Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

dichlorodioctylstannane

EC Number: 222-583-2

CAS Number: 3542-36-7

CLH-O-0000001412-86-230/F

Adopted

14 September 2018

Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Finland | Tel. +358 9 686180 | Fax +358 9 68618210 | echa.europa.eu

14 September 2018

CLH-O-0000001412-86-230/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: **dichlorodioctylstannane**

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

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

The proposal was submitted by **Sweden** and received by RAC on **6 July 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 **13/09/2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **30 October 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	050-021-00-4	dichlorodioctylstannane	222-583-2	3542-36-7	Acute Tox. 3 * STOT RE 1 Aquatic Chronic 3	H331 H372** H412	GHS06 GHS08 Dgr	H331 H372** H412			
Dossier submitters proposal	050-021-00-4	dichlorodioctylstannane	222-583-2	3542-36-7	**Retain** STOT RE 1 Aquatic Chronic 3 **Modify** Acute Tox. 2 **Add** Repr. 1B	**Retain** H372** H412 **Modify** H330 **Add** H360D	**Retain** GHS06 GHS08 Dgr	**Retain** H372** H412 **Modify** H330 **Add** H360D		Repr. 1B; H360D: C ≥ 0.03 %	
RAC opinion	050-021-00-4	dichlorodioctylstannane	222-583-2	3542-36-7	**Retain** STOT RE 1 Aquatic Chronic 3 **Modify** Acute Tox. 2 **Add** Repr. 1B	**Retain** H372** H412 **Modify** H330 **Add** H360D	**Retain** GHS06 GHS08 Dgr	**Retain** H372** H412 **Modify** H330 **Add** H360D		Inhalation: ATE = 0,098 mg/L (dust and mist) Repr. 1B; H360D: C ≥ 0.03 %	
Resulting Annex VI entry if agreed by COM	050-021-00-4	dichlorodioctylstannane	222-583-2	3542-36-7	Repr. 1B Acute Tox. 2 STOT RE 1 Aquatic Chronic 3	H360D H330 H372** H412	GHS08 GHS06 Dgr	H360D H330 H372** H412		Repr. 1B; H360D: C ≥ 0.03 % inhalation: ATE = 0.098 mg/L	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Dichlorodioctylstannane, further referred to as DOTC in this document, is an organotin compound with two octyl chains and two chlorine groups. Other organotin compounds previously assessed by RAC include dibutyltin dilaurate and dibutylbis(pentane-2,4-dionate-*O*,*O*)tin that contain shorter alkyl side chains. The RAC opinions on these compounds were in favour of amongst others, STOT RE 1 (immune system) and Repr. 1B; H360FD. One other dioctyltin compound previously assessed by RAC, dioctyltin bis(2-ethylhexylmercaptoacetate) was classified as Repr. 1B; H360D, which is also proposed for DOTC.

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

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute inhalation toxicity

Summary of the Dossier Submitter's proposal

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

Study 1 (Ciba-Geigy Ltd., 1976)

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

Study 2 (Hazelton Laboratories America, Inc. 1976)

Ten male SD rats/dose were exposed to DOTC in polyethylene glycol as aerosols at dose levels of 0, 0.11, 0.29, 0.44, 0.83 and 0.91 mg/L for 1 h. The nebulizer used was considered to deliver

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

Study 3 (Wells Laboratories, Inc. 1976)

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

The first of these three studies were reported by the registrants and SIDS to have a Klimisch reliability score of 2, while the third study had a lower score with the rationale that details of toxic effects were not reported other than lethal dose values in the SIDS Initial Assessment Report for SIAM 23 (2006). Several reporting deficiencies in the three available studies were noted by the DS, including lack of data on body weight, individual clinical signs, gross pathological findings, composition of the test substance and purity, and limited data on particle size distribution. Furthermore, all three studies predated GLP.

