Committee for Risk Assessment

RAC

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

14 September 2018

CLH-O-0000001412-86-223/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name:	**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]**

The proposal was submitted by **Sweden** and received by RAC on **14 August 2017.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Sweden has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **17 October 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **1 December 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Betty Hakkert**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **14 September 2018** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitter's proposal	050-RST-VW-Y	dioctyltin dilaurate; [1] stannane, dioctyl-, bis(coco acyloxy) derivs. [2]	222-883-3 [1] 293-901-5 [2]	3648-18-8 [1] 91648-39-4 [2]	Repr. 1B STOT RE 1	H360D H372 (immune system)	GHS08 Dgr	H360D H372 (immune system)			
RAC opinion	050-RST-VW-Y	dioctyltin dilaurate; [1] stannane, dioctyl-, bis(coco acyloxy) derivs. [2]	222-883-3 [1] 293-901-5 [2]	3648-18-8 [1] 91648-39-4 [2]	Repr. 1B STOT RE 1	H360D H372 (immune system)	GHS08 Dgr	H360D H372 (immune system)			
Resulting Annex VI entry if agreed by COM	050-RST-VW-Y	dioctyltin dilaurate; [1] stannane, dioctyl-, bis(coco acyloxy) derivs. [2]	222-883-3 [1] 293-901-5 [2]	3648-18-8 [1] 91648-39-4 [2]	Repr. 1B STOT RE 1	H360D H372 (immune system)	GHS08 Dgr	H360D H372 (immune system)			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Dioctyltin dilaurate, further referred to as DOTL in this document, is an organotin compound with two octyl chains and two laurate (C12) groups. The CLH proposal for DOTL embraces both the mono constituent substance (EC no. 222-583-2) and the UVCB substance (EC no. 293-901-5). According to the REACH lead registrant, the substance currently on the European market is the UVCB substance, although registered under EC no. 222-583-2 (October 2016). Dioctyltin substances may contain small amounts of monooctyltin and trioctyltin compounds as impurities. Although impurities are not defined for UVCB substances, the substances included in the current group entry are expected to have the same mono-/di-/tri-octyl ratios determined by the dioctyltin source. According to the dossier submitter (DS), the mono-/di-/tri-octyl ratios are not expected to affect the toxicity profile for the endpoints of interest and are not relevant for classification of the substances.

Other organotin compounds previously assessed by RAC include dibutyltin dilaurate and dibutylbis(pentane-2,4-dionate-O,O)tin that contain shorter alkyl side chains. The RAC opinions on these compounds concluded on classification as, amongst others, STOT RE 1 (immune system), Repr. 1B; H360FD and acute toxicity via inhalation. One other di-octyl tin compound previously assessed by RAC, dioctyltin bis(2-Ethylhexylmercaptoacetate), was classified as Repr. 1B; H360D, which was now also proposed for DOTL.

RAC evaluation on the proposed read across approach

Summary of the dossier submitter's proposal on the read across approach

Because no data is available on DOTL for the endpoints of interest (STOT RE, reproductive toxicity), a read across approach from dioctyltin dichloride (DOTC, EC 222-583-2) to DOTL was proposed based on hydrolytic and toxicokinetic behaviour, in accordance with the ECHA guidance document *Guidance on information requirements and chemical safety assessment, Chapter R.6: QSARs and grouping of chemicals (ECHA, 2008)*.

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

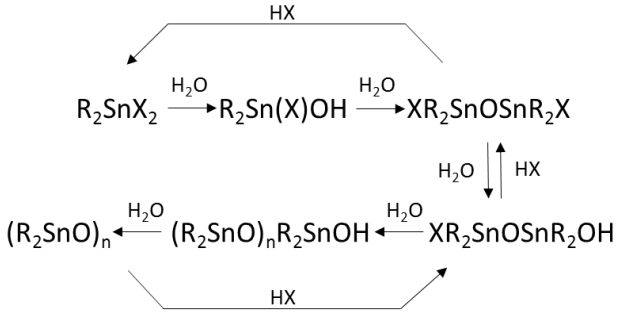


Figure: Hydrolysis scheme for dialkyltins (Davies, 2004; Aylett *et al.*, 1979)

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

- The hydrolytic behaviour at neutral and low pH supports the rapid formation of common intermediate(s). Subsequently, due to rapid hydrolysis at low pH there will be no systemic exposure to DOTL when administered orally.
- Hydrolysis studies at low pH for DOTC and DOTL show partial formation of the same species. The chemistry at low pH for DOTL is more complex compared to DOTC due to the binding nature of the carboxylate ligands, but the observed behaviour is in accordance with established chemistry for dialkyltins under aqueous conditions.
- In general, dioctyltin compounds are considered to have adverse effects on the immune system after repeated exposure. Acute toxicity data demonstrate that both the target substance and the source substance are of moderate toxicity.

