



Committee for Risk Assessment
RAC

Annex 1
Background document
to the Opinion proposing harmonised classification
and labelling at Community level of

2-ethylhexyl 10-ethyl-4,4-dimethyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate

EC Number: 260-829-0

CAS Number: 57583-35-4

CLH-O-0000001412-86-10/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
30 November 2012

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	2-ethylhexyl 10-ethyl-4,4-dimethyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate
EC number:	260-829-0
CAS number:	57583-35-4
Annex VI Index number:	-
Degree of purity:	> 80 % w/w
Impurities:	Residual reactants: 2-ethylhexyl mercaptoacetate; methyltin trichloride; dimethyltin dichloride; trimethyltin chloride

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, CLP Regulation	-	-
Current proposal for consideration by RAC	Repr. 2, H361d Acute Tox 4, H302 Skin Sens 1A, H317 STOT RE 1, H372 with nervous system as main target organ	Repr. Cat. 3, R63 Xn, R22 Xi, R43 T, R48/25.
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Repr. 2, H361d Acute Tox 4, H302 Skin Sens 1A, H317 STOT RE 1, H372 with nervous system as main target organ	Repr. Cat. 3, R63; Xn, R22 Xi, R43 T, R48/25.

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

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⁾
2.1.	Explosives	None		None	Not evaluated
2.2.	Flammable gases	None		None	Not evaluated
2.3.	Flammable aerosols	None		None	Not evaluated
2.4.	Oxidising gases	None		None	Not evaluated
2.5.	Gases under pressure	None		None	Not evaluated
2.6.	Flammable liquids	None		None	Not evaluated
2.7.	Flammable solids	None		None	Not evaluated
2.8.	Self-reactive substances and mixtures	None		None	Not evaluated
2.9.	Pyrophoric liquids	None		None	Not evaluated
2.10.	Pyrophoric solids	None		None	Not evaluated
2.11.	Self-heating substances and mixtures	None		None	Not evaluated
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	Not evaluated
2.13.	Oxidising liquids	None		None	Not evaluated
2.14.	Oxidising solids	None		None	Not evaluated
2.15.	Organic peroxides	None		None	Not evaluated
2.16.	Substance and mixtures corrosive to metals	None		None	Not evaluated
3.1.	Acute toxicity - oral	Acute tox 4 H302	Not applicable	None	
	Acute toxicity - dermal	None		None	Not evaluated
	Acute toxicity - inhalation	None		None	Not evaluated
3.2.	Skin corrosion / irritation	None		None	Not evaluated

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3.3.	Serious eye damage / eye irritation	None		None	Not evaluated
3.4.	Respiratory sensitisation	None		None	Not evaluated
3.4.	Skin sensitisation	Skin Sens 1A H317	None	None	
3.5.	Germ cell mutagenicity	None		None	Not evaluated
3.6.	Carcinogenicity	None		None	Not evaluated
3.7.	Reproductive toxicity	Repr. 2; H361d	Guidance currently not available	None	
3.8.	Specific target organ toxicity –single exposure	None		None	Not evaluated
3.9.	Specific target organ toxicity – repeated exposure	STOT RE 1 H372 with nervous system as main target organ	None	None	
3.10.	Aspiration hazard	None		None	Not evaluated
4.1.	Hazardous to the aquatic environment	None		None	Not evaluated
5.1.	Hazardous to the ozone layer	None		None	Not evaluated

¹⁾ Including specific concentration limits (SCLs) and M-factors

²⁾ Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: "danger"
Hazard statements: H302, H317, H361d, H372
Precautionary statements: not harmonised
Pictogram: GHS07, GHS08

Proposed notes assigned to an entry: None

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Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification ¹⁾	Reason for no classification ²⁾
Explosiveness	None		None	Not evaluated
Oxidising properties	None		None	Not evaluated
Flammability	None		None	Not evaluated
Other physico-chemical properties <i>[Add rows when relevant]</i>	None		None	Not evaluated
Thermal stability	None		None	Not evaluated
Acute toxicity	Xn; R22	None	None	
Acute toxicity – irreversible damage after single exposure	None		None	Not evaluated
Repeated dose toxicity	T; R48/25	None	None	
Irritation / Corrosion	None		None	Not evaluated
Sensitisation	Xi; R43	None	None	
Carcinogenicity	None		None	Not evaluated
Mutagenicity – Genetic toxicity	None		None	Not evaluated
Toxicity to reproduction – fertility	None		None	Not evaluated
Toxicity to reproduction – development	Repr. Cat. 3; R63	Guidance currently not available	None	
Toxicity to reproduction – breastfed babies. Effects on or via lactation	None		None	Not evaluated
Environment	None		None	Not evaluated

¹⁾ Including SCLs

²⁾ Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger: T
R-phrases: R22-R43-R48/25-R63
S-phrases: S36/37-S45

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

A classification proposal was submitted and discussed at ECB (TC C&L) for health endpoints in October 2006. Classification for health was concluded by TC C&L in October 2006. For information, discussions and conclusions of the TC C&L as reported in summary records of the corresponding meeting are presented in Annex I of the present report.

A new study (Ehman, 2007) has been published on developmental toxicity of dimethyltin dichloride (DMTC) since the TC C&L discussions and has been integrated in the present report.

Although it covers a different endpoint (developmental neurotoxicity), the results of this study are however consistent with the classification Repr. Cat. 3; R63 agreed by the TC C&L and proposed in the present dossier.

Registration dossiers are now available and have been reviewed to check whether additional information was available. A skin sensitisation study that was not previously identified was added but it does not impact significantly the proposed classification. No additional key studies have been identified.

2.2 Short summary of the scientific justification for the CLH proposal

A study of acute oral toxicity (Morton International, Inc. 1996) was performed on rats and showed a LD₅₀ of 1150 mg/kg bw, so a classification was proposed.

Two studies of skin sensitization are presented: a guinea pig optimization test (Ciba Geigy, 1975) showed a positive reaction of skin sensitization; in a Buehler test (Dow Chemical Company, 1989), sensitization was not induced. This last study comes from the registration dossier that was not available in the dossier reviewed by the TC C&L. The optimization test is considered as more sensitive due the use of adjuvant and a classification is therefore proposed.

The results of a simulated gastric hydrolysis study found that EHMA is rapidly released from dimethyltin bis (EHMA) and support the use of the chloride derivative, dimethyltin dichloride (DMTC) as the anchor compound for the repeated dose, reproductive, and developmental effects endpoints.

Two oral 90-day studies on DMTC indicate that the main target organ is the nervous system with deaths and severe neurological signs occurring from 75 ppm (5.2/6.7 mg/kg) and neuropathological lesions from 25 ppm (1.6/2.2 mg/kg). A classification for repeated toxicity is therefore proposed.

Studies of Noda show that DMTC is toxic for the prenatal development of the fetus. Severe maternal toxicity occurred at the high dose (deaths, severe thymus atrophy, vaginal bleeding, tremors, convulsions, ataxia and others clinical signs of toxicity). Fetotoxic responses observed at the high dose included total litter resorptions, cleft palate, and significantly decreased fetal body weight.

The results of the two new studies of Ehman indicated that exposure to dimethyltin dichloride (DMTC) decreased fluid consumption, depressed maternal weight gain, altered the spatial learning in the Morris water maze, decreased brain weight and altered levels of an apoptotic marker (DNA fragmentation). Moreover, in one of the experiment, neuropathological lesions were observed, as mild vacuolation of the neuropil in the cerebral cortex. Besides, DMTC altered litter weight and pup growth in experiment 2.

Based on the induction of cleft palate in some but not all experiments, reduced fetal weight in presence of maternal toxicity and observation of a developmental neurotoxic potential, classification in Repr 2 is therefore proposed.

2.3 Current harmonised classification and labelling

No current harmonised classification in Annex VI of CLP.

2.4 Current self-classification and labelling

The self-classifications that have been notified are given in the confidential Appendix II (separate file).

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The substance has CMR properties, i.e. reproductive toxicity that justifies harmonising its classification and labelling.

In this aim, a classification proposal was submitted and discussed at ECB (TC C&L) for health endpoints in October 2006.

In this context, all relevant information was collected for health endpoints. Available data indicate that classifications for acute oral toxicity, skin sensitization and repeated toxicity are also justified as recommended by TC C&L. It is noted that in the self classification notified by manufacturers and importers, the classifications for some of these endpoints differs between notifiers and with the proposed harmonized classification:

- For skin sensitisation, some notifiers apply no classification
- For repeated toxicity, some notifiers apply a classification in category 2

Classification for oral acute toxicity and reproductive toxicity are applied adequately by all notifiers.

Considering the recommendations of TC C&L and the absence of consensus on this classification as indicated by the classification notifications, harmonization of classification on this handover CLH dossier is considered to be required for the different endpoints concluded by the TC C&L.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

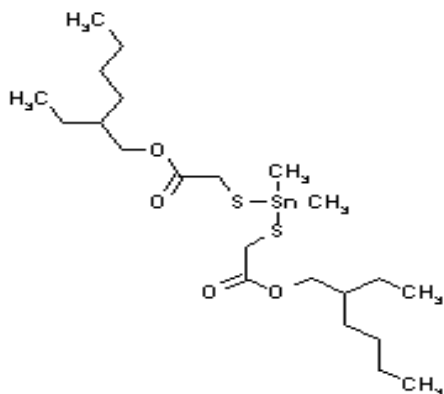
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	260-829-0
EC name:	2-ethylhexyl 10-ethyl-4,4-dimethyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate
CAS number (EC inventory):	57583-35-4
CAS number:	57583-35-4
CAS name:	8-Oxa-3,5-dithia-4-stannatetradecanoic acid, 10-ethyl-4,4-dimethyl-7-oxo-, 2-ethylhexyl ester
IUPAC name:	2-ethylhexyl 10-ethyl-4,4-dimethyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecan-1-oate
CLP Annex VI Index number:	-
Molecular formula:	C ₂₂ H ₄₄ O ₄ S ₂ Sn
Molecular weight range:	555.4 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
DMT(EHMA) CAS: 57583-35-4	82.7% (w/w)	80% - 100%	

Table 7: Impurities (non-confidential information)

Specific information available in the registration dossier of DMT(EHMA) is provided in a separate confidential appendix II.

Table 8: Additives (non-confidential information)

No data concerning the additives of DMT (EHMA) are available.

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 9: 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 at 20°C +/- 0.5°C and 1013 hPa Purity = 83.1% DMT(EHMA) and 14.1% tris(2-ethylhexylthioglycolate)methyltin	Harlan Laboratories; 2009	Measured
Melting/freezing point	-75 to -65 °C Purity = 98.1%	Crompton GmbH. 2001	Measured Differential Scanning Calorimetry* (*DSC)
Boiling point	>= 290 °C at 101325 Pa Purity = 98.1%	Crompton GmbH. 2001	Measured DSC
Relative density	1.18 g/cm ³ at 20 °C (density) Purity = 99.3% (w/w) pure (impurity = 0.7% (w/w) unknown) 1.17 g/cm ³ at 20°C (specific gravity) Purity = 60-100 wt%	BASF, 2000. Rohm and Haas Co. 2001.	Measured Measured
Vapour pressure	0.00441 hPa at 25 °C 0.81 Pa at 25°C	USEPA. 2000. Harlan laboratories, 2010.	Calculated Measured : effusion method: vapour pressure balance
Surface tension	Waived: Technical not feasible because the substance decomposes in water, so the testing cannot be performed.	-	-
Water solubility	Conc. at sat. (g/l) 3.75 mg/l Temperature (°C): 19.25 7.5 <= pH <= 8.1 The test item was concluded to hydrolyse fully within 6 minutes of contact with water; thus, it was decided that the water solubility	De Roode2004. Harlan laboratories, 2010	Estimated Not feasible.

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	test was not feasible.		
Partition coefficient n-octanol/water	8.48 Purity unknown	Witco GmbH. 1999.	Calculated
Flash point	156 +/- 2°C At 429.9 K At 101325 Pa Purity = 83.1% bis(2-ethylhexylthioglycolate) and 14.1% Tris(2-ethylhexylthioglycolate) methyltin	Harlan Laboratories; 2009	Measured Test used : closed cup (EU Method A.9)
Flammability/pyrophoricity	Non flammable Non pyrophoric Purity = 83.1% Bis(2-ethylhexylthioglycolate)dimethyltin and 14.1% tris(2-ethylhexylthioglycolate) methyltin	Harlan laboratories, 2009	Measured EU Method A.13 (Pyrophoric Properties of Solids and Liquids)
Explosive properties	Negative	Harlan laboratories, 2009	Estimated EU Method A.14 (Explosive properties)
Self-ignition temperature	Data lacking	-	-
Oxidising properties	Waived: Testing is technically not justified due to lack of atoms/functional groups associated with oxidizing potential	-	-
Granulometry	Not adequate	-	-
Stability in organic solvents and identity of relevant degradation products	10 g/l solutions in Tetrahydrofuran and Diethyl Ether for at least 32 days at 25 +/- 2 °C. Purity: 83.1% bis(2-ethylhexylthioglycolate) dimethyltin and 14.1% tris(2-ethylhexylthioglycolate)methyltin	Harlan laboratories, 2009	Measured Test used according to COUNCIL DIRECTIVE 98/8/EC, ANNEX III, 2
Dissociation constant	Waived: Technically not justified	-	-
Viscosity	Viscosity 66.3 mm ² /s at 20 deg C and 28.2 mm ² /s at 40 deg C Purity: 83.1% bis(2-ethylhexylthioglycolate) dimethyltin and 14.1% tris(2-ethylhexylthioglycolate)methyltin	Harlan laboratories, 2009	Measured OECD Test Guideline 114 (Viscosity of Liquids) EPA OPPTS 830.7100 (Viscosity)

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant in this dossier.

