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30.10.2017

Substance: Dichlorodioctylstannane
EC#: 222-583-2
CAS#: 3542-36-7
Index#: 050-021-00-4

The CLH proposal from KEMI for Dioctyltin dichloride contains in most cases an exemplary scientific discussion the facts.

Regrettably KEMI only used the new knowledge in immunotoxicity only for discussion of the OECD 443 study from Tonk and Menke, but not for a new assessment of immunotoxicity.

Toxicokinetics

In the Summary table of the endpoint are named the following studies:

- Penninks et al., 1987
- Study report, 1987. Reach registration dossier, public version (ECHA dissemination, 2016a)
- Naßhan, 2016

In the Endpoint of the registration dossier the following studies are named:

- Sagelsdorf et al., study report (1988)
- Ruthenberg et al. , study report (1988)

The study report from Ruthenberg et al. seems identical with the quoted study report from 1987 quoted by KEMI.

KEMI uses for assessment of toxicokinetic the unpublished and not by the lead registrant (dossier submitter) known study "Naßhan, H (2016) Dioctyltin dichloride [DOTC], CAS number: 3542-36-7. In-vitro metabolism study." In Annex I of the CLH proposal it was quote in an improper way the conclusion "*Dioctyltin dichloride is shown to be rapidly converted to ClOct2SnOSnOct2Cl under conditions representative of the mammalian stomach. The generation of a common intermediate, identical to one of the hydrolysis products of dioctyltin dilaurate (see 2.1.3), supports the analogue approach for read-across from DOTC to dioctyltin dilaurate.*"

The quoted report does neither refer to Dioctyltin dilaurate nor contains investigations on Dioctyltin dilaurate. The conclusion from the original study is: "*DOTC in-vitro metabolism can be monitored using 119Sn-NMR Spectroscopy.*"

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Under the simulated gastric conditions (0.1 M HCl/pH 1.2/37°C) the tin resonance at 125 ppm characteristic to DOTC decreases whereas two singlets at -92 / -145 ppm characteristic to the hydrolysis product (DOTCL)₂O increase with the same intensity.

The reaction is fast and reaches even after 30 seconds a hydrolysis rate of ~ 50 %. After 4 hours of incubation the in-vitro metabolism of DOTC reaches 90 % under the conditions of the study.”

Furthermore, KEMI used the study 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-butyltin dichloride and di-n-octyltin dichloride. Toxicol. Appl. Pharmacol. 42, 213–224 for other endpoints, but omitted the information, that Diocetyl tin dichloride or its metabolite is not transferred via milk.

Acute Toxicity – Inhalation

Agree to Acute Toxicity inhalation category 2, based on LC50(4h, rat) = 0.439 mg/L (Sachsse et al., 1979)

Assessment of immotoxicity and maternal toxicity

Immotoxicity is the essential effect of Diocetyl tin dichloride (DOTC). Studies on DOTC showed adverse effects relating to the thymus of animals (1,2,3,4,5). In rats it induce lymphocyte depletion in thymus and thymus-dependent areas of spleen and peripheral lymph nodes without signs of myelotoxicity or a generalized toxicity” (7).

The majority of the data are from reproduction and development studies respective from Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Tests. The adverse effects relating to the thymus (atrophy, weight decrease) are noted at the necropsy of the animals. Immune parameters were not measured while the testing excepted this from hematology, because parameters like interleukin are not part of the standard test protocols for repeated dose and /or development toxicity.

In general in this studies the noted depletion of the thymus of the test animals were below 80%, so this is no sufficient criteria for immunotoxicity (cp. ICH S8).

On the other side, as described in the registration dossier, there are many studies available, showing, that DOTC caused an acute effect in the immune system,

Seidler (8) showed already 1971, that a single oral administration of DOTC causes adverse effects in the thymus of rats.

Evidence that thymus atrophy caused by Diocetyl tin is an acute effect go back to 1975. Seinen 1 published a dose-response curve for the thymus weight of rats fed with DOTC. 48 hours after administration of 50 and 150 ppm DOTC there is a decrease of weight and the total number of “nucleated thymus cells.”

