Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

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

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

CLH-O-0000001412-86-223/F

Adopted

14 September 2018

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DIOCTYLTIN DILAURATE; [1] STANNANE, DIOCTYL-, BIS(COCO ACYLOXY) DERIVS. [2]

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: dioctyltin dilaurate; [1] stannane, dioctyl-, bis(coco acyloxy) derivs. [2]
CAS number: 3648-18-8 [1] 91648-39-4 [2]
EC number: 222-883-3 [1] 293-901-5 [2]
Dossier submitter: Sweden

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
28.11.2017	Germany		MemberState	1
Comment received				
In table 7 of the CLH Report for [1] Dioctyltin dilaurate, [2] Stannane, dioctyl-, bis(cocoacyloxy) derivs. one set of physico-chemical properties is stated. Unfortunately it is not stated for which of this two substance the given results are. Therefore, please clarify the test substances so that correct values are used for the evaluation.				
Dossier Submitter's Response				
Thank you for your comment. The following is stated in the list of reference for the reference [ECHA dissemination, 2016a] given to the data of the physicochemical properties in table 7 of the CLH report: ECHA dissemination (2016a). http://echa.europa.eu/sv/registration-dossier/-/registered-dossier/13131. According to the REACH lead registrant of DOTL, the substance currently on the European market is the UVCB substance although registered under EC no 222-583-2, the monosubstituent substance (October 2016). The physical-chemical data reported in the REACH registration dossier refers to data for the UVCB substance.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Belgium		MemberState	2
Comment received				
BECA considers the use of DOTC as a read-across from DOTL as justified since DOTC and DOTL are structurally similar, rapidly hydrolysed under low pH conditions and show common intermediates afterwards. The justification given regarding a similar structure as well as a similar hydrolytic and toxicokinetic behaviour is supported.				

Dossier Submitter's Response
Thank you for your support.
RAC's response
Your comment and support is noted.

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Germany		Individual	3
Comment received				
The registrants of the substance (DOTL CAS 3648-18-8) organized in the Organotin REACH Consortium appreciate and support the position forwarded by the lead registrant TIB Chemicals.				
Dossier Submitter's Response				
Please see response to comment number 7.				
RAC's response				
Please see response to comment number 7.				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Netherlands	PMC Vlissingen BV	Company-Importer	4
Comment received				
PMC Vlissingen BV supports the conclusions documented in the comments provided by TIB Chemicals AG.				
Dossier Submitter's Response				
Please see response to comment number 7.				
RAC's response				
Please see response to comment number 7.				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Germany	BNT Chemicals GmbH	Company-Manufacturer	5
Comment received				
The BNT Chemicals GmbH ist supporting the comment of TIB Chemicals AG.				
Dossier Submitter's Response				
Please see response to comment number 7.				
RAC's response				
Please see response to comment number 7.				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Luxembourg	<confidential>	Company-Manufacturer	6
Comment received				
We Support the comment/Statement of TIB Chemicals AG.				
Dossier Submitter's Response				
Please see response to comment number 7.				
RAC's response				
Please see response to comment number 7.				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Germany	TIB Chemicals AG	Company-Manufacturer	7

Comment received

See attached file

ECHA note – An attachment was submitted with the comment above. Refer to public attachment comment clh proposal dotl.pdf

Dossier Submitter's Response

Thank you for your comments.

DOTC hydrolyses under the conditions of the study by Naßhan (2015) (HCl, pH 1.2, 4 h, 37°C) to a dimeric form of the distannoxane ClOct2SnOSnOct2Cl to an extent of 90%, and with 10% DOTC unreacted. Hydrolysis of DOTL under the same conditions resulted in a complex mixture of tin containing substances and approx. 16% of the distannoxane ClOct2SnOSnOct2Cl was formed. Thus, a common hydrolysis products of DOTC and DOTL formed under the same conditions was detected.

We do not agree that there is evidence for stating that the toxicological effects of DOTC in vivo is not attributed to the dimeric distannoxane, but to the remaining unreacted (10%) DOTC. In absence of reliable data on in vivo transformation in the stomach and any in vivo toxicokinetics studies of the metabolism of the substances, we do not know what is actually formed in the stomach after oral administration of either DOTC or DOTL, or what happens in the intestines at neutral pH and what is actually absorbed and available systemically.
The dimeric structure of the distannoxane is well described in literature (see for instance, A. G. Davies, J. Chem. Res. 309- 314, 2004) although not discussed in the original study report on DOTL by Nasshan et al. The dimeric structures are usually observed in the solid state and may be preserved in non-coordinating solvents (like toluene-d8 that was used in the original study report on DOTL by Nasshan et al). However, there is evidence for dissociation in solution illustrating that the dimer with half the molecular weight (as compared to the dimer-of dimers) can be present in significant concentrations at equilibrium in solution (A.G. Davies, J. Chem. Res. 309- 314, 2004).