Comments received during public consultation

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

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

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

The other MSCA noted that transformation *in vivo* may be more complex due to (for example) the presence of enzymes. Further, if the common metabolite would be responsible for the observed

effects, it may not fall within the criteria for STOT RE 1 as the potency is then expected to be lower for DOTL. This MSCA also suggested taking into account other dioctyltin compounds with organic/carboxylate ligands that show similar effects.

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

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

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

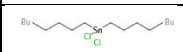
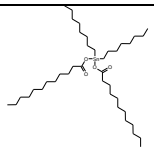
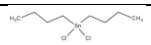
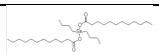
Assessment and comparison with the classification criteria

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

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

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

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

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

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

Additionally, effects on the immune system were also observed after exposure to other dioctyltin compounds with labile- or thioester ligands, as mentioned during the public consultation. All of these organotin compounds cause very similar (systemic) adverse effects. When there is

substance specific information available and/or a harmonised classification, these compounds all adversely affect the immune system and most of them also affect the reproductive system (similar effects include amongst others post-implantation loss, reduced postnatal viability, and skeletal effects).

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

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

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

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of reproductive toxicity

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

Summary of the Dossier Submitter's proposal

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

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

Study	Dosing	Results
Appel and Waalkens-Berendsen, 2004 OECD TG 421 (Combined reproductive screening test) GLP Wistar rats 10/sex/dose in main 13-week sub-chronic toxicity study 10 females/dose in satellite reproductive screening study	DOTC, 92.1% pure 0, 10, 100, 300 mg/kg diet/d (corresponding to approx. 0, 0.5-0.7, 4.2-6.2 and 8.4-17 mg/kg bw/d respectively). Main study animals were fed for 13 weeks daily. Females from the satellite groups were fed for 2 weeks premating, and continued until shortly after PND4. Main study males were mated with female from the satellite groups after 10 weeks premating.	F0 at 300 mg/kg diet unless otherwise stated: Gestation: females: ↓ bw (not corrected, -16% on GD21). Lactation: females: ↓ bw (-20% on PND4). Food consumption: females: ↓ (-18 to -68% and -10 to -15% at 100 mg/kg diet; -11% during GD7-14). Organs: ↓ absolute relative thymus weight (males: -73 to -75% and -47 to -48% at 100 mg/kg diet, females: -62 to -69% and -33 to -38% at 100 mg/kg diet and non-stat sign -23 to -24% at 10 mg/kg diet). ↑ Lymphoid depl, (males: 9/9 (moderate-severe) and 5/10 at 100 mg/kg diet (slight-moderate), females: 10/10 (severe-very severe in all groups) and 10/10 at 100 mg/kg diet and 5/10 at 10 mg/kg diet). No effects on fertility indices. Males: Stat. sign. changes in absolute/relative weight of spleen, kidney, liver and testes at highest dose. **Reproductive toxicity:** Strongly decreased (but not stat. sign. at 100/300 mg/kg diet): ↓ gestation index (71%/50% vs 86% in control), ↑ mean post-implantation loss (of 49%/70% vs 22% in control). ↓ live birth index (53%/60% vs 99% in control). Stat. sign. effects: ↓viability index PND 0-4 (74/12% vs 94% in control). F1: Foetal weight at PND1, (3.9 at 300 mg/kg diet vs 4.76 g in control). ↑ no. of runts (weight below 2 std. deviation vs. mean weight, at 10, 100 and 300 mg/kg diet: 7, 10 and 6, respectively vs. 1 in control). ↑ no. of cold pups at 300 mg/kg diet.
Tonk *et al*., 2011 OECD TG 443 – EOGRTS without cohorts 2/3 and extension of 1B. GLP unknown Wistar rats 24 females/group (20 in high dose group) Litters not standardised and pups weaned at PND21. Sexual maturation evaluated for 1 pup/litter, 8 F1 males/group for immune assessment	DOTC, purity unknown. 0, 3, 10, 30 mg/kg in diet (corresponding to F0 females: 0.17-0.21, 0.56-0.71 and 1.7-2.1 mg/kg bw/d during gestation and 0.27-0.55, 1.0-1.9, 2.9-5.2 mg/kg bw/d during lactation).	F0 females: ↓ bw (5%) during lactation at 10/30 mg/kg diet. No effects on fertility indices. No information on organ weights and histopathology of F0. **Development:** F1: At high dose only: ↓ mean no. of live pups/litter at PND4 (8.78 vs 10.48 in control). ↓absolute (-22%) & ↓ relative (-20%) thymus weight, ↓ thymus cellularity (-36% on PND42). Spleen at PND 42 (high dose only): ↓ absolute and relative No. of CD3+, CD3+CD4+ and CD3+CD8+ cells. ↓ T:B cell ratio. At PND70, CD3+CD4+ no longer stat. sign. reduced. Thymus at PND42 (high dose only): ↓ absolute no. CD4-CD8+, CD4+CD8+, immature (CD3low) and mature (CD3high) thymocytes. Not stat. sign. anymore at PND70. Delayed-type hypersensitivity (DTH): The DTH response at PND49 was stat. sign. ↑ at low/high dose (37% and 52%) and non-stat. sign. ↑ at mid dose. LOAEL: 30 mg/kg diet/d for developmental effects, NOAEL for F0 is 30 mg/kg diet/d in diet.
Study Report 2014 OECD TG 414 prenatal development toxicity study	DOTC, purity 97.7% 0, 10,100, 300 mg/kg diet from GD5-GD19	F0: ↓ bw on GD 20 (not corrected, -30% at high dose). ↓ bw gain on GD5-20 at mid- (-12%) & high dose (-31%). Organs: ↓ thymus size (7/25 mid dose, all at high dose), no details available.

