Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

Dioctyltin bis(2-Ethylhexyl mercaptoacetate)

EC Number: 239-622-4

CAS Number: 15571-58-1

ECHA/RAC/CLH-O-000000243-78-01/A1

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

8 June 2012

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Dioctyltin bis(2-ethylhexyl mercaptoacetate)
EC number:	239-622-4
CAS number:	15571-58-1
Annex VI Index number:	/
Degree of purity:	≥ 80% (w/w)
Impurities:	Mono-n-octyltin tris(2-ethylhexyl mercaptoacetate) (CAS N° 27107-89-7) < 20% (w/w);

Dioctyltin bis(2-ethylhexyl mercaptoacetate) [DOT(2-EHMA)] is often manufactured as a mixture with mono-octyltin tris(2-ethylhexyl mercaptoacetate) [MOT(2-EHMA), CAS No. 27107-89-7].

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI to CLP Regulation	/	/
Current proposal for consideration by RAC	Repr. 2 (H361d)	Repr. Cat. 3; R63
Resulting harmonised classification (future entry in Annex VI to CLP Regulation) based on the proposal by the dossier submitter	Repr. 2 (H361d)	Repr. Cat. 3; R63

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

The proposed harmonised classification is summarized in Tables 3 and 4.

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.7.	Reproductive toxicity	Repr. 2 H361d: Suspected of damaging the unborn child	/	/	

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Signal word: Warning
Hazard statements H361d: Suspected of damaging the unborn child
Precautionary statements P202: Do not handle until all safety precautions have been read and understood.
P280: Wear protective gloves/protective clothing/eye protection/face protection
P308+P313: IF exposed or concerned: Get medical advice/attention.

Proposed notes assigned to an entry: None

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Toxicity to reproduction – development	Repr. Cat. 3 R63: Possible risk of harm to the unborn child.	/	/	

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Indication of danger: -
R-phrases : R63: Possible risk of harm to the unborn child.
S-phrases S36/37/39: Wear suitable protective clothing, gloves and eye/face protection

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Not covered.

2.2 Short summary of the scientific justification for the CLH proposal

Toxicity for reproduction :

A two-generation study (Anonymous, 1997) was performed using mixture of DOT(isooctythioglycolate, (CAS No. 26401-97-8)/Octyltin tris (IOMA) (CAS No. 26401-86-5)) (78.8:16.9% mixture). Dioctyltin bis (IOMA) and dioctyltin bis (2-EHMA) are isomers of the same compound and are expected to be chemically and toxicologically equivalent.Under the experimental conditions of this two-generation study, the NOAEL for the F0 parental generation was 20 ppm (~1.5 mg/kg/bw), based on a reduction in the relative thymus weight of males at 60 ppm. The NOAEL for the F1 generation until weaning was 20 ppm (~1.6 mg/kg/bw/d), based on a decrease in relative thymus weight in male and female pups at 60 ppm. The NOAEL for the F1 generation post-lactation was 20 ppm, based on a slight decrease in the relative thymus weight of males and an increase in stillbirth at 60 ppm.

There is a GLP screening reprotoxicity study according to OECD guideline 421 (Appel and Waalkens, 2004) performed with the hydrolysis product Dioctyltin dichloride (3542-36-7). In this GLP key study, comparable effects were obtained with the 2-generation study, indeed thymus effect were also recorded. Dose-related effects were seen at 10, 100 and 300 mg/kg/day, with post-implantation losses in the top two dose-groups. The maternal LOAEL was set at 10 ppm diet (equivalent 0.7 mg/kg/bw for males and 0.5-0.7 mg/kg/bw for females) for treatment related effects to dams included lymphoid depletion.

There were relevant observed effects in the two generation study performed with DOT (IOMA):MOT(IOMA) (78.8: 16.9%) (anonymous, 1992) and the developmental reprotoxicity studies with DOT (IOMA):MOT(IOMA) 80:20% (Battenfeld, 1991, 1992), particularly the effects on pups such as increase in number of runts, decreased, fetal weight, decreased number of pups per litter, increased post-implantation loss, decrease thymus weight for the F0 parent and F1 progeny. In the developmental study in mice, significantly increased incidence of cleft palate in the fetuses exposed to 67 or100 mg/kg/day were observed, and incidences of bent forelimbs and exencephaly were significant in the fetuses exposed to 100 mg/kg/day. In addition, the screening reprotoxicity study with DOTC support also a part of these particular findings (increase post-implantation loss, decreased viability index, increase number of runts, decreased pups weights) and decrease absolute and relative thymus weight and lymphoid depletion in dams.

Based on these effects, DOT(2 -EHMA) is proposed to be classified with R63: 'Possible risk of harm to the unborn child' according to Directive 67/548/EEC and 'Reprotoxicity category 2', H361d according to regulation EC no.1272/2008 (CLP).

2.3 Current harmonised classification and labelling

The substance is not currently classified in Annex VI of Regulation EC N° 1272/2008.

2.4 Current self-classification and labelling

Industry self-classification is proposed for this substance for inclusion on the publicly available classification and labelling database

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Table 5: Self-classification and labelling according to CLP

Classification	
Acute Tox. 4 (H302) Skin Sens. 1 (H317) Repr. 2 (H361d) STOT-RE 1(H372) Aquatic acute & chronic 1 (H410)	
Labelling	
Signal word	Danger
Hazard statements	H302: Harmful if swallowed H317: May cause an allergic skin reaction H361d: Suspected of damaging the unborn child H372: Causes damage to organs (thymus) through prolonged or repeated exposure (oral) H410: Very toxic to aquatic life with long lasting effects
Precautionary statements	P202: Do not handle until all safety precautions have been read and understood. P260: Do not breathe dust/fume/gas/mist/vapours/spray. P273: Avoid release to the environment P280: Wear protective gloves/protective clothing/eye protection/face protection P308+P313: IF exposed or concerned: Get medical advice/attention. P501: Dispose of contents/container to licensed hazardous waste disposal agent/site in accordance with local, national and regional legislation..

2.4.2 Current self-classification and labelling based on DSD criteria

Table 6: Self-classification and labelling according to DSD

Classification	
Xn; R22 Xi; R38 R43 T; R48/25 Repr. Cat. 3; R63 N; R50/53	
Labelling	
Indication of danger	T: Toxic N: Dangerous for the environment
R-phrases	R22: Harmful if swallowed R48/25: Toxic, danger of serious damage to health by prolonged exposure if swallowed R38: Irritating to skin R43: May cause sensitization by skin contact R63: Possible risk of harm to the unborn child. R50/53: Very toxic to aquatic organisms may cause long-term adverse effects in the aquatic environment.
S-phrases	S24: Avoid contact with skin S36/37/39: Wear suitable protective clothing, gloves and eye/face protection S60 - This material and its container must be disposed of as hazardous waste S61: Avoid release to the environment. refer to special instructions/safety data sheets.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

According to article 36(1), a substance that fulfils the criteria set out in Annex I of the CLP regulation for the following shall normally be subject to harmonised classification and labelling in accordance with Article 37:

(d) reproductive toxicity, category 1A, 1B or 2 (Annex I, section 3.7).

According to Article 37, a manufacturer, importer or downstream user of a substance may submit to the Agency a proposal for harmonised classification and labelling of that substance and, where appropriate, specific concentration limits or M-factors, provided that there is no entry in Part 3 of Annex VI for such a substance in relation to the hazard class or differentiation covered by that proposal..

Currently, DOT(2-EHMA) fulfills criteria of both articles 36(1) & 37. In agreement with these articles, reproductive toxicity is proposed for harmonization in this dossier. Toxicokinetic information and other toxicological data are displayed for information so as to provide a general toxicological profile on DOT(2-EHMA) but are not proposed for harmonization.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

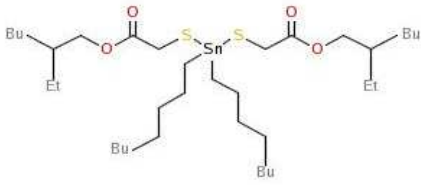
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 7: Substance identity

EC number:	239-622-4
EC name:	2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate
CAS number:	15571-58-1
CAS name	Dioctyltin bis(2-ethylhexyl mercaptoacetate)
IUPAC name:	2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecan-1-oate
CLP Annex VI Index number:	/
Molecular formula:	$C_{36}H_{72}O_4S_2Sn$
Molecular weight range:	751.7945

Structural formula:



1.2 Composition of the substance

Dioctyltin bis(2-ethylhexyl mercaptoacetate) [DOT(2-EHMA)] is always manufactured as a mixture with mono-octyltin tris(2-ethylhexyl mercaptoacetate) [MOT(2-EHMA), CAS No. 27107-89-7] as a highly efficient heat stabilizer in PVC. Moreover, it should be considered that the concentration ratio between [DOT(2-EHMA)] and [MOT(2-EHMA)] can differ depending on the manufacturer of the mixture.

The CLH report and classification and labelling proposal for DOTE have been established based on a purity of minimum 80% in reproductive toxicity studies. Regarding the substance identity, dioctyl bis(2-ethlhexyl mercaptoacetate) will be then considered as a mono-constituent substance.

Table 8: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Dioctyltin bis(2-ethylhexyl mercaptoacetate) EC no: 239-622-4		≥ 80 % (w/w)	

Current Annex VI entry: not relevant

Table 9: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Mono-n-octyltin tris(2-ethylhexyl ercaptoacetate) EC no.: 248-227-6		< 20 % (w/w)	
2-ethylhexyl mercaptoacetate EC no.: 231-626-4		0-0.5% (w/w)	
dichlorodioctylstannane EC no.: 222-583-2		0.-0.5% (w/w)	

Current Annex VI entry: not relevant

Table 10: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
/	/	/	/	/

Current Annex VI entry: not relevant

1.3 Physico-chemical properties

Table 11: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	Liquid, clear colourless to slightly yellow		
Melting/freezing point	-39°C		
Boiling point	No boiling point could be measured by DSC.		The substance decomposes at T >275°C and normal pressure without boiling.
Relative density	1.07 g/cm^3 at 20°C		
Vapour pressure	< 2.50 x 10^{-4} Pa		Due to the behaviour of the test material in the equipment, an exact value for the vapour pressure could not be calculated. Three tests were performed. Significant differences between the individual measurements were observed. The vapour pressure was therefore reported to be lower than the highest measured value at < 2.50 x 10^{-4} Pa
Surface tension	/		not technically feasible as the water solubility of the substance is less than 0.1mg/l.
Water solubility	The following statement was included in a physico-chemical properties study by Baltussen (2010) concerning the feasibility of a water solubility study on the test substance: "The test substance rapidly decomposes in contact with water forming a range of breakdown products. The test substance can only be analysed after derivatisation, but using derivatisation, a distinction between intact test substance and breakdown products can no longer be made. It is not possible to specifically analyse the intact test substance with any technique at low levels which is required due to the expected low water solubility of the test substance" It was concluded that the test on the water solubility of the test substance could not be performed		. study technically not feasible
Partition coefficient n-	A statement concerning the partition coefficient of the test material was		study technically not

octanol/water	included in the physico-chemical testing battery by Baltussen (2010): "The test substance rapidly decomposes in contact with water forming a range of breakdown products. The test substance can only be analysed after derivatisation, but using derivatisation, a distinction between intact test substance and breakdown products can no longer be made. It is not possible to specifically analyse the intact test substance with any technique at low levels which is required due to the expected low water solubility of the test substance." The author concluded that the study is not technically feasible.		feasible
Flash point	182°C		Pensky-Martens closed cup method.
Flammability	Not flammable		
Explosive properties	Not explosive		Expert judgement based on physico-chemical properties and the substance's structure
Self-ignition temperature	390 °C at 989.6 -999.2 hPa.		
Oxidising properties	No oxidising properties		Expert judgement based on physico-chemical properties and the substance's structure
Granulometry	Not relevant		

2 MANUFACTURE AND USES

2.1 Manufacture

Commercial stabilizers consisting of dioctyltin bis(2-ethylhexyl mercaptoacetate) and mono-octyltin tris(2-ethylhexyl mercaptoacetate) are produced from the corresponding mixture of dioctyltin/mono-octyltin chlorides, 2-ethylhexyl mercaptoacetate, and a base. Since the reaction is carried out in water, the organotin stabilizer is isolated by phase separation and eventually filtered to remove solids or stripped to remove volatile components.

