; test conc. not as high as the maximum of water solubility, test duration only 24h	Anonymous (1988a)
OECD 202 Vehicle DMSO	*Daphnia magna*	DOTE CAS 15571-58-1	48h-EC_{50} = 24.12 mg/L (nominal)	Rel. 1 GLP-study	Anonymous (2016a)

			48h-NOEC = 0.05 mg/L (nominal)		
EU Method C.2 vehicle	*Daphnia magna*	CAS 15571-58-1 TK 10315/A Irgastab 17 MOK-A	48h- EC_{50} = 0.17 mg/L (measured) 24h-EC_{50} = 0.32 mg/L (measured)	Rel.3 GLP study According to the updated registration dossier (2017-01), the test item contained 6-12% Ethylhexylthioglycolate (EHTG CAS 7659-86-1), which is more toxic than DOTE and makes the result unrepresentative. The study report itself contains no details on the content of EHTG but the registrant submitted details on the specifications of the test substance used in the test. According to these product specifications "TK 10315/A" and "Irgastab 17 MOK-A" contained 6 to 12% EHTG.	Anonymous (1993d)
OECD 201 Vehicle DMSO	*Pseudokirchneriella subcapitata*	CAS 15571-58-1 DOTE	72h-E_rC_{50} > 100 mg/L (nominal)	Rel. 1 GLP-study	Anonymous (2016b)
EU Method C.3 vehicle	*Desmodesmus subspicatus*	CAS 15571-58-1 TK 10315/A Irgastab 17 MOK-A	72h- E_rC_{50} = 0.17 mg/L (mean measured)	Rel.3 GLP study According to the updated registration dossier (2017-01), the test item contained 6-12% Ethylhexylthioglycolate (EHTG CAS 7659-86-1), which is more toxic than DOTE and makes the result unrepresentative. The study report itself contains no details on the content of EHTG but the registrant submitted details on the specifications of the test substance used in the test. According to these product specifications "TK 10315/A" and "Irgastab 17 MOK-A" contained 6-12% EHTG.	Anonymous (1993e)

11.5.1 Acute (short-term) toxicity to fish

A limit-test from the year 2004 using a WAF of 100 mg/L was conducted according to OECD Guideline 203 with *Danio rerio*. The concentration was analytically confirmed by measuring the total Sn and by using a conversion factor to conclude on the concentration of the parent substance DOT(2-EHMA). No vehicle was used. The semi-static test resulted in no effect at the limit concentration of 24.8 mg/L. The validity criteria were met.

There is also another acute toxicity test available. It is conducted in 1993 according to EU Method C.1 with *Danio rerio* under static conditions. The concentration was analytically confirmed but the method was not

specified. In addition, the purity of the test substance was only 70 %. Therefore, the study is evaluated with reliability 3. No effects occurred up to a concentration of 5.8 mg/L (measured at the end of the test).

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

The test conducted with DOTE from the year 2016 according to OECD Guideline 202 on *Daphnia magna*, used the vehicle DMSO (100 µL/L) and respectively a solvent control under static conditions. Four replicates per group consisting five Daphnids per replicate were used. The concentrations were analytically confirmed and remained within the acceptable range of ± 20 % of the nominal concentrations. The EC_{50} after 48 hours was 24.12 mg/L. The 48h-NOEC was 0.05 mg/L. At 0.1 mg/L immobility occurred (LOEC). The validity criteria were met.

Another acute toxicity test with *Daphnia magna* from the year 1993 used DOTE as test item. In the update of the registration dossier (2017-01), the registrant describes that the test item contained 6-12 % ethylhexylthioglycolate (EHTG CAS 7659-86-1) which shows an acute (48 hours) *Daphnia*-toxicity (EC_{50}) of 0.38 mg/L. Therefore, he assessed the study with reliability 3. From the study report itself it is not obvious that EHTG was a constituent of the test item as the test item description was "TK 10315/A" and "Irgastab 17 MOK-A". The registrant submitted product specifications from which show that the test item contained 9 ± 3% EHTG. The test was conducted with a vehicle and the test concentrations were confirmed analytically. The test was conducted under static conditions. The validity criteria were met. The test resulted in an EC_{50} of 0.17 mg/L (measured concentration). Because of the test item composition the test has to be evaluated with reliability 3.

