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**ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**REPORT ON THE PROPOSAL FOR CLASSIFICATION AND LABELLING (C&L) OF
DIMETHYLTIN DICHLORIDE**

**Series on Testing & Assessment
No. 247**

The corresponding annexe is available in the following cote : ENV/JM/MONO(2016)44/ANN1

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OECD Environment, Health and Safety Publications

Series on Testing & Assessment

No. 247

**REPORT ON THE PROPOSAL FOR CLASSIFICATION AND LABELLING (C&L) OF
DIMETHYLTIN DICHLORIDE**

**Joint Pilot Project of the OECD and the UN Sub-Committee of Experts on the Globally Harmonised
System of Classification and Labelling of Chemicals**



INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

A cooperative agreement among FAO, ILO, UNDP, UNEP, UNIDO, UNITAR, WHO, World Bank and OECD

**Environment Directorate
ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
Paris, 2016**

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FOREWARD

In 2014, the OECD Task Force on Hazard Assessment (TFHA) and the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology (JM) agreed to provide a coordination role for a pilot classification project upon invitation from the UN Sub-Committee of Experts on the Globally Harmonised System of Classification and Labelling of Chemicals (UNSCEGHS). A report of the Pilot Project of the OECD and the UN Sub-Committee of Experts on the Globally Harmonised System of Classification and Labelling of Chemicals detailing the process of the pilot project and learnings is published along with this report. (Report on the Pilot Project on Assessing the Potential Development of a Global List of Classified Chemicals. ENV/JM/MONO(2016)43, Series on Testing & Assessment No. 246). It also contains a template for Proposals for Classification and Labelling (Annex 1 to ENV/JM/MONO(2016)43/ANN1/PART1 & ENV/JM/MONO(2016)43/ANN1/PART1).

Accompanying the report are three case study chemicals where non-binding agreement on their classification have been reached. The results of this pilot project will be submitted to the UNSCEGHS for consideration in their deliberations on the potential development of a global list of classified chemicals.

This report on the Proposal for Classification and Labelling (C&L) of Dimethyltin Dichloride was prepared by the European Chemicals Agency, with review and input from the project team established for this pilot project under the OECD Task Force for Hazard Assessment. It contains a C&L report as well as an Annex with additional background information.

The following two reports on the Proposal for Classification and Labelling (C&L) are published with this report:

1. Report on the Proposal for Classification and Labelling (C&L) of Dicyclopentadiene ENV/JM/MONO(2016)45, Series on Testing & Assessment No. 248.
2. Report on the Proposal for Classification and Labelling (C&L) of Dibutyl Phthalate ENV/JM/MONO(2016)46, Series on Testing & Assessment No. 249.

This document is being published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology.

Proposal for Classification and Labelling (C&L)

**Based on the Globally Harmonized
System of Classification
and Labelling of
Chemicals (GHS)**

International Chemical Identification: Dimethyltin dichloride

CAS Number: 753-73-1

Contact details for dossier submitter:

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Finland**

Version number: 4

Date: 29/04/2016

Note on confidential information

Please be aware that this report is intended to be made publicly available. Therefore it does not contain any confidential information.

Disclaimer: This proposal for classification and labelling, is for a candidate substance as identified by the UN Sub-Committee on GHS and contributes to the Joint GHS Sub-Committee/OECD Pilot classification exercise (work plan in UN/SCEGHS/28/INF.22, Annex I). It is not intended for any other purposes. The proposal does not represent an official position of the European Chemicals Agency.

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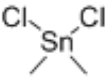
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1. IDENTIFY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

International Chemical Identification - Name(s) in the IUPAC nomenclature or other international chemical name(s)	Dimethyltin dichloride
Other names (usual name, trade name, abbreviation)	DMTC
ISO common name (if available and appropriate)	Not applicable.
CAS number (if available)	753-73-1
Other identifier(s) (if available)	EC number: 212-039-2
In case the substance is already included in a classification list - identifier of the entry	EU Index number in Annex VI, CLP Regulation: 050-029-00-8
Molecular formula	C ₂ H ₆ Cl ₂ Sn
Structural formula	
SMILES notation (if available)	Not available.
Molecular weight or molecular weight range	219.67 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable.
Degree of purity (%) (if relevant for the classification proposal)	90% < conc. >100%

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)
Dimethyltin dichloride	90% < conc. >100%

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	The impurity contributes significantly to the classification and labelling
Monomethyltin trichloride (MMTC) (CAS 993-16-8).	Not disseminated	The impurity has a mandatory classification in the EU as toxic for reproduction with regard to developmental effects, category 2 (Annex VI, CLP Regulation, EU).

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	The additive contributes significantly to the classification and labelling (yes/no)
No data available.			

Table 5: Test substances (non-confidential information)

Identification of test substance	Purity	Impurities and additives (identity, %, classification if available)	Other information	The study(ies) in which the test substance is used
Not considered useful for this dossier				

2. PROPOSED CLASSIFICATION AND LABELLING

2.1 Proposed classification and labelling according to the GHS criteria (GHS revision number 6)

Table 6: Proposed classification and reason for not proposing a classification for a hazard class

GHS chapter ref.	Hazard class or differentiation	Proposed classification - Hazard Class and Category Code(s); Hazard statement Code(s)	Proposed Specific Concentration Limits (SCL(s)) and M-factor(s)	Reason for no proposed classification
2.1	Explosives	No classification.		Data lacking
2.2	Flammable gases	No classification.		Hazard class not applicable
2.3	Aerosols	No classification.		Hazard class not applicable
2.4	Oxidising gases	No classification.		Hazard class not applicable
2.5	Gases under pressure	No classification.		Hazard class not applicable
2.6	Flammable liquids	No classification.		Hazard class not applicable
2.7	Flammable solids	No classification.		Data conclusive but not sufficient for classification
2.8	Self-reactive substances	No classification.		Data lacking
2.9	Pyrophoric liquids	No classification.		Hazard class not applicable
2.10	Pyrophoric solids	No classification.		Data lacking
2.11	Self-heating substances	No classification.		Data conclusive but not sufficient for classification
2.12	Substances which in contact with water emit flammable gases	No classification.		Data lacking
2.13	Oxidising liquids	No classification.		Hazard class not applicable
2.14	Oxidising solids	No classification.		Data lacking
2.15	Organic peroxides	No classification.		Hazard class not applicable
2.16	Corrosive to metals	No classification.		Data lacking
2.17	Desensitized explosives	No classification.		Hazard class not applicable
3.1	Acute toxicity - via oral route	Acute Tox. 3; H301		
	- via dermal route	Acute Tox. 3; H311		
	- via inhalation route	Acute Tox. 2; H330		
3.2	Skin corrosion/irritation	Skin Corr. 1; H314		
3.3	Serious eye damage/eye irritation	Eye Dam. 1; H318.		
3.4	Respiratory sensitisation	No classification.		Data lacking.

	Skin sensitisation	No classification.		Data inconclusive.
3.5	Germ cell mutagenicity	No classification.		Data conclusive but not sufficient for classification.
3.6	Carcinogenicity	No classification.		Data inconclusive.
3.7	Reproductive toxicity	Repr. 2; H361 (developmental toxicity)		
3.8	Specific target organ toxicity-single exposure	No classification.		Data conclusive but not sufficient for classification.
3.9	Specific target organ toxicity-repeated exposure	STOT RE 1; H372 (nervous system, immune system)		
3.10	Aspiration hazard	No classification.		
4.1	Hazardous to the aquatic environment	Aquatic Acute 3; H402 Aquatic Chronic 3; H412		
4.2	Hazardous to the ozone layer	No classification.		Hazard class not applicable

Proposed labelling

Pictogram Code(s): GHS05, GHS06, GHS08

Signal Word Code(s): Danger

Hazard statement Code(s): H301, H311, H330, , H314, H318, H361 (developmental), H372 (nervous system, immune system), H412

Supplemental information: The additional hazard statement “Corrosive to the respiratory tract” is suggested to be added in the labelling based on the classification of DMTC as acutely toxic by inhalation and as skin corrosive (see section 8.2)

3. IDENTIFIED USES

Used as an intermediate to make heat stabilizers for PVC. There is no known use for the general public.

4. DATA SOURCES

- EU REACH registration dossier on DMTC, published 25 June 2013, modified 3 August 2015, as disseminated on ECHA's webpage (<http://echa.europa.eu/information-on-chemicals>)*
- PubMed search on DTMC (date 8 May 2015)
- The opinion of the ECHA's Committee for Risk Assessment proposing harmonised classification and labelling at EU level of Dimethyltin dichloride, adopted 30 November 2012 (including its Annexes of which Annex I is based on a proposal for a EU harmonised classification from the French Competent Authority) (http://echa.europa.eu/opinions-of-the-committee-for-risk-assessment-on-proposals-for-harmonised-classification-and-labelling/-/substance-rev/2066/del/50/col/staticField_-104/type/asc/pre/2/view)

* Please note that the term "registrant" in the following text, and in Annex I, means the registrant that has submitted the registration dossier as required according to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

5. PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Colourless/white solid	Harper et al. (2005), and Summer et al. (2003), OECD SIDS (2006)	References were assessed with a reliability of 4 (Klimisch score).
Melting/freezing point	Melting point of test substance determined as 105 ± 0.5 °C	Author not disseminated (2013); report date 2013-01-28.	The key study was conducted in accordance with the accepted EU A.1 & OECD Guideline 102 test methods and GLP compliant.
Boiling point	189°C	Harper et al. (2005), Dobson et al. (2006), Author not disseminated (1994), Hoch (2001), Summer et al. (2003), Author not disseminated (2006); report date 2006-07-23, OECD SIDS (2006).	References were assessed with a reliability of 4 (Klimisch score). No single study was robust enough to be considered a key study.
Relative density	1.4 g/cm ³	Summer et al. (2003), Author not disseminated (1993); report date 1993-07-20, OECD SIDS (2006).	The density of the test substance was found to lie between 1 and 1.75 g/cm ³ . 1.4 g/cm ³ was chosen as the key value for chemical safety assessment as it is the midpoint of this range. References were assessed with a reliability of 4 (Klimisch score). No single study was robust enough to be considered a key study.
Vapour pressure	0.04 hPa at 20°C,	Dobson et al. (2006), van Dokkum and Huwer (2005) Summer et al. (2003) and OECD SIDS (2006).	References were assessed with a reliability of 4 (Klimisch score). No single study was robust enough to be considered a key study.
Surface tension	Not applicable.		
Water solubility	823.0 g/l at 20.0 ± 0.5 °C	Author not disseminated (2003); report date 2003-04-09.	The key study was a reliable study (Klimisch score 1), conducted in accordance with OECD TG 105 and GLP compliant. However, this analysis method only measures the amount of the alkyltin moiety, but cannot identify the other ligands attached to the tin.
Partition coefficient n-octanol/water	-2.18 ± 0.17 (22.0 ± 1.0 °C)	Author not disseminated (2003). Report date 2003-04-09.	The key study was a reliable study (Klimisch score 1), conducted in accordance with OECD TG 107 and GLP compliant. However, in water,

			DMTC undergoes rapid degradation by hydrolysis and is expected to hydrolyze within minutes. The reported value may not be due entirely to the named substance and any subsequent use of the partition coefficient to predict environmental behaviour of the named substance must carefully consider the limitations inherent in the measurement of this parameter.
Flash point	Not applicable		As the substance is a solid, testing is technically not feasible.
Flammability	Non flammable	Author not disseminated (2013). Report date 2013-01-21.	In a reported flammability study (Klimisch 1) conducted in accordance with EU Method A.10 and CLP compliant, no evidence of flammability was observed.
Explosive properties	Non explosive		There are no chemical groups associated with explosive properties present in the molecule.
Self-ignition temperature	The substance has been determined not to have a relative self-ignition temperature below its melting temperature.	Author not disseminated (2013). Report date 2013-01-21.	Testing was conducted using a procedure designed to be compatible with Method A16 Relative Self-Ignition Temperature for Solids of Commission Regulation (EC) No 440/2008 of 30 May 2008.
Oxidising properties			No studies are available.
Granulometry	Not applicable		The substance is marketed or used in a non solid or granular form.
Stability in organic solvents and identity of relevant degradation products			No studies are available.
Dissociation constant	pKa= 3.54	Vighi & Calamari, Chemosphere (1985).	No methodological information available. Klimisch 4 (not assignable).
Viscosity	Technically not feasible		The substance is unsuitable for viscosity testing as it is a solid.

6. EVALUATION OF PHYSICAL HAZARDS

6.1 Explosives

Table 8: Summary table of studies on explosive properties

Method	Results	Remarks	Reference
No studies are available.			

Short summary and overall relevance of the provided information on explosive properties

No studies are available.

According to the registrant it can be concluded from the structural formula that the substance is not explosive as it does not have functional groups associated with explosivity.

Comparison with the GHS criteria

A substance is not classified as explosive if there are no chemical groups associated with explosive properties present in the molecule (examples of groups which may indicate explosive properties are given in table A6.1 in Appendix 6 of the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria).

Conclusion on classification and labelling for explosive properties

From the structural formula it can be concluded that the substance is not explosive as it does not have functional groups associated with explosivity.

6.2 Flammable gases

Table 9: Summary table of studies on flammable gases

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on flammable gases

Not applicable.

Comparison with the GHS criteria

Not applicable.

Conclusion on classification and labelling for flammable gases

Not applicable.

6.3 Aerosols

Table 10: Summary table of studies on aerosols

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on aerosols

Not applicable.

Comparison with the GHS criteria

Not applicable.

Conclusion on classification and labelling for aerosols

Not applicable.

6.4 Oxidising gases

Table 11: Summary table of studies on oxidising gases

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on oxidising gases

Not applicable.

Comparison with the GHS criteria

Not applicable.

Conclusion on classification and labelling for oxidising gases

Not applicable.

6.5 Gases under pressure

Table 12: Summary table of studies on gases under pressure

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on gases under pressure

Not applicable.

Comparison with the GHS criteria

Not applicable.

Conclusion on classification and labelling for gases under pressure

Not applicable.

6.6 Flammable liquids

Table 13: Summary table of studies on flammable liquids

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on flammable liquids

Not applicable.

Comparison with the GHS criteria

Not applicable.

Conclusion on classification and labelling for flammable liquids

Not applicable.

6.7 Flammable solids

Table 14: Summary table of studies on flammable solids

Method	Results	Remarks	Reference
EU Method A.10	Not classified	The pile failed to ignite during the 2 minutes that the Bunsen flame was applied. The result of the preliminary screening test obviated the need to perform the main test.	Author not disseminated (2013), Report date 2013-01-21.

Short summary and overall relevance of the provided information on flammable solids

The test item has been determined to be not flammable as it failed to ignite in the preliminary screening test.

Comparison with the GHS criteria

If the test item does not ignite and propagate combustion either by burning with flame or smoldering along 200 mm within 4 minutes, the test item is not considered as highly flammable and no further testing is required. The flammability (solids) was determined by measuring the burning rate of test item prepared as a pile of set dimensions. The pile failed to ignite during the 2 minutes that the Bunsen flame was applied. The result of the preliminary screening test obviated the need to perform the main test.

The test item has been determined to be not highly flammable as it failed to ignite in the preliminary screening test.

Conclusion on classification and labelling for flammable solids

Not classified.

6.8 Self-reactive substances

Table 15: Summary table of studies on self-reactivity

Method	Results	Remarks	Reference
No studies are available.			

Short summary and overall relevance of the provided information on self-reactive substances

No studies are available.

Comparison with the GHS criteria

No studies are available.

Conclusion on classification and labelling for self-reactive substances

No studies are available.

6.9 Pyrophoric liquids

Table 16: Summary table of studies on pyrophoric liquids

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on pyrophoric liquids

Not applicable.

Comparison with the GHS criteria

Not applicable.

Conclusion on classification and labelling for pyrophoric liquids

Not applicable.

6.10 Pyrophoric solids

Table 17: Summary table of studies on pyrophoric solids

Method	Results	Remarks	Reference
No studies are available.			

Short summary and overall relevance of the provided information on pyrophoric solids

According to the registrant, in accordance with point 1, Annex XI of Regulation (EC) No. 1907/2006 (REACH), based on the known chemical and physical properties of the substance, its chemical structure and experience in handling the substance, testing in line with Method A13: Pyrophoric properties of solids and liquids of Commission Directive 92/69/EEC is expected to produce a negative results.

Comparison with the GHS criteria

No studies are available.

Conclusion on classification and labelling for pyrophoric solids

Not classified as no studies are available.

6.11 Self-heating substances

Table 18: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
A.16	The substance has been determined not to have a relative self-ignition temperature below its melting temperature		Author not disseminated (2013), Report date 2013-01-21.

Short summary and overall relevance of the provided information on self-heating substances

The substance has been determined not to have a relative self-ignition temperature below its melting temperature (105°C).

Comparison with the GHS criteria

A substance shall be classified as self-heating substance if a positive result at 140°C is obtained according to test method N4 described in the UN Recommendations on the TDG, Manual of tests and criteria, Part III, sub-section 33.1.6 used as the basis for the GHS criteria.

Not classified as the melting point temperature is below 140°C.

Conclusion on classification and labelling for self-heating substances

Not classified.

6.12 Substances which in contact with water emit flammable gases

Table 19: Summary table of studies on substances which in contact with water emit flammable gases

Method	Results	Remarks	Reference
No studies are available.			

Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

No studies are available.

The substance is known to be highly soluble in water.

According to the registrant, in accordance with point 1, Annex XI of Regulation (EC) No. 1907/2006 (REACH), based on the known chemical and physical properties of the substance, its chemical structure and experience in handling the substance, testing in line with Method A.12: Flammability (contact with water) of Commission Directive 92/69/EEC is expected to produce a negative results.

Comparison with the GHS criteria

No studies are available, but the substance is known to be highly soluble in water.

Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Not classified.

6.13 Oxidising liquids**Table 20: Summary table of studies on oxidising liquids**

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on oxidising liquids

Not applicable.

Comparison with the GHS criteria

Not applicable.

Conclusion on classification and labelling for oxidising liquids

Not applicable.

6.14 Oxidising solids**Table 21: Summary table of studies on oxidising solids**

Method	Results	Remarks	Reference
No studies are available.			

Short summary and overall relevance of the provided information on oxidising solids

No studies are available.

Comparison with the GHS criteria

No studies are available.

Conclusion on classification and labelling for oxidising solids

No studies are available.

6.15 Organic peroxides**Table 22: Summary table of studies on organic peroxides**

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on organic peroxides

Not applicable.

Comparison with the GHS criteria

Not applicable.

Conclusion on classification and labelling for organic peroxides

Not applicable.

6.16 Corrosive to metals**Table 23: Summary table of studies on the hazard class corrosive to metals**

Method	Results	Remarks	Reference
No studies are available.			

Short summary and overall relevance of the provided information on the hazard class corrosive to metals

No studies are available.

Comparison with the GHS criteria

No studies are available.

Conclusion on classification and labelling for corrosive to metals

No studies are available.

6.17 Desensitized explosives**Table 24: Summary table of studies on desensitized explosive properties**

Method	Results	Remarks	Reference
Not applicable.			

Short summary and overall relevance of the provided information on desensitized explosive properties

Not applicable.

Comparison with the GHS criteria

Not applicable.

Conclusion on classification and labelling for desensitized explosive properties

Not applicable.

7. TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 25: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
OECD TG 417, GLP compliant Rat SD, m/f, single dose, 10 mg/kg bw	Bioavailability: 0.52/0.71 m/f Mean (\pm SD) apparent volume of distribution: intravenous: 44.3 (\pm 26.7)/ 109 (\pm 92.9) L/kg, m/f oral: 52.7 (\pm 31.7)/138 (\pm 98.1) L/kg Renal clearance: intravenous: 0.105/0.559 L/h/kg, m/f oral: 0.0816/0.282 L/h/kg, m/f t_{max} ranged from 10 minutes to 4 hour post dosing. mean (\pm SD) C_{max} : 3834 (\pm 2983) / 1088 (\pm 656) μ g/mL m/f mean (\pm SD) $AUC_{0-\infty}$: 51.3 (\pm 53.7)/11.0 (\pm 0.31) μ g-hour/mL m/f	Deviations: Three deviations not affecting the quality of the study were reported.	Author not disseminated (2001) Report date 2001-09-05.
No guideline, GLP compliance not reported Rat SD, f 40 mg tin/L/d phases I and II in drinking water; 0.8 mL/100 g bw/d in phase III by gavage. Radioactive dimethyltin dichloride in phase III only	Dimethyltin dichloride was transferred to the pups mainly during the gestation period. Transfer during lactation was limited.		Noland <i>et al.</i> 1983
OECD TG 428, GLP compliant dimethyltin dichloride 89%, monomethyltin dichloride 11% dose: 100 μ g/cm ² as a 10 μ l/cm ² dose of a 10000 μ g/ml solution in ethanol	Human and rat skin, Wistar-derived rat strain. Dermal absorption was determined under occluded and unoccluded conditions. Percutaneous absorption rate at 24h: unoccluded human 20% and unoccluded rat 34%. In human skin the maximum absorption rate were 0.015 μ g/cm ² /h between 0-6h and 0.037 μ g/cm ² /h between 6-24h. In rat skin, the absorption rate was constant for the occluded skin (0.233 μ g/cm ² /h) during the whole exposure. While for the unoccluded skin the maximum rate was 1.07 μ g/cm ² /h, and the absorption was essentially complete within the first 3h.		Author not disseminated (1999) Report date: 1999-03-31.

Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

The absorption, distribution, metabolism and excretion (ADME) for dimethyltin dichloride was investigated in male and female Sprague-Dawley rats following administration of a single oral or intravenous dose of 10 mg/kg bw. Plasma and urine were collected at regular interval for analysis. Concentrations of tin were also generally higher in males than in females following the single oral dose. Smaller inter-animal variability was observed in the tin plasma concentrations following oral than intravenous administration. The distribution phase, for both routes of administration, lasted at least 8 hours. The tin plasma concentration decreased fast on the first 12 hour post-dosing, by approximately an order of magnitude, followed by a slower clearance rate. Tin plasma concentrations during the terminal elimination phase were higher for males than for females, but were similar between the two routes of administration.

The urinary clearance rate appeared to be influenced by the route of administration; biphasic after intravenous and triphasic after oral administration. Following the intravenous exposure, an initial faster clearance phase occurred within the first 24h, followed by slower elimination phase which coincided with the low plasma concentrations. After the oral dose, an additional phase, coinciding with the distribution phase, was observed.

Noland et al. (1983) investigated the dimethyltin dichloride gastrointestinal adsorption and trans placental transfer after oral administration in female Sprague-Dawley rats. The test substance was administrated to dams, two weeks prior breeding and continued through breeding and gestation, via drinking water on phases I and II and by gavage on phase III. The study showed that dimethyltin dichloride was able to cross the blood brain barrier and the placenta to reach the foetus. In pup the highest levels of tin were measured were measured at birth; tin levels were rapidly decreasing after birth demonstrating that exposure via lactation is limited.

Dermal absorption was investigated in vitro in human and rat skin. Percutaneous absorption rate in unoccluded and occluded human sample at 24 hours was 20% and 43%, respectively. While percutaneous absorption rate in unoccluded rat at 24 hours was 34%. For both species, the amount remaining in epidermis is considered potentially absorbable. After the 24h exposure, the overall recovery from the system was low: 55% and 67% of the occluded applied dose recovered in human and rat respectively while 31% and 48% was recovered from the unoccluded applications in human and rat respectively.

8. EVALUATION OF HEALTH HAZARDS

8.1 Acute toxicity - oral route

Table 26a: Summary table of animal studies on acute oral toxicity

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Value LD ₅₀	Reference
OECD TG 401 GLP compliant Oral gavage	Rat; Sprague-Dawley, 5 animals/sex and dose	Dimethyltin dichloride (DMTC)(85% DMTC: 15% monomethyltin trichloride). The [Di/Mono] Methyltin Chlorides Solution was a 50% organotin solution in an unspecified solvent.	Main study: 200, 300 and 500 mg/kg bw.	LD ₅₀ (male): 273 mg/kg bw (of 85% DMTC) LD ₅₀ (female): 164 mg/kg bw (of 85% DMTC) LD ₅₀ (male/female): 204.5 mg/kg bw (of 85% DMTC)	Elf Atochem NA, 1993
OECD TG 401 GLP compliance not reported Oral gavage	Rat; Sprague-Dawley, 5 animals/per sex and dose	dimethyltin dichloride 98.7%.	0, 100, 150, 200, 250 and 300 mg/kg bw	LD ₅₀ (male): 190 mg/kg bw LD ₅₀ (female): 160 mg/kg bw	Author not disseminated (1979). Report date: 1979-03-20.
Non-guideline GLP compliance not reported Oral gavage	Rat, Sprague-Dawley, 5 animals/per sex and dose	5% dimethyltin dichloride in corn oil (identity of test substance not the same as for substance defined in the SID part of registration dossier)	0.215, 0.464, 1.00, 2.15 and 4.64 mL/kg bw + controls	LD ₅₀ (male/female): 1.7 mL/kg bw	Author not disseminated (1978). Report date: 1978-05-25.
No guideline stated GLP compliance not reported Oral gavage	Rat, no strain data 6 males/dose	dimethyltin dichloride; purity not given	100, 200, 400, 800 and 1600 mg/kg bw No data on control animals.	LD ₅₀ (male): 141.4 mg/kg bw	Affiliated Medical Enterprises, 1971a .
No guideline stated. GLP compliance not reported (pre-GLP implementation) Oral gavage	Rat, Wistar, 10 males per dose	dimethyltin dichloride. Test material form: crystalline. 5% solution	48, 57, 69, 83, 100, 120 and 144 mg/kg bw No data on control animals.	LD ₅₀ (male): 73.86 mg/kg bw	Klimmer, O.R. 1971.

No methodological information. GLP compliance not reported. Oral	Rat, no further information given.	dimethyltin dichloride; purity not given	Not specified.	LD ₅₀ : 74 mg/kg bw	Hoch (2001) (from an overview in Applied Geochemistry)
No guideline followed, GLP compliance not reported. Acute oral test, with lower dosage groups repeated on 4th day. Animals observed for 10 days. Limited methodological information.	Rat, no data on strain, two females per dose.	dimethyltin dichloride. Test material form: crystalline.	Doses: 40, 80 and 160 mg/kg bw. 40 and 80 mg/kg bw given on 1st and 4th day; 160 mg/kg bw given on 1st day only.	Mortality: Both rats died in the 160 mg/kg bw group. LD ₅₀ to female rats was found to be between 80 and 160 mg/kg bw.	Barnes and Stoner (1958).
No guideline followed. Acute oral (gavage) LD50 determined as part of an in-vivo UDS study, in order to determine dosage levels for the study. GLP compliant.	Rat, Fischer 344, three males per dose.	Mixture of methyltin chloride compounds. Test material form: crystalline.	First range finding assay: 25, 50, 100, 200 and 400 mg/kg bw. Second range finding assay: 600 and 800 mg/kg bw.	LD ₅₀ : ca. 280 mg/kg bw (male).	Author not disseminated (1993). Report date: 1993-07-11.
Methodological information not available in English. GLP compliance not reported Route of administration: oral (unspecified).	Rabbit, no data on strain. Sex: male.	dimethyltin dichloride. Test material form: crystalline.	No data available.	Minimum lethal dose: 50 mg/kg bw (male).	Hashizume (1971).
No methodological information. GLP compliance not reported. Route of administration: oral (unspecified).	Rat, no data on strain or sex.	dimethyltin dichloride. Form: crystalline.	No data available.	No LD ₅₀ given.	Author not disseminated (1978b). Reference type: review article or handbook.

No methodological information available. Route of administration: oral (unspecified). GLP compliance not reported.	Rat, no data on strain or sex.	dimethyltin dichloride. Form: crystalline.	No data available.	LD ₅₀ : 88 - 119 mg/kg bw	van Dokkum, and Huwer (2005).
No methodological information available. Route of administration: oral (unspecified). GLP compliance not reported.	Rat, no data on strain or sex.	dimethyltin dichloride. Form: crystalline.	No data available.	LD ₅₀ : 237 mg/kg bw.	Author not disseminated (1978c).
No methodological information. Route of administration: oral (unspecified). GLP compliance not reported.	Rat, no data on strain or sex.	dimethyltin dichloride. Form: crystalline.	No data available.	LD ₅₀ : 90 mg/kg bw.	Author not disseminated (1978d). Reference type: review article or handbook.
No methodological information. Route of administration: oral (unspecified). GLP compliance not reported.	Rat, no data on strain or sex.	dimethyltin dichloride. Form: crystalline.	No data available.	LD ₅₀ : 74 mg/kg bw.	Dean (1976).
No methodological information. Route of administration: oral (unspecified). GLP compliance not reported.	Rat, no data on strain or sex.	dimethyltin dichloride. Form: crystalline.	No data available.	LD ₅₀ : 74 mg/kg bw.	Author not disseminated (1992).

Table 26b: Summary table of human data on acute oral toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 26c: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Short summary and overall relevance of the provided information on acute oral toxicity

The oral toxicity of dimethyltin dichloride was evaluated in two OECD TG 401 studies in rats. In both studies, a difference in toxicity between male and female was observed, females being more sensitive than males. In Elf Atochem (1993), gross pathology effects were observed in the lungs, thymus, kidneys and stomach. Under the conditions of this test, the acute oral LD₅₀ of [Di/Mono] Methyltin Chlorides Solution in the male rat was determined to be 546 mg/kg. In the female rat, the oral LD₅₀ was determined to be 328 mg/kg. In the sexes combined, the oral LD₅₀ was determined to be 409 mg/kg. According to the registrant, the [Di/Mono] Methyltin Chlorides Solution was a 50% organotin solution in an unspecified solvent and therefore the LD₅₀ value achieved should be halved. The LD₅₀ of 84.79 % pure Dimethyltin dichloride was therefore determined to be 273 mg/kg for male rats 164 mg/kg for female rats, with the combined LD₅₀ being 204.5 mg/kg bw.

In the second study, effects were observed in the lungs (hemorrhages) and in the stomach. The LD₅₀ were 190 and 160 mg/kg bw for male and female rats, respectively. The REACH registration dossier contains additional studies with reliability scores of 3 or 4 (non-reliable or not assignable), most of them performed prior to GLP. In these studies, the LD₅₀-values ranged from 73.86 to 280 mg/kg bw; these values can thus be used as supportive evidence of the LD₅₀ seen in the two OECD TG 401 studies in rats.

Comparison with the GHS criteria

In the two OECD TG 401 studies, the LD₅₀ values were in the range between 160 and 273 mg/kg bw being within the range of $50 \leq \text{ATE} < 300$ mg/kg bw and thus supporting classification with Acute toxicity Category 3. In addition, the LD₅₀ values ranged from 73.86 to 280 mg/kg bw in the non guideline studies providing further support for classification in Category 3.

Conclusion on classification and labelling for acute oral toxicity

Classification with category 3 is proposed for Acute toxicity via the oral route (Acute Tox. 3; H301).