2.2 Identified uses

Used as a heat stabilizer in PVC (Parametrix, Inc. 2000).

No use known for general public.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

An in vitro study was performed to assess gastric hydrolysis of DMT(EHMA) (ORTEP 2000).

A concentration of 2 µg/mL DMT(EHMA) (80% with 20% MMT(EHMA)) was tested under low pH (approximately 1-2) conditions (0.07 N HCl) at 37°C in order to simulate the possible hydrolytic action on mammalian gastric contents. Under acidic conditions it is expected that the tin-EHMA bond breaks, leading to formation of the corresponding alkyltin chloride and release of the ligand EHMA. The liberated EHMA may hydrolyse further, forming thioglycolic acid (TGA) and 2-ethylhexanol (EH). The degree of hydrolysis was studied by determination of the DMTC content in the water fraction after extraction of DMT(EHMA) in heptane. Methyltin chlorides are very water soluble while methyltin mercaptides are preferentially extracted by heptane.

Atomic absorption spectroscopy (AAS) data show more than 90% of the tin compounds in the water layer. Analysis of the water layer by tin specific GC-FPD shows only peaks with retention times of dimethyltin dichloride and monomethyltin trichloride. Total tin found by both AAS and GC-FPD are nearly the same, demonstrating that within experimental error all the tin species in the water are methyltin chlorides. By sulfur specific GC-FPD, approximately 75% of the theoretical total sulfur was found in the organic layer as the hydrolysate EHMA. Another sulfur bearing hydrolysate, thioglycolic acid, was identified but not quantified in the water layer. No other sulfur component was detected in the water. ICP -AES determined the remaining 25% of the total sulfur present in the water layer (presumed to be thioglycolic acid from GC-FPD, above).

The conversion of DMT(EHMA) to DMTC was rapid. The calculated percentage of hydrolysis was 103% after 0.5 hours. An apparent decrease of the percentage of hydrolysis in time was observed: 93% at 1h, 93% at 2h, 94% at 3h and 84% at 4h. This was thought to be due to a loss of EHMA (or 2-ethylhexanol) by adsorption or oxidation.

It should be noted that taken into account an hydrolysis of 100% and the respective molecular weight of DMT(EHMA) (555.4 g/mol) and of DMTC (220 g/mol), 1 mg DMTC is expected to be released from approximately 2.5 mg DMT(EHMA).

4.2 Acute toxicity

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Species	LD50 (mg/kg bw)	OBSERVATIONS AND REMARKS	Ref.
Rat 5/sex/dose	1150 mg/kg bw	<p>Test substance: DMT(EHMA): purity unknown. Test performed according to OECD 401. Doses: 625, 880, 1250, and 2500 mg/kg bw MORTALITY (dead/total number tested): 625 mg/kg bw: Males, 0/5; Females, 0/5 880 mg/kg bw: Males, 0/5; Females, 4/5 1250 mg/kg bw: Males, 1/5; Females, 4/5 2500 mg/kg bw: Males, 5/5; Females, 5/5 Gross necropsy findings of animals that died during the observation period included mottled lungs, liver and spleen; hemorrhagic lungs; reddened lungs; pale and/or congested liver; enlarged left kidney; stomach distended with gas; reddened intestines; intestines contained paste-like material; urinary bladder contained red or yellowish-red fluid; and external staining.</p>	Morton International, Inc. 1996.

4.2.1.2 Acute toxicity: inhalation

Not evaluated in this dossier.

4.2.1.3 Acute toxicity: dermal

Not evaluated in this dossier.

4.2.1.4 Acute toxicity: other routes

Not evaluated in this dossier.

4.2.2 Human information

No data available.

4.2.3 Summary and discussion of acute toxicity

In the oral acute toxicity, five rats on ten (one male and four females) died at the dose of 1250 mg/kg bw and the acute oral LD₅₀ was determined at 1150 mg/kg bw. At the dose of 880 mg/kg bw four out five females died indicating that the LD₅₀ for females is between 625 and 880 mg/kg bw. The LD₅₀ for either females or combined sexes are therefore between 300 and 2000 mg/kg bw.

4.2.4 Comparison with criteria

The acute oral LD₅₀ values for DMT(EHMA) are between 300 and 2000 mg/kg bw and a classification "**Acute Tox. 4, H302**" is proposed according to **CLP**.

The acute oral LD₅₀ values for DMT(EHMA) are between 200 and 2000 mg/kg bw and support a classification **Xn R22** according to Directive 67/548/EEC.

4.2.5 Conclusions on classification and labelling of acute toxicity

A classification Acute Tox 4 – H302 (Xn; R22) is proposed for acute toxicity as agreed at TC C&L of October 2006.

RAC evaluation of Acute toxicity

Summary of Dossier submitter's proposal

The CLH report includes one acute oral toxicity study, done according to OECD 401 with no reported deviations (Morton International, 1996). The combined LD₅₀ for both sexes was established at 1150 mg/kg and the dossier submitter proposed a classification of Acute Tox. 4 –H302 according to the CLP regulation and Xn R22 according to Directive 67/548/EEC.

Comments received during public consultation

Comments were received from two Member States during public consultation. One was in agreement with the proposed classification while another argued for additional classification of DMT(EHMA) for acute toxicity by the dermal and inhalation route. Further details can be found in the RCOM.

RAC assessment and comparison with criteria

In an oral acute toxicity study, five rats of ten (one male and four females) died at the dose of 1250 mg/kg bw and the acute oral LD₅₀ was determined at 1150 mg/kg bw. At the dose of 880 mg/kg bw four out of five females died indicating that the LD₅₀ for females is between 625 and 880 mg/kg bw. The LD₅₀ for either females or combined sexes are therefore between 300 and 2000 mg/kg bw, which according to the CLP regulation warrants a classification as Acute Tox. 4, H302. Falling between 200 and 2000 mg/kg bw, this therefore warrants a DSD classification of Xn; R22). RAC agrees with the proposal by the DS.

RAC did not evaluate acute dermal or inhalation toxicity or skin irritation, since the dossier submitted did not address these endpoints.

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

Not evaluated in this dossier.

4.4 Irritation

Not evaluated in this dossier.

4.5 Corrosivity

Not evaluated in this dossier.

4.6 Sensitisation

4.6.1 Skin sensitisation

4.6.1.1 Non-human information

Species	Type of test	No. of animals	Incidence of reactions observed	Ref.
Guinea pig	Guinea pig optimization test	10/sex	<p>Test substance: DMT(EHMA) ; purity not reported</p> <p>During the first week volume of 0.1 mL of the test substance in saline (0.1%) were injected intradermally at two sites (flank and back) and 2 and 4 days later at each one site on the back. During the second and third weeks of induction the substance was mixed with complete Freund's adjuvant in a 1:1 ratio. A total of 6 sensitising dose of 0.1 mL were injected subcutaneously. The test for skin sensitizations was performed two weeks after the last sensitizing treatment by intradermal injection of 0.1 ml of the test substance. Reaction was assessed twenty-four hours later.</p> <p>Under the experimental conditions employed, significant differences between the test group and the vehicle treated controls were found with the test substance. A positive reaction was observed with the following erythema scores (Draize scores), 24 hours after removal of dressing, by sex:</p> <p>Males: 4/10 had a score of 1</p> <p>Females: 7/10 had a score of 1</p>	CIBA-GEIGY, 1975

An additional study is available in the registration dossier that was not available in the dossier reviewed by the TC C&L:

Species	Type of test	No. of animals	Incidence of reactions observed	Ref.
Guinea pig	Buehler test	20/dose and 10 in	Test substance: DMT(EHMA); purity not	Dow Chemi

		controls	reported	cal Compa ny, 1989
			The potential of the test substance to produce delayed contact hypersensitivity in guinea pigs was evaluated using an adaptation of the Buehler method. A 50% w/v formulation in acetone was used for induction. Following primary challenge at 1% w/v in acetone there were no grades of 1 produced in the test or control animals. The incidence of a slightly patchy erythema response in the test group (10 of 20) was compared to that of the naive control group (6 of 10). The incidence and severity of these responses in the test group were essentially comparable to those produced by the naive control group indicating that sensitization had not been induced.	

4.6.1.2 Human information

No data.

4.6.1.3 Summary and discussion of skin sensitisation

A positive reaction was observed in a guinea pig optimization test. 24 hours after removal of dressing 4/10 males had an erythema score of 1 and 7/10 females had a score of 1. No reaction was observed in a modified Buehler test. However, due to the use of maximisation in the guinea pig optimization test, this test is considered more sensitive. Nevertheless, the severity of the effects has to be taken into consideration for the classification in "skin sensitisation" without considering the sensitivity of the tests used.

4.6.1.4 Comparison with criteria

Criteria of classification based on animal data according to 67/548/EEC regulation referred to cases where there were positive results from appropriate animal tests. Currently, the 2nd ATP to CLP brought in new criteria for classification of skin sensitisation, introducing subcategories 1A and 1B.

Sub-category 1B is defined for substances showing a low to moderate potency in animals, so this sub-category would not be well appropriate for the DMT(EHMA) because of the strong positive reaction observed in a guinea pig optimization test despite the fact that this test is known to be more sensitive than the others because of the maximization of the immune response with the adjuvant of Freund; however, there are sensitization effects that are not negligible although the Buehler test is negative.

Sub-category 1A (defined by a high potency in animals) would be more appropriate because of the strong severity of the reaction seen in the guinea pig optimization test.

With a concentration for intradermal induction of 0.1%, and an incidence sensitised guinea pigs of 55% (males and females), the potency calculated according to the table 3.4.2.3.4.2 in the guidance of the CLP criteria is considered as **strong**; so the classification in the sub-category 1A is justified. Moreover, positive effects seen in animals will normally justify classification (section 3.4.2.2.2.2 of the CLP).

4.6.1.5 Conclusions on classification and labelling of skin sensitisation

According to the 2nd ATP criteria, Skin Sens. 1A; H317 is proposed according to the CLP (**Xi; R43** according to the directive 67/548/EEC).

Guidelines to set specific concentration limit (SCL) for skin sensitization are based on guideline studies and it is therefore not possible to set SCL based on the positive result obtained in the guinea pig optimization test.

RAC evaluation of Skin Sensitisation

Summary of Dossier submitter's proposal

The CLH report includes two studies on skin sensitisation, one Guinea pig Maurer Optimization test (CIBA-GEIGY, 1975) and a modified Buehler test (Dow, 1989). The guinea pig optimization test showed a response in 55% of animals while the modified Buehler test showed no response. The dossier submitter proposes a classification of Skin Sens. 1B – H317 according to the 2nd ATP to the CLP regulation and DSD.

Comments received during public consultation

Two Member States submitted comments on skin sensitisation during public consultation. Both argued that given the strong positive reaction seen in the guinea pig optimization test, classification for Skin Sens. 1A – H317 should be considered. The dossier submitter agreed with the comments received and the changes can be seen in a revised version of the CLH report, supplied as an appendix to the RCOM.

RAC assessment and comparison with criteria

In the Maurer Guinea pig optimization test, 10 animals per sex were injected intra-dermally with 0.1 ml of the 0.1% test substance in saline. A total of 6 injections were applied during the 3 weeks of induction. Two weeks after the last injection, 0.1 ml of the test substance was applied intra-dermally. The reaction was assessed 24 hours later and a strong positive response was observed (Draize score 1 in 4/10 males and 7/10 females). A Buehler test in Guinea pigs revealed negative results.

A strong reaction was observed in the Guinea pig optimization test where 55% of animals showed an erythema score of 1 (Draize) 24 hours after removal of dressing. Although the Maurer optimization test is not guideline compliant, RAC considers that the Draize scores from this test may be used in the same way as those arising from the guideline compliant tests. Since $\geq 30\%$ of animals responded after a $\leq 0,1\%$ intradermal induction dose, the criteria for Skin Sens 1A - H317 according to CLP (DSD:Xi, R43) are met.

4.6.2 Respiratory sensitisation

Not evaluated in this dossier.

4.7 Repeated dose toxicity

Table 10: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Repeated dose toxicity study -during 13 weeks -on 15 rats/sex/dose. -3 doses: 25, 75, and 200 ppm -oral route, in drinking water	In the 200 ppm group: in the 5 first weeks, 21 females and 7 males died or were sacrificed, numerous clinical signs of toxicity and neuropathological lesions were observed. In the 75 ppm group: one male died, body weights were reduced, and neuropathological lesions were observed. In the 25 ppm group: food (males only) and water intake and neuropathological lesions. NOAEL < 25 ppm.	Reproductive organs have not been evaluated in this study.	Rohm and Haas Co. 1999.
Repeated dose toxicity study -during 13 weeks -on 10 rats/sex/dose -4 doses: 1, 6, 15, and 200 ppm -oral route, in diet	Deaths, severe neurological and neurobehavioural signs were observed at 200 ppm. Microscopic examination was not performed in lower dose animals. NOAEL = 15 ppm	Reproductive organs have not been evaluated in this study.	Elf Atochem NA. 1996.

