Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate; [DOTE]

EC Number: 239-622-4
CAS Number: 15571-58-1

CLH-O-0000001412-86-257/F

Adopted

30 November 2018

30 November 2018

CLH-O-0000001412-86-257/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: **2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate; [DOTE]**

EC Number: **239-622-4**

CAS Number: **15571-58-1**

The proposal was submitted by **Germany** and received by RAC on **16 October 2017.**

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

PROCESS FOR ADOPTION OF THE OPINION

Germany 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 **5 December 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **2 February 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Bert-Ove Lund**

Co-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 **30 November 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 state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	050-027-00-7	2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate; [DOTE]	239-622-4	15571-58-1	Repr. 1B	H360D	GHS08 Dgr	H360D			
Dossier submitter's proposal					**Add** STOT RE 1 Aquatic Chronic 2 **Modify** Repr. 2	**Add** H372 (thymus) H411 **Modify** H361d	**Retain** GHS08 Dgr **Add** GHS09	**Add** H372 (thymus) H411 **Modify** H361d			
RAC opinion					**Retain** Repr. 1B **Add** STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	**Retain** H360D **Add** H372 (immune system) H400 H410	**Retain** GHS08 Dgr **Add** GHS09	**Retain** H360D **Add** H372 (immune system) H410			
Resulting Annex VI entry if agreed by COM					Repr. 1B STOT RE 1 Aquatic Acute 1 Aquatic Chronic 1	H360D H372 (immune system) H400 H410	GHS08 GHS09 Dgr	H360D H372 (immune system) H410			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Substance abbreviations used throughout the text:

DOTE: dioctyltin bis(2-ethylhexyl mercaptoacetate); 2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate.
MOTE: monooctyltin tris(2-ethylhexyl mercaptoacetate
DOTI: dioctyltin bis(isooctyl mercaptoacetate)
MOTI: monooctyltin tris(isooctyl mercaptoacetate)
DOTC: dioctyltin dichloride
DOTEC: dioctyltinchloro 2-ethylhexyl mercaptoacetate

DOTE contains two stable octyl groups and two labile 2-ethylhexyl-mercaptoacetate groups potentially available to hydrolysis. Commercially produced DOTE may contain varying concentrations of MOTE as an impurity (Costlow, 2017). Some toxicological tests have also been conducted using DOTE containing 20-30% MOTE (e.g., DOTE:MOTE, 80:20). MOTE differs from DOTE by containing one less octyl group and one extra 2-ethylhexyl-mercaptoacetate group.

DOTE is a large molecule, and the same applies to the read-across substance DOTI. DOTI and DOTE are isomers differing only slightly in the structure of the C-8 alcohol of the mercaptoester ligand (either iso-octanol or 2-ethylhexanol, respectively). Since these alcohols are so close in structure, their respective mercaptoacetate esters are expected to have very similar physicochemical and toxicological properties, including hydrolysis products.

It has previously been assumed that both DOTE and DOTI quickly hydrolyse in the gastrointestinal (GI) tract to the dichloride DOTC, and that DOTC is the active metabolite of both substances. DOTE has therefore previously been assessed based on read-across to studies conducted on DOTC and DOTI:MOTI (RAC, 2012). A new study was conducted in order to specifically examine the hydrolysis of DOTE. This study reported that the monochloride ester (DOTEC; still containing one 2-ethylhexylmercaptoacetate group) was the only identifiable hydrolysis product after several days in 0.1 M HCl. Costlow *et al.* (2017) reported that DOTE hydrolysed to 70.8 Mol.% DOTEC, while 23 Mol.% remained unreacted and <2 Mol.% consisted of unidentified reaction products (Anonymous, 2015; later published as Costlow *et al*., 2017).

The dossier submitter (DS) stated that no DOTC is formed during *in vitro* hydrolysis of DOTE. It is noted that, the *in vivo* metabolism of DOTE and of its monochloride hydrolysis product (DOTEC) have not been studied, and the lack of information on further enzymatic metabolism, absorption, and potential toxicity, hamper the assessment of mode of action (MoA) and toxicity of DOTE. Likewise, the MoA for the toxicity of DOTC and DOTI are not fully known.

For these reasons, the new hydrolysis study describes the abiotic 'chemical' fate of DOTE at low pH, but does not inform about the *in vivo* fate of DOTE and its transformation products. Moreover, the results of the toxicity studies with DOTC, DOTI, and DOTE all show very similar adverse effects on the immune system.

RAC is of the view that studies on DOTE itself, DOTE:MOTE mixtures, and the structurally very similar analogues DOTI and DOTI:MOTI, should be considered in the hazard assessment of DOTE.

Studies cited in the CLH report are conducted using:

DOTE	90 days oral repeated dose toxicity study; similar to an OECD TG 408 study (Anonymous, 1970)
DOTE:MOTE	90 days oral repeated dose toxicity study; similar to an OECD TG 408 study (Anonymous, 1974)
DOTI:MOTI	OECD TG 416; Two-generation toxicity study (Anonymous, 1997)
DOTE	OECD TG 414; Developmental toxicity study in rabbits (Anonymous, 2014a)
DOTE	OECD TG 414; Developmental toxicity study in mice (Anonymous, 2014b)
DOTI:MOTI	Developmental toxicity study in rats; similar to an OECD TG 414 study (Battenfeld, 1991)
DOTI:MOTI	Developmental toxicity study in rabbits; similar to an OECD TG 414 study (Battenfeld, 1992)
DOTI:MOTI	Developmental toxicity study in mice; similar to an OECD TG 414 study (Faqi *et al*., 2001)
DOTC	OECD TG 421; Reproduction/developmental toxicity screening study (Appel & Waalkens-Berendsen, 2004)

HUMAN HEALTH HAZARD EVALUATION

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

Summary of the Dossier Submitter's proposal

Repeated dose toxicity was assessed based on two 90-d oral studies in rats, and two developmental toxicity studies in mice and rabbits, respectively. The relative thymus weight was reduced by 20-30% after 90 days exposure to 2-3 mg/kg bw/d in rats, and by 10-20% in the dams of the developmental toxicity studies in mice and rabbits after exposure to 20-30 mg/kg bw/d for 12 and 22 days, respectively.

