Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Adopted

30 November 2018

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2-ETHYLHEXYL 10-ETHYL-4,4-DIOCTYL-7-OXO-8-OXA-3,5-DITHIA-4-STANNATETRADECANOATE; [DOTE]

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: 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
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2018	Germany	Organotin REACH Consortium	Company-Manufacturer	1
Comment received				
General Comments The following are comments are submitted on behalf of the members of the Organotin Reach Consortium as part of the public consultation. The registrants of DOTE appreciate the submission of the CLH report on DOTE by the German Competent Authority, following Article 36(6) of the CLP-Regulation, after new data has been presented by the lead registrant of the substance. Read-across Relevant to the Toxicity Assessment for Reproduction The Read Across approach is discussed in Chapter 9, p10 and Chapter 10, pp 11-12 of the CLH Dossier. Metabolic Considerations Recent in vitro hydrolysis studies conducted by industry showed that, under the simulated gastric conditions of the test [Nasshan, 2014], DOTE breaks down to its monochloro ester, DOTEC. No DOTC was formed under the conditions of the study. These data were published [Costlow, Nasshan and Frenkel, 2017] and an identical independent study by a Member State confirmed the result [private communication]. In Chapter 9 and in Chapter 10 the dossier submitter states "However the study does not allow to conclude, if and which dioctyltin 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." While we appreciate that a single study cannot address all of the possible factors relevant to in vivo toxicokinetics of DOTE, we agree with the CLH. If DOTC is not formed in the stomach, then the oral exposure is not to DOTC and consideration of the available data strongly supports that a direct toxicological read-across to DOTE from DOTC feeding studies is not sufficient to assess the oral toxicity of DOTE.				

The hypothesis that DOTC is generated from DOTE or DOTEC after systemic uptake is, we believe, unlikely to be true to any significant extent because a metabolic transformation of this type, i.e. DOTE to DOTC, would most probably occur in the gastric milieu, if it occurs at all.
The metabolic formation of DOTC would require an enzymatic chlorination of the molecule. There is currently no evidence that non-gastric intermediary metabolic transformation of DOTE to DOTC occurs. While, enzymatic halogenation reactions are known; they are described, primarily in bacteria, and pursued for their purpose as chemical synthesis catalysts [Flecks, et al, 2008; van Pee, 2012]. They include haloperoxidases, oxygen-dependent enzymes and fluorinases, flavin-dependent halogenases, non-heme iron-dependent halogenases, and nucleophilic halogenases [Blasiak and Drennan, 2009]. All of these are quite rare. Further, the literature suggests the enzymes are FADH2-dependent, and there were no such reactions found in the literature for organotin compounds. Arguably, the most expected metabolism of this class of alkyltins would be a P450-dependent oxidation which is incapable of a chlorination reaction.
Given the metabolic considerations, the relevance of existing feeding studies with DOTC as a part of a weight-of-evidence assessment for DOTE should be diminished to a minimal level or considered nil.

Structural Considerations
The dossier submitter also used a structural read-across for the DOTE assessment. This was justified by the high structural similarity of DOTE and DOTI. In general, the registrants agree with this read-across, and note that the reproductive NOEL in the DOTI study is the high dose, i.e. no reproductive effects noted. The maternal and paternal NOEL is 20ppm (the low dose) which is driven by the effect of DOTI on the thymus gland. In the DOTI study there was no adverse effect on mating, fertility, pregnancy rates, gestation, litter size or neonatal body weight. The body weights of F1 and F2 animals showed no statistically significant differences from controls in either sex at birth, PND4, or PND7. Only at PND14 and after were there statistically significant decreases in pup body weights which persisted through PND21. This correlates exactly with the incidences of post-natal losses documented for the F1 and F2 animals.
This is probably not a reproductive effect, rather it is a likely consequence of impaired nursing behavior as a result of maternal toxicity and/or a consequence of a direct toxic effect 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.
For the reasons cited it is appropriate to use the DOTI study, with some diminished significance, for read-across to DOTE from DOTI, and it is clear that the DOTI outcome does not meet the GHS classification criteria of clear evidence for reproductive effects. This outcome, combined with the similarly non-adverse outcomes in the developmental toxicity studies noted below for rabbit and mouse with DOTE itself, supported the self-classification of DOTE as not toxic for reproduction under GHS.
The registrants agree with the CLH, i.e. data supporting DOTE are deemed adequate for all REACH reproductive endpoints. With the exception of the data from the 2-generation reproductive study with DOTI, no read-across of data from any other substance is necessary.

Considerations of Other Data
Other studies with DOTI exist, however, the OECD 414 developmental toxicity studies in mice, and rabbits have major methodological deficiencies and should be disregarded in the weight-of-evidence evaluation of teratogenicity of DOTE. Further, the OECD 414 developmental toxicity study with DOTI in rats was negative for developmental effects.

Mouse - developmental toxicity study (Faqi, 2001)
The registrants concluded that this DOTI study was flawed in its design and reporting. Interpretation of the data was compromised by lack of adequate documentation of maternal weights, lack of information on housing conditions, changes in food consumption, analytical confirmation of the test substance concentrations in gavage dosing solutions. In addition, fetal sex ratios, fetal weight by sex, and incidences of internal malformations were missing.
Dose-levels were 20, 30, 45 mg/kg/day in study #1, and 67 and 100 mg/kg/day in study #2
No individual animal or historical control data are available for the study. Dams were not weighed at any intermediate point during the dosing. The two experiments, with different control groups, were evaluated differently and statistically analyzed as separate studies rather than combined. Statistical analyses were not appropriate for a design in which individual experiments are clearly not independent. Pups were fixed in formalin, which is not a standard procedure and numerous skeletal parameters across doses were not reported.
The data from a new OECD 414 developmental toxicity study in mice with DOTE were clearly negative for developmental effects. This outcome in pregnant mice with DOTE itself, supported the self-classification of DOTE as not classified for developmental/reproductive toxicity under GHS.
In a weight-of-evidence assessment, the mouse data for the DOTE substance itself, from a GLP study uncompromised by flawed design and reporting, supersedes data from a study with a related substance. The new DOTE data must be considered as predominant for classification, even if ancillary data with a related substance are considered.

Rabbit - developmental toxicity study (Battenfeld 1992)
The registrants concluded that this DOTI study was seriously flawed because it was compromised by infectious disease in the test animals. A repeat of this study with DOTE was deemed necessary to produce relevant and convincing data for reclassification of DOTE.
The dose-levels of 1, 10 and 100 mg/kg/day were used.
It is highlighted in the report that "as a consequence of either pneumonia or enteritis, two dams in group 1 as well as three does in group 3 died after treatment had started". It is therefore questionable to fully attribute the observed fetal effects to the treatment because several deaths resulted from infectious disease which occurred during the study. Rabbits often abort litters as a maternal toxic response even in the absence of an effect on maternal weight. In the high dose of DOTI in this study there were abortions, but these high dose females were observed to have reduced or no food intake, and among surviving litters there was no increase in pre-implantation loss, dead fetuses, or decreased litter size. There was an increase in resorptions and a decrease in fetal body weight.
The data from a new OECD 414 developmental toxicity study in rabbits with DOTE were clearly negative for developmental effects. This outcome in pregnant rabbits with DOTE itself, supported the self-classification of DOTE as not classified for developmental/reproductive toxicity under GHS.
In a weight-of-evidence assessment, the rabbit data for the DOTE substance itself, from a GLP study unaffected by complications critical to the study's interpretation, supersedes data from a disease-compromised study with a related substance. The new DOTE data must be considered as predominant for classification, even if ancillary data with a related substance are considered.

Rat - developmental toxicity study (Battenfeld 1991)

The registrants conclude that value in this DOTI study was it demonstrated the rat was the least sensitive species for assessing possible developmental effects of DOTI. A repeat of this study with DOTE was deemed an inappropriate use of test animals because it would be unlikely to produce relevant and convincing data for reclassification of DOTE. The dose-levels of 1, 5 and 25 mg/kg/day were used. At the 1 and 5 mg/kg/day dose-levels
no substance related effects were observed. At the 25 mg/kg/day dose-level slight (~3%, not statistically significant) decreases in corrected body weight and corrected body weight of the dams occurred. This reduction was to a great extent due to the loss of corrected body weight in one single dam(No. 97). Seven dead fetuses were observed. All these dead fetuses descended from one dam (No. 97). Four early resorptions and an extremely low mean weight (2.2 g) of the two living fetuses were found in this animal. The marginal decrease of maternal body weight gain was secondary to the pup mortality observed in only one dam out of the 25 dams present in the group. No external, visceral or skeletal malformations were considered as treatment related in this study. These results support a conclusion that there are no adverse developmental effects in rats attributable to treatment with DOTI and that maternal toxicity is marginally present in the high dose dams. On the basis of this study alone, no classification would be proposed.