Furthermore Seinen (2) demonstrates clearly that DOTC produces effects in the thymus after a single administration (Figure 1).

In all treatment groups a dose related reduction of thymus weight, number and viability of thymus cells were observed.

Together with several other studies from Boyd (9), Henninghausen 10, 11, 12, Gruendel (13), Kishi (14),

Miller (15, 16) Penninks (17) and Volsen (18) demonstrating the acute adverse effect of Diocetyl tin: The chronology of the adverse effect after a single exposure to Diocetyl tin belonging to the immune system could be described as follows:

Figure 1 – Thymus weight, cell count and cell viability of rats after single intravenous administration of DOTC; data adopted from (2).

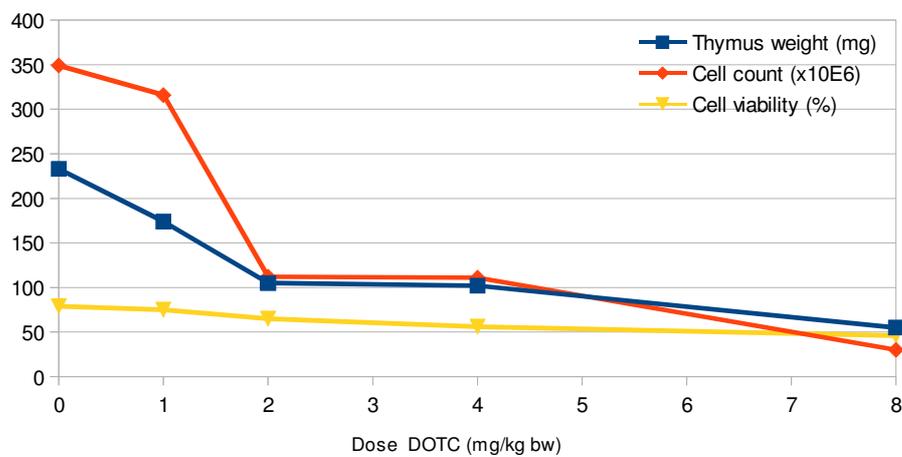


Table 1 – Chronology of the action of Dioctyltin in the immune system

	0 h	24 h	48 h	72 h
Decrease thymus weight	-	-	(X)	X
Decrease body weight	-	-	-	-
Decrease Thymocyte	-	X	X	X
Decrease IL-2	-	X	X	X
IgG ₁ (OX-18)	No data	X	No data	X
CD4 ⁺ CD8 ⁺	-	X	X	X

24 hours after a single administration of Dioctyltin to rats there is a decrease in thymocyte count, IL-2, IgG1 and CD4+ CD8+. The most studies report a decrease of the thymus weight after 72 hours, only one study report a decrease after 48 h. Body weight decreased only in relationship with general systemic toxicity.

So the adverse effect belonging to the immune system is more sensitive for maternal toxicity than decrease of body weight.

The doses in the studies uses for oral administration varied between 6.3 and 100 mg DOTC /kg bw. In the dose level 6.3 mg DOTC /kg bw there was registered a slight, but statistical significant reduction of the thymus (17).

Table 2 – Relative weight of thymus, liver and kidney 4 days a single administration of Dioctyltin dichloride (17)

Route	Dose (mg / kg bw)	Body wt (g)	Relative organ weight (g / 100 g bw)		
			Thymus	Liver	Kidney
Oral	0	119 ± 3	0.036 ± 0.02	4.83 ± 0.09	1.04 ± 0.04
	6.3	117 ± 4	0.03 ± 0.04 *	4.71 ± 0.10	1.08 ± 0.08

* p < 0.05

Relating to this data the LOAEL for a single administration is set to 0.5 mg DOTC /kg bw. In absence of data, a NOAEL is not indicated.

The adverse effect caused by Dioctyltin after single administration relating to the thymus was reversible within six weeks, relating to lymphocytes inhibition seems to cover less than eight days .

The effect of Dioctyltin to the immune system is similar of the effect of Corticosteroid, this can be explained by the interaction of Dioctyltin and Corticosteroid with Nr3C1 (glucocorticoid receptor) (14). This is one on the main difference to the besides the interaction of Dibutyl- and Tributyltin relating to the immune system.