2.2 Identified uses

Dioctyltin bis(2-ethylhexyl mercaptoacetate is mostly used as a stabiliser in plastic.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Table 12: Overview of experimental studies on absorption, metabolism, distribution and elimination

Method	Results	Remarks	Reference
in vitro study rat and human epidermis dermal Exposure regime: 24 hour(s) Doses/conc.: 17,007 ug tin/cm^2 OECD Draft Guideline for Dermal Delivery and Percutaneous Absorption: In Vitro Method [OECD TG 428]	Main ADME results: Absorption: Absorption of tin from DOT(EHMA) through rat epidermis significantly overestimates absorption through human epidermis. Evaluation of results: bioaccumulation potential cannot be judged based on study results	2 (reliable with restrictions) key study experimental result **Test material (EC name): 2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate**	Ward, R.J. (2003)
in vitro study no data in vitro A simulated gastric reaction study was performed.	Toxicokinetic parameters: Half-life 1st: Half-life 2nd: Metabolites identified: yes Details on metabolites: DOT(2-EHMA) readily hydrolyzed to DOTC under physiological conditions (pH 1 to 2).	2 (reliable with restrictions) key study experimental result **Test material (EC name): 2-ethylhexyl 10-ethyl-4,4-dioctyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate**	Anonymous (2000)

4.1.2 Human information

No data is available.

4.1.3 Summary and discussion on toxicokinetics

The results obtained from a in vitro gastric hydrolysis study (Yoder, 2000) support the use of DOTC as an appropriate surrogate for mammalian toxicology studies of the corresponding thioesters DOT(2-EHMA) /(IOMA) via the oral route as it was demonstrated that DOTE readily hydrolized to DOTC under physiological conditions (101% hydrolysis within 30 minutes). Thus, DOTC is an appropriate anchor compound and surrogate for the mammalian toxicology endpoints

of repeated dose, in vivo genotoxoxicity reproduction, and developmental effects, when they are assessed using oral administration. Acute toxicity, sensitization, irritation and in vitro genotoxicity are not covered under the category approach and were evaluated individually for each material. DOT (2 -EHMA) and the corresponding thioesters have been therefore joined into one family in a HPV program, presented and validated at OECD (see SIDS 2006, SIAM 23).

With respect to inhalation and dermal mammalian toxicity, the esters have much higher molecular weights and considerably lower volatility than the chloride. The high molecular weights of the esters reduce their potential for absorption via the dermal route, and their volatility reduces their potential for absorption via the inhalation route relative to the chloride.

The category approach was not used for the ecotoxicity and environmental fate endpoints. DOTC is not an appropriate surrogate for the thioesters for the ecotoxicity and environmental fate endpoints. The considerable difference in the structures of the labile ligands causes differences in water solubility between the alkyltin chloride and thioesters affecting their respective bioavailability and distribution in the environment. Furthermore, DOT(2-EHMA) and DOT(IOMA) will degrade in aqueous solution such that organisms will be exposed to the parent material and their different degradation products.

The absorption of DOT(2 -EHMA) was measured in vitro (Ward 2003) though 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 17,007 ug tin/cm^2 was determined to alter the barrier function of the epidermis. 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 ug/cm^2/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 ug/cm^2/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 ug/cm^2/h. From the unoccluded application, the maximum rate of tin absorption occurred during 12-24 hours of exposure and was 0.033 ug/cm^2/h. The mean rate of tin absorption over the whole 24-h exposure period was 0.025 ug/cm^2/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 ug/cm^2 (unoccluded) and 0.011 ug/cm^2 (occluded) through human epidermis and 0.641 ug/cm^2 (unoccluded) and 0.547ug/cm^2 (occluded) through rat epidermis.

These results show that the absorption of tin from dioctyltin bis(2-ethylhexylmercaptoacetate) through rat epidermis significantly overestimated absorption from human epidermis. 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.

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Table 13: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Rat (Tif:RAIf (SPF)) male/female Oral: unspecified Method: OECD Guideline 401	LD_{50}: 2000 mg/kg bw (male/female) LD_{50}: < 2000 mg/kg bw (female)	2 (reliable with restrictions) Key study	Anonymous (1992a)

(Acute Oral Toxicity)	LD_{50}: > 2000 mg/kg bw (male)	Experimental result **Test material:** **Dioctyltin bis(2-EHMA: Octyltin tris(2-EHMA) (purity 90:10% mixture)**	
Rat (Crj: CD(SD)) male/female Oral: gavage Method: EPA OPP 81-1 (Acute Oral Toxicity)	LD_{50}: 1800 mg/kg bw (male/female) LD_{50}: > 2500 mg/kg bw (male) (LD_{50} was estimated to be 3800 mg/kg; the 95% confidence limits were +- 4631 mg/kg and exceed the LD_{50} value because the dose response curve for males was extremely shallow) LD_{50}: 1150 mg/kg bw (female)	1 (reliable without restriction) Supporting study **Test material:** **Di(n-octyl)tin dichloride : tri-(n-octyl)tin chloride : n-octyltin trichloride, (purity 95.7: 2.3 :2.0% mixture)**	Auletta, C.S. and Daly, I.W. (1984)
Mouse ("H" (Czech. standard strain; Velaz Corp.)) male/female Oral: gavage Method not reported	LD_{50}: 2010 mg/kg bw (male/female)	2 (reliable with restrictions) Supporting study Experimental result **Test material:** **Dioctyltin bis(2-EHMA (reported as pure sample)**	Pelikan, Z. and E. Cerny (1970)

4.2.1.2 Acute toxicity: inhalation

No study is available for acute inhalation endpoint.

4.2.1.3 Acute toxicity: dermal

Table 14: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Rat (Tif:RAIf (SPF)) male/female Coverage: semiocclusive Method: OECD Guideline 402 (Acute Dermal Toxicity)	LD_{50}: > 2000 mg/kg bw (male/female)	1 (reliable without restriction) Key study Experimental result **Test material (mixture) :** **Dioctyltin bis(2-EHMA) [CAS No.**	Anonymous (1992)

		15571-58-1]:Octyltin tris(2-EHMA) [CAS No. 27107-89-7] (mixture 70:30%)	
Rat (Tif:RAIf (SPF)) male/female Coverage: semiocclusive Method OECD Guideline 402 (Acute Dermal Toxicity)	LD_0: > 2000 mg/kg bw (male/female) (no mortality)	1 (reliable without restriction) Key study Experimental result **Test material:** **Dioctyltin bis(2-EHMA) : Octyltin tris(2-EHMA) (purity 90:10% mixture)**	Anonymous (1992b)

4.2.1.4 Acute toxicity: other routes

No data is available.

4.2.2 Human information

No data is available.

4.2.3 Summary and discussion of acute toxicity

A robust acute oral toxicity rat study (OECD guideline 401) was carried out with a mixture of DOT (2 -EHMA) and MOT(2 -EHMA) (90:10%). Two doses (1000 and 200 kg/kg bw) were tested (single dose) with a 14 -days observation period. Animals in both dose groups exhibited clinical signs of toxicity and effects on mortality were observed. The LD_{50} was lower than 2000 mg/kg for female rats, the overall LD_{50} for males and females was 2000 mg/kg bw (lower 95% confidence limit= 1265 mg/kg/bw). More studies were available and included as supporting information.

A robust acute dermal toxicity rat study (OECD guideline 402) was carried out with a mixture of DOT(2 -EHMA) and Octyltin tris(2-EHMA) (90:10 % w/w). The test dose was 2000 mg/kg bw; the dose volume applied was 2 ml/kg bw. After 24 hours, the exposed skin was cleaned and the area of application was observed for 14 days. Due to the lack of observed mortality, the 14-day acute dermal LD_{50}s of the test substance were reported as: LD_{50} (both sexes) >2000 mg/kg bw. An other study (OECD 402) was carried out with a mixture of DOT (2 -EHMA) and MOT(2 -EHMA) (70:30%), the same result is observed : LD_{50} > 2000 mg/kg bw.

No information on inhalation toxicity was available.

Information on acute toxicity is reported here for information only, so as to provide a general toxicological profile on DOTE (EHMA). This endpoint is however not proposed for harmonisation.

4.3 Specific target organ toxicity – single exposure (STOT SE)

The acute oral and dermal studies didn't identify target organ toxicity in animals treated with DOTE.

4.4 Irritation

4.4.1 Skin irritation

4.4.1.1 Non-human information

Table 15: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Rabbit (New Zealand White) Coverage: semiocclusive (shaved) Method: OECD Guideline 404 (Acute Dermal Irritation / Corrosion) Observation period : 12 days	Moderately irritating (but not classified) Erythema score: 2.1 of max. 4 (mean (6 rabbits)) (Time point: 24-48-72 hours) (fully reversible within: 11 days) (Mean individual scores : 3-2-2-2-1.67-2) Edema score: 0.33 of max. 4 (mean (6 rabbits)) (Time point: 24-48-72 hours) (fully reversible) (Mean individual scores : 1-0-0.33-0-0.33-0.33)	1 (reliable without restriction) Key study Experimental result **Test material:** **Dioctyltin bis(2-EHMA) (purity > 98%)**	Varsho B.J. (1996)
Rabbit (New Zealand White) Coverage: (shaved) Method: OECD Guideline 404 (Acute Dermal Irritation / Corrosion) Observation period : 10 days	Moderately irritating (but not classified) Erythema score: 1.78 of max. 4 (mean (3 rabbits)) (Time point: 24-48-72 hours) (fully reversible within: 10 days) (Mean individual scores : 2 - 2 - 1.33) Edema score: 1.33 of max. 4 (mean (3 rabbits)) (Time point: 24-48-72 hours) (fully reversible within: 7 days) (Mean individual scores : 1.67 - 1 - 1.33)	1 (reliable without restriction) Key study Experimental result **Test material:** **Dioctyltin bis(2-EHMA) : Octyltin tris(2-EHMA) (purity 90:10% mixture)**	Anonymous (1992c)

4.4.1.2 Human information

No data is available.

4.4.1.3 Summary and discussion of skin irritation

One acute Dermal Irritation / Corrosion GLP test performed according to OECD 404 was carried out with DOT(2 - EHMA) (purity>98%). The test substance was applied undiluted on a patch on shaved rabbit skin. The test material induced slight to moderate erythema on all rabbits and very slight edema on four animals. Three rabbits had desquamation. There were no other dermal findings. All irritations were reversible and completely subsided at day 11 or earlier.

The Primary Irritation Index was calculated to be 2.2.

Information on skin irritation is reported here for information only, so as to provide a general toxicological profile on DOTE (EHMA). This endpoint is however not proposed for harmonisation.