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

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

The LC_{50} values from the second study were extrapolated from 1 h to 4 h exposure duration, while the LC_{50} reported in the first study was based on an actual 4 h exposure period resulting in an LC_{50} (0.439 mg/L) just below the criteria cut-off for category 2. The general ATE for Acute Tox. 2 is the same as the lower bound of the criteria (0.05 mg/L) according to Table 3.1.2 of the CLP regulation. The lowest LC_{50} reported was 0.0975 mg/L in the second study with a particle

size distribution (MMAD of 2.5-3.5 µm) that is within the recommended range of 1-4 µm for acute inhalation studies. Therefore, RAC considers this LC_{50} appropriate for ATE derivation. RAC notes that only male rats were used in this study, but the two other studies do indicate that male rats are equally or more sensitive as compared to females. The use of the LC_{50} calculated from a study with only males is therefore justified for derivation of an ATE.

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

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

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

***Table**. Summary of reproductive toxicity studies*

Study	Dosing	Results
Appel and Waalkens-Berendsen, 2004 OECD TG 421 (Combined reproductive screening test) GLP Wistar rats 10/sex/dose in main 13-week sub-chronic toxicity study 10 females/dose in satellite reproductive screening study	DOTC, 92.1 % pure 0, 10, 100, 300 mg/kg diet/d (corresponding to approx. 0, 0.5-0.7, 4.2-6.2 and 8.4-17 mg/kg bw/d respectively). Main study animals were fed for 13 weeks daily. Females from the satellite groups were fed for 2 weeks premating, and continued until shortly after PND4. Main study males were mated with female from the satellite groups	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

Study	Dosing	Results
	after 10 weeks premating.	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 GLP Sprague Dawley rats 25 mated females/group	DOTC, purity 97.7 % 0, 10,100, 300 mg/kg diet from GD5-GD19 Actual dose: 0, 0.8 ± 0.1, 7.2 ± 1.0, 22.4 ± 4.2 mg/kg bw/d	F0: ↓ bw on GD 20 (not corrected, -30 % at high dose). ↓ bw gain on GD5-20 at mid- (-12 %) & high dose (-31 %). Organs: ↓ thymus size (7/25 mid dose, all at high dose), no details available. **Development (F1):** ↑ Pre-implantation loss at mid (7 %) and high dose (10.4 %) vs. control (1.5 %). ↑ Post-implantation loss at low (6.8 %), mid (4.9 %) and high dose (6.9 %) vs. control (0.8 %). ↑ Skeletal malformations, predominantly missing bones in paws at mid (22) and high dose (47) vs. control (1). Increase also at low dose (11) but not stat. sign. ↑ Skeletal variations (predominantly poor ossification) at high dose (26 vs. 6 in control). Incidences at low/mid dose were 10/11 and not stat. sign. LOAEL for both maternal and developmental effects considered by the registrants to be 100 mg/kg diet or 7.2 mg/kg bw/d.

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

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

Based on skeletal malformations (missing bones, considered as rare findings) in the OECD TG 414 study, decreased live birth index along with increased number of stillborn pups at 7.2 and 22.4 mg/kg bw/day and increased post-implantation loss seen in multiple studies, the DS concluded that classification as Repr. 1B; H360D was warranted. The DS further proposed to add an SCL of 0.03 mg/kg bw/day since a 10 % increase in the incidence (ED_{10}) of total skeletal malformations was caused by about 0.8 mg/kg bw/day of test substance meeting the criteria for the high potency group ($ED_{10} \leq 4$ mg/kg bw/day) as outlined in the CLP guidance.

Comments received during public consultation

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

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

The DS replied that the authors and registrant(s) had categorized the findings as "malformations" and these could not be interpreted in another way as they did not have the raw data for review. According to the study authors, the malformations were associated with delayed foetal ossification. The DS interpreted this statement as that in addition to the missing bones, increased incidences of poor or incomplete ossification of sternum no. 5 and 6 (statistically significantly different in high-dose group compared to control) and metacarpal no. 5 in low, intermediate and high-dose groups were also evident. Furthermore, poor or incomplete ossification of proximal phalanx no. 3 and 4 were seen in all dose groups including the control group but they were not dose-dependent or statistically significant and the study authors therefore considered that these effects were not treatment-related. The DS further clarified that based on the cited text below from the report, it was interpreted that double staining was used and malformations like missing bones or variations such as delayed ossifications should have been picked up and reported:

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

The incomplete ossification of the same structures as the missing ones (proximal phalanx no. 3 and 4, metacarpal no. 5) were reported separately, therefore confirming that the staining

technique distinguishes between incomplete ossification and missing bone correctly and the malformations should be interpreted accordingly.

