APPLICATION FOR AUTHORISATION

DNEL SETTING FOR REPROTOXIC PROPERTIES OF

2-Ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate (DOTE)

SUBSTANCE NAME(S):	**2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate (DOTE)**
IUPAC NAME(S):	**2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecan-1-oate**
EC NUMBER(S):	**239-622-4**
CAS NUMBER(S):	**15571-58-1**

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Note of the Committee for Risk Assessment

APPLICATION FOR AUTHORISATION - DNEL SETTING FOR REPROTOXIC PROPERTIES OF DOTE

Pursuant to Article 77(3)(c) of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (the REACH Regulation), the Committee for Risk Assessment (RAC) has adopted a note on DNEL setting for reprotoxic properties of DOTE for the Application for Authorisation.

I PROCESS FOR ADOPTION OF THE OPINION

The Executive Director of ECHA in the mandate of 19.05.2022[1], requested RAC to prepare a note concluding on the DNEL setting for reprotoxic properties of DOTE for the Application for Authorisation.

Rapporteur, appointed by RAC: **Gerlienke Schuur**

Co-rapporteur, appointed by RAC: **Betty Hakkert**

The RAC note was adopted on **1 December 2022**.

The RAC note was adopted by consensus of all members present and having the right to vote.

[1] https://echa.europa.eu/documents/10162/17086/rac_mandate_art77_dnel_dote_en.pdf/67f39264-ab28-957f-3de9-4c61de7dff86?t=1654855596352

Preface

Reference values in the form of DNELs for threshold substances and/or dose- response relationships for non-threshold (mainly) carcinogens are often published in advance of applications for authorisation, so providing greater consistency and better use of the legally defined periods of opinion-development in the Committee for Risk Assessment (RAC)[2].

The derivation of the DNELs follows the method as specified in ECHA Guidance on Information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health.[3]

The DNEL and dose response relationships so derived serve as non-legally binding 'reference values'. They provide applicants with a clear signal as to how RAC is likely to evaluate these important elements of the risk assessment of applications for authorisation.

Summary

The reference DNELs for all routes of exposure of DOTE as agreed by RAC with regards to developmental effects as included in the candidate list provided for in Article 59(1) of the REACH Regulation are summarised in Table 1, e.g.:

Worker, long-term inhalation:	0.025 mg DOTE/m^3
Worker, long-term dermal:	1.8 mg/kg bw/day
General population, long-term oral:	0.0032 mg DOTE/kg bw/day

[2] At the 22nd meeting of the Committee for Risk Assessment (RAC) in September 2012, a proposal to set reference DNELs/DMELs and dose response relationships for substances prior to receiving applications for authorisation was introduced. Following a trial exercise, ECHA agreed to continue the practise, recognising its value to the authorisation process.

[3] https://echa.europa.eu/documents/10162/13632/information_requirements_r8_en.pdf/e153243a-03f0-44c5-8808-88af66223258

Table 1: Overview of derivation of reference DNELs for workers and general population exposed to DOTE by the inhalation, oral and dermal route

Point of departure for DNEL derivation by all routes		
NOAEL of about 1.6 mg DOTI : MOTI (80 : 20)/kg bw/day on increased stillbirths and decreased thymus weights from a two-generation reproduction toxicity study in rats.		
NOAEL (mg DOTE/kg bw/day)	**1.6**	
Inhalation absorption (%)	50	
Oral absorption (%)	20	
Dermal absorption (%)	0.1	
Derivation of Reference DNELs		
	WORKERS	GENERAL POPULATION
Assessment Factors		
Extrapolation LOAEL to NAEL	--	--
Interspecies, allometric scaling rat (not for inhalation)	4	4
Interspecies, remaining differences	2.5	2.5
Intraspecies	5	10
Exposure duration	1	1
Quality of whole database (read-across, lack of data on immune-developmental effects)	1, 5	1, 5
INHALATION		
Adjustment oral to inhalation	1/0.384	1/1.15
Correction for exposure regime (day/week)	7/5	7/7
Absorption percentage rat oral/human inhalation (%)	20/50	20/50
Breathing rate for workers light activity vs rest	6.7/10	--
Corrected NOAEC (mg DOTE/m^3)	**1.6**	**0.56**
Overall assessment factors	62.5	125
Reference DNEL INHALATION (mg DOTE/m^3)	**0.025**	**0.0045**
DERMAL		
Correction for exposure regime (day/week)	7/5	7/7
Absorption percentage rat or rabbit oral / human dermal (%)	20 /0.1	20/0.1
Corrected NOAEL (mg DOTE/kg bw/day)	**448**	**320**
Overall assessment factors	250	500
Reference DNEL DERMAL (mg DOTE/kg bw/day)	**1.8**	**0.64**
ORAL		
Correction for exposure regime (day/week)	—	7/7
Absorption percentage rat oral / human oral (%)	—	20/20
Corrected NOAEL (mg DOTE/kg bw/day)	—	1.6
Overall assessment factors	—	500
Reference DNEL ORAL (mg DOTE/kg bw/day)	—	**0.0032**

Annex: DNEL setting for the reproductive properties of DOTE

1. Relevant Endpoint(s)

For applicants applying for authorisation under Article 60(2) (adequate control route), in order to conclude whether the adequate control is demonstrated, only endpoints (i.e. properties of concern) for which the substance is included in Annex XIV need to be addressed in the hazard assessment. However, information on other endpoints might be necessary for comparing the risks with the alternatives.

For applicants aiming at authorisation based on Article 60(4) (socio-economic analysis route) Article 62(4)(d) also applies and the socio-economic analysis (SEA) route will as a consequence focus on the risks that are related to the intrinsic properties specified in Annex XIV. The SEA should in turn consider the impacts related to such risks. In practice the applicant is expected to provide this information in their Chemical Safety Report (CSR) for which an update may be advisable. However, for an authorisation to be granted, the applicant should also demonstrate that there are no suitable alternatives. In this latter analysis it may be the case that other endpoints than those for which the substance was listed in 'Annex XIV' become relevant in order to demonstrate that no suitable alternative is available.

DOTE (2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate or dioctyltin bis(2-ethylhexyl mercaptoacetate) is included on Annex XIV due to its reprotoxic properties (Article 57(c) of REACH). The basis for the identification of the substance as SVHC was its classification as toxic for reproduction category 1B (H360D). The DNELs proposed in the present document are based on the reprotoxic properties of this substance affecting developmental toxicity.

1.1. Reproductive toxicity / effects on developmental toxicity

DOTE is registered at > 1 000 tonnes per year. Mostly used as stabiliser in plastic.