*Acute toxicity - dermal route***Table 27a: Summary table of animal studies on acute dermal toxicity**

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, of duration exposure	Value LD ₅₀	Reference
OECD TG 402, EU Method B.3. GLP compliant. Deviations: The temperature and relative humidity of the animal room (66-72 °F and 30-60 %, respectively) exceeded the range specified in the protocol (61-70°F and 40-60%, respectively). The final body weight and method of euthanasia was not recorded for two males.	Rabbit, New Zealand White, male/female, 5 animals per sex and dose.	84.8 % DMTC in mixture with MMTC (Monomethyltin trichloride). Test material form: solution. Physical state: liquid.	200, 400 and 750 mg/kg bw. Occlusive, ca. 24 hours.	LD ₅₀ : 404 mg/kg bw (male/female).	Rush, E.R. (1993b).
No guideline followed. Very limited methodological information available. GLP compliance not reported.	Rat; no data on strain, 3 males per dose.	dimethyltin dichloride. Form: crystalline.	80 mg/kg applied on 5 successive days. Percutaneous application.	No LD ₅₀ determined.	Barnes and Stoner (1958).
OECD TG 402. Deviations: no information. GLP compliance not reported.	Rabbit: New Zealand White, male/female (six animals in total, no data on control animals).	Dimethyltin Dichloride: Methyltin Trichloride (90:10% mixture). Physical state: liquid.	2000 mg/kg bw.	LD ₅₀ > 2000 mg/kg.	Affiliated Medical Enterprises 1971b

Table 27b: Summary table of human data on acute dermal toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 27c: Summary table of other studies relevant for acute dermal toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Short summary and overall relevance of the provided information on acute dermal toxicity

Three acute dermal toxicity studies are available. In the most reliable study, the GLP-compliant OECD TG 402 study in rabbits using DMTC at 84.8% in a mixture with MMTC (monomethyltin trichloride) (Rush 1993b), an LD₅₀ value of 404 mg/kg bw has been determined. The other two studies have been insufficiently documented and are not considered sufficiently reliable for classification.

Comparison with the GHS criteria

The LD₅₀ value of 404 mg/kg is within the range of values ($200 \leq \text{ATE} < 1000$ mg/kg bw) warranting a classification in Category 3 for acute dermal toxicity according to the GHS criteria.

Conclusion on classification and labelling for acute dermal toxicity

Classification with Category 3 is proposed for Acute toxicity via the dermal route (Acute Tox. 3; H311).

Acute toxicity - inhalation route**Table 28a: Summary table of animal studies on acute inhalation toxicity**

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC ₅₀	Reference
Equivalent or similar to OECD TG 403. Inhalation (aerosol). GLP compliance not reported.	Rat, Tif: RAIf (SPF), 10 animals per sex and per dose.	dimethyltin dichloride. Form: crystalline. Aerosol.	44 ± 6, 90 ± 7, 121 ± 3 and 167 ± 23 mg/m ³ . Duration of exposure: 4 h.	LC ₅₀ : 115 mg/m ³ air (analytical).	Ciba-Geigy (1977)

<p>Equivalent or similar to OECD TG 403.</p> <p>Inhalation (aerosol).</p> <p>Deviations: only for 1 hour exposure.</p> <p>GLP compliance not reported.</p>	<p>Rat, no data on strain, 10 males per dose.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p> <p>Physical state: solid.</p>	<p>57.6 mg/l in air (with water as vehicle), 16.7 mg/l in air (with propylene glycol as vehicle).</p> <p>Duration of exposure: 1 h.</p>	<p>LC₅₀ > 56.7 mg/L air (nominal).</p> <p>Calculated LC50 on 4 hour using Haber laws and n=1: LC50 > 14.2 mg/L</p> <p>LC₅₀ > 16.7 mg/L air (nominal).</p> <p>Calculated LC50 on 4 hour using Haber laws and n=1: LC50 > 4.2 mg/L</p>	<p>International Bio-Research (1976).</p>
<p>Equivalent or similar to OECD TG 403.</p> <p>According to Federal Register August 12, 1961 et seq. FHSA.</p> <p>Deviations: no data.</p> <p>Inhalation (aerosol).</p> <p>GLP compliance not reported.</p>	<p>Rat, Wistar, 10 males per dose.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p>	<p>Concentrations: 100, 130 and 169 µl/l.</p> <p>Duration of exposure: 4 h.</p>	<p>LC₅₀: 139 µl/L air</p>	<p>Author not disseminated (1976).</p>
<p>Test type: standard acute method.</p> <p>GLP compliance not reported.</p> <p>Inhalation (aerosol).</p> <p>Duration of exposure: 1 h.</p>	<p>Rat, Sprague-Dawley, 10 males per dose.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p> <p>Aerodynamic mass median diameter (MMD): less than 5.0 µm in at least 80%.</p>	<p>For the first group the nominal aerosol concentration was 11.53 mg/litre. This aerosol delivery yielded a concentration of 5.77 mg/litre of test material.</p> <p>For the second group the nominal aerosol concentration was 15.02 mg/litre which was equivalent to 5.00 mg/litre of test material.</p>	<p>LC50 > 5.77 mg/L.</p> <p>Calculated LC50 on 4 hour using Haber laws and n=1: LC50 > 1.44 mg/L</p>	<p>Hazelton Laboratories 1976.</p>

Equivalent or similar to OECD TG 403. Deviations: only 1 hour exposure duration. Inhalation (aerosol). GLP compliance not reported.	Rat, no data on strain, 10 rats per dose, mixture of sexes.	dimethyltin dichloride. Form: crystalline. Physical state: solid.	50, 100, 200 and 300 mg/l/hour.	LC ₅₀ : 125 mg/L air (nominal). Calculated LC ₅₀ on 4 hour using Haber laws and n=1: LC₅₀=31.25 mg/L.	Wells Laboratories 1975
No methodological information. GLP compliance not reported. Inhalation (aerosol). Duration of exposure: 1 h.	Rat, no data on strain, male/female.	Monomethyltin trichloride + dimethyltin dichloride (21.5 : 78.5). Test material form: crystalline.	No information.	LC ₅₀ : 84 mg/L/hr.	Summer et al. (2003).
No methodological information available. Inhalation (vapour). GLP compliance not reported.	Species: rat. Strain: no data. Sex: no data.	dimethyltin dichloride. Form: crystalline.	No information.	LC ₅₀ : 1070 mg/L/hr.	Author not disseminated (1978e). Reference type: review article or handbook.
No methodological information available. Inhalation (aerosol). GLP compliance not reported.	Rat, male/female, no information on strain.	dimethyltin dichloride. Test material form: crystalline.	Concentrations: 1910 mg/m ³ . Duration of exposure: 1 h.	LC ₅₀ > 1910 mg/m ³ air	Author not disseminated (2006). Report date: 2006-07-23. Reference type: review article or handbook.
No methodological information available. Inhalation (vapour). GLP compliance not reported.	Species: rat. Strain: no data. Sex: no data.	dimethyltin dichloride. Test material form: crystalline.	No information.	LC ₅₀ > 1.91 mg/L/hour.	Author not disseminated (1978f). Reference type: review article or handbook.

Similar to OECD 403 with shorter duration of exposure (1h).	Rat. No information on sex or strain.	Test substance: dimethyltin dichloride. (purity not known).	Doses: 640, 1679, and 3012 mg/m ³ . 1 h exposure to aerosol.	LC ₅₀ 1632 mg/m ³ , 1.6 mg/L. Calculated LC ₅₀ on 4 hour using Haber laws and n=1: LC₅₀ = 0.4 mg/L.	Ciba-Geigy, 1977.
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Table 28b: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Case study of workers exposure. GLP compliance not reported.	mixture of half dimethyltin and half trimethyltin chloride vapour. Test material form: crystalline.	Route of administration: inhalation. Analytical verification of test atmosphere concentrations: no. Duration of exposure: 1.5 h. Remarks: over a 3 day working period.	1/6 workers died 12 days following the exposure. Symptoms preceding death: excretion of high levels of tin in the urine, respiratory depression, and coma. Autopsy results of the worker who died: massive fatty degeneration of liver cells and necrosis (shock kidneys i.e. proximal tubule degeneration). In two most severely affected surviving workers permanent neurological disabilities and non-persistent respiratory problems. All surviving workers had high tin concentrations in the urine with the highest levels occurring in the most severely affected.	Harper, C. et al. (2005).

Table 28c: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Short summary and overall relevance of the provided information on acute inhalation toxicity

Acute inhalation studies with exposure to DMTC aerosol of unknown purity for 1 and 4 hours have been performed in rats. The only studies using a 4-hour exposure resulted in LC₅₀ values of 0.115 mg/L and 0.139 mg/L. Both of these studies were equivalent or similar to OECD TG 403. The other LC₅₀ values are based on 1-hour exposures, which have been extrapolated to 4 hours according to Haber's law. Only the studies with sufficient methodological information are considered as sufficiently reliable and the resulting extrapolated 4-hour LC₅₀ values in these studies are 0.4, > 1.44 and 31.25 mg/L. The case study on workers is considered as a supporting study showing lethality in humans after an exposure to an unknown quantity of a mixture of dimethyltin and trimethyltin chloride vapour.

Comparison with the GHS criteria

The LC₅₀ values of 0.115, 0.139 and 0.4 mg/L warrant classification in Acute Toxicity Category 2 (the range of values for classification in Category 2 for dust/mist is $0.05 \leq \text{ATE} < 0.5$ mg/L), the value of > 1.44 at

most in category 4, and the highest LC₅₀ value of 31.25 mg/L warrants no classification. As three studies consistent with an OECD TG 403 warrant classification in Category 2, the Acute Toxicity Category 2 via inhalation route is proposed.

Conclusion on classification and labelling for acute inhalation toxicity

Classification with Category 2 is proposed for Acute Toxicity via inhalation route (Acute Tox. 2; H330).

8.2 Skin corrosion/irritation

Table 29a: Summary table of animal studies on skin corrosion/irritation

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
According to OECD TG 404 and Guideline EU Method B.4 (Acute Toxicity: Dermal Irritation / Corrosion). Deviations: The relative humidity of the animal room (26-53%) exceeded the range specified in the protocol (40-60%). GLP compliant.	Rabbit, New Zealand White, 4 males, 2 females.	dimethyltin dichloride. Test material form: no data. Physical state: liquid.	Applied volume or weight with unit: 0.5 mL. Duration of treatment / exposure: 4 hours. Observation period: 72 hours. Area of exposure: 1" x 1". Semi-occlusive binding. Residual test article was removed where practical using gauze moistened with distilled water.	Mean erythema score of 24, 48 and 72 hours: 4, max. score 4. Not reversible. Remarks: Same score for all rabbits at all time points. Mean edema score of 24, 48 and 72 hours: 3.94, max. score 4. Not reversible. Remarks: Same scores (4) for all rabbits at all time points, except of one score of 3 in one rabbit at 48 hours. Necrosis and blanching with severe edema on 6/6 test sites at the 1 hour scoring interval. The dermal irritation progressed to eschar on 3/6 test sites by the 72 hour scoring interval.	Rush (1993b).

<p>Type of method: in vivo.</p> <p>According to method described in 'Hazardous Substances Regulations' under the U.S. Federal hazardous Substances Labelling Act Sect. 191.11 (February 1965).</p> <p>Deviations: no data.</p> <p>GLP compliance not reported.</p>	<p>Rabbit, New Zealand White, no information on sex, 3 animals.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p>	<p>applied weight 0.5 g.</p> <p>Concentration: moistened with a 50% solution of polyethylene glycol in water.</p> <p>Duration of exposure: 24 hours.</p> <p>Area of exposure: 2.5 x 2.5 cm.</p> <p>Type of wrap: square gauze pad covered with aluminium foil secured with adhesive tape.</p> <p>No washing.</p>	<p>At 24 hours necrotic skin with deep fissuring into the subcutaneous tissue.</p> <p>No scores available as the test was ended early for humane reasons.</p>	<p>Author not disseminated (1973).</p> <p>Report date 1973-01-26.</p>
<p>Equivalent or similar to OECD TG 404 (Acute)</p> <p>GLP compliance not reported.</p>	<p>Rabbit, New Zealand, 6 males.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p>	<p>Amount applied: 0.5 g of test substance.</p> <p>Duration of treatment / exposure: 24 hours.</p> <p>Observation period: 24 and 72 hours.</p> <p>Area of exposure: 1" x 1"</p> <p>Standard patch test plasters.</p>	<p>Mean erythema score of 24 and 72 hours: 3, max. score 3.</p> <p>Reversibility: not reversible.</p> <p>Scores were the same for all animals and for intact and abraded skin.</p> <p>Mean edema score at 24 hours: 1, max. score 1.</p> <p>Reversibility: fully reversible within 72 hours.</p> <p>Primary dermal irritation index: 1.75.</p>	<p>Affiliated Medical Enterprises Inc. 1971c.</p>

Type of method: in vivo.	Rabbit, Russian, 3 males, 3 females.	dimethyltin dichloride. Form: crystalline.	Concentration: 50% in PEG. Duration of treatment / exposure: 24 hours. Observation period: 72 hours. Area of exposure: 2.5 x 2.5 cm. Type of coverage: occlusive.	Mean primary dermal irritation index (PDII) of 24 and 72 hours: 0.8. Reversibility: no data.	Author not disseminated (1973). Report date 1973-04-10.
According to Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1959) of the US Association of Food and Drug Officials. GLP compliance not reported.					

Table 29b: Summary table of human data on skin corrosion/irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 29c: Summary table of other studies relevant for skin corrosion/irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Short summary and overall relevance of the provided information on skin corrosion/irritation

In Rush (1993b), blanching and necrosis with severe oedema were observed on all dermal sites within 1 hour after a four-hour exposure time, with irritation progressing to eschar in 3 sites by termination of the observation period (at 72 hours). Under the conditions of the test, the substance would be considered to be corrosive to rabbit dermal tissue.

In the report dated 1973-01-26 (Author not disseminated (1973)), the skin over the test sites was necrotic after 24 hours, with deep fissuring into the subcutaneous tissue, and all animals were killed early for humane reasons. The exposure period of 24 hours in this study is too long for the data to be used for classification of DMTC for skin corrosion.

In Affiliated Medical Enterprises Inc. (1971c), very slight oedema was observed on all animals at both intact and abraded skin sites at 24 hours. No oedema was observed at 72 hours. The Primary Dermal Irritation Index is evaluated at 1.75. Moderate to severe erythema and eschar formation were observed on all animals, at both skin sites, at 24 and 72 hours. According to the evaluation of Draize, the substance would be considered a moderate irritant to the skin. The exposure period of 24 hours in this study is too long for the data to be used for classification of DMTC for skin corrosion.

In the report dated 1973-04-10 (Author not disseminated (1973)), the mean primary dermal irritation index (PDII) of 24 and 72 hours was 0.8 after a 24 h exposure. There was no information on the reversibility of the effect. The available information on this study does not meet the classification criteria for skin corrosion/irritation.

Comparison with the GHS criteria

Although corrosive responses were noted during the observation period of 1 hour after the four-hour exposure in Rush (1993b), classification in category 1C for skin corrosion is not justified because none of the studies provide sufficient information on whether corrosive effects occur after a shorter exposure (i.e., ≤ 3 min for subcategory 1A, or between 3 min and 1 hr for subcategory 1B). Thus, no differentiation between the subcategories can be made and classification in category 1 without a subcategory is warranted.

Conclusion on classification and labelling for skin corrosion/irritation

Classification Skin Corr. 1; H314 according to the GHS criteria is warranted.

Supplemental labelling information

In addition, based on the classification as skin corrosive and acutely toxic via inhalation it is appropriate to add the hazard statement “Corrosive to the respiratory tract” (in the EU: EUH071) in the labelling.

8.3 Serious eye damage/eye irritation

Table 30a: Summary table of animal studies on serious eye damage/eye irritation

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Equivalent or similar to OECD TG 405. GLP compliance not reported.	Rabbit, Russian, 3 males, 3 females.	dimethyltin dichloride. Form: crystalline.	0.1 g of test substance placed into left eye of rabbit with spatula. After application, the rabbits' eyelids were held open for a few seconds. Duration of treatment / exposure: 30 seconds. Observation period: 7 days.	(Results per animal in Annex I). Cornea score (mean of all time points): Score 80. Max. score 80. Reversibility: not reversible. Iris score (mean of all time points): Score 10. Max. score 10. Reversibility: not reversible. Conjunctivae score (mean of all time points): Score 12.5. Max. score 20. Reversibility: not reversible. 4/6 animals died during the 7-day observation period.	Author not disseminated (1973). Report date 1973-04-11.
Equivalent or similar to OECD TG 405. GLP compliance not reported.	Rabbit, New Zealand, 9 males.	dimethyltin dichloride. Form: crystalline.	Amount applied: 100 mg. Duration of treatment / exposure: 2 seconds. Removal of test substance: Washing. Time after start of exposure: three animals after two seconds; three animals after four seconds and three animals unwashed. Observation period: 72 hours. Scoring system: Draize (1956)	Cornea score (mean of 24, 48 and 72 hours): Score 4. Max. score 4. Reversibility: not reversible. Remarks: Score was the same for all rabbits at all time points. Iris score (mean of 24, 48 and 72 hours): Score 2. Max. score 2. Reversibility: not reversible. Remarks: scores were the same for all rabbits at all time points. Conjunctivae score (mean of 24, 48 and 72 hours): Score 3. Max. score 3. Reversibility: not reversible. Remarks: scores were the same for all rabbits at all time points. Chemosis score (mean of 24, 48 and 72 hours): Score 4. Max. score 4. Reversibility: not reversible. Remarks: scores were the same for all rabbits at all time points.	Author not disseminated (1971). Report date 1971-03-14.

Equivalent or similar to OECD TG 405. Deviations: no data. GLP compliance not reported.	Rabbit, New Zealand White, 3 males, 3 females.	dimethyltin dichloride. Form: crystalline powder. Physical state: solid.	Amount(s) applied: 100 mg. Duration of treatment / exposure: Eye held shut for 1 seconds, and then washed out after 30 seconds. Observation period: 1, 6 and 24 hours after application. The right eye served as a control. Removal of test substance: Washing with warm water.	At 1 h partial corneal destruction. Iris not visible in three animals and partly obscured in one. Marked chemosis and injection of the conjunctivae in all animals. Loss of sensation to touch over the cornea in five animals. Conjunctival mucosae of all rabbits was necrotic. At 24 hours moderate to severe necrosis of conjunctivae in all animals with severe periorbital oedema. Other effects: Instillation of the test compound caused pain. After 6 hours all animals appeared lethargic and had severe chemosis with blood stained discharge from the eyes. The cornea was completely opaque in five animals. Phlyctenar occurred on the cornea of one animal. An unpleasant odour was noted from all animals when examined at both 1 and 6 hours. Scores were not obtained as the animals were killed early for humane reasons.	Author not disseminated (1973). Report date 1973-01-26.
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Table 30b: Summary table of human data on serious eye damage/eye irritation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 30c: Summary table of other studies relevant for serious eye damage/eye irritation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Short summary and overall relevance of the provided information on serious eye damage/eye irritation

Dimethyltin dichloride is corrosive to skin and therefor deemed to cause serious eye damage. However, there are also three acute eye irritation / corrosion studies equivalent or similar to OECD TG 405. In all these studies dimethyltin dichloride caused irreversible effects on the eye within the observation period.

Comparison with the GHS criteria

A substance is classified for serious eye damage (Category 1) if the substance is corrosive to skin or if in at least one animal effects on the cornea, iris or conjunctiva are not expected to reverse or have not fully reversed within an observation period of normally 21 days or if corneal opacity ≥ 3 , and/or iritis > 1.5 calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material occur in at least 2 of 3 tested animals. The observation period in the available studies did not reach 21 days, but in the report dated 1973-01-26 (Author not disseminated (1973)), the severity of the effects suggest that the effects were irreversible. Also the cornea score 4 and iris score 2 in all tested animals meet the criteria for

serious eye damage. Dimethyltin dichloride is also corrosive to skin and therefore deemed to cause serious eye damage.

Conclusion on classification and labelling for serious eye damage/eye irritation

Classification Eye Dam. 1; H318 according to the GHS criteria is warranted.

8.4 Respiratory or skin sensitisation

Respiratory sensitisation

Table 31a: Summary table of animal studies on respiratory sensitisation

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
No data available.					

Table 31b: Summary table of human data on respiratory sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 31c: Summary table of other studies relevant for respiratory sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Short summary and overall relevance of the provided information on respiratory sensitisation

No data available.

Comparison with the GHS criteria

Not applicable because there is no data available.[]

Conclusion on classification and labelling for respiratory sensitisation

Not applicable because there is no data available.

*Skin sensitisation***Table 32a: Summary table of animal studies on skin sensitisation**

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, of duration exposure	Results	Reference
<p>Type of method: in vivo.</p> <p>Test system: Traditional sensitisation test.</p> <p>Type of study: Maurer optimisation test.</p> <p>Deviations: no information</p>	Guinea pig, Albino (type not specified), no data on sex, 10 animals per dose.	dimethyltin dichloride.	<p>Route of induction exposure: intradermal.</p> <p>Route of challenge exposure: intradermal.</p> <p>Vehicle: 1 part PEG to 7.3 parts water.</p> <p>Concentration: 0.1% solution.</p> <p>The first injection consisted of 0.05 mL, while the remaining nine were of 0.1 mL each. Ten sensitizing injections were given on alternative days during a three week period.</p> <p>Challenge controls: After an incubation period of two weeks, a final challenge dose of 0.05mL was injected.</p>	<p>From the first 10 induction exposures, the scores were as follows: Diameter: Average = 0.6, Maximum = 1.</p> <p>Colour: Average = 0.75, Maximum = 2 (in one animal only at one time point; all other scores were 1 or less).</p> <p>Height: Average = 0.72, Maximum = 1.</p> <p>From the challenge exposure after 2 weeks incubation, no score greater than 1 was seen in any animal for either diameter, colour or height.</p>	<p>Author not disseminated (1973).</p> <p>Report date 1973-04-11.</p>

Table 32b: Summary table of human data on skin sensitisation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 32c: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Short summary and overall relevance of the provided information on skin sensitisation

A Maurer optimisation test is available. The reactions elicited by the challenge dose were not substantially higher than the average of readings observed during the sensitizing period, and therefore the substance is not considered to have produced sensitization in the available test.

Comparison with the GHS criteria

Test methods for skin sensitisation are described in OECD TG 406, but other methods may be used provided that they are well-validated and scientific justification is given. The available Maurer optimisation test was negative.

Conclusion on classification and labelling for skin sensitisation

No classification is proposed for skin sensitisation as there are no positive studies available.

8.5 Germ cell mutagenicity

Table 33a: Summary table of mutagenicity/genotoxicity tests in vitro

Method, test guideline, and deviation(s) if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Bacterial reverse mutation assay OECD TG 472 No deviations. GLP-compliant.	Dimethyltin dichloride: Methyltin trichloride (72:28% mixture)	E. Coli WP2 uvr A; with and without metabolic activation Test concentrations: 10, 50, 100, 500, 1000, 5000 µg/plate	Genotoxicity: negative. Cytotoxicity: yes at 5000 µg/plate	Author not disseminated (1990). Report date 1990-07-24.
Chromosome aberration OECD TG 473 No deviations. GLP-compliant.	Mixes of methyltin compounds	Human peripheral lymphocytes. Test concentrations: Preliminary: 8, 40, 200, 1000, and 5000 µg/mL. Definitive experiment with metabolic activation: 10, 20, 40, 80, 160 µg/mL. Definitive experiment without metabolic activation: 2, 4, 8, 16, 32 µg/mL. Positive control, definitive experiment with metabolic activation: 11, 13, 15 µg/mL. Positive control, definitive experiment without metabolic activation: 150, 175, 200 µg/mL.	Genotoxicity: negative Cytotoxicity: yes, at ≥ 16 µg/mL	Author not disseminated (1990). Report date 1990-10-25.
Mammalian gene mutation assay OECD TG 476 No deviations. GLP-compliant.	Mixes of methyltin compounds	Chinese hamster ovary (CHO); with and without metabolic activation. Test concentrations: 0, 49, 61, 77, 96, 120 and 200 µg/mL (without metabolic activation); 0, 61.4, 76.8, 96, 120 and 150 µg/mL (with metabolic activation, replicate 1); 0, 31.4, 39.3, 49.2, 61.4, 76.8 and 96 µg/mL (with metabolic activation, replicate 2).	Genotoxicity: negative Cytotoxicity: yes	Author not disseminated (1990). Report date 1990-08-20.

<p>Bacterial reverse mutation assay</p> <p>OECD TG 471</p> <p>No deviations.</p> <p>GLP-compliant.</p>	<p>Dimethyltin dichloride:Methyltin trichloride (72:28% mixture)</p>	<p>Species/strain: <i>S. typhimurium</i> TA 1538; with and without metabolic activation</p> <p>Test concentrations: Range finding assay: 10, 50, 100, 500, 1000, and 5000 µg/plate.</p> <p>First mutagenicity assay: 10, 50, 100, 500, 1000, and 5000 µg/plate.</p> <p>Second mutagenicity assay: 5, 10, 50, 100, 500, and 1000 µg/plate.</p>	<p>Genotoxicity: negative Cytotoxicity: yes</p>	<p>Author not disseminated (1990).</p> <p>Report date 1990-07-18.</p>
<p>Spindle inhibition.</p> <p>No data on guideline or GLP-compliance.</p>	<p>Dimethyltin dichloride</p>	<p>Chinese hamster cells, without metabolic activation</p>	<p>Genotoxicity: no data Cytotoxicity: yes</p>	<p>Harper et al., 2005 (secondary source)</p>
<p>Three different genotoxicity tests.</p> <p>No data on guideline or GLP-compliance.</p>	<p>Dimethyltin dichloride</p>	<p>Three types of genotoxicity tests reported: 1) Induced mutation frequency (IMF) with <i>Salmonella typhimurium</i> TA100. 2) Rec assay with <i>Bacillus subtilis</i> H17 and M45 3) SOS chromotest with <i>E. coli</i> PQ37</p>	<p>Genotoxicity: negative Cytotoxicity: no data</p>	<p>Author not disseminated (1992).</p>
<p>Genotoxicity of organotin compounds in SOS chromotest and rec-assay / DNA damage and repair.</p> <p>No guideline followed.</p> <p>GLP compliance not reported.</p>	<p>Dimethyltin dichloride</p>	<p><i>E. coli</i>, other: PQ37; without metabolic activation</p> <p><i>Bacillus subtilis</i> (H17 Rec + and M45 Rec- strains); without metabolic activation</p>	<p><i>E. coli</i>: Genotoxicity: negative Cytotoxicity: no data</p> <p><i>Bacillus subtilis</i>: Genotoxicity: positive Cytotoxicity: no data</p>	<p>Hamasaki et al., 1992</p>

Study on induction of micronuclei, chromosome aberrations and sister chromatid exchanges. GLP compliance not reported.	Dimethyltin dichloride	Chinese hamster Ovary (CHO) cells without metabolic activation. Test concentrations: 0.5 µM - 1.0mM.	Positive at a cytotoxic concentration of 1mM (chromosomal aberrations and sister chromatid exchanges).	Dopp et al., 2007.
Cytogenicity No guideline followed. GLP compliance not reported.	Dimethyltin dichloride	Human peripheral lymphocytes	Genotoxicity: negative Cytotoxicity: no data	Jensen et al., 1991
Bacterial reverse mutation assay. Equivalent or similar to OECD 471. With deviations. GLP compliance not reported.	Dimethyltin dichloride	S. typhimurium TA 98 S. typhimurium TA 100	S. Typhimurium TA 98: Genotoxicity: negative Cytotoxicity: no data S. typhimurium TA 100: Genotoxicity: positive in the presence of cytotoxicity Cytotoxicity: no data	Hamasaki et al., 1993

Table 33b: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, test guideline, and deviation(s) if any	Test substance	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
<p>Unscheduled DNA synthesis (UDS).</p> <p>Equivalent of similar to OECD TG 486.</p> <p>With deviations.</p> <p>GLP-compliant.</p>	Mixture of methyltin chloride compounds	<p>Fischer 344 rats, males.</p> <p>Oral gavage.</p> <p>Doses:</p> <p>Actual ingested:</p> <p>First range finding study: 25, 50, 100, 200 and 400 mg/kg.</p> <p>Second range finding study: 600 and 800 mg/kg.</p> <p>First definitive UDS assay: 90, 175 and 350 mg/kg.</p> <p>Second definitive UDS assay: 50, 110 and 225 mg/kg.</p> <p>First range finding assay: 3 males at each dose.</p> <p>Second range finding assay: 3 males at each dose.</p> <p>First definitive UDS assay: 3 males at each dose, except negative control with 0.</p> <p>First definitive UDS assay: 3 males at each dose, except highest dosage group with 4 and positive control with 0.</p> <p>Second definitive UDS assay: 3 males at each dose, except negative control with 0.</p> <p>Second definitive UDS assay: 3 males at each dose, except highest dosage group with 4 and positive control with 0.</p>	<p>Genotoxicity: negative</p> <p>Toxicity: yes</p>	<p>Author not disseminated (1993).</p> <p>Report date 1993-07-11.</p>
<p>Chromosome aberration / micronucleus assay.</p> <p>No data on method or GLP-compliance.</p>	Monomethyltin trichloride and dimethyltin dichloride	<p>Swiss Webster, male/female, oral gavage.</p> <p>Doses:</p> <p>Actual ingested: 100, 200, 400 mg/kg bw in water, i.g.</p> <p>15 animals per sex and dose.</p>	<p>Genotoxicity: negative</p> <p>Toxicity: no data</p>	<p>Summer et al., 2003</p>

Table 33c: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data.				

Short summary and overall relevance of the provided information on germ cell mutagenicity

There are ten recorded mutagenicity or genotoxicity studies in vitro. All the Ames tests were negative. There was one positive chromosomal aberration test in human lymphocytes that shows positive results in the presence of metabolic activation. There is also one REC bacterial assay showing positive results. In vivo there is one UDS study (rat) and one poorly described MN study (mice) - both of which are negative.

Comparison with the GHS criteria

The GHS criteria requires positive results in vivo. No such evidence are seen although the data base is limited. The positive results seen in vitro is not of high concern, but it cannot be completely excluded that the substance may induce clastogenicity. However, in the in vivo clastogenicity study no evidence of effects were seen, although it should be noted that the study was not regarded as reliable.

Conclusion on classification and labelling for germ cell mutagenicity

No classification for germ cell mutagenicity is warranted as no positive animal or human data are available.

8.6 Carcinogenicity**Table 34a: Summary table of animal studies on carcinogenicity**

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
No data on method or GLP-compliance.	Rat, male/female, 20 animals	dimethyltin bis(2-ethylhexylthioglycolate) + monomethyltin tris(2-ethylhexylthioglycolate)	Oral (feed), two years, 100 ppm	No malignant tumours.	Summer et al., 2003

Table 34b: Summary table of human data on carcinogenicity

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data.				

Table 34c: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data.				

Table 34d: Are the following factors taken into consideration in the hazard assessment (yes/no)?

Reference	Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
No applicable.									

Short summary and overall relevance of the provided information on carcinogenicity

One negative study with low reliability is available for the substance. The data is scarce and no proper evaluation can be done.

Comparison with the GHS criteria

There is no data supporting classification for this hazard class.

Conclusion on classification and labelling for carcinogenicity

No classification for carcinogenicity is warranted as no positive animal or human data are available.

8.7 Reproductive toxicity***Adverse effects on sexual function and fertility***

No data on effects on fertility are available.

Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

No data on effects on fertility are available.

Comparison with the GHS criteria

No classification is proposed for fertility as no data on effects on fertility are available.