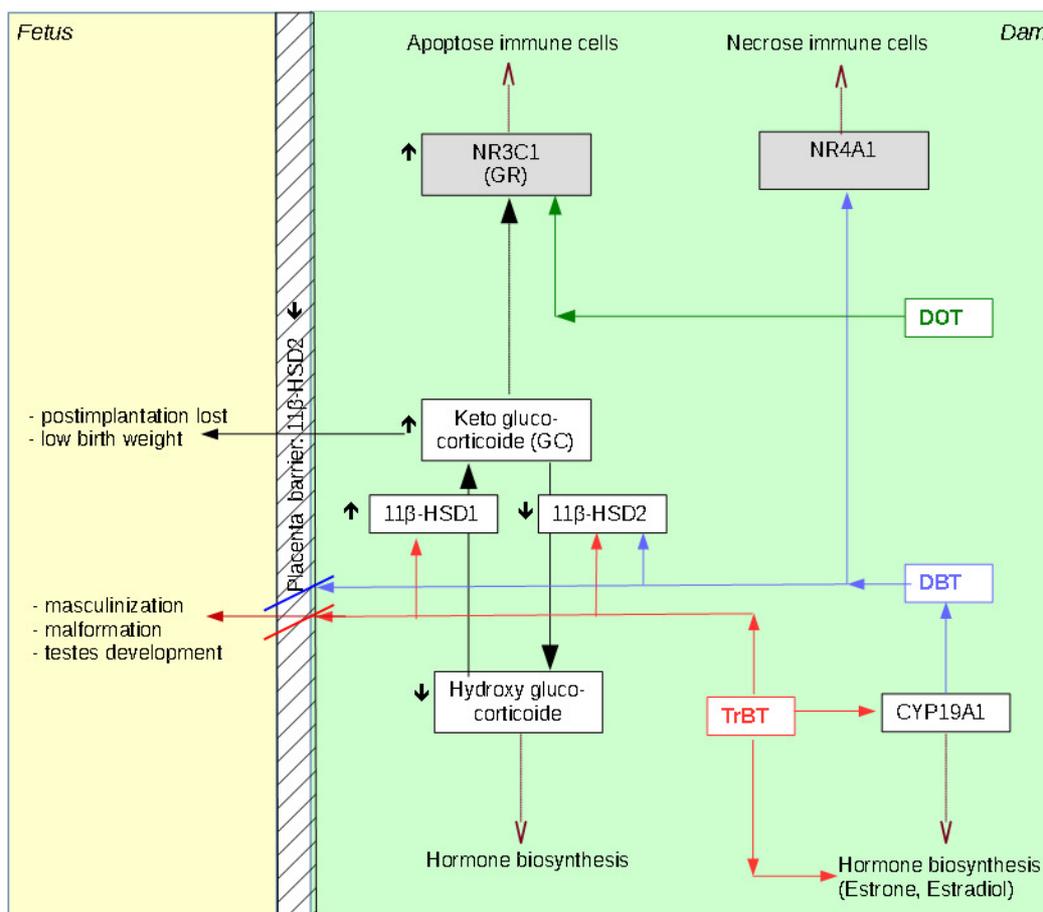
Dibutyltin causes necrosis of T-lymphocytes via activation of NR4A1 and Tributyltin causes apoptosis of T-lymphocytes (19). So via activation of the NR3C1 antagonist NR4A1 the glucocorticoid receptor function is disrupted. Dibutyltin blocks the glucocorticoid-induced expression of hepatic PEPCK and TAT, two enzymes with a key role in energy metabolism of the immune system and disrupts the glucocorticoid receptor-mediated regulation of gene transcription at the initial step of receptor activation by abolishing ligand binding to the receptor. Furthermore Dibutyltin is able to abolish the suppressive effect of glucocorticoids on the synthesis of the pro-inflammatory cytokines TNF- α and IL-6 by reducing the GR-dependent trans-repression of NF- κ B activation (20).