Classification in STOT RE Category 1 was considered applicable as significant toxic effects were observed in the 90-day oral studies in rats at or below the guidance value of 10 mg/kg bw/d for STOT RE 1. These observations were supported by significant toxic effects on thymus in mice and treatment-related as well as dose-dependent effects on thymus in rabbits, demonstrating specific target organ toxicity on thymus in several species. There were no studies using inhalation or dermal exposure. DOTE was thus proposed by the DS to be classified as STOT RE 1, H372: Causes damage to thymus through prolonged or repeated exposure.

Comments received during public consultation

Comments were recieved from three Member State Competent Authorities (MSCAs) and one industry organisation. All four comments supported classification with STOT RE 1, but the industry organisation proposed to postpone the decision on classification until the results are available from ongoing (single dose) studies aiming to determine whether STOT RE or STOT SE would be a more appropriate classification for the observed effects.

Assessment and comparison with the classification criteria

Both 90-d studies in rats are old (from 1970 and 1974), pre-dating OECD test guidelines and GLP, but they are similar to OECD TG 408 studies. Both were considered by the DS as reliable with restrictions. They are very briefly described in the CLH report, but they show consistent results with regard to thymus toxicity.

In one 90-d oral study, DOTE (97%) was administered via the diet at concentrations of 10, 25, 50, 100, 250, 500 and 1000 ppm to groups of male and female rats (Anonymous, 1970). The exposure roughly corresponded to 0.55, 1.3, 2.6, 5.3, 13, 26 and 53 mg/kg bw/d. Substantial mortality was observed from 500 ppm and the terminal body weight (magnitude not given) was decreased from 100 ppm in females and from 500 ppm in males, as compared to controls. At 100 ppm (approx. 5.3 mg/kg bw/d), almost complete depletion of lymphocytes and small thymus was reported in 2/5 females, effects that were seen in all males and females at 500 ppm. Thymus weight (absolute and/or relative weight not stated) was reduced by 20% also at 25 ppm (1-2 mg/kg bw/d).

In the other oral 90-d study (Anonymous, 1974), rats were given DOTE:MOTE 70:30% in the diet at concentrations of 25, 50, and 100 ppm (0, 1.6, 3.3 and 6.6 mg/kg bw/d). Effects on body weight gain were not given, but dose-dependent reduction of absolute and relative thymus weight was observed from 50 ppm. Thymus weight was completely recovered 15 days after cessation of exposure.

In addition, decreased thymus weights were observed in the two new developmental toxicity studies (with DOTE; Anonymous 2014a and 2014b) in mice and rabbits after 12 and 22 days exposure, respectively. The weight reduction was in the order of 10-20% at exposure levels of 30 and 20 mg/kg bw/d in mice and rabbits, respectively. As noted above, the exposure durations were very short and a comparison with the data from the 90-d studies or with the guidance value for a 90-d study is not appropriate. Significantly decreased relative thymus weights were also observed in the rat two-generation study (DOTI:MOTI; Anonymous, 1997), e.g., at 15-16 mg/kg bw/d in both sexes of the parental animals in the P0- and F1-generation.

RAC concludes, based on the above data on three species, and supported by the general knowledge of thymotoxicity being a characteristics of organotin compounds, that the thymus is a target organ after repeated exposure to DOTE, likely leading to an impaired function of the immune system. RAC suggests to specify the immune system (rather than the thymus) as the target organ in the hazard statement to be consistent with the target organ specification in the hazard statement for other organotins. Adverse effects occured below the guidance value for STOT RE of 10 mg/kg bw/d in the oral 90-d study on DOTE (Anonymous, 1970), and classification as STOT RE 1 is therefore supported by RAC. Thymus toxicity is also evident at low oral exposure levels in the 90-day study on DOTE:MOTE and in the two-generation study on DOTI:MOTI. No studies by the dermal or inhalation route exist, and although toxicity through these routes are likely to be lower than via the oral route, the lack of data precludes specifying the route in the hazard statement. Thus, RAC concludes in line with the DS proposal that **classification of DOTE as STOT RE 1; H372 (Causes damage to the immune system through prolonged or repeated exposure) is warranted**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Fertility

There was one oral two-generation reproduction toxicity study (OECD TG 416; Anonymous, 1997) performed with the read-across substance DOTI:MOTI (80:20) in rats. The dose levels in this study were 0, 20, 60, and 200 ppm (nominal in diet) (approximately 1.5, 4.4, and 15 mg test material/kg bw/d in P0 animals and 1.6, 4.7, 16 mg test material/kg bw/d in F1 animals).

The effects observed included slight decreases in maternal food consumption during lactation, slightly decreased pup body weights and lower thymus weight in both pups (F1 but not in F2) and parental animals. In the F1 generation at the high dose there was a statistically significant increase in stillbirths (26 vs. 5 in controls), and increased pup mortality after PND4, resulting in a lower viability and lactation indexes. No teratogenic effects were observed in this study.