*Adverse effects on development of the offspring***Table 35a: Summary table of animal studies on adverse effects on development of the offspring**

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
<p>US EPA, Developmental neurotoxicity study, Health effects TG, OPPTS 870.6300, EPA 712-C-98-239.</p> <p>No data on deviations or GLP compliance.</p> <p>Method: behavioural essays from PND 11 into adulthood and analysis of specific brain regions for apoptotic cell death and neuropathy.</p>	<p>SD rats.</p> <p>Experiment 1: 120 female rats (30 per dose)</p> <p>Experiment 2: 87 females (21 controls, 22 per dose)</p>	Dimethyl tin (DMT)	<p>Doses: 0, 3, 15 and 74 mg/L via drinking water.</p> <p>Exposure period:</p> <p>Experiment 1: 14 days pre-mating – PND 21</p> <p>Experiment 2: GD 6 – PND 21</p>	<p><u>Experiment 1:</u></p> <p>Maternal toxicity: ↓weight gain at highest dose.</p> <p>Fertility: low overall pregnancy rate (39%) in study but not treatment-related.</p> <p>Developmental toxicity: ↓brain weight (74 mg/L), mild vacuolation in the brain (all doses), slower learning in adult offspring in water maze (7 weeks old) maze (15 mg/L), no treatment-related difference in the incidence of non-learners in runway (PND 11).</p> <p><u>Experiment 2:</u></p> <p>Maternal toxicity: ↓body weight (lactation) as compared to controls (74 mg/L).</p> <p>Fertility: no effects seen.</p> <p>Developmental toxicity: ↓birth weight, ↓preweaning growth, ↓brain weight (only males, 74 mg/L), dose-dependent increase in no of animals that failed to learn to negotiate the runway (PND 11, n.s.) and in adult offspring (12 weeks old) water maze (15 mg/L)</p>	Ehman <i>et al.</i> , 2007

Similar to OECD TG 414; with deviations (exposure during GD 7-17, groups size below recommended in the TG); no data on GLP compliance	Wistar rats; Study I: 5 groups of 10 rats in each Study II: 8 groups of 8-11 rats in each.	Dimethyltin dichloride; purity \geq 99%	Study I: 0, 5, 10, 15 and 20 mg/kg on GD 7-17; Study II: 0, 20 and 40 mg/kg On GD 7-9, 10-12, 13-15 or 16-17; controls were given vehicle on GD 10-12	<p><u>Study I:</u></p> <p><i>20 mg/kg bw/d:</i></p> <ul style="list-style-type: none"> - maternal toxicity (vaginal bleeding, tremors, convulsions, ataxia, other clinical signs of toxicity, severe thymus atrophy) generally appearing after GD15. - death of 2/10 pregnant rats, treatment-related - total resorption in 1/8 living pregnant rats (the dam had all clinical signs of toxicity, see above). - cleft palate in 21 foetuses (22%) in 5/7 pregnant rats with living foetuses. <p><i>15 and 20 mg/kg/day:</i></p> <ul style="list-style-type: none"> - significant \downarrow mean body weight in living foetuses of both sexes, dose-dependent manner at. <p><i>15 mg/kg bw/day:</i></p> <ul style="list-style-type: none"> - two foetuses with omphalocele from one dam <p><u>Study II:</u></p> <p><i>40 mg/kg/day:</i></p> <ul style="list-style-type: none"> - slight maternal toxicity (reductions of the adjusted body weight gain and thymus weight) <p><i>All dose levels:</i></p> <ul style="list-style-type: none"> - no significant increase in the incidence of external, skeletal and visceral malformations at either dose in any group - no cleft palates - fetal body weight unaffected 	Noda, 2001
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<p>Teratogenicity study.</p> <p>Limited data on methodology and results.</p> <p>GLP compliance not reported.</p>	<p>Wistar rats, 10 pregnant females per sex and dose</p>	<p>Dimethyltin dichloride</p>	<p>0, 5, 10, 15 and 20 mg/kg bw; GD 7-17</p> <p>To clarify the teratogenic potential of DMTC, the compound was given at 20 or 40 mg/kg for 2 or 3 consecutive days at 4 different periods of gestation (GD 7-9, 10-12, 13-15 or 16-17).</p>	<p>Dosing on GD 7-17: Vaginal bleeding, convulsion, ↓ body weight gain and dead dams (2/10) at 20 mg/kg in the late stage of gestation. On GD 20 the incidence of fetuses with cleft plate was sign. ↑ at 20 mg/kg bw.</p> <p>Dosing on GD 7-9, 10-12, 13-15 or 16-17 (20, 40 mg/kg): No maternal toxicity and no fetus with cleft plate was observed in any DMTC-treated group.</p>	<p>Noda & Morita, 1994 (study source was brief summary).</p>
<p>Sub-acute and developmental toxicity study.</p> <p>Non-guideline.</p> <p>GLP compliance not reported.</p>	<p>SD rats, male/female, adult: 24 rats per sex and dose, developmental: 8-9 dams per dose</p>	<p>Dimethyltin dichloride</p>	<p>20 and 40 mg/L (in water)</p> <p>Actual ingested: Male: 0, 1.7 and 3.4 mg/kg bw; Female: 2.4 and 4.6 mg/kg bw; Females from parturition until weaning: 3.6 and 6.9 mg/kg bw</p>	<p>No effects on body weight or body weight gain, humoral immunity examinations, specific cell-mediated immunity or non-specific cell-mediated immunity.</p> <p>Developmental effects:</p> <ul style="list-style-type: none"> - at 40 mg/L, body weights of both male and female offspring was about 10% greater relative to controls on PND7-PND37 - body weights did not vary by dose for either sex when terminal weights were collected at PND44, 58, or 77 - pup mortality was limited to the 40 mg DMTC/L group; 2 pups from 1 litter died between PND14 and 17 and one pup from a second litter died on PND14. 	<p>DeWitt <i>et al.</i>, 2007</p>

<p><u>Step 1:</u> Study to determine whether DMTC was absorbed by the dam and transferred across the placenta to foetal blood and brain tissue.</p> <p><u>Step 2:</u> Cross-fostering study</p> <p><u>Step 3:</u> 14C-DMTC tracer study</p>	SD rats, female	Dimethyltin dichloride (DMTC)	<p><u>Step 1:</u> 3 groups. Group 1: 40 mg/L DMTC in drinking water (n = 13). Group 2: 40 mg/L tin as stannous chloride (n = 13) Group 3: distilled water/control group (n = 12)</p> <p><u>Step 2:</u> Group 1: 40 mg/L DMTC in drinking water Group 2: untreated control</p> <p><u>Step 3:</u> 14C-DMTC tracer administered by intubation to pregnant dams (GD 19)</p>	<p><u>Step 1:</u> - DMTC was absorbed by the rat dam, transferred across the placenta to the foetus and into the brain of the prenatal animal</p> <p><u>Step 2:</u> - significantly higher tin levels in pups from DMTC-exposed dams - highest levels of tin in blood at birth in gestationally exposed pups - DMTC mainly transferred to the pups during the gestation. - tin levels in brains of gestationally exposed pups highest at birth and different from controls - rapid clearance of the tin from the blood and brain in pups - ↓ DMTC concentration in pup brain and pup blood during post-natal period, indicating relatively unimpeded brain-to-blood transfer</p> <p><u>Step 3:</u> - DMTC was absorbed in the gastrointestinal tract of the dam and transferred across the placenta to fetal blood and brain tissue - majority of the tin was transferred from the pups prenatally, rather than during lactation</p>	Noland <i>et al.</i> , 1983
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Table 35b: Summary table of human data on adverse effects on development of the offspring

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 35c: Summary table of other studies relevant for adverse effects on development of the offspring

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
In vitro examination of neurotoxic potential. Non-guideline. GLP compliance not reported.	Dimethyltin dichloride	7, 10, 15, 20 and 50 µM DMT to primed PC12 cells, observation of neurite outgrowth and cell viability/cytotoxicity	<ul style="list-style-type: none"> - addition of DMT to primed PC12 cells inhibited neurite outgrowth and ↓ cell viability in a concentration dependent manner - total neurite outgrowth sign. inhibited by 7.0 µM (43%↓), 10 µM (52%↓), 20 µM (64%↓), and 50 µM (93%↓) - no sign. effect at < 7.0 µM - the absence of NGF decreased neurite outgrowth - neurite branching prevented in a concentration-dependent manner; sign. inhibition at 7.0 µM (46%↓), 10 µM (50%↓), 15 µM (58%↓), 20 µM (62%↓), and 50 µM (93%↓) - removal of NGF ↓ the number of branch points - segment length sign. ↓ by 20 µM (28%↓) and 50 µM (32%↓) DMT - no sign. effects detected on segment length at concentrations of ≤ 15 µM - % of cells staining positively for trypan blue ↑ from 7.0% in control cells to 20%, 19%, 24%, and 47% in the presence of 10, 15, 20, and 50 µM DMT, respectively 	Jenkins <i>et al.</i> , 2004

Short summary and overall relevance of the provided information on adverse effects on development of the offspring

Summary and discussion of reproductive toxicity

In the **Ehman (2007)** study, developmental neurotoxicity of DMTC in drinking water was evaluated in two experiments. The two experiments utilized the same doses and the same route of exposure and vehicle, but they utilized different dosing regimens and therefore experiments 1 and 2 are not replicates. In the first experiment, DMTC toxicity was expressed as depressed maternal weight gain (74 mg/l), and in the offspring as decreased brain weight (74 mg/l), mild vacuolation in the brain of adult offspring (all doses), and slower learning in the water maze (15 mg/L).

In the second experiment, the high concentration lead to depressed maternal weight as compared to controls at the high dose, decreased offspring birth weight and preweaning growth as compared to controls, and as decreased brain weight (males only high dose). Learning deficits were observed in the runway at postnatal day 11 (15, 74 mg/L, no statistical significance) and again in the adult offspring in the water maze (15 mg/L).

The effect observed in the runway testing was identified only in experiment 2 but it may be due to the lower number of tested pups in the first experiment. In the second experiment, learning was not observed during

any reinforced-trial blocks in the 15 mg/L group, but learning was achieved at the last set of trials in the 74 mg/L group. In addition, only the 15 mg/L dose-group showed a decreased latency during the non-reinforced (extinction phase), which seems to be an aberration. The absence of dose-response therefore questions the significance of this finding. It is however noted that the trial did not follow the OECD TG 426 on the neurotoxicity for the development, the test was performed only at PND 11 although it also has to be done at PND 25 and at adult age. Moreover, it is not known whether each tested pup comes from different litters as it is recommended in the OECD TG 426. It is therefore difficult to conclude on the presence or the absence of a neurotoxic effect based on this result.

In the Morris water maze, the 15 mg/L group shows longer latencies to reach the platform than the 74 mg/L group in the first week for the experiment 1 and in the second week for the experiment 2. Thus, the effect in the 15 mg/L group seems to be reproducible but it is not observed at the higher dose. In the both experiments, the 15 mg/L group spent more time in the outer zone than in the middle one. According to the OECD TG 426, 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 were only males rats, and a too small number of pups was tested (n= 11 control, 7 at 3 mg/L, 9 at 15 mg/L, and 11 at 74 mg/L). It may explain why the effect was not detected in experiment 1 at high dose. In experiment 2, the adequate number of pups was tested but it is not known whether they come from different litters.

Decreased brain weight was observed at 74 mg/L 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 mg/L and 20% of PND 22 rats at 74 mg/L. Evaluations of the lower dose groups (at 3 and 15 mg/L) showed similar vacuolation.

In the first study of **Noda, 2001**, severe maternal toxicity occurred at the high dose of 20 mg/kg bw/d. These clinical signs of toxicity are vaginal bleeding, tremors and convulsions [30%], ataxia and other clinical signs of toxicity (severe thymus atrophy) [100%] and they generally appear after the 15th day of gestation. Oral administration of DMTC at 20 mg/kg bw/d 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.

Administration of DMTC at 20 mg/kg bw/d caused cleft palate in a high incidence i.e. in 21 foetuses (22%) in 5 out of 7 pregnant dams with living foetuses. Furthermore, cleft palate is a rare and serious malformation in the rat, which generally cannot be considered to be a secondary non-specific consequence to maternal toxicity. However, according to the GHS criteria (GHS 3.7.2.4.4), maternal mortality greater than 10% is considered excessive and the data for that dose level should not normally be considered for further evaluation. In this case, the evidence on these serious malformations was not fully dismissed despite of $\geq 10\%$ maternal mortality, i.e. excessive maternal toxicity. There were also two foetuses with omphalocele from one dam exposed to DMTC at 15 mg/kg bw/day in study 1 and this effect was not associated with maternal death or severe maternal toxicity. However, no omphaloceles were detected at the high dose and the biological relevance of such low incidence and in one litter only is questionable. Moreover, mean body weight in living foetuses 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.

The reliability of **Noda and Morita (1994)** cannot be assessed due to limited details on methodology and results. It cannot be excluded that Noda and Morita (1994) and Noda (2001) refer to the same study.

The study by **Dewitt et al. (2007)** did not provide evidence of an impaired immune function in Sprague–Dawley rats exposed to dimethyltin dichloride (DMTC) during development.

The study of **Noland et al. (1983)** is included in the section on toxicokinetics in Annex I (study 2). This study is relevant for the developmental neurotoxicity analysis of dimethyltin dichloride (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.

In **Jenkins et al. (2004)** neurite outgrowth and cytotoxicity were examined in vitro using a split-plot design with subsampling. DMT inhibited neurite outgrowth and decreased cell viability in a concentration dependent manner, prevented neurite branching in a concentration-dependent manner, and decreased segment length.

Overall, DMTC induced a high incidence of cleft palates in rat foetuses at 20 mg/kg/day, a dose level which also caused severe maternal toxicity (20% mortality) (Noda, 2001, first study). There were also two foetuses with omphalocele from one dam exposed to DMTC at 15 mg/kg bw/day in study 1 and this effect was not associated with maternal death or severe maternal toxicity. No significant increase in the incidence of cleft palates or other external, skeletal or visceral malformations were observed in a second study (Noda, 2001, 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) study, which may be due to lower dosage (high dose between 4 and 12 mg/kg). In the study by Noda and Morita (1994) cleft palates were observed together with severe maternal toxicity. The reliability of the study cannot be assessed due to limited details on methodology and results, and it cannot be excluded that Noda and Morita (1994) and Noda (2001) refer to the same study. Altogether, the evidence on the serious malformations, i.e. cleft palates, was not fully dismissed despite of $\geq 10\%$ maternal mortality, i.e. excessive maternal toxicity. The biological relevance of two incidences of omphalocele from one dam at 15 mg/kg bw/day only in study 1 is questionable.

DMTC induced a decrease in the body weight of living foetuses at 15 and 20 mg/kg as compared to controls (Noda, 2001, first study). Also maternal body weight gain was significantly reduced in pregnant rats treated with 15 or 20 mg/kg/day of DMTC and the maternal body weight on GD 20 was also significantly lower in the high dose group as compared to the control. These foetal 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 pup 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.

DMTC showed developmental neurotoxic potential in the form of learning deficits consistently at the mid dose in water maze test in Ehman (2007). The maternal toxicity consisted on decreased maternal body weight gain only at the top dose (throughout the exposure in the first experiment and from lactation in the second experiment). There was also a dose-dependent but not statistically significant increase in the number of pups that failed to learn to negotiate the runway in one of two experiments in Ehman (2007). Developmental toxicity was also expressed in the offspring as a decreased brain weight (top dose only) and mild vacuolation in the brain (all doses, first experiment only) of adult offspring. DMTC inhibited neurite outgrowth and decreased cell viability in a concentration dependent manner, prevented neurite branching in a concentration-dependent manner, and decreased segment length in Jenkins et al. (2004) supporting the developmental neurotoxic potential. However, the absence of reproducibility and statistical significance of the effects observed in the runaway and the absence of effect at the top dose in water maze tests and the fact that the studies were not consistent with guideline requirements does not provide clear evidence of an adverse effect on development.

Comparison with GHS criteria

The GHS criteria for classification in Repr. 2 are as follows. Substances shall be 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, 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, and where the evidence is not sufficiently convincing to place the substance in Category 1. For instance, deficiencies in the study may make the quality of evidence less convincing, and in view of this, Category 2 would be the more appropriate classification.

DMTC induced cleft palates on the fetuses at 20 mg/kg/day, in the presence of severe maternal toxicity (20% mortality) at this high dose level (Noda 2001, Experiment 1). Also in the study by Noda and Morita (1994), cleft palates were observed together with severe maternal toxicity, but the reliability of the study cannot be assessed due to limited details on methodology and results, and it cannot be excluded that Noda and Morita (1994) and Noda (2001) refer to the same study. Cleft palates are rare and serious malformations in the rat and are not considered as secondary to maternal toxicity. Additionally, the evidence on the serious malformations, i.e. cleft palates, was not fully dismissed despite of $\geq 10\%$ maternal mortality, i.e. excessive maternal toxicity. The biological relevance of two incidences of omphalocele from one dam at 15 mg/kg bw/day only in study 1 is questionable. 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 experiments of Noda 2001, the cleft palates occurring at a high dose which caused excessive maternal toxicity (20% mortality) (and the low incidence of omphaloceles at the second highest dose only), the evidence is not considered sufficient to place the substance in category 1B.

DMTC induced a dose-dependent reduction in mean body weight of living fetuses (both sexes), that was significant at 15 and 20 mg/kg/day (respectively $3.5\text{g} \pm 0.20$, $3.4\text{g} \pm 0.22$, $3.2\text{g} \pm 0.25$, $2.9\text{g} \pm 0.16$, and $2.2\text{g} \pm 0.46$ in males and $3.3\text{g} \pm 0.16$, $3.2\text{g} \pm 0.20$, $3.2\text{g} \pm 0.27$, $2.8\text{g} \pm 0.15$, and $2.1\text{g} \pm 0.41$ in females of the control, 5, 10, 15 and 20 mg/kg) (Noda 2001, Experiment 1). 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 with 15 or 20 mg/kg/day of DMTC. Maternal body weight on GD 20 were also significantly lower in the high dose group (respectively $333\text{g} \pm 26.7$, $334\text{g} \pm 21.9$, $321\text{g} \pm 21.0$, $315\text{g} \pm 9.2$, and $252\text{g} \pm 41.1^{**}$ in the control, 5, 10, 15 and 20 mg/kg groups, respectively). 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 and the effect on foetal body weight is considered to be a secondary non-specific consequence of maternal toxicity not warranting classification.

DMTC showed developmental neurotoxic potential in the form of learning deficits consistently at the mid dose in water maze test in Ehman (2007). The maternal toxicity consisted on decreased maternal body weight gain only at the top dose (throughout the exposure in the first experiment and from lactation in the second experiment). There was also a dose-dependent but not statistically significant increase in the number of pups that failed to learn to negotiate the runway in one of two experiments in Ehman (2007). Developmental toxicity was also expressed in the offspring as a decreased brain weight (top dose only) and mild vacuolation in the brain (all doses, first experiment only) of adult offspring. DMTC inhibited neurite outgrowth and decreased cell viability in a concentration dependent manner, prevented neurite branching in a concentration-dependent manner, and decreased segment length in Jenkins et al. (2004) supporting the developmental neurotoxic potential. However, the absence of reproducibility and statistical significance of the effects observed in the runaway and the absence of effect at the top dose in water maze tests and the fact

that the studies were not consistent with guideline requirements does not provide clear evidence of an adverse effect on development.

Altogether, considering the findings on rare and serious malformations (cleft palates) and learning deficits, but taking into account the absence of reproducibility of cleft palates in both experiments of Noda (2001) and the high dose causing excessive maternal toxicity (20 % mortality) at which cleft palates were reported, the low incidence of omphaloceles at the second highest dose only, the absence of reproducibility and statistical significance of the learning effects observed in the runaway test, the absence of learning effect at the top dose in water maze tests and the fact that the studies were not consistent with guideline requirements, do provide some evidence of an adverse effect on development supporting classification in Category 2 for developmental toxicity.

Conclusions on classification and labelling

Classification as **Repr. 2; H361** (for developmental toxicity) is proposed (i.e. with H361: “Suspected of damaging the unborn child). Data are available only by oral route and the route of exposure cannot be specified in the hazard statement.

Adverse effects on or via lactation

Table 36a: Summary table of animal studies on effects on or via lactation

Method, test guideline, and deviation(s) if any	Species, strain, sex, no/group	Test substance	Dose levels, duration of exposure	Results	Reference
No data available.					

Table 36b: Summary table of human data on effects on or via lactation

Type of data/report	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Table 36c: Summary table of other studies relevant for effects on or via lactation

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Short summary and overall relevance of the provided information on effects on or via lactation

No data available.

Comparison with the GHS criteria

No data available.

Conclusion on classification and labelling for reproductive toxicity

Classification as **Repr. 2; H361** (for developmental toxicity) is proposed (i.e. with H361: “Suspected of damaging the unborn child). Data are available only by oral route and the route of exposure cannot be specified in the hazard statement.

8.8 Specific target organ toxicity-single exposure (STOT SE)

Table 37a: Summary table of animal studies relevant for STOT SE (oral route)

Method, test guideline, and deviation(s) if any, species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Effects	Reference
OECD TG 401 GLP compliant Oral gavage Rat; Sprague-Dawley, 5 animals/sex and dose	dimethyltin dichloride (85% dimethyltin dichloride: 15% monomethyltin dichloride). The [Di/Mono] Methyltin Chlorides Solution is a 50% organotin solution in an unspecified solvent. Main study: 200, 300 and 500 mg/kg bw.	Mortality: Males: 0/5, 1/5 and 2/5 at 200, 300 and 500 mg/kg bw, respectively. Females: 0/5, 3/5 and 4/5 at 200, 300 and 500 mg/kg bw, respectively. Clinical abnormalities: decreased activity, salivation, rough haircoat, mucoid/soft stools, fecal/urine stain, hunched posture, dehydration, dark material around the facial area, decreased defecation and food consumption, gasping and rales. Body weight gain in the majority of surviving animals. Dark red medulla of the kidney, dark red foci on the thymus, mottled lungs, abnormal coloured mucoid/fluid contents and eroded area(s), reddened mucosa and dark red linear striations on the stomach in animals that died.	Elf Atochem NA (1993).
OECD TG 401 GLP compliance not reported. Oral gavage Rat; Sprague-Dawley, 5 animals/per sex and dose	dimethyltin dichloride 98.7%. 0, 100, 150, 200, 250 and 300 mg/kg bw	LD ₅₀ (male): 190 mg/kg bw. LD ₅₀ (female): 160 mg/kg bw. Sedation, piloerection and ptosis on day 0. Piloerection, decreased locomotor activity, ataxia, ptosis, oily ventral surface, reddish nasal discharge and shallow respiration on days 1-4/death. Most animals surviving the observation period exhibited normal behaviour and appearance on days 5 through 14. Hemorrhagic lungs, and small and large intestines filled with yellow gelatinous material in animals that died. No outstanding gross pathological organ changes in surviving animals. Controls: abnormal defecation, piloerection, decreased locomotor activity and oily ventral surface on day 1 and normal behaviour and appearance on days 2-14. No outstanding gross pathological organ changes.	Author not disseminated (1979). Report date: 1979-03-20.

Non-guideline. GLP compliance not reported. Oral gavage. Rat, Sprague-Dawley, 5 animals/sex and dose.	5% dimethyltin dichloride in corn oil. 0.215, 0.464, 1.00, 2.15 and 4.64 mL/kg bw + controls.	LD ₅₀ (male/female): 1.7 mL/kg bw. 0.215 mL/kg bw: No deaths. 0.464 mL/kg bw: No deaths. 1.00 mL/kg bw: 1/5 females died. No male deaths. 2.15 mL/kg bw: 4/5 males and 3/5 females died. 4.64 mL/kg bw: All rats died. Males: 0.215 and 0.464 mL/kg bw: no effects. 1.00 mL/kg bw: depression, diarrhoea, bloody-appearing stains around the muzzle, urine stains, and piloerection. 2.15 mL/kg bw: depression, yellow mucoid diarrhoea, diarrhoea, bloody-appearing stains around the muzzle, urine stains, excessive nasal or salivation stains, piloerection, bloated appearance, emaciation, and mortality. 4.64 mL/kg bw: depression, depressed righting and placement reflexes, rapid respiration, shortness of breath, diarrhoea, bloody-appearing stains around the nose, comatose appearance, piloerection, hunched posture, emaciation, and shaking prior to complete mortality. Females: 0.215 and 0.464 mL/kg bw: no effects. 1.00 mL/kg bw: diarrhoea, depression, piloerection, urine stains and mortality. 2.15 mL/kg: depression, diarrhoea, piloerection, and mortality. 4.64 mL/kg bw: depression, diarrhoea, shortness of breath, and comatose appearance prior to complete mortality. Body weight gain in all animals. Gross pathology: Males: 2.15 mL/kg bw: Autolysis in 3/5 rats. 4.64 mL/kg bw: congested kidneys, stomach distended with gas, irritated intestines and mottled liver in 1/5 rats. Autolysis in 5/5 rats. Females: Autolysis in 1/5, 3/5 and 5/5 animals at 1.00, 2.15 and 4.64 mL/kg bw, respectively. 4.64 mL/kg bw: congested lungs, white lesions on lungs, congested kidneys, irritated intestines and intestines distended with gas in 1/5 rats.	Author not disseminated (1978). Report date: 1978-05-25.
No guideline stated. GLP compliance not reported. Oral gavage. Rat, no strain data 6 males/dose.	dimethyltin dichloride; purity not given. 100, 200, 400, 800 and 1600 mg/kg bw. No data on control animals.	LD ₅₀ (male): 141.4 mg/kg bw. Clinical signs: Depression, convulsions, death. Gross pathology: No significant findings.	Affiliated Medical Enterprises (1971a).

<p>No guideline stated.</p> <p>GLP compliance not reported (pre-GLP implementation).</p> <p>Oral gavage.</p> <p>Rat, Wistar, 10 males per dose.</p>	<p>dimethyltin dichloride.</p> <p>Test material form: crystalline.</p> <p>5% solution.</p> <p>48, 57, 69, 83, 100, 120 and 144 mg/kg bw</p> <p>No data on control animals.</p>	<p>LD₅₀ (male): 73.86 mg/kg bw.</p> <p>Preliminary study (if fixed dose study):</p> <p>100 mg/kg bw: 2/3 dead.</p> <p>200 mg/kg bw: 3/3 dead.</p> <p>Mortality:</p> <p>48 mg/kg bw: 1/10 dead.</p> <p>57 mg/kg bw: 2/10 dead.</p> <p>69 mg/kg bw: 5/10 dead.</p> <p>83 mg/kg bw: 6/10 dead.</p> <p>100 mg/kg bw: 8/10 dead.</p> <p>120 mg/kg bw: 8/10 dead.</p> <p>144 mg/kg bw: 10/10 dead.</p> <p>Clinical signs:</p> <p>Lassitude, hypokinesia, lack of appetite, thirstiness, unkempt fur, general weakness, and sometimes a tendency to lay on their sides and death. Recovery in the surviving rats within 4-6 days.</p> <p>No gross pathological signs.</p>	<p>Klimmer, O.R. 1971.</p>
<p>No methodological information.</p> <p>GLP compliance not reported.</p> <p>Oral</p> <p>Rat, no further information given.</p>	<p>dimethyltin dichloride; purity not given.</p>	<p>LD₅₀: 74 mg/kg bw.</p> <p>No available data on other effects.</p>	<p>Hoch (2001).</p>
<p>No guideline followed, GLP compliance not reported.</p> <p>Acute oral test, with lower dosage groups repeated on 4th day. Animals observed for 10 days.</p> <p>Limited methodological information.</p> <p>Rat, no data on strain, two females per dose.</p>	<p>dimethyltin dichloride.</p> <p>Test material form: crystalline.</p> <p>Doses: 40, 80 and 160 mg/kg bw.</p> <p>40 and 80 mg/kg bw given on 1st and 4th day; 160 mg/kg bw given on 1st day only.</p>	<p>Mortality: Both rats died in the 160 mg/kg bw group.</p> <p>LD₅₀ to female rats was found to be between 80 and 160 mg/kg bw.</p> <p>Clinical signs: Marked weakness in rats at 160 mg/kg bw.</p> <p>Body weight: No weight loss at 40 or 80 mg/kg bw.</p> <p>Gross pathology: No bile duct lesion at any dose.</p>	<p>Barnes and Stoner (1958).</p>

<p>No guideline followed.</p> <p>Acute oral (gavage) LD50 determined as part of an in-vivo UDS study, in order to determine dosage levels for the study.</p> <p>GLP compliant.</p> <p>Rat, Fischer 344, three males per dose.</p>	<p>Mixture of methyltin chloride compounds.</p> <p>Test material form: crystalline.</p> <p>First range finding assay: 25, 50, 100, 200 and 400 mg/kg bw.</p> <p>Second range finding assay: 600 and 800 mg/kg bw.</p> <p>Definitive in vivo-in vitro hepatocyte DNA repair (UDS) assay: 90, 175, and 350 mg/kg bw 2 or 16 hours before sacrifice.</p>	<p>LD₅₀: ca. 280 mg/kg bw (male).</p> <p>1st assay: 1 death at 400 mg/kg. Clinical signs of dosed rats: rough fur, diarrhoea, weakness, humped back, difficulty breathing, bloody nose, lacklustre eyes, and blood around eyes. Extreme weight loss in the surviving rats at 400 mg/kg.</p> <p>2nd assay: all rats died. Clinical signs at 600 or 800 mg/kg bw: rough fur, weakness, diarrhoea, lacklustre eyes, humped back, hypoactive, and blood around eyes.</p> <p>(UDS) assay: 3 rats from the 16 hour 350 mg/kg bw methyltin chloride dose group were found dead on the morning after dosing. All rats from the 16 hour 175 mg/kg bw dose-group had rough fur on the morning after dosing. 1 rat from the 2 hour 90 mg/kg bw dose-group and 2 rats from the 2 hour 350 mg/kg bw dose-group had diarrhoea. The surviving rat from the 16 hour 350 mg/kg bw dose-group had rough fur, humped back, diarrhoea, laboured breathing, and was hypoactive.</p>	<p>Author not disseminated (1993).</p> <p>Report date: 1993-07-11.</p>
<p>Methodological information not available in English.</p> <p>GLP compliance not reported.</p> <p>Route of administration: oral (unspecified).</p> <p>Rabbit, no data on strain.</p> <p>Sex: male.</p>	<p>dimethyltin dichloride.</p> <p>Test material form: crystalline.</p> <p>No data available.</p>	<p>Minimum lethal dose: 50 mg/kg bw (male).</p> <p>No available data on other effects.</p>	<p>Hashizume (1971).</p>
<p>No methodological information.</p> <p>GLP compliance not reported.</p> <p>Route of administration: oral (unspecified).</p> <p>Rat, no data on strain or sex.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p> <p>No data available.</p>	<p>No LD₅₀ given.</p> <p>Clinical signs: General uncharacteristic illness.</p> <p>Gross pathology: Inflammatory lesion of bile duct.</p>	<p>Author not disseminated (1978b).</p> <p>Reference type: review article or handbook.</p>

<p>No methodological information available.</p> <p>Route of administration: oral (unspecified).</p> <p>GLP compliance not reported.</p> <p>Rat, no data on strain or sex.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p> <p>No data available.</p>	<p>LD₅₀: 88 - 119 mg/kg bw.</p> <p>No available data on other effects.</p>	<p>van Dokkum, and Huwer (2005).</p>
<p>No methodological information available.</p> <p>Route of administration: oral (unspecified).</p> <p>GLP compliance not reported.</p> <p>Rat, no data on strain or sex.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p>	<p>LD₅₀: 237 mg/kg bw.</p> <p>No available data on other effects.</p>	<p>Author not disseminated (1978c).</p>
<p>No methodological information.</p> <p>Route of administration: oral (unspecified).</p> <p>GLP compliance not reported.</p> <p>Rat, no data on strain or sex.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p>	<p>LD₅₀: 90 mg/kg bw.</p> <p>No available data on other effects.</p>	<p>Author not disseminated (1978d).</p> <p>Reference type: review article or handbook.</p>
<p>No methodological information.</p> <p>Route of administration: oral (unspecified).</p> <p>GLP compliance not reported.</p> <p>Rat, no data on strain or sex.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p> <p>No data available.</p>	<p>LD₅₀: 74 mg/kg bw.</p> <p>No available data on other effects.</p>	<p>Dean (1976).</p>
<p>No methodological information.</p> <p>Route of administration: oral (unspecified).</p> <p>GLP compliance not reported.</p> <p>Rat, no data on strain or sex.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p>	<p>LD₅₀: 74 mg/kg bw.</p> <p>No available data on other effects.</p>	<p>Author not disseminated (1992).</p>