The hydrolytic behaviour of the substances at neutral and low pH supports the assumption that systemic exposure to the intact substances following oral administration is unlikely.

In analogy with the dioctyltins, recent hydrolysis studies of dibutyltins at low pH show that dibutyltin dichloride (DBTC) is rapidly hydrolysed to the distannoxane ClBu2SnOSnBu2Cl (Naßhan, 2015). DBTC and DBTL as well as DOTC and DOTL behave similarly in water at neutral pH and rapidly form oxides/hydroxides in good agreement with the expected lability of the ligands.

DBTC is also toxic to the immune system (STOT RE 1) and a reproductive toxicant (Repr. 1B, H360 FD), but appears to have higher acute toxicity (Acute Tox. 3*, H301; Acute Tox. 4*, H312; Acute Tox. 2*, H330) and reactivity (Skin Corr. 1B, H314; Muta. 2, H341) compared to DOTC with longer alkyl chains.

Importantly, comparative developmental toxicity data in the rat (Noda et al., 1993) of dibutyltins including dibutyltin dilaurate (DBTL) and DBTC at the same molar concentrations resulted in a comparable spectrum of foetal malformations mainly affecting the jaw (cleft mandible, cleft lower lip, ankyloglossia or schistoglossia) and exencephaly. Moreover, in repeated dose toxicity studies, for both DBTL and DBTC the immune system appears to be the most sensitive organ system with main findings in the thymus. In studies with repeated

exposure of DBTL atrophy and cell depletion in thymus and lymph nodes, with reduced organ size and cell counts have been described (Subramoniam et al., 1994). Studies with DBTC report thymus pathology with organ atrophy, shift in cell population and loss of structure in the organ (Waalkens-Berendsen 2003).

This information points to a common metabolite/intermediate in vivo and common biological targets for DBTL and DBTC. Moreover, this also further supports the applicability of read-across from DOTC to DOTL for reproductive toxicity and STOT RE since a similar relationship between DOTC and DOTL can be expected.

References:

Naßhan, H. (2016). Dibutyltin dichloride [DBTC], CAS number: 683-18-1. In-vitro metabolism study. Galata Chemicals GmbH, Lampertheim, Germany.

Noda T., Morita S., Baba A. (1993). Teratogenic effects of various di-n-butyltins with different anions and butyl(3-hydroxybutyl)tin dilaurate in rats. Toxicology 85(2-3), 149-160.

Subramoniam A, Khandelwal S, Dwivedi PD, Khanna S and Shanker R (1994). Dibutyltin dilaurate induced thymic atrophy and modulation of phosphoinositide pathway of cell signalling in thymocytes of rats. Immunopharmacol. Immunotoxicol., 16, 645–677.

Waalkens-Berendsen D. H. (2003). Reproductive/developmental toxicity screening study in the rat. Full report not available; study summary included in the REACH registration dossier for dibutyltin dichloride (ECHA, 2015d). TNO, The Netherlands. TNO Report V4906.

RAC's response

Thank you for you comments. RAC agrees with the dossier submitter and is of the opinion that the dimer is more likely (in part) bioavailable than DOTC. Because all closely related di-substituted tin compounds are classified to be toxic to the immune system and most of them are toxic to the developing organism, RAC considers the read across sufficiently justified.

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Austria	Environment Agency Austria	Please select organisation type..	8

Comment received

The present CLH proposal is based on the read across approach (analogue approach) to DOTC as target substance. It is hypothesised that the source and target compounds are structurally similar. The substances have a common dioctyltin (Oct2Sn-) group – considered to be the toxic component.
There is a difference in the species formation of DOTC and DOTL at low pH under the tested conditions of key studies (Naßhan, 2015, 2016). It is acknowledged in the CLH dossier that the target substances display a more complex chemistry during the specific experimental setup used in the simulated gastric hydrolysis study expected due to the coordinating carboxylate ligands which can bind to the tin moiety in various ways (monodentate, bidentate, bridging etc.). It has not been verified yet, whether these observed differences in a very limited test design (only in vitro hydrolysis studies) might have an impact on the toxicological outcome (e.g. severity of effect) and it is probably not possible to show this on the basis of present data. It is, however, demonstrated in the CLH report that there are partly identical species (distannoxanes) formed under these specific experimental conditions and thus it might be assumed that the toxicity pattern are indeed identical. It is thus agreed that the studies can be taken as support for the read across approach.
The toxicological data presented in the data matrix are limited; the only toxicological study for DOTL is an acute toxicity study. The toxicological data have been derived from REACH

