Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the 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

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: dichlorodioctylstannane
EC number: 222-583-2
CAS number: 3542-36-7
Dossier submitter: Sweden

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2017	Norway		MemberState	1
Comment received				
Norway would like to thank Sweden for the proposal for harmonised classification and labeling of dichlorodioctylstannane, CAS no. 3542-36-7.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
30.10.2017	Germany	BNT Chmeicals GmbH	Company-Downstream user	2
Comment received				
see attachment ECHA note – An attachment was submitted with the comment above. Refer to public attachment com clh dotc (1).pdf				
Dossier Submitter's Response				
Thank you for your comments. Response to general comments and comments on toxicokinetics are provided here. For comments on acute toxicity, maternal toxicity, immune toxicity, and reproductive toxicity – please refer to response to comments 6 and 8.				

General comments

- *"Regrettably KEMI only used the new knowledge in immunotoxicity only for discussion of the OECD 443 study from Tonk and Menke, but not for new assessment of immunotoxicity".* We have not assessed specific organ toxicity for effects on the immune system in this proposal and the hazard class was not open for comments during the public consultation. DOTC has a harmonised classification in STOT RE 1. The classification R48 was formerly concluded by Technical Committee for Classification and Labelling and hence included in Annex I of Directive 67/548/EEC (ATP 30, August 2008) and later translated and included in CLP Annex VI.

Toxicokinetics

- *"The study report from Ruthenberg et al. seems identical with the quoted study report from 1987 quoted by KEMI".* It is correct that the study referred to as Study report, 1987 in the CLH-report is the same as Ruthenberg et al., 1988 as cited in the REACH Registration dossier. Since the full reference was not made publically available in the public version of the REACH Registration at ECHA:s dissemination site we regarded the full reference as confidential at the time of preparation of the CLH-report.

- BNT Chemicals GmbH points to an irrelevant conclusion for the current proposal from the study by Naßhan 2016 in Annex I. We are thankful for noticing and bringing to our attention this copy-and paste error.

- The reference *Seinen, W., Vos, J.G., Van Krieken, R., Penninks, A., Brands, R., Hooykaas, H. (1977) Toxicity of organotin compounds III. Suppression of thymus-dependent immunity in rats by di-n-butyltindichloride and di-n-octyltindichloride. Toxicol. Appl. Pharmacol. 42, 213–224* is used in the CLH-report to discuss immunotoxicity. BNT Chemicals GmbH states in their comment that this study also provides information on DOTC and its metabolite not being transferred to pups via milk. However, no such data is provided to demonstrate this in the published article referred to. A statement without reference in the article indicates that it is the study author's understanding that the transfer of DOTC to milk is limited ("*As transplacental passage and secretion of DOTC in milk is limited, intubation experiments also were carried out."*).

RAC's response

Thank you for your comments. They are noted. Since no particular effects to the pups have been observed as a result of exposure via milk in the studies summarised in the CLH proposal, no classification is proposed for reproductive effects via lactation.

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
30.10.2017	France		MemberState	3
Comment received				
FR agrees with the classification proposal				
Dossier Submitter's Response				
Thank your for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
23.10.2017	Norway		MemberState	4
Comment received				
We support the classification of dichlorodioctylstannane with Repr 1B; H360D with a specific concentration limit of 0.03%. The evidence for developmental toxicity, expressed as a significant increase in the incidence of skeletal malformations (missing bones), is clear. The skeletal malformations start at 0.8 mg/kg bw/day, and therefore a specific concentration limit of 0.03% is warranted. We support that the observed effects are not linked to maternal toxicity.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Your support is noted.				