Table 37b: Summary table of animal studies relevant for STOT SE (dermal route)

Method, test guideline, and deviation(s) if any Species, strain, sex, no/group	Test substance, dose levels, duration of exposure	Effects	Reference
<p>OECD TG 402, EU Method B.3.</p> <p>GLP compliant.</p> <p>Deviations: The temperature and relative humidity of the animal room (66-72 °F and 30-60 %, respectively) exceeded the range specified in the protocol (61-70°F and 40-60%, respectively). The final body weight and method of euthanasia was not recorded for two males.</p> <p>Rabbit, New Zealand White, male/female, 5 animals per sex and dose.</p>	<p>84.8 % DMTC in mixture with MMTC.</p> <p>Test material form: solution.</p> <p>Physical state: liquid.</p> <p>200, 400 and 750 mg/kg bw.</p> <p>Occlusive, ca. 24 hours.</p>	<p>LD₅₀: 404 mg/kg bw (male/female).</p> <p>In the animals that died or were sacrificed moribund during the study, slight to severe dermal irritation at the site of the test material application, urine/fecal stain, soft stool/diarrhoea, decreased food consumption, decreased activity, pale eyes, decreased defecation, tremors, wobbly gait, respiratory abnormalities, mucoid stools, reddened iris, prostration, partial immobility of the hindlimbs, dark material and swelling around the facial area, convulsions, dehydration, emaciation, red ocular discharge, apparent hypothermia, raised area on the abdominal region and grinding of teeth. Clinical abnormalities in the animals surviving to study termination included slight to severe dermal irritation at the site of test material application, decreased defecation, decreased activity, pale eyes, dark material around the facial area, partial immobility of the hindlimbs, tremors, wobbly gait, soft stool, mucoid stools, fecal stain, reddened iris, apparent decreased food consumption and raised area on the abdominal region.</p> <p>Body weight Body weight loss during the day 0-7 in 1 male and 1 female at 400 mg/kg and in the one surviving male at 750 mg/kg (weight gain on day 7-14).</p> <p>Gross pathology Reddened mucosa in the digestive tract, petechial haemorrhages, abnormally coloured fluid/mucoid contents in the digestive tract, dark red foci on the mucosa of the stomach, mottled and/or dark red thymus, mottled and/or firm, consolidated lungs, clear amber or red fluid contents in the thoracic cavity and light red foamy contents in the trachea in animals that died. Cysts on the oviducts were noted in two surviving females.</p>	<p>Rush, E.R. (1993b).</p>
<p>No guideline followed. Very limited methodological information available. GLP compliance not reported.</p> <p>Rat; no data on strain, 3 males per dose.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p> <p>80 mg/kg applied on 5 successive days.</p> <p>Percutaneous application.</p>	<p>No LD₅₀ determined.</p> <p>Clinical signs: Necroses of superficial layer of skin with black eschar formation. No deep-seated inflammation.</p> <p>Body weight: Slight weight loss.</p> <p>Gross pathology: No bile duct lesions.</p>	<p>Barnes and Stoner (1958).</p>

OECD TG 402. Deviations: no data. GLP compliance not reported. Reliability score 4 (not assignable). Rabbit: New Zealand White, male/female (six animals in total, no data on control animals).	Dimethyltin Dichloride: Methyltin Trichloride (90:10% mixture). Physical state: liquid. 2000 mg/kg bw.	LD ₅₀ > 2000 mg/kg. No available data on other effects.	Affiliated Medical Enterprises 1971b.
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Table 37c: Summary table of animal studies relevant for STOT SE (inhalation route)

Method, test guideline, and deviation(s) if any Species, strain, sex, no/group	Test substance, form and particle size (MMAD), dose levels, duration of exposure	Effects	Reference
Equivalent or similar to OECD TG 403. Inhalation (aerosol). GLP compliance not reported. Reliability score 2 (reliable with restrictions). Rat, Tif: RAIf (SPF), 10 animals per sex and per dose.	dimethyltin dichloride. Form: crystalline. Aerosol. 44 ± 6, 90 ± 7, 121 ± 3 and 167 ± 23 mg/m ³ . Duration of exposure: 4 h.	LC ₅₀ : 115 mg/m ³ air (analytical). Mortality 44 ± 6 mg/m ³ : 0/10 males, 0/10 females within 14 days 90 ± 7 mg/m ³ : 3/10 males, 1/10 females within 14 days 121 ± 3 mg/m ³ : 5/10 males, 4/10 females within 14 days 167 ± 23 mg/m ³ : 10/10 males, 10/10 females within 14 days Clinical signs Within 2 hours after starting the exposure the animals in concentrations where mortalities occurred showed dyspnoea, ventral position, tremor and ruffled fur. These symptoms became more accentuated as the concentration was increased. After the 4 hour exposure period all animals showed, in addition to the above symptoms edema, in the head region. The surviving animals recovered within 6 to 7 days. They were submitted at random to a necropsy whenever they died, survivors at the end of the observation period.	Ciba-Geigy (1977).

<p>Equivalent or similar to OECD TG 403.</p> <p>Inhalation (aerosol).</p> <p>Deviations: only for 1 hour exposure.</p> <p>GLP compliance not reported.</p> <p>Rat, no data on strain, 10 males per dose.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p> <p>Physical state: solid.</p> <p>57.6 mg/l in air (with water as vehicle), 16.7 mg/l in air (with propylene glycol as vehicle).</p> <p>Duration of exposure: 1 h.</p>	<p>None of the animals died.</p> <p>Dimethyltin Dichloride in Distilled Water: Initial "excited" activity shown by all animals, the majority of the animals exhibited little or no activity throughout the period, depression, shallow respiration, serosanguineous stains around the nose, animals exhibited excessive masticatory movements, preening and lacrimation, and "squinting" of the eyes on occasion.</p> <p>Upon removal from the exposure chamber, all animals exhibited depression and shallow respiration, eight exhibited excessive lacrimation, nine exhibited serosanguineous stains around the nose and five exhibited wheezing. On the first post-exposure day, two exhibited serosanguineous stains around the nose and urine stains and one animal was wheezing. From the second day throughout termination of the study, all animals appeared grossly normal.</p> <p>An average bw gain of 82 g.</p> <p>No significant gross pathological alterations.</p> <p>Dimethyltin Dichloride in Propylene Glycol: Initial "excited" behaviour in all animals, little or no activity throughout the period, depression, laboured respiration, serosanguineous stains around the nose and/or mouth, excessive salivation, ataxia and damp haircoats in the majority of animals and occasional preening, excessive lacrimation, "squinting" of the eyes and masticatory movements.</p> <p>Upon removal from the exposure chamber, all animals exhibited depression, depressed righting and placement reflexes, excessive salivation and stains, damp haircoats, eight showed excessive lacrimation, seven serosanguineous stains around the nose and five exhibited wheezing. On the first and post-exposure day, two or three of the animals exhibited sero-sanguineous stains around the nose and one or two of the animals were wheezing. With the exception of one animal, which exhibited wheezing from day three through five, all animals exhibited normal appearance and behaviour until termination.</p> <p>An average bw gain of 107 g.</p> <p>No significant gross pathological alterations.</p>	<p>International Bio-Research (1976).</p>
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<p>Equivalent or similar to OECD TG 403.</p> <p>According to Federal Register August 12, 1961 et seq. FHSA.</p> <p>Deviations: no data.</p> <p>Inhalation (aerosol).</p> <p>GLP compliance not reported.</p> <p>Rat, Wistar, 10 males per dose.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p> <p>Concentrations: 100, 130 and 169 µl/l.</p> <p>Duration of exposure: 4 h.</p>	<p>LC₅₀: 139 µl/L air.</p> <p>Mortality: 100 µl/l : No mortalities within 14 days 130 µl/l : 4/10 mortalities within 24 hours; no further mortalities. 169 µl/l : 8/10 mortalities within 24 hours; no further mortalities.</p> <p>Clinical signs A slight - medium degree of depression of the respiration, slight apathia and disturbances in coordination. The reflexes, urine and fecal deposition of the survived animals were normalised 24 hours after exposition.</p> <p>Body weight During the observation period of 14 days all the surviving animals showed a normal gain of body weight.</p> <p>Gross pathology At terminal autopsy no macroscopic pathological changes were observed in the cranial, abdominal and chest cavities. The animals which died during or shortly after the exposure time showed lung oedemas and lung emphysemas of a slight to moderate degree.</p>	<p>Author not disseminated (1976).</p>
<p>Test type: standard acute method.</p> <p>GLP compliance not reported.</p> <p>Inhalation (aerosol).</p> <p>Duration of exposure: 1 h.</p> <p>Rat, Sprague-Dawley, 10 males per dose.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p> <p>Aerodynamic mass median diameter (MMD): less than 5.0 µm in at least 80%.</p> <p>For the first group the nominal aerosol concentration was 11.53 mg/litre. This aerosol delivery yielded a concentration of 5.77 mg/litre of test material.</p> <p>For the second group the nominal aerosol concentration was 15.02 mg/litre which was equivalent to 5.00 mg/litre of test material.</p>	<p>Mortality: No deaths occurred in either of the groups.</p> <p>Clinical signs: All rats in both exposure groups exhibited inactivity during the exposure period. At the termination of the exposures, all 5.77 mg/litre animals and several animals exposed to 5.00 mg/litre of test material had a slight to moderate discharge around the muzzle and nasal areas. All animals in both groups exhibited normal appearance and behaviour throughout the 14-day post-exposure observation period.</p> <p>Body weight: NDA.</p> <p>Gross pathology: NDA.</p>	<p>Hazelton Laboratories 1976.</p>

<p>Equivalent or similar to OECD TG 403.</p> <p>Deviations: only 1 hour exposure duration.</p> <p>Inhalation (aerosol).</p> <p>GLP compliance not reported.</p> <p>Rat, no data on strain, 10 rats per dose, mixture of sexes.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p> <p>Physical state: solid.</p> <p>50, 100, 200 and 300 mg/l/hour.</p>	<p>Mortality: 50 mg/l/hour: 2/10 100 mg/l/hour: 5/10 200 mg/l/hour: 7/10 300 mg/l/hour: 10/10</p> <p>Clinical signs: Rats exposed to lower dose of the test material experienced CNS depression. After recovery from the state of depression, the rats became very aggressive and fought with each other. They were also very sensitive to sound and touch. This behaviour of aggressiveness was only observed in the male rats.</p> <p>Body weight: NDA.</p> <p>Gross pathology: Gross findings at autopsy on dead rats showed blood in the lungs and heart failure. Fluid was in the chest cavity. The spleen was very dark and the stomach was filled with gas.</p>	<p>Wells Laboratories 1975</p>
<p>No methodological information.</p> <p>GLP compliance not reported.</p> <p>Inhalation (aerosol).</p> <p>Duration of exposure: 1 h.</p> <p>Rat, no data on strain, male/female.</p>	<p>Monomethyltin trichloride + dimethyltin dichloride (21.5 : 78.5).</p> <p>Test material form: crystalline.</p>	<p>LC₅₀: 84 mg/L/hr.</p> <p>No data available on other effects.</p>	<p>Summer et al. (2003).</p>
<p>No methodological information available.</p> <p>Inhalation (vapour).</p> <p>GLP compliance not reported.</p> <p>Species: rat. Strain: no data. Sex: no data.</p>	<p>dimethyltin dichloride.</p> <p>Form: crystalline.</p>	<p>LC₅₀: 1070 mg/L/hr.</p> <p>No data available on other effects.</p>	<p>Author not disseminated (1978).</p> <p>Reference type: review article or handbook.</p>
<p>No methodological information available.</p> <p>Inhalation (aerosol).</p> <p>GLP compliance not reported.</p> <p>Rat, male/female, no information on strain.</p>	<p>dimethyltin dichloride.</p> <p>Test material form: crystalline. Concentrations: 1910 mg/m3.</p> <p>Duration of exposure: 1 h.</p>	<p>Mortality: no deaths observed after 21 days.</p> <p>Body weight: normal body weight gain reported.</p> <p>No information available on other effects.</p>	<p>Author not disseminated (2006).</p> <p>Report date: 2006-07-23.</p> <p>Reference type: review article or handbook.</p>

No methodological information available. Inhalation (vapour). GLP compliance not reported. Species: rat. Strain: no data. Sex: no data.	dimethyltin dichloride. Test material form: crystalline.	LC ₅₀ > 1.91 mg/L/hour. No information available on other effects.	Author not disseminated (1978). Reference type: review article or handbook.
Similar to OECD 403 with shorter duration of exposure (1h). Rat. No information on sex or strain.	Test substance: dimethyltin dichloride. (purity not known). Doses: 640, 1679, and 3012 mg/m ³ . 1 h exposure to aerosol.	LC ₅₀ 1632 mg/m ³ , 1.6 mg/L. Calculated LC ₅₀ on 4 hour using Haber laws and n=1 as recommended in IR/CSA R7.4.4.1 for extrapolation to longer durations: LC₅₀ = 0.4 mg/L. No information available on other effects.	Ciba-Geigy, 1977.

Table 37d: Summary table of human data relevant for STOT SE

Type of data/report	Test substance	Route of exposure and relevant information about the study (as applicable)	Observations	Reference
Case study of workers exposure. GLP compliance not reported.	mixture of half dimethyltin and half trimethyltin chloride vapour. Test material form: crystalline.	Route of administration: inhalation. Analytical verification of test atmosphere concentrations: no. Duration of exposure: 1.5 h. Remarks: over a 3 day working period.	1/6 workers died 12 days following the exposure. Symptoms preceding death: excretion of high levels of tin in the urine, respiratory depression, and coma. Autopsy results of the worker who died: massive fatty degeneration of liver cells and necrosis (shock kidneys i.e. proximal tubule degeneration). In two most severely affected surviving workers permanent neurological disabilities and non-persistent respiratory problems. All surviving workers had high tin concentrations in the urine with the highest levels occurring in the most severely affected.	Harper et al. (2005), (study also referred to in section 8.9 STOT RE).

Table 37e: Summary table of other studies relevant for STOT SE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Test type: acute. No guideline followed. Immunotoxicity study looking at DT-diaphorase activity and thymus atrophy. Male Wistar rats dosed via subcutaneous injection. GLP compliance: not reported.	Identity of Dimethyltin dichloride. Test material form: crystalline.	Single exposure. Doses / concentrations (actual injected): 11 mg/kg bw (50 µmoles/kg bw). No. of animals per sex per dose: 4 male rats per dose. Control animals: no. After treatment with organotin animals were killed by decapitation, and thymus and spleen were dissected out, washed with saline, blotted, and weighed. The liver was perfused in situ with a cold 0.9% saline and then removed, washed, blotted and weighed.	NOAEL (male) \geq 11 11 mg/kg bw. Induction of heme oxygenase, peaking at 48 hours after treatment. No significant losses in cytochrome P-450. No significant changes in the DT-diaphorase activity at any time after injection. A good reciprocal correlation between DT-diaphorase activity and thymus weight ($r=0.73$, $n=12$).	Ariyoshi et al. (1991).

Short summary and overall relevance of the provided information on STOT SE**Oral route:**

Dimethyl dichloride is classified as Acute Tox. 3; H301, and although some gross pathological organ changes were reported in animals that died, there is no clear evidence for a specific organ toxicity in the absence of lethality. Furthermore, the adaptive responses such as decreased activity, salivation, rough haircoat, mucoid/soft stools, fecal/urine stain, hunched posture, dehydration, dark material around the facial area, decreased defecation and food consumption, gasping and rales are not considered toxicologically relevant and do not warrant classification for STOT SE.

Dermal route:

Dimethyl dichloride is classified as Acute Tox. 3; H311 and Skin Corr. 1; H314, and apart from cysts on the oviducts in two female rabbits surviving to study termination (Author not disseminated (1993), report date 1993-07-25), there is no evidence for a specific organ toxicity in the absence of lethality or that would not be related to corrosive properties of the substance. However, cysts on the oviducts are not considered to warrant classification for STOT SE since they are commonly observed in New Zealand White rabbits.

Inhalation route:

The reversible activity changes, excessive salivation, ataxia and damp haircoats, depression, shallow respiration, serosanguineous stains around the nose, excessive masticatory movements, preening and lacrimation, and "squinting" of the eyes on occasion in the absence of lethality are considered adaptive responses that are not toxicologically relevant and do not warrant classification for STOT SE. Dimethyl dichloride is classified as Acute Tox. 2; H330, and no specific target organ toxicity was reported in the animal studies in the absence of lethality. In the human case study on six workers, unknown level of exposure over three days to a mixture of half dimethyltin and half trimethyltin chloride vapour caused death that was preceded by high levels of tin in the urine, respiratory depression, and coma, massive fatty degeneration of liver cells and necrosis or proximal tubule degeneration. The surviving workers, who were the most severely affected, developed permanent neurological disabilities, but respiratory problems did not

persist. Care should be taken not to assign classification for Acute Toxicity and STOT SE for the same effect. Specific target organ toxicity (single exposure) is defined as specific, non-lethal target organ toxicity arising from a single exposure to a substance, whereas acute toxicity classification is generally assigned on the basis of evident lethality or where the potential to cause lethality can be concluded from evident toxicity. In this case study on workers, unknown exposure levels over three days to a mixture of half dimethyltin and half trimethyltin chloride vapour caused one death and permanent neurological disabilities in the surviving workers. This human evidence is considered rather as contributing to the weight of evidence assessment the available information on animal studies supporting the classification as Acute Tox. 2; H330.

Other routes:

No evidence for immunotoxicity was observed in Ariyoshi et al. (1991).

Comparison with the GHS criteria

As dimethyl dichloride is classified as Acute Tox. 3; H301, Acute Tox. 3; H311 and Acute Tox 2; H330, and there is no clear evidence for a specific organ toxicity in absence of lethality, no classification for STOT SE is warranted.

Conclusion on classification and labelling for STOT SE

No classification is warranted for STOT SE.

8.9 Specific target organ toxicity-repeated exposure (STOT RE)

Table 38a: Summary table of animal studies relevant for STOT RE

Method, test guideline, and deviation(s) if any, species, strain, sex, no/group	Test substance route of exposure, dose levels, duration of exposure	Results	Reference
<p>90-day repeated dose oral study</p> <p>Equivalent or similar to OECD TG 408.</p> <p>GLP-compliant.</p> <p>SD rats, male/female</p> <p>15 animals per sex and dose in main study; 15 per sex and dose in neurotoxicity study.</p>	<p>methyltin chlorides (mixture of dimethyltin dichloride/methyltin trichloride).</p> <p>Oral in drinking water.</p> <p>0, 25, 75, 200 ppm (reduced to 150 during 5th week of treatment and terminated during 6th treatment week).</p>	<p>NOAEL (males) < 25 ppm or 1.6 mg/kg bw/d NOAEL (females) < 25 ppm or 2.2 mg/kg bw/d</p> <p><i>200 ppm:</i></p> <ul style="list-style-type: none"> - 7 males and 21 females died or were sacrificed between days 18 and 34, with severe treatment-related clinical signs - remaining animals sacrificed by day 36 of the study - sign. ↓ body weight - sign. ↓ food consumption - marked changes in various blood biochemical parameters - at day 92, the biochemical parameters were essentially the same between the control and treated groups - changes in various organ weights, including ↓ thymus and spleen weight at the interim sacrifice (males) - various gross pathology findings - in several preterminal non-perfused animals small thymus and/or spleen <p><i>Neurobehaviour:</i></p> <p>Week 4: one male with slight ataxia ➤ slight overall gait incapacity, slight tremors at the limbs and clonus of the jaws (chomping), 3 females with slight/moderate tremors at the limbs or head and clonic convulsions, 2 animals with hunched posture and a severe overall gait incapacity attributed to a severe ataxia in one animal + red liquid material at the urogenital region.</p> <ul style="list-style-type: none"> - incidence of a rearing body position in the home cage as well as average number of rearing incidents in the arena sign. reduced - hindlimb grip strength sign. decreased and body temperature sign. reduced in females <p><i>75 ppm:</i></p> <ul style="list-style-type: none"> - 1 male found dead on day 41, with e.g. tremors, hypersensitivity and thin dehydrated body condition - thin and dehydrated appearance for another male - abnormal gait/behaviour for a third male - one female with hypersensitivity, convulsions and reduced activity - changes in various organ weights, including ↓ absolute and relative thymus and/or spleen weight at the terminal sacrifice and ↑ brain weight (males, at end of recovery period it was comparable to controls) - histopath. changes in brain tissue - terminal evaluations indicated possible treatment-related lymphoid atrophy of the thymus <p><i>Neurobehaviour:</i></p> <p><i>FOB:</i></p> <p>Week 4: one male with severe ataxia including falling over which resulted in a severe overall incapacity + several other neurological signs throughout the study</p> <ul style="list-style-type: none"> - sign. lower body temperature <p>Week 8 and 13: sign. decrease in rearing incidents in the arena (females)</p> <p><i>Motor activity:</i></p> <ul style="list-style-type: none"> - sign. reductions in total activity counts <p><i>25 ppm:</i></p> <ul style="list-style-type: none"> - no mortalities - absolute and relative kidney weights (female) - treatment-related findings limited to reduced food (male) and water intake, neuropathological lesions and vacuolisation of white matter in the brain and spinal cord. 	Rohm and Haas (1999).

<p>90-day oral study in rat.</p> <p>OECD TG 408, with deviations.</p> <p>GLP-compliant.</p> <p>Wistar rat, male/female, 10 per sex and dose (main study), 6 per sex and dose (satellite groups).</p>	<p>MMTTC/DMDTC (30/70).</p> <p>Oral (feed).</p> <p>0, 1, 6, 15 and 200 ppm.</p> <p>Actual ingested:</p> <p>Males: 0, 0.06, 0.39, 0.98, and 16.81 mg/kg bw/d.</p> <p>Females: 0, 0.07, 0.41, 1.02, and 17.31 mg/kg bw/d.</p>	<p>NOAEL (males) 15 ppm or 0.98 mg/kg bw/d NOAEL (females) 15 ppm or 1.02 mg/kg bw/d</p> <p>Both NOAELs based on neurological effects.</p> <p>*(actual dose received) based on test material</p> <p>200 ppm: - neurological signs around week 4, including increased activity, rearing, convulsive activity (females), increased landing footsplay (males) - 3 females in the main group died towards the end of the first month and most of the females and a number of males showed severe neurological signs (incl. tremors and convulsions) ➤ all animals were considered moribund and killed for humane reasons - 1 animal in the subgroup found dead and the others killed for humane reasons (due to convulsions, blepharospasm, tremors) - treatment-related cell death, neuronal death (more pronounced in females) in various areas and submeningeal oedema in the brain - treatment-related tubular dilatation in the kidneys - females had an increased incidence of corticomedullary haemorrhage in the thymus. Most of the high-dose females also showed cortical lymphoid depletion in the thymus. - all animals showed decreased accumulation of brown pigment in the spleen.</p> <p>15 ppm: - no clinical signs - no histopath. changes</p> <p>6 ppm: - no clinical signs</p> <p>1 ppm: - no clinical signs</p>	<p>Elf Atochem NA (1996).</p>
<p>7-day study to evaluate the palatability of a methyltin chloride mixture</p> <p>No guideline available.</p> <p>GLP-compliant.</p> <p>SD rats, female, 5 per dose</p>	<p>methyltin chlorides (mixture of dimethyltin dichloride/methyltin trichloride).</p> <p>Oral in drinking water.</p> <p>0, 250 and 500 ppm.</p> <p>7 days.</p>	<p>- no mortality/early sacrifices - signs of dehydration commencing from day 4 - no other clinical signs - changes in body weight - changes in food and water consumption</p>	<p>Author not disseminated (1997).</p> <p>Report date: 1997-08-26.</p>
<p>Subacute, 14-day study</p> <p>Non-guideline</p> <p>GLP-compliant.</p> <p>SD rats, male/female, 5 per sex and dose</p>	<p>methyltin chlorides (mixture of dimethyltin dichloride/methyltin trichloride).</p> <p>Oral in drinking water.</p> <p>0, 25, 75, 150 and 200 ppm.</p>	<p>- no mortalities/early sacrifices - no clinical signs related to treatment - slightly lower (n.s.) mean body weight at the end of the study (200 ppm males, all dose group females) - some changes in food and water consumption - sign. (relative to bw) ↓ in kidney weight in 200 ppm females</p>	<p>Author not disseminated (1997).</p> <p>Report date: 1997-08-28.</p>
<p>90-day study in rats.</p> <p>OECD TG 408.</p> <p>GLP compliance not reported.</p> <p>Wistar rat, male/female, 10 per sex and dose</p>	<p>1175-114</p> <p>Read across from 78/22 Mono/Di-methyltin dichloride.</p> <p>Oral (feed).</p> <p>0, 20, 100 or 500 ppm.</p>	<p>- no deaths or signs of intoxication - treatment-related histopath. changes: hyperplasia of the urinary bladder epithelium (moderate to slight) in most animals at 500 ppm, in a number of animals at 100 ppm and 2 females at 20 ppm; slightly enlarged pale nuclei and foamy cytoplasm of the epithelial cells of the proximal tubules in the intercortico-medullary region of the kidney in 4 males and 1 female of the highest dose group.</p>	<p>Author not disseminated (1978).</p> <p>Report date: 1978-11-20.</p>

Table 38b: Summary table of human data relevant for STOT RE

Type of data/report	Test substance	Route of exposure	Relevant information about the study (as applicable)	Observations	Reference
Poisoning incident report; secondary source.	Dimethyltin dichloride and trimethyltin chloride vapours		Occupational, unintentional exposure, inhalation	Two chemists who had been intermittently exposed to vapours of dimethyltin dichloride and trimethyltin chloride for about 3 months abruptly developed a status of mental confusion with generalized epileptic seizures. Before the acute episode, the subjects had complained of headaches, pain in various organs, and psychological disturbances such as memory defects, vigilance loss, insomnia, anorexia, and disorientation. Both patients recovered completely following removal from exposure.	Harper et al., 2005
Poisoning incident report; case study of workers exposure; secondary source.	Mixture of half dimethyltin and half trimethyltin chloride vapour		Occupational exposure, 6 workers (male and female), inhalation. Maximum exposure was a total of 1.5 hours over a 3 day working period. No estimates of exposure levels were given.	Six chemical workers exposed to methyltins primarily by inhalation experienced headache, tinnitus, deafness, impaired memory, disorientation, aggressiveness, psychotic and other severe neuropsychiatric behaviour, syncope and loss of consciousness as symptoms of exposure; one subject died. The two surviving workers with the highest urinary tin levels exhibited fixed neurological effects which were not resolved more than six years after exposure. The remaining three survivors returned to work, but had memory loss, which persisted for six months.	Harper et al., 2005 (study also referred to in section 8.8 STOT SE)

Table 38c: Summary table of other studies relevant for STOT RE

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Publication/ literature paper.	dimethyltin dichloride	Investigating relationship between chemical structure of organotin compounds and thymolytic activity. Data considered in the review include: oral treatment studies, intravenous treatment studies and in vitro experiments. An intravenous treatment study and in vitro experiment included the assessment of dimethyltin dichloride Female rats, no strain data given; 0, 12, 24, 48 mg/kg bw/d i.v., 5-8 animals per dose. No further data on in vitro experiment methodology.	Intravenous Experiment: - DMTC did not affect the thymus. - No effects were seen on any of the lymphoid organs. - A single injection of 48 mg/kg bw caused 3 of the 8 animals to die within 2 days. - No liver or bile duct changes were found. - No other treatment-related histopathological changes were noted. In vitro experiment: - No effect in the cell count or viability was observed.	Seinen et al., 1977.
Subacute study; secondary source. No data on methodology or GLP compliance	dimethyltin dichloride	Rat, 5 mg/kg bw/d	NOAEL (no data on sex) \geq 5 mg/kg bw/d based on test material. NOAEL based on thymus weight.	Dobson et al., 2006.

Short summary and overall relevance of the provided information on STOT RE

Two 90-day studies are considered as reliable: Rohm and Hass (1999) and Elf Atochem NA (1996).

In Rohm and Hass (1999) rats were treated with a methyltin chloride mixture containing 90% dimethyltin dichloride at concentrations of 25 (1.6 mg/kg bw/day in males; 2.2 mg/kg bw/day), 75 (5.2 mg/kg bw/day in males; 6.7 mg/kg bw/day in females) or 200 ppm (15.50 mg/kg bw/day in males; 19.5 mg/kg bw/day in females). At 200 ppm, 7 males and 21 females died or were sacrificed during the first 5 weeks due to poor condition. All remaining animals were sacrificed by Week 6. Animals in this group 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. At 75 ppm 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. At 25 ppm 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.

Functional Observational Battery (FOB) tests indicated several findings, primarily affecting the 200 ppm group. 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 region. Significant findings for the 75 ppm group 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 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 females relative to the control group.

There were also significant blood biochemical changes for males in the 200 ppm group 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 had an elevated urine pH at week 4. 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 at termination sacrifice. Absolute heart weight was decreased significantly for 25 and 75 ppm females at interim sacrifice, but not at terminal sacrifice.

Absolute and relative kidney weights were significantly increased for 25 ppm and 75 ppm females at terminal sacrifice. Gross pathological findings for preterminal animals included small thymus and/or spleen, emaciated carcass, dilation of digestive tract/discooured 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 and at the terminal evaluation for the 75 ppm group. Results of the histopathological examinations indicated clear treatment related nervous system lesions for preterminal 200 ppm animals in various regions of 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 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 showed slight to moderate vacuolization in brain and spinal cord tissue at the terminal examination.

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, behavioural effects, and neuropathological lesions at 200 ppm (equivalent to 15.5 and 19.5 mg/kg/day for males and females, respectively). 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. 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.

In Elf Atochem NA (1996), 1, 6, 15, and 200 ppm (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 bw/day in females) of Dimethyltin Dichloride: Methyltin Trichloride (66.5:33.5% mixture) was given by oral route in diet. Three females of the 200 ppm group 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 were sacrificed. Mean body weight for males of the 200 ppm group on days 7 and 28 were significantly lower. Food consumption on day 7 was significantly decreased in animals (both sexes) of the 200 ppm group 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) on day 6 only. Mean intake of the test substance in animals receiving 1, 6, 15, or 200 mg/kg diet 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. The specific gravity of urine was significantly increased in females of the 6 ppm dose group.

Upon microscopic examination, treatment-related histopathological changes were observed in the brain, the kidneys, and the thymus of animals treated with 200 ppm of the test substance. Macroscopic pathological observations showed some gross skin changes at 200 ppm that were probably treatment-related. Animals below 15 ppm were not examined microscopically because no microscopic changes were found at 15 ppm as compared to the control group. Females at 200 ppm (17.31 mg/kg bw/day) had an increased incidence of corticomedullary haemorrhage in the thymus and most of these high-dose females also showed cortical lymphoid depletion in the thymus. All females and males (16.81 mg/kg bw/day) treated with 200 ppm showed decreased accumulation of brown pigment in the spleen. Neuropathological examinations showed that animals in the high dose group showed signs of convulsions, tremors, blepharospasm, and hunched posture, and microscopic observations showed pronounced neuronal death in a number of areas of the cerebellum (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. Also a slight increase in swollen axons in the spinal cord was observed in this high dose group. Based on the effects described above, particularly the neurotoxic effects observed in the high dose group, the NOAEL was placed at 15 ppm 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 dimethyltin dichloride component of the mixture.