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

A simulated gastric hydrolysis study of dimethyltin bis(EHMA) found that under low pH conditions (similar to mammalian gastric systems) all of the available EHMA ligands had been released and there was complete hydrolysis of the test substance (103%) (see 4.1). The results of this simulated gastric hydrolysis study of dimethyltin bis(EHMA) support the use of the chloride derivative, dimethyltin dichloride, as the anchor compound for the dimethyltin family of compounds to the extent possible for the repeated dose, reproductive, and developmental effects endpoints (ORTEP, 2000). Available data on EHMA are also presented.

Species	Dose, ppm in the diet (mg/kg body weight)	Duration of treatment	Observations and Remarks	Ref.
Sprague-Dawley Rats (15/sex/dose for the	25, 75, and 200 ppm in drinking water	13 weeks with 2-week neurotoxi	Test substance: Dimethyltin Dichloride: Methyltin Trichloride (90:10% mixture) Test similar to OECD Guideline 408 (Repeated dose 90-Day oral toxicity in rodents) and 424	Rohm and Haas Co.

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<p>main study and 15/sex/dose for the neurotoxicity component)</p>	<p>(equivalent to 1.6, 5.2 and 15.50 mg/kg bw/day in males and 2.2, 6.7 and 19.5 mg/kg bw/day in females)</p> <p>By oral route, in drinking water</p>	<p>city recovery group</p>	<p>(Neurotoxicity in rodents).</p> <p>Reproductive organs have not been evaluated in this study.</p> <p>General toxicity:</p> <p>200 ppm (15.50 mg/kg bw/day in males; 19.5 mg/kg bw/day in females): During the first 5 weeks, 7 males and 21 females in the 200 ppm group (15.50 mg/kg bw/day in males; 19.5 mg/kg bw/day in females) died or were sacrificed, due to poor condition. All remaining animals were sacrificed by Week 6. Animals in the 200 ppm group (15.50 mg/kg bw/day in males; 19.5 mg/kg bw/day in females) showed clinical signs of toxicity, including tremors, convulsions and aggression/hypersensitivity/difficulty when handled. Animals appeared to be weak, thin, and dehydrated, were cold to touch, were observed lying on their sides, and had decreased home-cage activity levels. An increased incidence of fur staining was also noted. Body weights and food intake were significantly lower at all intervals.</p> <p>75 ppm (5.2 mg/kg bw/day in males; 6.7 mg/kg bw/day in females): One male was found dead during Week 6 (no other deaths occurred). Abnormal clinical signs were limited to tremors, hypersensitivity (difficulty when handled), a thin dehydrated body condition for the male that died, a transitory dehydrated appearance for another male, and hypersensitivity, convulsions, and reduced activity for one female. Body weights and food consumption were significantly lower for males at most intervals measured following treatment.</p> <p>25 ppm (1.6 mg/kg bw/day in males; 2.2 mg/kg bw/day): Water consumption significantly decreased for all treated groups during most intervals measured; however, following treatment termination, water consumption values were generally comparable between the treated groups and the control.</p> <p>Functional Observational Battery (FOB) tests indicated several findings, primarily affecting the 200 ppm group (15.50 mg/kg bw/day in males; 19.5 mg/kg bw/day in females). At Week 4, females showed significantly reduced rearing, lower hindlimb grip strength, and decreased body temperature. Ataxic gait was observed for one male, along with tremors and clonus of jaws. Three females showed tremors and clonic convulsions. Hunched posture was observed for one male, and for one female ataxia was noted, as well as a red liquid material at the urogenital</p>	<p>1999.</p>
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		<p>region.</p> <p>Significant findings for the 75 ppm group (5.2 mg/kg bw/day in males; 6.7 mg/kg bw/day in females) were limited to lower body temperature of females. One male showed ataxia and unusual hind limb movements (which were also observed for this animal on subsequent testing occasions). At Weeks 8, 13, and following recovery, rearing was significantly decreased for the 75 ppm (6.7 mg/kg bw/day in females) females and their body temperature was significantly decreased at the Week 13 assessment. At Week 13, the rate of linear decrease was significantly lower for the 75 ppm (6.7 mg/kg bw/day in females) females relative to the control group.</p> <p>There were significant blood biochemical changes for males in the 200 ppm group (15.50 mg/kg bw/day in males) at Week 4, which included increases in BUN, creatinine, and phosphorus and decreases in potassium levels. Many of the animals sacrificed by Week 6 showed marked changes in various blood biochemical parameters, including increases in BUN, creatinine, AST, ALT, and phosphorus. Males in the 200 ppm group (15.50 mg/kg bw/day in males) had an elevated urine pH at Week 4.</p> <p>Absolute and relative thymus weights were significantly decreased for the 200 ppm (15.50 mg/kg bw/day in males) males at the interim sacrifice (Week 4) and 75 ppm males (5.2 mg/kg bw/day in males) at termination sacrifice. Absolute heart weight was decreased significantly for 25 and 75 ppm (2.2 mg/kg bw/day and 6.7 mg/kg bw/day in females, respectively) females at interim sacrifice, but not at terminal sacrifice. Absolute and relative kidney weights were significantly increased for 25 ppm and 75 ppm (2.2 mg/kg bw/day and 6.7 mg/kg bw/day in females, respectively) females at terminal sacrifice.</p> <p>Gross pathological findings for preterminal animals included small thymus and/or spleen, emaciated carcass, dilation of digestive tract/discolored digestive material, and dark areas on the stomach and/or lungs. A small thymus was also seen at the interim evaluation for 200 ppm males (15.50 mg/kg bw/day in males) and at the terminal evaluation for the 75 ppm group (5.2 mg/kg bw/day in males; 6.7 mg/kg bw/day in females). Results of the histopathological examinations indicated clear treatment related nervous system lesions for preterminal 200 ppm (15.50 mg/kg bw/day in males; 19.5 mg/kg bw/day in females) animals in various regions of</p>	
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			<p>the brain and spinal cord, characterized by slight to mild ventricular dilation, mild to moderate neuronal necrosis, and slight to mild white matter vacuolization. Nervous system changes were observed for 75 ppm (5.2 mg/kg bw/day in males; 6.7 mg/kg bw/day in females) animals at terminal examination (although slight and less frequent) and possible treatment-related lymphoid atrophy was observed for this group. Animals in the 25 ppm group (1.6 mg/kg bw/day in males; 2.2 mg/kg bw/day) showed slight to moderate vacuolization in brain and spinal cord tissue at the terminal examination.</p> <p>Overall, treatment of male and female rats with a 90:10% mixture of dimethyltin: monomethyltin chloride (administered in drinking water) resulted in death, reduced body weight, decreased food and water intake, blood biochemical changes, behavioral effects, and neuropathological lesions at 200 ppm (equivalent to 15.5 and 19.5 mg/kg/day for males and females, respectively).</p> <p>At 75 ppm (equivalent to 5.2 and 6.7 mg/kg/day for males and females, respectively), one male died, body weights were reduced (males only), food and water intake were decreased, motor activity was reduced (females only), and neuropathological lesions were observed.</p> <p>For the 25 ppm group (equivalent to 1.6 and 2.2 mg/kg/day for males and females, respectively), no mortality occurred and treatment-related findings were limited to reduced food (males only) and water intake and neuropathological lesions. The no-observed-adverse-effect level (NOAEL) was considered to be less than 25 ppm.</p>	
Wistar Rats 10/sex/dose	<p>1, 6, 15, and 200 ppm in food or mg/kg diet</p> <p>By oral route in diet.</p> <p>(equivalent to 0.06, 0.39, 0.98 and 16.81 mg/kg bw/day in males and 0.07, 0.41, 1.02 and 17.31 mg/kg</p>	13 weeks	<p>Test substance: Dimethyltin Dichloride: Methyltin Trichloride (66.5:33.5% mixture).</p> <p>Test similar to OECD Guideline 408 (Repeated dose 90-Day oral toxicity in rodents).</p> <p>Reproductive organs have not been evaluated in this study.</p> <p>General toxicity: Three females of the 200 ppm group (17.31 mg/kg bw/day in females) died during the first month and most males, and remaining females in this group showed severe neurological and neurobehavioural signs, including tremors, convulsions, and increased footsplay. All remaining animals of the 200 ppm group (16.81 mg/kg bw/day in males; 17.31 mg/kg bw/day in females) were sacrificed.</p> <p>Mean body weight for males of the 200 ppm group (16.81 mg/kg bw/day in males) on Days 7 and 28</p>	Elf Atochem NA., 1996.

	<p>bw/day in females)</p>	<p>were significantly lower. Food consumption on Day 7 was significantly decreased in animals (both sexes) of the 200 ppm group (16.81 mg/kg bw/day in males; 17.31 mg/kg bw/day in females) and increased in females on Day 28. Food conversion efficiency was significant only for high-dose males on Day 21. Mean water consumption was significantly reduced in females (6 ppm group [0.41 mg/kg bw/day in females]) on Day 6 only. Mean intake of the test substance in animals receiving 1, 6, 15, or 200 mg/kg diet (0.06, 0.39, 0.98 and 16.81 mg/kg bw/day in males and 0.07, 0.41, 1.02 and 17.31 mg/kg bw/day in females, respectively) were 0.06, 0.39, 0.98, and 16.81 mg/kg bw/day in males and 0.07, 0.41, 1.02, and 17.31 mg/kg bw/day in females. There was a significant increase in alanine aminotransferase and aspartate aminotransferase in males of the 1 ppm group (0.06 mg/kg bw/day in males). The specific gravity of urine was significantly increased in females of the 6 ppm dose group (0.41 mg/kg bw/day in females).</p> <p>Upon microscopic examination, treatment-related histopathological changes were observed in the brain, the kidneys, and the thymus of animals treated with 200 ppm (16.81 mg/kg bw/day in males; 17.31 mg/kg bw/day in females) of the test substance. Macroscopic pathological observations showed some gross skin changes that were probably treatment-related (200 ppm [16.81 mg/kg bw/day in males; 17.31 mg/kg bw/day in females]). Animals in the lower dose groups were not examined microscopically. Neuropathological examinations showed that animals in the high dose group (200 ppm [16.81 mg/kg bw/day in males; 17.31 mg/kg bw/day in females]) showed signs of convulsions, tremors, blepharospasm, and hunched posture. Microscopic observations showed pronounced neuronal death in a number of areas of the cerebellum in the 200 ppm group (16.81 mg/kg bw/day in males; 17.31 mg/kg bw/day in females) (more pronounced in females). The areas with predominant lesions were the hippocampal region, the piriform, entorhinal, and perirhinal cortices, the amygdala, the olfactory nuclei and the tenia tecta. A slight increase in swollen axons in the spinal cord was observed in the 200 ppm group (16.81 mg/kg bw/day in males; 17.31 mg/kg bw/day in females). Based on the effects described above, particularly the neurotoxic effects observed in the high dose group, the NOAEL was placed at 15 ppm. This was equivalent to 0.98 mg/kg bw/day (males) and 1.02 mg/kg bw/day (females) of the test mixture or 0.62 mg/kg bw/day (males) and 0.65 mg/kg bw/day (females) for the</p>	
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ANNEX 1 – BACKGROUND DOCUMENT TO RAC OPINION ON 2-ETHYLHEXYL 10-ETHYL-4,4-DIMETHYL-7-OXO-8-OXA-3,5-DITHIA-4-STANNATETRADECANOATE

			dimethyltin dichloride component of the mixture.	
Rat 5/sex/dose	0, 0.05, 0.1 and 0.2% (actual dose received: males: 42, 82, and 168 mg/kg/day; females: 45, 87, and 173 mg/kg/day)	28 days	<p>Substance tested: 2-ethylhexyl mercaptoacetate;</p> <p>Purity > 98%; Reproductive organs have not been evaluated in this study.</p> <p>- Body weight: There were no statistically significant differences between the bodyweights of EHMA treated groups.</p> <p>- Food consumption: There were no statistically significant differences between the food intakes of male control rats and male rats treated with the three dose levels of EHMA. Female rats treated with EHMA consistently consumed more diet than the control group throughout the study. These differences were statistically significant for female rats treated with 0.05 % EHMA on study days 20-24 and 24-27 respectively, and for animals given 0.1 % EHMA on study days 20-24.</p> <p>- Clinical signs: no abnormalities of condition or behavior related to treatment were seen in rats given EHMA.</p> <p>- Hematological findings: The white blood cell and lymphocyte counts for EHMA treated male rats were lower than the controls, and these differences were statistically significant for the 0.1 % and 0.2 % EHMA dose groups. The mean cell haemoglobin for male rats treated with 0.2 % EHMA was statistically significantly lower than the controls. Female rats treated with 0.2% EHMA had statistically higher haematocrit values, mean cell volumes and platelet counts than the control animals.</p> <p>- Clinical biochemistry findings: There were no statistically significant differences between control and EHMA treated female rats, in the three dose groups, for any of the serum chemical measurements. For male rats the only statistically significant difference between control and EHMA treated animals was an increase in aspartate aminotransferase activity in the 0.2 % EHMA group.</p> <p>- Mortality: none</p> <p>- Gross pathology incidence: no change related to treatment.</p> <p>- Organ weight changes: Male rats administered 0.05 % and 0.2% EHMA had statistically significantly higher relative kidney weights than</p>	BIBRA (1998)