1.1.1. Closely related substances

In several RAC opinions[4,5,6] on classification of dioctyltin substances, information from studies performed with analogous substances is used in support for classification. DOTE itself contains two stable octyl groups and two labile 2-ethylhexylmercaptoacetate groups. The closely related substance DOTI (dioctyltin bis(isooctyl mercaptoacetate) is an isomer of DOTE, with a slight difference in structure of the C8-alcohol. MOTE (monooctyltin tris(2 ethylhexyl mercaptoacetate) contains one octyl group less and one extra 2-ethylhexylmarcaptoacetate group compared to DOTE, and MOTI (monooctyltin tris(isooctyl mercaptoacetate) is an isomer of MOTE. DOTC (dioctyltin dichloride) contains two octyl groups and two chlorine groups. In general, dioctyltin

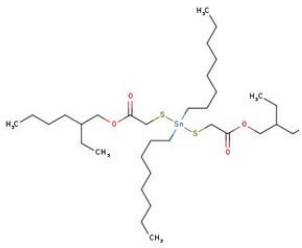
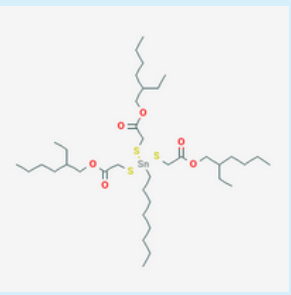
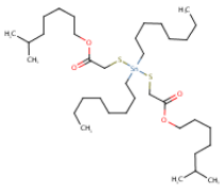
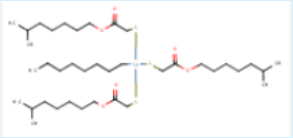
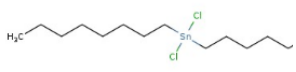
[4] RAC CLH opinion (2012) on DOTE: https://echa.europa.eu/documents/10162/66e6b196-74e2-618f-21ce-8b513c2de8e6
[5] RAC CLH opinion (2018) on DOTE: https://echa.europa.eu/documents/10162/012baffd-3879-dc4c-4f6b-d507e91c6724
[6] RAC CLH opinion (2018) on DOTC: https://echa.europa.eu/documents/10162/7d5c6cee-658c-3dfa-b588-778b180e2fd9

compounds are considered to have adverse effects on the immune system after repeated exposure, demonstrated amongst others as decreased thymus weights.

In the REACH registration dossier on DOTE, the lead registrant submitted for the endpoint developmental toxicity studies performed with the substance DOTE as well as studies performed with the closely related substance DOTI : MOTI (80 : 20).

In 2012, the RAC CLH opinion on DOTE was based entirely on read-across. RAC also considered studies with DOTC relevant in the CLH opinions (2012, 2018). In the following Table 2 the dioctyltin substances addressed in this document are listed with their acronyms, names, and identifiers.

Table 2: Dioctyltin substances considered with regards to their developmental effects

Acronym	Substance name	EC number	CAS number	MW[1]	Chemical structure
DOTE	dioctyltin bis (2-ethylhexyl mercaptoacetate)	239-622-4	15571-58-1	751.8	
MOTE	monooctyltin tris(2-ethylhexyl mercaptoacetate)	248-227-6	27107-89-7	841.9	
DOTI	dioctyltin bis(isooctyl mercaptoacetate	247-666-0	26401-97-8	751.8	
MOTI	monooctyltin tris(isooctyl mercaptoacetate	247-665-5	26401-86-5	841.9	
DOTC	Dioctyltin dichloride	222-583-2	3542-36-7	416.1	

[1] data from ECHA webpage was used in case available

In the CLH opinion DOTE (2018), RAC was of the view that studies on DOTE itself, DOTE : MOTE mixtures, and the structurally very similar substances DOTI and DOTI : MOTI mixtures, should be considered in the hazard assessment of DOTE:

"*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."

1.1.2. Developmental studies

Available studies are summarised in Table 8 in the Annex.

Two pre-natal developmental toxicity (PNDT) studies are available with DOTE, one with rabbits, one with mice. Studies with rats, as well as a generation reproduction toxicity study are lacking. The following information is based on the RAC CLH opinion DOTE (2018).

Studies with DOTE

A PNDT (OECD TG 414) study was performed in rabbits with doses of 0, 4, 20, 80 mg DOTE/kg bw/day. Maternal effects consisted of a dose-dependent decrease 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. Fetal 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 LOAEL was considered to be 80 mg/kg bw/day for both maternal and developmental toxicity. The NOAEL is therefore 20 mg DOTE/kg bw/day.

It should be noted though that the control group in the rabbit PNDT study 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 incidences 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.

The second PNDT study (OECD TG 414) was performed in mice with doses of 0, 15, 30, 60 mg DOTE/kg bw/day. Maternal effects consisted of a decrease in thymus weight at the mid- and high dose groups (23 and 35 %, respectively). 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 LOAEL and NOAEL for maternal effects are 30 and 15 mg/kg bw/day. For developmental effects no LOAEL could be derived (although there is a statistically positive trend in percentage post-implantation loss). The NOAEL is 60 mg DOTE/kg bw/day. However, it should be noted that the highest dose in this study (60 mg/kg bw/day) was notably lower compared to the highest doses in the studies with the closely related substances, and similar to the dose of DOTI : MOTI where the dose-response for reproductive toxicity started.

Studies with other dioctyltin substances

The results from studies investigating developmental toxicity of related dioctyltin (DOT) substances described in the CLH background document and opinion on DOTE (2018) are summarised below.

With the mixture DOTI : MOTI (about 80 : 20) four relevant studies are available (three TG 414 PNDT studies, with rabbits, mice and rats, and a TG 416 2-generation study with rats). Information is derived from the RAC CLH opinion on DOTE (2018).

The first PNDT study with DOTI : MOTI (Battenfeld, 1991) was performed in rats with doses of 1, 5, and 25 mg/bw/day. Both maternal and developmental effects occurred only at the top dose of 25 mg/kg bw/day. 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 NOAEL for developmental toxicity stated in the CLH background document (2018) is 5 mg/kg bw/day.

The second PNDT study with DOTI : MOTI (Battenfeld, 1992) was performed in rabbits at 1, 10, and 100 mg/kg bw/day. 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. The NOAEL for developmental toxicity was reported in the CLH background document (2018) as 10 mg/kg bw/day, however a NOAEL of 1 mg/kg bw/day was stated by WHO (2006, 2020) based

on marginal retardation of fetal development and by MAK (2009) based on increased non-ossified sections in skull (not significant).