GLP Sprague Dawley rats 25 mated females/group	Actual dose: 0, 0.8 ± 0.1, 7.2 ± 1.0, 22.4 ± 4.2 mg/kg bw/d	**Development (F1):** ↑ Pre-implantation loss at mid (7%) and high dose (10.4%) vs. control (1.5%). ↑ Post-implantation loss at low (6.8%), mid (4.9%) and high dose (6.9%) vs. control (0.8%). ↑ Skeletal malformations, predominantly missing bones in paws at mid (22) and high dose (47) vs. control (1). Increase also at low dose (11) but not stat. sign. ↑ Skeletal variations (predominantly poor ossification) at high dose (26 vs. 6 in control). Incidences at low/mid dose were 10/11 and not stat. sign. LOAEL for both maternal and developmental effects considered by the registrants to be 100 mg/kg diet or 7.2 mg/kg bw/d.

PND=postnatal day

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

Adverse effects on development were observed in the prenatal developmental study and the combined reproductive screening study. Maternal toxicity in the form of lower body weight and effects on the immune system (thymus) were noted. However, the DS argued that the lower maternal body weight is limited and there is no established link between the effects on the maternal thymus and developmental toxicity. Therefore, the developmental effects should be regarded as relevant.

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

Comments received during public consultation (DOTC)

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

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

In reply, the DS argued that the reported malformations cannot be interpreted in another way. The malformations are, according to the study authors, associated with delayed foetal ossification. The DS believed that this means that in addition to the missing bones, increased incidences of poor or incomplete ossification of sternum no. 5 and 6 (statistically significantly different

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

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

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

Comments received during public consultation (DOTL)

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

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

Assessment and comparison with the classification criteria

Fertility

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

Development

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

concluded that based on the information available, no direct relationship between the effects on the maternal thymus and effects on development can be established.