In addition, there was a reproductive toxicity screening study (OECD TG 421; Appel & Waalkens-Berendsen, 2004) with DOTC. The dose levels in this study were 0, 10, 100 and 300 ppm (corresponding to 0.5-0.7, 4.2-6.2 and 8.4-17.0 mg DOTC/kg bw/d). The main effect in the dams was lymphoid depletion in the thymus, which occurred at all doses with a dose-dependent increase in severity.

No effects were observed on the mating index, pre-coital time, female fecundity index, female fertility index or male fertility index. The gestation index was 86, 100, 71 and 50 % in the control, 10, 100 and 300 ppm groups, respectively. The live birth index was 99, 95, 53 and 60% in the control, 10, 100 and 300 ppm groups, respectively. Post-implantation loss was 22.3, 21.0, 49.2 and 70% for the control, 10, 100 and 300 ppm groups, respectively.

As no effects were observed on fertility, no classification for fertility was proposed by the DS.

Development

Five prenatal developmental toxicity studies (PNDT, OECD TG 414) were presented in the CLH report, two performed with DOTE (Anonymous, 2014a (rabbit) and 2014b (mouse)) and three with DOTI/MOTI 80:20 (called DOTI in the rest of the opinion; Battenfeld, 1991 (rat) and 1992 (rabbit); Faqi *et al*., 2001 (mouse)).

The first study with DOTE was performed in rabbits with doses of 0, 4, 20, 80 mg/kg bw/d. The LOAEL was 80 mg/kg bw/d for both maternal and developmental toxicity. Maternal effects consisted of a dose-dependent depression in maternal thymus weight compared to controls (5.1, 9.6 and 12.8% in the low-, mid-, and high dose group, respectively), which was considered biologically relevant at the high dose. Foetal effects at the high dose included decreased body weight (-11.9%) and crown-rump length (-10.7%) compared to controls, and one high dose litter with total foetal loss.

The second PNDT with DOTE was performed in mice with doses of 0, 15, 30, 60 mg/kg bw/d. Maternal effects consisted of a depression in thymus weight at the mid- and high dose groups (23 and 35%, respectively). Also, a statiscally non-significant decrease in maternal body weight gain was observed at the high dose. The only developmental effect was a statistically significant trend in the percentage of post-implantation loss (0.0 ± 0.0 in controls, 0.9 ± 2.8 at low-, 1.5 ± 4.9 at mid-, and 2.6 ± 5.6 at high dose).

The first PNDT with DOTI was performed in rats with doses of 1, 5, and 25 mg/kg bw/d. Both maternal and developmental effects occurred only at the top dose of 25 mg/kg bw/d. Maternal toxicity consisted of a slight, non-significant decrease in corrected body weight and body weight

gain. There was a significant increase in the percentage of dead foetuses, however, all dead foetuses were from a single dam.

The second study with DOTI was performed in rabbits at 1, 10, and 100 mg/kg bw/d. Effects at the high dose consisted of an increased incidence of abortions, post-implantation loss, minor visceral anomalies, minor skeletal head anomalies, skeleletal variations of the sternum and feet bones, and a significant reduction in foetal body weight. As maternal disease was noted in some animals, the DS considered that this study was less significant in the assessment compared to the new DOTE study.

The third study with DOTI was performed in mice at 20, 30, or 45 mg/kg bw/d (group 1); and at 67 or 100 mg/kg bw/d (group 2). Maternal toxicity consisting of a significant decrease in thymus weight occurred from 45 mg/kg bw/d. At 67 mg/kg bw/d an increase in foetal incidence of cleft palates (+5.5%) was observed. The DS considered that this study was less significant than the new DOTE study as detailed information on several endpoints was not reported.

The studies with DOTE itself in mice and rabbits showed some developmental effects, as well as slight maternal toxicity. The DS noted that the highest dose tested might have been too low to enable the detection of a dose-response relationship and thus it might only reflect the starting point of a potential dose-related response. The studies with the read-across substance DOTI as well as with DOTC consistently showed developmental effects, including post-implantation loss, retardations of foetal development and decreased foetal weight.

In the oral two-generation reproduction toxicity study in rats (OECD TG 416; Anonymous, 1997) performed with the read-across substance DOTI:MOTI (80:20), slightly decreased pup body weights and lower thymus weight in F1 pups (but not in F2) were seen. In the F1 generation at the high dose there was a statistically significant increase in stillbirths (26 vs. 5 in controls), resulting in a lower viability index. No teratogenic effects were observed in this study.

As the studies with DOTE itself showed only some evidence of developmental toxicity and its potency seemed to be lower than that of DOTI, the DS proposed classification as Repr. 2; H361d.

Lactation

In the two-generation study with DOTI/MOTI, postnatal deaths occurred during the lactation period. However, the DS concluded that a contribution of the test substance in milk for the observed effects could not be estimated, and only the (assumed) presence of substance in milk was not sufficient justification for classification for effects on/via lactation. No classification for effects on or via lactation was proposed by the DS.

Comments received during public consultation

Five comments that addressed toxicity to reproduction were received during the public consultation.

One was from the Organotin REACH Consortium, which argued that only the two PNDT studies with DOTE itself, and the two-generation study with DOTI should be considered for classification for reproductive toxicity of DOTE, as these were the most relevant studies. According to the registrants, these studies did not warrant classification for reproductive toxicity.

The other four comments were from MSCAs, which all opposed the proposed revision of the current harmonised classification from Repr. 1B to Repr. 2 for development. The main arguments provided were:

- The effects observed in the studies with DOTE are consistent with those induced by DOTI and DOTC;

- As maternal toxicity was very limited, the developmental effects could not be considered secondary to maternal toxicity;
- The new studies had too low dose levels, but the effects observed at the high doses supported classification;
- Differences in the potency between DOTE and DOTI did not result in different hazard categories for reproductive toxicity.