The third PNDT study with DOTI : MOTI (Faqi, 2001) was performed in mice at 20, 30, or 45 mg/kg bw/day (group 1); and at 67 or 100 mg/kg bw/day (group 2). Maternal toxicity consisting of a significant decrease in thymus weight occurred from 45 mg/kg bw/day. At 67 mg/kg bw/day an increase in the percentage of resorptions/implantations was observed, as well as an increase in foetal incidence of cleft palates (+ 5.5 %) and a decrease in fetal weight. Skeletal anomalies (supernumerary lumbar ribs) were observed at all groups, but relevance was questioned (MAK, 2009). Further, it should be noted that detailed information on some parameters (such as food consumption, analysis of dosing formulations) was not reported. The NOAEL for developmental toxicity reported in the CLH background document (2018) and by MAK (2009) is 45 mg/kg bw/day.

An oral two-generation reproduction toxicity study in rats (OECD TG 416; Anonymous, 1997) performed with DOTI : MOTI with parental dosing of about 1.5, 4.4, 15 mg/kg bw/day and F1 dosing of about 1.6, 4.7, 16 mg/kg bw/day. In the parent animals, the relative thymus weights were statistically significant decreased at the highest dose, and in males slightly decreased at the middle dose. At the highest dose, there was a slight decrease in pup mortality, significant difference in viability index at day 4 of lactation. Pup body weights were significantly decreased for males and females after 14 and 21 days lactation. In the F1 generation, there was an increase in the number of stillbirths (26 vs 5 in the control group), and a slight increase at the middle dose. At the middle dose, there was a slight decrease in the relative thymus weights in females. At the highest dose, the relative thymus weight was significantly decreased in both sexes and in females also the relative spleen weight was decreased. In the F2 generation, no differences were found. As noted in the CLH background document (2018) and registration dossier, the NOAEL for the F1 generation until weaning was ~1.6 mg/kg bw/day, based on a decrease in relative thymus weights in male and female pups at 4.7 mg/kg bw/day. The NOAEL for the F1 generation post lactation was ~1.6 mg/kg bw/day, based on a slight decrease in the relative thymus weight of males and an increase in stillbirths at 4.7 mg/kg bw/day.

In addition, further studies on DOTC in rats from the CLH background document on DOTC[7] were added.

In a PNDT study (OECD TG 414; Study report 2014) rats were administered 0, 10, 100 or 300 mg DOTC in the diet (ca. 0, 0.8; 7.2; 22.4 mg DOTC/kg bw/day). Maternal toxicity consisted of a significant decrease in thymus weight and reduced body weight changes at 7.2 mg/kg bw/day. At the mid and high dose an increase in foetuses with skeletal malformations was observed. Increased pre-implantation losses were reported with 1.5, 7.0 and 10.4 % for control, mid-, and high dose, respectively. The NOAEL was 0.8 mg/kg bw/day.

A repeated dose 90-day oral toxicity study (OECD TG 408) combined with a reproduction/ developmental screening test (OECD TG 421) (Appel & Waalkens, 2004) was performed in rats with about 0, 0.5 - 0.7, 5.0 – 6.2, 8.4 - 16.6 mg DOTC/kg bw/day. Statistically non-significant but high incidences of post-implantation losses werer observed with 50 % and 70 % in the mid and high dose groups, respectively. The lack of statistical significance is likely due to high variation in some animals and a single dam in the control group with only implantation sites,

[7] https://echa.europa.eu/documents/10162/00e4969a-9026-1080-ef4c-6a01ebb06daa

resulting in a high control incidence of post-implantation loss (23 %). The median post-implantation loss was 7, 11, 50 and 95 % in the control, low, mid and high dose, respectively, and thus indicates a dose-response relationship. The post-implantation loss was accompanied by a statistically significant decrease in live birth index (53 and 60 % in mid and high dose groups compared to 99 % in the control), followed by a 22 and 87 % reduction in postnatal viability at postnatal day (PND) 1 - 4 in the mid and high dose groups, respectively. The pup weight was statistically significantly lower at PND 1 in the high-dose group (3.9 g vs 4.76 g in control), the number of runts was increased in a non-dose dependent manner in all dose groups and the number of cold pups was increased in the high dose group. NOAEL was stated (CLH background document on DOTC, 2018) as 0.5 - 0.7 mg/kg bw/day.

An OECD TG 443, extended one-generation reproductive toxicity study, was performed in rats, with dietary administration of 0.17 - 0.55, 0.6 - 1.9, or 1.7 - 5.2 mg DOTC/kg bw/day (Tonk et al., 2011 a). The highest dose resulted in a non-significant increase in post-implantation loss and small but significant increase in postnatal viability. Effects on the developing immune system observed included changes in thymus weight at the highest dose only, which corresponds to 1.7 - 2.1 mg/kg bw/day during gestation and to 2.9 - 5.2 mg/kg bw/day during lactation. The T-cell–dependent antibody response to keyhole limpet haemocyanin (KLH) was evaluated on PND 21 and 35, and the delayed-type hypersensitivity response against KLH was evaluated on PND 49. No effects were found on PND 21. Effects on lymphocyte subpopulations in the thymus and spleen were observed only at 30 mg/kg diet on PND 42 and 70 (spleen only). The delayed-type hypersensitivity (DTH) response, evaluated at PND 49, was increased in all dose groups with statistical significance in the low and high-dose groups. The increased DTH response and lower thymus weight in the pups at dose levels up to 5.2 mg/kg bw/day confirm adverse effects on the immune system also in developing animals (CLH background document DOTC, 2018).

In a further study, juvenile rats were dosed with DOTC by gavage from PND 10 to PND 21 (0, 0.15, 0.3, 0.5, 1.0, 1.5, 3.0 or 5.0 mg/kg bw/day) and after weaning (0, 3, 6, 10, 20, 30, 60 or 100 mg/kg feed) until time of sacrifice (Tonk et al., 2011 b). Effects included a dose-dependent decrease in F1 body weight, a dose-dependent decrease in absolute and relative thymus weights (BMDL 0.1 mg/kg bw/day), a decrease in thymus cellularity and relative thymic cell count at all time points evaluated, and a dose-dependent decrease in absolute and relative spleen weights and spleen cellularity. Immune effects were more pronounced on PND 21 and 42 than on PND 70 and were observed at lower doses than other developmental effects. The most sensitive immune parameters affected included T-cell–dependent antibody response (TDAR) parameters and thymocyte subpopulations. The KLH-stimulated lymphoproliferative response showed a dose-dependent increase, with a BMDL of 0.003 mg/kg bw/day (Tonk et al., 2011 b).

Tonk et al. notes that the effects on the most sensitive immune parameters occurred at doses well below those showing other developmental effects. The most sensitive developmental immune effect was KLH-specific lymphoproliferative response for which the BMDL was estimated at 0.06 mg/kg feed (about 0.003 mg/kg bw/day) on the basis of extrapolation below the lowest dose tested. For reduced offspring body weight the BMDL was 30.2 mg/kg feed (about 1.5 mg/kg bw/day); the BMDLs differed more than two orders of magnitude, illustrating the selective developmental immunotoxic potential of DOTC in the study design with juvenile rats. This is also the case when compared with the OECD TG 421 with DOTC where stillbirths were noted from circa 5 mg DOTC /kg bw/day.