Together, these two oral 90-day studies on DMTC indicate that the main target organ is the nervous system. Deaths and severe neurological signs occurred from 75 ppm (5.2/6.7 mg/kg) in Rohm and Hass (1999) and at 200 ppm (16.81/17.31 mg/kg) in Elf Atochem NA (1996). Besides, neuropathological lesions were observed from the lowest dose of 25 ppm (1.6/2.2 mg/kg) in Rohm and Hass (1999) as evidenced by moderate vacuolization in the brain and spinal cord tissue and ventricular dilation and neuronal necrosis at highest doses. Neuronal death was found at 200 ppm (16.81/17.31 mg/kg), but no dose-response was observed in neuronal micro-vacuolization or degeneration in Elf Atochem NA (1996). The absence of neurological findings in the shorter-term studies and in the unreliable 90-day study do not decrease the concern for clear adverse effects on the nervous system supported also by human data. In the poisoning incident report on six workers (Harper et al., 2005), unknown exposure to a mixture of half dimethyltin and half trimethyltin chloride vapour over three days caused death that was preceded by high levels of tin in the urine, respiratory depression, and coma, massive fatty degeneration of liver cells and necrosis or proximal tubule degeneration. The surviving workers, who were the most severely affected, developed permanent neurological disabilities, but respiratory problems did not persist. In the other poisoning incident report reviewed in Harper et al. (2005), two chemists who had been intermittently exposed to vapours of dimethyltin dichloride and trimethyltin chloride for about 3 months abruptly developed a status of mental confusion with generalized epileptic seizures. Before the acute episode, the subjects had complained of headaches, pain in various organs, and psychological disturbances such as memory defects, vigilance loss, insomnia, anorexia, and disorientation. Both patients recovered completely following removal from exposure. No estimates of exposure levels were given in the poisoning incident reports (Harper et al., 2005), but the observed neurological effects contribute to the weight of evidence assessment of the available information on animal studies supporting a classification with STOT RE 1 with the nervous system as the target organ.

Also, the absolute and relative weights of the thymus were reduced in the 90-day oral studies at 75 ppm in males (about 5 mg/kg bw/day), indications of a possible treatment-related lymphoid atrophy of the thymus were observed in animals in the mid dose group of 75 ppm (below 10 mg/kg bw/day), and a smaller spleen was also observed in females in the mid and low groups (Rohm and Haas 1999). In Elf Atochem NA (1996), females at 200 ppm (17.31 mg/kg bw/day) had an increased incidence of corticomedullary haemorrhage in the thymus and most of these high-dose females also showed cortical lymphoid depletion in the thymus. All females and males (16.81 mg/kg bw/day) treated with 200 ppm showed decreased accumulation of brown pigment in the spleen. Accumulation of brown pigment is a normal phenomenon in rats, which gradually increases with age. The 200 ppm animals were killed at an early stage of the study, at which they had not yet accumulated pigment in the spleen. Therefore, the decreased splenic pigment was not considered to be an effect of the treatment, but rather a consequence of the difference in age between these animals and those of

the other treatment groups and the controls, that were killed two months later. No histochemical changes were observed at the lower dose of 1 mg/kg bw/day in Elf Atochem NA (1996). However, the effects on thymus observed at 75 ppm in Rohm and Haas (1999) supported by the effects on spleen justify the STOT RE 1 classification also for the immune system as this dose level is within the range of guidance values for category 1 classification. The effects observed on the thymus and spleen are consistent with a known class effect of organotins on the immune system, and it is to be noted that reduced thymus weights (atrophy) were also observed in the two prenatal developmental rat studies (Noda 2001) on day 20 of gestation of females treated at 15 and 20 mg/kg although these dose levels are above the guidance value range for classification in Category . No examination of spleen was conducted.

Comparison with the GHS criteria

According to the GHS criteria in Figure 3.9.1 in section 3.9.2.1, classification in category 1 is justified if: “substances that have produced significant toxicity in humans, or that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to produce significant toxicity in humans following repeated exposure”. The guidance dose value for this category for a rat 90 day study is ≤ 10 mg/kg bw/day. The effects seen in the thymus (and to a lesser extent in the spleen) as well as in the nervous system in the two rat 90-day studies justify classification in STOT RE Category 1 according to the GHS criteria with the central nervous system and the immune system as the specific target organs/systems (see Note to Figure 3.9.1). The observed neurological effects in humans contribute to the weight of evidence assessment of the available information on animal studies supporting a classification with STOT RE 1 with the nervous system as the target organ.

Conclusion on classification and labelling for STOT RE

Classification as STOT RE 1; H372 (nervous system, immune system) is considered warranted. No specific concentration limit is proposed.

8.10 Aspiration hazard

Table 39: Summary table of evidence for aspiration hazard

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data available.				

Short summary and overall relevance of the provided information on aspiration hazard

Not applicable as there is no data available.

Comparison with the GHS criteria

Not applicable as there is no data available.

Conclusion on classification and labelling for aspiration hazard

Not applicable as there is no data available.

9. EVALUATION OF ENVIRONMENTAL HAZARDS

9.1 HAZARDOUS TO THE AQUATIC ENVIRONMENT

9.1.1 Rapid degradability of organic substances

Table 40: Summary of relevant information on rapid degradability

Method, test guideline, and deviation(s) if any	Results	Remarks	Reference
OECD Guideline 111 (Hydrolysis as a Function of pH)	Hydrolysis DT ₅₀ at 25°C: pH 4 > 1 year pH 7 > 1 year pH 9 > 1 year	Several deviations from the guideline but considered not to impact the validity of the study	Author not disseminated (2004). Report date 2004-06-17.
Secondary source with no methodological information	In a literature source with limited detail, the photodegradation constant was reported to be 1.8×10^{-12} cm ³ / molecule/second (25 °C).		Secondary source (Dobson et al (2006)).
OECD Guideline 301 F (Ready Biodegradability: Manometric Respirometry Test)	The determination was performed with a single concentration of dichlorodimethylstannane: 14-d biodegradation: 3% 28-d biodegradation: 3% 35-d biodegradation: 3% Not readily biodegradable	Minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of the relevant results	Author not disseminated (2003). Report date 2003-06-22.

Hydrolysis

Hydrolytic stability of dimethyltin dichloride was tested according to the OECD TG 111 at three pHs (4, 7, 9). The study can be considered as a valid test in spite of several deviations from the TG protocol. The result of the study showed that dimethyltin dichloride is hydrolytically stable.

Photochemical degradation

The included photo degradation information originates from secondary source and the applied methodology in the study is not known. Based on this information it can be concluded that there is no indication of photo degradation of dimethyltin dichloride.

Ready biodegradability

Ready biodegradation of dimethyltin dichloride was tested according to the OECD TG 301 F (Manometric respiratory test) and only minor deviations to the TG protocol were reported. For example, additional NaNO₃ was added to the mineral medium to prevent nitrogen limitation during the test. The test was performed at one concentration (0.111 mg/L) in three replicate flasks and oxygen concentration was measured every 4 hours. Inoculum activity and toxicity controls contained 100 mg/L of reference substance (two replicates for each). After 28 days of incubation it was decided to extend the test with two weeks because the oxygen consumption had not reached the plateau phase. The actual termination was after 35 days.

Very low degradation (3%) of dimethyltin dichloride was observed through the study. Based on this, the substance has not achieved oxygen depletion of more than 60% within 28 days and cannot, be considered as readily and, thus, rapidly degradable in the environment in line with the GHS criteria.

BOD₅/COD

Not available.

Aquatic simulation tests

Not available.

Field investigations and monitoring data (if relevant for C&L)

Not available.

Inherent and Enhanced Ready Biodegradability tests

Not available.

Soil and sediment degradation data

Not available.

9.1.2 Environmental transformation of metals or inorganic metal compounds

Summary of data/information on environmental transformation

Not relevant for an organic tin compound.

9.1.3 Environmental fate and other relevant information

Not considered in this document.

9.1.4 Bioaccumulation

Table 41: Summary of relevant information on bioaccumulation

Method, test guideline, and deviation(s) if any	Species	Results	Remarks	Reference
A non-guideline study	Natural community of algae	BCF after five (5) days exposure = 1.6 - 5.9.	Limited information provided on the study methods and results to conclude on the validity of the provided information.	Hall & Pinkey (1985)
A non-guideline study	<i>Artemia franciscana</i> (Crustacea)	BCF (24h) at 10 mg Sn/L exposure = 50 BCF (24h) at 100 mg Sn/L = 6	Limited information provided on the study methods and results to conclude on the validity of the provided information.	Hadjispyrou et al. (2001)
EPIWIN estimate		BCF = 3.16 L/kg	Low reliability as the EPIWIN model has not been developed and validated for organometallic substances	Review article or handbook (2006): 2006-07-23

Estimated bioaccumulation

An EPIWIN estimation is available that derived a BCF value of 3.16 L/kg. The results are not relevant for classification purposes.

Measured partition coefficient and bioaccumulation test data

Dimethyltin dichloride has a very low octanol-water partition coefficient, i.e. $\log K_{ow} = -2.18$ measured at 22°C (OECD TG 105, Spruit & Schilt 2003).

Two experimental aquatic bioaccumulation studies are considered. A non-guideline study on bioaccumulation of dimethyltin dichloride in algae resulted a BCF ranging from 1.6-5.9 (Hall & Pinkey, 1985). The available summary of the study in Annex I does not provide sufficient details to evaluate its reliability and therefore the study is considered as a supportive study only, for classification purposes.

Another non-guideline bioaccumulation study tested bioaccumulation of dimethyltin dichloride to an aquatic crustacean *Artemia franciscana* (Hadjispyrou et al. 2001). The test was performed at two concentrations (10 and 100 mg/L). The BCF factor of dimethyltin dichloride was reported to be 50 at 10 mg Sn/L to 6 at 100 mg Sn/L (Sn = tin). Also this study is not reported in detail and it is considered as a supportive study for classification purposes.

9.1.5 Acute aquatic hazard

Table 42: Summary of relevant information on acute aquatic toxicity

Method, test guideline, and deviation(s) if any	Species	Test material	Results	Remarks	Reference (NA = not available)
<i>Fish</i>					
OECD TG 203	<i>Danio rerio</i> (formerly <i>Brachydanio rerio</i>)	Dimethyltin dichloride	The 96h LC ₅₀ and 96h NOEC were determined to be >100 and ≥ 100 mg/l, Based on nominal concentrations (measured concentrations were >80% of nominals and therefore no reason to use measured concentrations)	GLP, minor deviations	Author not disseminated (2003). Report date 2003-09-14.
OECD TG 203	red killifish <i>Oryzias latipes</i>	Dimethyltin dichloride	LC ₅₀ (48h): 6 mg/L; not known if the values were based on nominal or measured conc.	GLP compliance not reported; methods not reported in detail	Nagase et al. (1991)
Review article with no methodological information.	<i>Misgurnus fossilis</i>	Dimethyltin dichloride	Maximum acceptable concentration for development: 100-1000 mg/L; not known if the values were based on nominal or measured conc.	NA, No GLP	Summer et al. (2003)
<i>Invertebrates</i>					
OECD TG 202	<i>Daphnia magna</i>	Dimethyltin dichloride	EC ₅₀ (48h): 17 mg/L. The value is based on nominal concentrations (measured concentrations were >80% of nominals and therefore no reason to use measured concentrations)	GLP compliant	Author not disseminated (2003). Report date 2003-09-14.
Proposed OECD Acute Immobilization Test (1981)	<i>Daphnia magna</i>	Dimethyltin dichloride	EC ₅₀ (24h): 88 mg/L. Based on nominal concentrations.	No GLP compliance	Vighi & Calamari (1985)
A non-guideline study	<i>Daphnia magna</i>	Dimethyltin dichloride	EC ₅₀ (24h): 19.27 mg/L. Based on nominal concentrations.	GLP compliance not reported.	Kungolos et al. (2004)
A non-guideline study conducted in accordance with generally accepted scientific principles	<i>Artemia franciscana</i>	Dimethyltin dichloride	LC ₅₀ (24h): 80.7 mg/L.	GLP compliance not reported.	Hadjispyrou et al. (2001)
A result quoted in a review paper	Aquatic crustacea: <i>Rithropanopeus harrisi</i>	Dimethyltin dichloride	LC ₅₀ (14d): 10-20 mg/L.	GLP compliance not reported.	Eisler (1989)
no information on method (value taken from a review)	<i>Brachionus plicatilis</i>	Dimethyltin dichloride	LC ₅₀ (24h): 74 mg/L.	No GLP compliance	Summer et al. (2003)

paper)					
OECD TG 201	<i>Desmodesmus subspicatus</i>	Dimethyltin dichloride	ErC ₅₀ (72h): 37 mg/L ErC ₉₀ (72h): >110 mg/L EbC ₅₀ (72h): 11.8 mg/L EbC ₉₀ (72h): 47 mg/L All values based on measured (geom. mean) concentrations.	GLP compliant	Author not disseminated (2003). Report date 2003-09-08.
Non-standard test method	<i>Scenedesmus obliquus</i> and <i>Platyrnonas</i> sp.	Dimethyltin dichloride	<u><i>S. obliquus</i></u> : ErC ₅₀ (96h) = 805.84 ng/L 0.000806 mg/L <u><i>Platyrmonas</i> sp.</u> : ErC ₅₀ (96h) = 988.69 ng/L 0.000988 mg/L Based on nominal concentrations.	No GLP compliance	Huang et al. (1996)
Non-standard test method	<i>Scenedesmus quadricauda</i> , <i>Ankistrodesmus falcatus</i> and <i>Anabaena flos-aquae</i>	Dimethyltin dichloride	<u><i>S. quadricauda</i></u> : IrC ₅₀ (4h) = 4.1 mg/L <u><i>A. falcatus</i></u> : IrC ₅₀ (4h) = 21 mg/L <u><i>A. flos-aquae</i></u> : IrC ₅₀ (4h) > 5 mg/L	No GLP compliance	Wong et al. (1982)
OECD TG 201	<i>Skeletonema costatum</i>	Dimethyltin dichloride	EbC ₅₀ (72h) > 9.8 mg/L (cell number) EbC ₅₀ (96h) > 9.8 mg/L (cell number) ErC ₅₀ (72h) > 9.8 mg/L (growth rate) ErC ₅₀ (96h) > 9.8 mg/L (growth rate) Based on nominal concentrations.	GLP compliant	Author not disseminated (1996). Report date 1996-03-12.
Non-standard test method	<i>Scenedesmus obliquus</i>	Dimethyltin dichloride	ErC ₅₀ (96h) = 1118.4 µg/L = 1.118 mg/L based on nominal conc.	No GLP compliance	Huang et al. (1993)
No methodological information reported	<i>Skeletonema costatum</i> and <i>Thalassiosira pseudonana</i>	Dimethyltin dichloride	EC ₅₀ (72h) > 0.93 mg/L	GLP compliance not reported	based on secondary source (Dobson et al. (2006))

The study results that were the basis for the proposed classification are highlighted in bold letters in the Table above.

Acute (short-term) toxicity to fish

Three acute toxicity studies in fish are available.

The study in zebrafish (*D. rerio*) was performed according to the OECD TG 203 and GLP principles. Several minor deviations from the standard protocol were reported (deviations in aeration regime, storage, temperature, etc.) but were assumed to have no major impact on the results of the study. Only one concentration of the test substance was applied and no mortality was observed in control or at 100 mg/L exposure to dimethyltin dichloride during the 96-h study. The test fulfilled the validity criteria as required in the relevant OECD Guideline.

The acute toxicity study in red killifish (*Oryzias latipes*) was also performed according to OECD TG 203. The fish were exposed to five concentrations of dimethyltin dichloride for 96 h. The LC₅₀ (48 h) was reported to be 6 mg/L. For unknown reasons, the 96-h LC₅₀ value was not reported. The overall documentation and reporting of the study methods and results was not sufficient, namely information on

exposure conditions, data on controls, analytical monitoring and maintenance of test concentrations, etc. is missing. Furthermore, both section 4.1.1.3 of the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and the relevant OECD Guideline 203 indicate that a 96 hour LC₅₀ value should be reported. As such, the study results were not considered relevant for classification purposes.

The third acute toxicity study in fish (*Misgurnus fossilis*) was not performed according to any standard protocols and GLP principles. The test type, exposure duration, analytical monitoring methods have not been described, whilst no LC₅₀ value was reported. As such, the study results were not considered relevant for classification purposes.

Acute (short-term) toxicity to aquatic invertebrates

Six studies are available on acute toxicity of dimethyltin dichloride to aquatic invertebrates.

One study followed the OECD Guideline 202 and GLP principles. The study is well reported and considered as fully reliable and EC₅₀(48h) for *D. magna* was 17 mg/L.

Due to methodological and reporting deficiencies (e.g. shorter test duration, no information on analytical monitoring, etc.) the results from the other five studies on aquatic invertebrates have only been considered in a supportive capacity, for classification purposes. It is noted that the EC₅₀ values derived from these studies ranged between 10 and 88 mg/L.

Acute (short-term) toxicity to algae or aquatic plants

Two available algae studies were performed according to the OECD Guideline 201 and GLP principles. Both studies have been considered as relevant for classification purposes

Firstly, in the 72-h study, *Desmodesmus subspicatus* was exposed to five concentrations (0, 1.1, 3.6, 11, 36 and 111 mg/L) of dimethyltin dichloride. The resulting toxicity values were ErC₅₀(72h): 37 mg/L (growth rate) and EbC₅₀(72h): 11.8 mg/L (biomass) based on the mean measured concentration of the substance.

Secondly, in the 96-h study, *Skeletonema costatum* was exposed to five nominal concentrations (0.098, 0.49, 0.98, 4.9, and 9.8 mg/L) of dimethyltin dichloride. The recorded acute toxicity was too low to define reliable EC₅₀ values (both cell numbers and growth rate).

The remaining algae studies were not performed according to internationally recognised study guidelines, there was no analytical monitoring, no information on results other than the toxicity values and their reporting was limited. Consequently the study results were not considered relevant for classification purposes. It is noted that the EC₅₀ values derived from these studies ranged between 0.8 µg/L and 4.1 mg/L.

Acute (short-term) toxicity to other aquatic organisms

Not available.

9.1.6 Long-term aquatic hazard

Table 43: Summary of relevant information on chronic aquatic toxicity

Method, test guideline, and deviation(s) if any	Species	Test material	Results	Remarks	Reference
OECD TG 201	<i>Desmodesmus subspicatus</i>	Dimethyltin dichloride	NOErC (72h): 1.1 mg/L ErC ₁₀ (72h): 5.7 mg/L EbC ₁₀ (72h): 2.9 mg/L NOEC not defined for the biomass endpoint. All values based on measured (geom. mean) concentrations.	GLP compliant	Author not disseminated (2003). Report date 2003-09-08.
OECD TG 201	<i>Skeletonema costatum</i>	Dimethyltin dichloride	NOEbC(72h) = 4.9 mg/L (cell number) NOEbC(96h) = 0.98 mg/L (cell number) NOErC(72h) = 4.9 mg/L (growth rate) NOErC(96h) = 4.9 mg/L (growth rate) Based on nominal concentrations.	GLP compliant	Author not disseminated (1996). Report date 1996-03-12.

Chronic toxicity to fish

Not available.

Chronic toxicity to aquatic invertebrates

Not available.

Chronic toxicity to algae or aquatic plants

NOEC and ErC₁₀ values are available for two algae studies performed according to the OECD Guideline 201 and GLP principles (the same studies as for acute toxicity). For *Desmodesmus subspicatus* the defined NOErC (72h) was 1.1 mg/L and ErC₁₀ (72h) was 5.7 mg/L (growth rate). For *Skeletonema costatum* NOErC (72h) was 4.9 mg/L based on nominal concentration. Both studies are considered relevant to be used for classification purposes.

Chronic toxicity to other aquatic organisms

Not available.

9.1.7 Comparison with the GHS criteria for hazardous to the aquatic environment

Acute aquatic hazard

There are several acute toxicity studies available for all trophic levels (see section 9.1.5 and Table 42 for more details).

In fish, only the study performed according to the OECD TG 203 and GLP principles and a reported 96h LC50 value of above 100 mg/L was deemed relevant to be used for classification purposes.

In crustacea, only the study performed according to OECD TG 202 and GLP principles and a reported 48h EC50 value of 17 mg/L in *Daphnia magna* was deemed relevant to be used for classification purposes. This was also the basis for the proposed acute classification.

In algae, two studies were performed according to OECD TG 201. The lowest concentration for which toxicity was observed was 37 mg/L.

Comparing the information derived from the most reliable and fully documented acute aquatic toxicity studies available with the GHS criteria (Table 4.1.1.(a)), a classification as Category Acute 3 [acute aquatic toxicity value above 10 but below (or equal to) 100 mg/L] applies.

Long-term aquatic hazard (including bioaccumulation and degradation)

Bioaccumulation

The available information on bioaccumulation (experimental log Kow = -2.18; two non-guideline studies showing BCF from 1.6 to 50) indicate that dimethyltin dichloride has low potential for bioaccumulation since the substance does not meet the GHS criteria (log Kow \geq 4 or BCF \geq 500) to be considered potentially bioaccumulative.

Rapid degradation

The available studies on hydrolysis (DT₅₀ > 1 year) and ready biodegradability (degradation ~ 3% in the 35 d extended test according to OECD Guideline 301F) indicate that dimethyltin dichloride should be considered as not rapidly degradable according to the GHS criteria.

Chronic aquatic toxicity

As mentioned in section 9.1.6, chronic aquatic toxicity of dimethyltin dichloride is available only for one trophic level: algae. As both studies were deemed as relevant for classification purposes, the results from the most sensitive one [NOErC (72h) = 1.1 mg/L] were considered in classification for long-term aquatic hazard. This results in no classification for long-term aquatic hazards.

Table 4.1.1 (b) (i) of GHS applies and based on this no chronic classification should be appointed. However, since there are no adequate chronic data available for fish and crustacea, the surrogate approach should be considered for those trophic levels. Based on the acute toxicity in *D. magna* (EC50(48h) = 17 mg/L) and non-rapid degradation, the classification based on Table 4.1.1 (b) (iii) applies and the substance should be classified in Category Chronic 3. Since this is more stringent than the classification based on the available chronic data, dimethyltin dichloride should be classified as Category Chronic 3 for long term (chronic) hazard according to GHS.

9.1.8 Conclusion on classification and labelling for hazardous to the aquatic environment

Based on the presented information classification as Category Acute 3 and Category Chronic 3 are proposed for dimethyltin dichloride.

9.2 HAZARDOUS TO THE OZONE LAYER

9.2.1 Conclusion on classification and labelling for hazardous to the ozone layer

Dimethyltin dichloride is not included in *The Montreal Protocol on Substances that Deplete the Ozone Layer* and therefore it is not proposed to be classified in this hazard class.

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JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY**

**ANNEX 1 TO THE PROPOSAL FOR CLASSIFICATION AND LABELLING (C&L) OF
DIMETHYLTIN DICHLORIDE**

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**Joint Pilot Project of the OECD and the UN Sub-Committee of Experts on the Globally Harmonised
System of Classification and Labelling of Chemicals**



INTER-ORGANIZATION PROGRAMME FOR THE SOUND MANAGEMENT OF CHEMICALS

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ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT
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FOREWORD

This document is Annex 1 to the Report on the Proposal for Classification and Labelling (C&L) of Dimethyltin Dichloride.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

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AIM OF ANNEX I TO THE C&L REPORT

The aim of Annex I is to provide detailed study summaries, transparently and objectively as in the original data source, without subjective interpretations.

Please note that the term “registrant” in the following text, means the registrant that has submitted the registration dossier as required according to Regulation (EC) No 1907/2006 of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

For data sources, please see section 4 in the C&L report.

1. PHYSICAL HAZARDS

1.1.Explosives

[Study 1]

Study reference:

No studies are available.

According to the registrant it can be concluded from the structural formula that the substance is not explosive as it does not have functional groups associated with explosivity.

Detailed study summary and results:

Not applicable as no studies are available.

1.2.Flammable gases

[Study 1]

Study reference:

Not applicable.

Detailed study summary and results:

Substance is a solid.

1.3.Aerosols

[Study 1]

Study reference:

Not applicable.

Detailed study summary and results:

Substance is a solid.

1.4.Oxidising gases

[Study 1]

Study reference:

Not applicable.

Detailed study summary and results:

Substance is a solid.

1.5.Gases under pressure

[Study 1]

Study reference:

Not applicable.

Detailed study summary and results:

Substance is a solid.

1.6.Flammable liquids

[Study 1]

Study reference:

Not applicable.

Detailed study summary and results:

Substance is a solid.

1.7.Flammable solids

[Study 1]

Study reference:

Author not disseminated (2013). Report date 2013-01-21. REACH registration dossier.

Detailed study summary and results:

Identification: Dimethyltin dichloride

Description: white solid block

Expiry / Retest Date: 01 June 2013

Storage Conditions: room temperature in the dark.

Study Summary: A mould (250 mm long x 20 mm wide x 10 mm high) was loosely filled with test item (previously broken up to form a white powder). A non-combustible, non-porous board was placed onto the mould which was then inverted. The mould was removed and an air-rich Bunsen burner flame applied to one end of the pile for two minutes.

If the test item does not ignite and propagate combustion either by burning with flame or smoldering along 200 mm within 4 minutes, the test item is not considered as highly flammable and no further testing is required.

If the test item propagates burning of a 200 mm length in less than 4 minutes, the main test is performed.

Material and methods:

- Preliminary test.

Test substance 94.5% purity.

Testing was conducted using a procedure designed to be compatible with Method A10 Flammability (Solids) of Commission Regulation (EC) No 440/2008 of 30 May 2008. The screening test for EU A.10 requires that the flame is applied for a maximum of 2 minutes, the same as the test method N1 described in the UN Recommendations on the TDG, Manual of tests and criteria, Part III, sub-section 33.2.1 used as the basis for the GHS criteria.

Results:

The flammability (solids) was determined by measuring the burning rate of test item prepared as a pile of set dimensions. The pile failed to ignite during the 2 minutes that the Bunsen flame was applied. The result of the preliminary screening test obviated the need to perform the main test.

The test item has been determined to be not highly flammable as it failed to ignite in the preliminary screening test.

1.8. Self-reactive substances

[Study 1]

Study reference:

No studies are available.

Detailed study summary and results:

No studies are available.

1.9. Pyrophoric liquids

[Study 1]

Study reference:

Not applicable.

Detailed study summary and results:

Substance is a solid.

1.10. Pyrophoric solids

[Study 1]

Study reference:

No studies are available.

Detailed study summary and results:

According to the registrant, in accordance with point 1, Annex XI of Regulation (EC) No. 1907/2006 (REACH), based on the known chemical and physical properties of the substance, its chemical structure and experience in handling the substance, testing in line with Method A13: Pyrophoric properties of solids and liquids of Commission Directive 92/69/EEC is expected to produce a negative results.

1.11. Self-heating substances

[Study 1]

Study reference:

Author not disseminated (2013). Report date 2013-01-21. REACH registration dossier.

Detailed study summary and results:

Testing was conducted using a procedure designed to be compatible with Method A.16 Relative Self-Ignition Temperature for Solids of Commission Regulation (EC) No 440/2008 of 30 May 2008. The test item has been determined not to have a relative self-ignition temperature below its melting temperature.

The test item was heated in a fine mesh stainless steel cube that was suspended in an oven. The temperature/time-curves of both test item and oven were recorded.

Apparatus: Oven: A temperature programmed laboratory oven with a Eurotherm temperature controller. Wire mesh cube: A piece of stainless steel wire mesh with 0.042 mm openings was cut, folded and secured with wire into an open topped cube with dimensions of 2 x 2 x 2 cm. Thermocouples: Type K thermocouples. Recorder: Graphtec GL200a data logger

Performance of the Test: The cube was completely filled with the test item and placed in the center of the oven at room temperature. A thermocouple was placed in the center of the sample and another in the oven. The oven temperature was programmed to increase from ambient to 117 °C (which was approximately 10 °C higher than the melting temperature supplied by the Sponsor) at a rate of 0.5 °C/min. The temperature/time curves relating to the condition in the center of the sample and the oven were recorded on a data logger. Before and after the test the appearance of the test item was recorded.

Data Handling: The self-ignition temperature, as obtained by this method, is the minimum ambient temperature expressed in [°C] at which a certain volume of a substance ignites under defined conditions. Temperature measurement data and any heat effects observed between room temperature and about 117°C were evaluated. If an exothermic reaction of the test item appears, the sample thermocouple shows a sharp temperature rise above the oven temperature. The temperature of the oven at which the sample reaches 400 °C by c-heating is stated as the self-ignition temperature of the test item.

1.12. Substances which in contact with water emit flammable gases

[Study 1]

Study reference:

No studies are available.

According to the registrant, in accordance with point 1, Annex XI of Regulation (EC) No. 1907/2006 (REACH), based on the known chemical and physical properties of the substance, its chemical structure and experience in handling the substance, testing in line with Method A.12: Flammability (contact with water) of Commission Directive 92/69/EEC is expected to produce a negative results.

Detailed study summary and results:

Not applicable as no studies are available.

1.13. Oxidising liquids

[Study 1]

Study reference:

Not applicable.

Detailed study summary and results:

Substance is a solid.

1.14. Oxidising solids

[Study 1]

Study reference:

No studies are available.

Detailed study summary and results:

Not applicable as no studies are available.

1.15. Organic peroxides

[Study 1]

Study reference:

Not applicable.

Detailed study summary and results:

Substance is not an organic peroxide.

1.16. Corrosive to metals

[Study 1]

Study reference:

No studies are available.

Detailed study summary and results:

No studies are available.

1.17. Desensitized explosives

[Study 1]

Study reference:

Not applicable.

Detailed study summary and results:

Not applicable.

2. TOXICOKINETICS

[Study 1]

Study reference:

Reference type: study report.

Author not disseminated (2001).

Report date 2001-09-05.

Materials and methods

Type of method: In vivo.

Objective of study: Toxicokinetics.

Test guideline: OECD TG 417 (Toxicokinetics).

Principles of method if other than guideline: To determine the absorption and urinary elimination profiles of dimethyltin dichloride in rats after a single oral dose as compared to an intravenous dose.

Three protocol deviations occurred during the study:

1. The protocol stated that the temperature and relative humidity ranges for the animal rooms would be 72° ± 4°F and 30% to 70%, respectively. On four days, a temperature of 78-79°F was measured. On six days a relative humidity of 71-74% was measured.
2. The protocol stated that feeders will be changed and sanitized at least once per week. There were two weeks where this was not documented.
3. Several blood samples were collected at times that differed from the target times by more than 10%. For these samples, the actual time of collection was used in calculating individual AUC values, however, mean plasma concentrations were based on the target collection times.

These deviations did not negatively impact the quality or integrity of the data nor the outcome of the study.

GLP compliance: Yes.

Test materials

Identity of test material: dimethyltin dichloride.

Test material form: Crystalline.

Radiolabelling: No.

Test animals

Species: Rat.

Strain: Sprague-Dawley.

Sex: Male/female.

Details on test animals and environmental conditions:

TEST ANIMALS

- Source: Charles River Laboratories, Raleigh, NC
- Age at study initiation: 8 weeks (males); 9 weeks (females)
- Weight at dose administration: 226-247 g (males); 212-245 g (females)
- Housing: individually in suspended wire-mesh cages
- Individual metabolism cages: yes (animals designated for urine collection)
- Diet (e.g. ad libitum): PMI Nutrition International, Inc. Certified Rodent LabDiet® 5002 ad libitum.
- Water (e.g. ad libitum): Tap water ad libitum
- Acclimation period: approximately 1 week.

ENVIRONMENTAL CONDITIONS

- Temperature (°F): 71-79
- Humidity (%): 48-71
- Air changes (per hr): at least 10
- Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration: oral and intravenous.

Details on exposure:

PREPARATION OF DOSING SOLUTIONS:

The dosing formulation was prepared by dissolving 0.25 g of the test substance in 50 mL of sterile water for injection, U.S.P. in a plastic, autoclaved injection vial. The vial was sealed with a septum cap and inverted until the solution appeared uniform. Three 1 mL samples were removed from the vial, placed in plastic sample containers, and transferred to the Analytical Chemistry Department at WTI, Research for analysis of total tin. The formulation was homogeneous and contained the appropriate concentration of tin (94.5% of target).