registration. Since more information on toxicological properties and or MoA is of interest to substantiate the read across, maybe other information sources should be considered. E.g. organotins are known/suspected to have endocrine disruptor properties. Maybe there is further data available e.g. in regard to underlying MoA of toxicity, from which more information on DOTL and DOTC toxicological properties can be derived. This information might be helpful to substantiate the read across. Furthermore, the toxicity data of dibutyltin dilaurate (DBTL) (RAC opinion available dealing also inter alia with the classification endpoints - reproductive toxicity and STOT RE) might be worth to be considered to substantiate the hazardous properties of the octyl form, since the proposed classification is also partly based on read across to DBTC. For DBTL there are in contrast to DOTL repeated dose toxicity studies available.
Although there is limited toxicological data available for establishing a comprehensive data matrix for read across, the read across hypothesis - as based on the currently available evidence - is considered as plausible. Inclusion of additional information would be helpful to further reduce the uncertainties related to the application of the read across.

Dossier Submitter's Response

Thank you for your support and constructive comments.

AT asked for more information on toxicological properties and/or MoA of interest to substantiate the read-across, and suggested other information sources that could be considered on endocrine disrupting properties, underlying MoA of toxicity, and toxicity data of dibutyltin dilaurate (DBTL).

There is, to our knowledge, no information on potential endocrine disrupting properties or MoA of toxicity of DOTL that could be used to substantiate and strengthen the justification of read-across from DOTC. There is sparse data on DOTC on endocrine disrupting properties and underlying MoA of developmental toxicity or immunotoxicity. Overall, studies of endocrine disrupting properties of other organotins than tributyltin chloride are very few. In a comparative in vitro study of aromatase activity of various organotins Cooke (2002) found that tributyltin chloride and dibutyltin dibromide inhibited aromatase activity (a commercial preparation), but not DOTC, monobutyltin trichloride, monooctyltin trichloride or trioctyltin chloride at the concentrations tested. This study also suggested that tributyltin was more potent than dibutyltin and that there could be a structure-activity relationship for organotin inhibition of aromatase activity.

As AT point out, including information on DBTC and DBTL could be worth to consider in comparison to substantiate the read-across between the corresponding dioctyltins. For this comment, please also see our reasoning in response to comment number 7.
Furthermore, in an in vitro study comparing effects of tributyltin chloride and dibutyltins, DBTC and DBTL were found to be partial antagonists of PPARγ and RXRα (Milton et al., 2017). In addition, DBTC and DBTL were both reported to induce adipogenesis in a PPARγ-dependent manner and to partially repress inflammatory genes in preadipocyte and macrophage cell lines. This would suggest that DBTC and DBTL may act via a similar mode of action, at least on effects mediated via activation of nuclear receptors PPARγ and RXRα. Accordingly, it is reasonable to assume a similar functional relationship between DOTC and DOTL. In addition, as stated above in response to comment number 7, for both DBTL and DBTC the immune system appears to be the most sensitive organ system with main findings in the thymus in repeated dose toxicity studies. Albeit there could potentially be some differences in potency, DOTC and DBTC appears to have similar immunosuppressive properties in their ability to selectively supress T-lymphocyte activity in rats (Seinen, Vos, Krieken et al., 1977). Moreover, studies of DBTC and DOTC also point to antiproliferative effects and a preferential depletion of immature thymoblasts (Pieters et al., 1992, Penninks

et al., 1985, Seinen and Willems, 1976). There is some evidence that synthesis of IL-2, a factor involved in early thymocyte activation, is downregulated at mRNA level by DOTC (Volsen et al., 1989). An early indication of glucocorticoids not being involved in the effects of DOTC on the thymus came from Seinen and Willems (1976) that concluded in their dietary study of DOTC in adrenalectomized rats that the selective effects of DOTC on thymus was not induced by stress related release of glucocorticoids. There are however no comparable mechanistic data on DOTL.

References:

Cooke GM. 2002. Effect of organotins on human aromatase activity in vitro. Toxicol Lett 126:121130.