These effects of DOTC on the developing immune system are also found in comparing rat studies

with DOTC by Smialowicz et al. (2002, 1988). Dosing with 20, 30, 40 or 50 mg DOTC/kg bw/day during prenatal and/or postnatal period resulted in no consistent alteration in immune function in pups. When pups were directly dosed three times a week with 5, 10 or 15 mg DOTC/kg bw/day from PND 3 to 24, a suppression of the lymphoproliferative response to T- and B-cell mitogens was found up to PND 60. Dosing of young adult rats (8 weeks old) with 10 or 20 mg DOTC/kg bw/day showed no effect on lymphoproliferative response. These studies also showed that the developing immune system is more susceptible to DOTC than the mature immune system.

Comparison of the studies

To be able to more quantitatively compare the effects of the different dioctyltin compounds, the doses used in the studies are calculated with regards to the dioctyltin (DOT) content. The molecular weights of the DOT substances and the resulting factors used for adjustment of the doses are the following: DOTC: 0.83; DOTE: 0.459; DOTI : MOTI (80 : 20): 0.448. More detailed information can be found in Table 7 in the Annex.

The available studies with DOTE, DOTI : MOTI (80 : 20), and DOTC indicate:

- The common maternal effect of DOTE, DOTI : MOTI and DOTC is thymus toxicity, reported as decreased thymus weights. Effects on thymus weight were also observed in offspring in the generation study with DOTI : MOTI. Furthermore, developmental immune effects were found in the F1 generation study with DOTC. In addition to decreased absolue and relative thymus and spleen weights, changes in lymphocyte subpopulations were noted at PND 42 in spleen and thymus as well as an increased delayed-type hypersensitivity response.
- Developmental effects frequently observed with this group of substances are decreased fetal body weights, increased pre- and or post-implantation loss in PNDT studies and decreased live pups and/or pup survival (PND 1 - 4) in generation studies.
- Skeletal malformations were observed in studies with DOTC[8] (rats) and DOTI : MOTI (in rabbits and mice). No (statistically significant) skeletal malformations were reported with DOTE; however, as noted in the CLH opinion and background document on DOTE (2018), the highest dose of DOTE was similar to the dose of DOTI (expressed in DOT) where the dose-response for reproductive toxicity started.

It has to be noted that the PNDT studies in rabbits and mice performed with DOTE have relevant shortcomings (e.g., less sensitive species used, not sufficiently high doses applied). A developmental effect relevant for DNEL derivation for DOTE would be an increase in post-implantation loss observed in the PNDT study with mice (Anonymous, 2014b), that showed a dose-related significant trend. In the absence of a NOAEL or LOAEL for this effect, RAC considered BMD modelling. However, the data of the PNDT study was not suitable for this purpose because of the low dosing. No studies have been conducted to examine the effects of DOTE on the developing immune system, which is considered the target organ of the substance.

In the following Table 3, the NOAELs and LOAELs from the developmental studies are summarized with doses related to the **DOT** content of the individual substance.

[8] https://echa.europa.eu/documents/10162/00e4969a-9026-1080-ef4c-6a01ebb06daa

Table 3: NOAEL and LOAEL for (developmental) effects observed with DOT substances

Dose (mg DOT/kg bw/day)		OECD TG	study type, developmental effects, reference	Species	Substance
NOAEL	LOAEL				
9	37	414	PNDT study: reduced foetal crown-rump length, reduced foetal weights (Anonymous, 2014a)	rabbit	DOTE
28	-	414	PNDT study: post-implantation loss (sign. trend; Anonymous, 2014b)	mouse	DOTE
4.5	45	414	PNDT study: reduced foetal weights, increased abortions, increased post-implantation losses, multiple skeletal/visceral abnormalities (Battenfeld, 1992)	rabbit	DOTI : MOTI (80 : 20)
20	30	414	PNDT study: reduced foetal body weights, increased percentage resorptions/ implantations, increased incidence of malformations such as cleft palates, bent ribs (Faqi et al., 2001)	mouse	DOTI : MOTI (80 : 20)
2	11	414	PNDT study: increased post-implantation loss (one litter) (Battenfeld, 1991)	rat	DOTI : MOTI (80 : 20)
0.7	2	416	Two-generation reproduction toxicity study: increased stillbirths, decreased pup body weight during lactation, slight decrease in the relative thymus weights (Anonymous, 1997)	rat	DOTI : MOTI (80 : 20)
0.7	6	414	PNDT study increased skeletal malformations, increased pre-implantation loss (not sign.) (Study report, 2014)	rat	DOTC
0.5	4.5	421	Screening study for reproductive and developmental toxicity: sign. trend for post-implantation losses; increased stillbirths, reduced mean viability index PND 1 - 4 (Appel and Waalkens-Berendsen, 2004)	rat	DOTC

Dose (mg DOT/kg bw/day)		OECD TG	study type, developmental effects, reference	Species	Substance
NOAEL	LOAEL				
		443	Extended one-generation reproductive toxicity (EOGRT) study: reduced number of live pups at high dose (ca. 3 mg DOT/kg bw/day); effects on delayed-type hypersensitivity already apparent at lowest dose level (ca. 0.3 mg DOT/kg bw/day) (Tonk et al., 2011 a)	rat	DOTC
			Study with juvenile rats, dosing by gavage from PND 10 through PND 21 and after weaning from PND 21 via the feed to sacrifice at PND 42, 70 and 90): effect on thymus weights noted at a BMDL of 0.1 mg DOTC/kg bw/day (PND 21), effects on TDAR parameters noted at BMDLs of 0.003 - 0.014 mg DOTC/kg bw/day (Tonk et al., 2011 b)	rats	DOTC

- The results from the PNDT studies with DOTI : MOTI (80 : 20) indicate that rats might be more sensitive (NOAEL 2 mg DOT/kg bw/day) compared to rabbits (NOAEL 4.5 mg/kg bw/day) and even more when compared to mice (NOAEL 20 mg/kg bw/day).
- The rat study with DOTI : MOTI (80 : 20) results in a NOAEL of 0.7 mg DOT/kg bw/day for developmental effects based on a two-generation reproduction toxicity study (Anonymous, 1997). In the two-generation reproduction toxicity study increased stillbirth in F1 was observed at the high dose. The same NOAEL was observed for a decrease in relative thymus weight in both parental animals and pups.
- The studies with DOTC provided NOAELs for offspring mortality (and malformations in the PNDT study) of 0.5, 0.7 and 1.0 mg DOT/kg bw/day for the OECD TG 421 screening study (Appel and Waalkens-Berendsen, 2004) and the PNDT study (Study report, 2014). In the studies from Tonk et al. (2011 a, b), functional immune effects (TDAR and DTH respons in pups/juveniles occurred at much lower dose levels than effects on thymus weight or other developmental effects (about 10-fold lower).
- The lowest NOAELs (expressed in DOT) for effects on post-implantation loss and increases in stillbirths for DOTI : MOTI (80 : 20) of 0.7 mg DOT/kg bw/day and for DOTC of 0.5 mg DOT/kg bw/day from rat studies are very similar.
- The studies from Tonk et al. with DOTC indicate that effects on the developing immune system such as DTH response and TDAR parameters occurred at lower dose levels than other developmental effects such as post-implantation loss and stillbirth.