4.4.2 Eye irritation

4.4.2.1 Non-human information

Table 16: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Rabbit (New Zealand White) TSCA Health Effects Test Guidelines, 40 CFR 798.4500 Method : OECD Guideline 405 (Acute Eye Irritation / Corrosion)	not irritating Cornea score: Cornea opacity score : 0 of max. 4 (mean (6 rabbits)) (Time point: 24-48-72 hours) (All mean individual score is 0) Cornea area score: 0 of max. 4 (mean (6 rabbits)) (Time point: 24-48-72 hours) (All mean individual score is 0) Iris score: 0 of max. 2 (mean (6 rabbits)) (Time point: 24-48-72 hours) (All mean individual score is 0) Conjunctivae score: (Redness) 0.5 of max. 3 (mean (6 animals)) (Time point: 24-48-72 hours) (fully reversible within: 4 days) (Mean individual scores : 0.67-0.67-0.33-1.33-0-0) (Chemosis) 0.22 of max. 4 (mean (6 rabbits)) (Time point: 24-48-72 hours) (fully reversible within: 4 days) (Mean individual scores : 0-0.33-0-1-0-0) (Discharge) 0 of max. 3 (mean (6 rabbits)) (Time point: 24-48-72 hours) (All mean individual score is 0)	1 (reliable without restriction) Key study Experimental result **Test material:** **Dioctyltin bis(2-EHMA (purity>98%)**	Varsho, B.J. (1996)

4.4.2.2 Human information

No data is available.

4.4.2.3 Summary and discussion of eye irritation

One in vivo rabbit eye irritation GLP study performed according to OECD 405 was carried out with DOT(2 -EHMA) (purity>98%). The test substance was instilled undiluted in the right lower conjunctival sac. Minor conjunctival

irritation was observed, and no iris or corneal effects. Effects were fully reversible within 96h. The test substance was not considered as an eye irritant.

Information on eye irritation is reported here for information only, so as to provide a general toxicological profile on DOTE (EHMA). This endpoint is however not proposed for harmonisation.

4.4.3 Respiratory tract irritation

No data is available.

4.5 Corrosivity

No data is available.

4.6 Sensitisation

4.6.1 Skin sensitisation

4.6.1.1 Non-human information

Table 17: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Guinea pig (Pirbright White Strain (Tif: DHP)) male/female Guinea pig maximisation test Induction: intradermal and epicutaneous Challenge: epicutaneous, occlusive Method: OECD Guideline 406 (Skin Sensitisation)	Sensitising (according to the Regulation EC no.1272/2008 (CLP)) No. with positive reactions: 1st reading: 0 out of 10 (Control group (induction with vehicle)); 24 h after chall.; dose: 30% 2nd reading: 0 out of 10 (Control group (induction with vehicle)); 48 h after chall.; dose: 30% 1st reading: 9 out of 10 (Control group (induction with test article)); 24 h after chall.; dose: 30% 2nd reading: 9 out of 10 (Control group (induction with test article)); 48 h after chall.; dose: 30% 1st reading: 0 out of 20 (Test group (induction with vehicle));	1 (reliable without restriction) Key study Experimental result **Test material:** **Dioctyltin bis(2-EHMA) :Octyltin tris(2-EHMA) (purity 90:10% mixture)**	Anonymous (1993)

	24 h after chall.; dose: 30% 2nd reading: 0 out of 20 (Test group (induction with vehicle)); 48 h after chall.; dose: 30% 1st reading: 18 out of 20 (Test group (induction with test article)); 24 h after chall.; dose: 30% 2nd reading: 20 out of 20 (Test group (induction with test article)); 48 h after chall.; dose: 30% rechallenge: 0 out of 10 (Control group (induction with vehicle)); 24 h after chall.; dose: 10% rechallenge: 0 out of 10 (Control group (induction with vehicle)); 48 h after chall.; dose: 10% rechallenge: 0 out of 10 (Control group (induction with test article)); 24 h after chall.; dose: 10% rechallenge: 0 out of 10 (Control group (induction with test article)); 48 h after chall.; dose: 10% rechallenge: 0 out of 20 (Test group (induction with vehicle)); 24 h after chall.; dose: 10% rechallenge: 0 out of 20 (Test group (induction with vehicle)); 48 h after chall.; dose: 10% rechallenge: 17 out of 20 (Test group (induction with test article)); 24 h after chall.; dose: 10% rechallenge: 16 out of 20 (Test group (induction with test article)); 48 h after chall.; dose: 10%		
Guinea pig (Pirbright White Strain (Tif: DHP)) male/female Guinea pig maximisation test Induction: intradermal and epicutaneous Challenge: epicutaneous, occlusive Method : OECD Guideline 406	Sensitising No. with positive reactions: 1st reading: 0 out of 10 (Control group (induction with vehicle)); 24 h after chall.; dose: 50% 2nd reading: 0 out of 10 (Control group (induction with vehicle)); 48 h after chall.; dose:	2 (reliable with restrictions) Supporting study Experimental result **Test material: Dioctyltin bis(2-EHMA) : Octyltin tris(2-EHMA)**	Anonymous (1993)

(Skin Sensitisation)	50% 1st reading: 3 out of 10 (Control group (induction with test article)); 24 h after chall.; dose: 50% 2nd reading: 5 out of 10 (Control group (induction with test article)); 48 h after chall.; dose: 50% 1st reading: 0 out of 20 (Test group (induction with vehicle)); 24 h after chall.; dose: 50% 2nd reading: 0 out of 20 (Test group (induction with vehicle)); 48 h after chall.; dose: 50% 1st reading: 17 out of 20 (Control group (induction with test article)); 24 h after chall.; dose: 50% 2nd reading: 20 out of 20 (Control group (induction with test article)); 48 h after chall.; dose: 50% rechallenge: 0 out of 10 (Control group (induction with vehicle)); 24 h after chall.; dose: 20% rechallenge: 0 out of 10 (Control group (induction with vehicle)); 48 h after chall.; dose: 20% rechallenge: 0 out of 10 (Control group (induction with test article)); 24 h after chall.; dose: 20% rechallenge: 0 out of 10 (Control group (induction with test article)); 48 h after chall.; dose: 20% rechallenge: 0 out of 20 (Test group (induction with vehicle)); 24 h after chall.; dose: 20% rechallenge: 0 out of 20 (Test group (induction with vehicle)); 48 h after chall.; dose: 20% rechallenge: 17 out of 20 (Test group (induction with test article)); 24 h after chall.; dose: 20% rechallenge: 15 out of 20 (Test group (induction with test	**(purity 70:30% mixture)**	

	article)); 48 h after chall.; dose: 20%		

4.6.1.2 Human information

No data is available.

4.6.1.3 Summary and discussion of skin sensitisation

A GLP guinea pig maximization test (OECD Guideline 406) was carried out with a mixture of DOT(2 -EHMA) and Octyltin tris(2-EHMA) (70:30% w/w). For induction treatment test substance was formulated in peanut oil (5%) or an adjuvant/saline mixture (intradermal); or in vaseline (5%), epidermal.

85 and 80% of animals in the test group exhibited erythema at 24 and 48 hours respectively; 1/5 females exhibited very slight edema at 48 h. Induction treatment was intradermal and epicutaneous. Challenge treatment was epicutaneous (occlusive).

The test substance showed an extremegrade of skin sensitizing potential in albino guinea pigs.The test substance showed an extreme grade of skin sensitizing potential in albino guinea pigs.

A second GLP guinea pig maximization test (OECD Guideline 406) was carried out with a mixture of DOT(2 -EHMA) and Octyltin tris(2-EHMA) (90:10% w/w). The test substance was induced intradermal and epicutaneous (two stages). The test substance showed an extreme grade of skin sensitizing potential in albino guinea pigs.

Information on skin sensitization is reported here for information only, so as to provide a general toxicological profile on DOTE (EHMA). This endpoint is however not proposed for harmonisation.

4.6.2 Respiratory sensitisation

No data is available.

4.7 Repeated dose toxicity

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Table 18: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Rat (Wistar) male/female Subchronic (oral: feed) 10, 100, 300 mg DOTC/kg diet (0.7, 6.5-6.8, and 19.3-19.8 mg DOTC/kg bw/day) (nominal in diet) Exposure: 13 weeks (daily) Method: OECD Guideline 408	LOAEL: 0.7 mg/kg bw/day (nominal) (male/female) based on: test mat. (based on effect on thymic weight. This level was equivalent to 10 mg DOTC/kg in diet (in males and females).) BMDL05: 0.45 mg/kg bw/day (nominal) (female) based on: test mat. (The BMDL of mg/kg/day is recommended as a	1 (reliable without restriction) Key study Read-across from supporting substance (structural analogue or surrogate) **Test material:**	Appel MJ and Waalkens-Berendsen DH. (2004) Kim J (2004)

(Repeated Dose 90-Day Oral Toxicity in Rodents)	surrogate for a NOAEL for the effect of dioctyltin dichloride on absolute and relative thymus weight) BMD: 0.5 mg/kg bw/day (nominal) (female) based on: test mat. (for decreased absolute and relative thymus weights.)	**Read-across with Dichlorodioctylstannane (CAS no 3542-36-7) (purity 94.1%)**	
Rat (Sprague-Dawley) male/female Subchronic (oral: feed) 25, 50, and 100 ppm (0, 1.6, 3.3, and 6.6 mg/kg bw/day) (nominal in diet) Exposure: 90 days (continuously) Method equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	NOAEL: 25 ppm (male/female) based on: test mat. (At 50 and 100 ppm : significant dose-related reduction in absolute and relative thymus gland weights. 25 ppm is equivalent to 1.25 mg/kg/day, based on a food factor of 0.05.)	2 (reliable with restrictions) Supporting study Experimental result **Test material: Dioctyltin bis(2-EHMA) : Octyltin tris(2-EHMA) (purity 70:30% mixture)**	Anonymous (1974)
rat (Wistar) male/female subchronic (oral: feed) 100, 500, and 1000 ppm (experiment 1) (nominal in diet) 50 and 250 ppm (experiment 2) (nominal in diet) 10 and 25 ppm (experiment 3) (nominal in diet) Exposure: 90 days (continuously) equivalent or similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)	NOAEL: 10 ppm (male/female) based on: test mat. (reduced thymus weight (10 ppm is equivalent to 0.5 mg/kg/bw/day))	2 (reliable with restrictions) Supporting study Experimental result **Test material: Dioctyltin bis(2-EHMA) : Octyltin tris(2-EHMA) : Trioctyltin (2-EHMA) (purity 97: 0.3 : 2.17% mixture)**	Anonymous (1970)

4.7.1.2 Repeated dose toxicity: inhalation

No data is available.

4.7.1.3 Repeated dose toxicity: dermal

No data is available.

4.7.1.4 Repeated dose toxicity: other routes

No data is available.

4.7.1.5 Human information

No data is available.

4.7.1.6 Other relevant information

No data is available.

4.7.1.7 Summary and discussion of repeated dose toxicity

The key study (Apple and Waalkens, 2004) was carried out with the hydrolysis product DOTC (94.1% of purity) according to GLP and OECD 408. The data of the latter study was used for "read across" to evaluate repeated exposure with Dioctyltin bis (EHMA) (CAS N0 15571-58-1). Indeed, DOT(2-EHMA) was demonstrated that it readily hydrolysed to Dichlorodioctyltstanane (CAS no.3542-36-7) under physiological conditions (see IUCLID section 7.1.1). Thus DOTC(Dichlorodioctylstannane) was considered to be an appropriate anchor compound and surrogate for the mammalian toxicology endpoints of repeated dose, in vivo genetic toxicity, reproduction and developmental effects, when they are assessed using oral administration.