Regarding the two PNDT studies with DOTI, it was also stated that the previous RAC opinion for DOTE considered these studies sufficiently reliable for classification.

An additional notion was that a comparison of ED10 between DOTE and its analogues could be useful for a comparison of their potency.

Assessment and comparison with the classification criteria

Fertility

There is no reproductive toxicity study available with DOTE itself that includes information on effects on sexual function and fertility. However, a two-generation study (OECD TG 416) with DOTI/MOTI (80:20) and a reproductive toxicity screening study (OECD TG 421) with DOTC are presented in the CLH dossier. The DOTI/MOTI study is relevant, but it is not clear to what extent the DOTC data can be read across to DOTE considering the new *in vitro* data showing no transformation of DOTE to DOTC *in vitro*.

No effects were observed on the reproductive organs or fertility indices in these studies. In the two-generation study, a significant increase in stillbirths occurred in the F1-generation. In the screening study on DOTC, the mid- and high dose induced an increase in post-implantation loss. However, as all effects occurred post-implantation, RAC agrees with the DS that they should be considered as developmental effects.

As there were no effects on sexual function and fertility observed in the two-generation study with the read-across substance DOTI/MOTI (80:20), **RAC concludes that no classification is warranted for effects on sexual function and fertility**.

Development

In 2012, DOTE was classified by RAC as Repr. 1B; H360D, based on read-across from DOTI and DOTC. The dossier included the same five read-across studies as the current dossier, but lacked the PNDT studies with DOTE itself.

The classification at that time was based on the following effects, exerted by DOTI:

- reduction in foetal body weight in rabbits and mice;
- increased post-implantation loss in rabbits;
- abortions in rabbits;
- increased number of stillbirths in rats;
- increased rate of pup mortality in rats (PND 4 > PND 1, reduced lactation index
- (PND 21));
- increased incidences of minor visceral anomalies, skeletal head anomalies, and skeletal variations in rabbits;
- increased incidences of skeletal variations, skeletal abnormalities, cleft palate, and exencephaly in mice;
- reduced thymus weights in F1 pups (indicative for developmental immunotoxicity ≥60 ppm (≥4.4 mg/kg bw/d, two-generation study, rat));

In addition, the classification opinion referred to these effects seen after exposure to DOTC:

- increased post-implantation loss in rats and mice;
- reduced T-cell mitogen response (indicative for an immunosupressive effect) in directly dosed weanlings (rats) on PND 3-24, indicating that weanlings are more sensitive than young adults.

RAC considered these effects as clear evidence of developmental toxicity in three species, while there were no or only slight signs of maternal (thymo-)toxicity (RAC, 2012). Regarding the limited reporting of the PNDT study with DOTI in mice, RAC stated: "*Details may be lacking since data requirements for a full study report to achieve compliance to testing guidelines are higher. Nevertheless, there is no obvious reason to question the results of this published study. Dose dependency of effects and consistency with other studies support the reliability of the study.*"

As to the incidence of infectious disease in the PNDT in rabbits with DOTI, RAC noted: "*Industry concluded that robustness of this study was compromised by infections. RAC did not share this view: The original study did not report other animals to be affected by infectious diseases.*" Also Industry's view is not in agreement with the overall conclusion of the study author in the original study report: "*At the high dose level of 100 mg/kg/d, clear-cut embryotoxic effects, i.e. an increased rate of abortions and embryolethal effects as well as marked retardations of fetal development, were induced by the test substance.*" and "*marginal retardation effects on fetal development could be attributed to treatment with the intermediate dose of 10 mg/kg/day*".

RAC also concluded that the observed developmental toxicity was not considered to be a secondary non-specific consequence of the (thymo-)toxicity.

In the two-generation study (OECD TG 416; Anonymous, 1997) performed with the read-across substance DOTI:MOTI, a statistically significant increase in stillbirths occurred in the F1-generation, leading to a lower viabilitiy index. In the screening study on DOTC, the mid- and high dose induced a reduced live birth index and an increase in post-implantation loss.

Considering the previous RAC opinion, the main question is whether the two new PNDT studies with DOTE show a qualitative difference in the developmental toxicity of DOTE compared to DOTI and DOTC, which would invalidate the previous conclusion.

As described previously in this opinion (see RAC general comment), new *in vitro* hydrolysis data indicate that DOTC is not formed from DOTE, although the exact *in vivo* metabolism of both DOTE and DOTC is still unclear. DOTC and DOTE induce similar thymotoxicity, which indicates they share similarities in their toxicity profiles. There is no data on the MoAs of these organotins for either reproductive toxicity or thymotoxicity, i.e. it is not known if it is the parent substance or active metabolites that exert the toxicity. For these reasons, RAC considers that the DOTC data cannot be ignored and should be used in a weight of evidence (WoE), but not necessarily in a strict read across approach. In fact, it is noted that the effects seen in the studies with the close analogue DOTI are already sufficient to support for classification and labelling, whitout the need for considering DOTC data.

It is difficult to assess whether there are qualitative differences between these substances based on the available database, but small differences would not affect the classification.

The PNDT study with DOTE in rabbits showed a significant negative trend on foetal weight and crown-rump length (see table below, and also table 3 in the response to comments document). In the rabbit PNDT study with DOTI there was also a reduction in foetal weight at the high dose (100 mg DOTI/kg bw/d). The crown-rump length was not mentioned, but there were multiple skeletal/visceral abnormities that indicate a disturbance/delay in foetal development. The study

with DOTI also showed an increase in post-implantation loss at 100 mg/kg bw/d. This effect was not seen in the study with DOTE, however, there was one litter with only dead foetuses.