DOSE ADMINISTRATION:

On the day of dose administration, each animal was weighed. The amount of dosing formulation needed for each animal was calculated based on a dose volume of 2 mL/kg. The dose for Groups 1 and 2 was administered as a bolus intravenous dose into a tail vein; the dose for Groups 3 and 4 was administered orally by gavage.

Duration and frequency of treatment / exposure: Single dose.

Doses / concentrations: 10 mg/kg bw (orally and intravenously).

No. of animals per sex per dose: Three.

Control animals: Yes.

Details on dosing and sampling:

TOXICOKINETIC STUDY (Absorption, distribution, excretion)

- Tissues and body fluids sampled: plasma, urine.
- Time and frequency of sampling: Groups 1 (intravenous) and 3 (oral), approximately 0.3 mL of blood was collected from a tail vein of each animal at 10 and 30 minutes and 1, 2, 4, 8, 12, 18, 24, 48, 72, and 96 hours after dose administration. For Groups 2 (intravenous) and 4 (oral), urine was collected from each animal over the following intervals after dose administration: 0-8, 8-16, 16-24, 24-48, 48-72 and 72-96 hours.

Statistics: All calculations for this report were performed with Microsoft® Excel spreadsheets using full floating decimal point calculations. Equations other than those used for toxicokinetic data analysis and for standard statistical parameters (e.g., mean, standard deviation [SD], percent coefficient of variation [%CV], and linear regression) are presented with the tables. Slightly different results can be expected if calculations are based on the values as presented in the tables because some numbers have been rounded for display.

Results and discussions

Main ADME results:

Type

Absorption Based on the mean $AUC_{0-\infty}$ values, comparative bioavailability was 0.52 for males and 0.71 for females.

Distribution For males, mean (\pm SD) apparent volume of distribution was 44.3 (\pm 26.7) L/kg and 52.7 (\pm 31.7) L/kg following intravenous and oral administration, respectively. For females, mean (\pm SD) values were 109 (\pm 92.9) L/kg and 138 (\pm 98.1) L/kg.

Excretion For males, renal clearance was 0.105 and 0.0816 L/h/kg following intravenous and oral administration, respectively. For females, renal clearance was 0.559 and 0.282 L/h/kg following intravenous and oral administration, respectively.

Toxicokinetic studies

Details on absorption:

Concentrations of tin were generally higher in males than in females following the single intravenous dose. Inter-animal variability in the plasma concentrations of tin was high, with coefficients of variation frequently near or exceeding 100%. Concentrations of tin in both males and females typically fluctuated during the first 8 hours after dose administration; t_{max} ranged from 10 minutes to 4 hour post dosing. Mean (\pm SD) C_{max} values were 3834 (\pm 2983) μ g/mL for males and 1088 (\pm 656) μ g/mL for females. Mean (\pm SD) $AUC_{0-\infty}$ values were 51.3 (\pm 53.7) μ g-hour/mL for males and 11.0 (\pm 0.31) μ g-hour/mL for females.

Concentrations of tin were also generally higher in males than in females following the single oral dose. Inter-animal variability in the plasma concentrations of tin tended to be less following oral administration than following intravenous administration. There was also less fluctuation in the concentrations of tin following dosing although the range for t_{max} was high: 10 minutes to 8 hour post-dosing. Mean (\pm SD) C_{max} values were 1255 (\pm 917) μ g/mL for males and 481 (\pm 189) μ g/mL for females. Mean (\pm SD) $AUC_{0-\infty}$ values were 26.7 (\pm 4.3) μ g-hour/mL for males and 7.87 (\pm 0.79) μ g-hour/mL for females. Based on the mean $AUC_{0-\infty}$ values, comparative bioavailability was 0.52 for males and 0.71 for females.

Details on distribution in tissues:

Following the distribution phase, which lasted at least 8 hours regardless of the route of administration, plasma concentrations of tin at 12 hour post-dosing had decreased by approximately an order of magnitude. After this rapid clearance phase, tin was cleared from plasma more slowly. Plasma concentrations of tin during the terminal elimination phase were higher for males than for females, but were similar between the two routes of administration. Terminal elimination rate constants were similar between the two routes of administration. For males, mean (\pm SD) terminal half-lives were 184 (\pm 73) hours and 173 (\pm 80) hours following intravenous and oral administration, respectively. For females, mean (\pm SD) terminal half-lives were 157 (\pm 137) hours and 146 (\pm 114) hours following intravenous and oral administration, respectively. The rapid decrease after the distribution phase and the long terminal half-life suggests that some fraction of tin is retained within the animal that is capable of maintaining an equilibrium with the plasma, at least over the duration examined in this study.

Clearance and apparent volume of distribution were lower for males than for females, but were similar between the two routes of administration. The differences in these parameters between the sexes are consistent with the differences in the $AUC_{0-\infty}$ values. For males, mean (\pm SD) clearance was 0.203 (\pm 0.153) L/hour/kg and 0.204 (\pm 0.033) L/hour/kg following intravenous and oral administration, respectively. For females, mean (\pm SD) clearance was 0.486 (\pm 0.014) L/hour/kg and 0.685 (\pm 0.069) L/hour/kg following intravenous and oral administration, respectively. For males, mean (\pm SD) apparent volume of distribution was 44.3 (\pm 26.7) L/kg and 52.7 (\pm 31.7) L/kg following intravenous and oral administration, respectively. For females, mean (\pm SD) apparent volume of distribution was 109 (\pm 92.9) L/kg and 138 (\pm 98.1) L/kg following intravenous and oral administration, respectively.

Details on excretion:

Following the single intravenous dose, mean (\pm SD) percent of dose (as total tin) eliminated in the urine was 103 (\pm 29.9)% for males and 112 (\pm 43.4)% for females. Urinary elimination of tin appeared to be biphasic, with the initial phase occurring through 24 hour post-dosing. The terminal phase was slower than the initial phase and coincided with the low, relatively constant terminal plasma concentrations.

Following the single oral dose, mean (\pm SD) percent of dose (as total tin) eliminated in the urine was 40.1 (\pm 4.08)% for males and 42.0 (\pm 4.66)% for females. Percent of dose eliminated via the urine following oral dosing was lower than would be expected based on the comparative bioavailability of approximately 50–70%. Urinary elimination of tin following oral dosing appeared to be triphasic: the first phase coincided with the distribution phase, the second phase was similar to the initial phase following intravenous administration, and the third phase was similar to the terminal phase following intravenous administration.

The terminal elimination rate constants for urinary elimination of tin were similar between the sexes and routes of administration. For males, the urinary half-life, based on mean amounts of urine eliminated, was 13 and 11 hours following intravenous and oral administration, respectively. For females, the urinary half-life, based on mean amounts of

urine eliminated, was 10 hours regardless of route of administration. Renal clearance was lower in males than in females and lower following oral administration than following intravenous administration because of the differences in the $AUC_{0-\infty}$ values. For males, renal clearance was 0.105 and 0.0816 L/hour/kg following intravenous and oral administration, respectively. For females, renal clearance was 0.559 and 0.282 L/hour/kg following intravenous and oral administration, respectively.

Toxicokinetic parameters:

C_{max} : Intravenous dosage to males: 3834 (± 2983) ng/ml.

C_{max} : Intravenous dosage to females: 1088 (± 656) ng/ml.

C_{max} : Oral dosage to males: 1255 (± 917) ng/ml.

C_{max} : Oral dosage to females: 481 (± 189) ng/ml.

AUC: Intravenous dosage to males: 51327 (± 53673) ng-hr/ml.

AUC: Intravenous dosage to females: 11032 (± 306) ng-hr/ml.

AUC: Oral dosage to males: 26675 (± 4301) ng-hr/ml.

AUC: Oral dosage to females: 7868 (± 794) ng-hr/ml.

Half-life 1st: Intravenous dosage to males: 184 (± 73) hr⁻¹.

Half-life 1st: Intravenous dosage to females: 157 (± 137) hr⁻¹.

Half-life 1st: Oral dosage to males: 173 (± 80) hr⁻¹.

Half-life 1st: Oral dosage to females: 146 (± 114) hr⁻¹.

Metabolite characterisation studies

Metabolites identified: not measured.

Bioaccessibility

Any other information on results incl. tables:

Table 1: Summary of Pharmacokinetic Parameters for Tin in Rat Plasma following Administration of 10 mg/kg Dimethyltin Dichloride

C_{max}(ng/mL)				
	Intravenous		Oral	
	Mean	SD	Mean	SD
Males	3834	2983	1255	917
Females	1088	656	481	189

AUC_{0-∞}(ng-hr/mL)				
	Intravenous		Oral	
	Mean	SD	Mean	SD
Males	51327	53673	26675	4301
Females	11032	306	7868	794

Elimination Rate Constant(hr⁻¹)				
	Intravenous		Oral	
	Mean	SD	Mean	SD
Males	0.00412	0.00133	0.00449	0.00208
Females	0.00712	0.00620	0.00685	0.00536

Half Life(hr)				
	Intravenous		Oral	
	Mean	SD	Mean	SD
Males	184	73	173	80
Females	157	137	146	114

Clearance(L/hr/kg)				
	Intravenous		Oral	
	Mean	SD	Mean	SD
Males	0.203	0.153	0.204	0.0328
Females	0.486	0.0135	0.685	0.0691

Apparent Volume of Distribution(L/kg)				
	Intravenous		Oral	
	Mean	SD	Mean	SD
Males	44.3	26.7	52.7	31.7
Females	109	92.9	138	98.1

Table 2: Mean Tin Plasma Concentrations and Pharmacokinetic Parameters Based on Mean Values in Rats following Intravenous or Oral Administration of 10 mg/kg Dimethyltin Dichloride

Group Summary				
Route	Intravenous		Oral	
Sex	Male	Female	Male	Female
Mean (\pmSD) Plasma Concentrations (ng/mL)*				
10 min	2955 (3705)	334 (228)	702 (570)	387 (259)
30 min	1593 N/A	680 (455)	533 (406)	301 (67.1)
1 hr	2057 (2126)	330 (32.5)	547 (333)	327 (101)
2 hr	2213 (1889)	1032 (749)	685 (459)	376 (85.6)
4 hr	1545 (1616)	695 (468)	1396 (628)	382 (89.5)
8 hr	3874 (3883)	508 (262)	1169 (852)	285 (43.1)
12 hr	75.1 (55.3)	20.1 (3.14)	73.0 (65.3)	19.9 (2.12)
18 hr	94.1 (86.5)	21.4 (21.2)	77.0 (74.0)	21.7 (6.27)
24 hr	87.8 (63.7)	27.0 (9.69)	78.0 (62.5)	26.1 (18.5)
48 hr	51.9 (27.1)	13.5 (12.4)	71.7 (61.9)	16.9 (5.90)
72 hr	104 (112)	11.9 (10.3)	60.8 (49.4)	16.7 (8.55)
96 hr	59.2 (48.6)	5.94 (10.3)	59.2 (39.1)	15.3 (3.77)
Linear Regression of log Plasma Concentrations vs. Time**				
Slope	-0.00146	-0.00745	-0.00168	-0.00253
Y-intercept (ng/mL)	91.2	34.1	84.1	25.6
Coefficient of Determination (r^2)	0.130	0.919	0.943	0.745
Pharmacokinetic Parameters				
C _{max} (ng/mL)	3874	1032	1396	387
t _{max} (hr)	0.5	2	4	0.17
AUC _{0-∞} (ng-hr/mL)	50710	7941	31896	7557
Comparative Bioavailability	N/A	N/A	0.63	0.95
K _{el} (hr ⁻¹)	0.00337	0.0172	0.00387	0.00583
Half-life (hr)	206	40	179	119
Cl (L/hr/kg)	0.106	0.675	0.168	0.709
V _d (L/kg)	31.4	39.4	43.5	122

* Individual values below the limit of quantification were treated as zero

N/A = not applicable

** For terminal phase: 18 hr post dosing to last point above the quantification limit

Table 3: Summary of Percent of Dose Eliminated in Urine following Administration of 10 mg/kg Dimethyltin Dichloride to Rats*

INTRAVENOUS		
Collection Interval	Mean % of Dose (\pm SD)	
	Males	Females
0 – 8 hr	82.2 (27.7)	84.9 (38.3)
8 – 16 hr	17.8 (4.20)	18.0 (0.77)
16 – 24 hr	2.51 (1.21)	7.65 (4.40)
24 – 48 hr	0.71 (0.18)	1.08 (0.37)
48 – 72 hr	0.27 (0.10)	0.36 (0.10)
72 – 96 hr	BLQ N/A	BLQ N/A
TOTAL:	103 (29.9)	112 (43.4)
ORAL		
Collection Interval	Mean % of Dose (\pm SD)	
	Males	Females
0 – 8 hr	13.0 (3.80)	17.8 (3.13)
8 – 16 hr	20.6 (0.33)	19.1 (3.15)
16 – 24 hr	5.75 (1.35)	4.01 (0.58)
24 – 48 hr	0.60 (0.05)	0.83 (0.17)
48 – 72 hr	0.17 (0.04)	0.20 (0.11)
72 – 96 hr	BLQ N/A	BLQ N/A
TOTAL:	40.1 (4.08)	42.0 (4.66)

* Means based on 3 animals per sex. BLQ = below the limit of quantification (5 ng/mL). N/A = not applicable

Table 4: Kinetics of the Urinary Elimination of Tin following Intravenous Administration of 10 mg/kg Dimethyltin Dichloride to Rats

Amount Eliminated per Interval (µg)*								
	Males				Females			
Collection Interval	50040	50048	50053	Mean (±SD)	50058	50069	50071	Mean (±SD)
0 – 8 hr	664	1041	1348	1018 (343)	1526	997	591	1038 (469)
8 – 16 hr	164	266	232	220 (52.0)	227	223	209	220 (9.4)
16 – 24 hr	48.3	20.6	24.4	31.1 (15.0)	156	66.4	58.7	93.5 (53.8)
24 – 48 hr	6.26	9.60	10.3	8.74 (2.18)	17.2	8.39	14.2	13.3 (4.47)
48 – 72 hr	2.14	3.28	4.53	3.32 (1.20)	5.79	3.78	3.85	4.39 (1.22)
72 – 96 hr	BLQ	BLQ	BLQ	BLQ N/A	BLQ	BLQ	BLQ	BLQ N/A
Amount Remaining To Be Eliminated (ARE; µg)								
0 hr	884	1340	1619	1281	1932	1299	877	1369
8 hr	221	299	271	264	406	301	286	331
16 hr	56.7	33.5	39.3	43.2	178	78.6	76.4	111
24 hr	8.40	12.9	14.9	12.1	23.0	12.2	17.8	17.6
48 hr	2.14	3.28	4.53	3.32	5.79	3.78	3.58	4.39
Elimination Kinetics (Linear Regression of log ARE vs. Time from 24-48 hr)								
Slope (b)	-0.0247	-0.0247	-0.0215	-0.0233	-0.0249	-0.0212	-0.0290	-0.0290
Y-Intercept (µg)	33.0	50.6	48.9	43.8	91.0	39.2	88.4	88.4
Coefficient of Determination (r ²)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Elimination rate Constant (hr ⁻¹)	0.0570	0.0570	0.0495	0.0537	0.0574	0.0487	0.0668	0.0668
Half-life (hr)	12	12	14	13	23	14	10	10
Cl _r (L/hr/kg)**	N/A	N/A	N/A	0.105	N/A	N/A	N/A	0.559

* BLQ = below the limit of quantification (5 ng/mL).

** Renal clearance only calculated using mean values

N/A =not applicable

Table 5: Kinetics of the Urinary Elimination of Tin following Oral Administration of 10 mg/kg Dimethyltin Dichloride to Rats

Amount Eliminated per Interval (µg)*								
	Males				Females			
Collection Interval	50040	50048	50053	Mean (±SD)	50058	50069	50071	Mean (±SD)
0 – 8 hr	186	113	206	168 (49.1)	255	206	181	214 (37.6)
8 – 16 hr	264	263	271	266 (4.27)	258	187	244	230 (37.8)
16 – 24 hr	54.4	81.6	86.8	74.2 (17.4)	41.5	55.4	47.6	48.2 (6.98)
24 – 48 hr	7.62	8.57	7.25	7.82 (0.68)	9.15	8.44	12.2	9.93 (2.01)
48 – 72 hr	1.59	2.52	2.56	2.22 (0.55)	3.17	3.20	0.98	2.45 (1.28)
72 – 96 hr	BLQ	BLQ	BLQ	BLQ N/A	BLQ	BLQ	BLQ	BLQ N/A
Amount Remaining To Be Eliminated (ARE; µg)								
0 hr	513	468	573	518	567	460	486	504
8 hr	327	355	367	350	312	254	305	290
16 hr	63.6	92.7	96.6	84.3	54	67.1	60.8	60.6
24 hr	9.22	11.1	9.8	10.0	12.3	11.6	13.2	12.4
48 hr	1.59	2.52	2.56	2.22	3.17	3.20	0.98	2.45
Elimination Kinetics (Linear Regression of log ARE vs. Time from 24-48 hr)								
Slope (b)	-0.0318	-0.0268	-0.0243	-0.0273	-0.0246	-0.0233	-0.0471	-0.0293
Y-Intercept (µg)	53.3	48.9	37.6	45.3	47.9	42.3	178	62.6
Coefficient of Determination (r ²)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Elimination rate Constant (hr ⁻¹)	0.0732	0.0618	0.0560	0.0628	0.0566	0.0537	0.109	0.0675
Half-life (hr)	9.5	11	12	11	12	13	6.4	10
Cl _r (L/hr/kg)**	N/A	N/A	N/A	0.0816	N/A	N/A	N/A	0.282

* BLQ = below the limit of quantification (5 ng/mL).

** Renal clearance only calculated using mean values

N/A =not applicable

Reliability score 1 (reliable without restriction) given by the registrant because the study was conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of the relevant results.

[Study 2]

Study reference:

Reference type: publication.

Author: Noland, E.A., McCauley, P.T. & Bull, R.J.

Year: 1983.

Title: Dimethyltin dichloride: Investigations into its gastrointestinal absorption and transplacental transfer.

Bibliographic source: Journal of Toxicology and Environmental Health, 12:89-98.

Materials and methods

Type of method: in vivo.

Objective of study: absorption, distribution.

Test guideline: no guideline followed.

Principles of method if other than guideline:

The present work was undertaken to determine whether the organic tin was absorbed by the dam and crossed the placenta to pup blood and brain, and to determine when and how rapidly tin passes from dam to pup. This was accomplished in three phases:

- 1.a comparison of absorption of organic and inorganic tin,
- 2.a comparison of prenatal and postnatal levels of tin in the pups, and
- 3.a tracer study to determine if the organic tin passed to the pup in-tact.

GLP compliance: no according to the registrant.

Test materials

Identity of test material: dimethyltin dichloride.

Test material form: crystalline.

Radiolabelling: yes, phase III only.

Details on test material:

- Radiochemical purity (if radiolabelling): 100 μ Ci.
- Specific activity (if radiolabelling): 6.9 mCi/mM.
- Locations of the label (if radiolabelling): methyl 14 C.

Test animals

Species: rat.

Strain: Sprague-Dawley.

Sex: female.

Details on test animals and environmental conditions:

TEST ANIMALS:

- Source: Charles Rivers breeding laboratory.
- Age at study initiation: 70 days old.
- Housing: animals were housed one per cage with sawdust bedding in a room.
- Individual metabolism cages: yes.
- Diet (e.g. ad libitum): Purina Lab Chow ad libitum.
- Water (e.g. ad libitum): ad libitum.

ENVIRONMENTAL CONDITIONS:

- Temperature: 22 °C.
- Humidity: 54 %.

Administration / exposure

Route of administration: drinking water (phases I and II); oral: gavage (phase III).

Vehicle: water.

Duration and frequency of treatment / exposure: Two weeks prior to breeding and continued through breeding and gestation.

Doses / concentrations:

40 mg tin/L (Phases I and II)

0.8 mL/100 g bw (Phase III)

No. of animals per sex per dose: 13 females (phases I and II); 19 pregnant females (phase III).

Control animals: yes, concurrent vehicle.

Details on study design: In Phases I and II animals received test material as 40 mg/L in solution two weeks prior to breeding and through breeding and gestation.

In Phase III animals received a 0.9 M NaCl solution containing 100 µCi/L. Four animals were sacrificed by decapitation at each of 5, 15 and 30 minutes, 1, 2, 6 and 24 hours after dosing.

Details on dosing and sampling:

TOXICOKINETIC STUDY (Absorption, distribution, excretion):

- Tissues and body fluids sampled: blood and brains from pups removed from dams and blood from dams from phases I and II; brains and blood from dams and foetuses from phase III.

- Time and frequency of sampling: pups removed from dams prior to first nursing in both phases.

Statistics:

Data for the first phase were analysed using a Kruskal-Wallis test, and the means were compared with a non-parametric multiple comparisons procedure based on Kruskal-Wallis ranked sums. Data for the cross-fostering phase were analysed using an analysis of variance (ANOVA). Drinking-water consumption data were analysed using a two-way nested ANOVA.

Results and discussions

Details on distribution in tissues:

The majority of the tin received as dimethyltin is transferred to the pups during gestation rather than lactation.

Transfer into organs:

Transfer type: blood/brain barrier. Observation: distinct transfer.

Transfer type: blood/placenta barrier. Observation: distinct transfer.

Metabolite characterisation studies

Metabolites identified: not measured.

Bioaccessibility

Phase I

In the dams' blood there were significant differences among dosage groups. A non-parametric multiple comparison showed the tin content from treated animals given DMDC was significantly higher than the control. Analysis of pup blood and brain yielded similar results. The tin content in blood from pups of treated dams was significantly higher than blood from pups of the control. The tin content from the brains of pups of treated dams was significantly higher than controls. Dam body weights did not differ throughout the experiment. Dams exposed to test material drank significantly less than controls (see Table 1).

Phase II

The level of tin in the blood of dams remained constant and the levels in test material exposed dams was significantly higher than that of the controls. The highest levels of tin in blood at any time in any age group were demonstrated at birth in gestationally exposed animals. By 10 day post-natal age, blood tin levels decreased rapidly in both prenatally exposed groups. Pups exposed only postnatally (CT/DM) had blood levels of tin significantly lower than the DM/DM and the DM/CT animals. Both the CT/CT and CT/DM groups were found to be statistically lower than the other two groups but not different from each other. At 21 days, all four groups were different from each other.

Tin levels in the brains of prenatally exposed pups were again highest at birth and different from controls. At 10 days, the DM/DM pups demonstrated significantly higher levels of tin in the brain than the other groups. The DM/CT group was also different from the other groups. At 21 days, the DM/DM group was significantly higher than the CT/DM group, which was higher than either the CT/CT or DM/CT group.

Phase III

The highest measured level of labelled material in the dam blood was recorded at 1 hour, while the level in the brain continued to increase through 24 hours when measurements were stopped. The label in the pup blood reached its highest measured level at 6 hours and in the brain the highest concentration measured was the last sample taken at 24 hours after exposure. The level of DPM/g in dam brain at 24 hours post dosing were equivalent to DPM/g in pup brain at 6 hour post dose.

Table 1: Results of the Kruskal-Wallis Multiple Comparison for Tissue Tin Content

Dose (mg tin/l)	Dam blood		Pup blood		Pup brain	
	Mean (µg/g)	Group ^a	Mean (µg/g)	Group ^a	Mean (µg/g)	Group ^a
Dimethyltin dichloride (40 mg/l)	10.68	A	14.63	A	1.17	A
Distilled water control	0.14	B	0.20	B	0.32	B
Overall	$\chi^2 = 23.8$, df = 2, p < 0.0001		$\chi^2 = 24.7$, df = 2, p < 0.0001		$\chi^2 = 23.1$, df = 2, p < 0.0001	

^a Means with the same group letter are not statistically different

Reliability score 2 (reliable with restrictions) given by the registrant because the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

[Study 3]

Study reference:

Reference type: study report.

Author not disseminated (1999).

Report date: 1999-03-31.

Materials and methods

Type of method: in vitro.

Test guideline: according to OECD TG 428 (Skin Absorption: In Vitro Method) Draft dated 1996.

Deviations: no data.

GLP compliance: yes (incl. certificate).

Test materials

Radiolabelling: no.

Identity of test material: Purity: 89% dimethyltin dichloride, 11% monomethyltin dichloride.

Test material form: crystalline.

Details on test material: Methyltin chloride mixture containing 11% monomethyltin trichloride and 89% dimethyltin dichloride (DMTC) - tin content: 53.5% w/w.

Test animals

Species: human skin and rat skin.

Strain: Sprague-Dawley.

Sex: male.

Details on test animals and environmental conditions:

Human skin:

Extraneous tissue was removed from human whole skin samples. The skin samples were immersed in water at 60°C for 40-45 seconds and the epidermis teased off the dermis. Each epidermal membrane was given an identifying number and stored frozen on aluminium foil until required for use.

Rat skin:

Skin was from male rats of the Wistar-derived strain (supplied by Charles River UK Ltd, Margate, Kent, UK.), aged 28 days \pm 2 days. Fur from the dorsal and flank region was carefully shaved using animal clippers, ensuring that the skin was not damaged. The clipped area was excised and any subcutaneous fat removed. The skins were soaked for approximately 20 hours in 1.5M sodium bromide then rinsed in distilled water. The epidermis was carefully peeled from the dermis. Each epidermal membrane was given an identifying number and stored frozen on aluminium foil until required for use.

Administration / exposure

Type of coverage: dermal absorption was determined under both occluded and unoccluded conditions.

Vehicle: ethanol.

Duration of exposure: 24 hrs.

Doses: Based on results of three experiments to determine a non-damaging dosage, the methyltin chloride mixture was applied to both human and rat epidermal membranes at a rate of 100 µg/cm² as a 10 µl/cm² dose of a 10000 µg/ml solution in ethanol. This application was equivalent to a nominal dose rate for tin of 53.5 µg/cm², based upon a tin content of 53.5% w/w in the methyltin chloride mixture, or 89 µg/cm² based on DMTC content.

No. of animals per group: 6 cells per species.

Details on in vitro test system (if applicable):

MEASUREMENT OF MEMBRANE INTEGRITY:

Samples of epidermis were mounted in glass diffusion cells with an exposed area of 2.54 cm². The cells were placed in a water bath maintained at 32 ± 1 °C.

The integrity of the membranes was determined by measurement of their electrical resistance across the skin membrane. Membranes with a measured resistance <10 kΩ (human) or <2.5 kΩ (rat) were regarded as having a lower integrity than normal and not used for exposure to the test material.

ASSESSMENT OF SKIN BARRIER DAMAGE:

This assessment was only performed for human epidermis.

The receptor chambers of 3 cells containing intact membranes were filled with a recorded volume of receptor fluid (water) and the cells placed in a waterbath maintained at 32 ± 1°C. The methyltin chloride mixture was applied undiluted to these cells at a rate of 0.1 g/cm² and the cells occluded for the entire exposure period (24h). Concurrently with these experiments, 2 untreated control cells were assessed under identical conditions as the treated cells.

After the 24h contact period, the donor chamber were emptied and the surface of the membrane decontaminated by flushing with water (5 x 5 mL volumes), followed by physiological saline (5 mL). The receptor chambers were emptied and rinsed with physiological saline (5 mL).

The procedure used for the assessment of skin integrity was repeated and a damage ratio calculated by dividing the pre-treatment resistance measurement by the post treatment resistance measurement. If the membrane has been damaged by the test material, the electrical resistance falls, compared to the initial measurement. When the change to the barrier properties of the membrane is more than 3-fold, this is regarded as significant impairment. Damage ratios <3 are regarded as insignificant. In this experiment with the methyltin chloride mixture, the mean damage ratio for the treated cells was 24 (versus a control value of 1.4), which clearly indicated significant damage to the barrier function of the epidermis.

The experiment was repeated using the minimum amount that could practically weighed onto the cells (0.002 g/cm²). This dose level gave a mean damage ratio of 6.8 (versus a control

value of 1), which again was regarded as have significantly damaged the epidermal barrier.

In a further attempt to find a non-damaging dose to human epidermis, a third series of experiments were designed. Three solutions of the methyltin chloride mixture (10000, 50000 and 100000 µg/mL) were prepared in ethanol and each applied to three replicate epidermal membranes at a rate of 10 µl/cm² (a, 100, 500 or 1000 µg methyltin chloride mixture /cm²). For control purposes, ethanol only was applied to 2 cells at a rate of 10 µl/cm². The applications were not occluded until the ethanol vehicle had evaporated naturally and then remained covered until the end of the 24h contact period, when the cells were decontaminated and post-treatment resistance measurements taken, as previously described. The determined mean damage ratios were 1.33, 5.1 and 9.7 for the 100, 500 or 1000 µg methyltin chloride mixture /cm² applications respectively (versus 0.93 for the control applications), indicating that a non-damaging dose of methyltin chloride mixture would lie between 100 and 500 µg/cm².

MEASUREMENT OF TEST SUBSTANCE ADSORPTION:

The receptor chambers of cells containing intact human and rat epidermal membranes (6 cells per species) were filled with a recorded volume of receptor fluid (water) and placed in a water bath maintained at 32 ± 1°C. A pre-treatment sample (0.5 mL) was taken from each receptor chamber for analysis by ICP-MS. An equal volume of fresh receptor fluid was added to each receptor chamber to replace the volume removed.

From information gained from the above experiments to define a non-damaging dose of the test material, the methyltin chloride mixture was applied to both human and rat epidermal membranes at a rate of 100 µg/cm² as a 10 µl/cm² dose of a 10000 µg/mL solution in ethanol. This application was equivalent to a nominal dose rate for tin of 53.5 µg/cm², based upon a tin content of 53.5% w/w in the methyltin chloride mixture. For the cells designated to be occluded, as soon as the ethanol vehicle had evaporated naturally, the cells were occluded for the remainder of the 24h contact period.

At recorded intervals, samples (0.5 mL) of the receptor fluid were taken for analysis by ICP-MS. The volume of fluid in the receptor chamber was maintained by the addition of (0.5 mL) of fresh receptor fluid to the chamber immediately after the removal of each sample.

MASS BALANCE DETERMINATION:

After the final sample of receptor fluid had been taken at the end of the exposure period, the remaining fluid in the receptor chamber was discarded and the chamber rinsed with fresh receptor fluid (5 mL) which was also discarded. The donor chambers were carefully removed and washed with water (10 mL) and the washings retained for analysis.

The surface of the epidermis was rinsed with water (5 x 5 mL) and the rinsings were combined prior to analysis.

The epidermis was carefully removed from the receptor chamber and placed in a glass

scintillation vial.

All samples were stored refrigerated while awaiting analysis.

Results and discussions

Absorption in different matrices:

Human skin - unoccluded application (Mean of 6 replicates - % of dose recovered)

Donor chamber 2.43

Skin wash 8.02

Epidermis 20.15

Absorbed (receptor fluid) 0.25

TOTAL RECOVERY 30.85

Human skin - occluded application (Mean of 5 replicates - % of dose recovered)

Donor chamber 6.71

Skin wash 3.45

Epidermis 43.26

Absorbed (receptor fluid) 1.39

TOTAL RECOVERY 54.80

Rat skin - unoccluded application (Mean of 5 replicates - % of dose recovered)

Donor chamber 2.19

Skin wash 11.89

Epidermis 24.18

Absorbed (receptor fluid) 9.95

TOTAL RECOVERY 48.21

Rat skin - unoccluded application (Mean of 6 replicates - % of dose recovered)

Donor chamber 2.41

Skin wash 4.01

Epidermis 51.08

Absorbed (receptor fluid) 10.04

TOTAL RECOVERY 67.54

Total recovery:

Due to volatility of the test substance (25.1 Pa at 25 °C) and the use of ethanol, the overall recovery of tin from the test system was low.