		<p>the controls, but these differences were not apparent from the statistical analysis of the absolute kidney weights. Female rats treated with 0.1 % EHMA had significantly higher absolute and relative kidney weights than the control animals. The absolute kidney weights of female rats treated with 0.05 % EHMA were also statistically significantly higher than those of the control females. Male liver weights were not affected by treatment with EHMA. In the EHMA treated female groups, the only statistically significant effect on liver weights was an increase in the relative liver weights of animals given 0.1 % EHMA, compared with controls. These changes did not exhibit a dose-response relationship and are not considered to be biologically relevant.</p> <p>- <u>Biochemical examination of the liver:</u> Hepatic protein concentrations were slightly higher than controls in male and female rats treated with the two top doses of EHMA (0.1 % and 0.2 %), though the differences were not statistically significant. Treatment with EHMA did not produce any statistically significant increases in cyanide-insensitive palmitoyl-CoA oxidation or lauric acid 11- and 12-hydroxylation in male or female rats. There were no significant changes in microsomal protein concentrations of EHMA treated male and female rats compared with the control group.</p> <p>- <u>Histopathology:</u> Histopathological examination of the kidneys from female rats treated with the three dose-levels of EHMA showed a statistically significant incidence of nephrocalcinosis. Nephrocalcinosis was not seen in male ratstreated with EHMA. Nephrocalcinosis is not uncommon in female rats and is not considered to be treatment related. Examination of the lungs from animal numbers 1 and 34 showed interstitial pneumonitis and perivascular cuffing. Microgranuloma was also seen in the lungs from animal n°1. Haematoxylin and eosin stained liver sections from male and female rats treated with EHMA did not show any significant histological changes compared with the control groups. Oil red O stained sections of control livers from both sexes showed large amounts of fat in the periportal areas.</p> <p>Treatment with 0.2 % (168 mg/kg/day in males; 173 mg/kg/day in females) EHMA and 0.05% (42 mg/kg/day in males; 45 mg/kg/day in females) EHMA produced little reduction in the amount of periportal fat in the livers from male and female rats compared with the controls. Treatment with 0.1 % (82 mg/kg/day in males; 87 mg/kg/day in females) EHMA gave a variable reduction of</p>	
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			<p>periportal fat. In some male and female rats a slight reduction was seen, while in others no change occurred. Statistical analysis showed no significant trend for the reduction in periportal fat with increasing dose of EHMA.</p> <p>- <u>Electron microscopic examination:</u> Peroxisomes in liver cells from control male rats appeared as small spherical structures of fairly regular size scattered throughout the cytoplasm of the hepatocytes. Most contained a prominent electron dense core and the number and morphology of peroxisomes appeared similar in both portal and centrilobular areas. Administration of 0.2 % EHMA to male rats appeared to have little effect on the peroxisome population of hepatocytes. The peroxisomes remained small and were scattered throughout the cytoplasm but showed a variation in the numbers per cell. Portal and centrilobular areas were similar and most peroxisomes appeared to have electron dense cores. In livers from control female rats the appearance of peroxisomes was similar to those of the male rats. The peroxisomes were small and scattered throughout the cytoplasm. The appearance of electron dense cores in the peroxisomes was variable, and the nature of peroxisome size and distribution was similar in portal and centrilobular areas.</p> <p>The administration of up to 0.2 % EHMA in the diet of rats for 28 days does not lead to a proliferation of hepatic peroxisomes, and does not produce any treatment-related effects.</p>	
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4.7.1.2 Repeated dose toxicity: inhalation

Not evaluated in this dossier.

4.7.1.3 Repeated dose toxicity: dermal

Not evaluated in this dossier.

4.7.1.4 Repeated dose toxicity: other routes

Not evaluated in this dossier.

4.7.1.5 Human information

No data

4.7.1.6 Summary and discussion of repeated dose toxicity

In these three studies (BIBRA, Elf Atochem, Rohm and Haas), reproductive organs have not been evaluated.

The results of a simulated gastric hydrolysis study of dimethyltin bis (EHMA) demonstrated rapid hydrolysis to dimethyltin dichloride and EHMA. This result supports the use of the parent chloride, dimethyltin dichloride, as an appropriate surrogate compound for classification of the bis (EHMA) ester for endpoints related to *in vivo* mammalian toxicity. This presumption justifies extrapolating *in vivo* mammalian toxicity data on dimethyltin dichloride to other ester compounds in the dimethyltin family, particularly for repeated dose, reproductive, and developmental effects endpoints. It should be noted that taken into account an hydrolysis of 100% and the respective molecular weight of DMT(EHMA) (555.4 g/mol) and of DMTC (220 g/mol), 1 mg DMTC is expected to be released from approximately 2.5 mg DMT(EHMA). The lowest dose (0.05% of EHMA, corresponding to 42 mg/kg/day of EHMA or 16.8 mg/kg/day of DMTC in the males and 45 mg/kg/day of EHMA or 18 mg/kg/day of DMTC in the females) leads to the little reduction in the amount of periportal fat in the livers from male and female rats and higher relative kidney weight for the males (at 42 mg/kg/day of EHMA or 16.8 mg/kg/day of DMTC).

Besides, registration data on EHMA has been published on the ECHA website and a reproductive and developmental toxicity screening study (GLP, OECD TG421, of reliability 1) on EHMA is exhibited, showing that there is no need for classification of EHMA for the reproductive and developmental toxicity. However, the toxicity evaluation of the DMT(EHMA) by read across from DMTC may be taken with some limitations and uncertainties because of the potential underestimation of the hazard.

Together, these two oral 90-day studies on DMTC indicate that the main target organ is the nervous system. The overall NOAEL for neuropathology is considered to be 0.6 mg/kg body weight for the dimethyltin dichloride component of the mixture (Elf Atochem, feeding study). Marginal effects were seen at 1.4 and 2 mg/kg body weight for males and females respectively, in the drinking water study (Rohm and Haas) and clear effects at 4.6 and 6 mg/kg bw for males and females respectively in the drinking water study (Rohm and Haas).

Deaths and severe neurological signs occurred from 75 ppm (5.2/6.7 mg/kg) in Rohm and Haas 1999 and at 200 ppm (16.81/17.31 mg/kg) in Elf Atochem 1996. Besides, neuropathological lesions were observed from the lowest dose of 25 ppm (1.6/2.2 mg/kg) in Rohm and Haas 1999 as evidenced by moderate vacuolization in the brain and spinal cord tissue and ventricular dilation and neuronal necrosis at highest doses. Similar lesions were found at 200 ppm (16.81/17.31 mg/kg) in Elf Atochem 1996 but histopathology was not performed at lower doses.

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

Under DSD, the following effects are considered as serious damage:

- (a) substance-related deaths;
- (b) (i) major functional changes in the central or peripheral nervous systems, including sight, hearing and the sense of smell, assessed by clinical observations or other appropriate methods (e.g. electrophysiology);
 - (ii) major functional changes in other organ systems (for example the lung);
- (c) any consistent changes in clinical biochemistry, haematology or urinalysis parameters which indicate severe organ dysfunction. Haematological disturbances are considered to be particularly important if the evidence suggests that they are due to decreased bone marrow production of blood cells;

- (d) severe organ damage noted on microscopic examination following autopsy:
- (i) widespread or severe necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity (e.g. liver);
 - (ii) severe morphological changes that are potentially reversible but are clear evidence of marked organ dysfunction (e.g. severe fatty change in the liver, severe acute tubular nephrosis in the kidney, ulcerative gastritis); or
 - (iii) evidence of appreciable cell death in vital organs incapable of regeneration (e.g. fibrosis of the myocardium or dying back of a nerve) or in stem cell populations (e.g. aplasia or hypoplasia of the bone marrow).

In the available 90-day studies study DMTC induced:

- Deaths from 75 ppm (5.2/6.7 mg/kg) in the drinking water study (Rohm and Haas, 1999) relevant for criteria (a)
 - Histopathological lesions in the brain from 25 ppm (1.6/2.2 mg/kg) in the drinking water (Rohm and Haas, 1999) study relevant for criteria (d) (ii)
 - The main effects target the nervous system.
- In the read across study, the lowest doses of 0.05% EHMA equivalent to 42 mg/kg/day of EHMA or 16.8 mg/kg/day of DMTC for the males and 45 mg/kg/day of EHMA or 18 mg/kg/day of DMTC for the females, leads to the little reduction in the amount of periportal fat in the livers from male and female rats and higher relative kidney weight for the males (at 42 mg/kg/day of EHMA or 16.8 mg/kg/day of DMTC).

4.7.1.8 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

By oral route, substances shall be classified as toxic under DSD when they cause serious damage at levels of order ≤ 50 mg/kg in a 90-day study.

Both critical effects identified above in the 90-day studies and in the read across (16.8 mg/kg/day and 18 mg/kg/day of DMTC respectively for males and females) from DMTC occur below the threshold of 50 mg/kg.

4.7.1.9 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

A classification T; R48/25 is proposed according to the Directive 67/548/EEC.

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

Under CLP, the following effects are considered as relevant:

- (a) morbidity or death resulting from repeated or long-term exposure. Morbidity or death may result from repeated exposure, even to relatively low doses/concentrations, due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites;

(b) significant functional changes in the central or peripheral nervous systems or other organ systems, including signs of central nervous system depression and effects on special senses (e.g. sight, hearing and sense of smell);

(c) any consistent and significant adverse change in clinical biochemistry, haematology, or urinalysis parameters;

(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination;

(e) multi-focal or diffuse necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity;

(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction (e.g., severe fatty change in the liver);

(g) evidence of appreciable cell death (including cell degeneration and reduced cell number) in vital organs incapable of regeneration.

In the available 90-day studies study DMTC induced:

- Deaths from 75 ppm (5.2/6.7 mg/kg) in the drinking water (Rohm and Haas, 1999) study relevant for criteria (a)
- Histopathological lesions in the brain from 25 ppm (1.6/2.2 mg/kg) in the drinking water (Rohm and Haas, 1999) study relevant for criteria (d) and (f)
- The main effects target the nervous system.

In the read across study, the lowest doses of 0.05% EHMA equivalent to 42 mg/kg/day of EHMA or **16.8 mg/kg/day of DMTC** for the males and 45 mg/kg/day of EHMA or **18 mg/kg/day of DMTC** for the females, leads to the little reduction in the amount of periportal fat in the livers from male and female rats and higher relative kidney weight for the males (at 42 mg/kg/day).

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

By oral route, substances shall be classified under CLP in category 1 when they cause significant and/or severe toxic effects of relevance to human health at levels of order ≤ 10 mg/kg in a 90-day study.

Both critical effects identified above in the 90-day studies occur below the threshold of 10 mg/kg. However, the read across study from DMTC does not show effects occurring below the threshold of 10 mg/kg (16.8 and 18 mg/kg/day respectively for the males and the females).

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

A classification **STOT RE 1- H372** is proposed according to the CLP.

The main target organ identified is the central nervous system and it is proposed to add nervous system to the hazard statement.

A SCL is allocated if the effective dose level or concentration is 10 times below the guidance values according to the CLP, that corresponds to an effective dose below 1 mg/kg bw). So no SCL is determined for DMT(EHMA) according to the CLP regulation.

RAC evaluation of Specific target organ toxicity/Repeated dose toxicity

Summary of Dossier submitter's proposal

No repeated dose toxicity studies are available on DMT(EHMA). The dossier submitters presented the results of a simulated gastric hydrolysis study, which demonstrated the rapid hydrolysis of DMT(EHMA) to dimethyltin dichloride (DMTC) and 2-ethylhexyl mercaptoacetate (EHMA). The DS therefore proposes to use read-across from DMTC to classify for DMT(EHMA). Two repeated dose toxicity studies on DMTC are presented in the CLH report, one 90-day oral (drinking water) repeated dose study in rats similar to OECD 408 and OECD 424 (neurotoxicity in rodents) (Rohm and Haas, 1999) and one 90-day oral (diet) repeated dose study in rats similar to OECD 408 (Elf Atochem, 1996). In addition, one 28-day repeated dose study using EHMA was included (BIBRA, 1998). The DS concludes that the DMT moiety is of greater toxicological concern than EHMA and proposes classification as STOT RE 1 according to the CLP Regulation (DSD: T; R48/25).

Comments received during public consultation

Two comments from Member States were received during public consultation. One supported the classification proposal while another argued that the effects were seen at dose levels which are borderline between STOT RE 1 and STOT RE 2 and requested further analysis on the EHMA moiety. Further details can be found in the RCOM.

RAC assessment and comparison with criteria

Since no studies on DMT(EHMA) are available, the DS referred to the studies on DMTC and EHMA. In an *in vitro* study (ORTEP 2000) a concentration of 2 µg/mL DMT(EHMA) (80% with 20% MMT(EHMA)) was tested at a pH of approximately 1-2 (0.07 N HCl) at 37°C in order to simulate the possible hydrolytic action on mammalian gastric contents. The degree of hydrolysis was studied by determination of the DMTC content in the water fraction after extraction of DMT(EHMA) in heptane. The conversion of DMT(EHMA) to DMTC was rapid. The calculated percentage of hydrolysis was 103% after 0.5 hours. This supports the use of data from DMTC to evaluate DMT(EHMA). It is estimated that approximately 1mg of DMTC is released from every 2.5 mg of DMT(EHMA)

In the two oral 90-day studies on DMTC the main target organ was the nervous system. Deaths and severe neurological signs occurred from 75 ppm (5.2/6.7 mg/kg/day) in Rohm and Haas (1999), as evidenced by moderate vacuolisation in the brain and spinal cord tissue and ventricular dilation and neuronal necrosis at highest doses. At 25 ppm (equivalent to 1.6 and 2.2 mg/kg/day for males and females, respectively), no mortality occurred and treatment-related findings were limited to reduced food (males only) and water intake and neuropathological lesions with moderate vacuolization in brain and spinal cord tissue. The NOAEL was considered to be less than 25 ppm. In the Elf Atochem (1996) study deaths and severe neurological signs occurred at 200 ppm (16.81/17.31 mg/kg/day),

with similar lesions to those found in the Rohm and Haas (1999) study. Histopathology was not performed at lower doses. The overall NOAEL for neuropathology was 0.6 mg/kg bw/day for the dimethyltin dichloride component of the mixture.