Date	Country	Organisation	Type of Organisation	Comment number
30.10.2017	Germany	Galata Chemicals GmbH	Company-Manufacturer	5
Comment received				
Dear Sirs, We appreciate the assessment by KEMI of the toxicity of DOTC to reproduction based on scientific facts which says in part. "A statistically significant increase in pre-implantation loss was observed in the high dose group compared to control (10.4% compared to 1.5%, $p<0.05$), however it is noted that the incidence in the control group is unusually low. No clinical signs of toxicity or mortality of the dams were noted at any dose. A statistically significant decrease (6.5-8.8%) in body weight (without a concurrent effect on food consumption) was reported towards the end of the gestation in the high dose group compared to control and consequently a decreased body weight gain (28-48 % decrease as compared to control) during gestation (GD 0-20) was recorded. The corrected body weight change GD 5-20 was also statistically significantly reduced in the 300 mg/kg dose group compared to control (5.85 g versus 23.94 g in control, $p<0.001$) but the corrected body weight was only slightly reduced in high dose group compared to the control group (-6.8%). The weight of uteri in high dose dams (59.1 g) was 10.86 g (16%) lower compared to control (69.96 g), however, since the difference cannot be accounted for by differences in fetal weight (approx. 4 g in all groups) and the slight difference in mean litter size (10.1 compared to 11.4 fetuses in control), there appears to be some toxicity to the uterus. In conclusion, malformations (mainly missing bones in the forepaws) was seen at all dose levels with incidences increased in a dose response manner (and the dossier submitter considers that no NOAEL can be identified in the study) with or without maternal toxicity in the form of effects on body weight. In addition, effects on the degree of ossification (without a concurrent effect on fetal weight) were also recorded at these dose levels. The maternal effects on the thymus is not considered to cause the observed malformations." This assessment is in general agreement with the study authors which says in part: ☐ Decreased maternal body weight in Group 4 ☐ Decreased thymus size; clear in Group 4, probable in Group 3; ☐ Post-implantation loss is not statistically significant [NSS] in any group, but Group 4 correlates with fetal skeletal results showing reduced ossification; Table 9 ☐ No SS in utero maternal reproductive effect; Table 9 ☐ No apparent effect on fetal growth; weights and C-R length NSS; Table 10 ☐ No treatment-related external or visceral malformations				

☐ No biologically relevant increase in visceral variations
☐ Skeletal malformations [missing metacarpals and phalanges] shows a dose-response, accompanied by an increased incidence of skeletal variations [decreased ossification in sternum, fore limb and caudal vertebrae at the high dose,

KEMI poses the question, are the skeletal effects a retardation of ossification or true malformations? The former are quite common in the presence of maternal or fetal toxicity whereas the latter are rare. In the case of DOTC, the skeletal effects are believed to be retardation of ossification [i.e. Alizarin red S staining is not visible] and not true malformations [i.e. total ablation of the phalanx]? The OECD 414 report did not characterize the specific anatomy of the "missing" phalanges, however the study protocol suggests that this question can be answered. Half of the fetuses were stained with BOTH alizarin Red S [for ossified tissue] and Alcian blue [for cartilaginous tissue]. The inference, not specifically stated in the report, is that the phalanges are anatomically present, but not ossified, suggesting the reported findings are delays in ossification of phalanges which are anatomically present.
We understand that photographs of the reported observations and/or the preserved fetal specimens may be available, so we will pursue a further evaluation of the raw data to document the fetal outcomes and provide a clear and full explanation of the observations.

The evaluating member state, without access to the raw data of the study, understandably considered the skeletal malformations in the most severe way, as missing bones of the forelimb and the main adverse effect of developmental toxicity with dose-dependent incidence. This interpretation of the skeletal results which cannot be explained as secondary to maternal toxicity would warrant a classification as Toxic to reproduction category 1B.
The way the study monitor has interpreted the data deviates from the member state interpretation. The retardation of ossification is presented as a known and fully reversible skeletal effect.
A review of any available photographs of the malformations in question and any retained fetal specimens should be able to verify the nature of the effect and which interpretation of the "missing" phalanges is the more accurate.

ECHA note – An attachment was submitted with the comment above. Refer to public attachment CLH_DOTC_Comment_301017.doc

Dossier Submitter's Response

Thank you for your comments.

Firstly, we would like to clarify that we did not pose the question "*are the skeletal effects a retardation of ossification or true malformations?*" in the CLH-report as stated in the comment above.

Since we do not have the raw data and the study authors as well as the Registrant(s) categorizes the findings as "malformations" we cannot interpret them in any other way. The malformations are according to the study authors associated with delayed fetal ossification. We interpret this as that in addition to the missing bones, increased incidences of poor or incomplete ossification of sternum no. 5 and 6 (statistically significant different compared to control in high dose group) and metacarpal no. 5 in low, intermediate and high dose groups are also evident. Poor or incomplete ossification of proximal phalanx no. 3 and 4 were also seen in all dose groups including 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.