The study conducted in 1988 with *Daphnia magna* had deficiencies in the documentation. Therefore the reliability was assessed with 3 and the study cannot be used for classification.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

One study from the year 2016 with the algae *Pseudokirchneriella subcapitata* (72h exposure) was conducted according to OECD Guideline 201 and GLP with a vehicle (0.1 % DMSO) and the test substance DOTE. It was a limit test under static conditions. Up to a concentration of nominal 100 mg/L no effects occurred. The validity criteria were met.

Another study conducted in the year 1993 with the algae *Desmodesmus subspicatus* and a vehicle according to the EU Method C.3 under static conditions resulted in an E_rC_{50} of 0.17 and a NOE_rC of 0.04 mg/L (measured end concentrations). Similar to the acute toxicity study with *Daphnia magna* the registrant assessed this study with reliability 3 as the test item contained 6-12 % ethylhexylthioglycolate (EHTG CAS 7659-86-1) as EHTG shows an acute (72 hours) algae toxicity (EC_{50}) of 0.41 mg/L, according to the registrant. This test substance composition is not obvious from the study report itself as there was only the description "TK 10315/A" and "Irgastab 17 MOK-A" for the test substance. The registrant submitted product specifications which show that the test item contained 9 ± 3% EHTG. The validity criteria were met but the results have to be related to mean measured concentrations instead of measured end concentration. The E_rC_{50} based on mean measured concentrations (0.03 – 0.17 – 0.69 – 3.29 – 11.17 mg/L) is 2.16 mg/L. Because of the test item composition the test has to be evaluated with reliability 3.

The study conducted in 1988 with *Desmodesmus subspicatus* had deficiencies in the documentation. Therefore, the reliability was assessed with 3 and the study cannot be used for classification.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

Not available.

11.6 Long-term aquatic hazard

Table 21: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
OECD 211	*Daphnia magna*	CAS 15571-58-1 DOT(EHMA)	21d-$NOEC_{reproduction}$ = 1.448 mg/L (mean measured) 21d-$NOEC_{parental\ survival+mobile\ offspring+body\ length}$ = 0.286 mg/L (mean measured)	Rel. 1 GLP-study	Anonymous (2004b)
EU Method C.3 vehicle	*Desmodesmus subspicatus*	CAS 15571-58-1 TK 10315/A Irgastab 17 MOK-A	72h- NOE_rC = 0.04 mg/L (mean measured)	Rel. 3 GLP study According to the updated registration dossier (2017-01), the test item contained 6-12% Ethylhexylthioglycolate (EHTG CAS 7659-86-1), which is more toxic than DOTE and makes the result unrepresentative. The study report itself contains no details on the content of EHTG but the registrant submitted details on the specifications of the substance used in the test. According to these product specifications "TK 10315/A" and "Irgastab 17 MOK-A" contained 6-12% EHTG.	Anonymous (1993e)
OECD 201	*Desmodesmus subspicatus*	TK 10974 (Sn-content is 23.68%)	72h- NOE_rC = 0.12 mg/L (initial)/ 0.057 mg/L (mean measured)	Rel. 3 Composition and purity of TS not reported	Anonymous (1988b)
OECD 201	*Pseudokirchneriella subcapitata*	CAS 1557-58-1 DOTE	72h-NOE_rC > 100 mg/L (nominal)	Rel. 1 GLP-study	Anonymous (2016b)

11.6.1 Chronic toxicity to fish

Not available.

11.6.2 Chronic toxicity to aquatic invertebrates

The test from the year 2004 according to OECD Guideline 211 with *Daphnia magna* was conducted under semi-static conditions. Water Accommodated Fractions (WAF) from DOTE were used but the test concentrations were analytically confirmed nonetheless. No vehicle was used. The validity criteria were met. After 21 days the test resulted in a NOEC for parental survival, reproduction of mobile offspring and body length of 0.286 mg/L based on the arithmetic mean of the measured concentrations. The test fulfils the validity criteria.

11.6.3 Chronic toxicity to algae or other aquatic plants

One study from the year 2016 over 72 hours with the algae *Pseudokirchneriella subcapitata* was conducted according to OECD Guideline 201 and GLP with a vehicle (0.1 % DMSO) and the test substance DOTE. It was a limit test under static conditions. Up to a concentration of nominal 100 mg/L no effects occurred.