Dioctyltin do not disrupt the regulation of GC in contrast to Di- and Tributyltins.

11 β - hydroxysteroid dehydrogenase type 1 (11 β -HSD1) catalyzes the conversion of inactive glucocorticoid (GC) in the active species, the type 2 the different way. Thereby the access of GC to the glucocorticoid receptor (GR) and the mineralocorticoid receptors (MR) is regulated, additional to the rendering specificity of MR for aldosterone 21.

Tributyltin activates 11 β -HSD1 22, Di- and Tributyltin 11 β -HSD2 23. This results in an increase of GC. In case of the Dioctyltins there is no increase of GC levels 2. The change in the GC levels by Dibutyltin (inhibition of deactivation) is too weak to causes apoptosis pathway (24).

Figure 2 – Action of DBT, TrBT and DOT belonging to the glucocorticoid system and consequence



So the activation of NR3C1 via Dioctyltin 14 causes a suppression of immune system after one time administration.

So it is clearly shown that DOTC causes an acute adverse effect in the immune system. The degree of thymus depletion depends on the dose, this among other the Reproductive/developmental toxicity screening study on DOTC from Appel MJ and Waalkens-Berendsen DH. (2004).

For immunotoxicity of DOTC a LOEL of 0.5 mg/kg bw/day was derived

Assessment of toxicity for reproduction and development

Important information by assessor / new lead registrant:

The lead registrant of DOTC was changed end of August 2017. First after this change (mid of October 2017) it was possible for the new lead registrant to access and evaluate the OECD 414 study used in the dossier by the former lead registrant. The CLH proposal was performed by KEMI before the change of the lead registrant.

Prenatal developmental toxicity study of DOTC administered orally in diet to Sprague Dawley rat

This study was rated in the registration dossier with Klimisch 1 and the most serious reason for KEMI for its justification to classify DOTC as toxic for reproduction category 1B is the OECD 414 study showing serious skeletal effects:

“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.”

Skeletal Examination

Malformations:

Statistically significant and treatment related increases were observed in the percentage of malformations of missing metacarpal No. 5 (11.4 and 34.6 % at 100 and 300 mg/kg, respectively, as compared to 0.8 % in the control), proximal phalanx No. 3 bilateral (14.3 and 28.0 % at 100 and 300 mg/kg, respectively, as compared to 0.8 % in the control) and proximal phalanx No. 4 (13.3 and 27.1 % at 100 and 300 mg/kg, respectively, as compared to 0.8 % in the control).

Observations of skeletal malformations were:

- 1 incidence in 132 foetuses (1 of 22 litters affected) at 0 mg/kg
- 11 incidences in 115 foetuses (8 of 21 litters affected) at 10 mg/kg
- 22 incidences in 105 foetuses (11 of 20 litters affected) at 100 mg/kg
- 47 incidences in 107 foetuses (19 of 20 litters affected) at 300 mg/kg

Split thoracic vertebra centrum no. 12 was noted as a single occurrence in a single litter at 10 ppm. Missing caudal vertebral arch no. 2 on both sides was noted in 2 litters (2 fetuses) at 10 ppm and 2 litters (3 fetuses) at 300 ppm. Both these observations were judged to be incidental occurrence.

Variations:

Statistically significant and treatment related increases were observed in the percentage variations of poor ossification of sternum No. 5 (6.5 % at 300 mg/kg as compared to 0 % in the control) and sternum No. 6 (14.0 % at 300 mg/kg as

compared to 0 % in the control). A dose dependent and treatment related increase in poor ossification of metacarpal No. 5 was observed (1.0 and 3.7 % at 100 and 300 mg/kg, respectively, as compared to 0 % in the control).

Observations of skeletal variations were:

- 6 incidences in 132 fetuses (5 of 22 litters affected) at 0 mg/kg
- 11 incidences in 115 fetuses (7 of 21 litters affected) at 10 mg/kg
- 10 incidences in 105 fetuses (4 of 20 litters affected) at 100 mg/kg
- 26 incidences in 107 fetuses (12 of 20 litters affected) at 300 mg/kg

For the skeletal analysis, the fetuses were not double-stained for bone and cartilage (a bone precursor in utero), so "missing" may not really be missing, just delayed. One very plausible explanation for the skeletal effects may be a delay in maturation which is sufficient to decrease ossification [a clearly reversible fetal effect], but not of sufficient severity to reduce fetal body weight.