1.1.1. Point of departure for DNEL derivation

With regards to DNEL derivation for DOTE, there are two scenario's:

Scenario 1: The NOAEL of **20 mg DOTE/kg bw/day** derived from the PNDT study with DOTE in rabbits (Anonymous, 2014a) with the following justification:

- The study is performed with DOTE itself.
- The NOAEL from the rabbit PNDT study is the lowest NOAEL from the studies with DOTE.
- The developmental effects in the rabbit study (reduced fetal weight, reduced fetal crown-rump length) are less severe compared to effects in studies with closely related substances. They are, however, comparable with the effects in studies with closely related substances (reduced fetal weight in the rabbit PNDT study with DOTI : MOTI).

Scenario 2: the NOAEL of about 1.6 mg DOTI : MOTI/kg bw/day (**1.6 mg DOTE/kg bw/day**) derived from the two-generation reproduction toxicity study in rat with DOTI : MOTI (80 : 20; Anonymous, 1997) based on increased stillbirth in F1 as well as (slight) decreases in thymus weights is used as PoD with the following justification:

- Rats seem to be the most sensitive species with regard to embryomortality. No rat studies on reproductive or developmental toxicity with DOTE are available.
- DOTE and DOTI are isomers differing only slightly in the structure of the C-8 alcohol of the mercaptoester ligand (either iso-octanol or 2-ethylhexanol, respectively).
- Support from the PNDT study with DOTI : MOTI in rabbits, providing a NOAEL of 1 or 10 mg/kg bw/day based on reduced fetal weights, increased abortions and post-implantation loss and multiple skeletal and visceral abnormalities at 100 mg/kg bw/day, and a slight, not statistically significant, increase in non-ossified sections in the skull at 10 mg/kg bw/day, as well as from the PNDT with DOTI : MOTI in rats, providing a NOAEL of 5 mg/kg bw/day on post-implantation loss (one litter).

For further information:

- DOTC studies in rats focussing on immune function in the developing animal do indicate these effects occur at about 5 fold lower effect levels than observed for the other effects (stillbirth and post-implantation loss).

1.2. Bioavailability

Oral

In the CSR (2017) the registrant proposed 100 % absorption following oral administration.

No substance specific information is available on absorption via the oral route.

With DOTC, a toxicokinetic study is available (Penninks et al., 1987) in which rats were administered a single dose of radio-active labelled DOTC orally or intravenously. After oral treatment, 80 % of the radioactivity was already excreted in the feces during the first day of treatment. The absorbed dose was calculated to be about 20 %.

DOTE is a somewhat larger molecule then DOTC, the oral absorption might even be lower. RAC proposes to use 20 % for oral absorption (also taken into account the assumption of 50 % for inhalation).

Inhalation

In the CSR (2017) the registrant proposed 100 % absorption following inhalation exposure.

No substance specific information is available on absorption via the inhalation route.

RAC will consider a more substance-specific absorption taking into account the data on oral and dermal absorption, both being relatively low. Therefore, 100 % absorption (default according to the R8 guidance) is considered to be too high, RAC proposes to use a lower value. Based on the proposal for the oral absorption a value of 50 % for the inhalation route is considered proportionate.

Dermal

In the CLH background document for DOTE (2018) the following is reported:

"The absorption of DOTE was measured in vitro (Ward, 2003) through both occluded and unoccluded human and rat epidermis. The absorption through rat epidermis was much faster than through human epidermis:

HUMAN EPIDERMIS: A dose of undiluted liquid DOTE, corresponding to 17 007 µg tin/cm² was determined to slightly reduce the measured electrical resistance across rat skin. Electrical resistance is one indicator of the integrity of the barrier function of the epidermis. The measured reduction was minimal [3.13 ohms versus < 3.00 ohms indicative of undamaged skin]. Because human skin is typically more robust than rat skin, the authors chose to continue with the 17 007 µg tin/cm² dose, which was the highest dermal dose achievable, and both the rat and the human skin samples were judged to be entirely adequate for the integrity of the dermal penetration test.

From the occluded and unoccluded applications, the rates of tin absorption over the 0-24 h exposure period were below the limit of quantification (0.001 µg/cm²/h). In terms of percent applied tin, 0.0001% was absorbed from the occluded dose, and 0.0001% was absorbed from the unoccluded dose after 24 hours of exposure.

RAT EPIDERMIS: Absorption of tin through rat epidermis was much faster than through human epidermis. From the occluded application, the maximum rate of tin absorption (0.035 µg/cm²/h) occurred during 16-24 hours of exposure, and the mean rate of tin absorption over the whole

24-h exposure period was 0.021 µg/cm²/h. From the unoccluded application, the maximum rate of tin absorption occurred during 12-24 hours of exposure and was 0.033 µg/cm²/h. The mean rate of tin absorption over the whole 24-h exposure period was 0.025 µg/cm²/h. In terms of percent applied tin, 0.003% was absorbed from the occluded dose, and 0.004% was absorbed from the unoccluded dose after 24 hours of exposure. The overall recovery of tin from the test system after 24-h exposure was low and may be due to adsorption of the test substance to the glass equipment used. The recovery was 45.5% (human) and 25.2% (rat) of the applied occluded doses, and 29.6% (human) and 30.5% (rat) were recovered from the unoccluded test systems. Of the recovered tin, 2.1% (human) and 5.5% (rat) were obtained from the surface of the epidermis and donor chamber. The mean amounts of tin absorbed by 24 hours were 0.010 µg/cm² (unoccluded) and 0.011 µg/cm² (occluded) through human epidermis and 0.641 µg/cm² (unoccluded) and 0.547 µg/cm² (occluded) through rat epidermis.

These results show that the absorption of tin from DOTE through rat epidermis significantly overestimated the absorption from human epidermis. However, by 24 hours only a small amount of the applied tin (3% in human and 1% in the rat) is associated with the epidermis and is not regarded as systemically available. Thus, based on the low recovery the reliability of the study is highly questionable."