Miller K, Scott MP. Immunological consequences of dioctyltin dichloride (DOTC)-induced thymic injury. Toxicol Appl Pharmacol. 1985 May;78(3):395-403

Milton FA, Lacerda MG, Sinoti SBP, Mesquita PG, Prakasan D, Coelho MS, de Lima CL, Martini AG, Pazzine GT, Borin MF, Amato AA, Neves FAR. Dibutyltin Compounds Effects on PPARγ/RXRα Activity, Adipogenesis, and Inflammation in Mammalians Cells. Front Pharmacol. 2017 Aug 2;8:507.

Penninks A, Kuper F, Spit BJ, Seinen W. On the mechanism of dialkyltin-induced thymus involution. Immunopharmacology. 1985 Aug;10(1):1-10.

Pieters RH, Bol M, Seinen W, Penninks AH. Cellular and molecular aspects of organotin-induced thymus atrophy. Hum Exp Toxicol. 1994 Dec;13(12):876-9.

Seinen W, Willems MI. Toxicity of organotin compounds. I. Atrophy of thymus and thymus-dependent lymphoid tissue in rats fed di-n-octyltindichloride. Toxicol Appl Pharmacol. 1976 Jan;35(1):63-75.

Volsen SG, Barrass N, Scott MP, Miller K. Cellular and molecular effects of di-n-octyltin dichloride on the rat thymus. Int J Immunopharmacol. 1989;11(6):703-15.

RAC's response

Thank you for your comments. RAC considers the information on DBTC and DBTL valuable for supporting the read-across. Further, DOTL may indeed be less bioavailable compared to DOTC. In view of the limited information, RAC is of the opinion that the classification for DOTC should be directly applied to DOTL. Further, STOT RE 1 is considered more appropriate than STOT RE 2 regarding potential potency differences. In addition, no SCL can be derived.

Date	Country	Organisation	Type of Organisation	Comment number
30.10.2017	France		MemberState	9

Comment received

DOTC is currently classified STOT RE 1 due to its immunotoxic properties and subjected to a harmonisation proposal as Repro 1B – H360B, both of which are supported by FR.

Dioctyltin moiety is expected to be the toxic component responsible for reprotoxic as well as immunotoxic properties of dioctyltin compounds and the demonstration that common metabolites are formed from both DOTL and DOTC is therefore key for the proposed read-across.
The studies available for DOTC are performed by oral route so that hydrolysis under gastric conditions are more relevant to inform on this point than hydrolysis at neutral pH. Gastric hydrolysis studies indeed show formation of a common metabolite for DOTC and DOTL, although in quite different proportions, respectively >90% from DOTC and 15% from DOTL. These raise some uncertainties on the application of the read-across. In this regard, it is noted that :

- The conclusions are based on simulated gastric hydrolysis and may not reflect the complexicity of the in vivo situation in which gastric enzymes may also interfere with the transformation of the substances. Is there information on in vivo metabolites formed from octyltincarboxylates or other organotincarboxylates ? It would help to understand how carboxylate side chain are transformed or removed.
- It is stated in several places of the proposal « In general, dioctyltin compounds are ascribed as having immunotoxic properties via the thymus gland. The use of dioctyltin compounds is therefore restricted according to REACH (EC) No 1907/2006 Annex XVII, entry 20 in a number of consumer articles (≥ 0.1 % by weight of tin). » Data on immuno- or reproductive toxicity for other dioctyltin compounds and in particular dioctyltin with organic ligands or carboxylate ligands would provide further support for the read-across.

With these uncertainties in mind, it is noted that :
- The developmental effects of DOTC are observed from 0.8 mg/kg and developmental effect are usually tested in prenatal studies up to limit dose of 1000 mg/kg so that even if a small amount of the common metabolite to DOTC is formed from DOTL, it will induce developmental effects at DOTL doses that are relevant for classification and not irrealistic due to its low acute toxicity.
- For STOT RE, effects of DOTC starts from 0.7 mg/kg and STOT RE in category 1 apply below 10 mg/kg (maximal ratio of 14). Gastric hydrolysis data (ratio of 10 for production of a common metabolite on a mass basis) are in favour of a classification in category 1 but with a reduced margin to consider potential uncertainties on the in vivo situation. The most appropriate classification STOT RE 1 or STOT RE 2 should therefore be discussed with regard to the level of uncertainty in the effective DOTL doses.