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

Assessment and comparison with the classification criteria

Fertility

Two reproduction studies were available, one reproduction screening study with doses up to 8.4-17 mg/kg bw/day and an EOGRT study using very low doses (up to 1.7-2.1 mg/kg bw/day). In neither of these studies, were effects observed that would support classification for fertility. However, in the EOGRT study no effects were seen in parental animals and therefore, adverse effects on fertility at higher concentrations cannot be excluded. The EOGRT study was primarily conducted to assess developmental immunotoxicity. In addition, a reproduction screening study cannot be used to exclude effects on fertility, amongst others due to the limited endpoints and power of the experimental design. **As a consequence, RAC proposes not to classify DOTC for adverse effects on sexual function and fertility because there is a lack of relevant data.**

Development

In the single pre-natal development study available (2014) performed with SD rats, skeletal malformations were seen in the form of missing bones predominantly at metacarpal no. 5 and proximal phalange no. 3, in the forepaws of foetuses. The most important adverse effects are summarized in the table below. The malformations at metacarpal no. 5, proximal phalange no. 3 and no. 4 were all statistically significantly increased at the mid and high doses in a dose-dependent manner. Skeletal variations in the form of poor or incomplete ossification of sternum no. 5, 6 and metacarpal no. 5 were significantly increased in the high dose group. Additionally, poor and incomplete ossification was also observed in the proximal phalange no. 3 and no. 4 (not shown in table below), although not in a dose-dependent way. As suggested by the DS, RAC considers it possible that these skeletal variations may be milder forms of the malformations (missing bones) in the same position.

***Table**. Results summary of the OECD TG 414 Pre-natal development toxicity study (2014)*

Test substance intake	0 ± 0.0 mg/kg bw/d	0.8 ± 0.1 mg/kg bw/d	7.2 ± 1.0 mg/kg bw/d	22.4 ± 4.2 mg/kg bw/d
Foetal data				
Malformations (total)				
Foetal basis, no. (%)	1 (0.8)	11 (9.6)	22** (21.0)	47*** (43.9)
Litter basis, no. (%)	1 (4.5)	8 (38.0)	11 (55.0)	19 (95.0)
Metacarpal no. 5 bilateral				
Foetal basis, no. (%)	1 (0.8)	3 (2.6)	12 (11.4*)	37 (34.6*)
Litter basis, no. (%)	1 (4.5)	3 (14.3)	6 (30.0)	18 (90.0)
Proximal phalanx no. 3 bilateral				
Foetal basis, no. (%)	1 (0.8)	9 (7.8)	15 (14.3*)	29 (28.0*)

Litter basis, no. (%)	1 (4.5)	7 (35.0)	10 (50.0)	16 (80.0)
Proximal phalanx no.4 bilateral				
Foetal basis, no. (%)	1 (0.8)	8 (7.0)	15 (13.3*)	29 (27.1*)
Litter basis, no. (%)	1 (4.5)	6 (28.6)	9 (45.0)	16 (80.0)
Variations (total)				
Foetal basis, no. (%)	6 (4.5)	11 (9.6)	10 (9.5)	26* (24.3)
Litter basis, no. (%)	5 (22.7)	7 (33.3)	4 (20.0)	12 (60.0)