In the above study, tested dose levels were 10, 100, 300 mg DOTC/kg diet (0.7, 6.5-6.8, and 19.3-19.8 mg DOTC/kg bw/day). No treatment-related changes were observed in clinical signs, food conversion, neurobehavioural testing, ophtalmoscopy and urinary volume and density. The decreased body weight associated with reduced food consumption in males and females of the 300 mg/kg/day group was most probably due to reduced palatability of the test item. A number of treatment related changes were observed (decreased in haemoglobin, packed cell volume, mean corpuscular haemoglobin, total white blood cells, absolute numbers of lymphocytes and an increase in prothrombin time). These changes involved the 300 mg/kg/day group and were considered toxicologically relevant. Furthermore, a number of treatment-related clinical chemistry changes were observed (decreases in total protein and calcium and increases in alkaline phosphatase, albumin to globulin ratio, bilirubin and bile acids). These changes involved the 100 and 300 mg/kg/day groups and were considered toxicologically relevant.

A number of treatment related changes in organ weights were observed (a decrease in thymus weights and increases in kidney and liver weights). These changes involved all dose groups.

The decreased absolute and relative thymus weights observed at all dose-levels was correlated with histopathological effects observed in the 100 and 300 ppm dose groups and were considered adverse effects. The decreased absolute and relative thymus weights in females of the 10 ppm group, although not accompanied by histopathological changes, they were also considered toxicologically relevant. It was considered to reflect a toxicologically-relevant change in the thymus, which was in accordance with the shown toxicity profile of the test substance (i.e. thymotoxicity). A NOAEL for subchronic toxicity was not established for this study. The LOAEL was determined to be 10 mg DOTC/kg diet or 0.7 mg DOTC/kg bw/d.

The two old subchronic studies (Anonymous, 1974 and 1970) with mixtures of DOT(2-EHMA)(CAS No. 15571-58-1) and MOT(2-EHMA) (CAS No. 27107-89-7) at 70/30% Dioctyltin (2 -EHMA) /Monooctyltin (2 -EHMA) and 97:2.17 % Dioctyltin (2 -EHMA) and Monooctyltin (2 -EHMA) demonstrated that the substance causes clear target effects substantiated by thymus lymphocyte depletion.

1/In the first subchronic diet non GLP study (Anonymous, 1970), rats were given 100, 500 and 1000 ppm (test 1), 50, 250 ppm (test 2), 10, 25 ppm (test 3) of a mixture of 97:2.17 % Dioctyltin (2 -EHMA) and Monooctyltin (2 -EHMA) during 90 days. the following effects were observed:

- Mortality: 9/15 males and 4/15 females died in the 500 ppm diet group; 15/15 males and 14/15 females died in the 1000 ppm diet group;
- Food consumption and food efficiency: slightly, but not significantly reduced at 500 and 1000 ppm.

Haematology:

- Significant decrease of RBC at 100 ppm diet for males, and at 500 ppm diet for females (week 6).
- Significant decrease in percentage of lymphocytes and neutrophils at 500 ppm diet (both sexes) (weeks 6 and 12).
- Significant decrease in hemoglobin content at 100 ppm diet for males (week 12), and at 500 ppm diet for females (weeks 6 and 12).

  - Significant decrease in percentage of packed cell volume at 100 ppm diet for males and females (week 12), and at 500 ppm diet for females (week 12).

- Urinalysis: Specific gravity of the urine was significantly decreased and UGOT levels were significantly increased at 500 ppm diet (both sexes). Specific gravity of the urine of females at 100 ppm diet was also significantly decreased.
- Biochemical: The sugar content of the blood was significantly decreased in males and females at 500 ppm diet. SGOT levels were significantly increased in females at 10 ppm diet. SGPT levels were significantly increased in females at 10 ppm diet and in males at 500 ppm diet. SAP levels were significantly increased at 100 and 500 ppm diet for both sexes.
- The water content of the brain was significantly decreased at 500 ppm diet.
- Organ weights: The following statistically significant changes were observed:

  - Terminal body weight: decreased in females at 100 ppm diet, and in males and females at 500 ppm diet;
  - Relative heart weight: increased in females at 500 ppm diet;
  - Relative kidney weight: increased in males and females at 500 ppm diet;
  - Relative liver weight: increased in males at 10 ppm diet and in females at 500 ppm diet;
  - Relative spleen weight: increased in females at 500 pm diet;
  - Relative brain weight: increased in males and females at 500 ppm diet;
  - Relative gonads weight: increased in males at 500 ppm diet;
  - Relative thymus weight: decreased in males and females at 100 and 500 ppm diet

- Histopathology: 2/5 females at 100 ppm diet, and 5/5 males and 5/5 females at 500 ppm diet had almost complete depletion of lymphocytes resulting in a very small thymus with a uniform picture of the remaining reticula parenchyma, which hardly permitted a distinction between cortex and medulla. This damage of the thymus was occasionally accompanied with little active lymph nodes and a slight reduction of splenic lymphoid cells. In the kidney, 3/5 males and 2/5 females exhibited swollen tubular epithelial cells containing a granular or finely vacuolated cytoplasm.

The NOAEL was determined to be 10 ppm diet (equivalent to 0.5 mg/kg/bw/day), on the basis of reduced thymus weight at 25 ppm diet. The LOAEL was determined to be 25 ppm diet (calculated as 1.07-1.24 mg/kg bw/day in males and 1.46-1.51 mg/kg bw/day in females). Calculation of dosage was performed using body weights of 340 g (males) and 200 g (females), and average food consumption of 14.6-16.8 g/rat/day (males) and 11.7-12.1 g/rat/day (females).

2/ <u>In the second subchronic old study (not GLP) (Anonymous, 1974),</u> rats were given mixture of 70/30% Dioctyltin (2 -EHMA) /Monooctyltin (2 -EHMA) at 25, 50 and 100 ppm in diet (equivalent to an average daily intake of 0, 1.6, 3.3 and 6.6 mg/kg/day during 90 days. The following relevant effects were observed:

Significant dose-related reduction in absolute and relative thymus weights in the 50 ppm (3.3 mg/kg/day) and 100 ppm (6.6 mg/kg/day) dose groups.

The NOAEL was determined to be 25 ppm in the diet (calculated as 1.25 mg/kg/day, based on a food factor of 0.05)

The reports on these two tests do not contain information on the test substance homogeneity and stability. However, the observed effects are comparable to the results of a reliable 90 days repeated dose toxicity study performed with Dioctyltindichloride, the gastric hydrolysis product of DOTC (Appel and Waalkens, 2004): In the latter 90 day repeated dose study, the decreased absolute and relative thymus weights observed at all dose-levels (10, 100 and 300 mg/kg diet) and was correlated with histopathological effects observed in the 100 and 300 ppm dose groups considered as adverse effects. The decreased absolute and relative thymus weights in females of the 10 ppm group, although not accompanied by histopathological changes was also considered toxicologically relevant. It was considered to reflect a toxicologically-relevant change in the thymus, which was in accordance with the shown toxicity profile of the test substance (i. e. thymotoxicity).

The data of the latter study was used for “read across” to evaluate the dose toxicity of repeated exposure with DOT(2-EHMA). This study is used for read across for DOT(2-EHMA) as it was demonstrated that it readily hydrolysed to Dichlorodioctyltilstanane (CAS no.3542-36-7) under physiological conditions (see section 7.1.1). Thus DOTC (Dichlorodioctylstannane) was considered to be an appropriate anchor compound and surrogate for the mammalian toxicology endpoints of repeated dose, in vivo genetic toxicity, reproduction and developmental effects, when they are assessed using oral administration.

A NOAEL for subchronic toxicity was not established for this study. The LOAEL was determined to be 10 mg DOTC/kg diet or 0.7 mg DOTC/kg bw/d, based on effects on the thymus.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

The evaluation of the repeated dose toxicity was based on three studies:

- Two subchronic oral toxicity tests (rat) with mixtures containing a high concentration of DOT(2 -EHMA) (70 and 97% purity)- no guideline studies;

- One subchronic toxicity test performed according to OECD 408 guideline with the hydrolysis product dioctyltin dichloride (92 % purity) (Appel and Waalkens, 2004).

The use of DOTC study as an appropriate read-across for mammalian toxicology studies of DOT(2-EHMA)/(IOMA) via the oral route is supported based on a simulated gastric reaction study which has shown readily gastric hydrolysis of DOT(EHMA) readily hydrolized to DOTC under physiological conditions, Thus, data on DOTC are relevant and adequate for DOT(2-EHMA) hazard assessment regarding endpoints of repeated dose, in vivo genetic toxicity, reproduction, and developmental effects, when they are assessed using oral administration.

Read across is therefore applied using a valid repeated dose toxicity study performed with DOTC (92%).

No data on dermal or inhalatory repeated dose toxicity are available.

Information on repeated toxicity exposure is reported here for information only, so as to provide a general toxicological profile on DOTE(EHMA). This endpoint is however not proposed for harmonisation.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

The evaluation of the repeated dose toxicity was based on three studies:

- Two subchronic oral toxicity tests (rat) with mixtures containing a high concentration of DOT(2 -EHMA) (70 and 97% purity)- no guideline studies;

- One subchronic toxicity test performed according to OECD 408 guideline with the hydrolysis product dioctyltin dichloride (92 % purity) (Appel and Waalkens, 2004).

The use of DOTC study as an appropriate read-across for mammalian toxicology studies of DOT(2-EHMA)/(IOMA) via the oral route is supported based on a simulated gastric reaction study which has shown readily gastric hydrolysis of DOT(EHMA) readily hydrolized to DOTC under physiological conditions, Thus, data on DOTC are relevant and adequate for DOT(2-EHMA) hazard assessment regarding endpoints of repeated dose, in vivo genetic toxicity, reproduction, and developmental effects, when they are assessed using oral administration.

Read across is therefore applied using a valid repeated dose toxicity study performed with DOTC (92%).

No data on dermal or inhalatory repeated dose toxicity are available.

Information on repeated toxicity exposure is reported here for information only, so as to provide a general toxicological profile on DOTE(EHMA). This endpoint is however not proposed for harmonisation.