The critical effects (death and histopathological lesions in the brain) identified in the 90-day studies occurred between 1.6 and 6.7 mg/kg bw/day DMTC, which corresponds to 4 and 16.75 mg/kg bw/day of DMT(EHMA).

The RAC notes that absolute and relative weights of the thymus have been reduced in a 90-day oral study, with effect levels at about 5 mg/kg bw/day in males (Rohm and Haas, 1999), and in another 90-day oral study about 15 mg/kg bw/day in both sexes (including histopathological lesions) (Elf Atochem 1996). Since no histochemical analysis has been performed at the lower dose of 1 mg/kg/day in the latter study it remains unclear whether effects on the thymus at this dose can be excluded, and the effect on the thymus at 5 mg/kg/day in the 90 days oral Roehm and Haas (1999) study is considered to be relevant for a hazard statement.

Reduced thymus weights have also been observed in the two developmental studies (Noda, 2001) on day 20 of gestation at 15 and 20 mg/kg/day. The effects observed on the thymus are consistent with a class effect of organotins on the immune system.

The threshold level for classification as toxic under DSD is 5 mg/kg/day. A classification of T; R48/25 according to Directive 67/548/EEC is therefore supported.

Substances that cause significant and/or severe toxic effects of relevance to human health at ≤ 10 mg/kg/day in a 90-day study shall be classified under CLP in Category 1. Since effects are seen below that level, classification as **STOT RE 1- H372** is warranted. The main target organs identified are the central nervous system and the immune system, therefore **nervous system and immune system** should be added as target organs to the hazard statement. A specific concentration limit is not warranted, because the effective dose level or concentration is not 10 times below the guidance value of ≤ 10 mg/kg according to the CLP.

4.9 Germ cell mutagenicity (Mutagenicity)

Not evaluated in this dossier.

4.10 Carcinogenicity

Not evaluated in this dossier.

4.11 Toxicity for reproduction

Table 11: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
First prenatal development study	Dimethyltin dichloride toxic to dams and fetuses at 15 mg/kg/day. Under the conditions of the first prenatal development study, the NOAEL for maternal and fetal toxicity was considered to be 10 mg/kg/day.	Similar to TG OECD 414.	Noda, T. 2001.
Second prenatal development study	Adverse effects to dams were reported at both 20 and 40 mg/kg/day on days 10-12 of gestation. At 40 mg/kg/day, maternal body weight gain and maternal thymus weights were significantly reduced. Maternal thymus weights and adjusted body weight gain were also significantly reduced at 20 mg/kg/day of days 10-12 of gestation.	Similar to TG OECD 414.	Noda, T. 2001.
A first developmental neurotoxicity study	In Ehman's study, developmental neurotoxicity of dimethyl tin dichloride (DMTC) was evaluated in two experiments. In the first study, female Sprague-Dawley rats were exposed via drinking water to DMTC before mating and throughout gestation and lactation. DMTC toxicity was expressed as depressed maternal weight gain, and in the offspring, decreased brain weight, decreased apoptosis and mild vacuolation in the brain of adult offspring, and slower learning in the water maze.	Similar to the neurotoxicity study of EPA OPPTS 870.6300	Ehman K.D., 2007.
A second developmental neurotoxicity study	In a second study, DMTC exposure occurred from gestational day 6 to	Similar to the neurotoxicity study of EPA	Ehman K.D., 2007.

	weaning. The high concentration again depressed maternal weight gain, decreased offspring birth weight and preweaning growth, and decreased brain weight. Learning deficits were observed in the runway at postnatal day 11 (15, 74 ppm) and again in the adult offspring in the water maze (15 ppm).	OPPTS 870.6300	
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4.11.1 Effects on fertility

Not evaluated in this dossier.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

The following data were included in the classification proposal discussed by TC C&L.

A simulated gastric hydrolysis study of dimethyltin bis(EHMA) found that under low pH conditions (similar to mammalian gastric systems) all of the available EHMA ligands had been released and there was >100% hydrolysis of the test substance (see 4.1). The results of this simulated gastric hydrolysis study of dimethyltin bis(EHMA) support the use of the chloride derivative, dimethyltin dichloride, as the anchor compound for the dimethyltin family of compounds to the extent possible for the repeated dose, reproductive, and developmental effects endpoints, (ORTEP, 2000).

Species Cell culture	Route	Dose	Exp. time	Exp. period	Observations and Remarks	Ref.
Rat First study: 5 groups of 10 pregnant rats each. Second study: 8 groups of 8-11 pregnant rats each: 3 groups	Gavage Saline	First study: 5, 10, 15, and 20 mg/kg bw/day Second study: 20 or 40 mg/kg bw/day	-	First study: GD 7-17 Second study: one of four different periods of gestation (days 7-9, 10-12, 13-15, or 16-17).	First prenatal development study: Test substance: DMTC, (Purity > 99.0%) Test similar to OECD 414 but conducted using less than the recommended number of animals per dose group (10 instead of 20). Maternal toxicity data in study 1: -significant reduction in the maternal body weight gain in a dose dependent manner; -significant reduction of maternal food intake; -significant reduction of maternal thymus weights in a dose dependent manner. There was a dose-dependent reduction of maternal body weight gain of pregnant rats treated orally with DMTC during days 7-17 of gestation. Maternal body weight gain was significantly reduced in pregnant rats treated	Noda, T. 2001.

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<p>at 20 mg/kg bw/day during 3 periods of gestation (days 7-9, 13-15, 16-17); 4 groups at 40 mg/kg bw/day during 4 periods of gestation (days 7-9, 10-12, 13-15, 16-17) and one control group.</p>					<p>with 15 or 20 mg/kg/day of DMTC (data shown as a graph in the publication). Maternal body weight on GD 20 were also significantly lower in the high dose group (respectively 333g ±26.7, 334g ±21.9, 321g ±21.0, 315g ±9.2, and 252g ±41.1** in the control, 5, 10, 15 and 20 mg/kg groups). Maternal food intake was also significantly reduced by DMTC-treatment at 20 mg/kg/day at GD 11 and between GD 13 and 17 (data shown as a graph in the publication). No other significant differences in maternal food intake were present in the remaining dose groups.</p> <p>Two animals receiving DMTC-treatment at 20 mg/kg/day died, one on day 18 of gestation and the other on day 19 of gestation. These animals exhibited clinical signs of toxicity, including piloerection, ataxia, perinasal and periocular staining, vaginal bleeding, tremor, and convulsion, for about four days prior to their deaths. No gross pathological changes in the organs of the dead dams were noted at necropsy. No other mortalities were observed in either the control or DMTC-treated groups.</p> <p>Mainly after the day 15 of the gestation, all the animals treated by DMTC at 20 mg/kg/day showed clinical signs of toxicity, including perinasal and periocular staining, piloerection, and ataxia. Three pregnant animals from this group exhibited vaginal bleeding, tremor, and convulsion and two pregnant rats from this group died in the late stage of gestation. No clinical signs of toxicity were observed in the other DMTC-treated groups. There was a dose-dependent reduction in maternal thymus weights, with a significant reduction at 15 and 20 mg/kg/day on day 20 of gestation (respectively 196mg ±37.5, 190mg ±32.6, 168mg ±26.8, 151mg ±13.6* and 45mg ±21.7** on GD20 in the control, the 5, 10, 15 and 20 mg/kg). Maternal brain weight was not affected in any DMTC-treated group.</p> <p>Total resorption was observed in one of the eight living pregnant rats at 20 mg/kg/day on day 20 of gestation. There was a dose-dependent reduction in mean body weight of living fetuses (both sexes), that was significant at 15 and 20 mg/kg/day (respectively 3.5g ±0.20, 3.4g ±0.22, 3.2g ±0.25, 2.9g ±0.16, and 2.2g ±0.46 in males and 3.3g ±0.16, 3.2g ±0.20, 3.2g ±0.27, 2.8g ±0.15, and 2.1g ±0.41 in females of the control, 5, 10, 15 and 20 mg/kg). There was no significant difference in the number of</p>	
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					<p>corpora lutea, number of implants, number of living fetuses, or the incidence of post-implantation loss and sex ratio.</p> <p>The incidence of external malformations increased in fetuses from dams exposed to DMTC at 20 mg/kg/day during the days 7-17 of gestation (respectively 0, 0, 0, 2.5 and 22.5% of fetuses at 0, 5, 10, 15 and 20 mg/kg/d). Cleft palate was observed in 21 fetuses (22%) from five of seven pregnant rats with living fetuses on day 20 of gestation. In addition to cleft palate, one fetus was associated with general edema and pes varus, and one with general edema. Omphalocele was observed in two fetuses from one dam exposed to 15 mg/kg/day; however, the incidence was not statistically significant. No other external malformations were observed in either the control or DMTC-treated groups.</p> <p>There was a statistically significant increase in the number of fetuses with dilation of the renal pelvis from dams exposed to DMTC at 20 mg/kg/day during days 7-17 of gestation (respectively 0, 0, 1, 4, and 5 fetuses at 0, 5, 10, 15 and 20 mg/kg of DMTC). No other visceral malformations observed were statistically significant. No statistically significant difference in the incidence of skeletal malformations and skeletal variations were observed in either the control or DMTC-treated groups.</p> <p><u>Second prenatal development study:</u> Test substance: DMTC, (Purity > 99.0%).</p> <p>Maternal toxicity data in study 2: -significant reduction of maternal body weight gain; -significant reduction of maternal food intake; Significant reduction of maternal thymus weight and adjusted body weight gain.</p> <p>Significant reductions in maternal body weight gain on days 13, 16, and 17 of gestation and food intake on the consecutive days of gestation after day 12, were reported for pregnant rats exposed to DMTC at 40 mg/kg/day on days 10-12 of gestation (data not shown in the publication). Maternal body weight gain was not significantly reduced in the other DMTC-treated groups or over the whole gestation period (body weight gain of 112±0.16 in controls, 110 ±12.5, 107±18.0, 111±9.0 at 20 mg/kg on GD 7-9, 10-12 and 13-15 and 110±13.1, 95±17.8, 103±12.4 and 102±22.6 at 40 mg/kg on GD 7-9, 10-12,</p>
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				<p>13-15 and 16-17). Adjusted body weight gain was significantly decreased in rats exposed to DMTC at 20 mg/kg during GD 10-12 and in all rats exposed to 40 mg/kg (47 ± 7.0 in controls, 40 ± 7.4, $35 \pm 9.6^*$, $42 \pm 6.5^*$ at 20 mg/kg on GD 7-9, 10-12 and 13-15 and $35 \pm 6.0^{**}$, $30 \pm 9.2^{**}$, $33 \pm 6.6^{**}$ and $31 \pm 13.3^{**}$ at 40 mg/kg on GD 7-9, 10-12, 13-15 and 16-17). General behavior among groups, including the control group, was not significantly different.</p> <p>Maternal thymus weights and adjusted body weight gain in pregnant rats exposed to 20 mg/kg/day on days 10-12 (maternal thymus weights for the control group: 253 mg ± 45.1,) and in every treatment group at 40 mg/kg/day were significantly reduced (maternal thymus weights of respectively 253 mg ± 45.1 in the control group, and 220 mg ± 22.7, 207 mg $\pm 39.9^*$, and 217 mg ± 15.3 at 20 mg/kg during GD 7-9, 10-12, and 13-15, and 208 mg $\pm 37.6^*$, 190 mg $\pm 36.9^{**}$, 182 mg $\pm 32.7^{**}$, 172 mg $\pm 45.1^{**}$ at 40 mg/kg during GD 7-9, 10-12, 13-15 and 16-17). Gravid uterus and maternal body weights were not affected in either the 20 or 40 mg/kg/day groups. Total resorption was observed in one of 10 dams exposed to 40 mg/kg/day during days 7-9 of gestation. Mean fetal body weight was reduced in females from dams exposed to 40 mg/kg/day during days 7-9 (data not shown). No significant differences were reported in the number of corpora lutea, implants, or living fetus. The incidence of post-implantation loss, the sex ratio, and male fetal body weight were not significant in any treatment group (data not shown).</p> <p>There was no significant increase in the incidence of external, skeletal, or visceral malformations at either 20 or 40 mg/kg/day. Cleft palate was not observed in fetuses from dams exposed to DMTC at 20 or 40 mg/kg/day on days 7-9, 10-12, 13-15, or 16-17 of gestation. The numbers of fetuses with skeletal variations, cervical ribs, and/or splitting of the first cervical vertebra arch increased significantly in the groups treated with DMTC at 40 mg/kg/day on days 7-9 and/or days 13-15 of gestation (0, 5* and 4* fetuses with cervical rib respectively in the control group and the groups treated with DMTC at 40 mg/kg/day on days 7-9 and 13-15; 0 and 6* fetuses with splitting of the first cervical vertebra arches respectively in the control group and in the 40 mg/kg/day group at days 7-9). Fetuses with kinked ureter</p>
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					significantly increased in the group treated with DMTC at 40 mg/kg/day on days 16-17 of gestation (0, 3, 1, 2 and 6* fetuses respectively in the control group and in the 40 mg/kg/day group treated on days 7-9, 10-12, 13-15, 16-17).	
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***p<0.05, ** p<0.01**

A new study was published (Ehman K.D., 2007) since the discussion of TC C&L, in which developmental neurotoxicity of dimethyltin dichloride (DMTC) is evaluated. Two types of experiments were performed. According to the registration dossier, this study is similar to the developmental neurotoxicity study of EPA OPPTS 870.6300.