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

In the repeated dose 90-day oral toxicity study (OECD TG 408) combined with a reproduction/developmental screening test (OECD TG 421) (2004), a statistically non-significant, but high incidence of post-implantation loss was observed (50 % and 70 % in the mid and high dose groups, respectively; results summarized in table below). The lack of statistical significance is likely due to high variation in some animals and a single dam in the control group with only implantation sites, resulting in a high control incidence of post-implantation loss (23 %). As noted by the DS, the median values rather than the mean reflect the actual data better because of the high variation in some animals. The median post-implantation loss was 7, 11, 50 and 95 % in the control, low, mid and high dose, respectively, and thus indicates a dose-response relationship. The post-implantation loss was accompanied by a statistically significant decrease in live birth index (53 and 60 % in mid and high dose groups compared to 99 % in the control), followed by a 22 and 87 % reduction in postnatal viability (PND1-4) in the mid and high dose groups, respectively. The pup weight was statistically significantly lower at PND1 in the high-dose group (3.9 g vs 4.76 g in control), the number of runts was increased in a non-dose dependent manner in all dose groups and the number of cold pups was increased in the high dose group (incidence not provided in the CLH report).

***Table**. Results summary of the Combined reproductive screening test (2004)*

Dose level	Control	10 mg/kg diet	100 mg/kg diet	300 mg/kg diet
Test substance intake	0 mg/kg bw/d	0.5-0.7 mg/kg bw/d	4.2-6.2 mg/kg bw/d	8.4-17 mg/kg bw/d
Number of pregnant females	7	8	7	8
Mean number of implantations	12.6	13.4	11.3	10.3
Number of dams with only implantation sites observed at necropsy	1	0	0	3
Post-implantation loss (%) Mean value Median value	 22.33 ± 13.16 7	 20.98 ± 7.11 11	 49.23 ± 17.45 50	 69.99 ± 14.71 95[£]
Pups delivered (total) (N)	70	88	72	43
Pups delivered (live + dead mean) [N= number of 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 CLH report. However, RAC notes that the lower body weights in the high dose group were at least in part due to the high incidence in post implantation losses and to the reduced pup/foetal weights.

Moreover, maternal body weight was not significantly lower in the mid dose group as compared to the controls while the increase in post-implantation loss and the decrease in live birth index were already statistically significant at this dose level. RAC concludes that the effects seen in the mid and high dose groups are not secondary to effects on maternal body weight or weight gain. Thymus weight of parental animals was statistically significantly lower in high and mid dose groups compared to the control animals and accompanied by significant lymphoid depletion in both sexes (see the table under the heading "Summary of the Dossier Submitter proposal"). During the lactation period, one female in the control group, three females in the intermediate dose group and two females in the high dose group also displayed other clinical effects: thin, pale appearance, piloerection and/or blepharospasm. For the majority of these dams there was no correlation between onset of clinical signs and intrauterine or postnatal death of pups.

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

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

Effects on the developing immune system observed included changes in thymus weight and in immunologic cell populations in the pups (see the table under the heading "Summary of the DS's proposal"). Significant changes in immunologic cell populations and thymus weight were observed at the highest dose only, which corresponds to 1.7-2.1 mg/kg bw/day during gestation and to 2.9-5.2 mg/kg bw/day during lactation. The delayed type hypersensitivity (DTH) response, evaluated at PND49, was increased in all dose groups with statistical significance in the low and high-dose groups. The increased DTH response and lower thymus weight in the pups at dose levels up to 5.2 mg/kg bw/day confirm adverse effects on the immune system also in developing animals. At slightly higher dose levels (4.2-6.2 and 7.2 mg/kg bw/day), effects on thymus weights were also observed in some maternal animals of the reproductive screening study and of the pre-natal development study. Based on the available information, RAC agrees with the DS that the pups may be more sensitive compared to parental animals, but the available study is not robust enough for definite conclusions. In conclusion, the effects on the developing immune system are supportive, but not clear evidence for effects on development.

Comparison with the criteria

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

These adverse effects are:

- Skeletal malformations (missing bones, dose dependent) at the mid and high dose groups in the absence of significant maternal toxicity (mid dose group)

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

Further effects observed that are considered as supportive evidence include: reduced ossification partially in the same position as the missing bones (at lower concentrations), small increase in post implantation loss and postnatal viability and an increase in DTH response in the EOGRT study. Reduced pup weight, increased number of runts (not dose-dependent) and cold pups in the combined reproductive screening test.

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

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

Lactation

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

Conclusion

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

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).