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

4.9.1.1 In vitro data

Table 19: Summary table of relevant in vitro mutagenicity studies

Method	Results	Remarks	Reference
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) Salmonella typhimurium strains TA98, TA1535, TA1537, and TA1538; Saccharomyces cerevisiae D4 (met. act.: with and without) Doses: 0.005, 0.01, 0.1, 1.0, 5.0, and 10.0 ul/plate (20.0 ul/plate was used for strain TA1537 without activation) equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results: negative Test results: negative for Salmonella typhimurium strains TA98, TA1535, TA1537, and TA1538; Saccharomyces cerevisiae D4(all strains/cell types tested); met. act.: with and without; cytotoxicity: yes (The test substance was found to be toxic to the strain TA1537 at 10 and 20 ul/plate and to the strains TA1538 and D4 at 10 ul/plate.)	2 (reliable with restrictions) supporting study Experimental result **Test material:** **Dioctyltin bis(2-EHMA) : Octyltin tris(2-EHMA) (purity 70:30% mixture)**	Anonymous (1978a)
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538 (met. act.: with and without) Doses: 300, 900, 2700, 8100, and 24,300 µg/0.1 ml equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Evaluation of results: positive negative for S. typhimurium, other: TA98, TA1535 and TA1538(strain/cell type: TA98, TA1535 and TA1538); met. act.: with and without; cytotoxicity: yes positive (at 300 and 2700 ug/1 ml) for S. typhimurium TA 1537(strain/cell type: TA 1537); met. act.: with; cytotoxicity: yes negative for S. typhimurium TA 1537(strain/cell type: TA 1537); met. act.: without; cytotoxicity: yes negative for S. typhimurium TA 100(strain/cell type: TA 100); met. act.: with; cytotoxicity: yes positive (at 2700 ug/1 ml) for S. typhimurium TA 100(strain/cell type: TA 100); met. act.: without; cytotoxicity: yes	2 (reliable with restrictions) supporting study experimental result **Test material:** **Dioctyltin bis(2-EHMA) : Octyltin tris(2-EHMA) (purity 70:30% mixture)**	Anonymous. (1983)
Bacterial reverse mutation assay	Evaluation of results: negative	2 (reliable with	Anonymous

(e.g. Ames test) (gene mutation) S. typhimurium TA 1535, TA 1537, TA 98 and TA 100 (met. act.: with and without) Doses: 15, 45, 135, 405, and 1215 µg/0.1 ml equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	Test results: negative for S. typhimurium TA 1535, TA 1537, TA 98 and TA 100(all strains/cell types tested); met. act.: with and without	restrictions) key study experimental result **Test material:** **Dioctyltin bis(2-EHMA) : Octyltin tris(2-EHMA) (purity 70:30% mixture)**	(1979)
Bacterial reverse mutation assay (e.g. Ames test) (gene mutation) S. typhimurium TA 100 (met. act.: without) Doses: 0.005, 0.01, 0.1, 1.0, 5.0, and 10 ul/plate The test was performed in accordance with the method of Ames et al. (1975)	Test results: positive for S. typhimurium TA 100(all strains/cell types tested (Salmonella typhimurium strain TA100)); met. act.: without	2 (reliable with restrictions) supporting study experimental result **Test material:** **Dioctyltin bis(2-EHMA) : Octyltin tris(2-EHMA) (purity 70:30% mixture)**	Anonymous. (1978b)

4.9.1.2 In vivo data

Table 20: Summary table of relevant in vivo mutagenicity studies

Method	Results	Remarks	Reference
Micronucleus assay (chromosome aberration) Rat (Wistar outbred Crl) male Oral: gavage 500, 1000, 2000 mg/kg bw (actual ingested (Just before dosing, the animals were weighed and the test substance was dissolved and diluted in corn oil at concentrations of 25, 50 and 100 mg/ml. The orally (by gavage) given dosing volume was 20 ml/kg bw.)) Method: OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	Evaluation of results: negative Test results: Genotoxicity: negative (Dichlorodioctylstannane reached the bone marrow in this micronucleus test. The results did not indicate any chromosomal damage and or damage to the mitotic apparatus of the target cells in the bone marrow.) (male/female); toxicity: no effects	1 (reliable without restriction) Key study Read-across from supporting substance (structural analogue or surrogate) **Test material:** **Read-across with Dichlorodioctylstannane (CAS no 3542-36-7) (purity > 99.1%)**	Krul, C.A.M. (2003)
Micronucleus assay (chromosome aberration) Mouse (CFLP) male/female	Evaluation of results: negative Test results: Genotoxicity: negative	2 (reliable with restrictions) Supporting study	Hossack D.J.N, Richold, M. and Richardson, J.C. (1980)

Oral: gavage 2250, 4500, and 9000 mg/kg bw (actual ingested) Method equivalent or similar to OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	(male/female); toxicity: yes (bone marrow depression)	Experimental result **Test material:** **Dioctyltin bis(IOMA) [CAS no. 26401-97-8]:Octyltin tris(IOMA) [CAS no.26401-86-5] (purity 80:20% mixture)**	

4.9.2 Human information

No data is available.

4.9.3 Other relevant information

No data is available.

4.9.4 Summary and discussion of mutagenicity

In vitro studies: Ames tests

In the key study (1979), an Ames test was carried out with a mixture of 70% dioctyltin bis(2-ethylhexylmercaptoacetate) and 30% mono-octyltin tris(2-ethylhexylmercaptoacetate). This mixture was tested in strains of S. typhimurium (TA 1535, TA 1537, TA 98 and TA 100), with or without S9, and there are positive and negative controls. No mutagenic activity was observed in this test.

Others studies were used as supporting studies because they are less complete than the key study. All these studies used the same mixture as the key study, DOTE: MOTE, 70:30%. One of these studies gave negative results, and two old studies showed a (weak) positive response without metabolic activation.

In vitro studies: Mouse lymphoma assay

A GLP study guideline (OECD 473) was available. DOTE was examined for its potential to induce gene mutations at the TK-locus of cultured mouse lymphoma L5178Y cells, in both the absence and the presence of a metabolic activation system (S9-mix). DOTE was cytotoxic in both the absence and presence of S9-mix.

In the absence of S9-mix no increase in mutant frequency was observed at any test substance concentration evaluated. In the presence of S9-mix at 72 μg/ml the mutant frequency was significantly increased by 238 mutants per 1,000,000 clonable cells compared to the negative control. Since relatively small intervals (0.85) were used and the increase was observed at a single concentration causing more than 90% cytotoxicity compared to six concentrations causing 50-70% cytotoxicity which showed no increase in mutant frequency, it is concluded that this increase is not indicative for mutagenicity.

It is concluded that under the conditions used in this study, the test substance DOTE is not mutagenic at the TK-locus of mouse lymphoma L5178Y cells.

In vivo studies

Three micronucleus tests were available. The key study (Krul 2003) was a guideline study (OECD 474), and the test substance was DOTC(CAS no. 3542-36-7), the hydrolysis product (read-across approach). No chromosomal damage and/or damage to the mitotic apparatus of the target cells in the bone marrow was observed. The dose of 2000 mg/kg bw was cytotoxic (reduced number of PE per number of erythrocytes), which is an evidence that DOTC reached the bone marrow.

This supports the conclusion that DOTC does not induce chromosomal damage or damage to the apparatus of bone marrow cells in mammals.

This result is confirmed in the supporting study (Hossack 1980): a mixture of DOT(IOMA): MOT(IOMA), 80:20% failed to show any evidence of mutagenic potential when administered orally. Dioctyltin bis (IOMA) and dioctyltinnbis (2-EHMA) are isomers of the same compound and are expected to be chemically and toxicologically equivalent (read-across approach). However, evidence of bone marrow depression was observed, whichis an evidence that test substance reached the bone marrow.

Others in vivo studies: DOTC, at dose-levels up to 5000 µg/kg bw, did not increase the number of sister chromatid exchanges in somatic cells of male and female chinese hamsters (1983). A dose of 1.2 mg/l of DOTC gave no indication of genotoxicity in vivo in a covalent DNA binding assay (1988).

Information on mutagenicity is reported here for information only, so as to provide a general toxicological profile on DOTE(EHMA). This endpoint is however not proposed for harmonisation.

4.10 Carcinogenicity

No data is available.

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Table 21: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Rat (Sprague-Dawley) male/female two-generation study oral: feed 20, 60, and 200 ppm (nominal in diet) Exposure: Duration of dosing of F0 generation males - 10 weeks prior to mating, during mating (3 weeks), and post mating until sacrifice; females - 10 weeks prior to mating and during mating. Mated females continued to receive test diets during gestation and lactation; unmated females received test diets until sacrifice. Test diets were prepared weekly and analyzed for homogeneity and stability. Duration of dosing of F1 generation:	NOAEL (P): 20 ppm (male/female) (based on a reduction in the relative thymus weight of males) NOAEL (F1): 20 ppm (male/female) (The NOAEL for the F1 generation until weaning was 20 ppm (~1.6 mg/kg bw/d), based on a decrease in relative thymus weights in male and female pups at 60 ppm. The NOAEL for the F1 generation post lactation was 20 ppm, based on a slight decrease in the relative thymus weight of males and an increase in stillbirths at 60 ppm.)	1 (reliable without restriction) Key study read-across from supporting substance (structural analogue or surrogate) **Test material:** **Dioctyltin bis(IOMA) [CAS no. 26401-97-8]:Octyltin tris(IOMA) [CAS no. 26401-86-5] (purity 78.8 : 16.9% mixture)**	Anonymous (1997)

males - 14 weeks (starting at the end of lactation prior to mating), during mating (3 weeks), and post mating until sacrifice; females - 14 weeks (starting at the end of lactation prior to mating) and during mating (3 weeks). (continuously (in diet)) Method: OECD Guideline 416 (Two-Generation Reproduction Toxicity Study)			

4.11.1.2 Human information

No data is available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Table 22: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Rat (Han-Wistar SPF) Oral: gavage 1, 5, and 25 mg/kg/day (actual ingested) Exposure: days 6-15 of gestation (once/day x 10 days) Method equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	NOAEL (maternal toxicity): 5 mg/kg bw/day (slight but nonsignificant decrease in corrected body weight and corrected body weight gain of the dams indicating a marginal maternal toxic effect of the test substance) NOAEL (developmental toxicity): 5 mg/kg bw/day (significant increase in the percentage of dead fetuses)	1 (reliable without restriction) Key study Read-across from supporting substance (structural analogue or surrogate) **Test material:** **Dioctyltin bis(IOMA) [CAS no. 26401-97-8]:Octyltin tris(IOMA) [CAS no. 26401-86-5] (purity 80:20% mixture)**	Battenfeld, R. (1991)
Rabbit (New Zealand White) Oral: gavage 1.0, 10, and 100 mg/kg/day (actual ingested) Exposure: From day 6 through day 18 of gestation, groups of dams (23-24 per treatment group) were intragastrically treated once per day with the test substance	NOAEL (developmental toxicity): 10 mg/kg bw/day (10 mg/kg/day: Slight non-significant increase in minor skeletal head anomalies (incompletely ossified bones in the skull). 100 mg/kg/day: Significantly increased incidence of abortions, post implantation	1 (reliable without restriction) Key study Read-across from supporting substance (structural analogue or surrogate) **Test material:**	Battenfeld, R. (1992)

administered in peanut oil. (once/day x 13 days) Method equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	loss, minor visceral anomalies (severely dilated renal pelves and additional small vessels originating from the aortic arch), minor skeletal head anomalies (incompletely ossified bones in the skull), and skeletal variations of the sternum and feet bones (not or incompletely ossified sternebrae and feet bones); and a significant reduction in fetal body weight.)	**Dioctyltin bis(IOMA) [CAS no. 26401-97-8]:Octyltin tris(IOMA) [CAS no. 26401-86-5] (purity 80:20% mixture)**	
Mouse (NMRI) oral: gavage 20, 30, or 45 mg/kg (group 1); 67 or 100 mg/kg (group 2) (actual ingested) Exposure: days 6-17 of gestation (once/day x 12 days) Method equivalent or similar to OECD Guideline 414 (Prenatal Developmental Toxicity Study)	NOAEL (maternal toxicity): 30 mg/kg bw/day (Based on a significant decrease in thymus weight at 45 mg/kg/day.) NOAEL (developmental toxicity): 45 mg/kg bw/day (based on an increased incidence of cleft palate in fetuses from dams exposed to 67 mg/kg/day.)	2 (reliable with restrictions) Supporting study Read-across from supporting substance (structural analogue or surrogate) **Test material:** **Dioctyltin bis(IOMA) [CAS no. 26401-97-8]:Octyltin tris(IOMA) [CAS no. 26401-86-5] (purity 80:20% mixture)**	Faqi, A.S., H. Schweinfurth, and I. Chahoud (2001)

4.11.2.2 Human information

No data is available.