Another study conducted in 1993 with the algae *Desmodesmus subspicatus* and a vehicle according to the EU Method C3 under static conditions resulted in an E_rC_{50} of 0.17 and a NOE_rC of 0.04 mg/L (measured end concentrations). Similar to the acute toxicity study with *Daphnia magna* the registrant assessed this study with

reliability 3 as the test item contained 6-12 % ethylhexylthioglycolate (EHTG CAS 7659-86-1) as EHTG shows an acute (72 hours) algae toxicity (EC_{50}) of 0.41 mg/L, according to the registrant. This test substance composition is not obvious from the study report as there was only the description "TK 10315/A" and "Irgastab 17 MOK-A" for the test substance. The registrant submitted product specifications from which show that the test item contained 9 ± 3% EHTG. The validity criteria were met but the results have to be related to mean measured concentrations. Therefore the NOE_rC based on mean measured concentrations (0.03 – 0.17 – 0.69 – 3.29 – 11.17 mg/L) is 0.17 mg/L. Because of the test item composition the test has to be evaluated with reliability 3.

The study conducted in 1988 with *Desmodesmus subspicatus* had deficiencies in the documentation. Therefore, the reliability was assessed with 3 and the study cannot be used for classification.

11.6.4 Chronic toxicity to other aquatic organisms

Not available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

Table 22: Comparison with criteria for acute aquatic hazards

	Criteria for environmental hazards	**DOTE**	**Conclusion**
Acute Aquatic Toxicity	Cat. 1: $LC_{50}/EC_{50}/ErC_{50} \leq 1$ mg/L	Fish: 96h-LC_{50} > 24.8 mg/L (measured) Invertebrates: 48h- EC_{50} = 24.12 mg/L (measured) Algae: 72h- E_rC_{50} > 100 mg/L (nominal)	**Not Aquatic acute 1**

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Table 23: Comparison with criteria for long-term aquatic hazards

	Criteria for environmental hazards	DOTE	Conclusion
Rapid Degradation	Half-life hydrolysis < 16 days Readily biodegradable in a 28-day test for ready biodegradability (> 70 % DOC removal or > 60 % $ThCO_2$, ThOD)	Half-life hydrolysis > 1 year 11-43 % after 28 days (ThCO2 and BOD) => not readily biodegradable	**Not rapidly degradable**
Bioaccumulation	BCF > 500 or log Kow ≥ 4	Log Kow = 15.35	**Low potential for bioaccumulation** (based on Weight-of-Evidence: high molecular weight, very high log Kow, very low water solubility)
Aquatic Toxicity	Table 4.1.0 (b)(iii): As there is no long-term fish toxicity test available based on acute data: Cat. 1: E(L)C_{50} ≤ 1 mg/L Cat. 2: E(L)C_{50} >1 to ≤ 10 mg/L Cat. 3: E(L)C_{50} >10 to ≤ 100 mg/L Table 4.1.0 (b)(i): Cat. 1: NOEC ≤ 0.1 mg/L Cat. 2: NOEC ≤ 1 mg/L	Fish: 96h-LC_{50} > 24.8 mg/L (measured) Fish: no long-term toxicity test available Invertebrates: 21d-NOEC = 0.286 mg/L (mean measured) Algae: 72h- NOE_rC > 100 mg/L (nominal)	**Based on Table 4.1.0 (b)(iii):** **No aquatic chronic classification** **Based on Table 4.1.0 (b)(i):** **Aquatic chronic 2, H411** (based on 21d-$NOEC_{invertebrates}$ = 0.286 mg/L)

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

DOTE is not rapidly degradable and therefore, with NOEC of 0.286 mg/L fulfils the classification criteria for hazardous to the aquatic environment “Aquatic Chronic 2”. The hazard statement code is H411.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter’s proposal

DOTE is used as a stabiliser in plastic and currently has no classification for hazards to the aquatic environment in Annex VI to CLP.

The DS proposed classification as Aquatic Chronic 2. DOTE was considered not rapidly degradable based on its hydrolysis rate and results from three OECD TG 301 degradation tests. A reliable study on the BCF of DOTE is not available but on the basis of a log K_{ow} of 15.35 (calculated) and the high weight of the molecule (751.8 g/mol) and poor water solubility (0.001 pg/L, calculated), DOTE was considered to have a low potential for

bioaccumulation. Acute aquatic data are available for fish, invertebrates and algae. Invertebrates are the most sensitive trophic level with a 48-h EC_{50} of 24.12 mg/L for *Daphnia magna* that is based on nominal concentrations. This value is higher than the calculated water solubility of DOTE. Chronic aquatic data that are considered reliable are only available for daphnids and algae. The lowest chronic endpoint is a 21-d NOEC of 0.286 mg/L for *Daphnia magna* that is based on measured test concentrations. On the basis of this endpoint classification as Aquatic Chronic 2 was proposed.