References and Abstracts
Costlow, RD, H Nasshan, and P Frenkel [2017]. Simulated gastric hydrolysis and developmental toxicity of dioctyltin bis(2-Ethylhexylthioglycolate) [DOTE] in rabbits and mice, Regulatory Toxicology and Pharmacology 87 (2017) 23-29.
Nasshan, H [2014]. Dioctyltin bis(2-Ethylhexylthioglycolate) [DOTE], CAS No. 15571-58-1, In Vitro Metabolism Study, Galata Chemicals, GmbH Report
Flecks, S, EP Patallo, X Zhu, AJ Ernyei, G Seifert, AC Dong, JH Naismith and Karl-Heinz van Pée [2008] New Insights into the Mechanism of Enzymatic Chlorination of Tryptophan, Angew Chem Int Ed Engl. 2008; 47(49): 9533–9536.
van Pée, K-H [2012] Enzymatic chlorination and bromination, Methods in Enzymology 516:237-57.
Blasiak, LC and CL Drennan [2009]. Structural Perspective on Enzymatic Halogenation, Accounts of Chemical Research 42(1) January 2009 147-155.

Dossier Submitter's Response

Read-across
Metabolic Considerations

From the *in vitro* hydrolysis study it cannot be concluded which dioctyl tin species is formed *in vivo* after systemic uptake of DOTE in the organism, therefore formation of DOTC, or a common intermediate, cannot be excluded. In this case, i*n vivo* metabolites or the toxicologically active species *in vivo*, are unknown.
Structural considerations
In the two generation study with DOTI used in a structural read-across for the DOTE assessment no indication of impaired nursing or maternal toxicity was observed. Regarding the endpoint developmental toxicity the applied dose regime for DOTE was rather low and did not produce minimal observable toxic effects for at least one intermediate dose level as requested by the OECD TG 414. Besides some developmental and/or maternal toxicity are aimed to be induced at the highest dose within OECD TG 414, effects at this dose level did not reach significancy. Based on the information from rather low doses read across appears to be necessary.
Considerations of other Data
For the structurally highly similar DOTI (isooctyl- vs. ethylhexyl side chain) clear evidence of developmental effects in rabbits and mice was observed, although more detailed

information would have been beneficial. Nevertheless, RAC (2012) stated, there is no obvious reason to question the results of the published mouse study with DOTI: Dose-dependency of effects and consistency with other studies support the reliability of the study. Furthermore, RAC (2012) does not share the view that robustness of the rabbit study with DOTI was compromised by infections. The new developmental toxicity studies show that DOTE interferes with gestational integrity in mice and the fetal development in rabbits. At the doses chosen developmental effects appear marginal at the highest dose, but statistically significance in trend tests suggests treatment-related beginning of a dose-response relationship.
RAC's response
RAC supports the response from the dossier submitter, and further notes that the different tin-substances discussed above all are thymotoxic, despite different structures, and that the MoA is unknown. Also for developmental toxicity, the MoA is unknown, and the closely related structural analogue DOTI is causing developmental toxicity. The new studies on DOTE suggest similar developmental toxicity, but the doses of DOTE used are too low to fully confirm or contradict such developmental toxicity.

Date	Country	Organisation	Type of Organisation	Comment number
02.02.2018	Sweden		MemberState	2
Comment received				
We are aware that the following comments are not within the scope of CLP and the process of harmonised classification, but we find it notable that the two new developmental toxicity studies (2014 a and b) were performed after the RAC-opinion of DOTE as Repr. 1B was adopted (in June 8, 2012) and included in CLP Annex VI in October 2013, and a non-EU branch of a new Registrant sponsored the studies (Costlow, Nasshan, Frenkel, 2017; and REACH registration, public version (ECHA dissemination, 2018)). It is also noted that the new hydrolysis study using NMR (2015), with results questioning the read-across approach, were performed only after the two new developmental toxicity studies (2014) were performed. Thus, at the time when the two new developmental toxicity studies were performed, no data gap for developmental toxicity due to invalidation by the Registrants of the read-across approach was evident. Moreover, the Registrant(s) still uses the two-generation reproductive toxicity study of DOTI/MOTI for fulfilling the data requirement of an EOGRTS (REACH registration, public version (ECHA dissemination, 2018)). This means that in practice there was never any data gap for developmental toxicity since already existing data from two developmental toxicity studies in mouse and rabbit from 1992 and 2001 of DOTI/MOTI together with the two-generation reproduction toxicity study of DOTI/MOTI are sufficient to warrant classification in Repr. 1B for adverse effects on the development of the offspring. This strategy that have been used by the Registrant(s) is clearly not in line with what is accepted with regards to generating new (vertebrate) data and testing proposals for registered substances in the EU, and in particular not for substances with harmonised classification in Repr. 1B. We are of the opinion that such approaches should not be accepted in attempts to circumvent regulatory measures (such as CLH and candidate listing) in the EU.				
Dossier Submitter's Response				
Thank you for your comments.				
RAC's response				
The comment is noted.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
30.01.2018	France		MemberState	3

Comment received

- Effects reported with related substances DOTI/MOTI in a 2-generation study in rat ((maternal effects on the thymus as a critical toxic effect for the adult), increased incidence of postnatal losses (correlated with a statistically significant decreased in body weight in both male and females)) are consistent with the observed effects on reproduction and development in the new studies realized with DOTE substance itself in mice and in rabbits.

- FR considers there is clear evidence of developmental toxicity considering the strong effects on the foetuses (decreased foetal crown-rump length in rabbits and increased post implantation losses in mice) in both newer studies realized with DOTE on the most sensitive species mice and rabbits. These effects cannot be considered secondary to maternal toxicity. Thus, FR considers that a harmonized classification in category 1B of the developmental toxicity is more relevant than a category 2. Such classification is so far supported based on these findings and the applied read-across among 3 chemicals (DOTE, DOTI, DOTC) in the CLH dossier of 25 March 2011 and the RAC opinion of 8 June 2012.

• Dossier submitter made a direct comparison of the studies using DOTI/MOTI with the newer studies on the substance itself for a conclusion on classification and labelling of DOTE. Dossier submitter concludes that the recently conducted developmental toxicity studies with DOTE in two species (mouse and rabbit) suggest that DOTI might have a higher toxicological potency with respect to developmental toxicity as supported by the Summary Tables of Dose-Response (Table 14 and Table 15) in Chapter 10.10.10. Based on the hypothesized low potency of DOTE, dossier submitter proposes a classification as Repr. 2 H316d. In comparison to other studies on analogues the highest dose tested using DOTE was too low to detect a dose-responses relationship thus the highest dose tested might rather reflect the starting point of a potential dose-response relationship. The developmental effects reported with DOTE are sufficient to classify as Repro. 1B. If DOTE is considered of low potency, FR proposes to the dossier submitter to check by calculations if specific concentration limits could be applicable for DOTE. In addition, a comparison of ED10 between DOTE and analogues could be useful to compare their potency.

• FR concludes that the available data are sufficient to propose a classification of the substance in category 1B for developmental toxicity. It is proposed that dossier submitter look at the possibility to set SCL for DOTE.

Dossier Submitter's Response

Worth to be discussed at RAC.

RAC's response

RAC shares the view that the highest dose tested using DOTE was too low to detect a dose-responses relationship, and that the highest dose tested might rather reflect the starting point of a potential dose-response relationship. Based on the DOTI data, RAC is of the opinion that Category 1B is still appropriate.

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2-ETHYLHEXYL 10-ETHYL-4,4-DIOCTYL-7-OXO-8-OXA-3,5-DITHIA-4-STANNATETRADECANOATE; [DOTE]

Date	Country	Organisation	Type of Organisation	Comment number
02.02.2018	Belgium		MemberState	4

Comment received

Fertility
There is no data highlighting any concern for fertility effects. Therefore, no classification is required.