4.11.3 Other relevant information

Table 23: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Rat (Wistar) female Oral: feed 10, 100, 300 mg dichlorooctylstannane/kg diet (nominal in diet) Exposure: Duration of exposure: daily for 2 consecutive weeks during the premating period, daily during gestation (up to 26 days after study initiation) and up to euthanasia at or shortly after postnatal day (PN) 4. (daily)	NOAEL (reproduction toxicity): 0.5 — 0.7 mg/kg bw/day (female) (Based on reproductive and developmental effects: animals showing only implantations at necropsy, animals delivering only dead pups, decreases in gestation, live birth and viability indices and increases in post-implantation loss and number of runts) LOAEC (general toxicity): 0.5 — 0.7 mg/kg bw/day (female) (decreases in absolute and	1 (reliable without restriction) Key study Read-across from supporting substance (structural analogue or surrogate) **Test material:** **Read-across with Dichlorodioctyllstannane (CAS no 3542-36-7) (purity**	Appel, M.J. and D.H. Waalkens-Berendsen. (2004)

Method: OECD Guideline 421-reproduction/ developmental screening study	relative thymus weights associated with treatment related lymphoid depletion at 10, 100 and 300 mg/kg/day groups)	**94%)**	

4.11.4 Summary and discussion of reproductive toxicity

Effects on fertility

In the two generation study performed under GLP and according to OECD 416 (Anonymous, 1997), the mixture Dioctyltin bis(IOMA) [Cas No. 26401 -97 -8]: Octyltin tris(IOMA) [Cas No. 26401 -86 -5] (78.8:16.9%) were administered to the F0 generation 10 weeks prior to mating, during mating (3weeks) and post-mating. Dioctyltin bis (IOMA) and dioctyltin bis (2-EHMA) are isomers of the same compound and are expected to be chemically and toxicologically equivalent The F1 generation was treated 14 weeks during premating, 3 weeks during mating. Females continued to receive the test material during gestation and lactation.

The following treatment-related effects were observed:

F0 generation:

- Mortality: 1 male died at 200 ppm diet
- Absolute food consumption reduced in females at 200 ppm diet (-6% on lactation days 7-14, -9% on lactation days 14-21)
- Viability index slightly reduced at 200 ppm (96.2% vs. 98.6% in the controls).
- Lactation index significantly decreased at 200 ppm diet (88.6% vs. 94.4% in controls) after 21 days lactation.
- Slight increase in pup mortality at 200 ppm diet.
- Pup body weights significantly decreased at 200 ppm diet in both sexes after 14 and 21 days lactation.
- Slight delay in vaginal opening at 200 ppm diet.
- Slight decrease in relative thymus weight in males at 60 ppm diet; significant decrease in relative thymus weight in both sexes at 200 ppm diet.
- Increased incidence of thymic involution at 200 ppm diet (significant for males only).
- Microscopic examination of the organs found no substance-related changes.

F1 generation:

- No mortality.
- Body weight: significant reduction in males at 200 ppm diet.
- Food consumption: reduced in females at 200 ppm diet; significant on lactation days 14-21.
- Increased number of stillbirths at 200 ppm diet (26 vs. 5 in controls).
- Viability index: decreased at 200 ppm (82.0% vs. 95.7% in controls).
- Pup mortality: increased at 200 ppm diet from day 4-21 of lactation.
- Lactation index: decreased at 200 ppm diet (82.3% vs. 94.4%).
- Pup body weight: significantly reduced at 200 ppm for males and females on days 4, 7, 14, and 21 of lactation.
- Morphological changes: pinna unfolding, eye and ear opening were slightly delayed at 200 ppm diet.
- Relative thymus weight: significantly decreased in males and females at 200 ppm diet and at 60 ppm for females only
- Relative spleen weight: significantly decreased in females at 200 ppm diet.
- Increased incidence of thymic involution at 200 ppm (significant for males).

The NOAEL for F0 males and females was 20 ppm diet (approx. 1.5 mg/kg bw/day) based on a slightly reduced relative thymus weight for males at 60 ppm (approx. 4.4 mg/kg bw/day).

The NOAEL for the F1 generation was 20 ppm diet (approx. 1.6 mg/kg bw/day), based on a reduction in relative thymus weights for males and females at 60 ppm diet (approx. 4.7 mg/kg bw/day).

No teratogenic effects were observed in this study.

Comparable effects on the thymus were observed in the 13 consecutive weeks study combined with the reprotox screening assay performed according to OECD 421 with the hydrolysis product DOTC (Appel and Waalkens, 2004) (purity>94%):

At 10 ppm (equivalent 0.7 mg/kg/bw for males and 0.5-0.7 mg/kg/bw for females), treatment-related effects to dams included lymphoid depletion were observed in dams.

At 100 ppm (equivalent to 6.8 -6.8 mg/kg/bw/day, treatment-related effects included increased post-implantation loss (49%), decreased gestation index (71%) decreased live birth index (53%), decreased viability index (74%), increased number of runts, increased pup mortality (PN1 and 4), and decreased absolute and relative thymus weights and lymphoid depletion in the dams.

At 300 ppm (equivalent to 19.3 -19.8 mg/kg/bw/day), treatment-related effects included increased in post-implantation loss (70%), decreased gestation index (50%), decreased live birth index (60%) decreased viability index (12%), increased number of runts, decreased pups weights (PN 1and 4), increased pup mortality (PN 1 and 4), and decreased absolute and relative thymus weights and lymphoid depletion (dams).

Based on reproductive and developmental effects in the screening reprotox assay (particularly severe post-implantation losses and fetal losses) observed after mating of 100 and 300 mg/kg female of the satellite groups with male animals of the main study, the low dose level of 10 mg Dichlorodioctylstannane/kg diet (equivalent to 0.7 mg/ kg body weight/day in males and 0.5-0.7 mg/kg body weight for females) can be considered as a NOAEL for fertility and developmental effects.

Based on the treatment related histological changes in the thymus (lymphoid depletion) of the 10 mg/kg female animals of the satellite groups, 10 mg Dichlorodioctylstannane/ kg diet (equivalent to 0.5-0.7 mg/kg body weight/day) was considered to be a LOAEL for maternal toxicity.

Summary for effects on fertility

Under the experimental conditions of this two generation study, the NOAEL for the F0 parental generation was 20 ppm (~1.5 mg/kg/bw), based on a reduction in the relative thymus weight of males at 60 ppm. The NOAEL for the F1 generation until weaning was 20 ppm (~1.6 mg/kg/bw/d), based on a decrease in relative thymus weight in male and female pups at 60 ppm. The NOAEL for the F1 generation post-lactation was 20 ppm, based on a slight decrease in the relative thymus weight of males and an increase in stillbirth at 60 ppm.

There is a GLP screening reprotoxicity study according to OECD guideline 421 (Appel and Waalkens, 2004) performed with the hydrolysis product dioctyltin dichloride (3542-36-7) and described in section 7.8.3. In this GLP key study, comparable effects were obtained with the 2-generation study, indeed thymus effect were also recorded. Dose-related effects were seen at 10, 100 and 300 mg/kg/day, with post-implantation losses in the top two dose groups. The maternal LOAEL was set at 10 ppm diet (equivalent 0.7 mg/kg/bw for males and 0.5-0.7 mg/kg/bw for females) for treatment related effects to dams included lymphoid depletion.

Developmental toxicity

1/In the developmental toxicity study in rats (Battenfeld, 1991), dams were treated with mixture of DOT (IOTG) and MOT(IOTG) (80:20%) at 1, 5 and 25 mg/kg/day during day 6 -15 of gestation. Alopecia was observed in single animals of all four groups and was not attributed to treatment. There was a slight (non significant) decrease in corrected body weight and corrected body weight gain from day 6 to day 21 at 25 mg/kg/day dose.
This reduction was attributed largely to one single dam. There was a statistically increase in the percentage of dead fetuses at 25 mg/kg/day. The seven dead fetuses concerned only on litter. Though clear-cut effects were found in only one dam in 25 mg/kg/day dose group, the test substance was considered to induce marginal maternal toxicity at 25 mg/kg/day. The dose-level without maternal and/or embryofetotoxicity was 5 mg/kg/day (equivalent to 0.77 mg Sn/kg b. w/day).

2/In the mice developmental rabbits study (Faqi, 2001), dams were given mixture of DOT(IOTG) and MOT(IOTG) at 23, 30, 45, 67 and 100 mg/kg/day durin day 6 to 17 of pregnancy. There was a dose dependent decrease in maternal body weight gain, but differences were not significant in mice exposed to the test substance. No signs of toxicity were

observed with the exception of one dam in the 100 mg/kg dose group that died. Pregnancy rates were comparable between treated groups and the control groups.

Maternal effects:

The mean maternal thymus weights in the 45 and 100 mg/kg dose groups, but not the 67 mg/kg dose group, were significantly lower than the control groups. Maternal liver weights were significantly lower in the 100 mg/kg dose group. The number of implantations per litter was comparable between treated groups and the control groups. Resorption rates were significantly increased in mice treated with 67 or 100 mg/kg/day.

Fetal observations:

Fetal weights were significantly decreased in the 67 and 100 mg/kg/day groups. There were no dead fetuses in any of the treated groups. There were no external malformations reported in the fetuses exposed to 20, 30, or 45 mg/kg/day however a significantly increased incidence of cleft palate in the fetuses exposed to 67 or100 mg/kg/day were observed, and incidences of bent forelimbs and exencephaly were significant in the fetuses exposed to 100 mg/kg/day. Skeletal variations reported in the low dose groups included unossified digit and supernumerary cervical ribs (significantly increased at 20 and 45 mg/kg, but not at 30 mg/kg); hindpaw incompletely ossified, Os frontale misshapened, and interparietale incompletely ossified (significantly increased at 45 mg/kg); and supernumerary lumbar or cervical ribs (significantly increased at 20, 30, and/or 45 mg/kg). There was a significant increase in skeletal abnormalities in the fetuses of dams exposed to 67 or 100 mg/kg/day. Skeletal abnormalities reported in these dose groups included bent forelimbs, bent hindlimbs, dislocated sternum, fused or bent ribs, or bent vertebral column. Skeletal variations were observed in the low dose groups (20, 30, or 45 mg/kg/day). However, in the high dose groups (67 or 100 mg/kg/day), oral administration of the test substance resulted in a significantly increased incidence of fetuses with malformations (i. e., cleft palate, bent forelimbs, exencephaly) and increased resorption rates. The authors defined malformations as a permanent or irreversible structural change that is likely to adversely affect survival or health. The authors reported a no-observed-adverse-effect-level (NOAEL) for each endpoint examined, i. e., malformations, variations, organ toxicity.

- The embryo-fetal NOAEL for malformations was reported as 45 mg/kg/day, based on an increased incidence of cleft palate in fetuses from dams exposed to 67 mg/kg/day.
- A NOAEL for skeletal variations could not be determined, but would be expected to be < 20 mg/kg/day, based on an increased incidence of supernumerary lumbar ribs observed at 20 mg/kg/day.
- The authors reported that the NOAEL for organ toxicity was 30 mg/kg/day, based on a significant decrease in thymus weight at 45 mg/kg/day.

3/In the rabbit embryotoxicity study (Battenfeld, 1992), dams were given mixture of DOT(IOTG) and MOT(IOTG) (80:20%) during day 6 -18 of pregnancy at 1, 10 and 100 mg/kg/day.

Maternal effects:
No differences between treatment groups were observed for maternal body weight gain. The high incidence of abortion in the 100 mg/kg/day group was considered to result "at least partly from a slight maternal toxic effect of the test compound."