In a first experiment, one hundred and twenty Sprague-Dawley female rats were exposed to DMTC via drinking water two weeks before mating and throughout gestation and lactation. The day of birth was designated postnatal day 0 (PND). Three DMTC treated-groups (n=9 at 3 ppm and 74 ppm and n=10 at 15 ppm) and one control (n=13) were formed (3 ppm = 0.28 to 0.57 mg/kg/day; 15 ppm = 1.16 to 2.67 mg/kg/day; 74 ppm = 4.38 to 12.2 mg/kg/day). Only one male offspring from each litter, were tested in the different neurobehavioral tasks. Each pup was evaluated in only one test.

Trials performed in the Experiment 1:

1) Maternal fluid intake and weights:

Overall, consumption increased among all dose groups throughout gestation and lactation. During the first two weeks of exposure (pre-mating), water consumption was significantly decreased in all DMTC-exposed groups (time-by-dose $F_{(9, 87)} = 37.71$, $p < 0.0001$). During the first half of gestation, only the high concentration decreased consumption (data not shown, time-by-dose $F_{(18, 180)} = 1.98$, $p = 0.013$) but these differences were not significantly different from control throughout the rest of exposure.

Only the high concentration of DMTC altered maternal body weight gain, which was significantly lower than the control group for the duration of the exposure (time-by-dose $F_{(18, 180)} = 3.58$, $p < 0.0001$).

2) Reproductive parameters:

The overall pregnancy success rate was very low (47 of 120 rats, 39%). Nevertheless, there was no treatment effect on the number of pregnancies (n=10 control, n=14 at 3 and 15 ppm and n=9 at 74 ppm).

3) Offspring number and growth:

The total number of live pups per litter was: control, 14.4 ± 1.0 ; 3 ppm, 13.3 ± 0.7 ; 15 ppm, 15.0 ± 0.9 ; and 74 ppm, 12.8 ± 0.8 . Three litters in the high-concentration group had one to two dead pups, whereas control litters had none; however this difference was not significant. After culling at PND 1, three litters (one control, two at 74 ppm) lost three to four more pups each.

Average male pup weight per litter was not significantly altered by DMTC exposure (data not shown). Pups weighed at the time of testing for the runway, motor activity and water maze showed no differences from control.

4) Neurobehavioral assessments:

- a) *The runway learning test:* PND11 pups were food-deprived 10h prior to testing in their dark cycle and were trained to negotiate a runway for a dry suckling reward from its anesthetized mother in the goal box and latency is recorded. Acquisition consisted of 25 alternating reinforced (R), (15 s of dry suckling) and non-reinforced (N), (placement in a holding cage for 15 s) trials. If the pup failed to find the dam within the allotted time, the experimenter guided it down the runway (R) or the animal was immediately placed in the holding cage (N). Extinction, i.e. the blocked access to the dam, immediately followed acquisition.

Several pups in each treatment group failed to learn the task, using the criterion of having at least one latency less than the maximum time of 120 s. However there was no treatment-related difference in the incidence of the non-learners (control: 3 of 12; at 3 ppm: 1 of 9; at 15 ppm: 0 of 10 and at 74 ppm: 1 of 9).

Then, extinction trials began on the 26th trial and the maximum time was set at 100 s. When pups reached the criterion of two consecutive 100-second trials, they were no longer tested. The median number of trials required for each dose group was: control: 18; at 3 ppm: 13; at 15 ppm: 15.5; and at 74 ppm: 24). Although higher in the high-dose group, there were no significant differences in trials to extinction.

- b) *Motor activity* data were collected using automated figure-eight chambers. Photocell interruptions (counts) were recorded over 5 minutes intervals of the 30 minutes test session. In experiment 1, motor activity was assessed in males at PNDs 13, 17 and 21 (n=14 control, n=9 at 3 ppm, n=10 at 15 ppm and n=9 at 74 ppm). Total motor activity counts during 30 minutes sessions showed an age-related increase from PND 13 (average of all dose groups: 27.1), PND 17 (average: 80.4) and PND 21 (average: 116.9), but were no treatment-related differences. Analysis of the within-session activity (in 5 minutes intervals) showed that habituation was not evident until PND 21 in all treatment groups.
- c) *Morris water maze* is a spatial memory trial where adolescents/young adults' rats (7 weeks old) have to find a hidden platform. Dependant variables included swim speed, latency and path length to find the platform and time spent in the outer edge of the tank or one of the three concentric zones. Only males were tested in the water maze (n=11 control, n=7 at 3 ppm, n=9 at 15 ppm and n=11 at 74 ppm).

For spatial training, rats learned the fixed position of the platform during 2 trials a day for 9 days. The maximum trial time was 60 s, after which time the observer guided the rat to the platform. The middle dose group showed significantly longer latencies during the first week of training (dose $F_{(3, 31)} = 3.57$, $p=0.025$). In the second week, the low and middle dose groups spent significantly less time in the middle zone (dose $F_{(3, 31)} = 5.79$, $p=0.003$), and in addition, the 15 ppm group spent more time in the outer zone (dose $F_{(3, 31)} = 5.52$, $p=0.004$). The high dose group showed no differences on any of these parameters.

On the 10th day, the platform was removed for a probe trial and the time spent to search in the correct quadrant was measured over 60 s. A visible probe trial was also conducted using a raised platform of a contrasting color to confirm that the tested animals were not visually impaired. There were no effects on swim speed or search parameters during the probe trials (both the memory probe, platform removed, and the visual trial with the raised platform).

5) Neuropathology:

Neuropathological evaluations were performed in brain male rats (n=6-8/dose at PND 1, n=5-8/dose at PND 12, n=5-9/dose at PND 22, and n=5/dose at adult age, 80-90 days old), in each sections (e.g., olfactory bulb, striatum, cerebral cortex, hippocampus, thalamus, hypothalamus, brainstem, cerebellum). Histopathological alterations were noted in the cerebral cortex of rats sacrificed at PND 22 and as adults.

Three of five (60%) adult offspring at 74 ppm and one of five (20%) PND 22 rats at 74 ppm had slight/mild vacuolation of the neuropil of the gray matter of the cerebral cortex. In adults, the lower dose groups evaluations showed similar vacuolation at 15 ppm (1 of 5 adults) and 3 ppm (1 of 5 adults). There were no lesions in the offspring at PND 1 or 12, or in the offspring at the lower doses at PND 22. There were no histopathological findings in any major brain region other than the cerebral cortex, and no such findings were observed in control rats at any age.

The cerebral cortical lesion was characterized by 2-4 micron diameter, round vacuoles in the gray matter neuropil in the region of the orbital cortex. On a score of 1 (minimal) to 5 (severe), the rats in the lower dose groups received scores of 1, whereas the high-dose rats received scores of 2 (slight/mild).

6) Brain weights:

PND 1, 12, 22 male brain rats and adults were analysed (n=4-11/dose/age). From PND 12, all subjects came from different litters. Analysis of brain weights revealed an overall effect of dose ($F_{(3, 88)} = 3.61, p < 0.016$) but no interaction with age. The data showed significant decreases in the low- and high-dose groups. The low dose was 4% lower and the high dose 8% lower, than controls. The mid dose group average was equal to the control mean.

7) Apoptosis assessment:

Apoptosis was quantified using a Cell Death ELISA procedure slightly modified, which uses antibodies to bind fragmented DNA characteristic of apoptotic cell death. The bound fragments (i.e. nucleosomes) are then quantified photometrically.

After weighing, brains were dissected into the following regions: brainstem, neocortex, hippocampus and cerebellum, frozen and stored at -70°C . ELISA assays were conducted only on tissues collected at PND 22 (n=3-7/dose/region) and as adults (n=2-4/dose/region).

Significant dose-by-age interactions were observed for the cerebellar (dose-by-age $F_{(3, 27)} = 2.93, p = 0.05$) and cortical (dose-by-age $F_{(3, 27)} = 5.79, p = 0.003$) DNA fragmentation data. Significant decreases of DNA fragmentation were observed only at PND 22 in the cerebellum (15 and 74 ppm) and cortex (all doses). However, these changes did not show a clear dose-response. No differences were seen in adult tissues, but in some cases the sample sizes were not optimal (n=2-4/dose).

Trials performed in Experiment 2:

In the second experiment, eighty-seven pregnant female rats (Sprague-Dawley) (n=21 control, n=22 per DMTC dose groups: 3, 15, and 74 ppm) were treated from gestational day 6 (GD6) throughout gestation and lactation. Both male and female offspring (one from each litter) were tested in the different neurobehavioral tasks with the exception of the *runway task*, in which only males were tested.

1) Maternal fluid intake and weights:

From the beginning of exposure to the end of gestation, fluid intake was significantly lower in the 15 and 74 ppm dose groups. Only during the second week of exposure, the 3 ppm dose group showed decreased consumption. Intake returned to control levels in all except the high-concentration group during lactation.

No reductions in body weight were evident until lactation, at which time the high-concentration body weight was significantly lower than controls..

2) Reproductive parameters:

All of the timed-pregnant females delivered with the exception of one 74 ppm DMTC female. Additionally, one 74 ppm female delivered only six pups and was not used. All of the deliveries occurred when expected.

3) Offspring number and growth:

Five litters had one or two dead pups, but this finding was not related to dose; one litter at 3 ppm, and four litters at 15 ppm.

Body weight pups changes during the lactation period showed a significant dose-by-sex interaction ($F_{(3, 69)} = 3.0, p=0.037$). Males in the high-concentration group weighed significantly less than controls throughout lactation, and in the same group, the females weighed less with significance only at PND 17 and PND 21. In contrast, no treatment effect on body weight was measured weekly after weaning, or at the time of behavioural testing. Thus, the high concentration suppressed growth during lactation in the second study, but not the first study.

4) Neurobehavioral assessments:

a) *Runway testing*: A different training schedule was used in Experiment 2. Pups ($n=20$ /dose except $n=19$ at 74 ppm) were food-deprived for 8 h and then tested during their light cycle. Testing began with a preliminary training session of 5 massed reinforced (R) trials, followed by a 2 min retention interval in the holding cage. There were then 25 acquisition trials in which reinforced (R) and non-reinforced (N) trials alternated in blocks of 5 trials. The maximum time allowed for each trial was 100 second.

The percentage of pups that failed to learn to negotiate the runway increased in a dose-dependent manner (control: 3 of 20; 3 ppm: 5 of 20; 15 ppm: 6 of 20; and 74 ppm, 6 of 19), with no statistical significance.

The control and the low-dose group showed significant decreasing slopes (p 's < 0.04 for all) for each R-trials. In contrast, the 15 ppm dose group did not show decreased latencies on any of the R-trials and the high-dose group showed a decreasing slope ($p=0.008$) only during the last block of R-trials.

b) *Motor activity*:

Only PND 17 male and female offspring were tested (one male and one female from each litter; $n=21$ for the control and at 15 ppm, $n=20$ at 3 ppm, $n=17$ at 74 ppm). There were no group differences in PND 17 motor activity and no interactions with sex. Total counts for each group (sexes combined, mean \pm SEM) were: control, 115.4 ± 7.8 ; 3 ppm, 114.2 ± 5.6 ; 15 ppm, 121.5 ± 9.2 ; and 74 ppm, 119.0 ± 8.8). Unlike in Experiment 1, habituation was evident in all treatment groups at PND 17.

c) *Spontaneous alternation*:

Spontaneous alternation was measured on PND 25 using a Plexiglass T-shaped apparatus. Pups were placed in the stem for a 30 s acclimation, after which time, the gate was raised allowing the rat to enter either arm, and to explore only the 2 opposing arms for 5 min. All arm entries were counted as the measure of motor activity, whereas alternation was considered when the rat left one arm and entered the other. Both males and females were tested at 10/dose.

The overall dose effect did not reach significance (dose $F_{(3, 36)} = 2.48, p=0.077$). The number of arm visits was (in average): male and female control group: 10.5; males at 15 ppm: 6.7 ± 1.6 and males at 74 ppm: 11.7 ± 0.7 . The percent of alternations showed no significant difference across groups or gender (mean \pm SEM): control, 84.5 ± 2.9 ; 3 ppm, 84.3 ± 2.5 ; 15 ppm, 86.1 ± 2.4 ; and 74 ppm, 78.7 ± 2.6 .

d) *Morris water maze:*

Unlike in Experiment 1, 12 weeks old rats were tested. Both males and females (one from each litter) performed the trial (n=10/sex/dose).

As in Experiment 1, the 15 ppm (middle) dose group had significantly higher latencies to learn the platform position (dose $F_{(3, 71)} = 3.1$, $p=0.032$).

In terms of the spatial pattern of swimming on days 2 and 3, all dose groups spent significantly more time in the outer zone than in the middle zone. This propensity for the outer zone persisted in the middle dose group into the second week of training (dose $F_{(3, 71)} = 7.44$, $p=0.0002$), and was significant for both males and females. The tracings from middle dose rats showed they spent more time in outer zone, and less time in the middle zone of the tank. Analysis of the memory probe again revealed less time in the middle zone (dose $F_{(3, 71)} = 3.21$, $p=0.028$) in both males and females of the middle dose group. There were no differences across dose groups in swim speed or latency to find the visible platform.