It should be noted though that the control group in the rabbit PNDT with DOTE had relatively small litters (mean size 4.9±1.4 in controls, no comparison with historical control data (HCD) was available) and high incidence of skeletal malformations/variations, which decreases the chances of finding a statistically significant effect. Moreover, only slight maternal toxicity was observed at the top dose (thymus weight decreased by 13% as compared to controls), which indicates that the dose levels were too low to really study developmental toxicity and to determine a dose-response relationship in this study.

Nevertheless, the effects on foetal weight and crown-rump length confirm that DOTE interferes with foetal development in rabbits.

***Table**: Summary of results in the rabbit PNDT study with DOTE*

	0 mg/kg bw/d	4 mg/kg bw/d	20 mg/kg bw/d	80 mg/kg bw/d[1]
Maternal thymus weight (g)	2.24 (100%)	2.12 (94.9%)	2.02 (90.4%)	1.95 (87.2%)
Implantation sites	5.1	6.0	5.5	5.0
Live foetuses	4.9	5.7	5.0	4.6
Dead foetuses	0	2	2	3
Gravid uterus w	230.1	309.5	253.7	196.9
Pre-implantation loss (%)	0.9	0.8	2.3	4.9
Post-implantation loss (%)	3.1	3.5	6.4	5.7
Foetal weight (g)	36.6	37.3	35.5	32.3
Crown rump length (mm)	92.1	91.1	89.3	82.3*
Sternum No 5 absent	7 (7.6%)	4 (3.4%)	4 (4.2%)	5 (4.3%)
Sternum No 5 poor ossification	4 (4.4%)	1 (0.8%)	4 (4.2%)	10 (8.7%)
Sternum No 6 poor ossification	4 (5.3%)	1 (0.8/%)	3 (3.2%)	10 (8.7%)

[1]One litter with foetal loss excluded

*P<0.05

In the PNDT with DOTE in mice, the only developmental effect was a significant positive trend in the percentage of post-implantation loss (0.0±0.0, 0.9±2.8, 1.5±4.9, 2.6±5.6% in control, low-, mid- and high dose, respectively). However, as noted by the DS and several member states, the highest dose in this study (60 mg/kg bw/d) was notably lower compared to the highest doses in the studies with the analogues. The maternal effects observed at 60 mg DOTE/kg bw/d were restricted to a numerically decreased corrected body weight gain, but the decrease was not statistically significant.

According to the registration dossier of DOTE, the dose selection rationale of this study was based on the PNDT study with DOTI. The choice of 60 mg/kg bw/d was based on the maternal and developmental effects observed with DOTI at 67 and 100 mg/kg bw/d:

"*Therefore, the high dose chosen for this study is 60 mg/kg, to reflect a dioctyltin dose with minimal maternal and foetal toxicity as the upper bound. It is anticipated that this dose will meet the developmental toxicity test guideline criteria of producing some maternal toxicity without compromising the survival of the pregnant dam, the integrity of pregnancies to Day 18, or the survival of the developing foetuses*."

The use of such dose levels might have been justified if there would have been overt maternal toxicity in the PNDT study with DOTI. However, as stated by RAC in 2012, maternal toxicity caused by the dose levels used (67 and 100 mg/kg bw/d) can be characterised as slight (mainly decreased thymus weight). Moreover, if the goal is to determine whether a substance is toxic for development, dose levels minimising the chances to observe adverse effects on the integrity of pregnancies or the survival of the developing foetuses may not be appropriate.

Thus, RAC notes that the highest dose of DOTE was similar to the dose of DOTI where the dose-response for reproductive toxicity started.

The trend to an increase in post-implantation loss does indicates that DOTE induces foetal mortality in mice, similar to DOTI.

Considering the outcome of the PNDT studies with DOTE, RAC considers it justified to assume that DOTE induces developmental effects similar to DOTI and DOTC. The low dose levels in the studies with DOTE limits the opportunity to perform a direct comparison of the quantitative differences in the potency. There is no ground to derive a specific concentration limit for DOTE.

As there are no data in humans, classification in Category 1A is not warranted.

The differentiation between Category 1B and Category 2 depends on the strength of the evidence, including the nature of the effects observed, the quality of the data, and the relevance of the effect for humans.

The DS proposed Category 2 for development, based on the two PNDT studies with DOTE that show only slight adverse effects on development. However, considering the marginal maternal toxicity at the highest dose levels (60-80 mg/kg bw/d) and the significant trends in developmental toxicity seen, it is highly probable that DOTE would show clear evidence of adverse effects on development at higher dose levels. This is also supported by the results of the studies with the closely related substance DOTI, which included increased post-implantation loss, increase incidence of resorption, increase pup mortality. Given the close structural similarity between DOTE and DOTI, the clear evidence of developmental toxicity in the studies with DOTI and the outcome of the PNDT studies with DOTE which indicate that DOTE has comparable effects, RAC considers that there is sufficient evidence to **retain the current harmonised classification of DOTE as Repr. 1B (H360D)**.

Lactation

In the two-generation study with DOTI, there was an increase in growth retardation and pup losses between PND4 and PND21 in all dose groups in both F1 and F2 generations (dead pups: 0, 4, 11, and 20 in the F1 and 9, 26, 29, and 22 in the F2 at 0, 1.6, 4.7, 16 mg/kg bw/d). The explanation presented by the DS was that pups experience a higher exposure as they are exposed through both maternal milk and from the diet, although exposure via milk was only assumed and not measured. This explanation seems plausible, however RAC notes that although pup body weight was not significantly decreased compared to controls at birth (3-4%), whereas it was significantly decreased (19-21%) by PND14, no further decrease in pup body weight was noted from PND14 to 21 when additional exposure via food becomes more important. Although the

lactational period seems the most sensitive for pup toxicity, the concentration of DOTI in maternal milk and the (relative) exposure of the pups through milk were not determined. Also, it is possible that the growth retardation and pup mortality were late effects of the developmental toxicity experienced during gestation, which RAC already concluded that DOTE warrants classification for.