Development
BECA is of the opinion that it is not a clear-cut case. We would have appreciated to have the data presented in a table, with the values for the control group which are lacking for some endpoints (post-implantation loss for example) in the study with DOTE on mice (Anonymous, 2014b).
We are concerned about the findings in the studies Anonymous (2014b and 2014a), particularly when considering the post-implantation loss percentage and the crown-rump lengths (CRLs). The fact that all these effects were seen in two species (mice and rabbits), probably affecting both sexes as it not specified otherwise, and with a higher severity as the dose rises, increases the concern about DOTE.
Indeed, in mice, a significant positive trend in the percentage of post-implantation loss was detected with 0.9 ± 2.8, 1.5 ± 4.9 and 2.6 ± 5.6 (%) at 15, 30 and 60 mg/kg bw/d, respectively. In rabbits, mean post-implantation losses were found to be of 3.1, 3.5, 6.4 and 5.7, at 0, 4, 20 and 80 mg/kg bw/d, respectively.
Mean CRLs slightly decreased at the highest dose in mice (23.2, 24.0, 23.3 and 22.9 mm, in the control, low, intermediate and high dose groups, respectively). However, in rabbits, mean CRLs were significantly decreased at the highest dose (92.1, 91.1, 89.3 and 82.3 mm, at 0, 4, 20 and 80 mg/kg bw/d, respectively).
For these reasons, BECA does not support the modification of the current entry of DOTE in Annex VI and would rather support the maintenance of the actual classification as Repr. 1B (H360D).

Dossier Submitter's Response

Anonymous, 2014a and b (equal to Costlow et al. 2017): Tables

Table 2
Maternal thymus weights for rabbits and mice.

Rabbit DOTE dose [mg/kg/day]	Rabbit Thymus weight [g]	Mouse DOTE dose [mg/kg/day]	Mouse Thymus weight [g]
0	2.235 ± 0.674	0	0.0400 ± 0.0097
20	2.121 ± 0.660	15	0.0385 ± 0.0064
40	2.021 ± 0.469	30	0.0306 ± 0.0089 *
80	1.950 ± 0.478	60	0.0260 ± 0.0097 *

Mean and Standard Deviation of maternal thymus weights [g] of rabbits and mice exposed to DOTE at the indicated doses are presented.
(*) indicates a statistically significant difference ($p < 0.05$).

Table 3
Maternal reproductive success and fetal development after DOTE exposure in rabbits.

DOTE Dose [mg/kg bw/day] Rabbit	0	4	20	80
Rabbits confirmed mated	24	24	24	24
Confirmed pregnant	19	21	19	20
Body weight gain [kg] GD29-GD6	0.511	0.584	0.520	0.472
Corrected weight gain[a] [g]	277.7	274.5	266.6	274.8
Maternal Thymus Weight [g]	2.235	2.121	2.021	1.950
Mean Corpora Lutea	5.2	6.1	5.6	5.3
Mean Implantation Sites	5.1	6.0	5.5	5.0
Live Fetuses/Litter	4.89	5.67	5.00	4.55
Non-viable Fetuses (Litters)	0 (0)	2 (2)	2 (2)	3 (1)
Implantation Losses	4.3%	5.5%	9.6%	9.0%
Mean Fetal Body Weight [g] (±SD)	36.6 (4.3)	37.3 (7.6)	36.0 (6.0)	32.3 (8.9)
Mean Crown-Rump Length [mm] (±SD)	92.1 (5.8)	91.1 (7.9)	89.3 (8.7)	82.3 (10.4)
Sex Ratio [M/F]	1.0	1.7	1.0	1.1
Fetuses examined [Litters]	93 [19]	119 [21]	95 [19]	91 [20]
Fetuses examined externally	93	119	95	91
Fetuses examined viscerally	93	119	95	91
Fetuses examined skeletally	93	119	95	91
Fetuses examined by Head Section	42	54	43	40
No. of External Malformations [Litters]	0 [0]	0 [0]	0 [0]	0 [0]
No. of Visceral Malformations [Litters]	0 [0]	0 [0]	0 [0]	0 [0]
No. of Skeletal Malformations [Litters]	9 [6]	3 [2]	4 [3]	9 [7]
No. of External Variations [Litters]	0 [0]	0 [0]	0 [0]	0 [0]
No. of Visceral Variations [Litters]	5 [4]	4 [3]	6 [5]	3 [3]
No. of Skeletal Variations [Litters]	4 [3]	6 [6]	7 [4]	14 [9]
Visceral Variations				
Dilated renal pelvis [Litters]	4 [3]	3 [2]	3 [3]	2 [2]
Small gall bladder [Litters]	1 [1]	2 [1]	3 [2]	1 [1]
Skeletal Malformations				
Absent 5th sternebral ossification center [Litters]	7 [5]	4 [3]	4 [3]	5 [4]
Absent 1st proximal phalanx [Litters]	2 [2]	1 [1]	1 [1]	5 [5]
Absent 5th proximal phalanx [Litters]	2 [2]	1 [1]	1 [1]	5 [5]
Skeletal Variations				
Poor ossification of parietal bone [Litters]	0 [0]	1 [1]	0 [0]	3 [3]
Poor ossification of inter-parietal bone [Litters]	0 [0]	1 [1]	0 [0]	1 [1]
Supplementary rib 1st lumbar vertebrae bilateral [Litters]	0 [0]	1 [1]	2 [1]	1 [1]
Rudimentary rib 1st lumbar vertebrae unilateral [Litters]	0 [0]	2 [2]	0 [0]	0 [0]
Poor Ossification of 5th sternebral center [Litters]	4 [3]	1 [1]	4 [3]	10 [6]
Poor Ossification of 6th sternebral center [Litters]	4 [3]	1 [1]	3 [3]	10 [6]

[a] Corrected body weight gain is (GD29 minus GD6 weight) minus GUW; where GUW = Gravid Uterine Weight.

Table 4
Maternal reproductive success and fetal development after DOTE exposure in mice.

DOTE Dose [mg/kg bw/day] Mice	0	15	30	60
Mice confirmed mated	25	25	25	25
Confirmed pregnant	21	21	20	20
Body weight gain [g] GD18-GD5	20.07	19.89	19.50	17.77
Corrected weight gain[a] [g]	3.19	2.62	2.66	2.34
Maternal Thymus Weight [g]	0.040	0.038	0.031*	0.026*
Mean Corpora Lutea	10.1	10.7	10.7	10.0
Mean Implantation Sites	10.1	10.7	10.6	10.0
Live Fetuses/Litter	10.1	10.6	10.5	9.7
Non-viable Fetuses (Litters)	0 (0)	0 (0)	0 (0)	0 (0)
Post-implantation Losses[b]	0.0%	0.9%	1.5%	2.6%
Mean Fetal Body Weight [g] (±SD)	1.349 (0.162)	1.372 (0.121)	1.297 (0.164)	1.308 (0.153)
Mean Crown-Rump Length [mm] (±SD)	23.23 (2.32)	24.03 (2.09)	23.28 (2.30	22.90 (3.06)
Sex Ratio [M/F]	1.2	1.3	1.4	1.2
Fetuses examined [Litters]	212 [21]	222 [21]	209 [20]	194 [20]
Fetuses examined externally	212	222	209	194
Fetuses examined viscerally	100	105	98	92
Fetuses examined skeletally	112	117	111	102
No. of External Malformations [Litters]	0 [0]	0 [0]	0 [0]	0 [0]
No. of Visceral Malformations [Litters]	0 [0]	0 [0]	0 [0]	0 [0]
No. of Skeletal Malformations [Litters]	0 [0]	0 [0]	0 [0]	2 [2]
No. of External Variations [Litters]	0 [0]	0 [0]	0 [0]	0 [0]
No. of Visceral Variations [Litters]	4 [4]	5 [3]	3 [5]	6 [3]
No. of Skeletal Variations [Litters]	6 [3]	3 [6]	2 [4]	14 [9]
Visceral Variations				
Dilated renal pelvis; unilateral- right [Litters]	2 [1]	3 [2]	2 [2]	2 [2]
Pale colored kidneys [Litters]	2 [1]	2 [2]	1 [1]	4 [2]
Skeletal Malformations				
Fused 2nd and 3rd sternebral ossification centers [Litters]	0 [0]	0 [0]	0 [0]	1 [1]
Short 13th rib unilateral [Litters]	0 [0]	0 [0]	0 [0]	1 [1]
Skeletal Variations				
Poor ossification of frontal bone [Litters]	0 [0]	1 [1]	1 [1]	1 [1]
Poor ossification of parietal bone [Litters]	2 [2]	1 [1]	1 [1]	1 [1]
Poor ossification of inter-parietal bone [Litters]	2 [2]	1 [1]	1 [1]	1 [1]
Ossification site 1st lumbar vertebrae unilateral [Litters]	4 [4]	0 [0]	0 [0]	7 [6]
Ossification site 1st lumbar vertebrae bilateral [Litters]	0 [0]	2 [2]	1 [1]	4 [4]
Poor ossification 11th rib unilateral [Litters]	0 [0]	2 [2]	0 [0]	1 [1]
Supplementary rib 1st lumbar vertebrae bilateral [Litters]	0 [0]	0 [0]	0 [0]	2 [2]
Discontinuous cartilage 13th rib unilateral [Litters]	0 [0]	0 [0]	0 [0]	1 [1]