We do not see how Galata Chemicals can interpret the information in the study report as "[…] *the phalanges are anatomically present, but not ossified, suggesting the reported findings are delays in ossification of phalanges which are anatomically present*." based on the information that the foetuses were stained with both Alizarin Red S [for ossified tissue] and Alcian blue [for cartilaginous tissue]. On the contrary, if the findings, currently reported as malformations, would rather be delays in ossification and not true malformations (missing bone) then these structures would have been stained with Alcian blue and reported as such. Indeed, findings of poor or incomplete ossification of the same structures as the the missing ones (proximal phalanx no. 3 and 4, and metacarpal no. 5) are also reported in various pups. Importantly, reportings of both "missing" and "poorly ossified" of different structures but in the same pup are frequent in the high dose group, thus confirming that the staining technique can distinguish between malformations (missing, not stained with Alizarin Red S or Alcian blue) and variations (poorly ossified, stained with Alcian blue).

[Of note from the reference used in the study report, Tyl and Marr. Chapter 7. Developmental toxicity testing – Methodology. In Developmental and Reproductive Toxicology – A practical approach, Second edition, Edited by Ronald D. Hood: *In a double-stained skeleton, the ossified bone will be stained red to purple and the cartilage stained blue. The staining of the cartilage allows the examiner to ascertain whether the underlying structure is present (i.e. is the bone merely not yet ossified, although the cartilage anlage is present, or is the underlying cartilage actually missing?)*].

In the REACH registration the following conclusion is made: "*The NOAEL and the LOAEL for developmental toxicity was also determined to be 10 ppm (0.8 mg/kg bw/day) and 100 ppm (7.2 mg/kg bw/day), respectively, based on a statistically significant and treatment-related increase in the percentage of skeletal malformations associated with delayed fetal ossification. As the observed skeletal malformation associated with delayed fetal ossification was only noted at maternally toxic doses DOTC is regarded as not teratogenic in the rat but as fetotoxic at a maternally toxic doses*."
Both the Registrant(s) and study authors considers that DOTC is not teratogenic because "*the observed skeletal malformation associated with delayed fetal ossification was only noted at maternally toxic doses*" and not because of incorrect denomination of the finding (i.e. variation rather than true malformation). Thus we agree that "*The way the study monitor has interpreted the data deviates from the member state interpretation*".
However, we would like to emphasize that this is due to different view on the influence of maternal toxicity on the observed developmental toxicity and not because of diverging view on the findings being true malformation or not.
Moreover, the presence of both skeletal malformations (missing bones) and variations (poor ossification) does not render the malformations of less concern. Substance-related skeletal effects covering the the whole spectrum of effects is possible: from delayed ossification to missing bone.

RAC's response

Thank you for your comment. RAC concurs with the dossier submitter that the missing bones should be regarded as true malformations based on the staining and reported information available to the dossier submitter. Further, the missing bones should be considered concerning regardless of their origin (including a possible result of delayed ossification at the end of gestation).

Date	Country	Organisation	Type of Organisation	Comment number
30.10.2017	Germany	BNT Chmeicals GmbH	Company-Downstream user	6

Comment received

Deteiled discussion in attached file

ECHA note – An attachment was submitted with the comment above. Refer to public attachment com clh dotc (1).pdf

Dossier Submitter's Response

Thank you for your comments.

Specific responses are provided below and in comment no. 2 and 8.

Assessment of immunotoxicity and maternal toxicity

Thank you for detailing the mechanism of the immuntoxicity of DOTC. We have not re-assessed the harmonised classification for specific organ toxicity (STOT RE 1) and it is not within the scope of the public consultation. Thus, we have not discussed any additional information from studies on immune toxicity other than the information from the available studies on reproductive toxicity and repeated dose toxicity studies that are considered to be relevant for the assement of reproductive toxicity.

There are no doubts that DOTC is causing adverse effects on the immune system, however, it is not demonstrated that the observed developmental effects (malformations, post-implantation loss, pup viability) are secondary and unspecific to the immune toxicity in the dams/pregnant animals. We agree that effects on the immue system appears at lower doses compared to the general maternal toxicity, such as decrease in body weight, but there is no evidence to demonstrate a link between the effects on thymus and the observed developmental toxicity at the same dose levels.

In the TG 414 study, the only investigated effect on the thymus/immunesystem in the dams was the gross pathological examination where a reduction in size of the thymus were reported at intermediate and high dose. No information on grading of severity, organ weights or histopathology is available to the dossier submitter. At intermediate dose 7 out of 25 female rats (28%) had reduced thymus size, all of these 7 females were pregnant and in 4 out of the resulting 7 litters there were increased incidences of skeletal malformations or variations. In total there were 12 (out of 20) litters with skeletal malformations or variations at intermediate dose.
At high dose 100% (20/20) of the dams had reduced thymus size and 19 out of 20 litters had increased incidences of malformations and/or variations.