The water solubility of DOTE could not be determined experimentally and was calculated as 0.001 pg/L. The vapour pressure was reported as $<2.50 \times 10^{-4}$ Pa due to significant differences between individual measurements. Data on sorption is not available but based on the estimated high log K_{ow} value (15.35) adsorption to sediment and soil is expected. In the past, many studies were performed with DOTE that contained 6-12% of an impurity (ethylhexylthioglycolate, EHTG; CAS 7659-86-1), which is more toxic than DOTE. Consequently, these studies were not used for classification purposes.

Degradation

The hydrolysis of DOTE was tested according to OECD TG 111. The half-life for pH 4, 7 and 9 was calculated to be >1 year. Ready biodegradation was tested following OECD TG 301F (one test) and OECD TG 301B (two tests). The degradation in these three tests varied from 11 to 43% over 28 days, indicating that DOTE was not readily biodegradable. On the basis of this information DOTE was considered not rapidly degradable for classification purposes.

Bioaccumulation

A log K_{ow} of 15.35 was calculated for DOTE using Kowwin v1.68. One bioaccumulation study according to OECD TG 305 was available. The test was performed in a flow-through system at exposure concentrations of 0.25 and 2.5 µg/L. The reported BCF values were 1294 L/kg and 99 L/kg for the two concentrations respectively. The values are based on the limit of quantification as DOTE could not be detected in the fish tissue. Since the exposure concentrations are above the reported water solubility, the study is considered unreliable. On the basis of the estimated log K_{ow} of 15.35 and the weight of the molecule of 751.8 g/mol, DOTE was considered to have a low potential for bioaccumulation.

Aquatic toxicity

Valid acute aquatic toxicity data are available for fish, invertebrates and algae with invertebrates being the most sensitive trophic level. For chronic toxicity data, valid data are available for invertebrates and algae. In all studies actual test concentrations were determined and endpoints are based on mean measured concentrations or based on nominal where the measured concentrations were within 20% of nominal. The test concentrations in the acute fish test and the chronic invertebrate test were prepared from Water Accommodated Fractions (WAF), and the concentration of the test substance was analytically confirmed. All relevant information on aquatic toxicity considered reliable is presented in the following table:

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/L)	
OECD TG 203/EU C.1 Limit test GLP study Purity not reported WAF used	*Danio rerio*	n.r.	*Semi-static*	96 h	LC_{50}	>24.8 (nominal)	Anonymous (2004a)
OECD TG 202 Vehicle DMSO GLP study	*Daphnia magna*	immobility	*Static*	48 h	EC_{50}	24.12 (nominal)	Anonymous (2016a)
OECD TG 211	*Daphnia magna*	reproduction parental survival, mobile offspring, body length	*Semi-static*	21 d	NOEC NOEC	1.448 (mean measured) 0.286 (mean measured)	Anonymous (2004b)
OECD TG 202 Limit test Vehicle DMSO GLP study	*Pseudokirchneriella subcapitata*	growth rate	*Static*	72 h	EC_{50} NOEC	>100 (nominal) ≥100	Anonymous (2016b)

An acute limit test (according to OECD TG 203) was performed with *Danio rerio*. The test solution (renewed daily) was generated from a Water available fraction (WAF) and the actual concentration was determined by measuring the total Sn and calculating this back to DOTE. The purity of the test substance was 87.5 %. No effects were observed at the limit concentration of 24.8 mg/L.

A static acute study (according to OECD TG 202) with *Daphnia magna* was performed. For preparation of the test solution, DMSO was used at a concentration of 0.01%. The purity of the test substance was 99%. The measured concentrations were within 20% of nominal and endpoints are based on nominal concentrations. Effects were observed and an EC_{50} of 24.12 mg/L was reported. No effects were observed in the solvent control.