[a] Corrected body weight gain is (GD18 minus GD5 weight) minus GUW; where GUW = Gravid Uterine Weight.
[b] Pre-implantation losses were 0% for all groups.

Development Worth to be discussed at RAC. Values for the control group for postimplantation loss are 0,0±0,0. Note: Mean CRLs show only a statistically significant negative trend.
RAC's response
RAC shares the view expressed by the BE CA.

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2018	Germany	Organotin REACH Consortium	Company-Manufacturer	5

Comment received

Overall Assessment of Toxicity to Reproduction
The registrants share the view presented by the dossier submitter in the CLH for DOTE.
The recently-conducted GLP developmental toxicity studies [OECD 414] in the most sensitive species mouse and rabbit with DOTE produced no statistically significant adverse effects on fetal morphology, either for the skeletal elements or soft tissue elements, even in the presence of maternal toxicity.
The registrants note also that there is no evidence that DOTE or DOTI had an adverse effect on a related parameter of reproduction, fertility. Mating behavior (monitored as time to mate), pregnancy rates, duration of gestation, number of implantations sites, and number of live fetuses were unaffected by treatment in the relevant studies.
The dossier submitter notes in section 10.10.6 that in comparison to other studies on analogues, the highest dose tested using DOTE may have been too low to produce an effect. The registrants note that a memorandum was written in advance of each of the definitive studies which analyzed the available developmental and other toxicity data for DOTE and related substances as well as data from range-finding studies with DOTE. The data cited in the memoranda were used to select doses for the definitive DOTE studies in compliance with the selection criteria noted in OECD Guideline 414. This dose selection rationale has been documented in the DOTE dossier under section 7.8.2 / details on study design. Further a side-by-side comparison of the doses from all studies with mice and rabbits appears in section 10.10.10 of the CLH dossier.
In the opinion of the registrants, the side-by-side comparison of the dose rates and total doses of the mouse and rabbit studies [Tables 14 and 15 of the CLH] show that the non-lethal doses used in the studies with DOTE were comparable or higher than the studies with DOTI in mice [high dose of 57.7 mg/kg DOTE versus 53.6 mg/kg DOTI] and in rabbits [high dose of 76.9 mg/kg DOTE versus 80.0 mg/kg DOTI]. We conclude from this that some criticism of the dose selection in the DOTE studies may be justified, but the comparison of the responses in both species are not an artifact of the dose-selection. It follows that the conclusions for DOTE classification are fully valid.
In summary, it is the position of the LR and the co-registrants that only the mouse and rabbit studies [OECD 414] with DOTE itself, and the 2-generation study [OECD 416] with DOTI MOTI 80:20 should be considered for the classification of the toxicity to reproduction of DOTE. Based on these studies alone, a classification as not toxic for reproduction is warranted.

Dossier Submitter's Response

There is a statistically significant negative trend on foetal weight and on foetal crown-rump length in rabbits and a statistically significant positive trend on percentage of post implantation loss in mice. Neither the side-by-side comparison of the dose rates and total doses of the mouse and rabbit studies nor the memorandum refute the criticism on the

dose selection, e.g. no comparable dose with DOTE was given for the high dose of DOTI in mice.
RAC's response
RAC shares the view that the highest dose tested using DOTE was too low to detect a dose-response relationship, and that the highest dose tested might rather reflect the starting point of a potential dose-response relationship. Based on the DOTI data, RAC is of the opinion that Category 1B is still appropriate. The dose-selection rationale is indeed explained ("*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*."), but RAC questions why at all conducting studies only using dose levels that are known to cause minimal effects.

Date	Country	Organisation	Type of Organisation	Comment number
02.02.2018	Austria		MemberState	6
Comment received				
Seven reproductive toxicity studies have been considered for the developmental toxicity endpoint: two new studies (2014) carried out with DOTE itself (two TG 414 (mice, rabbit) and four studies with DOTI/MOTI (three TG 414 studies (mice, rabbit, rat), one TG 416, two generation reproduction toxicity study (rat)) and one study with DOTC (CAS: 3542-36-7, TG 421, Reproduction / Developmental Toxicity Screening Test). DOTI has structural similarity to DOTE and thus it is important to include DOTI/MOTI observations. DOTC data have been included since no in vivo ADME studies are available and it cannot unambiguously excluded that DOTC is not formed after systemic uptake of DOTE and DOTI. Thus, it is appreciated that DOTI and DOTC data are considered in a weight of evidence approach. The same studies carried out with DOTI/MOTI and with DOTC have been considered in the classification proposal of DOTE in the year 2012, the assessment of these data – at that time no data with DOTE itself were available - resulted in a harmonised classification of DOTE as Repr. 1B D. Based on the results of the new TG 414 studies with DOTE compound, the Dossier submitter (DS) proposes to classify DOTE into Repr. 2d. It is argued in the CLH report that despite of the results with DOTI/MOTI (which the dossier submitter considers as toxicologically relevant) there is only some limited evidence of developmental toxicity for DOTE in two species with relatively low toxicological significance (trend only). However, it is clearly described and indicated in the CLH proposal that the outcome of the new TG414 studies indicates that DOTE interferes with gestational integrity in mice (post-implementation loss) and the embryological development in rabbits (fetal body weight, mean fetal crump-length) indicating adverse effects on the development. These observations are statistically significant. Moreover, it is stated in the CLH report that there is some evidence that DOTE interferes with the morphology of skeletal elements. The DS does conclude that these effects can be considered as incidental, however, considering the raw data (see confidential annex), we conclude that more pronounced effects are found at the highest dose level, which is indicative for a dose relationship and that the effects are due to the treatment. In interpreting the data it needs to be considered that the applied dose-regime is not full in accordance with TG 414 requirements and must be considered as too low as e.g. in the rabbit study only marginal deviations in the thymus weight had been observed (no				

statistical significant difference, thymus weight can be effected by pregnancy). Thus, we agree with the assumption of the DS, that the study results might only reflect the starting point of potential dose response relationships and, therefore, the results cannot be considered as negative.
We consider the new studies as providing further evidence for developmental effects of DOTE, and thus confirming the need to classify DOTE for its developmental toxic effects.
The toxicity profile of DOTE in the two studies (although doses are considered inappropriate) are in accordance with previous observations with DOTI/MOTI, DOTC: e.g. decrease in fetal body weight, post-implantation loss, interference with the morphology of skeletal elements.
The differences in potency of DOTI/MOTI and DOTE have been described in the CLH report, however differences in potency do not result in different hazard categories for reproductive toxicants (1A, 1B or 2) (CLP guidance 3.7.2.6 and Annex VI).
On the basis of the above described arguments the Austrian CA is of the opinion that the classification of DOTE as Repr. 1B D should not be changed based on the new available data

ECHA note – An attachment was submitted with the comment above. Refer to confidential attachment Confidential_annex_Dote_02022018.docx

Dossier Submitter's Response
Worth to be discussed at RAC. Note: Only trends are statistically significant.
RAC's response
RAC is of the opinion that Category 1B is still appropriate.