Finally, based on coconut fatty acids composition, only half of the UVCB composition is expected to be DOTL. The other half is composed of octyltins with other carboxylate ligands of longer or shorter chains. It is expected that the hydrolysis or metabolism scheme of the carboxylate bound will be similar to DOTL and produce similar metabolites when both carboxylate ligands are removed. Slower or quicker absorptions of these compounds may however influence their toxicity. As the side chain length of fatty acids is distributed around C12 (laurate), it will probably have no significant impact on the overall absorption but it also add some uncertainties as discussed below.

In conclusion, the proposed classification Repro 1B-H360D, without SCL, can therefore be supported for DOTL and the UVCB. STOT RE classification is also supported but the most appropriate category, STOT RE 1 or STOT RE 2, should be further discussed with regard to the level of uncertainties in the effective DOTL/UVCB doses.

Dossier Submitter's Response

Thank you for your comments and your support for Repr. 1B H360D and STOT RE classification. Specific response to issues raised is found below.

- *The conclusions are based on simulated gastric hydrolysis and may not reflect the complexity of the in vivo situation in which gastric enzymes may also interfere with the transformation of the substances. Is there information on in vivo metabolites formed from octyltincarboxylates or other organotincarboxylates? It would help to understand how carboxylate side chain are transformed or removed.*

We indeed agree that the *in vivo* situation are more complex than what is observed under *in vitro* conditions. We are not aware of any *in vivo* studies determining the metabolites of octyltincarboxylates. Alkyltin compounds can be dealkylated and hydroxylated, and the alkyl residues further oxidized. These reactions occur particularly in the liver and involvement of cytochrome P450 enzymes have been suggested. Still, it has been argued that dioctyltins are probably hardly metabolized and that hydroxylation and dealkylation *in vivo* of octyltin compounds is less than of butyltins (Penninks et al., 1987, Appel, K. E. 2004). However, it

is important to emphasize that these studies followed the distribution of radioactivity, i.e. the 14C label was traced and not the organotin moiety as such.

DBTC on the other hand, have been shown to be metabolised in the rat following intraperitoneal injection (4 mg/kg bw) by hydroxylation of the butyl groups (butyl(3-hydroxybutyl)tin dichloride, butyl(4-hydroxybutyl)tin dichloride) and by formation of monobutyltin trichloride (Ishizaka et al., 1989). In a microsomal metabolism study in vitro of dibutyltin (di)acetate (DBTA), formation of dibutyl and monobutyl species were seen. Hydrolysis and formation of dibutyltin and liberation of acetate was indicated in vivo in mouse after oral administration of DBTA with a proportion of non-metabolised DBTA and other dibutyltin derivatives detected in faeces (Kimmel et al., 1977).

- *It is stated in several places of the proposal « In general, dioctyltin compounds are ascribed as having immunotoxic properties via the thymus gland. The use of dioctyltin compounds is therefore restricted according to REACH (EC) No 1907/2006 Annex XVII, entry 20 in a number of consumer articles (≥ 0.1 % by weight of tin). » Data on immuno- or reproductive toxicity for other dioctyltin compounds and in particular dioctyltin with organic ligands or carboxylate ligands would provide further support for the read-across.*

In a Supplement 2009 for n-Octyltin Compounds for the MAK-Collection Part I, MAK Value Documentations 2015 the majority of studies on repeated dose toxicity, adverse effects on fertility and sexual function, and on adverse effects on the development of the offspring are done on DOTC, Mono-n-octyltin trichloride (MOTC), Di-n-octyltin-bis(2-ethylhexyl mercaptoacetate) (DOTE), Mono-n-octyltin tris (2-ethylhexyl mercaptoacetate) (MOTE), Di-n-octyltin-bis(isooctyl mercaptoacetate) (DOTI), and Mono-n-octyltin-tris(isooctyl-mercaptoacetate) (MOTI).

DOTE/MOTE and DOTI/MOTI are organotin thiolglycolates with thioester ligands and it is unclear to what extent these octyltin compounds can be taken into account for read-across purposes for dialkyltins with labile carboxylate ligands. The thioglycolates presumably have additional potentially toxic metabolites that needs to be taken into account in assessment of systemic toxicity.

In general, the MAK document notes that the lowest effects level (about 0.18 mg tin/kg bw/day) in repeated dose toxicity studies were observed in studies with DOTC and DOTI for effects on thymus (reduced relative thymus weight and lymphoid depletion). For effects on reproductive toxicity, most studies are performed with MOTI, MOTI/DOTI, DOTC and MOTC/DOTC. Common findings were increased incidence of post-implantation loss, decreased gestation index, decreased litter size, increased number of stillbirths and increased postnatal mortality. For pre-natal developmental toxicity, only studies on DOTI/MOTI were included in the MAK-document, however, there are more recent studies available for DOTE (as reported in the recent proposal for revising the existing harmonised classification) and DOTC (reported in the current CLH-report). In available studies, developmental effects included cleft palate, effects on ossification, skeletal abnormalities/variations and decreased foetal viability.