Percutaneous absorption rate (unoccluded human) at 24 hours 20%. The amount remaining in epidermis considered potentially absorbable.

Percutaneous absorption rate (unoccluded rat) at 24 hours 34%. The amount remaining in epidermis considered potentially absorbable.

Any other information on results:

Preliminary Study:

During preliminary assessments, the direct application of a non-damaging occluded dose of the methyltin chloride mixture to the epidermis could not be achieved, however a dose of $100 \mu\text{g}/\text{cm}^2$ (applied as a $10000 \mu\text{g}/\text{mL}$ solution in ethanol at a rate of $10 \mu\text{l}/\text{cm}^2$ and occluded only after the ethanol had evaporated), was determined not to damage the epidermis (damage ratio = 1.33). Ethanol itself was determined not to affect the barrier function of the epidermis.

This same regime was used in experiments to determine the absorption through human and rat epidermis and the distribution of tin within the test system. Similar experiments were also carried out where the applications were left unoccluded throughout the 24h exposure period.

Absorption through human epidermis:

From the occluded applications to human epidermis, an initial rate of tin absorption of $0.015 \mu\text{g}/\text{cm}^2/\text{h}$ measured during the first 6h of exposure. Between 6-24h a maximum absorption rate of $0.037 \mu\text{g}/\text{cm}^2/\text{h}$ was achieved. From the unoccluded application a similar pattern of absorption was seen, except that the tin absorption rates were 5-6 times slower during the 0-6h and 6-24h periods ($0.003 \mu\text{g}/\text{cm}^2/\text{h}$ and $0.006 \mu\text{g}/\text{cm}^2/\text{h}$). In terms of percent of applied tin, 1.4% was absorbed from the occluded dose, while only 0.25% was absorbed from the unoccluded dose after 24h exposure.

Absorption through rat epidermis:

Absorption of tin through rat epidermis was much faster than through human epidermis. From the occluded application, tin absorption maintained an essentially constant rate ($0.233 \mu\text{g}/\text{cm}^2/\text{h}$) throughout the entire 24h exposure. From the unoccluded application, the absorption process was essentially complete within the first 3h of exposure, with a maximum mean absorption rate of $1.07 \mu\text{g}/\text{cm}^2/\text{h}$ during this period. From both applications, 10% of the applied tin was determined to have been absorbed by 24h after dosing.

Distribution of dose and mass balance:

The overall recovery of tin from the test system after 24h exposure was low with 55% and 67% of applied tin being recovered from the occluded applications to human and rat epidermis respectively, while only 31% and 48% was recovered from the unoccluded applications. Since (a) more tin was recovered from the occluded experiments than from the unoccluded exposures and (b) the methyltin chloride mixture is volatile, the assumption is that the losses of tin from the occluded experiments (essentially a sealed system) occurred during the period that the ethanol vehicle was evaporating prior to occlusion. If this is the case, then recovery would be expected to be lower from the unoccluded experiments compared to those that were occluded. This hypothesis is also supported by the fact that tin absorption is faster through rat epidermis than through human epidermis, thus allowing less time for tin to volatilise from the surface of rat epidermis during this early stage of the absorption process, giving rise to the higher recovery values from the rat experiments.

A high proportion of the recovered tin was present in the epidermis for both human and rat. For the occluded applications this amounted to about half of the dose and about fifth of that applied to unoccluded experiments. The amounts determined to have been absorbed into the

receptor fluid were low (0.25% and 1.4% from human experiments and 10% from both rat experiments). Approximately 10% for human and 6.4% - 14% for rat was washed off the surface of the epidermis and the donor chamber.

3. HEALTH HAZARDS

3.1. Acute toxicity

3.1.1. Acute oral toxicity

Acute oral toxicity - animal data

[Study 1]

Study reference:

Elf Atochem NA, 1993

Test type:

Equivalent or similar to OECD Guideline 401 (Acute Oral Toxicity), GLP compliant. The study was conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of the relevant results.

CLINICAL OBSERVATIONS

LD₅₀ study animals were observed for clinical abnormalities a minimum of two times on study day 0 (postdose) and daily thereafter (days 1-14). A mortality check was performed twice daily, in the morning and afternoon.

BODY WEIGHTS

Individual body weights were obtained for the LD₅₀ study animals prior to fasting (day -1), prior to dosing on day 0 and for all surviving animals on days 7 and 14.

GROSS NECROPSY

All LD₅₀ study animals which died spontaneously during the study or were euthanized (carbon dioxide inhalation) at study termination (day 14) were necropsied. Body cavities (cranial, thoracic, abdominal and pelvic) were opened and examined. No tissues were retained.

Necropsy of survivors performed: yes

Reliability score given by the registrant: 1 (reliable without restriction).

Test substance:

Test material (EC name): dimethyltin dichloride (85% Dimethyltin dichloride: 15% monomethyltin dichloride).

Form: solution.

Storage condition of test material: room temperature.

Test animals:

Rat (Sprague-Dawley) male/female.

No. of animals per sex per dose: 5 males and 5 females.

Source: Charles River Laboratories, Inc., Portage, Michigan.

Age at study initiation: Young adult.

Weight at study initiation: Males: 229 - 261 g Females: 231 - 273 g.

Fasting period before study: Overnight.

Housing: The animals were housed individually in suspended stainless steel cages.

Diet (e.g. ad libitum): Municipal tap water treated by reverse osmosis or deionization (back-up system) was available to the animals ad libitum throughout the study.

Water (e.g. ad libitum): Purina Certified Rodent Chow #5002 was provided ad libitum to the animals throughout the study (except during fasting).

Acclimation period: minimum of 5 days.

Environmental conditions:

Temperature: 64-79°F.

Humidity (%): 35 – 58.

Photoperiod: 12-hour light/12-hour dark cycle.

IN-LIFE DATES: From: 24 February 1993 To: 15 April 1993.

Administration/exposure:

Oral: gavage.

The solvent in the 50% solution was not stated.

Preliminary Study: 100, 300, 500, 1000, 2000, 3000, 5000 mg/kg bw.

Main study: 200, 300 and 500 mg/kg bw.

DOSING

On day -1, the animals chosen for the LD50 study were weighed and fasted overnight. On day 0, the test material was administered orally as a single dose using a ball tipped stainless steel gavage needle attached to a syringe. Individual doses were calculated based on the animal's fasted (day 0) body weight. Animals were returned to ad libitum feeding after dosing.

Statistics

The LD₅₀ and 95% confidence intervals were calculated separately for males, females and the combined sexes (when possible) using a computer adaption of the method of Litchfield and Wilcoxon. Body weight means and standard deviations were calculated separately for males and females for each dose level administered.

Results:

LD₅₀: 273 mg/kg bw (male) based on: 85% Dimethyltin dichloride: 15% monomethyltin dichloride, 95% CL 141 - 528.5.

LD₅₀: 164 mg/kg bw (female) based on: 85% Dimethyltin dichloride: 15% monomethyltin dichloride, 95% CL 104.5 - 258.

LD₅₀: 204.5 mg/kg bw (male/female) based on: 85% Dimethyltin dichloride: 15% monomethyltin dichloride, 95% CL 152.5 - 273.5.

Mortality

No animals in the 200 mg/kg bw group died.

1 male and 3 females in the 300 mg/kg bw group died.

2 male and 4 females in the 500 mg/kg bw group died.

Under the conditions of this test, the acute oral LD50 of [Di/Mono] Methyltin Chlorides Solution in the male rat was determined to be 546 mg/kg. In the female rat, the oral LD50 was determined to be 328 mg/kg. In the sexes combined, the oral LD50 was determined to be 409 mg/kg. The [Di/Mono] Methyltin Chlorides Solution is a 50% organotin solution in an unspecified solvent and therefore the LD50 value achieved should be halved according to the registrant. The LD50 of 84.79 % pure Dimethyltin dichloride would therefore be 273 mg/kg for male rats 164 mg/kg for female rats, with the combined LD50 being 204.5 mg/kg bw.

All mortality occurred by study day 4.

Clinical signs

The most notable clinical abnormalities observed included decreased activity, salivation, rough haircoat, mucoid/soft stools, fecal/urine stain, hunched posture, dehydration, dark material around the facial area, decreased defecation and food consumption, gasping and rales.

Body weight

Body weight gain was noted for the majority of surviving animals during the test period.

Gross pathology

The most notable gross internal findings were observed in the animals that died and included dark red medulla of the kidney, dark red foci on the thymus, mottled lungs, abnormal coloured mucoid/fluid contents and eroded area(s), reddened mucosa and dark red linear striations on the stomach.

[Study 2]

Study reference:

Author not disseminated (1979).

Report date 1979-03-20.

Test type:

OECD Guideline 401 (Acute Oral Toxicity), not GLP-compliant. Study conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

Reliability score given by the registrant: 2 (reliable with restrictions).

Test substance:

Test material (EC name): dimethyltin dichloride.

Test material form: crystalline.

Analytical purity: 98.7%.

Physical state: solid.

Test animals:

Species: rat

Strain: Sprague-Dawley

Sex: male/female

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Ace Animals, Inc., Boyertown, Pennsylvania
- Age at study initiation: Weanling
- Weight at study initiation: 35 and 85 g
- Housing: Five per cage per sex, in standard polypropylene cages with stainless steel lids
- Diet (e.g. ad libitum): Free access to Purina Rat Chow
- Water (e.g. ad libitum): Free access to water
- Acclimation period: NDA

ENVIRONMENTAL CONDITIONS

- Temperature: 74 ± 2°F
- Humidity: 45 - 55 %
- Photoperiod (hrs dark / hrs light): 12 hours light/dark

IN-LIFE DATES: From: 26 October 1978 To: 23 November 1978

Administration/exposure:

Route of administration: oral: gavage

Vehicle: corn oil

VEHICLE

- Concentration in vehicle: 1% w/v
- Amount of vehicle (if gavage): 10 - 30 mL/kg
- Justification for choice of vehicle: NDA

MAXIMUM DOSE VOLUME APPLIED: 30 mL/kg dosage groups, or 55 mL/kg in the vehicle control

DOSAGE PREPARATION (if unusual): All solutions were mixed thoroughly on a stir plate prior to and during administration.

Doses: 0, 100, 150, 200, 250 and 300 mg/kg bw

No. of animals per sex per dose: 5 males and 5 females

Control animals: yes

Duration of observation period following administration: 14 days

Statistics

The LD₅₀ and its confidence limits were computed in accordance with the method of J.T. Litchfield, Jr. and F. Wilcoxon, 'A Simplified Method of Evaluating Dose-Effect Experiments', J. Pharm. Exp. Therap., 96:99-115, 1949.

Results:

LD₅₀: 190 mg/kg bw (male) based on: test mat. 95% CL 162.4 – 222.3

LD₅₀: 160 mg/kg bw (female) based on: test mat. 95% CL 100 - 256

Mortality

100 mg/kg bw: No deaths occurred.

150 mg/kg bw: 2 males and 3 females died on day 2.

200 mg/kg bw: 2 females died on day 1; 2 males and 1 female died on day 2; 1 male died on day 3.

250 mg/kg bw: 4 males and 5 females died on day 2.

300 mg/kg bw: 1 female died on day 1; 4 males and 3 females died on day 2; 1 male died on day 6.

Clinical signs

Test animals exhibited sedation, piloerection and ptosis on day 0 (within three hours following intubation). Piloerection, decreased locomotor activity, ataxia, ptosis, oily ventral surface, reddish nasal discharge and shallow respiration were observed primarily on days one through four or until death occurred. Most animals surviving the observation period exhibited normal behavior and appearance on days 5 through 14. Control animals received the vehicle (corn oil) alone at 55 mL/kg bw). They exhibited abnormal defecation, piloerection, decreased locomotor activity and oily ventral surface on day one and normal behavior and appearance on days 2 through 14.

Gross pathology

Autopsies of those animals found dead revealed lungs hemorrhagic, and small and large intestines filled with yellow gelatinous material. Autopsies of surviving animals, including control animals, revealed no outstanding gross pathological organ changes.

[Study 3]

Study reference:

Author not disseminated (1978).

Report date: 1978-05-25.

Test type:

Oral gavage study in rats. This study was conducted to evaluate the acute oral toxicity potential of the test material in accordance with the techniques specified in the Regulations for the Enforcement of the Federal Hazardous Substances Act (Code of Federal Regulations, Title 16, Chapter II, 1976). Not GLP-compliant.

Test substance:

Details on test material: 5% Dimethyltin dichloride in corn oil (identity of test material not the same as for substance defined in SID part).

Test material form: crystalline.

Test animals:

Species: rat

Strain: Sprague-Dawley

Sex: male/female

Food was withheld from the rats for approximately 18 hours prior to dosage. Following dosage, food consisting of Purina Laboratory Chow and water were available ad libitum. The rats were housed in groups in stainless steel wire mesh cages suspended above the droppings. The animals were housed under a 12-hour light/12-hour dark cycle.

The weight range for the male rats was 160 to 325 g and for the female rats was 157 to 254 g.

Administration/exposure:

Route of administration: oral: gavage

Vehicle: corn oil

The sample was administered undiluted or as volume per volume solutions in corn oil (Mazola) at the dosage levels. Dilution necessitated use of the indicated dosage factors which were calculated by the following formula to enable administration of the desired dose of compound.

Doses: 0.215, 0.464, 1.00, 2.15 and 4.64 mL/kg bw

No. of animals per sex per dose: 5 males and 5 females

Control animals: yes

All animals were observed closely for gross signs of systemic toxicity and mortality at frequent intervals during the day of dosage, and at least once daily thereafter, for a total of 14 days. Gross necropsies were performed on the animals that died. At the end of the 14-day observation period the surviving rats were weighed, sacrificed by CO₂ inhalation and gross necropsies were performed.

Statistics

Statistical analysis of the mortality data was by the moving average method and probit analysis.

Results and discussion:

LD₅₀: 1.7 mL/kg bw (male/female) based on: test mat. 95% CL 1.25 - 2.34.

Mortality

0.215 mL/kg bw: No deaths.

0.464 mL/kg bw: No deaths.

1.00 mL/kg bw: 1/5 females died. No male deaths.

2.15 mL/kg bw: 4/5 males and 3/5 females died.

4.64 mL/kg bw: All rats died.

Clinical signs

At the 0.215 and 0.464 mL/kg bw levels for males, all rats appeared normal on the day of dosage and throughout the 28-day observation period.

At the 1.00 mL/kg bw level for males, signs of systemic toxicity included depression, diarrhea, bloody-appearing stains around the muzzle, urine stains, and piloerection.

At the 2.15 mL/kg bw level for males, signs of systemic toxicity included depression, yellow mucoid diarrhea, diarrhea, bloody-appearing stains around the muzzle, urine stains, excessive nasal or salivation stains, piloerection, bloated appearance, emaciation, and mortality.

At the 4.64 mL/kg bw level for males, signs of systemic toxicity noted prior to complete mortality included depression, depressed righting and placement reflexes, rapid respiration, shortness of breath, diarrhea, bloody-appearing stains around the nose, comatose appearance, piloerection, hunched posture, emaciation, and shaking.

At the 0.215 and 0.464 mL/kg bw levels for females, all rats exhibited normal appearance on the day of dosage and throughout the 28-day observation period.

At the 1.00 mL/kg bw level for females, signs of systemic toxicity included diarrhea, depression, piloerection, urine stains and mortality.

At the 2.15 mL/kg level for females, signs of systemic toxicity included depression, diarrhea, piloerection, and mortality.

At the 4.64 mL/kg bw level for females, signs of systemic toxicity noted prior to complete mortality included depression, diarrhea, shortness of breath, and comatose appearance.

Body weight

Body weight gain occurred in all animals examined.

Gross pathology

No effects in males were seen in the 0.215, 0.464 and 1.00 mL/kg bw dosage groups. Autolysis was noted in 3/5 animals in the 2.15 mL/kg bw group. In the highest dosage group, congested kidneys, stomach distended with gas, irritated intestines and mottled liver were all noted in 1/5 rats, and autolysis was noted in all of the animals at this dosage.

No effects in females were seen in the 0.215 and 0.464 mL/kg bw dosage groups. Autolysis was noted in 1/5 animals in the 1.00 mL/kg bw group 3/5 animals in the 2.15 mL/kg bw group and in all animals in the 4.64 mL/kg bw group. In the highest dosage group, congested lungs, white lesions on lungs, congested kidneys, irritated intestines and intestines distended with gas were all noted in 1/5 rats.

Reliability score given by the registrant: 3 (not reliable). Test material was a 5% dilution in corn oil with results given in mL/kg bw of the solution.

[Study 4]

Study reference:

Affiliated Medical Enterprises (1971a).

Test type:

Test material was administered by oral intubation to six male rats. The animals were observed for 28 days after dosing.

Oral gavage study in rats.

Reliability score 4 (not assignable) was given by the registrant since there were very little information on methodology, impurities or results. No mention of a followed guideline. Not conducted to GLP.

Test substance:

Test material (EC name): dimethyltin dichloride

Test material form: crystalline

Name of test material (as cited in study report): DM-8121.

Test animals:

Species: rat

Strain: no data

Sex: male

The rats were fasted for 12 hours prior to dosing; the initial body weights ranged from 113 to 268 grams.

Administration/exposure:

Route of administration: oral: gavage

Vehicle: water

Details on oral exposure:

The test material was prepared in distilled water to yield a concentration of 100 mg/mL and administered by oral intubation.

Doses: 100, 200, 400, 800 and 1600 mg/kg bw.

No. of animals per sex per dose: Six males

Control animals: no data

Details on study design:

The animals were observed during the day of dosing and daily thereafter for 28 days.

Decedents during the study were examined for gross lesions.

Statistics: LD₅₀ calculated by the Thompson Moving Average Method.

Results and discussion:

LD₅₀: 141.4 mg/kg bw (male) based on: test mat. 95% CL 100.2 - 196.1.

Mortality

100 mg/kg bw: 1/6 died

200 mg/kg bw: 5/6 died

400 mg/kg bw: 6/6 died

800 mg/kg bw: 6/6 died

1600 mg/kg bw: 6/6 died

All deaths occurred within 4 days of dosing, apart from one rat at the 200 mg/kg bw level died at 21 days. No information available for the timing of the mortality of the rat at 100 mg/kg bw.

Clinical signs: Depression, convulsions, death.

Gross pathology: No significant findings.

[Study 5]

Study reference:

Klimmer, O.R. 1971.

Test type:

Oral gavage study in rats. Very brief study report, with little methodological information. No reference to any guideline followed. Pre-dates GLP.

Test substance:

Test material (EC name): dimethyltin dichloride.

Test material form: crystalline.

Test animals:

Species: rat

Strain: Wistar

Sex: male

The rats used were male Wistar rats. The rats, which had come from a recognized breeder, weighed 120-140 grams and had not eaten since the previous day. The rats were kept two to a cage and fed ad libitum with water and "ssniff R", the standard rat feed in pellet form.

Administration/exposure:

Route of administration: oral: gavage

Vehicle: 1:1 Tween 80 and peanut oil

Details on oral exposure:

The 10% solution of the test substance in "Tween 80" was diluted 1:1 with pure peanut oil because only a small volume was necessary for the test. The resulting 5% solution was administered once to male Wistar rats by gavage.

Doses: 48, 57, 69, 83, 100, 120 and 144 mg/kg bw

No. of animals per sex per dose: 10 males

Control animals: no data.

Details on study design: The rats were observed daily for 14 days after exposure. The rats that died spontaneously and those that were killed at the end of the experiment were dissected.

Statistics

The Probit method was used to calculate an acute oral LD₅₀.

Results and discussion:

Preliminary study (if fixed dose study):

100 mg/kg bw: 2/3 dead.

200 mg/kg bw: 3/3 dead.

LD₅₀: 73.86 mg/kg bw (male) based on: test mat. 95% CL 64.42 - 84.7.

Mortality

48 mg/kg bw: 1/10 dead.

57 mg/kg bw: 2/10 dead.

69 mg/kg bw: 5/10 dead.

83 mg/kg bw: 6/10 dead.

100 mg/kg bw: 8/10 dead.

120 mg/kg bw: 8/10 dead.

144 mg/kg bw: 10/10 dead.

Clinical signs

Within 2-3 hours of the application, the animals began to show signs of the usual uncharacteristic symptoms of dialkyltin poisoning. They showed lassitude, hypokinesia, lack of appetite, thirstiness, unkempt fur, general weakness, and sometimes a tendency to lay on their sides, as well as death within 24-72 hours. Recovery in the surviving rats took place within 4-6 days.

Gross pathology

No obvious signs of toxicity.

The Probit method was used to calculate an acute oral LD₅₀ of 73.86 mg/kg rat (64.42-84.7 mg/kg) from the mortality rates.

The necropsy results were uncharacteristic.

[Study 6]

Study reference:

Hoch, M., 2001.

Title: Organotin compounds in the environment - an overview.

Bibliographic source: Applied Geochemistry 16 (2001) 719-743. Primary source: Bulten, E.J., Meinema, H.A., 1991. In: Merian, E. (Ed), Metals and Their Compounds in the Environment. VCH. Weinheim, pp. 1245-1259.

Test type:

Oral study. No methodological information. No information on GLP compliance. Reliability score 4 (not assignable) was given by the registrant as the reference is a secondary source with no methodological information.

Test substance:

Dimethyltin dichloride, crystalline.

Test animals:

Species: rat.

Strain: no data.

Sex: no data.

Administration/exposure:

Route of administration: oral: unspecified.

Control animals: no data.

Results and discussion:

The LD₅₀ of dimethyltin dichloride to rats was 74 mg/kg bw.

[Study 7]

Study reference:

Barnes, J.M. & Stoner, H.B., 1958.

Title: Toxic properties of some dialkyl and trialkyl tin salts.

Bibliographic source: Brit. J. Industr. Med. 15, 15.

Reliability score 4 (not assignable) was given by the registrant because of limited methodological information, only two animals were used, limited details of the toxic effects reported and acute lethal doses were not reported.

Test type:

No guideline followed, not GLP-compliant.

Acute oral test, with lower dosage groups repeated on 4th day. Animals observed for 10 days. Limited methodological information.

Test substance:

Test material (EC name): dimethyltin dichloride.

Test material form: crystalline.

Test animals:

Species: rat.

Strain: no data.

Sex: female.

Administration/exposure:

Route of administration: oral: unspecified.

Details on oral exposure: 40 and 80 mg/kg bw given on 1st and 4th day; 160 mg/kg bw given on 1st day only.

Doses: 40, 80 and 160 mg/kg bw.

No. of animals per sex per dose: Two females.

Control animals: no data.

Details on study design: 10 day observation period.

Results and discussion:

The LD₅₀ to female rats was found to be between 80 and 160 mg/kg bw.

Mortality: Both rats died in the 160 mg/kg bw group.

Clinical signs: Marked weakness in rats at 160 mg/kg bw.

Body weight: No weight loss at 40 or 80 mg/kg bw.

Gross pathology: No bile duct lesion at any dose.

[Study 8]

Study reference:

Author not disseminated (1993).

Report date: 1993-07-11.

Test type:

Test type: dose finding as part of UDS study.

No guideline followed. Acute oral LD₅₀ determined as part of an in-vivo UDS study, in order to determine dosage levels for the study. GLP compliant.

Reliability score 4 (not assignable) was given by the registrant.

Test substance:

Name of test material (as cited in study report): mixture of methyltin chloride compounds (identity of test material not the same as for substance defined in SID part).

Test material form: crystalline.

Test animals:

Species: rat.

Strain: Fischer 344.

Sex: male

Details on test animals and environmental conditions: Animals were received from Charles River Laboratories, Raleigh, NC, USA. The weights of ten randomly selected animals ranged from 106.1 to 116.8 g at the time the animals were received. Rats used in the dose range-finding assay weighed between 161.9 and 199.9 g and were approximately 8 weeks old when they were dosed with the test material.

Temperature range: 58 to 78°F.

Humidity range: 32 to 89%.

Light cycle: 12 hours light/12 hours dark.

Cage specification: Rats were housed no more than 3 per cage in polycarbonate cages containing hardwood-chip bedding.

Food: Purina Certified Rodent Chow No. 5002 ad libitum.

Administration/exposure:

Route of administration: oral: gavage.

Vehicle: no data.

Doses:

First range finding assay: 25, 50, 100, 200 and 400 mg/kg bw.

Second range finding assay: 600 and 800 mg/kg bw.

No. of animals per sex per dose: Three males per dosage group.

Control animals: yes.

Statistics: The LD₅₀ was calculated by an SRI generated program on the VAX-3100 (LD50) that uses a point estimate of the linear interpolation of the log doses.

Results and discussion:

LD50: ca. 280 mg/kg bw (male) based on: test mat.

In the first range-finding assay, one animal in the 400 mg/kg dose group died on the second day after dosing. Clinical signs of rats dosed with methyltin chloride included rough fur, diarrhea, weakness, humped back, difficulty breathing, bloody nose, lacklustre eyes, and blood around eyes. The two surviving rats in the high-dose group had extreme weight loss (rats weighed 52 and 66% of their day 0 weight when they were sacrificed on day 7).

All of the rats in the second range-finding assay died before their scheduled sacrifice. Clinical signs of rats dosed with 600 or 800 mg/kg bw methyltin chloride included rough fur, weakness, diarrhea, lacklustre eyes, humped back, hypoactive, and blood around eyes. The LD₅₀ of methyltin chloride was estimated at approximately 443 mg/kg bw by summarising results of both range-finding assays together. Dose levels for the first definitive in vivo-in vitro hepatocyte DNA repair (UDS) assay were set at 90, 175, and 350 mg/kg bw (approximately 20, 40, and 80% of the LD₅₀). Rats were dosed orally, three per group, except for the 16 hour high dose, which was dosed four per group, with the test article solution, negative (vehicle) control, or positive control 2 or 16 hour before sacrifice. The negative control group received water 16 hour before sacrifice. The positive control group received dimethylnitrosamine (DMN, 10 mg/kg bw) dissolved in water 2 hour before sacrifice. Three rats from the 16 hour 350 mg/kg bw methyltin chloride dose group were found dead on the morning after dosing. All rats from the 16 hour 175 mg/kg bw dose-group had rough fur on the morning after dosing. One rat from the 2 hour 90 mg/kg bw dose-group and two rats from the 2 hour 350 mg/kg bw dose-group had diarrhea. The surviving rat from the 16 hour 350 mg/kg bw dose-group had rough fur, humped back, diarrhea, labored breathing, and was hypoactive.

The first definitive UDS assay was terminated after the first eight test animals produced insufficient viable cells for evaluation of UDS. It was suspected this was because of a technical error in the preparation of the cell culture medium. A second definitive UDS assay was conducted using a new batch of cell culture medium. The LD₅₀ was adjusted downward to approximately 278 mg/kg bw methyltin chloride based on the pattern of death observed in the first definitive assay.

[Study 9]

Study reference:

Hashizume, M. 1971.

Title: Experimental Studies on the Toxicity of Dimethyltin Dichloride Through Digestive Tract.

Bibliographic source: Tokyo Ika Daigabu Zasshi 29 (1).

Test type:

Methodological information not available in English. Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Test material form: crystalline.

Test animals:

Species: rabbit.

Strain: no data.

Sex: male.

Administration/exposure:

Route of administration: oral: unspecified.

Vehicle: no data.

Control animals: no data.

Results and discussion:

Minimum lethal dose: 50 mg/kg bw (male) based on: test mat.

Interpretation of results: Not possible to classify based on the results available.

Reliability score 4 (not assignable) was given by the registrant.

[Study 10]

Study reference:

Author not disseminated (1978b).

Reference type: review article or handbook.

Test type:

No methodological information. Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Test animals:

Species: rat.

Strain: no data.

Sex: no data.

Administration/exposure:

Route of administration: oral: unspecified.

Vehicle: no data.

Control animals: no data.

Results and discussion:

Clinical signs: General uncharacteristic illness.

Gross pathology: Inflammatory lesion of bile duct.

Reliability score 4 (not assignable) was given by the registrant.

[Study 11]

Study reference:

Reference type: review article or handbook.

Author: van Dokkum, H.P. and Huwer, S.L.

Title: Tiered Environmental Risk Assessment of Methyltins from Heat Stabilizers in Rigid PVC in Sweden.

Bibliographic source: Regulatory Toxicology and Pharmacology 41 (2005) 73-81. Original source: Morton, 1998. Environmental risk assessment of methyltin heat stabilisers in rigid PVC. Morton International.

Test type:

No methodological information available. GLP compliance not known.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Test animals:

Species: rat.

Strain: no data.

Sex: no data.

Administration/exposure:

Route of administration: oral: unspecified.

Vehicle: no data.

Control animals: no data.

Results and discussion:

LD50: 88 - 119 mg/kg bw based on: test mat.

Reliability score 4 (not assignable) was given by the registrant.

[Study 12]

Study reference:

Author not disseminated (1978c).

Reference type: review article or handbook

Test type:

No methodological information available. Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Test animals:

Species: rat.

Strain: no data.

Sex: no data.

Administration/exposure:

Route of administration: oral: unspecified.

Vehicle: no data.

Control animals: no data.

Results and discussion:

The acute oral LD50 of dimethyltin dichloride is reported to be 237 mg/kg bw.

Reliability score 4 (not assignable) was given by the registrant.

[Study 13]

Study reference:

Author not disseminated (1978d).

Reference type: review article or handbook.

Test type:

No methodological information. Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Test animals:

Species: rat.

Strain: no data.

Sex: no data.

Administration/exposure:

Route of administration: oral: unspecified.

Vehicle: no data.

Control animals: no data.

Results and discussion:

The acute oral LD50 of dimethyltin dichloride is reported to be 90 mg/kg bw.

Reliability score 4 (not assignable) was given by the registrant.

[Study 14]

Study reference:

Author: Dean, R.R.

Year: 1976.

Title: Tin and Its Uses - A New Type of Organotin Stabilizer for PVC.

Bibliographic source: Quarterly Journal of the Tin Research Institute. No. 107 (1976).

Test type:

No methodological information. Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Test animals:

Species: rat.

Strain: no data.

Sex: no data.

Administration/exposure:

Route of administration: oral: unspecified.

Vehicle: no data.

Control animals: no data.

Results and discussion:

The acute oral LD50 of dimethyltin dichloride is reported to be 74 mg/kg bw.

Reliability score 4 (not assignable) was given by the registrant.

[Study 15]

Study reference:

Author not disseminated (1992).

Test type:

No methodological information. Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Test animals:

Species: rat.

Strain: no data.

Sex: no data.

Administration/exposure:

Route of administration: oral: unspecified.

Vehicle: no data.

Control animals: no data.

Results and discussion:

The acute oral LD50 of dimethyltin dichloride is reported to be 74 mg/kg bw.

Reliability score 4 (not assignable) was given by the registrant.

Acute oral toxicity - human data

No data.

Acute oral toxicity - other data

No data.

3.1.2. Acute dermal toxicity

Acute dermal toxicity - animal data

[Study 1]

Study reference:

Rush, E.R. (1993b).

Test type:

Guideline: OECD Guideline 402 (Acute Dermal Toxicity).

Deviations yes.

Guideline EU Method B.3 (Acute Toxicity (Dermal)).

Deviations yes.

Principles of method if other than guideline: The temperature and relative humidity of the animal room (66-72 °F and 30-60 %, respectively) exceeded the range specified in the protocol (61-70°F and 40-60%, respectively) during this study. The final body weight and method of euthanasia was not recorded for animal #6636/M and #6640/M, respectively. These occurrences are considered to have had no adverse effect on the outcome of this study.

GLP compliant.

Test substance:

Test material (EC name): 84.8 % DMTC in mixture with MMTC.