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

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

Test substance intake	**0 ± 0.0 mg/kg bw/d**	**0.8 ± 0.1 mg/kg bw/d**	**7.2 ± 1.0 mg/kg bw/d**	**22.4 ± 4.2 mg/kg bw/d**
Foetal data				
Malformations (total) Foetal basis, no. (%) Litter basis, no. (%)	 1 (0.8) 1 (4.5)	 11 (9.6) 8 (38.0)	 22** (21.0) 11 (55.0)	 47*** (43.9) 19 (95.0)
Metacarpal no. 5 bilateral Foetal basis, no. (%) Litter basis, no. (%)	 1 (0.8) 1 (4.5)	 3 (2.6) 3 (14.3)	 12 (11.4*) 6 (30.0)	 37 (34.6*) 18 (90.0)
Proximal phalanx no. 3 bilateral Foetal basis, no. (%) Litter basis, no. (%)	 1 (0.8) 1 (4.5)	 9 (7.8) 7 (35.0)	 15 (14.3 *) 10 (50.0)	 29 (28.0*) 16 (80.0)
Proximal phalanx no.4 bilateral Foetal basis, no. (%) Litter basis, no. (%)	 1 (0.8) 1 (4.5)	 8 (7.0) 6 (28.6)	 15 (13.3*) 9 (45.0)	 29 (27.1*) 16 (80.0)
Variations (total) Foetal basis, no. (%) Litter basis, no. (%)	 6 (4.5) 5 (22.7)	 11 (9.6) 7 (33.3)	 10 (9.5) 4 (20.0)	 26* (24.3) 12 (60.0)

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

In the combined reproductive screening test by Appel and Waalkens-Berendsen (2004), a non-statistically significant, but high incidence of post-implantation loss was observed (~50% and 70% in the mid and high dose groups, respectively; results summarised in the table below). The lack of statistical significance is likely due to high variation in some animals and a single dam in the control group with only implantation sites, resulting in a high control incidence of post-implantation loss (23%). As noted by the DS, the median values rather than the mean reflect the actual data better because of the high variation in some animals. The median post-implantation loss was 7%, 11%, 50% and 95% in the control, low, mid and high doses, respectively, and thus indicates a dose-response relationship. The post-implantation loss was accompanied by a statistically significant decrease in live birth index (53% and 60% in mid and high dose groups compared to 99% in the control), followed by a reduction in postnatal viability (PND1-PND4) in the mid- and high dose groups of -22% and -87%, respectively. The pup weight was statistically significantly lower at PND1 in the high dose group (3.9 g vs 4.76 g in control), the number of runts was increased in a non-dose-dependent manner in all dose groups and the number of cold pups was increased in the high dose group (amount not mentioned in the CLH report).

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

Dose level	**Control**	**10 mg/kg diet**	**100 mg/kg diet**	**300 mg/kg diet**
Test substance intake	0 mg/kg bw/d	0.5-0.7 mg/kg bw/d	4.2-6.2 mg/kg bw/d	8.4-17 mg/kg bw/d
Number of pregnant females	7	8	7	8
Mean number of implantations	12.6	13.4	11.3	10.3
Number of dams with only implantation sites observed at necropsy	1	0	0	3
Post-implantation loss (%) Mean value Median value	 22.33 ± 13.16 7	 20.98 ± 7.11 11	 49.23 ± 17.45 50	 69.99 ± 14.71 95[£]
Pups delivered (total) (N)	70	88	72	43
Pups delivered (live + dead mean) [N= number of litters]	11.67 ± 0.80 N=6	11.00 ± 0.71 N=8	10.29 ± 052 N=7	8.60 ± 1.21 N=5
Mean viable litter size PND 1 [N= number of litters]	11.50 ± 0.72 N=6	10.50 ± 0.95 N=8	7.60 ± 1.63 N=5	6.50 ± 2.22 N=4
Total no. of live born pups[f] (Live birth index)	69 (99)	84 (95)	38[#] (53)	26[#] (60)