A few repeated dose toxicity studies on di-n-octyltin maleate (DOTM) are noted but they were not adequately documented and were excluded from further assessment in the MAK-document. One 3-generation reproductive toxicity study in rat of DOTM from 1968 was included that reported degeneration of testes in F0 generation, and decreased litter size and survival rate of foetuses in F1 at the highest dose tested (approx. 28.1 mg/kg bw/day).

- *For STOT RE, effects of DOTC starts from 0.7 mg/kg and STOT RE in category 1 apply below 10 mg/kg (maximal ratio of 14). Gastric hydrolysis data (ratio of 10 for production of a common metabolite on a mass basis) are in favour of a classification in category 1 but with a reduced margin to consider potential uncertainties on the in vivo situation. The most appropriate classification STOT RE 1 or STOT RE 2 should therefore be discussed with regard to the level of uncertainty in the effective DOTL doses.*

We agree that difference in molecular weight means that the mass proportion of DOTL or the stannane, dioctyl-, bis(coco acyloxy) derivs generated by hydrolysis will vary; this may be reflected in toxic potency differences compared to the source substance DOTC. Moreover, we agree that the in vivo situation is unknown. Therefore, we are happy to get this issue discussed by RAC, however, we also would like to emphasize that STOT RE 1 classification is based on potency and not on strength of evidence. Although, of course expert judgement can be applied in the total weight of evidence assessment and in applying the guidance values for category 1.

- *Finally, based on coconut fatty acids composition, only half of the UVCB composition is expected to be DOTL. The other half is composed of octyltins with other carboxylate ligands of longer or shorter chains. It is expected that the hydrolysis or metabolism scheme of the carboxylate bound will be similar to DOTL and produce similar metabolites when both carboxylate ligands are removed. Slower or quicker absorptions of these compounds may however influence their toxicity. As the side chain length of fatty acids is distributed around C12 (laurate), it will probably have no significant impact on the overall absorption but it also add some uncertainties as discussed below.*

Toxicokinetic differences following the generation of a common intermediate in the gastrointestinal system are not relevant, as the toxicokinetics behaviour of DOTC, DOTL and the stannane, dioctyl-, bis(coco acyloxy) derivs will be the same, regardless of the substances dosed. Based on this, exposure of the biological targets can be assumed.

References:

Ishizaka T., Suzuki T., Saito Y. (1989). Metabolism of Dibutyltin Dichloride in Male Rats. J. Agr. Food Chem. 37(4), 1096-1101.

Kimmel EC, Fish RH, Casida JE. Bioorganotin chemistry. Metabolism of organotin compounds in microsomal monooxygenase systems and in mammals. J Agric Food Chem. 1976 Jan-Feb;25(1):1-9

RAC's response

RAC appreciates the comment and additional input from the dossier submitter. RAC also considers the information on other organotin compounds valuable to strengthen the read across proposal. The proposed read across is accepted based on the provided information in the CLH report and the additional comparison with other closely related dibutyltin compounds. The classification as STOT RE for DOTC will therefore apply to DOTL, based on the read across approach. However, applying an SCL to DOTL is not considered justified by RAC because of uncertainty about the potency of DOTL.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Belgium		MemberState	10
Comment received				
Fertility : There is no data highlighting any concern for fertility effects. Therefore, no classification is warranted. Development : BECA considers that the available data warrant a classification for DOTL as Repr. 1B – H360D. Indeed, in line with our comment for DOTC, in an OECD TG 414 study (Study report, 2014), a dose-dependent increase in the number of skeletal malformation was observed (0.8, 9.6, 21 and 43.9 % of fetuses exposed to 0, 10, 100 and 300 mg/kg diet were affected, respectively. Results were significant at the intermediate and the highest dose). Malformed fetuses were observed in more than 50 % of the intermediate dose litters and in 95 % of the litters at the highest dose. Skeletal variations numbers were increased as well and significant at the intermediate and the highest doses. In the screening test (Appel et al., 2004), an increase in the post-implantation losses was seen at the intermediate and highest doses (100 and 300 mg/kg diet). Moreover, a dose-dependent increase in the number of dead pups at PND 0 was also noted. This was also seen in the one-generation reproductive toxicity study (Tonk et al., 2011) where a significant decrease in the mean number of live pups per litter at PND4 was noted at 30 mg/kg diet (highest dose). Effects on the thymus relative and absolute weights and/or lymphoid depletion were observed in the parental and/or F1 generation of the different studies, and it seems to be a consistent effect. For all these reasons, BECA support the dossier submitter's proposal to classify the substance and his justification to warrant a cat. 1B for the development.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted. RAC agrees with the proposed classification for DOTC and thus also for DOTL based on the read across approach. However, applying an SCL to DOTL is not considered justified by RAC because of uncertainty about the potency of DOTL.				