Overall, in a very preliminary assessment is that this substance produced clear maternal toxicity effects [body weight; thymus size] in G4 and this was accompanied by marginal embryo/fetal effects [skeletal variations] at this dose. There is no evidence of a teratogenic response in rats. The adverse effect on the maternal thymus in G3 is probably real and would likely be proven if histopathology were performed on this maternal organ. This is a target organ-specific maternally toxic effect. Therefore the apparent NOEL is G2 based on the thymus effect in the dams in G3 with no adverse effect on the fetuses at this dose".

So there is an evidence the missing forepaws and ribs are no malformation, but rather cartilage (retarded ossified bones) not "pictured" by the uses staining method. This is also supported by evaluation by KEMI :*"the predominant finding was poor ossification."*

Based the deviation in staining fetus skeletons it is not possible to make a conclusion on teratogenic effects based on this study.

In the conclusion by KEMI is pointed out, that the *"mainly missing bones in the forepaws was seen at all dose levels"* and *"effects on the degree of ossification"*. This conclusion also supports that there is an important deficits in the study in evaluation of the stained fetus skeleton.

Additional there are no data for historical control groups are included into the study and that postimpantation lost in the control group are remarkable low. Notably the following passage in the study report:

"Treatment related increase in percentage of malformations was noted at mid and high dose, the differences were statistically significant.

Occasional statistically significant differences that did occur between groups treated with the test item and vehicle control showed no dose dependency and were caused by higher as well as lower stages of development and therefore considered not to be treatment related",

This triggers our concern that similar findings are not extraordinary in historical control groups.

Based on the deviation in the study, the study have be to categorize as Klimisch 2.

The lead registrant will be updated the registration dossier as soon as possible in this important issue.

Furthermore all noted effects are occurs at levels above the LOAEL of 0.5 mg/kg bw/day for immunotoxicity.

OECD 421 - Appel MJ and Waalkens-Berendsen DH. (2004). Dichlorodioctylstannane

There may be a calulation error in the post –impantation lost in the mid and the high dose in table 5:

Table 5: Summary of pup data

Dose level	Control	10 mg/kg diet	100 mg/kg diet	300 mg/kg diet
Number of pregnant females	7	8	7	8
Mean number of implantations	12.6	13.4	11.3	10.3
Post implantation loss (%)				
Mean value	22.33 ± 13.159	20.98 ± 7.114	49.23 ± 17.453	69.99 ± 14.713
Median value	7	11	50	95 ^f
[N = number of females]	N=7	N=8	N=7	N=8
Pups delivered (total) (N)	70	88	72	43

In case of a median of 95 % nearly all fetus have been lost. This was not the case. Furthermore if we compare the values for post implantation lost and pups delivered between the control group and the mid dose group (absolute values), it seems that there is a error in calculation.

Also in this study all noted effects are occur at levels above the LOAEL of 0.5 mg/kg bw/day for immunotoxicity.

Conclusion:

In all studies effects occur only in dose above the LOAEL for immunotoxicity. Only in the study of Tonk et al. was used a dose below the LOAEL for immunotoxicity respective maternal toxicity (~0.20 mg/kg bw/day); in this dose no adverse effects were determined.

The most concerning study for a classification of DOTC as toxic for reproduction by KEMI was the OECD 414 study in rats. Based on the serious deficits of the study (no historical control data, implication of retarded ossification in historical control) there is in our opinion no justification to classify DOTC as toxic for reproduction category 1B. Furthermore the OECD 443 study do not show any adverse effects in the mid and high dose, effects in juvenile animals (high dose) are caused by the acute nature of the immune suppressive effect of DOTC. The dossier submitter as well as KEMI stated that juvenile animals are not more sensitive than adult animals.

Under the precautionary principle we understand, that the substance DOTC should be classified as toxic for reproduction category 2.

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