A static algal growth inhibition limit test (according to OECD TG 201) with *Pseudokirchneriella subcapitata* was performed. For preparation of the test solution, DMSO was used at a concentration of 0.01%. The purity of the test substance was 99%. The measured concentrations were within 20% of nominal and endpoints are based on nominal concentrations. No effects were observed with an EC_{50} of >100 mg/L and NOEC ≥100 mg/L reported. No effects were observed in the solvent control.

A chronic toxicity test (according to OECD TG 211) with *Daphnia magna* was performed. The test solution (renewed daily) was prepared by the Water available fraction (WAF) approach and the actual concentration was determined by measuring the total Sn and calculating this

back to DOTE. The purity of the test substance was 87.5 %. Effects were observed with a $NOEC_{reproduction}$ of 1.448 mg/L reported. For parental survival, mobile offspring and body length a NOEC of 0.286 mg/L was reported.

Based on the available information for aquatic toxicity, the DS concluded that DOTE does not meet the criteria for Aquatic Acute 1. For chronic toxicity, the DS concluded that DOTE meets the criteria for Aquatic Chronic 2 - H411 based on the NOEC of 0.286 mg/L.

Comments received during public consultation

Five MSCAs and one Company-Manufacturer commented during the public consultation. Two of the MSCAs supported the proposed classification by the DS.

One MSCA supported the classification as Aquatic Chronic 2 proposed by the DS but did not agree with the conclusion that the substance does not fulfil the criteria for Aquatic Acute 1. They also asked for clarification as to whether the endpoint from the acute Daphnia study was based on nominal or measured concentrations. For the conclusion on Aquatic Acute 1, the DS replied that there are no results from a reliable short-term test meeting the classification criteria. Considering the nominal or measured concentrations, the DS clarified that the endpoint was based on nominal concentrations as the measured concentrations were within 20% of nominal.

One MSCA requested additional study details on the hydrolysis study because the conclusions would be contradictory when compared with previous assessments. For the bioaccumulation the MSCA commented that in an earlier PBT assessment on the substance and ECHAs conclusion for transitional substances, data uncertainties and interpretation with the B/vB criteria were considered and that it is unclear whether the bioaccumulation would be below 500 L/kg. The MSCA requested further data relating to the molecular weight which would result in DOTE not meeting the bioaccumulation criteria. For the hydrolysis, the DS provided additional study details supporting their conclusion on hydrolysis. For bioaccumulation, the DS replied that in the ECHA conclusion it was stated that no definitive BCF value is available and that there were some uncertainties regarding the interpretation. The BCF of the dioctyltin substances would be perhaps around a maximum 1000 L/kg but probably much lower. The DS mentioned that they based their conclusion on the weight of evidence (log K_{ow}, molecular weight and water solubility).

One MSCA supported aquatic chronic classification in at least Category 2. However, they also stated that they are of the opinion that classification in Category 1 cannot be excluded because of the limited dataset. The DS replied that new tests cannot be requested and that the proposal is based on the available data.

A Company-Manufacturer explained about the process-related impurity, the ligand EHTG (EC 231-626-4). They stated that this contaminant, that is (self-)classified as H410, has caused or influenced the observed effects in the toxicity tests, especially the key study. Studies where a more purified sample of DOTE was used showed much lower or no toxicity compared to older tests where DOTE was used that contained 6-12% EHTG. The manufacturer proposed that a future classification should be based on the mixture rules for CLP. It was furthermore commented that the test waters were prepared utilising the WAF procedure. As EHTG has a much higher water solubility, the EHTG would have been disproportionally solubilised compared to DOTE itself. Furthermore, the analytical method used to determine the DOTE content in the test media would, during analysis, have dissolved previously undissolved DOTE, as such, the actual concentration of DOTE would have been

over estimated. The manufacturer proposes to repeat the chronic daphnia study with purified DOTE. The DS replied that by the WAF method applied in the chronic daphnia study, the disproportional solution of EHTG was minimised. Furthermore, the concentration of the EHTG in the DOTE used would correspond to the levels of EHTG in DOTE currently available. Due to the analytical confirmation, the solution method, relevance for currently available DOTE, and fact that impurities hazardous to the environment present at concentrations >0.1% should be taken into account for the purpose of classification, the chronic Daphia study is considered appropriate for evaluating the ecotoxicological effects of DOTE.