Date	Country	Organisation	Type of Organisation	Comment number
30.10.2017	France		MemberState	11
Comment received				
See general comment				
Dossier Submitter's Response				
Thank you for your support and your comments. Please our response to comment number 9.				
RAC's response				
Please see the response to comment number 9.				

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON DIOCTYLTIN DILAURATE; [1] STANNANE, DIOCTYL-, BIS(COCO ACYLOXY) DERIVS. [2]

Date	Country	Organisation	Type of Organisation	Comment number
28.11.2017	Germany		MemberState	12
Comment received				
Classification as Repr. 1B, H360D based on read across is supported.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Luxembourg	<confidential>	Company-Manufacturer	13
Comment received				
We Support the comment/Statement of TIB Chemicals AG.				
Dossier Submitter's Response				
Please see response to comment number 7.				
RAC's response				
Please see the response to comment number 7.				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Germany	TIB Chemicals AG	Company-Manufacturer	14
Comment received				
see attached file mentionend in general comments ECHA note – An attachment was submitted with the comment above. Refer to public attachment comment clh proposal dotl.pdf				
Dossier Submitter's Response				
Please see response to comment number 7.				
RAC's response				
Please see the response to comment number 7.				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Austria	Environment Agency Austria	Please select organisation type..	15
Comment received				
Assuming that the read across argumentations are valid (see general comment) the reproductive classification into Repr. 1B H360D based on toxicological observation with DOTC is supported.				
Dossier Submitter's Response				
Thank you for your support. Please see comment number 8 for our response to your general comment.				
RAC's response				
Noted, see also the response to comment number 8.				

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Belgium		MemberState	16
Comment received				
BECA supports the proposal of classification and agrees with the justification and conclusion given by the Swedish Chemicals Agency. Dose-dependent effects on the thymus weight (decrease) and lymphoid depletion, both detected after exposure to very low dose of substance (such as 0.5-0.7 mg/kg bw/d), were detected consistently between studies. We agree this requires a classification for STOT RE cat 1and we accept to not specify the route of exposure as well.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
30.10.2017	France		MemberState	17
Comment received				
See general comment				
Dossier Submitter's Response				
Thank you for your comments and support of STOT RE classification. Please see our response to your previous comment number 9.				
RAC's response				
Noted. Please see the response to comment number 9.				

Date	Country	Organisation	Type of Organisation	Comment number
28.11.2017	Germany		MemberState	18
Comment received				
With respect to classification as STOT RE 1 based on an oral 90-d-study a significant effect at concentrations equal to or lower than 10 mg/kg bw/day is required. The classification proposal is based on read across to the source substance DOTC. Compared to DOTC a 10-fold lower potency of DOTL is derived in the CLH-report (chapter 10.12.2, p 46/47). In consequence the LOAEL of the oral 90-d-study with DOTC (0.7 mg/kg bw/day) is converted into a presumed LOAEL of 7 mg/kg bw/day (10 x 0.7 mg/kg bw/day) for DOTL. This concentration is below the dose of 10 mg/kg bw/day and thus fulfils the conditions relevant for STOT RE 1 classification. However the effects at this dose were limited to a reduced thymus weight of 14% only in females. It would be helpful to include in chapter 10.12.2 a more detailed description which effects occurred in the several studies below the respective concentration relevant for classification.				
Dossier Submitter's Response				
Thank you for comments. Effects relevant for classification for STOT RE 1 classification with thymus/immune system as target organ at dose levels equal to or lower than the guidance value of 10 mg/kg bw/day (90-day, oral, rat) or equal to or lower than the guidance value of 30 mg/kg bw/day (28-day, oral, rat):				