5) Neuropathology:

Brains were prepared and examined as described for Experiment 1, but only adult rats (both males and females) were used (n=10/dose/sex, except n=9 in the 15 ppm males group).

One male offspring at 74 ppm had a single neuron in the midbrain with central chromatolysis. There were no others axonal lesions and no others rats at this dose level exhibited the same lesions. Thus, significance of this finding in a single neuron in a single treated rat remains undetermined.

6) Brain weight:

PND12, 22 and adults male brain rats were analysed (n=7-9/dose/age). From PND12 on, all subjects came from different litters. As in the first experiment, there was an overall effect of dose ($F_{(3, 67)} = 4.05$, $p=0.01$) in male rats. Collapsed across age, only the high dose group showed a significant decrease of 4%.

7) Apoptosis:

Tissues were collected from male rats only as described for experiment 1 on PND 12, 22 and as adults, with n=6-8/dose/region (brainstem, neocortex, hippocampus and cerebellum).

No treatment effect at PND 12 was observed for the brainstem, but significant increases relative to control at PND22 and decreases in adults (dose-by-age interaction ($F_{(6, 60)} = 11.90$, $p<0.0001$)). These significant effects were seen in the mid and high dose groups. In addition, cerebellar data revealed small significant increase in DNA fragmentation (dose-by-age $F_{(6, 60)} = 3.36$, $p=0.006$) at PND 12, but only at the high dose (means \pm SE: control, 1.0 ± 0.07 ; 3 ppm, 1.30 ± 0.07 ; 15 ppm, 1.06 ± 0.04 ; 74 ppm, 1.07 ± 0.08).

4.11.2.2 Human information

No data available.

4.11.3 Other relevant information

The study of Noland et al. (1983) was not included in this dossier at the time of the proposal for the harmonised classification and labelling to the TC C&L in October 2006. Nevertheless, this study is informative for the developmental neurotoxicity analyse of dimethyltin dichloride.

Studies were conducted with seventy-day-old Sprague-Dawley females' rats to determine whether DMTC was absorbed by the dam and transferred across the placenta to foetal blood and brain tissue.

In the first study, three groups were formed. The first group received the DMTC at 40 mg/L in their drinking water (n = 13). The second group received identical levels of tin as stannous chloride (n = 13) and a third group received distilled water (n = 12), and served as the control group. The exposure began 2 weeks prior to breeding and continued through gestation. At birth and prior to the first nursing, the pups were removed from the dams and sacrificed. Blood samples pups were taken and brains pups were removed. Dams weight gain and water consumption were measured and blood samples were taken.

The tin content of blood from control dams and from dams given stannous chloride was not different while the tin content from those given DMTC was significantly higher than both of the other two groups ($X^2 = 24.7$, $df = 2$, $p < 0.0001$). Similarly, the tin content in blood from pups of dams given DMTC was significantly higher than blood from pups of either of the other two groups ($X^2 = 24.7$, $df = 2$, $p < 0.0001$). In the pup brain of dams exposed to DMTC, the tin level was significantly higher than controls or those given stannous chloride ($X^2 = 23.1$, $df = 2$, $p < 0.0001$).

DMTC is therefore absorbed by the rat dam, transferred by placenta to the foetus and arrives in the brain of the prenatal animal.

In a second step, a cross-fostering study was conducted. Two groups of rats were tested, one treated with DMTC in drinking water (40 mg tin/L) and the non treated control. At birth, before first nursing, pups born to DMTC-exposed dams were placed either with control dams for rearing (n = 8) (DM/CT) or with another DMTC-exposed dams (n = 8) (DM/DM). Pups born to control dams were placed either with DMTC-exposed dams (n = 8) (CT/DM) or with another control dam (n = 8, CT/CT). Brain and blood samples were collected from the remaining control (n = 4) and DMTC-exposed litters (n = 4) on post natal days (PND) 10 and 21.

The levels of tin in pups from DMTC-exposed dams ($5.88 \mu\text{g/g} \pm 0.83$) was significantly higher than that of controls (0.115 ± 0.04 ; $p = 0.005$).

The highest levels of tin in blood were reached at birth in gestationally exposed pups. Then, at PND 10, blood tin levels decreased rapidly in both prenatally exposed groups (i.e. in DM/DM and DM/CT groups). Pups exposed only postnatally (CT/DM) had blood levels of tin significantly lower than the DM/DM and the DM/CT animals. This results show that DMTC is mainly transferred to the pups during the gestation.

Tin levels in the brains of gestationally exposed pups were again highest at birth and different from controls ($p = 0.004$). At PND 10, the DM/DM pups demonstrated significantly higher levels of tin in the brain than the other groups ($f = 19.42$; $df = 3.12$; $p = 0.01$). At PND 21, the DM/DM group was significantly higher than the CT/DM group, which was higher than either the CT/CT or the DM/CT group ($f = 9.445$; $df = 3.21$; $p = 0.05$).

The greatest decrease seen in the pups from the DM/CT group, indicate a rapid clearance of the tin from the blood and brain.

During the postnatal period, the DMTC follows the same pattern of decreasing concentration in pup brain and pup blood, indicating relatively unimpeded brain-to-blood transfer, as is expected in animals with immature blood-brain barriers.

In the third study, a ¹⁴C-DMTC tracer was administered by intubation to the 19-day pregnant dams. Then, dams were sacrificed at 5 minutes, 15 min, 30 min, 1h, 2h, 6h and 24h (n = 4 at each interval). Brains and blood samples were taken for analysis. The fetuses were removed in order to take blood and brain samples.

The highest measured levels of ¹⁴C-DMTC in the blood were recorded at 1h for the dams and at 6h for the pups while the levels in the brain continued to increase through 24h for both the dams and the pups. The ¹⁴C-DMTC was readily absorbed into both dam blood and brain and fetal blood and brain. However, at 6h after exposure, the fetal blood ¹⁴C level represented 16% of dam blood level although fetal brain ¹⁴C levels represented 167% of dam brain ¹⁴C concentrations. This 10-fold higher brain/blood ratio for fetal might be explained by the immaturity of the "blood-brain barrier" or by the transfer of a more available ¹⁴C-non-Sn-containing metabolite than ¹⁴C-DMTC.

The results of this study have demonstrated that DMTC is absorbed in the gastrointestinal tract of the dam and DMTC is transferred across the placenta to fetal blood and brain tissue. The majority of the tin is transferred from the pups prenatally, during gestation rather than lactation.

4.11.4 Summary and discussion of reproductive toxicity

In the first study of Noda (oral treatment on days 7-17 of gestation), severe maternal toxicity occurred at the high dose of 20 mg/kg/day. These clinical signs of toxicity are vaginal bleeding, tremors and convulsions [30%], ataxia and other clinical signs of toxicity [100%] (severe thymus atrophy) and they generally appear after the 15th day of gestation. Oral administration of DMTC at 20 mg/kg/day resulted in the death of two pregnant rats [20%], caused by the DMTC treatment. Total resorption was observed in one of eight living pregnant rats, which exhibited all these clinical signs of toxicology at this level dose in the late stage of gestation.

Besides, administration of DMTC at 20 mg/kg/day caused cleft palate in 21 fetuses (22%). The teratogenicity of DMTC can be discussed because of the severe maternal toxicity at this dose level. As cleft palates are a rare and serious malformation, it cannot be considered as secondary to maternal toxicity and it cannot be discarded. Moreover, mean body weight in living fetuses of both sexes decreased in a dose-dependent manner with significance at 15 and 20 mg/kg/day.

In order to reduce maternal toxicity, shorter periods of DMTC treatment (two or three consecutive days at one of four different periods of gestation) and relative high doses of DMTC were chosen in a second study. The highest dose (40 mg/kg/day) of DMTC caused slight maternal toxicity as indicated by the reductions of the adjusted body weight gain and the thymus weight. No significant increase in the incidence of external, skeletal and visceral malformations were observed at either dose in any treatment period group, and no cleft palate was found. Fetal body weight was also unaffected.

In Ehman's study, developmental neurotoxicity of dimethyl tin (DMTC) was evaluated in two experiments. In the first study, female Sprague-Dawley rats were exposed via drinking water to DMTC before mating and throughout gestation and lactation. DMTC toxicity was expressed as depressed maternal weight gain, and in the offspring, decreased brain weight, decreased apoptosis and mild vacuolation in the brain of adult offspring, and slower learning in the water maze.

In a second study, DMTC exposure occurred from gestational day 6 to weaning. The high concentration again depressed maternal weight gain, decreased offspring birth weight and preweaning growth, and decreased brain weight. Learning deficits were observed in the runway at postnatal day 11 (15, 74 ppm) and again in the adult offspring in the water maze (15 ppm).

The effect observed in the runway testing was identified only in experiment 1 but it may be due to the lower number of tested pups. In the second experiment, the 74 ppm dose-group succeed in learning as there are decreased latencies in the last block of reinforced-trials, although the 15 ppm dose-group failed to learn in all the reinforced trials. However, only the 15 ppm dose-group shows a decreased latency during the non-reinforced (extinction phase), which seems to be an aberration. *So, learning was not observed during any reinforced-trial blocks in the 15 ppm group, but was only achieved at the last set of trials in the 74 ppm group.* The absence of dose-response therefore questions the significance of this finding. It is however noted that the trial does not follow the test guideline 426 of the OECD on the neurotoxicity for the development: on the one hand, the test was performed only at PND 11 although it also has to be done at PND 25 and at adult age; on the other hand, a too small number of pups was tested in the experiment 1 (n= 11 control, 9 at 3 and 74 ppm, and 10 at 15 ppm) instead of 10 rats by sex. Moreover, it is not known whether each tested pup comes from different litters as it is recommended in the test guideline 426. It is therefore difficult to conclude on the presence or the absence of a neurotoxic effect.

In the Morris water maze, the 15 ppm-group shows longer latencies to reach the platform than the 74 ppm-group in the first week for the experiment 1 and in the second week for the experiment 2. Thus, the effect in the 15 ppm group seems to be reproducible but it is not observed at the higher dose. In the both experiments, the 15 ppm group spent more time in the outer zone than in the middle one. According to the TG 426 (OECD), the trials have to be performed on 10 animals by sex and by litter, at PND 25 and adult age. However, in the experiment 1, there are only males rats, and a too small number of pups was tested (n= 11 control, 7 at 3 ppm, 9 at 15 ppm, and 11 at 74 ppm). It may explain that effect at higher may not have been detected in experiment 1. In experiment 2, the adequate number of pups was tested but it is not known whether they come from different litters. These uncertainties make it difficult to come to a clear conclusion.

DNA fragmentation due to apoptosis was lower at PND 22 in the experiment 1 whereas an increase was observed at PND 22 (in 15 and 74 ppm groups) in the experiment 2. However, in the experiment 1, the sample sizes were not optimal (n= 2-4/dose), and in the experiment 2, only males are tested. The between-subject variability observed could be caused by the extended study across the ages.

Decreased brain weight was observed at 74 ppm in the both experiment although only males were affected in the second experiment. Histopathological alterations in the brain of offspring of dams exposed to DMTC were noted in the cerebral cortex of rats sacrificed at PND 22 and as adults in experiment 1. Slight/mild vacuolation of the neuropil of the gray matter of the cerebral cortex were observed in 60% of adult offspring at 74 ppm and 20% of PND 22 rats at 74 ppm. Evaluations of the lower dose groups (at 3 and 15 ppm) showed similar vacuolation.

Moreover, in the repeated dose toxicity study in rats, neurobehavioral effects and similar neuropathological lesions were observed at 25 ppm, 75 ppm and 200 ppm (Elf Atochem, 1996), and at 200 ppm in rats (Rohm & Haas Co, 1999).

Overall, the results of both experiments demonstrate a reproducible effect of 15 ppm perinatal DMTC exposure on spatial learning. Changes in expression of apoptosis, brain weight and the occurrence of neuropathological lesions also indicate potential neurotoxicity of DMTC.

The study of Ehman does not follow the TG 426 of the OECD concerning the number and the gender of animals tested and the neurobehavioural effects observed in both the runway testing and the morris water maze have to be confirmed by other studies.

4.11.5 Comparison with criteria

The CLP criteria for classification in Repr.2 are as follow:

*"Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or **experimental animals**, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or **on development**, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects."*

Overall, based on animal studies:

- DMTC induced cleft palates on the fetuses at 20 mg/kg/day, in presence of severe maternal toxicity at this high dose level (Noda 2001, first experiment). Cleft palates are a rare and serious malformation and it is therefore not considered as secondary to maternal toxicity. However, no significant increase in the incidence of cleft palates or other external, skeletal and visceral malformations were observed in a second study at similar or higher dose levels although the substance was administered for shorter durations but covering the whole embryogenesis period. Malformations were also not observed in Ehman 2007 but it may be due to lower dosage (high dose between 4 and 12 mg/kg). Therefore, considering the absence of reproducibility in both experiment of Noda 2001, the evidence is not considered sufficient to place the substance in category 1B.
- DMTC induced a decrease in fetal body weight at 15 and 20 mg/kg (Noda 2001, first experiment). At these doses, maternal toxicity was also observed but the magnitude of fetal weight decrease (-17% and -37% in male pups and -15% and -34% in female pups) was more important than the magnitude of maternal weight decrease (-5% and -24%). No effect on fetal body weight was observed in a second study at similar or higher dose levels although the substance was administered for shorter durations and induced maternal toxicity as evidenced by significant decrease in maternal adjusted body weight gain. In Ehman 2007, a decrease in fetal body weight was observed only at high dose (7-12 mg/kg) in the second experiment during lactation when maternal weight was also significantly decreased. The link between foetotoxicity and maternal toxicity is therefore likely but cannot be totally excluded. Therefore, the evidence is not considered sufficient to place the substance in category 1B.
- DMTC showed a developmental neurotoxic potential in Ehman 2007. The absence of reproducibility of the effects observed in the runaway and water maze tests does not allow giving a clear conclusion. Besides, the study is not consistent with guideline requirement, which raise further uncertainties on the significance of the result. However, it is noted that histopathological lesions observed only in experiment 1 are similar to those reported in adult exposed to DMTC and in fetuses exposed to MMTC, which support that this effect is specific to methyltin exposure and is treatment-related. However, due to the uncertainties discussed above, the evidence is not considered sufficient to place the substance in category 1B.