Hence, RAC agrees with the DS that there is at present **insufficient evidence to justify classification for effects on or via lactation**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

DOTE is used as a stabiliser in plastic and currently has no classification for hazards to the aquatic environment in Annex VI to CLP.

The DS proposed classification as Aquatic Chronic 2. DOTE was considered not rapidly degradable based on its hydrolysis rate and results from three OECD TG 301 degradation tests. A reliable study on the BCF of DOTE is not available but on the basis of a log K_{ow} of 15.35 (calculated) and the high weight of the molecule (751.8 g/mol) and poor water solubility (0.001 pg/L, calculated), DOTE was considered to have a low potential for bioaccumulation. Acute aquatic data are available for fish, invertebrates and algae. Invertebrates are the most sensitive trophic level with a 48-h EC_{50} of 24.12 mg/L for *Daphnia magna* that is based on nominal concentrations. This value is higher than the calculated water solubility of DOTE. Chronic aquatic data that are considered reliable are only available for daphnids and algae. The lowest chronic endpoint is a 21-d NOEC of 0.286 mg/L for *Daphnia magna* that is based on measured test concentrations. On the basis of this endpoint classification as Aquatic Chronic 2 was proposed.

The water solubility of DOTE could not be determined experimentally and was calculated as 0.001 pg/L. The vapour pressure was reported as $<2.50 \times 10^{-4}$ Pa due to significant differences between individual measurements. Data on sorption is not available but based on the estimated high log K_{ow} value (15.35) adsorption to sediment and soil is expected. In the past, many studies were performed with DOTE that contained 6-12% of an impurity (ethylhexylthioglycolate, EHTG; CAS 7659-86-1), which is more toxic than DOTE. Consequently, these studies were not used for classification purposes.

Degradation

The hydrolysis of DOTE was tested according to OECD TG 111. The half-life for pH 4, 7 and 9 was calculated to be >1 year. Ready biodegradation was tested following OECD TG 301F (one test) and OECD TG 301B (two tests). The degradation in these three tests varied from 11 to 43% over 28 days, indicating that DOTE was not readily biodegradable. On the basis of this information DOTE was considered not rapidly degradable for classification purposes.

Bioaccumulation

A log K_{ow} of 15.35 was calculated for DOTE using Kowwin v1.68. One bioaccumulation study according to OECD TG 305 was available. The test was performed in a flow-through system at exposure concentrations of 0.25 and 2.5 µg/L. The reported BCF values were 1294 L/kg and 99 L/kg for the two concentrations respectively. The values are based on the limit of quantification as DOTE could not be detected in the fish tissue. Since the exposure concentrations are above

the reported water solubility, the study is considered unreliable. On the basis of the estimated log K_{ow} of 15.35 and the weight of the molecule of 751.8 g/mol, DOTE was considered to have a low potential for bioaccumulation.

Aquatic toxicity

Valid acute aquatic toxicity data are available for fish, invertebrates and algae with invertebrates being the most sensitive trophic level. For chronic toxicity data, valid data are available for invertebrates and algae. In all studies actual test concentrations were determined and endpoints are based on mean measured concentrations or based on nominal where the measured concentrations were within 20% of nominal. The test concentrations in the acute fish test and the chronic invertebrate test were prepared from Water Accommodated Fractions (WAF), and the concentration of the test substance was analytically confirmed. All relevant information on aquatic toxicity considered reliable is presented in the following table:

Guideline / GLP status	Species	Endpoint	Exposure		Results		Reference
			Design	Duration	Endpoint	Toxicity (mg/L)	
OECD TG 203/EU C.1 Limit test GLP study Purity not reported WAF used	*Danio rerio*	n.r.	*Semi-static*	96 h	LC_{50}	>24.8 (nominal)	Anonymous (2004a)
OECD TG 202 Vehicle DMSO GLP study	*Daphnia magna*	immobility	*Static*	48 h	EC_{50}	24.12 (nominal)	Anonymous (2016a)
OECD TG 211	*Daphnia magna*	reproduction parental survival, mobile offspring, body length	*Semi-static*	21 d	NOEC NOEC	1.448 (mean measured) 0.286 (mean measured)	Anonymous (2004b)
OECD TG 202 Limit test Vehicle DMSO GLP study	*Pseudokirchneriella subcapitata*	growth rate	*Static*	72 h	EC_{50} NOEC	>100 (nominal) ≥100	Anonymous (2016b)

An acute limit test (according to OECD TG 203) was performed with *Danio rerio*. The test solution (renewed daily) was generated from a Water available fraction (WAF) and the actual concentration was determined by measuring the total Sn and calculating this back to DOTE. The purity of the test substance was 87.5 %. No effects were observed at the limit concentration of 24.8 mg/L.

A static acute study (according to OECD TG 202) with *Daphnia magna* was performed. For preparation of the test solution, DMSO was used at a concentration of 0.01%. The purity of the test substance was 99%. The measured concentrations were within 20% of nominal and

endpoints are based on nominal concentrations. Effects were observed and an EC_{50} of 24.12 mg/L was reported. No effects were observed in the solvent control.

A static algal growth inhibition limit test (according to OECD TG 201) with *Pseudokirchneriella subcapitata* was performed. For preparation of the test solution, DMSO was used at a concentration of 0.01%. The purity of the test substance was 99%. The measured concentrations were within 20% of nominal and endpoints are based on nominal concentrations. No effects were observed with an EC_{50} of >100 mg/L and NOEC ≥100 mg/L reported. No effects were observed in the solvent control.