In the CSR (2017), the registrant used a value for dermal absorption of 0.004 % for DNEL derivation.

However, from the available information it is difficult to make a firm conclusion on the percentage of absorption. According to OECD TG 428[9] recovery should be 100 ± 10 % of the radioactivity and any deviation should be justified. The OECD Guidance Notes on dermal absorption[10] indicate that "*Low recoveries raise the concern that the value for absorbed dose could be lower than that which would be achieved from a study where the recoveries were within the guideline range*." As recovery of the substances in these investigations was within a range of 25.2 to 45.5 %, this study is not suitable to quantify penetration. Moreover, it seems that the applied dose used was high, probably to assess rates at infinite dose levels. Such conditions are not suited to assess the percentage of dermal absorption. In conclusion, the dermal absorption does not seem to be very high, but the current information does not allow derivation of a well-defined, substance specific percentage. A similar study with DOTC was also reported to have a low recovery (MAK, 2009).

RAC acknowledges that dermal absorption seems to be (very) low for DOTE. However, the value of 0.004 % derived in the *in vitro* study with DOTE is not reliable and might be too low due to the low recovery in the experiments and the rather high concentration applied. In the absence of information, R8 guidances proposes a default value of 10 % dermal absorption for high MW substances like DOTE. In the current case there is substance specific information, although of poor quality, that points towards very low dermal absorption. In view of that RAC proposes not to use the default value but to use a value of 0.1 % which in between the default percentage and the percentage derived from the poor quality study.

[9] https://www.oecd-ilibrary.org/docserver/9789264071087-en.pdf?expires=1655713244&id=id&accname=guest&checksum=75B39038E57289E7E8267DFEFB01CA31

[10] https://www.oecd.org/chemicalsafety/testing/48532204.pdf

1.3. Mode of Action (MoA)

In the CLH opinion on DOTE (RAC 2018), the following is concluded with regards to the Mode of Action of dioctyltin substances:

"*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*."

2. Derived No Effect Levels (DNELs)

2.1. Corrections and assessment factors

Scenario 1:

The **NOAEL of 20 mg DOTE/kg bw/day** derived from the PNDT study with rabbits (Anonymous, 2014a) is used as PoD for DNEL derivation for developmental effects.

In addition, RAC proposes absorption percentages of 50 % for inhalation, 20 % for the oral route, and 0.1 % for the dermal route.

Corrections

Exposure-related corrections include considerations of inhalation rate (higher in workers than in resting rabbits) and exposure frequency.

In the PNDT study with rabbits used as PoD, administration was from GD 6 - 28, and consequently RAC applied adjustment for 5 days per week for workers.

Assessment factors

Inter- and intraspecies variation

Regarding the assessment factors for inter- and intraspecies variation, the default factors as described in the ECHA Guidance R.8 are used: For intraspecies differences AF 5 for workers and AF 10 for the general population, and for interspecies differences AF 2.4 for allometric scaling (rabbits, only when relevant) and AF 2.5 for remaining differences.

LOAEL/NAEL extrapolation

Not required since a NOAEL is available.

Quality of the whole data base

There is some uncertainty with regards to the data on DOTE. In the PNDT rabbit study, the reductions in fetal body weight and crown-crump length were slight (significant trend for fetal body weight, statisticaly sifnficant reduction in the high dose group, but only - 10.7 %). In the mice study, a dose-related statistically significant trend for an increased incidence of post-implantation loss was observed. From the studies with DOTI : MOTI, it can be concluded that rats might be the most sensitive species for effects on the foetus, compared to rabbits and mice.

For this difference in sensitivity, and a PoD based on the rabbit, a factor of 4 is included.

Furthermore, the developmental immune effects reported in the EOGRTS and study with juvenile rats with DOTC (Tonk et al., 2011 a, b) show functional immune effects (TDAR and DTH response in pups/juveniles) at lower levels than effects on post-implantation loss or stillbirth. These effects are indicative of a suppressed immune function. They also occur at lower levels than effects on thymus weights in parental animals or pups. For DOTE, there is a lack of robust data on immune function in the developing animal.

RAC notes that the (developing) immune function/parameters have not been examined in the available DOTE (and DOTI : MOTI) studies and proposes an assessment factor of 5 to compensate for this information gap. The exact figure for the factor is difficult to derive, because of the low dosing and small dose spacing in the Tonk et al. study and a large set of different immune parameters.

Exposure duration

The NOAEL was derived from a PNDT study. Therefore, no AF for exposure duration to address developmental effects is applied.

Conclusion on assessment factors

The total assessment factors are:

- Inhalation: 250 for workers and 500 for the general population.
- Dermal: 600 for workers and 1 200 for the general population.
- Oral: 1 200 for the general population.

Scenario 2:

A **NOAEL of 1.6 mg DOTE/kg bw/day** derived from a two-generation reproduction toxicity study in rats with DOTI : MOTI (80 : 20) (Anonymous, 1997) is used as PoD for DNEL derivation for developmental effects.

In addition, RAC proposes absorption percentages of 50 % for inhalation, 20 % for the oral route, and 0.1 % for the dermal route.

Corrections

Exposure-related corrections include considerations of inhalation rate (higher in workers than in resting rats) and exposure frequency.

In the two-generation reproduction toxicity study in rats with DOTI : MOTI (80 : 20) that is used as PoD, administration was continuously via the diet, and consequently RAC applied adjustment for 5 days per week for workers.

Assessment factors

Inter- and intraspecies variation

Regarding the assessment factors for inter- and intraspecies variation, the default factors as described in the ECHA Guidance R.8 are used: For intraspecies differences AF 5 for workers and AF 10 for the general population, and for interspecies differences AF 4 for allometric scaling

(when relevant) and AF 2.5 for remaining differences.

LOAEL/NAEL extrapolation

Not required since a NOAEL is available.

Quality of the whole data base

There are no data available for rats with DOTE and DOTI : MOTI to compare. Comparison of the mice and rabbit data do indicate that DOTE and DOTI : MOTI behave in a similar way. Comparison of the DOTI : MOTI data for rats, rabbits and mice indicate rats might be more sensitive. Comparing the studies, all converted to dioctyltin, provides comparable NOAELs/LOAELs in the same species. Taking into account the close analogy from DOTE to DOTI, an assessment factor of 1 for read-across is considered.

Furthermore, the developmental immune effects shown in the EOGRTS and study with juvenile rats with DOTC (Tonk et al., 2011 a, b) show that these effects occur at lower doses compared to effects on post-implanation loss or stillbirth. They also occur at lower levels than effects on thymus weights in parental animals or pups. For DOTE, there is a lack of robust data on immune function in the developing animal.