Fetal observation:
Total fetal death was found only in the controls and in the 100 mg/kg/day dose group. In both groups, total post-implantational loss occurred in 3 dams. Percentages of implantations per group were 17.7% (control), 10.5% (1 mg/kg/day), 5.7% (10 mg/kg/day), and 28.4% (100 mg/kg/day). External examination revealed two nasal clefts and an encephalocele in one fetus of group 2. Umbilical hernia was found in one fetus of the control group and in one fetus each in Groups 3 and 4. These were not associated with treatment. Other findings, such as malformations of the vertebral column (one animal in Group 4) and absence of the right kidney and adrenal gland (one animal in Group 4) were regarded as chance findings and not attributed to treatment due to their single occurrence and because they represented totally different types of malformations. The lack of a statistically significant difference to the control group and inconsistency regarding the type of anomaly found did not "point towards a compound-related effect. " Fetuses with minor external anomalies (flexion of digits and limbs, open eyelids, shortened tail) were observed in all four groups, and not attributed to the test substance. Minor visceral anomalies found included severly dilated renal pelves and additional small vessels originating from the aortic arch. The statistically significant increase in the incidence of visceral anomalies of fetuses in Group 4 is an indication of retardation in fetal development. Individual body weights of the fetuses in Group 4 with minor visceral anomalies were approximately 40% lower than the mean weight of control fetuses. Suspected or definite compound-related changes noted included:
-1 mg/kg/day: No substance-related effects.

-10 mg/kg/day: Slight non-significant increase in minor skeletal head anomalies (incompletely ossified bones in the skull).
-100 mg/kg/day: clear substance-related embryotoxic effects were noted i. e. significantly increased incidence of abortions, post-implantational loss, minor visceral anomalies (severely dilated renal pelves and additional small vessels originating from the aortic arch), minor skeletal head anomalies (incompletely ossified bones in the skull), and skeletal variations of the sternum and feet bones (not or incompletely ossified sternebrae and feet bones); and a significant reduction in fetal body weight.

In conclusion, the author of the rabbit developmental study reported that the evaluation of reproduction data and fetal weights indicated a slight embyrolethal and moderate retardative effect (with regard to fetal development) at the high dose level (100 mg/kg/day).

Both the available developmental toxicity studies in mice and rabbits and the 2 -generation study with a mixture of DOT(IOMA) /MOT(IOMA) (78.8%/16.9%) show serious effects on fetal weight. In the 2 -generation study in rats the F1 and F2 pup viability is also seriously affected. These effects are not inconsistent with what is seen in the reprotox screening assay (OECD 421) in rats with DOTC (CAS no. 3542-36-7) particularly the increase in post-implantation loss, which confirms that read-across from DOTC is justified. The developmental study in rats of the DOT(IOMA) /MOT(IOMA) showed also an increase in the number of dead fetuses at 25 mg/kg/day.

Serious skeletal malformations are seen in mice (bent forelimbs, bent hindlimbs, dislocated sternum, fused or bent ribs and bent vertebral column) and rabbits (not or incompletely ossified sternebrae and feet bones) but not in rats. However, it is important to note that these effects occur at dose levels where the maternal animals showed thymic atrophy which may be evidence of maternal toxicity.

Summary for developmental toxicity

There were three developmental studies in rat, mice and rabbits. The NOAEL for maternal toxicity and embryofetal development in the rat study were set at 5 mg/kg/day (based on decrease in maternal body weight gain and increase in the percentage of dead fetuses at 25 mg/kg/day).

In the mice study, the embryofetal NOAEL for malformations was reported at 45 mg/kg/day based on an increased incidence of clef palate in fetuses from dams given 67 mg/kg/day. A NOAEL for skeletal variations could not be determined, but would be expected to be <20 mg/kg/day, based on an increased incidence of supernumerary lumbar ribs observed at 20 mg/kg/day

In the rabbit study, the NOEL for developmental and maternal toxicity was set at 1 mg/kg/day The evaluation of reproduction data and fetal development indicated a slight embryofetal and moderate retardative effect at 100 mg/kg/day (signficantly increased incidence of abortion, increase incidence of post-implantation losses, increased incidence of external and visceral malformation) while maternal toxicity was very slight.

Toxicity to reproduction: other studies

The gastric hydrolysis rates support the conclusion that dioctyltin dichloride (DOTC) (Cas No. 3542 -36 -7) is the toxophore in the oral studies, due to rapid gastric hydrolysis of the dioctyltin thioglycolate ester to the chloride. DOT(IOMA) (Cas No 26401 -97 -8) is an isomer of (DOT(2 -EHMA) (CAS No. 15571 -58 -1) that is considered to behave similarly.

1/The lowest NOAEL (actually 0.5 -0.7 mg/kg bw/d) was found in the combined repeated dose and reproduction/developmental toxicity test with DOTC (Apple and Waalkens, 2004). At the higher dose levels effects on pups such as increase in number of runts, increased number of cold pups, number of pups per litter, were observed. Based on the observed histological changes in the thymus (lymphoid depletion) of the 10 mg/kg females, the low dose of 10 mg dichlorooctylstannane/kg diet (equivalent to 0.5-0.7 mg/kg bw/day for females) was considered to be a LOAEL for maternal toxicity.

4.11.5 Comparison with the criteria

There were relevant observed effects in the two generation study performed with DOT (IOMA): MOT(IOMA) (78.8: 16.9%) and the developmental reprotoxicity studies with DOT (IOMA): MOT(IOMA) 80:20%, particularly the effects on pups such as increase in number of runts, decreased, fetal weight, decreased number of pups per litter, increased

post-implantation loss, decrease thymus weight for the F0 parent and F1 progeny. In addition, the screening reprotoxicity study with DOTC support also a part of these particular findings (increase post-implantation loss, decreased viability index, increase number of runts, decreased pups weights) and decrease absolute and relative thymus weight and lymphoid depletion in dams.

Based on these effects, DOT(2 -EHMA) is classified with R63: 'Possible risk of harm to the unborn child' according to Directive 67/548/EEC and 'Reprotoxicity category 2 (H361) according to CLP.

4.11.6 Conclusions on classification and labelling

Directive 67/548/EEC	CLP
Reprotoxicity category 3 R63: possible risk of harm to the unborn child	Reprotoxicity category 2 H361d: Suspected of damaging the unborn child

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The CLH report presents 5 studies relevant for the assessment of reproductive toxicity. Although more detailed information would have been beneficial, the data give clear evidence of developmental toxicity in three different species.

The findings of developmental effects include:

- reduction in fetal body weight in rabbits and mice
- increased post-implantation losses in rats, rabbits and mice
- abortions in rabbits
- increased number of stillbirths in rats
- increased rates of pup mortalities 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 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, 2-gen study, rat))
- reduced T-cell mitogen response (indicative for an immunosupressive effect) in directly dosed weanlings (rats) from PND 3-24, indicating that weanlings are more sensitive than young adults

The effects occur at daily doses of 23-100 mg/kg/day, and for some of the effects with clear dose-response. At these dose levels, there are signs of maternal thymotoxicity in some of the studies (signs in rats and in mice (mice are based on a LOAEL comparison 50-fold less sensitive than rats), no signs in rabbits), and the maternal toxicity caused by these dose levels can therefore according to RAC be characterised as slight.

The dossier submitter proposed that the maternal thymotoxicity should be characterised as moderate maternal toxicity, and that the developmental effects could have been secondary to the maternal toxicity, warranting classification for developmental toxicity in Repr. 2 (H361d) (CLP)

and Repr. Cat 3; R63 (DSD). Visceral abnormalities in rabbits were considered to be related to fetal growth retardation.

Comments received during public consultation

No new information was received during the public consultation. Six Member States questioned that the maternal thymotoxicity is moderate and that the developmental effects could have been secondary to the maternal toxicity, and were rather of the opinion that the substance should be classified in Repr. 1B (H360D) (CLP) and Repr. Cat 2; R61 (DSD). More details on the studies reported and an overview table on substances used for testing were added by the dossier submitter in a revised version of the CLH report submitted after public consultation. This version can be found attached to the RCOM.

There were also requests for more detailed data from the studies, discussion on developmental immunotoxicity and effects on fertility. The thymus is clearly a target organ in the developing animal as well as in adults, and there is some evidence to suggest that young animals are more sensitive than adults. However, the available data do not allow RAC to make a firm conclusion on this.

RAC assessment and comparison with the criteria

The major difference in the assessment made by the dossier submitter and RAC concerns whether the developmental effects could have been secondary to the observed maternal (thymo-)toxicity.

In agreement with comments received during public consultation, RAC also finds that the signs of maternal thymotoxicity are rather to be characterised as slight. Furthermore, RAC notes that the developmental toxicity studies are rather short (10-13 days), with some effects (post-implantation losses) likely to have occurred after just a few days of exposure, and that the maternal thymotoxicity may not have been implemented as functional effects on the immune system after such short exposure periods. In addition, there is no plausible link between the thymotoxicity and the different types of developmental effects observed in three species. The strongest thymocytic (T-cell suppressive) effect was observed in the rat, however higher level of evidence for developmental toxicity came from mice and rabbits, which were much less sensitive to maternal thymotoxicity.

To clarify the potential contribution of maternal toxicity the following observations are informative: In mice (Faqi *et al*., 2001) that received a mixture (80:20%) of DOT(IOMA) (diisooctyl 2,2'-[(dioctylstannylene)bis(thio)]diacetate, CAS No. 26401-97-8, EC no 247-660-0) and MOT(IOMA) (triisooctyl 2,2',2''-[(octylstannylidyne)tris(thio)]triacetate, CAS no. 26401-86-5, EC no. 247-665-5) on GD 6-17, skeletal variations were significantly increased at dose levels (≥20 mg/kg bw/d) below those causing thymus weight effects (45 mg/kg bw/d). Significant increases of skeletal abnormalities were seen at ≥67 mg/kg bw/d while no signs of maternal toxicity were recorded except decreased liver weight and one dead dam at 100 mg/kg bw/d. Significant maternal toxicity was also absent in rabbits at doses where abortions, fetolethality and skeletal /visceral abnormities were seen (Battenfeld, 1992). RAC is therefore of the opinion that the maternal thymotoxicity has no bearing on the reproductive toxicity observed in these species, and supports the view expressed by the six Member States during public consultation on this issue.

According to the RAC, there is clear evidence of developmental toxicity in three different species, while there are no or only slight signs of maternal (thymo-)toxicity. The observed developmental toxicity is not considered to be a secondary non-specific consequence of the (thymo-)toxicity. Additionally, there is no mechanistic information that raises doubt about the relevance of the

developmental effects for humans. Classification in Reproductive Toxicity Category 1B (H360D) according to the CLP criteria is therefore appropriate. The corresponding classification under DSD is Repr. Cat. 2; R61.

Repr. 1A (CLP) is not appropriate in view of the lack of human data. Repr. 2 should be chosen if there is only some evidence or the quality of evidence is less convincing. In this case, there is clear evidence of developmental toxicity occurring in three different species, where the evidence comes from convincing studies.

Regarding effects on fertility, the available data indicate that all toxic effects occur post-implantation, however RAC noted that the proposal was targeted at developmental effects. It is concluded that data may not be sufficiently detailed or complete for a comprehensive evaluation for the endpoint fertility. Thus no decision is taken with regard to this endpoint..

RAC remarks on read-across and category approach to a common metabolite

None of the studies of concern for reproductive toxicity were conducted on the DOT(2-EHMA) (Dioctyltin bis(2-ethyhexyl mercaptoacetate), which is proposed for classification. The key studies referred to in the proposal used Dioctyltin bis(IOMA) [CAS no. 26401-97-8]:Octyltin tris(IOMA) [CAS no. 26401-86-5] mixture (≥80:<20%) (2 rat studies, 1 study in rabbits, 1 study in mice) and DOTC (Dioctyltin dichloride, EC no. 222-583-2, CAS no. 3542-36-7))(1 rat study).