Date	Country	Organisation	Type of Organisation	Comment number
02.02.2018	Sweden		MemberState	7

Comment received

The SE CA does not support the proposal to change the current harmonised classification of DOTE to Repr 1B, H361d.

The harmonised classification of DOTE as adopted by the RAC in 2012 were based on findings from studies of the source substances DOTI/MOTI and DOTC: one two-generation reproductive toxicity study in rat (Anonymus, 1997) and two prenatal developmental studies in mouse and rabbit (Faqi et al 2001, Battenfeld 1992) of DOTI/MOTI, and on one reproductive toxicity screening study of DOTC in rat (Appel and Waalkens-Berendsen, 2004).
The new pre-natal reproductive toxicity studies (2014a and b) cannot, in our opinion, dismiss the results from these studies on DOTI/MOTI and DOTC that the current harmonised classification is based on.
Firstly, we agree with the conclusion in the CLH-report regarding the recent in vitro hydrolysis study under simulated mammalian gastric conditions using 119-Sn-NMR that the study does not allow to conclude, if and which other dioctyltin species might be formed after systemic uptake of DOTE and /or DOTEC under in vivo conditions. The new hydrolysis study does not exclude that DOTC, or a common intermediate, can be formed in vivo. Consequently, read-across to DOTC cannot be disregarded based on the results of this hydrolysis study.
Moreover, DOTI and DOTE are isomers differing only slightly in the structure of the mercaptoester ligand (iso-octanol or 2-ethylhexylhexanol). 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. Therefore, read-across from studies of DOTI/MOTI are appropriate and justified and the available read-across data from DOTI/MOTI should be included in the total weight of evidence assessment to justify classification.

It is acknowledged that the prenatal developmental toxicity study of DOTI/MOTI in mouse by Faqi, 2001 study has some flaws in its reporting, however, reiterating the earlier RAC-assessment of the study in the opinion from 2012: "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."
Regarding the prenatal developmental toxicity study of DOTI/MOTI in rabbit by Battenfeld, 1992 and interpretation of results in relation to infectious disease, RAC stated the following: "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 compliant to 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"."
Thus, the relevance and reliability of the findings of developmental toxicity in these two studies is not compromised as was pointed to in the current CLH-report.

Regarding the two new prenatal developmental toxicity studies of DOTE in mouse and rabbit we consider that it is not possible with confidence to conclude that they were negative for any developmental effects since the dosing was not optimal.
The rationale for the highest dose selected in the mouse developmental study was according to the study author that "The risk of maternal deaths and increased resorptions in addition to potentially compromised maternal liver and thymic function at 100 mg/kg bw/day (80 mg/kg DOTI) was deemed unacceptably high for pregnant animals. Therefore, the high dose chosen for the DOTE study is 60 mg/kg to reflect a dioctyltin dose with minimal maternal and fetal toxicity as the upper bound."
However, in the RAC opinion of DOTE (June 8, 2012) the only sign of maternal toxicity at 100 mg/kg/d noted was reduced thymus weight (-27%, $p<0.05$) and reduced corrected body weight gain (2.9±4.8 g versus 5.1±7.0 g in control, (-44%) not statistically significant different from control).
Regarding maternal thymotoxicity and developmental toxicity being a secondary consequence of this, RAC made the following points in the RAC opinion:
- Developmental toxicity was seen at doses without thymus effects
- No specific mode of action has been identified to show that developmental effects can be caused by a specific thymus (T-lymphocyte)-related mechanism.
- Even in case a specific mode of action demonstrates that developmental effects is secondary to that specific mode, downgrading of the classification category can only be justified if non-relevance for humans has been demonstrated.

One death of a pregnant dam at 100 mg/kg/d was reported, but in absence of any other adverse clinical signs or severe effects on body weights, this cannot be considered substance treatment-related (no individual data available on maternal body weights, body weight gains etc.). The incidence of resorptions was reported to be increased to 16% ($p\leq0.05$ compared to 8% in control). Thus, the rationale for selecting high dose -

unacceptably high maternal toxicity and increased resorptions at 100 mg/kg bw/day - in the study by Faqi et al 2001, cannot be supported. The dose 100 mg/kg bw/day refers to the test material DOTI/MOTI 80:20 and the dose can be adjusted to approx. 80 mg DOTI/kg bw/day.
At 67 mg/kg bw/day in the study by Faqi et al 2001, there was no maternal toxicity except for minimal effects on thymus (thymus weight -13%) and body weight gain (-10%). A statistically significant increase in the incidence of resorptions was reported (13% versus 8% in control). This dose should therefore have been selected as an intermediate dose (according to OECD TG 414: At least one intermediate dose level should produce minimal observable toxic effects) and not as high dose (according to OECD TG 414: the highest dose should be chosen with the aim to induce some developmental and/or maternal toxicity (clinical signs or a decrease in body weight) but not death or severe suffering).
Importantly, the adverse effects on the development of the offspring evident from the Faqi study were concluded by RAC to be the following: Dose-dependent developmental effects were observed in pups: Skeletal variations were seen from 20 mg/kg/d onwards, post implantation losses, skeletal abnormalities and cleft palates at 67 mg/kg/d and above, and in addition at 100 mg/kg/d increased incidence of exencephaly resulting from the treatment.
Therefore, including the higher dose also in the new study would have increased the possibility to detect a dose-dependent effect.

In the new prenatal developmental toxicity study in rabbit (2014a) the high dose (80 mg/kg bw/day) induced no (minor) maternal toxicity as a 12.8% decrease in thymus weight. There were no statistical significant effects nor a dose-related pattern of maternal body weight or body weight gain (uncorrected or corrected).
In the study by Battenfeld 1992, there was no statistical significant difference in body weight or body weight gain between groups and there were no data available on effects on the thymus. Clinical signs of bloody outflow in three dams in the high dose (100 mg/kg bw/day) group were reported. In one of these three animals, bloody vaginal outflow could be correlated with abortion the following day. In total there were four abortions in the high dose group and all occurred after the termination of treatment (GD 18). We have no access to the individual data of the does and it is not reported in the study summary if the bloody outflow was transient or persistent. In a dose-range finding study (8 animals per group, oral gavage daily from day 6 through day 18 p.c. and animals terminated day 28 p.c.), prior to the aforementioned study, at 100 mg/kg bw/day a moderate reduction of body weight (no data reported) during the treatment period and a lower corrected body weight on day 28 was recorded in this group. This finding was considered compound related although the difference to the control group was not statistically significant. Moreover, no clinical symptoms that were substance related were reported. In conclusion, there was no to mild maternal toxicity at 100 mg/kg bw/day.
The rationale for the highest dose selected in the rabbit developmental according to the study "The risk of maternal deaths and abortions with DOTI/MOTI at 100 mg/kg was deemed to be unacceptably high for the rate of loss of pregnant animals in PNDT study if it could be avoided" therefore appears not to be valid if following the recommendation of the OECD TG 414.

The adverse effects on the development of the offspring evident from the Battenfeld (1992) study were concluded by RAC to be the following: "Increased rates of post-implantation losses seen at 100 mg/kg/d are consistent with findings in mice and in rats in the reproductive toxicity screening study (Appel and Waalkens-Berendsen, 2004). Increased rates of skeletal variations, minor visceral and skeletal head abnormalities indicating retardation of fetal development were seen in pups from surviving dams that

received 100 mg/kg/d. The increase in the minor skeletal head abnormalities appeared to be dose-related: non-significantly increased incidences at 10 mg/kg/d and significantly increased incidences at 100 mg/kg/d were indicating that this effect was test-substance related and occurred in absence of any indication of maternal toxicity." From the two-generation reproductive toxicity study of DOTI/MOTI in rats, RAC concluded in its opinion on DOTE: "As reported in the CLH dossier, consistent pup effects were seen at 200 ppm in the F0 and F1 generation (pup lethality and impairment of postnatal viability, reductions in pup body weights indicative of postnatal growth retardation and thymus atrophy indicating developmental immunotoxicity). Indicative for development retardation, a delayed vaginal opening were seen in F1 pups whereas delayed pinna unfolding, eye and ear opening were reported for F2 pups. Increases in stillbirths in F2 pups was the only finding that was observed in addition to the range of consistent findings in pups of both generations. There was no indication of maternal toxicity that may have influenced the pup effects." And finally, in support of the statement that a common intermediate of DOTE (or DOTI) and DOTC cannot be excluded by the results of the new hydrolysis study there is evidence of similar toxicological properties from the Reproductive toxicity screening study of DOTC in rat. The following conclusion was made by RAC: "Reproductive findings from the DOTC study are consistent with findings on DOT(IOMA) in rats (no comparison possible for other species). This indicates that these structurally similar substances either have the same inherent reproductive toxicity or form a common hydrolysis product (e.g. DOTC) which is a reproductive toxicant." To conclude, the SE CA does not support the proposal to change the current harmonised classification of DOTE to Repr 1B, H361d, and is of the opinion that Repr. 1B, H360D should be retained.
Dossier Submitter's Response
Worth to be discussed at RAC. DOTE (or DOTI) and DOTC might have a common metabolic intermediate, but DOTC is not considered structurally similar to the other two substances.
RAC's response
RAC is of the view that the new studies on DOTE are conducted using too low dose levels to be informative concerning developmental toxicity, and supports to retain the Category 1B classification, based on in particular the DOTI data.