RAC notes that the (developing) immune function/parameters have not been examined in the available DOTE (and DOTI : MOTI) studies and proposes an assessment factor of 5 to compensate for this information gap. The exact figure for the factor is difficult to derive, because of the low dosing and small dose spacing in the Tonk study and a large set of different immune parameters.

Exposure duration

The NOAEL was derived from a 2-generation reproduction toxicity study with DOTI : MOTI (80 : 20). Therefore, no AF for exposure duration to address developmental effects is applied.

Conclusion on assessment factors

The total assessment factors are:

- Inhalation: 62.5 for workers and 125 for the general population.
- Dermal: 250 for workers and 500 for the general population.
- Oral: 500 for the general population.

2.2. DNEL overview

In the following tables the DNELs derived by RAC (addressing reproductive (developmental) toxicity as identified in Annex XIV, Article 57(c) of REACH) are summarised.

Calculations of inhalation DNELs for workers and the general population covering developmental effects are summarised in Table 4, dermal DNELs for workers and the general population in Table 5, and oral DNELs for the general population in Table 6.

2.2.1. Inhalation DNELs

Table 4: DNELs for inhalation, systemic, long-term

DNELs inhalation, systemic, long-term				
	NOAEL of 20 mg DOTE/kg bw/day, based on reduced fetal crown-rump-length and weight in rabbit PNDT study		NOAEL of about 1.6 mg DOTI : MOTI (80 : 20)/kg bw/day on increased stillbirths and decreased thymus weights from a two-generation reproduction toxicity study in rats	
Point of Departure (PoD)	**NOAEL of 20 mg DOTE/kg bw/day**		**NOAEL of 1.6 mg DOTE/kg bw/day**	
CORRECTION	Workers	General population	Workers	General population
Correction for exposure regime rabbit or rat/human (days/week)	7/5	7/7	7/5	7/7
Adjustment route of exposure (rabbit/rat oral to human inhalation): Worker (8 h): 0.072 or 0.384 m^3/kg bw/8 h General population (24 h): 0.216 or 1.15 m^3/kg bw/24 h	1/0.072	1/0.216	1/0.384	1/1.15
Route-specific bioavailability: 20 % oral rat/50 % inhalation humans	20/50	20/50	20/50	20/50
Activity driven differences: At rest 6.7 m^3, light activity 10 m^3	6.7/10	--	6.7/10	--
Corrected PoD for human inhalation (mg/m^3)	**104.2**	**37.0**	**1.6**	**0.56**
ASSESSMENT FACTORS (AFs)	Workers	General population	Workers	General population
LOAEC to NAEC (in case no NOAEC)	—	—	--	--
Interspecies, allometric scaling	—	—	—	—
Interspecies, remaining differences	2.5	2.5	2.5	2.5
Intraspecies	5	10	5	10
Duration	1	1	1	1
Quality of database	4x 5	4 x 5	1 x 5	1 x 5
Remaining	1	1	1	1
Overall AF	**250**	**500**	**62.5**	**125**
Reference DNEL, inhalation, systemic, long-term (mg/m^3)	**0.42**	**0.074**	**0.026**	**0.0044**

2.2.2. Dermal DNELs

Table 5: DNEL for dermal, long-term

DNELs dermal, systemic, long-term				
	NOAEL of 20 mg DOTE/kg bw/day, based on reduced fetal crown-rump-length and weight in rabbit PNDT study		NOAEL of about 1.6 mg DOTI : MOTI (80 : 20)/kg bw/day on increased stillbirths and decreased thymus weights from a two-generation reproduction toxicity study in rats	
Point of Departure (PoD)	**NOAEL of 20 mg DOTE/kg bw/day**		**NOAEL of 1.6 mg DOTE/kg bw/day**	
CORRECTION	Workers	General population	Workers	General population
Correction for exposure regime rabbit or rat / human (days/week)	7/5	7/7	7/5	7/7
Route-specific bioavailability: 20 % oral rat/0.1 % dermal humans	20/0.1	20/0.1	20/0.1	20/0.1
Corrected PoD for human dermal (mg/kg bw/day)	**5 600**	**4 000**	**448**	**320**
ASSESSMENT FACTORS (AFs)	Workers	General population	Workers	General population
LOAEC to NAEC (in case no NOAEC)	—	—	--	--
Interspecies, allometric scaling	2.4	2.4	4	4
Interspecies, remaining differences	2.5	2.5	2.5	2.5
Intraspecies	5	10	5	10
Duration	1	1	1	1
Quality of database	4 x 5	4 x 5	1 x 5	1 x 5
Remaining	1	1	1	1
Overall AF	**600**	**1 200**	**250**	**500**
Reference DNEL, dermal, systemic, long-term (mg/kg bw/day)	**9.3**	**3.3**	**1.8**	**0.64**

2.2.3. Oral DNELs

Table 6: DNEL for oral, long-term

DNELs oral, systemic, long-term				
	NOAEL of 20 mg DOTE/kg bw/day, based on reduced fetal crown-rump-length and weight in rabbit PNDT study		NOAEL of about 1.6 mg DOTI : MOTI (80 : 20)/kg bw/day on increased stillbirths and decreased thymus weights from a two-generation reproduction toxicity study in rats	
Point of Departure (PoD)	**NOAEL of 20 mg DOTE/kg bw/day**		**NOAEL of 1.6 mg DOTE/kg bw/day**	
CORRECTION		-		-
Correction for exposure regime rabbit or rat/human (day/week)		7/7		7/7
Route-specific bioavailability: 20 % oral rat or rabbit/20 % oral humans		20/20		20/20
PoD for human oral (mg/kg bw/day)		**20**		**1.6**
ASSESSMENT FACTORS (AFs)	Workers	General population	Workers	General population
LOAEC to NAEC (in case no NOAEC)	—	—	—	--
Interspecies, allometric scaling	—	2.4	—	4
Interspecies, remaining differences	—	2.5	—	2.5
Intraspecies	—	10	—	10
Duration	—	1	—	1
Quality of database	—	4 x 5	—	1 x 5
Remaining	—	1	—	1
Overall AF	—	**1 200**	—	**500**
Reference DNEL, oral, systemic, long-term (mg/kg bw/day)	**Not relevant**	**0.017**	**Not relevant**	**0.0032**

2.2.4. DNEL conclusion

RAC concludes on the lowest DNELs derived in the two scenario's.