Total no. of stillborn pups[f] (% stillborn)	1 1.4	4 4.5	34[#] 47	17[#] 40
Total number of dead pups PND 0 to PND 4[f]	4	7	10[**]	23[#]
Total number of pups dying perinatally	5	11	44	40
Mean viability index PND 1-4	94	92	74	12
Mean viable litter size PND 4 [N= number of litters]	10.83 ± 0.60 N=6	11.00 ± 0.79 N=7	9.33 ± 0.67 N=3	3.00 ± 0.00 N=1
Pup weight (g) PND 1 (all viable pups)	4.76 ± 0.23	4.74 ± 0.23	4.19 ± 0.35 (-12%)	3.90 ± 0.09 (-18%)
Pup weight gain (g) PND 1 to PND 4	2.17 ± 0.26	1.86 ± 0.38	1.41 ± 0.58	-0.57 ± 0.00
Total number of runts ǂ [N= number of litters]	1 N=1	7 N=3	10 N=3	6 N=1

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

Maternal toxicity was observed in the form of lower body and thymus weight compared to the controls. The maternal body weight was 16% lower at GD21 and 20% lower at PND4 in the high dose group compared to the control. No corrected body weights were provided in the report. However, RAC notes that the lower body weights in the high dose groups were at least in part due to the high post-implantation losses and the reduced pups/foetal weights. Moreover, no significant lower maternal body weight was observed at the mid dose group while the median post-implantation loss and decreased live birth index were already statistically significantly increased at this dose level. RAC concludes that the effects seen in the mid and high doses are not secondary to effects on maternal body weight or body weight gain.

Thymus weights of parental animals were significantly lower compared to the control animals and were accompanied by significant lymphoid depletion in both sexes. During the lactation period, one female in the control group, three females in the intermediate dose group and two females in the high dose group also displayed other treatment related clinical effects: thin, pale appearance, piloerection and/or blepharospasm. For the majority of these dams, there was no correlation between onset of clinical signs and intrauterine or postnatal death of pups.

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

The third study summarised by the DS was an EOGRTS similar to OECD TG 443 (Tonk *et al*., 2011). However, the animals were dosed at low concentrations that resulted in limited post-implantation loss (non-significant increase) and postnatal viability (small but significant increase). It is to be

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

Comparison with the criteria

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

These adverse effects are:

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

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

Lactation

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

Conclusion

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

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

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

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

Summary of the Dossier Submitter's proposal

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

In the combined developmental and sub-chronic toxicity study by Appel and Waalkens-Berendsen (2004), treatment related changes were seen including decreased haemoglobin, packed cell volume, total white blood cells, absolute number of lymphocytes and an increase in prothrombin time that was considered biologically relevant at the highest dose (8.4-17 mg/kg bw/d). A dose-dependent decrease in absolute and relative thymus weights (-14% to -73%) were observed at all dose levels, being statistically significant in the mid- and high-dose levels. These effects were correlated with histopathological findings, including a high incidence of lymphoid depletion (mid/high dose groups) and decreased size of thymic lobules resembling thymus atrophy as described in the literature for organotin compounds. A non-statistically significant lymphoid depletion was also observed at the low dose in females, indicating a toxicologically relevant treatment related effect. Similarly, 7/25 dams from the developmental toxicity study in the 7.2 and 22 mg/kg bw/d dose groups had reduced thymus sizes.

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

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

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

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

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

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

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

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

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

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

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

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

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

The DS concluded that adverse effects on the immune system, predominantly lymphoid depletion and reduced thymus sizes, were seen with DOTC at dose levels well below the guidance values for STOT RE 1, supporting classification in STOT RE 1 for DOTL. Regarding possible potency differences, the DS noted that adverse effects are already observed at dose levels around 0.5-0.7 mg/kg bw/d of DOTC in the 90-d sub-chronic (combined) toxicity study. Based on 20% absorption after oral administration, it may be assumed that almost 20% of the distannoxane dimer is absorbed since this is the dominant (90%) hydrolysis product indicated by gastric simulation. This same dimer is formed at a smaller fraction of around 15% (and thus ~6x lower) after hydrolysis at acidic pH of DOTL. If, hypothetically, this would be the only toxic tin moiety that is bioavailable for DOTL, an almost 10-fold higher dose (corrected for differences in molecular weight, 743 g/mol for DOTL and 416 g/mol for DOTC) is required to obtain the same adverse effects as for DOTC. This would still be below the cut-off criteria for STOT RE 1 (<10 mg/kg bw/d).

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

Comments received during public consultation

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

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

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

Assessment and comparison with the classification criteria

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

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

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

the same classification. RAC considers the information as insufficient for derivation of an SCL for DOTL.

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

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).