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
30.01.2018	France		MemberState	8
Comment received				
FR agrees with STOT RE 1 H 372 classification in regard to the read-across with structural analogues and the data available with DOTE substance in animal studies. The criteria for classification are fulfilled.				
Dossier Submitter's Response				
Thank you for your comment.				
RAC's response				
RAC agrees that the criteria for STOT RE 1 are fulfilled.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON 2-ETHYLHEXYL 10-ETHYL-4,4-DIOCTYL-7-OXO-8-OXA-3,5-DITHIA-4-STANNATETRADECANOATE; [DOTE]

Date	Country	Organisation	Type of Organisation	Comment number
02.02.2018	Belgium		MemberState	9
Comment received				
BECA supports the proposal of classification and agrees with the justifications given by BAuA. Effects on the thymus weight (decrease) were detected consistently between studies, in different species (rat, mouse, rabbit) and at very low doses (less than 10 mg/kg bw/d).				
Dossier Submitter's Response				
Thank you for your comments.				
RAC's response				
RAC agrees that the criteria for STOT RE 1 are fulfilled.				

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2018	Germany	Organotin REACH Consortium	Company-Manufacturer	10
Comment received				
The LR and co-registrants agree with the dossier submitter that the predominant effect in feeding studies with DOTE or the structural surrogate DOTI is decreased weight and lymphoid depletion of the thymus gland. The registrants have self-classified DOTE as STOT RE 1, H372 for this reason. It is important to document that there are no studies with DOTE conducted by the registrants or others which can differentiate the effect on the thymus as one requiring a repeated dose [STOT RE] versus one requiring a single dose [STOT SE]. An accurate GHS classification of DOTE for this outcome is highly desirable and the registrants petition the dossier submitter to (a) reconsider this STOT classification at a later time, pending definitive data on DOTE voluntarily submitted by the registrants, or (b) make a specific request of the registrants to generate the definitive comparative data on this effect for DOTE. Studies on DOTC [Kishi et al., 2006] show that the thymic effect can occur after a single exposure to DOTC and is fully reversible. So for DOTC, a STOT SE classification would be the most appropriate. The DOTE registrants agree entirely that the effect on the thymus is an outcome of essentially all of the repeated-dose studies with DOTC. The registrants do not suggest here that a read-across to DOTC for this effect is justified. They do suggest that there is a high degree of uncertainty around a STOT RE classification for DOTE because there are no studies which compare an acute response to a repeated-dose response. The literature also shows this thymic effect occurs normally, though to a lesser extent, during gestation [Smialowicz et al., 1988], and a similar outcome can be induced by merely under-feeding female rats compared to controls. On the basis of new data with the DOTE substance itself, it would be possible to accurately determine the nature of the specific organ toxicity, for single or repeated exposure, based on substance-specific scientific evidence. For the reasons given above the LR and co-registrants propose that the harmonization of the specific organ toxicity for DOTE be briefly deferred until the data from on-going test programs are available. The nature of the specific organ toxicity can be accurate and based on the appropriate comparative data. References and Abstracts Kishi, H, Nemoto, M., Enomoto, M., Shinoda, M., Kawanobe, T., and Matsui, H. [2006]. Acute Effects of Dioctyltin on the Immune System of Rats. Env. Tox. Pharm. 22, 240-247. Smialowicz RJ, MM Riddle, RR Rogers, DG Rowe, RW Luebke, LD Fogelson, CB Copeland				

(1988). Immunologic effects of perinatal exposure of rats to dioctyltin dichloride, J Toxicol Environ Health 25(4):403-22.

Dossier Submitter's Response

For DOTE no data are available to allow for a STOT SE classification. Underlying studies with DOTE are repeated dose studies and new animal data should not be generated for the purpose of classification.

RAC's response

RAC agrees that the criteria for STOT RE 1 are fulfilled. Should new data indicate a need to classify for STOT SE, industry is encouraged to self-classify DOTE for STOT SE.

Date	Country	Organisation	Type of Organisation	Comment number
02.02.2018	Sweden		MemberState	11

Comment received

The SE CA supports the classification proposal of DOTE as STOT RE 1 and consider it appropriate to state the immune system as target organ instead of the thymus to be consistent with existing entries of (dioctyl) organotin compounds in Annex VI.

Effects on the thymus starting from 1.25 mg/kg bw/day in the repeated dose 90-day oral toxicity study of DOTE by Anonymous (1970) are relevant for classification in STOT RE 1. Decreased relative thymus weights in males and females starting from 1.25 mg/kg bw/day (approx. 20%) and statistically significant from 5 mg/kg bw/day were reported. In addition, 2/5 females at 5 mg/kg bw/day and all males (5/5) and females (5/5) at 25 mg/kg bw/day had almost complete depletion of lymphocytes.
In the Repeated dose 90-day oral toxicity study in rat by Anonymous, 1974 the test material DOTE/MOTE in doses up to and including 6.6. mg/kg bw/day were used. We note that the test material is a mixture at 70:30% DOTE:MOTE. However, since an available 90-day repeated dose oral toxicity study of MOTE in rat at doses up to and including 82-91 mg/kg bw/day (Study report, 2014 in the publically disseminated REACH registration at ECHA) showed no effects, it can be assumed that the observed effects on the thymus in the study from 1974 can be attributed to DOTE and not MOTE. The effects relevant for STOT RE 1 classification were statistically significant and dose-dependent increase in absolute and relative weight of the thymus (approx. 25-35%) starting from 3.3 mg/kg bw/day. It is not clear to us if adverse histopathological findings in the thymus were evident at the end of the treatment or not, since this was not reported in the CLH-report. However, it is stated that there were no adverse histopathological effects at the end of the 15 or 30-day recovery period.
In the pre-natal developmental toxicity study (2014b) in mouse of DOTE the pregnant animals were reported to have dose-dependent and statistically significant reduction in thymic weight from 30 mg/kg bw/day (-24%), a dose that after adjustment for exposure length would be below the guidance value for STOT RE 1 in rat. No histopathological assessment was done to confirm if the reported effects on the weight of thymus was correlated with significant organ damage.

Since studies of DOTI/MOTI are included in the weight of evidence assessment of reproductive toxicity, and in absence of any evidence showing that organ damage after repeated exposure is not a suitable endpoint for read-across approach for DOTE we consider that information on thymus effects from the two-generation reproductive toxicity study of DOTI/MOTI could be included in the weight of evidence assessment of STOT RE 1 as well to be consistent.

Dossier Submitter's Response
Data from the two-generation reproductive toxicity study of DOTI/MOTI could be included in the weight of evidence assessment of STOT RE 1 classification of DOTE but are not considered necessary.
RAC's response
RAC agrees that the criteria for STOT RE 1 are fulfilled, and that the immune system should be highlighted as the affected target organ.