In the TG 421 study lymphoid depletion was observed in 5/10 animals of the 10 mg/kg group and in all animals of the 100 and 300 mg/kg groups. Severity score was severe to very severe in all groups. In the dose group of 10 mg/kg bw/day there were four dams with incidences of post-implantation loss higher than the mean value of the control group. Two of the four dams did not have any effects on thymus but the incidences of postimplantation losses were 42% and 31% respectively. Overall, no clear correlation between lymphoid depletion in the dams and post-implantation loss was observed. Nonetheless, no causal relationship between lymphoid depletion and post-implantation loss has been demonstrated.

Assessment of developmental toxicity

OECD TG 414

We would like to reiterate that we do not consider that the observed maternal effects of the thymus is causing the fetal skeletal malformations.

Setting NOAEL for maternal toxicity based on effects on thymus at 10 mg/kg diet as argued by BNT Chemicals does not justify less stringent classification in category 2 since there is no evidence for the developmental toxicity being secondary to these effects.

Regarding the statement by BNT Chemicals that the staining for skeletal analysis in the study is deviating and that therefore *"it is not possible to make a conclusion on teratogenic effects based on this study"* we would like to remind that in OECD TG 414 there is no detailed guidance on staining of cartilage and bone and no requirement on performing double staining. In OECD Guidance Document 43 staining of foetal cartilage is recommended in addition to staining the ossified bone, and double-staining techniques with Alizarin red S and alcian blue may be used. Nevertheless, quoting the study report *"Skeletal examination: 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 prescence or absence of skeletal malformation (variations)."* From our understanding, double staining was indeed performed. For further discussion on the interpretation of the results, please see response to comment no. 5

OECD TG 421

We would like to confirm that the calculation of median values of incidences of post-implantation loss in intermediate and high dose groups as presented in table 5 in the CLH-report is correct. It is true that nearly all foetuses were lost in high dose group, and it should be noted that the extract from table 5 of the CLH-report in the attached document by BNT Chemicals GmbH (CLH_DOTC_Comment_301017.doc) shows the total (viable + dead) number of pups delivered. Further down in table 5 in the CLH-report there is information on the number of viable and dead pups delivered, respectively. Total number of stillborn pups were 1, 4, 34, 17 (1.4, 4.5, 47, 40%) in control, low, intermediate and high dose respectively. It should be noted that in high dose group there were also 3 dams out of 8 with total intrauterine death (=100% post-implantation loss). The other four dams had incidences of 0, 15.4, 54.5, 90, 100% post-implantation loss. At intermediate dose there were two dams with only stillborn pups (=100% post-implantation loss), the other five dams had incidences of 0, 0, 10, 50, 84.6% post-implantation loss. The median values of the incidences of post-implantation loss (50% and 95% respectively) in the intermediate and high dose groups are thus reflecting this.

RAC's response

In the pre-natal development toxicity test, developmental effects were observed in the absence of immune toxicity. There is no information available to RAC that is able to link the observed toxicity to the immune system to the reproductive effects. Further, RAC agrees with the dossier submitter that double staining seems to be used and as noted in response to comment 5, the missing bones should be regarded as malformations and concerning regardless of their origin.

OTHER HAZARDS AND ENDPOINTS – Acute Toxicity

Date	Country	Organisation	Type of Organisation	Comment number
30.10.2017	France		MemberState	7
Comment received				
FR agrees with the classification proposal for acute toxicity.				
Dossier Submitter's Response				
Thank you for your support.				
RAC's response				
Noted.				

Date	Country	Organisation	Type of Organisation	Comment number
30.10.2017	Germany	BNT Chmeicals GmbH	Company-Downstream user	8
Comment received				
Deteiled discussion in attached file ECHA note – An attachment was submitted with the comment above. Refer to public attachment com clh dotc (1).pdf				
Dossier Submitter's Response				
Thank you for your support for Acute Tox. 2, H330 based on LC50 (4h, rat) = 0.439 mg/L. Responses to additional issues in the attached document are provided in response to comments no. 2 and 6.				
RAC's response				
Thank you for your comments.				

PUBLIC ATTACHMENTS
1. CLH_DOTC_Comment_301017.doc [Please refer to comment No. 5]
2. com clh dotc (1).pdf [Please refer to comment No. 2, 6, 8]