Assessment and comparison with the classification criteria

Degradation

DOTE is hydrolytically stable at environmentally relevant pHs (4, 7 and 9). The half-life for hydrolysis was estimated at >1 year. Three studies on ready biodegradability are available, one according to OECD TG 301F and two according to OECD TG 301B. In the 301F study, 29-43% degradation was observed after 28 days and 36-68% after 74 days. The criteria for ready biodegradability were not fulfilled because of high differences between the replicates. The two 301B studies showed 23 and 19% degradation after 28 days, therefore also not fulfilling the criteria for ready biodegradability. It should be noted that the hydrolysis tests as well as the biodegradation screening tests were performed at exposure concentrations exceeding the water solubility, this could have suppressed the transformation rates since most of the substance was probably not dissolved and as such not available for hydrolysis or biotransformation. Future experiments with lower exposure concentrations might indicate a different outcome. Despite this uncertainty, there is currently no information showing rapid degradation of DOTE under environmental conditions.

In conclusion, RAC agrees with the DS proposal to consider DOTE as not rapidly degradable for the purpose of classification and labelling.

Aquatic bioaccumulation

A study on the bioaccumulation of DOTE is available in the dossier but this study is considered unreliable because the exposure concentrations greatly exceeded the water solubility of DOTE. DOTE was not detected in fish tissue above the limit of detection. RAC notes that the reported limit values may actually be underestimated since the actual dissolved concentrations are not known. The log K_{ow} reported as 15.35 (KOWWIN v1.68; EPI Suite) should be considered unreliable because it is a QSAR-estimated value (KOWWIN v1.68; EPI Suite) and substances with such a high hydrophobicity are generally out of the domain of the calculation programs and the maximum log K_{ow} value in the training set is around 10. The log K_{ow} is therefore considered as >10.

The conclusions of the DS on potential for bioaccumulation are based on the log K_{ow} of 15.35 and the high molecular weight of 751.8 g/mol. There is no guidance that links the log K_{ow} and molecular weight to the BCF cut-off value of 500 L/kg in the CLP criteria. The log K_{ow} of >10 could be used in a weight of evidence approach to suggest that the BCF could be lower than 2000 L/kg (REACH PBT Guidance, R.11 - Appendix R.11-1). However, the available estimated log K_{ow} by itself is considered to be insufficient for a weight of evidence assessment. Therefore, it cannot be excluded that the BCF will be higher than 2000 L/kg and consequently it also cannot be excluded that it will be higher than 500 L/kg.

On this basis, RAC disagrees with the DS's proposal to consider DOTE as a substance with a low potential to bioaccumulate and considers DOTE to have a potential for bioaccumulation.

Aquatic toxicity

Short-term aquatic toxicity

Short-term toxicity data for DOTE that are considered reliable for classification purposes are available for fish, invertebrates and algae (see table). The acute endpoints for fish and algae lie above the tested concentration. In these tests, it can be concluded that there are no effects at the maximum water soluble concentration. For *Daphnia magna*, the EC_{50} reported is 24.12 mg/L, although this value lies above the threshold for an aquatic acute classification (1 mg/L). However, this value is higher than the maximum water solubility of 0.001 pg/L reported in the CLH report for DOTE. The endpoints available are based on mean measured concentration or are nominal concentrations, confirmed by measurements. It is possible that the test material was not fully dissolved (despite the filtering of the WAF) and probably included in the chemical analysis as well as any material adsorbed on to the test vessel walls. Toxicity testing with poorly water soluble and hydrophobic substances like DOTE would be better performed in flow-through exposure systems. Nevertheless, effects were observed and the reported concentrations are higher than the estimated water solubility.

The value for the water solubility presented in the CLH report (0.001 pg/L) is a QSAR-estimated value (WSKOWWIN v1.42; EPI Suite) based on a log K_{ow} of 15.35. RAC notes that this value should be used with care since the log K_{ow} in itself has a limited reliability and the maximum log K_{ow} in the training set of the water solubility QSAR is 8.27. RAC estimated the water solubility in the same calculation program with a log K_{ow} of 10, which resulted in a water solubility of 46 pg/L. Even so, EPI Suite has not been validated for chemicals that contain metal in their molecular structure and consequently the water solubility estimates are considered unreliable. Nonetheless, in the absence of an experimentally derived water solubility, the solubility estimate, whilst of limited reliability, provides an indication regarding the water solubility of DOTE. In this context, DOTE can be considered as a poorly water-soluble substance (<1mg/L).