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
30.01.2018	France		MemberState	12
Comment received				
France supports the proposal to classify the substance as Aquatic Chronic 2, H411. According to data DOTE is not ready biodegradable and based on the long term test with Daphnia magna (21d-NOEC = 0.286 mg/L), DOTE fulfills the classification criteria for Aquatic chronic 2. Page 41-43 According to the table 20, the only reliable test with Daphnia magna shows a 48h-EC50 of 24.12 mg/L (nominal concentration). However, the table 22 (page 45) which summarize the information for acute aquatic hazards, presents the same result indicating that it is expressed as measured concentrations. This point needs to be clarified. Furthermore, we do not agree with the conclusion formulated in the table 22 (page 45), which refers that DOTE does not fulfil with criteria for classification as Aquatic acute 1. We believe that more reliable information is necessary in order to conclude about the acute aquatic hazards of the substance.				
Dossier Submitter's Response				
Thank you for your support for the Aquatic Chronic 2 classification. Concerning your comment on table 20 and 22: In the acute toxicity test on *Daphnia magna* an analytical confirmation was performed and the concentrations remained within the acceptable range of +/- 20%. Therefore, the concentrations were also given as nominal concentrations and should be in both tables. Thank you for your hint. For the comparison of data with criteria for acute aquatic hazards: There is no result from a reliable short-term toxicity test available, making a classification with Aquatic Acute 1 necessary. We considered all available data for the decision and quoted these in the CLH report.				
RAC's response				
Agreed with the reply from the DS that the endpoint from the *D. magna* study is based on nominal concentration because the measured concentration were within 20% of nominal. From the comment about the conclusion it is unclear why the available information for DOTE would not be sufficient for conclusions about the acute aquatic hazard. Classification should be based on the available data, it is not possible to request more data for CLP purposes and according to the Annex VI report, reliable data is available for all three taxonomic levels. Please note that the interpretation of the studies for classification purposes is further discussed in the opinion.				

Date	Country	Organisation	Type of Organisation	Comment number
02.02.2018	Belgium		MemberState	13
Comment received				
BE CA supports the proposal to classify DOTE for the aquatic environment with Aquatic Chronic 2, H411.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
26.01.2018	United Kingdom		MemberState	14
Comment received				
Hydrolysis: Section 11.1.3 includes details of a 2015 in-house, non-GLP hydrolysis study following OECD TG 111. The study reports that less than 10% DOTE was hydrolysed at 50 oC over 5 days at each pH 4, 7 and 9. This appears to contradict previous assessments which considered that DOTE would hydrolyse rapidly to either dioctyltin hydroxide/oxide or octyltin hydroxide/oxide (OECD SIDS and ECHA PBT / Transitional Substances evaluation). It would be useful to provide further study details to confirm the analytical method and consider if it was sufficiently accurate / sensitive to determine concentrations of DOTE and/or hydrolysis products. Bioaccumulation: Table 11.7.2 includes the log Kow for DOTE but not measured BCF data which are presented in section 11.4. The table also includes the following statement 'Low potential for bioaccumulation (based on Weight-of-Evidence: high molecular weight, very high log Kow, very low water solubility)'. The classification bioaccumulation assessment should consider the historic PBT assessment for the substance and ECHAs conclusion for transitional substance for this substance with CAS: 3542-36-7 and CAS: 27107-89-7. These assessments considered data uncertainties and interpretation with the conclusion that DOTE did not meet the B/vB criteria. However, it is unclear if the BCF would be below the classification bioaccumulation criteria of 500L/kg. Given the statement in the table, are there further data relating to molecular weight which would result in DOTE not meeting the classification bioaccumulation criteria?				
Dossier Submitter's Response				
Hydrolysis: In OECD SIDS for DOTE, OECD SIDS for dioctyltin dichloride and selected thioesters as well as the results of substance evaluation for transitional dossiers rapid hydrolysis of DOTE within 10 minutes forming dioctyltin oxide/dioctyltin hydroxide was reported. It was also expected that the labile ligands can be displaced by other anion in the medium and the displaced thioester ligands can also undergo further hydrolysis of the ester linkage to form thioglycolic acid and 2-ethylhexanol. Due to problems with standard OECD tests,				

electrospray ionisation mass spectrometry was used as testing method. Nevertheless, it was not mentioned under which test conditions (e.g. temperature and pH) the test was performed.

Details of OECD TG 111 (registration dossier):
1g (1.3 mMol) of the test item was added to 100 ml of the respective buffer solution (pH 4.0 HCl/NaCl/Citric acid; pH 7.0 Na_2HPO_4/NaH_2PO_4; pH 9.0 H_3BO_3/KCl/NaOH in a 250 ml Erlenmeyer flask, which was closed and heated for 5 days at 50°C. Thereafter the solution was allowed to cool down to room temperature and the mixture was extracted with 20 ml hexane and the samples were analysed by 119Sn-NMR. After 5 days less than 10% DOTE was hydrolysed at the investigated pH values.

Study details cited from Costlow et al. 2017:
Hydrolytic stability of DOTE was conducted in accordance with OECD Guideline Number 111 measured at pH 1.2 & 37 _C and at pH 4, 7, and 9 & 50 _C in buffer solutions from VWR International GmbH [pH 1.2 ¼ HCl 0.1 M; pH 4.0 ¼ HCl/NaCl/Citric acid; pH 7.0 ¼ Na2HPO4/NaH2PO4; pH 9.0 ¼ H3BO3/KCl/NaOH].119Sn-NMR Instrument: Bruker Advance 200;; sample preparation: 370 ml/330 ml toluene-d8 (10 mg/ml CrAcAc)).
Sample preparation was as follows: a 1 g (1.3 mMol) DOTE in 100ml of the respective buffer solution, heated for up to 120 h at 37 or 50 °C with stirring. The reaction mixtures were extracted with hexane; after removal of the solvent remaining residues were analyzed by 119Sn-NMR. All samples were run in duplicate.
Aqueous phases were analyzed for Total Organic Carbon [TOC]
using Shimadzu TOC-L at ambient temperature; injection Volume 50 ml].

Furthermore, hydrolysis of DOTE under simulated gastric conditions (0.1 M HCl, pH 1.2, 37°C) was investigated and resulted in a half-life < 1 minute and dioctyltin chloro 2-ethylhexylmecaptoacetate as transformation product. In the study also TOC analysis has been conducted to ensure completeness of the analysis and recover all organic carbon in aqueous phases including water-soluble organotin substances and their breakdown products. Some organic carbon (1.4 – 4.2% of the total available organic carbon) was detected in the aqueous phase. The authors assumed that these traces could be attributed to 2-EHTG (a hydrolysed ligand of DOTE) and its breakdown products ethylhexanol and thioglycolic acid. Therefore, they concluded that dioctyltin chloro 2-ethylhexylmecaptoacetate is the only tin-containing transformation product of DOTE under simulated gastric conditions.

Bioaccumulation:
As mentioned in chapter 11.4.2 the BCF-study did not fulfil the validity criteria defined in Guideline OECD 305. Thank you for your support.Hence, this study, which is the only BCF-study in the registration dossier, could not be used for the comparison with the CLP criteria in chapter 11.7.2 (Table 23).

ECHA conclusion of substance evaluation for transitional dossiers considered the above mentioned BCF-study. It was highlighted that no definitive BCF value is available and that there were some uncertainties around interpretation. In the fact sheet it was concluded that available fish bioconcentration results are strongly suggestive: BCF of the dioctylin substances is perhaps around 1000 as a maximum but more probably much lower (~100 or less).
As no valid/high quality BCF value is available, the log K_{ow} should be used for classification. While the log K_{ow} (15.35) meets the criterion (log $K_{ow} \geq 4$) it is suggested that the substance has a low potential for bioaccumulation based on weight of evidence

(very high log K_{ow} (15.35), high molecular weight (751.79 g/mol) and very low water solubility (0.001 pg/L)).
RAC's response
Hydrolysis: The DS provided the requested additional details. Bioaccumulation: The DS has provided all data available for the conclusions on bioaccumulation. RAC agrees that DOTE does not meet the criteria for bioaccumulation.