A classification Repr. 2 H361d" is proposed for DMTC (see separate CLH dossier).

Classification in Repr. Cat.1A is not appropriate as it should be based on human data and no human data specific of DMTC are available.

Considering the rapid gastric hydrolysis of DMT(EHMA) into DMTC, the same effects are expected further to oral exposure and a classification "Repr. 2 H361d" is proposed for DMT(EHMA) (Reprotox Cat 3 Xn R63 according to Directive 67/548/EEC).

4.11.6 Conclusions on classification and labelling

A classification "**Repr. 2 H361d**" is proposed (**Repr. Cat 3, Xn R63** according to Directive 67/548/EEC).

Data are available only by oral route and the route of exposure cannot be specified in the hazard statement.

RAC evaluation of Reproductive toxicity

Summary of Dossier submitter's proposal

No reproductive toxicity studies on DMT(EHMA) are available so the DS applied read-across from DMTC. Please see section on repeated dose toxicity for further details. Two prenatal developmental studies in rats (gavage) similar to OECD 414 (with some deviations on group size and exposure) were evaluated in the CLH report (Noda *et al.*, 2001). In addition, two developmental neurotoxicity studies in rats (drinking water) similar to EPA OPPTS 870.6300 were presented (Ehman, 2007). One supporting study (Noland, 1983) is included to demonstrate transfer of DMTC to blood and brain of foetuses from exposed mothers during gestation. Based on effects seen in the prenatal development and neurotoxicity studies, the DS proposed a classification of Repr. 2 – H361d according to the CLP Regulation (DSD: Repr. Cat. 3; R63). Effects on fertility were not examined in the CLP report.

Comments received during public consultation

Comments were received from four Member States during public consultation. Two member states supported the proposal while one suggested considering classification as Repr. 1B – H360D. The fourth Member State suggested no classification was warranted. Further details, including the dossier submitter's response, can be found in the RCOM.

RAC assessment and comparison with criteria

No data regarding developmental toxicity are available for DMT(EHMA). RAC considers read-across from DMTC to be acceptable; please see section on repeated dose toxicity for further details.

Evaluation of toxicity for reproduction of DMT(EHMA) is based on the two prenatal development studies (both published in Noda, 2001) and two developmental neurotoxicity studies (both in Ehman, 2007). In the first study of Noda (2001) (oral treatment on days 7-17 of gestation at 0, 5, 10, 15, and 20 mg/kg bw/day), severe maternal toxicity occurred at the high dose of 20 mg/kg/day. These clinical signs of toxicity were vaginal bleeding, tremors and convulsions (30%), ataxia and other signs of toxicity (severe thymus atrophy) (100%) and they generally appeared after the 15th day of gestation. Oral administration of DMTC

at 20 mg/kg/day resulted in the death of two pregnant rats (20%). At this dose, total absorption was observed in one of eight living pregnant rats, which exhibited all these clinical signs of toxicity in the late stages of gestation. DMTC at 20 mg/kg/day also caused cleft palate in 21 fetuses (22%). The teratogenicity of DMTC occurred in the presence of severe maternal toxicity at this dose level. Mean body weight in living fetuses of both sexes decreased in a dose-dependent manner with statistical significance at 15 and 20 mg/kg/day.

In the second study of Noda (2001), shorter periods of DMTC treatment (two or three consecutive days at one of four different periods of gestation) and daily doses of 20 or 40 mg DMTC/kg bwt were chosen in order to reduce maternal toxicity. The highest dose (40 mg/kg/day) caused slight maternal toxicity as indicated by the reductions of the adjusted body weight gain and the thymus weight. No significant increase in the incidence of external, skeletal or visceral malformations were observed at either dose in any treatment period group, and no cleft palate was found. Foetal body weight was also unaffected.

In the developmental neurotoxicity studies (Ehman, 2007) the effect of DMTC in drinking water was evaluated in two experiments. In the first, female Sprague-Dawley rats were exposed via drinking water to 0, 3, 15, and 74 ppm DMTC daily before mating and throughout gestation and lactation. Reduced maternal weight gain occurred at the highest dose. In the offspring, decreased brain weight, decreased apoptosis and mild vacuolation in the brain of adult offspring, and slower learning in the water maze have been observed. In a second study, DMTC via drinking water has been provided from gestational day 6 to weaning. The high concentration depressed maternal weight gain, decreased offspring birth weight and pre-weaning growth, and decreased brain weight. Learning deficits were observed in the runway at postnatal day 11 at 15, 74 ppm and again in the adult offspring in the water maze at 15 ppm, although the latter has not been seen at the highest concentration. However, these effects occurred either in one study only, had no dose response relationship or, occurred in the presence of maternal toxicity.

During public consultation one Member State recommended to evaluate the Reprotoxicity studies on EHMA, which are described in the published registration data. After reviewing these data the DS concluded that the reproductive and developmental screening test published on the ECHA website (registration data) does not show any effects justifying classification for these endpoints. The RAC agreed with this conclusion. In the OECD 421 screening assay male and female rats received 0, 10, 50 and 150 mg EHMA/kg bwt. No test article-related effects on male and female mating indices, male copulation or female conception index were shown. There were no effects on gestation length or on numbers of corpora lutea or implantation sites at the lower doses. Slight reductions in the mean numbers of corpora lutea and implantation sites and decreased numbers of pups born corresponded to reduced maternal body weight gain late in gestation. Since these effects were attributed primarily to a single female with only 6 corpora lutea the effects have not been considered test article-related. The decreased viability and growth of F1 animals through post-partum day 4 also occurred at the 150 mg/kg bwt/day dose at which maternal toxicity occurred.

In conclusion

- DMTC induced cleft palates in the fetuses at 20 mg/kg/day, in presence of severe maternal toxicity at this high dose level (Noda, 2001, first study). No significant increase in the incidence of cleft palates or other external, skeletal or visceral malformations were observed in a second study at similar or higher dose levels although the substance was administered for shorter durations but covering the whole embryogenesis

period. Maternal toxicity and malformations were not observed in the Ehman (2007) studies, which may be due to lower dosage (high dose between 4 and 12 mg/kg). Therefore, considering the absence of reproducibility in both studies in Noda (2001) and since no skeletal malformations seen in the Ehman (2007) studies, the occurrence of cleft palate in one study in the presence of severe maternal toxicity is not considered sufficient to place the substance in category 1B.

- DMTC induced a decrease in foetal body weight at 15 and 20 mg/kg (Noda, 2001, first study). At these doses, maternal toxicity was also observed but the magnitude of foetal weight decrease (-17% and -37% in male pups and -15% and -34% in female pups) exceeded the magnitude of maternal weight decrease (-5% and -24%). These effects did not occur in the second study at similar or higher dose levels although the substance induced significant decrease in maternal adjusted body weight gain. In Ehman (2007), a decrease in foetal body weight was observed only at high dose (7-12 mg/kg) in the second experiment during lactation when maternal weight was also significantly decreased. The link between foetotoxicity and maternal toxicity is therefore likely and cannot be totally excluded. Therefore, the evidence is not considered sufficient to place the substance in category 1B.
- DMTC showed developmental neurotoxic potential in Ehman (2007). The absence of reproducibility of the effects observed in the runaway and water maze tests does not permit a clear conclusion to be drawn. Besides, the studies are not consistent with guideline requirements, which raises further uncertainties on the significance of the results. Due to these uncertainties, the evidence is not considered sufficient to place the substance in category 1B.

The effects reported above support classifying DMTC as a reproductive toxicant for effects seen on development. Due to the inconsistencies in these effects, RAC agrees with the DS proposal and considers classification of DMT(EHMA) in category Repr. 2 - H361d justified (DSD: Repr. Cat 3 Xn R63).

As the dossier submitted did not address the fertility endpoint, RAC did not evaluate this aspect of reproductive toxicity.

4.12 Other effects

Not evaluated in this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier.

6 OTHER INFORMATION

No other information relevant.

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8 ANNEXES

ANNEX I

Collection of discussions of DMTC and DMT(EHMA) classifications at ECB

For health effects, DMTC and DMT(EHMA) classifications were discussed and concluded at the Technical Committee of Classification and Labelling (TC C&L) in October 2006.

Environmental effects were not discussed at ECB.

Extract from document ECBI/13/07 Rev. 2 - Draft Summary Record - Meeting of the Technical Committee C&L on the Classification and Labelling of Dangerous Substances - Arona, 4-5 October 2006

Dimethyltin dichloride, DMTC (F048) [1]

EC number: 212-039-2, CAS number: 753-73-1

Classification proposal : [Repr. Cat. 3; R63 - T+; R26 - T; R25 - Xn; R21 - T; R48/23/24/25 - C; R34 - R52/53]

ECBI/25/06 French C&L proposal, as prepared by IND, for Dimethyltin dichloride, DMTC

FR presented the classification proposal.

DE asked about the substances, whether these complex substances were already diluted in solution in the studies and whether you need to go back and understand the toxicity of the complex substance itself.

IND replied that the CAS numbers are for the purified substances. All these substances are produced as mixtures and we have used the historical data as if it was the purified substance. We understand this might be a discussable method, but it is pragmatic and we assume that at least this would never lead to under classification and that is the reason why we have decided to use it.

Acute toxicity:

The **TC C&L experts** agreed to classify DMTC with T+; R26, T; R25 and Xn; R21

Corrosivity:

The **TC C&L experts** agreed to classify DMTC with C; R34

Long term toxicity:

T; R48/23/24/25 was proposed. **DE** supported classification for the oral route only as the other routes were read across from acute toxicity and the substance was corrosive. Especially that made it questionable to include longer term effects by inhalation. **NL** did not agree to the reading across for the dermal route.

BE and **DE** did not support reading across from acute to long term toxicity for the inhalation route.

It was agreed not to classify with T; R48/23/24.

The **TC C&L experts** agreed to classify DMTC with T; R48/25.

Reprotoxicity:

There was a proposal to classify for developmental toxicity: Repr. Cat. 3; R63 based on evidence for the substance and as presented by **F**.

R63 was agreed without a long discussion. **N** and **DE** expressed agreement, no MS expressed disagreement.

Conclusion:

The TC C&L agreed to classify **Dimethyltin dichloride, DMTC** with **Repr.Cat. 3; R63 – T+; R26 – T; R25- 48/25 - Xn ; R21 – C; R34**. The corresponding labelling would then be the symbol: T+, and the R-phrases: 21-25-26-34-48/25-63 and the S-phrases: 26-28-36/37/39-45. (*Classification for environmental effects has to be discussed in January 2007.*)

[...]

Dimethyltin bis(2-ethylhexyl- mercaptoacetate, DMT (EHMA) (F050) [3]

EC number: 260-829-0, CAS number: 57583-35-4

Classification proposal : [Repr. Cat. 3; R63 - T; R24 - Xn; R22 - T; R48/23/24/25 - Xi; R43 - R52/53]

ECBI/24/06 French C&L proposal, as prepared by IND, for Dimethyltin bis(2-ethylhexyl-mercaptoacetate, DMT (EHMA)

ECBI/24/06 Rev. 1 French revised C&L proposal for Dimethyltin bis(2-ethylhexyl-mercaptoacetate, DMT (EHMA)

Acute toxicity:

The T; R24 proposal was withdrawn due to availability of additional data supporting no classification for the dermal route.

Xn; R22 was agreed.

Sensitisation:

The **TC C&L experts** agreed to classify DMT (EHMA) with R43 without further discussion.

Long term toxicity:

The **TC C&L experts** agreed to classify DMT (EHMA) with T; R48/25 based on the DMTC data due to the DMTC being a hydrolysis product of DMT (EHMA). The long-term toxicity proposed by the other routes was not agreed.

Reprotoxicity:

Repr. Cat. 3; R63 was proposed on basis of the DMTC data because DMTC is a hydrolysis product of DMT (EHMA). This was agreed by the TC C&L.

Conclusion:

The **TC C&L** agreed not to classify **Dimethyltin bis(2-ethylhexyl-mercaptoacetate, DMT (EHMA)** T; R24; T; R48/23 and T; R48/24. The TC C&L agreed to classify **Dimethyltin bis(2-ethylhexyl-mercaptoacetate, DMT (EHMA)** with Repr. Cat. 3; R63 - T; R48/25 - Xn; R22 - R43. The corresponding labelling would then be the symbol: T, and the R-phrases: 22-43-48/25-63 and the S-phrases: 36/37-45. (*Classification for environmental effects has to be discussed in January 2007.*)