A chronic toxicity test (according to OECD TG 211) with *Daphnia magna* was performed. The test solution (renewed daily) was prepared by the Water available fraction (WAF) approach and the actual concentration was determined by measuring the total Sn and calculating this back to DOTE. The purity of the test substance was 87.5 %. Effects were observed with a $NOEC_{reproduction}$ of 1.448 mg/L reported. For parental survival, mobile offspring and body length a NOEC of 0.286 mg/L was reported.

Based on the available information for aquatic toxicity, the DS concluded that DOTE does not meet the criteria for Aquatic Acute 1. For chronic toxicity, the DS concluded that DOTE meets the criteria for Aquatic Chronic 2 - H411 based on the NOEC of 0.286 mg/L.

Comments received during public consultation

Five MSCAs and one Company-Manufacturer commented during the public consultation. Two of the MSCAs supported the proposed classification by the DS.

One MSCA supported the classification as Aquatic Chronic 2 proposed by the DS but did not agree with the conclusion that the substance does not fulfil the criteria for Aquatic Acute 1. They also asked for clarification as to whether the endpoint from the acute Daphnia study was based on nominal or measured concentrations. For the conclusion on Aquatic Acute 1, the DS replied that there are no results from a reliable short-term test meeting the classification criteria. Considering the nominal or measured concentrations, the DS clarified that the endpoint was based on nominal concentrations as the measured concentrations were within 20% of nominal.

One MSCA requested additional study details on the hydrolysis study because the conclusions would be contradictory when compared with previous assessments. For the bioaccumulation the MSCA commented that in an earlier PBT assessment on the substance and ECHAs conclusion for transitional substances, data uncertainties and interpretation with the B/vB criteria were considered and that it is unclear whether the bioaccumulation would be below 500 L/kg. The MSCA requested further data relating to the molecular weight which would result in DOTE not meeting the bioaccumulation criteria. For the hydrolysis, the DS provided additional study details supporting their conclusion on hydrolysis. For bioaccumulation, the DS replied that in the ECHA conclusion it was stated that no definitive BCF value is available and that there were some uncertainties regarding the interpretation. The BCF of the dioctyltin substances would be perhaps around a maximum 1000 L/kg but probably much lower. The DS mentioned that they based their conclusion on the weight of evidence (log K_{ow}, molecular weight and water solubility).

One MSCA supported aquatic chronic classification in at least Category 2. However, they also stated that they are of the opinion that classification in Category 1 cannot be excluded because of the limited dataset. The DS replied that new tests cannot be requested and that the proposal is based on the available data.

A Company-Manufacturer explained about the process-related impurity, the ligand EHTG (EC 231-626-4). They stated that this contaminant, that is (self-)classified as H410, has caused or influenced the observed effects in the toxicity tests, especially the key study. Studies where a

more purified sample of DOTE was used showed much lower or no toxicity compared to older tests where DOTE was used that contained 6-12% EHTG. The manufacturer proposed that a future classification should be based on the mixture rules for CLP. It was furthermore commented that the test waters were prepared utilising the WAF procedure. As EHTG has a much higher water solubility, the EHTG would have been disproportionally solubilised compared to DOTE itself. Furthermore, the analytical method used to determine the DOTE content in the test media would, during analysis, have dissolved previously undissolved DOTE, as such, the actual concentration of DOTE would have been over estimated. The manufacturer proposes to repeat the chronic daphnia study with purified DOTE. The DS replied that by the WAF method applied in the chronic daphnia study, the disproportional solution of EHTG was minimised. Furthermore, the concentration of the EHTG in the DOTE used would correspond to the levels of EHTG in DOTE currently available. Due to the analytical confirmation, the solution method, relevance for currently available DOTE, and fact that impurities hazardous to the environment present at concentrations >0.1% should be taken into account for the purpose of classification, the chronic Daphia study is considered appropriate for evaluating the ecotoxicological effects of DOTE.

Assessment and comparison with the classification criteria

Degradation

DOTE is hydrolytically stable at environmentally relevant pHs (4, 7 and 9). The half-life for hydrolysis was estimated at >1 year. Three studies on ready biodegradability are available, one according to OECD TG 301F and two according to OECD TG 301B. In the 301F study, 29-43% degradation was observed after 28 days and 36-68% after 74 days. The criteria for ready biodegradability were not fulfilled because of high differences between the replicates. The two 301B studies showed 23 and 19% degradation after 28 days, therefore also not fulfilling the criteria for ready biodegradability. It should be noted that the hydrolysis tests as well as the biodegradation screening tests were performed at exposure concentrations exceeding the water solubility, this could have suppressed the transformation rates since most of the substance was probably not dissolved and as such not available for hydrolysis or biotransformation. Future experiments with lower exposure concentrations might indicate a different outcome. Despite this uncertainty, there is currently no information showing rapid degradation of DOTE under environmental conditions.

In conclusion, RAC agrees with the DS proposal to consider DOTE as not rapidly degradable for the purpose of classification and labelling.

Aquatic bioaccumulation

A study on the bioaccumulation of DOTE is available in the dossier but this study is considered unreliable because the exposure concentrations greatly exceeded the water solubility of DOTE. DOTE was not detected in fish tissue above the limit of detection. RAC notes that the reported limit values may actually be underestimated since the actual dissolved concentrations are not known. The log K_{ow} reported as 15.35 (KOWWIN v1.68; EPI Suite) should be considered unreliable because it is a QSAR-estimated value (KOWWIN v1.68; EPI Suite) and substances with such a high hydrophobicity are generally out of the domain of the calculation programs and the maximum log K_{ow} value in the training set is around 10. The log K_{ow} is therefore considered as >10.