Thus:

Worker, long-term inhalation: 0.025 mg DOTE/m^3

Worker, long-term dermal: 1.8 mg/kg bw/day

General population, long-term oral: 0.0032 mg DOTE/kg bw/day

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4. Annex

Table 7: Names, identifiers and molecular weights of the DOT substances

Substance	EC	CAS	MW[1]	MW (DOT/DOT substance)
DOT	--	15231-44-4, 94410-05-6	345.2 345.142	1
DOTC	222-583-2	3542-36-7	416.061	0.830
DOTE	239-622-4	15571-58-1	751.804	0.459
DOTI : MOTI (80 : 20)			769.823 (601.442 + 168.381)	0.448
DOTI	247-666-0	26401-97-8	751.803	
MOTI	247-665-5	26401-86-5	841.905	

[1]According to ECHA database

Table 8: Results from studies related to developmental toxicity for DOTE, DOTI : MOTI and DTOC

Substance	Study type and details, doses, reference	Dose level (mg/kg bw/d)	
		Maternal	Offspring
Rabbit			
DOTE	OECD TG 414, PNDT study, rabbits, GD6-28; 0, 4, 20, 80 mg DOTE/kg bw/day (Anonymous, 2014a) = 0, 2, 9, 37 mg DOT/kg bw/day	20: NOAEL 80: reduced thymus weights (-12.8% in highest dose, dose-dependent)	20: NOAEL 80: reduced foetal crown-rump length (-11.9%), reduced foetal weights (not sign. but > 10% compared to control)
DOTI : MOTI (80 : 20)	OECD TG 414, PNDT study, rabbits, GD6-18; 0, 1, 10, 100 mg DOTI : MOTI/kg bw/day (Battenfeld, 1992) = 0, 0.5, 4.5, 45 mg DOT/kg bw/day	10: NOAEL 100: increased incidence of abortions (4 dams)	1 or 10: NOAEL 10: not significant increased non-ossified sections in skull 100: reduced foetal weights, increased abortions, increased post-implantation loss (28.4 %), multiple skeletal/visceral abnormalities
Mouse			
DOTE	OECD TG 414, PNDT study, mice, GD5-17; 0, 15, 30, 60 mg DOTE/kg bw/day, (Anonymous, 2014 b), = 0, 7, 14, 28 mg DOT/kg bw/day	15: NOAEL ≥30: reduced thymus weights	≥ 60: NOAEL dose-related increased in post-implantation loss (trend statistically sign.: 0.0, 0.9, 1.5, 2.6 %)

Substance	Study type and details, doses, reference	Dose level (mg/kg bw/d)	
		Maternal	Offspring
DOTI : MOTI (80 : 20)	OECD TG 414, PNDT study, mice, GD6-17; 1st: 0, 20, 30, 45 mg DOTI : MOTI/kg bw/day; 2nd: 0, 67, 100 mg DOTI : MOTI/kg bw/day (Faqi et al., 2001), study not reliable: some detailed info missing; = 0, 9, 13, 20, 30, 45 mg DOT/kg bw/day	30: NOAEL 45: reduced thymus weights (- 15 %) ≥ 67: increased percentage resorptions/implantations 100: 1 death, reduced liver weights	45: NOAEL ≥ 67: reduced foetal body weights, increased resorptions/ implantations, increased incidence of malformations such as cleft palates, bent ribs
Rat			
DOTI : MOTI (80 : 20)	OECD TG 414, PNDT study, rats, GD6-15; 0, 1, 5, 25 mg DOTI : MOTI/kg bw/day (Battenfeld, 1991) = 0, 0.5, 2, 11 mg DOT/kg bw/day	5: NOAEL 25: slight non-sign. reduced corrected body weight (attributed to one single dam)	5: NOAEL 25: significant increase in percentage of dead foetusses (one litter)
DOTI : MOTI (80 : 20)	OECD TG 416, two-generation reproduction toxicity study, rats; 0, 20, 60, 200 ppm DOTI : MOTI in diet; ca. 0, 1.6, 4.7, 15.9 mg DOTI : MOTI/ kg bw/day (F1) (Anonymous, 1997) = ca. 0, 0.7, 2, 7 mg DOT/kg bw/day (F1)	1.6: NOAEL 4.7: reduced relative thymus weights (slight; sign. at 15.9) 15.9: increased thymic involution (sign. in males; P and F1); viability index decreased (F1 only)	1.6: NOAEL 4.7: reduced thymus weights in pups; slight increase in stillbirth (F1) 15.9: increased stillbirths and pup mortality during lactation (in F1), reduced pup body weights (P and F1 gen.)
DOTC	OECD TG 414, PNDT study, rats, GD5-19, 0, 10, 100, 300 mg in diet = 0, 0.8, 7.2, 22 mg/kg bw/day (Study report, 2014) = 0.7, 6, 18 mg DOT/kg bw/day	0.8: NOAEL ≥ 7.2: reduced thymus weights, reduced body weight changes	0.8: NOAEL ≥ 7.2: increased skeletal malformations (sign.), increased pre-implantation losses (not sign.) 22: increased pre-implantation losses (sign.)

Substance	Study type and details, doses, reference	Dose level (mg/kg bw/d)	
		Maternal	Offspring
DOTC	OECD TG 421, screening study, rats, 0, 10, 100, 300 ppm in diet = 0, 0.5-0.7, 5.0–6.2, 8.4-16.6 mg/kg bw/day (Appel and Waalkens-Berendsen, 2004) = ca. 0; 0.5 (0.4-0.6); 4.5 (4–5); 11 (7–14) mg DOT/kg bw/day	Ca. ≥ 0.5-0.7: reduced thymus weights, lymphoid depletion	Ca. 0.5-0.7: NOAEL Ca. ≥ 5.0-6.2: significant trend for post-implantation losses; increased number of stillborn pups, reduced mean viability index PND 1 - 4
DOTC	OECD TG 443, extended one-generation reproductive toxicity study, rats, 0, 3, 10, 30 mg in diet = 0.17 - 0.55, 0.6 - 1.9, 1.7 - 5.2 mg/kg bw/day (Tonk et al., 2011 a) = ca. 0; 0.3 (0.14 - 0.46); 1.0 (0.5 – 1.6); 3 (1.4 - 4.3) mg DOT/kg bw/day	(effects on thymus and hypersensitivity not investigated)	≥ 0.17 - 0.55: increased delayed-type hypersensitivity 1.7 – 5.2: reduced number of live pups, reduced thymus weights
DOTC	Study with juvenile rats, dosing by gavage from PND 10 through PND 21 with 0, 0.15, 0.3, 0.5, 1.0, 1.5, 3.0, or 5.0 mg DOTC/kg bw/day and after weaning from PND 21 via the diet with 0, 3, 6, 10, 20, 30, 60, or 100 mg DOTC/kg feed until time of sacrifice (up to PND 90)	(not investigated)	BMDL for decreased thymus weight at PND 21 was 0.10 mg/kg bw/day, BMDLs on functional immune parameters and thymocyte subset ranging from 0.003 - 0.014 mg/kg bw/day