Date	Country	Organisation	Type of Organisation	Comment number
01.02.2018	Finland		MemberState	15
Comment received				
Toxicity tests with crustacea are recommended for classification purposes of aquatic hazards. The key study for this proposal is Daphnia magna reproduction test (OECD 211). According to the study, the chronic toxicity NOEC value for DOTE is 286 µg/l. The substance is not considered rapidly degradable and predicted to have a low bioaccumulation potential. Based on the available information and the classification criteria FI CA supports the proposed classification of Aquatic Chronic 2, H411 for DOTE.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
31.01.2018	Germany	Organotin REACH Consortium	Company-Manufacturer	16
Comment received				
DOTE contains, as a process-related impurity, the ligand EHTG (EC 231-626-4). The ligand is classified with H410 [very toxic to aquatic life with long lasting effects]. This classification is based on an old testing paradigm, i.e. commercial products were the most common test materials. This practice was not wrong, but it was deficient in many cases because it could not determine which component(s) of a commercial product contributed to the overall observed effect. In some cases the entire outcome could be attributed to a single component versus the commercially-active component. For DOTE, it is a highly credible hypothesis that the effects of commercial samples of DOTE on the aquatic toxicity are likely caused, or at least significantly exacerbated, by the EHTG impurity. To demonstrate this, and test the hypothesis, the lead registrant conducted comparative studies on daphnids and algae with a highly purified sample of DOTE. The 48-hour EC50 for purified DOTE in the more sensitive species, the daphnids, was 24.12 mg/L. This was more than two orders of magnitude higher than the equivalent EC50, determined in an identical test, with a test material which contained between 6-12 % EHTG. In algae, DOTE, at a concentration of 100 mg/L (nominal), produced no effects, i.e. the NOEC >100 mg/L for the purified material. This comparison of studies conducted on purified DOTE versus a commercial DOTE containing >6% EHTG supported the hypothesis. The EHTG contributed disproportionately				

to the overall aquatic toxicity assessment in both species with the commercial DOTE. Therefore, a fully justified aquatic toxicity classification of the product is both possible, and far more appropriate, if any future classification is based on the mixture rules for CLP, assuming the aquatic toxicity for the impurity and the purified substance are available. This approach was discussed, before conducting the trial studies, between the LR and the German CA. The approach was appreciated and preferred by the CA.
The proposal for the harmonized classification of DOTE in the CLH dossier is based on the 21d-NOEC for daphnia, determined in an OECD TG 211 study [Anonymous, 2004b] together with the non-readily biodegradability of DOTE. The DOTE test sample used for the aquatic study contained EHTG as an impurity. At the time of the study the EHTG impurity specified as <3 %. However, the study report does not provide specific information about the EHTG content in this DOTE test sample. We infer, therefore, that the EHTG impurity could have been as much as 2-4 times higher [6-12%].
The OECD 211 study utilized the WAF procedures to prepare the test waters for the daphnia; the study did not use co-solvents. Considering the demonstration project noted above, the WAF approach could reasonably create a dramatic testing artifact and produce a substantial impact on the measured outcome because of the differential physical properties of DOTE and EHTG. The solubility of DOTE is far below the observed LOEC of 1.448 mg/l and even below the NOEC of 0.286 mg/L. In addition to their hydrophobic properties, organotin compounds are known to have a high affinity for solids and thus can readily adsorb to the walls of glass vessels. EHTG is much more soluble in water [hydrophilic]; thus less likely to adsorb to the walls of glass vessels and be more bioavailable in an aquatic test system. It follows that a WAF, particularly a WAF prepared with no co-solvent, is likely to disproportionately solubilize the EHTG impurity versus the DOTE, creating a testing artifact, a substantial impact on the measured outcome, and a misclassification of the DOTE substance.
Adding to the difficulties for interpretation of tests with organotin thioglycolate esters in aquatic systems, the measured concentrations of DOTE, the test item in the WAFs for the commercial DOTE aquatic tests, were determined by quantifying the tin content using Atomic Absorption Spectroscopy [AAS]. This analytical technique requires the WAFs be strongly acidified to bring the tin-containing molecule into a soluble form. This acidification will also dissolve some or all of the fraction of DOTE previously adsorbed to the solid materials of the test containers. This creates an analytical artifact which increases the actual dose of DOTE available within the aqueous milieu of the test system. Based on preliminary data, the LR and the co-registrants believe that the toxic effects observed in daphnia in the existing tests with commercial DOTE samples on aquatic species can reasonably, and to a large extent, be attributed to the EHTG impurity. It is proposed to repeat the OECD 211 daphnia study with a purified DOTE sample as a test item, rather then to harmonize the aquatic toxicity based on results which are an artifact of the method used to create the WAF. The OECD 211 daphnia study cited in the CLH document has essentially no accurate chemical analytical evidence on which to document or properly evaluate the presence or absence of an artifact. Given that a correctly designed study could eliminate the testing artifact by using the appropriate DOTE test sample and a proper analytical approach, a repeat of the OECD 211 study is entirely appropriate. A new test will critically examine the hypothesis and simultaneously provide the necessary evidence for a correct and unambiguous classification.

References and Abstracts
Anonymous (2004b). 8-Oxa-3,5-dithia-4-stannatetradecanoic acid, 10-ethyl-4,4-dioctyl-7-oxo-, 2-ethylhexyl ester [Dioctyltin bis(2-ethylhexylmercaptoacetate), CAS. No. 15571-58-1]: Daphnia magna, reproduction test (semi-static). Testing laboratory: NOTOX B.V. Report no. NOTOX Project 375097. TNO Study No. 5312/02. Owner company:

Parametrix, Inc.
Dossier Submitter's Response
WAF procedure means that no serial dilution was done. The test substance was directly dissolved in test medium. The undissolved fraction was separated from the water fraction by pipetting the water fraction and filtering it over a glass filter. Therefore, it seems that the unproportional solution of EHTG (2-ethylhexyl mercaptoacetate; CAS 7659-86-1) was minimised. Concerning the low water solubility of DOTE: in contrast to the normal WAF procedure, in this test an analytical confirmation of the test concentrations was prepared. The measured concentrations were used to describe the effect levels. Therefore, the study and the effects occurring from the exposition of *Daphnia magna* with the test substance are appropriate for classification and labelling. When correctly understood, the current EHTG impurity concentration of DOTE corresponds to the one of the OECD 211 daphnia study. Therefore, this study is considered appropriate to evaluate the ecotoxicological effects of DOTE. Additionally, according to CLP regulation Article 11, an impurity (here: EHTG) which is present in another substance (here: DOTE) equal or above a threshold (according to Annex I Table 1.1 0.1 % for Hazardous to Aquatic Environment Acute Cat. 1 or Chronic Cat. 1) shall be taken into account for the purposes of classification. This supports the use of the OECD 211 daphnia study in the assessment of the necessary classification of DOTE.
RAC's response
In the comment it is stated that the DOTE tested in the key study contained a contaminant (EHTG) that could have caused the effects observed. Especially since 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. In this, references 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 replied that the unproportional 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. RAC considers that the water solubility of DOTE (0.0012 pg/L) 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 unproportional solution this cannot have been excluded. Nevertheless, in none of the studies available, the concentration of EHTG has been determined in the test concentrations, therefore any conclusion on the actual extent of the contribution of EHTG to the effects observed cannot be drawn since its actual presence 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. But also in this study, the actual exposure level of EHTG is unknown. Therefore, the claim that effects observed are caused by the EHTG impurity is considered to be unsubstantiated. Also considering the fact that the EHTG impurity in the DOTE tested is representative for the current levels in DOTE, RAC agrees with the DS that the OECD TG 211 Daphnia study can be used in the classification of DOTE.

Date	Country	Organisation	Type of Organisation	Comment number
02.02.2018	Sweden		MemberState	17
Comment received				

The Swedish Chemicals Agency supports the DS proposal for harmonized classification of DOTE as Aquatic Chronic in at least category 2. Category 2 is justified since DOTE is not rapidly degradable (11-43% degradation (BOD) after 28 days) and there is reliable chronic toxicity data from Daphnia magna (21 d NOEC =0.286 mg/l). However, due to the limited database (no chronic toxicity studies are available for fish or algae) classification in category 1 cannot be excluded.
Dossier Submitter's Response
Thank you for your support. Requirement of chronic data (especially animal tests) for classification and labelling is not possible. If there are only adequate chronic toxicity data for one or two trophic levels available, the substance should be assessed by the most stringent outcome of (a) chronic toxicity data and (b) acute toxicity data (Figure 4.1.1 of CLP regulation). Based on the currently available data and the most stringent outcome of the assessment of this data the substance should be classified as Aquatic Chronic 2.
RAC's response
The absence of chronic data is no reason for a higher classification other than the method described in the guidance (surrogate method). Nevertheless, please note that this is further discussed in the opinion.

CONFIDENTIAL ATTACHMENTS
1. Confidential_annex_Dote_02022018.docx [Please refer to comment No. 6]