According to the Guidance on the Application of the CLP criteria (p. 561; version 5.0, July 2017), where the acute toxicity is recorded at levels in excess of the water solubility, the $L(E)C_{50}$ for classification purposes may be considered to be equal to or below the measured water solubility. In such circumstances, it is likely that Category 1 for Aquatic Chronic and/or Aquatic Acute should be applied. In making this decision, due attention should be paid to the possibility that the excess undissolved substance may have given rise to physical effects on the test organisms. On the basis of the available data in the study report, we can't rule out the possibility that the substance was in suspension, there is however also no obvious evidence of physical effects (coating/entrapment, etc.) either. Therefore, the outcome from the test is considered reliable and with that it is presumed that physical effects have not occurred. As indicated above, there is no experimentally derived water solubility value. Therefore, the actual water solubility to be used for the classification is unknown. Nevertheless, as mentioned above the calculated water solubility sufficiently indicates that the actual water solubility will be <<1 mg/L.

On this basis, RAC disagrees with the DS and concludes that DOTE warrants classification as Aquatic Acute 1. No M-factor is derived due to the uncertainties in reliably determining the water solubility.

Long-term aquatic toxicity

Long-term toxicity data for DOTE that are considered reliable for classification purposes are available for invertebrates and algae (see table). The key value is a NOEC of 0.286 mg/L for *Daphnia magna*. In the public consultation it was commented that the effects observed are potentially or likely to be caused by the impurity EHTG. However, there are insufficient data to confirm this claim. Furthermore, the level of EHTG in the DOTE tested in the chronic Daphnia study is representative for the currently available DOTE. Therefore, it is concluded that the study is suitable for classification purposes. Also in this case, the reported endpoint highly exceeds the calculated water solubility for DOTE and the same guidance applies as cited above for the acute classification. Since the test concentration is made from the water available fraction where undissolved test substance is removed from the solution, RAC concludes that undissolved substance, and therefore physical effects, are unlikely.

On this basis, RAC disagrees with the DS and concludes that DOTE warrants classification as Aquatic Chronic 1. No M-factor is derived due to the uncertainties in reliably determining the water solubility.

Conclusions for classification

RAC concludes that DOTE fulfils the CLP criteria for **classification as Aquatic Acute 1; H400 and Aquatic Chronic 1; H410.** RAC notes that in the absence of an M-factor, according to Article 10(4) of the CLP Regulation, the manufacturer, importer or downstream user is to set the M-factor based on available data for the substance. When in the future experimental data on the water solubility of DOTE become available, an M-factor might be proposed for inclusion in Annex VI of the CLP.

Supplemental information - In depth analyses by RAC

DOTE contains a process-related impurity, the ligand EHTG (EC 231-626-4), generally in a concentration between 3 and 12%. It was suggested during the public consultation that this impurity might have caused or influenced the observed effects in the toxicity tests, especially the key study. It was also noted that the WAF procedure, used to generate the test solutions, would have disproportionally solubilised the impurity. As such, the endpoint would not be suitable for classification. Reference is made to an acute Daphnia study where a purified sample of DOTE is tested containing 0.1% of EHTG resulting in a much lower EC_{50} than determined in a test where unpurified DOTE was tested. The DS states that for the long-term Daphnia test presented for CLP, the disproportional solution of EHTG is minimised by the procedure followed and that the level of the EHTG impurity in the DOTE tested is representative for the current levels in DOTE.

Despite the inherent uncertainties, RAC considers that the estimated water solubility of DOTE is much lower than that of the impurity EHTG (4.73 mg/L according the REACH dossier on the ECHA dissemination site). In fact, the difference in water solubility is more than 12 orders of magnitude (4.73×10^{12}). This indicates that even with minimisation of disproportional solution this cannot be excluded. Nevertheless the concentration of EHTG has not been determined in any of the studies available. Therefore, any conclusion on the

actual extent of the contribution of EHTG to the effects observed cannot be drawn since its actual presence and concentration is unknown. Even in the study where purified DOTE was tested, the level of EHTG was still 0.1% and the presence of EHTG in the test solutions could still be very high considering the difference in water solubility of the two substances. However, in this study, the actual exposure level of EHTG is also unknown. RAC concludes that it is not possible to draw a conclusion on the potential effects of the impurity and that the observed effects are presumed to be caused by DOTE.

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this dossier.

13 REFERENCES

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14 ANNEXES

conf. Annex I