The dossier submitter's view in the original CLH dossier was that DOTC is an appropriate surrogate for the mammalian toxicity of the corresponding thioesters DOT(2-EHMA/(IOMA) due to its 100% hydrolysis in simulated mammalian gastric contents within 30 min, and RAC shares this view.

Reproductive findings from the DOTC study are consistent with findings on DOT(IOMA) in rats (no comparison possible for other species). This indicates that these structurally similar substances either have the same inherent reproductive toxicity or form a common hydrolysis product (e.g. DOTC) which is a reproductive toxicant.

This category concept is internationally accepted for the oral route (see OECD SIAR on dioctyltin compounds http://webnet.oecd.org/Hpv/UI/SIDS_Details.aspx?id=FA10501B-95AD-42C8-8873-42AC7BB34E9E.

In conclusion DOTC is considered as the active moiety causing developmental effects in mammalian species. DOTC is a hydrolysis product of DOT(2-EHMA) and of DOT (IOMA), which are structurally similar and which immediately form DOTC at comparable hydrolysis rates after oral administration Therefore read-across from DOT(IOMA) and DOTC to DOT(2-EHMA) appears to be justified.

In absence of any reasons that may indicate significantly (significant in the meaning of qualitative difference) lower toxicity of DOT(2-EHMA) than of the other members of this dioctyl tin group, similar reproductive effects are expected for DOT(2EHMA) as for the tested substances Thus, the read across to DOT(2EHMA) is fully justified.

If in future, new data may show that there are quantitative differences in the potency of DOTC, DOT(IOMA) and DOT(2-EHMA), these might be relevant when considering specific concentration limits (when agreed and adopted) for this endpoint. but not for classification.

RAC recommendations

While the present CLH dossier proposes classification of DOT(2-EHMA) only, RAC encourages a

Member State to consider the preparation of classification dossiers on DOTC and DOT(IOMA) in order to achieve a consistent classification of these category members.

In depth analysis by RAC addressing issues discussed during RAC-19 and ETINSA position proposal dated 24th Nov. 2011

RAC supports that dioctyltin bis(2-ethylhexyl mercaptoacetate) (DOT(2-EHMA)) is a developmental toxicant, but finds that the evidence fits the criteria for classification in Repr. 1B (H360D), rather than the category proposed by the dossier submitter (Repr. 2 (H361d)).

Opposed to the draft opinion discussed at RAC-19, the dossier submitter considered their original proposal for classification Repro. 2 – H361d still appropriate due to the following reasons:

1. Read-across from other substances adds uncertainties, which reduce the quality of evidence (see RAC remarks above).

2. Shortcomings in the studies reduce the quality of evidence (see a).

3. The developmental effects can possibly be caused by a secondary, specific, maternally-mediated mechanism (see b)

Taking the discussion at the RAC 19 meeting and Industry's comments (ETINSA position proposal, 24th Nov. 2011) into account, the RAC opinion on points 2 and 3 above is as follows:

a) Strength of evidence from the available studies

The CLH dossier reported all key studies as equivalent or similar to the respective OECD testing guidelines. All studies were regarded as reliable without restriction (class 1) except the mouse developmental studies judged as reliable with some restrictions (class 2).

<u>2-Generation study in rats (0, 20, 60, 200 ppm) Anonymous, 1997</u>

As reported in the CLH dossier, consistent pup effects were seen at 200 ppm in the F0 and F1 generation (pup lethality and impairment of postnatal viability, reductions in pup body weights indicative of postnatal growth retardation and thymus atrophy indicating developmental immunotoxicity). Indicative for development retardation, a delayed vaginal opening were seen in F1 pups whereas delayed pinna unfolding, eye and ear opening were reported for F2 pups. Increases in stillbirths in F2 pups was the only finding that was observed in addition to the range of consistent findings in pups of both generations. There was no indication of maternal toxicity that may have influenced the pup effects.

Thus, RAC can not agree on a 'low strength of evidence' from this study as suggested in the position paper (date 2011-11-24) where Industry did not consider the effect as an adverse reproductive outcome. Industry suggested that the 'post-natal effects are direct toxic effects to the F1 generation which are carried over to animals selected as parents for the F2 generation.'

Consistency of effects among F1 and F2 pups confirm that pre- and postnatal toxicity is a direct developmental effect where maternal toxicity is of no significance.

<u>Developmental study in mice (GD 6-17: 0, 20, 30, 45, 67, 100 mg/kg/d) (Faqi et al., 2001)</u>

Except for one death of a pregnant dam at 100 mg/kg/d test substance, the only signs of maternal

toxicity were reduced thymus weight observed at 45 mg/kg/d (-15%) and 100 mg/kg/d (-27%) and lower liver weight at 100 mg/kg/d (-19%). The original publication documented that maternal weight gain at GD 18 was non-significantly reduced at 67 mg/kg and 100 mg/kg/d. However, after correction of uterus weight, maternal weight gain was reduced at 100 mg/kg/d. No sign of maternal effect was seen at doses of 20 and 30 mg/kg/d.

Dose-dependent developmental effects were observed in pups: Skeletal variations were seen from 20 mg/kg/d onwards, post implantation losses, skeletal abnormalities and cleft palates at 67 mg/kg/d and above, and in addition at 100 mg/kg/d increased incidence of exencephaly resulting from the treatment.

In the absence of any significant maternal toxicity at 67 mg/kg and below and questioning the relevance of the reduced thymus weight at 45 mg/kg/d, developmental effects can not be attributed to maternal toxicity. Although a number of details were criticised in the position paper to be lacking in the publication, the observations in pups cannot be dismissed because treatment-related developmental effects occurred at doses below 100 mg/kg/d without any or minor (thymus weight) maternal toxicity.

RAC can not follow the conclusion of Industry in their position paper that fetal observations are always associated with maternal toxicity. There was also a conclusion that maternal-fetal causality is difficult to assess because of study design deficiencies. 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.

<u>Developmental study (GD 6-18: 0, 1, 10, 100 mg/kg/d) in rabbits (Battenfeld, 1992)</u>

Out of 22-24 dams, deaths due to pneumonia or enteritis and clinical signs of nasal haemorrhage were reported for three dams of the high dose (100 mg/kg/d) and two dams of the 1 mg/kg/d group. No significant effect on growth or other sign of maternal toxicity were observed.

In contrast to the dossier submitter who attributed abortions ‘at least partly to a slight maternal toxic effect’ and the Industry position which interpreted abortions as stress-related, RAC considered the significant increase in abortions at the high dose in the absence of any other sign of maternal toxicity and the absence of any other ‘stress’-related effect not as an effect of maternal toxicity. Increased rates of post-implantation losses seen at 100 mg/kg/d are consistent with findings in mice and in rats in the reproductive toxicity screening study (Appel and Waalkens-Berendsen, 2004). Increased rates of skeletal variations, minor visceral and skeletal head abnormalities indicating retardation of fetal development were seen in pups from surviving dams that received 100 mg/kg/d. The increase in the minor skeletal head abnormalities appeared to be dose-related: non-significantly increased incidences at 10 mg/kg/d and significantly increased incidences at 100 mg/kg/d were indicating that this effect was test-substance related and occurred in absence of any indication of maternal toxicity.

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 compliant to 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”.

Developmental study (GD 6-15: 0, 1, 5, 25 mg/kg/d) in rats (Battenfeld, 1991)

Indeed there is only weak evidence on developmental effects from this rat study since dead fetuses were seen only from one dam.

Rat 13 week diet study with satellite group for reproductive toxicity screening (Appel and Waalkens, 2004) (Original data not available) (0, 10, 100 and 300 ppm, treatment per group: 10 females were exposed from 2 weeks premating until PND 4, and 10 males were exposed 13 weeks premating)

In this reproduction/developmental toxicity screening study, postimplantation loss and reduced live birth index, pup lethality and impairment of postnatal viability and runts indicative for developmental retardation were observed at 100 ppm (6.5-6.8 mg/kg/d) and 300 ppm (19.3-19.8 mg/kg/d). Dose-dependent reductions in relative thymus weight (-23% and -62%) and lymphoid depletion in rats of the 100 ppm and 300 ppm groups were also observed: Lymphoid depletion is a microscopic finding likely to be associated to thymus atrophy.

No other sign of maternal toxicity was reported. Lymphoid depletion to an extent leading to about 20-30% weight decrease of thymus alone is not considered to represent a maternal effect of marked toxicity. It is indicating a minor systemic toxic effect. Causality to the observed developmental toxicity is speculative due to the low extent of thymus atrophy and clear evidence of embryo/fetotoxicity at the 100 ppm dose (6.5-6.8 mg/kg/d). Similar pup effects were also observed in mice and rabbits without immunotoxic effects in these species.

b) Evidence for a secondary mode of action causing developmental effects

The position paper states that “although the mechanism of action of thymus involution on embryo development is still unclear, it is possibly a secondary specific maternally-mediated mechanism and can not be reliably extrapolated to humans which, according to CLP criteria, suggest classification in category 2 for reproductive toxicity”.

If choosing Repr. 2 rather than 1B, CLP criteria require identification of a mode of action that gives clear evidence that developmental toxicity is a secondary consequence of thymotoxicity.

Opposite to the suggestion of Industry that such a specific mode is possible, although not yet found and characterised, the proof of evidence requires 1) identification of a specific mode of action and 2) proof that this mode of action is not relevant for humans.

With respect to the discussed mode of action and regarding the available key studies in rats, mice and rabbits, the RAC points out the following points of importance that support the classification of DOT(2EHMA) as Repr. 1B (H360D):

- Consistent and dose-dependent effects of embryo- and fetotoxicity, reduced pup viability, retardation effects on pup growth and maturation were found in three test species.
- The strongest effect on thymus weight was observed in rats. Most concern for developmental toxicity came from the studies in mice and rabbits, and it is noted that mice were much less sensitive than rats to the thymotoxicity and that no thymus effect was seen in the rabbit developmental studies.
- Developmental toxicity was seen at doses without thymus effects.
- No specific mode of action has been identified to show that developmental effects can be caused by a specific thymus (T-lymphocyte)-related mechanism.

- Even in case a specific mode of action demonstrates that developmental effects is secondary to that specific mode, downgrading of the classification category can only be justified if non-relevance for humans has been demonstrated.
- Effects on maternal weight gain were absent or of minor extent in the relevant studies. In the mouse developmental study (Faqi et al., 2004), where the original report documented lower (corrected) body weight gain at the high dose, developmental toxicity was also observed at lower doses without effects on body weight. Thus, high doses may induce maternal toxicity, which exacerbates the pup effects (e.g. pups from pregnant mice that received 100 mg/kg/d in the Faqi study). Minor and non-significant depression in body weight gain may also be contributable to resorptions; hovever data are insufficient to estimate corrected body weight gain. Based on the data available, maternal weight gain suppression was not a precondition for the developmental toxicity.
- In rats, reduction of thymus weight was observed in dams (and in non-pregnant adults after repeated/chronic administration of DOT(2-EHMA, see section 4.7: Repeated dose toxicity in BD). Moreover reduced thymus weight was also seen in F1 and F2 pups at doses of 60 ppm (4.7 mg/kg/d) and above (Anonymous, 1997). Significant suppression of T-cell mediated immune function was observed in 10-week old pups that were directly dosed at PND 3-24 (Smialowicz, 1988). RAC considered reduced thymus weight in pups a direct developmental immunotoxic effect after prenatal or postnatal exposure that as such would require classification as Repr. 1B (H360D).

4.12 Other effects

No data is available.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier.

6 OTHER INFORMATION

Not relevant.

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