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

- Decreased absolute and relative thymus weights in males in all treated groups in a dose-response manner, statistically significant at **4.2-6.2 mg/kg bw/day** (-47/-48%) and at **8.4-17 mg/kg bw/day** (-75/-73%) compared to control.
- Increased incidence of lymphoid depletion at **4.2-6.2 mg/kg bw/day** (5/10 males, severity score slight to moderate) and at **8.4-17 mg/kg bw/day** (9/10 males, severity score, moderate to severe).
- Decreased absolute thymus weight in females all treated groups in a dose-dependent manner: -14% ($p<0.05$), -68%, ($p<0.01$), -73% ($p<0.01$) at 0.7, **6.5-6.8**, and 19.3-19.8 mg/kg bw/day respectively compared to control.
- Decreased relative thymus weight in females in all treated groups in a dose-dependent manner: -14% $p<0.05$), -69% ($p<0.01$), -70% ($p<0.01$) at 0.7, **6.5-6.8**, and 19.3-19.8 mg/kg bw/day respectively compared to control.
- Increased incidence of lymphoid depletion in females (severity score was slight to very severe) at **6.5-6.8 mg/kg bw/day** (10/10 females) and at 19.3-19.8 mg/kg bw/day (9/10 females).
- Decreased absolute and relative thymus weight in pregnant females in all treated groups in a dose-dependent manner (-23/-24%, -38/-33% and -69/-62% at 0.5-0.7, **4.2-6.2, and 8.4-17 mg/kg bw/day** respectively compared to control), but only stat. sign. at 4.2-6.2 and 8.4-17 mg/kg bw/day.
- Increased incidence of lymphoid depletion (severity score was severe to very severe) in pregnant females in all groups: 5/10, 10/10 and 10/10 at **0.5-0.7, 4.2-6.2, and 8.4-17 mg/kg bw/day** respectively.

Repeated dose 14-day oral toxicity study in young male rats (Penninks & Seinen, 1982):

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

Repeated dose 6-week oral toxicity study in male and female rats, 4-week study in male rats, and a time-response study up to 28 days in female rats (Seinen and Willems, 1976):

- Thymic atrophy and lymphocyte depletion at 50 ppm (estimated to be **6 mg/kg/day**) and at 150 ppm DOTC in the diet (estimated to be **18 mg/kg bw/day**). All DOTC-fed animals showed atrophy of the thymus. At 150 ppm, the cortex was almost completely depleted of lymphocytes. At 50 ppm DOTC in the diet, lymphocytes depletion of the thymus was less pronounced.
- Decreased thymus weight: -51/-67% and -73%/-75% at 50 and 150 ppm respectively, in males/females.
- Total thymocyte counts diminished to 33 and 6 % of the control value at week 4 in animals fed 50 or 150 ppm DOTC in the diet, respectively.
- Thymus cell viability was significantly decreased at day 14 in the 150 ppm group ($p < 0.05$) and at day 28 at both 50 and 150 ppm ($p < 0.001$).

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

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

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

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

In conclusion, the effects observed on the immune system including thymus atrophy with lymphoid depletion were clearly dose related and were observed at dose levels starting from 0.5-0.7 mg/kg bw/day, and adverse at dose levels below the guidance value 10 mg/kg bw/day.

RAC's response

Thank you for the request, and for provided summary on the observed effects of DOTC below the classification criteria for STOT RE. This is helpful and a modified version of this overview is included in the "Dossier submitter's summary" in the RAC opinion. RAC considers the effects below 1 mg/kg bw/d sufficient for classification because of the dose-response observed at higher dose levels.

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Luxembourg	<confidential>	Company-Manufacturer	19
Comment received				
We Support the comment/Statement of TIB Chemicals AG.				
Dossier Submitter's Response				
Please see response to comment number 7.				
RAC's response				
Please see response to comment number 7.				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Germany	TIB Chemicals AG	Company-Manufacturer	20
Comment received				
see attached file mentionend in general comments ECHA note – An attachment was submitted with the comment above. Refer to public attachment comment clh proposal dotl.pdf				
Dossier Submitter's Response				
Please see response to comment number 7.				
RAC's response				
Please see response to comment number 7.				

Date	Country	Organisation	Type of Organisation	Comment number
01.12.2017	Austria	Environment Agency Austria	Please select organisation type..	21
Comment received				
Assuming that the read across argumentations are valid (see general comment) the reproductive classification into STOT RE1 (thymus/immune system) based on toxicological observation with DOTC is supported.				

Dossier Submitter's Response
Thank you for your support. Please see comment number 8 for our response to your general comment.
RAC's response
Noted, see also the response to comment number 8.

PUBLIC ATTACHMENTS
1. comment clh proposal dotl.pdf [Please refer to comment No. 7, 14, 20]