The conclusions of the DS on potential for bioaccumulation are based on the log K_{ow} of 15.35 and the high molecular weight of 751.8 g/mol. There is no guidance that links the log K_{ow} and molecular weight to the BCF cut-off value of 500 L/kg in the CLP criteria. The log K_{ow} of >10 could be used in a weight of evidence approach to suggest that the BCF could be lower than 2000 L/kg (REACH PBT Guidance, R.11 - Appendix R.11-1). However, the available estimated log K_{ow}

by itself is considered to be insufficient for a weight of evidence assessment. Therefore, it cannot be excluded that the BCF will be higher than 2000 L/kg and consequently it also cannot be excluded that it will be higher than 500 L/kg.

On this basis, RAC disagrees with the DS's proposal to consider DOTE as a substance with a low potential to bioaccumulate and considers DOTE to have a potential for bioaccumulation.

Aquatic toxicity

Short-term aquatic toxicity

Short-term toxicity data for DOTE that are considered reliable for classification purposes are available for fish, invertebrates and algae (see table). The acute endpoints for fish and algae lie above the tested concentration. In these tests, it can be concluded that there are no effects at the maximum water soluble concentration. For *Daphnia magna*, the EC_{50} reported is 24.12 mg/L, although this value lies above the threshold for an aquatic acute classification (1 mg/L). However, this value is higher than the maximum water solubility of 0.001 pg/L reported in the CLH report for DOTE. The endpoints available are based on mean measured concentration or are nominal concentrations, confirmed by measurements. It is possible that the test material was not fully dissolved (despite the filtering of the WAF) and probably included in the chemical analysis as well as any material adsorbed on to the test vessel walls. Toxicity testing with poorly water soluble and hydrophobic substances like DOTE would be better performed in flow-through exposure systems. Nevertheless, effects were observed and the reported concentrations are higher than the estimated water solubility.

The value for the water solubility presented in the CLH report (0.001 pg/L) is a QSAR-estimated value (WSKOWWIN v1.42; EPI Suite) based on a log K_{ow} of 15.35. RAC notes that this value should be used with care since the log K_{ow} in itself has a limited reliability and the maximum log K_{ow} in the training set of the water solubility QSAR is 8.27. RAC estimated the water solubility in the same calculation program with a log K_{ow} of 10, which resulted in a water solubility of 46 pg/L. Even so, EPI Suite has not been validated for chemicals that contain metal in their molecular structure and consequently the water solubility estimates are considered unreliable. Nonetheless, in the absence of an experimentally derived water solubility, the solubility estimate, whilst of limited reliability, provides an indication regarding the water solubility of DOTE. In this context, DOTE can be considered as a poorly water-soluble substance (<1mg/L).

According to the Guidance on the Application of the CLP criteria (p. 561; version 5.0, July 2017), where the acute toxicity is recorded at levels in excess of the water solubility, the $L(E)C_{50}$ for classification purposes may be considered to be equal to or below the measured water solubility. In such circumstances, it is likely that Category 1 for Aquatic Chronic and/or Aquatic Acute should be applied. In making this decision, due attention should be paid to the possibility that the excess undissolved substance may have given rise to physical effects on the test organisms. On the basis of the available data in the study report, we can't rule out the possibility that the substance was in suspension, there is however also no obvious evidence of physical effects (coating/entrapment, etc.) either. Therefore, the outcome from the test is considered reliable and with that it is presumed that physical effects have not occurred. As indicated above, there is no experimentally derived water solubility value. Therefore, the actual water solubility to be used for the classification is unknown. Nevertheless, as mentioned above the calculated water solubility sufficiently indicates that the actual water solubility will be <<1 mg/L.

On this basis, RAC disagrees with the DS and concludes that DOTE warrants classification as Aquatic Acute 1. No M-factor is derived due to the uncertainties in reliably determining the water solubility.

Long-term aquatic toxicity

Long-term toxicity data for DOTE that are considered reliable for classification purposes are available for invertebrates and algae (see table). The key value is a NOEC of 0.286 mg/L for *Daphnia magna*. In the public consultation it was commented that the effects observed are potentially or likely to be caused by the impurity EHTG. However, there are insufficient data to confirm this claim. Furthermore, the level of EHTG in the DOTE tested in the chronic Daphnia study is representative for the currently available DOTE. Therefore, it is concluded that the study is suitable for classification purposes. Also in this case, the reported endpoint highly exceeds the calculated water solubility for DOTE and the same guidance applies as cited above for the acute classification. Since the test concentration is made from the water available fraction where undissolved test substance is removed from the solution, RAC concludes that undissolved substance, and therefore physical effects, are unlikely.

On this basis, RAC disagrees with the DS and concludes that DOTE warrants classification as Aquatic Chronic 1. No M-factor is derived due to the uncertainties in reliably determining the water solubility.

Conclusions for classification

RAC concludes that DOTE fulfils the CLP criteria for **classification as Aquatic Acute 1; H400 and Aquatic Chronic 1; H410.** RAC notes that in the absence of an M-factor, according to Article 10(4) of the CLP Regulation, the manufacturer, importer or downstream user is to set the M-factor based on available data for the substance. When in the future experimental data on the water solubility of DOTE become available, an M-factor might be proposed for inclusion in Annex VI of the CLP.

Additional references

Costlow *et al*., 2017, "Simulated gastric hydrolysis and developmental toxicity of dioctyltin bis(2-ethylhexylthioglycolate) [DOTE] in rabbits and mice". Regulatory Toxicology and Pharmacology 87:23-29. Cited as Anomymous, 2015 in the CLH report.

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).