Test material form: solution.

Physical state: liquid.

Storage condition of test material: room temperature.

Test animals:

Species: rabbit.

Strain: New Zealand White.

Sex: male/female.

Source: Mohican Valley Rabbitry, Loudonville, OH.

Age at study initiation: NDA.

Weight at study initiation: Male: 2.662 - 2.893 kg. Female: 2.436 - 2.601 kg.

Housing: The animals were housed individually in suspended stainless steel cages.

Diet (e.g. ad libitum): Purina Certified Rabbit Chow #5322 ad libitum.

Water (e.g. ad libitum): Municipal tap water treated by reverse osmosis or deionization. (back-up system) ad libitum.

Acclimation period: minimum of 5 days.

ENVIRONMENTAL CONDITIONS

Temperature (°F): 66 – 72.

Humidity (%): 30 - 60 %.

Air changes (per hr): 10 – 12.

Photoperiod (hrs dark / hrs light): 12-hour light/12-hour dark cycle.

IN-LIFE DATES: From: 2nd March 1993 To: 12th April 1993.

Administration/exposure:

Type of coverage: occlusive.

Vehicle: unchanged (no vehicle).

Test site

- Area of exposure: Not specified, but covered with 4" x 8" gauze dressing.
- % coverage: ≥ 10%.
- Type of wrap if used: plastic.

Removal of test substance

- Washing (if done): Residual test material was removed using gauze moistened with distilled water.
- Time after start of exposure: 24 hours.

Test material

- Amount(s) applied (volume or weight with unit): NDA.
- Concentration (if solution): 100%.

Duration of exposure: ca. 24 hours.

Doses: 200, 400 and 750 mg/kg bw.

No. of animals per sex per dose: 5 males, 5 females per dose.

Control animals: no.

Details on study design

- Duration of observation period following administration: 14 days.
- Frequency of observations and weighing: Observations were performed daily.
- Bodyweights recorded on days 0, 7 and 14.
- Necropsy of survivors performed: yes.
- Other examinations performed: Clinical signs, gross necropsy.

Statistics

The LD50 and 95% confidence intervals were calculated separately for males, females and the combined sexes (when possible) using a computer adaption of the method of Litchfield and Wilcoxon. Body weight means and standard deviations were calculated separately for males and females for each LD50 level administered.

Results and discussion:

LD50: 404 mg/kg bw (male/female) based on: test mat.
95% CL 287 - 568

Mortality:

200 mg/kg: 0/5 males, 0/5 females
400 mg/kg: 4/5 males, 2/5 females
750 mg/kg: 4/5 males, 5/5 females

Clinical signs:

Clinical abnormalities were observed during the LD50 study in the animals that died during the study were sacrificed moribund and in animals surviving to study termination. In the animals that died or were sacrificed moribund during the study, clinical abnormalities included slight to severe dermal irritation at the site of the test material application, urine/fecal stain, soft stool/diarrhea, decreased food consumption, decreased activity, pale eyes, decreased defecation, tremors, wobbly gait, respiratory abnormalities, mucoid stools, reddened iris, prostration, partial immobility of the hindlimbs, dark material and swelling around the facial area, convulsions, dehydration, emaciation, red ocular discharge, apparent hypothermia, raised area on the abdominal region and grinding of teeth. Clinical abnormalities noted in the animals surviving to study termination included slight to severe dermal irritation at the site of test material application, decreased defecation, decreased activity, pale eyes, dark material around the facial area, partial immobility of the hindlimbs, tremors, wobbly gait, soft stool, mucoid stools, fecal stain, reddened iris, apparent decreased food consumption and raised area on the abdominal region.

Body weight

Although, body weight loss was noted during the day 0-7 interval for 1 male and 1 female in the 400 mg/kg dose level and for the one surviving male in the 750 mg/kg dose level, these animals did gain weight during the day 7-14 interval. Body weight gain was noted for the remaining surviving animals during the test period.

Gross pathology

The most notable gross internal findings were observed in the animals that died and included reddened mucosa in the digestive tract, petechial hemorrhages, abnormally coloured fluid/mucoid contents in the digestive tract, dark red foci on the mucosa of the stomach, mottled and/or dark red thymus, mottled and/or firm, consolidated lungs, clear amber or red fluid contents in the thoracic cavity and light red foamy contents in the trachea. Cysts on the oviducts were noted in two female rabbits surviving to study termination. However, this observation was not considered significant since it is commonly observed in this strain of rabbit.

Reliability score 1 (reliable without restriction) was given by the registrant. The study was conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which did not affect the quality of the relevant results.

[Study 2]

Study reference:

Barnes, J.M. & Stoner, H.B., 1958.

Title: Toxic properties of some dialkyl and trialkyl tin salts.

Bibliographic source: Brit. J. Industr. Med., 1958, 15, 15.

Test type:

No guideline followed. Very limited methodological information available. Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Test animals:

Species: rat.

Strain: no data.

Sex: male.

Administration/exposure:

Type of coverage: no data.

Vehicle: dimethyl phthalate (dissolved in 0.1 mL dimethyl phthalate).

Duration of exposure: Applied on 5 successive days.

Doses: 80 mg/kg.

No. of animals per sex per dose: 3 males.

Control animals: no data.

Details on study design: Rats were observed for 12 days and at necropsy, the skin lesions and condition of the bile duct were examined.

Results and discussion:

Clinical signs: Necroses of superficial layer of skin with black eschar formation. No deep-seated inflammation.

Body weight: Slight weight loss.

Gross pathology: No bile duct lesions.

No LD50 was determined.

Reliability factor 4 (not assignable) was given by the registrant, because publication was with very limited detail and application was percutaneous rather than topical.

[Study 3]

Study reference:

Affiliated Medical Enterprises (1971b).

Test type:

Guideline OECD Guideline 402 (Acute Dermal Toxicity).

Deviations: no data.

Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Details on test material:

Name of test material (as cited in study report): Dimethyltin Dichloride [CAS No. 753-73-1]:Methyltin Trichloride [CAS No. 993-16-8] (90:10% mixture).

Physical state: liquid.

Test animals:

Species: rabbit.

Strain: New Zealand White.

Sex: male/female.

Administration/exposure:

Type of coverage: no data.

Vehicle: polyethylene glycol.

Doses: 2000 mg/kg bw.

No. of animals per sex per dose: six animals in total.

Control animals: no data.

Results and discussion:

Mortality: No mortality occurred.

The acute dermal LD50 of dimethyltin dichloride was reported to be > 2000 mg/kg.

Reliability score 4 (not assignable) was given by the registrant because the data was secondary source information from a review dossier.

Acute dermal toxicity - human data

Not available.

Acute dermal toxicity - other data

Not available.

3.1.3. Acute inhalation toxicity

Acute inhalation toxicity - animal data

[Study 1]

Study reference:

Ciba-Geigy (1977).

Test type:

Equivalent or similar to Guideline OECD Guideline 403 (Acute Inhalation Toxicity).

The test was carried out in accordance with the below method.

K. Sachsse, L. Ullmann, G. Voss and R. Hess: Measurement of inhalation toxicity of aerosols in small laboratory animals. In: Proceedings of the Europ. Soc. for the Study of Drug Toxicity. Vol. XV, pp. 239-251, Zurich, June 1973.

Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Test animals:

Species: rat.

Strain: Tif: RAIf (SPF).

Sex: male/female.

Details on test animals and environmental conditions:

TEST ANIMALS

- Source: Raised on testing lab premises.
- Weight at study initiation: 170 to 200 g.
- Housing: Macrolon cages, type 4, (10 animals to a cage).
- Diet (e.g. ad libitum): NAFAG, Gossau SG ad libitum.
- Water (e.g. ad libitum): ad libitum.
- Acclimation period: minimum of 4 days.

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 22 ± 1.
- Humidity (%): 55 ± 5.
- Photoperiod: 14 hrs dark / 10 hrs light.

Administration/exposure:

Route of administration: inhalation (aerosol).

Type of inhalation exposure: nose only.

Vehicle: air.

Details on inhalation exposure

For inhalation the rats were kept on separate PVC tubes positioned radially around the exposure chamber such that snout and nostrils of the animals only were exposed to the aerosol.

The aerosol was generated by injecting a 50 % aqua test solution of the test material at a rate of 12, 30 and 60 mL/hour into an air stream which was discharged into the exposure chamber through a spray nozzle under a pressure of 2 atm. at a rate of 10 l/min.

The concentration and the particle size distribution of the aerosol in the vicinity of the animals was monitored at 1 hour intervals throughout the aerosol exposure. The concentration was determined gravimetrically by sampling the test atmosphere through a selectron filter of 50 mm diameter and with a pore size of 0.2 µm at an air flow rate of 10 l/min. The size distribution of the aerosol particles was measured with a Cascade Impactor with selectron filters of 25 mm diameter and with a pore size of 0.2 µm at an air flow rate of 17.5 l/min.

Analytical verification of test atmosphere concentrations: yes.

Duration of exposure: 4 h.

Concentrations: 44 ± 6, 90 ± 7, 121 ± 3 and 167 ± 23 mg/m³.

Average concentration, gravimetrically determined.

No. of animals per sex per dose: 10 males, 10 females.

Control animals: no.

Details on study design

- Duration of observation period following administration: 14 days.

- Other examinations performed: physical condition and incidence of death.

Statistics:

LC 50 including 95 % confidence limits was calculated by probit analysis method.

Results and discussion:

Sex male/female

LC50: 115 mg/m³ air (analytical) (based on act. ingr.)

95% CL 105 – 126.

Exp. Duration: 4 h.

Mortality

44 ± 6 mg/m³ : 0/10 males, 0/10 females within 14 days

90 ± 7 mg/m³ : 3/10 males, 1/10 females within 14 days

121 ± 3 mg/m³ : 5/10 males, 4/10 females within 14 days

167 ± 23 mg/m³ : 10/10 males, 10/10 females within 14 days

Clinical signs

Within 2 hours after starting the exposure the animals in concentrations where mortalities occurred showed dyspnoea, ventral position, tremor and ruffled fur. These symptoms became more accentuated as the concentration was increased. After the 4 hour exposure period all animals showed, in addition to the above symptoms edema, in the head region. The surviving animals recovered within 6 to 7 days. They were submitted at random to a necropsy whenever they died, survivors at the end of the observation period.

Body weight: NDA.

Gross pathology:

Dead Animals: Hemorrhages in the lungs were observed.

Sacrificed Animals: No substance related gross organ changes were seen.

Reliability score 2 (reliable with restrictions) was given by the registrant, because study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

[Study 2] etc.

Study reference:

International Bio-Research 1976.

Test type:

Test type: standard acute method.

Limit test: yes.

According to Regulations for the Enforcement of the Federal Hazardous Substances Act (Revised, Federal Register, September 17, 1964).

Deviations: no data.

Equivalent or similar to Guideline OECD Guideline 403 (Acute Inhalation Toxicity)

Deviations: yes; only for 1 hour exposure.

Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Physical state: solid.

Test animals:

Species: rat.
Strain: no data.
Sex: male.

Details on test animals and environmental conditions

TEST ANIMALS

- Source: Harlan industries
- Weight at study initiation: 249 - 300 g
- Diet and water: Commercial pellets and water were available to the animals ad libitum.

Administration/exposure:

Route of administration: inhalation (aerosol).
Type of inhalation exposure: no data.
Vehicle: water or propylene glycol in air.

Details on inhalation exposure

The concentration of the test material in the chamber atmosphere was determined by the following formula:

$$A / (B \times C) = D$$

Where A = Weight of sample in mg used during the exposure period

B = Air flow (litres/minute)

C = Duration of exposure (minutes)

D = Concentration of sample in chamber atmosphere (mg/litre)

In the study with water, A was 34,000 mg, B was a total of 10 litres/minute, C was 60 minutes and D was 57.6 mg/l. In the study with propylene glycol, A was 10,000 mg, B was a total of 10 litres/minute, C was 60 minutes and D was 16.7 mg/l.

The chamber used in this study consisted of an 18.5-liter glass cylinder. The lid contained air intake and exit tubes positioned at the top and bottom of the chamber, respectively. Air was supplied from a compressed air line, pressure regulated and filtered, and then delivered to a Vapo-Nephron nebulizer where the sample formed into respirable, micron-sized droplets or vapour. The vapour was then passed into the top of the exposure chamber and was uniformly distributed throughout the chamber and past the breathing zone of the animals.

Analytical verification of test atmosphere concentrations: no.

Duration of exposure: 1 h.

Concentrations: 57.6 mg/l in air (with water as vehicle), 16.7 mg/l in air (with propylene glycol as vehicle).

No. of animals per sex per dose: 10 males per dose.

Control animals: no.

Details on study design:

At the conclusion of the one-hour exposure periods, the animals were removed from the chamber and housed by groups in wire mesh cages elevated above the droppings.

Observations were made of the appearance and behaviour of the animals continuously during the exposure period and at frequent intervals thereafter for a total of 14 days. At the end of

the observation period, the animals were weighed, sacrificed by cerebral concussion, and gross necropsies were performed.

Results and discussion:

Sex: male.

LC50 > 56.7 mg/L air (nominal) based on test mat.

Exp. Duration: 1 h.

Mortality:

No mortalities occurred in animals exposed to the test material in distilled water or propylene glycol.

Clinical signs:

Dimethyltin Dichloride in Distilled Water:

Observations noted during the exposure period included initial "excited" activity shown by all of the animals, the majority of the animals exhibited little or no activity throughout the period, depression, shallow respiration, serosanguineous stains around the nose, animals exhibited excessive masticatory movements, preening and lacrimation, and "squinting" of the eyes on occasion.

Upon removal from the exposure chamber, all of the animals exhibited depression and shallow respiration, eight exhibited excessive lacrimation, nine exhibited serosanguineous stains around the nose and five exhibited wheezing. On the first post-exposure day, two exhibited serosanguineous stains around the nose and urine stains and one animals was wheezing. From the second day throughout termination of the study, all of the animals appeared grossly normal.

Dimethyltin Dichloride in Propylene Glycol:

Observations noted during the exposure period included initial "excited" behaviour in all of the animals, little or no activity throughout the period, depression, laboured respiration, serosanguineous stains around the nose and/or mouth, excessive salivation, ataxia and damp haircoats in the majority of animals and occasional preening, excessive lacrimation, "squinting" of the eyes and masticatory movements.

Upon removal from the exposure chamber, all of the animals exhibited depression, depressed righting and placement reflexes, excessive salivation and stains, damp haircoats, eight showed excessive lacrimation, seven serosanguineous stains around the nose and five exhibited wheezing. On the first and post-exposure day, two or three of the animals exhibited sero-sanguineous stains around the nose and one or two of the animals were wheezing. With the exception of one animals, which exhibited wheezing from day three through five, all of the animals exhibited normal appearance and behaviour until termination.

Body weight

DIMETHYLTIN DICHLORIDE IN DISTILLED WATER

The animals showed an average body weight gain of 82 g; this weight gain was within the normal limits for the rats of the age, sex and strain used in this study.

DIMETHYLTIN DICHLORIDE IN PROPYLENE GLYCOL

The animals from this study showed an average body weight gain of 107 g; this weight gain is within the normal limits for the rats of the age, sex, and strain used in this study.

Gross pathology

Gross necropsies performed at termination revealed no significant gross pathological alterations in animals exposed to the test material in distilled water or propylene glycol.

Reliability score 2 (reliable with restrictions) was given by the registrant, because study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

[Study 3]

Study reference:

Author not disseminated (1976).

Test type:

Test type: standard acute method.

Limit test: no.

According to Federal Register August 12, 1961 et seq. FHSA.

Deviations: no data.

Equivalent or similar to Guideline OECD Guideline 403 (Acute Inhalation Toxicity).

Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Test animals:

Species: rat.

Strain: Wistar.

Sex: male.

Details on test animals and environmental conditions

TEST ANIMALS

- Source: not stated.
- Weight at study initiation: 200 - 210 g.
- Housing: individual cages.
- Diet (e.g. ad libitum): Standard laboratory diet (Ssniff/Intermast) ad libitum.
- Water (e.g. ad libitum): ad libitum.
- Acclimation period: 4 days.

ENVIRONMENTAL CONDITIONS

- Temperature: 22 °C.

- Humidity (%): 50 – 60.
- Photoperiod (hrs dark / hrs light): 12/12.

Administration/exposure:

Route of administration: inhalation (aerosol).

Type of inhalation exposure: no data.

Vehicle: water.

Details on inhalation exposure

The rats were immobilized in special cages in the inhalation chamber for four hours, so that only their noses came in contact with the aerosol. The chamber used in this study consisted of a 20 l plastic cylinder with an air change of once every two minutes. Air was supplied from a compressed air line into a nebulizer. On a flow meter connected after the nebulizer the exact air flow of 10 l/minute was determined. The nebulizer produced an aerosol with particle sizes of less than 5 microns. The sample was injected at a constant rate into the nebulizer, mixed with the air in the mixing area and then passed into the top of the exposure chamber and was then uniformly distributed throughout the chamber and past the breathing zone of the animals.

The concentration of the test material in the chamber atmosphere was determined by the following formula

$$A / (B \times C) = D$$

A = Weight of sample in µl used during the exposure period.

B = Air flow (l/min.).

C = Duration of exposure (min).

D = Concentration of sample in chamber atmosphere (µl/l).

Analytical verification of test atmosphere concentrations: no data.

Duration of exposure: 4 h.

Concentrations: 100, 130 and 169 µl/l.

No. of animals per sex per dose: 10 males per dose. No female rats were used.

Control animals: no.

Details on study design:

At the conclusion of the four hour exposure period the animals were removed from the chamber, their heads carefully cleaned of any material and housed in individual cages. Observations were made continuously of the appearance and behaviour of the animals during, the exposure period and at frequent intervals thereafter for a total of 14 days. At the end of the observation period the rats were weighed, sacrificed and gross autopsies were performed.

Results and discussion:

LC50: 139 µl/L air based on act. ingr.
95% CL 125 - 154.6.

Exp. duration 4 h.

Mortality:

100 µl/l : No mortalities within 14 days

130 µl/l : 4/10 mortalities within 24 hours; no further mortalities.

169 µl/l : 8/10 mortalities within 24 hours; no further mortalities.

Clinical signs

During the exposure time and several hours thereafter the animals showed a slight - medium degree of depression of the respiration, slight apathia and disturbances in coordination. The reflexes, urine and fecal deposition of the survived animals were normalised 24 hours after exposition.

Body weight

During the observation period of 14 days all the surviving animals showed a normal gain of body weight.

Gross pathology

At terminal autopsy no macroscopic pathological changes were observed in the cranial, abdominal and chest cavities. The animals which died during or shortly after the exposure time showed lung edemas and lung emphysemas of a slight to moderate degree.

Reliability score 2 (reliable with restrictions) was given by the registrant because study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

[Study 4]

Study reference:

Hazelton Laboratories (1976).

Test type:

Test type: standard acute method.

Limit test: yes.

Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Test animals:

Species: rat.
Strain: Sprague-Dawley.
Sex: male.

TEST ANIMALS:

- Weight at study initiation: 238-276 g.

ENVIRONMENTAL CONDITIONS:

- No data available.

Administration/exposure:

Route of administration: inhalation: aerosol.

Type of inhalation exposure: no data.

Vehicle: air.

Details on inhalation exposure:

The first group was exposed to the test material dissolved in equal parts of solvent (w/w) composed of equal parts of propylene glycol and distilled water (w/w) in a 38-liter glass inhalation chamber. The second group was similarly exposed to an aerosol of one part test material dissolved in two parts distilled water (w/w). Both solutions were the maximum concentrations achievable.

The stock solutions for both groups were each placed in a glass nebulizer (refluxing type with internal impactor) which was designed to deliver particles with an aerodynamic mass median diameter (MMD) in the range of 2.5 to 3.5 μ , with at least 80% less than 5.0 μ MMD. For the first group, the nebulizer was operated for 60 minutes with five litres/minute airflow at 30 psi and an additional one litre/ minute airflow through the chamber. The nebulizer for the second group was operated for 60 minutes with six litres/minute airflow. Total airflow for both groups was six litres/minute. The nebulizers were pre-weighed after charging and post-weighed after each 60-minute generation period. The loss of weight divided by the total airflow during each exposure (360 litres) gave the nominal aerosol concentration for that exposure.

All animals were group-housed in stainless steel mesh cages on one level during each exposure. At the termination of the exposure period, each chamber was allowed to remain sealed for another 30 minutes with an airflow of 15 litres/minute.

Analytical verification of test atmosphere concentrations: no.

Duration of exposure: 1 h.

Concentrations:

For the first group the nominal aerosol concentration was 11.53 mg/litre. This aerosol delivery yielded a concentration of 5.77 mg/litre of test material. For the second group the nominal aerosol concentration was 15.02 mg/litre which was equivalent to 5.00 mg/litre of test material.

No. of animals per sex per dose: 10 male rats per dose.

Control animals: no.

Details on study design:

All animals were observed for pharmacotoxic manifestations and mortality periodically throughout the one-hour exposure period, and daily for 14 days after the termination of exposure. All rats were sacrificed on the 15th day.

Results and discussion:

Mortality: No deaths occurred in either of the groups.

Clinical signs: All rats in both exposure groups exhibited inactivity during the exposure period. At the termination of the exposures, all 5.77 mg/litre animals and several animals exposed to 5.00 mg/litre of test material had a slight to moderate discharge around the muzzle and nasal areas. All animals in both groups exhibited normal appearance and behaviour throughout the 14-day post-exposure observation period.

Body weight: NDA.

Gross pathology: NDA.

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

[Study 5]

Study reference:

Wells Laboratories (1975).

Test type:

Test type: standard acute method.

Limit test: no.

Equivalent or similar to Guideline OECD Guideline 403 (Acute Inhalation Toxicity)

Deviations: yes, only 1 hour duration.

Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride

Form: crystalline

Name of test material (as cited in study report): dimethyltin dichloride 1120-98

Physical state: solid

Test animals:

Species: rat.
Strain: no data.
Sex: male/female.

Administration/exposure:

Route of administration: inhalation: aerosol.

Type of inhalation exposure: no data.

Vehicle: air.

Details on inhalation exposure: Groups of 10 rats, both male and female, inhaled the test material within a closed chamber of known volume. Test material was dissolved in water and the pH of the solution was determined (1.25-1.50). Solution was sprayed into the chamber by means of an atomizer with nozzle size to provide droplets in the 3 to 10 micron range which animals inhaled for one hour.

Analytical verification of test atmosphere concentrations: no.

Duration of exposure: 1 h.

Concentrations: 50, 100, 200 and 300 mg/l/hour.

No. of animals per sex per dose: 10 rats per dose, mixture of sexes.

Control animals: no.

Results and discussion:

Sex: male/female.
LC50: 125 mg/L air (nominal) based on test mat.
95% CL 83.3 - 187.5.
Exp. Duration: 1 h.

Mortality:
50 mg/l/hour: 2/10
100 mg/l/hour: 5/10
200 mg/l/hour: 7/10
300 mg/l/hour: 10/10

Clinical signs:

Rats exposed to lower dose of the test material experienced CNS depression. After recovery from the state of depression, the rats became very aggressive and fought with each other. They were also very sensitive to sound and touch. This behaviour of aggressiveness was only observed in the male rats.

Body weight: NDA.

Gross pathology: Gross findings at autopsy on dead rats showed blood in the lungs and heart failure. Fluid was in the chest cavity. The spleen was very dark and the stomach was filled with gas.

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which did not affect the quality of the relevant results.

[Study 6]

Study reference:

Reference type: review article or handbook.

Author: Summer K.H., Klein, D. & Greim, H.

Year: 2003.

Title: Ecological and Toxicological Aspects of Mono and Disubstituted Methyl-, Butyl-, Octyl-, and Dodecyltin Compounds - Update 2002.

Bibliographic source: Organotin Environmental Programme (ORTEP) Association, The Hague, The Netherlands. Primary source: Wells Laboratories, Inc. (1976) Report on Inhalation LC50 in Rats Using 25% Mono/75% Dimethyltin Chloride. Laboratory No. G-1404.

Test type:

Limit test: no.

Principles of method if other than guideline: No methodological information.

GLP compliance: no according to the registrant data.

Test substance:

Name of test material (as cited in study report): monomethyltin trichloride + dimethyltin dichloride (21.5 : 78.5) (identity of test material not same as for substance defined in section 1 (if not read-across)).

Test material form: crystalline.

Test animals:

Species: rat.
Strain: no data.
Sex: male/female.

Administration/exposure:

Route of administration: inhalation: aerosol.
Type of inhalation exposure: no data.
Vehicle: no data.
Analytical verification of test atmosphere concentrations: no data.
Duration of exposure: 1 h.
Control animals: no data.

Results and discussion:

Sex: male/female.
LC50: 84 mg/L/hr based on test mat.
Exp. Duration: 1 h.

Reliability score 4 (not assignable) was given by the registrant because the information source was a review article with no methodological information.

[Study 7]

Study reference:

Author not disseminated (1978e).

Reference type: review article or handbook.

Test type:

Materials and methods: No methodological information available.

GLP compliance: no according to the registrant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Test animals:

Species: rat.
Strain: no data.
Sex: no data.

Administration/exposure:

Route of administration: inhalation: vapour.
Vehicle: no data.
Analytical verification of test atmosphere concentrations: no data.
Control animals: no data.

Results and discussion:

Sex: no data.
LC50: 1070 mg/L/hr.
Based on: no data.

Reliability score 4 (not assignable) was given by the registrant because the study is from a review article with no methodological information.

[Study 8]

Study reference:

Reference type: review article or handbook.

Author not disseminated (2006).

Report date: 2006-07-23.

Test type:

Limit test: no.

No methodological information available.

GLP compliance: no according to the registrant data.

Test substance:

Test material (EC name): dimethyltin dichloride.

Test material form: crystalline.

Test animals:

Species: rat.

Sex: male/female.

Administration/exposure:

Route of administration: inhalation: aerosol.

Type of inhalation exposure: no data.

Vehicle: no data.

Analytical verification of test atmosphere concentrations: no data.

Duration of exposure: 1 h.

Concentrations: 1910 mg/m³.

Control animals: no data.

Results and discussion:

Sex male/female

LC50 > 1910 mg/m³ air based on test mat.

Exp. Duration: 1 h.

Mortality: no deaths observed after 21 days.

Body weight: normal body weight gain reported.

Reliability factor 4 (not assignable) was given by the registrant because the information was a secondary source information from review dossier.

[Study 9]

Study reference:

Author not disseminated (1978f).

Reference type: review article or handbook.

Test type:

Principles of method if other than guideline: no methodological information available.

GLP compliance: no according to the registrant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Test material form: crystalline.

Test animals:

Species: rat.
Strain: no data.
Sex: no data.

Administration/exposure:

Route of administration: inhalation: vapour.
Type of inhalation exposure: no data.
Vehicle: no data.
Analytical verification of test atmosphere concentrations: no data.
Control animals: no data.

Results and discussion:

LC50 > 1.91 mg/L/hour.

Reliability factor 4 (not assignable) was given by the registrant because the information source is a review article with no methodological information.

[Study 10]

Study reference:

Ciba-Geigy, 1977.

Test type:

Similar to OECD 403 with shorter duration of exposure.

Test substance:

Test substance: DMTC (purity not known).

Test animals:

Rat.

Administration/exposure:

Doses: 640, 1679, and 3012 mg/m³.

1 h exposure to aerosol.

Results and discussion:

LC50 1632 mg/m³, 1.6 mg/L.

Calculated LC50 on 4 hour using Haber laws and n=1 as recommended in IR/CSA R7.4.4.1 for extrapolation to longer durations: LC50 = 0.4 mg/L.

Acute inhalation toxicity - human data

[Study 1]

Study reference:

Reference type: secondary source.

Author: Harper, C. et al.

Year: 2005.

Title: Toxicological Profile for Tin and Tin Compounds.

Bibliographic source: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Primary Source: Rey C. et. al. (1984) Methyltin intoxication in six men: Toxicologic and clinical aspects. Vet Hum Toxicol 26: 121-122.

Test type:

Limit test: no.

Case study of workers exposure.

GLP compliance: no according to the registrant.

Test substance:

Test material: mixture of half dimethyltin and half trimethyltin chloride vapour.

Test material form: crystalline.

Administration/exposure:

Route of administration: inhalation.

Type of inhalation exposure: no data.

Analytical verification of test atmosphere concentrations: no.

Duration of exposure: 1.5 h.

Remarks: over a 3 day working period.

Results and discussion:

One of six workers died 12 days following exposure to a mixture of half dimethyltin and half trimethyltin chloride vapour that occurred during the cleaning of a caldron at a chemical plant. Maximum exposure was a total of 1.5 hours over a 3 day working period. No estimates of exposure levels were given. The symptoms preceding death included excretion of high levels of tin in the urine, respiratory depression, and coma.

Autopsy of the chemical worker who died following exposure to a combination of methyltin salts revealed massive fatty degeneration of liver cells and necrosis.

Although the two surviving workers, who were the most severely affected, developed permanent neurological disabilities, respiratory problems did not persist.

Autopsy of the one chemical worker who died following exposure to the combination of the methyltin salts revealed shock kidneys (i.e., proximal tubule degeneration), which represents serious tubule damage. The other five exposed men had high tin concentrations in the urine with the highest levels occurring in the most severely affected.

Acute inhalation toxicity - other data

No data.

3.2.Skin corrosion/irritation

Skin corrosion/irritation - animal data

[Study 1]

Study reference:

Rush, R.E. (1993b).

Test type:

According to Guideline OECD Guideline 404 (Acute Dermal Irritation / Corrosion).

Deviations: yes.

According to Guideline EU Method B.4 (Acute Toxicity: Dermal Irritation / Corrosion).

Deviations: yes.

Principles of method if other than guideline: The relative humidity of the animal room (26-53%) exceeded the range specified in the protocol (40-60%) during this study. These occurrences are considered to have had no apparent impact on the outcome of this study.

GLP compliance: yes.

Test substance:

Test material (EC name): dimethyltin dichloride.

Test material form: no data.

Physical state: liquid.

Storage condition of test material: room temperature.

Test animals and administration/exposure:

Species: rabbit.

Strain: New Zealand White.

TEST ANIMALS

- Source: Mohican Valley Rabbitry, Loudonville, OH.
- Age at study initiation: NDA.
- Weight at study initiation: 2 - 3.5 kg.
- Housing: The animals were housed individually in suspended stainless steel cages.
- Diet (e.g. ad libitum): Purina Certified Rabbit Chow #5322 was provided ad libitum.
- Water (e.g. ad libitum): ad libitum.
- Acclimation period: minimum of 5 days.

ENVIRONMENTAL CONDITIONS

- Temperature (°F): 61 – 70.
- Humidity (%): 26 - 53%.
- Air changes (per hr): NDA.
- Photoperiod (hrs dark / hrs light): 12-hour light/12-hour dark cycle.

IN-LIFE DATES: From: 1st March 1993 To: 4th March 1993

Type of coverage: semiocclusive.

Preparation of test site: shaved.

Vehicle: no data.

TEST MATERIAL

Amount(s) applied (volume or weight with unit): 0.5 mL.

Duration of treatment / exposure: 4 hours.

Observation period: 72 hours.

Number of animals: 6 (4 male, 2 female).

Control animals: no.

TEST SITE

- Area of exposure: 1" x 1".

- % coverage: NDA.

- Type of wrap if used: Gauze patch held in place at the cut edges with nonirritating tape. An elastic wrap was placed over the trunk and secured at both ends with tape to prevent removal and ingestion of the test material (semi-occlusive binding).

REMOVAL OF TEST SUBSTANCE:

- Washing (if done): Residual test article was removed where practical using gauze moistened with distilled water.
- Time after start of exposure: 4 hours.

SCORING SYSTEM:

The data for each animal was individually analysed based on the definitions presented below:

1. Non-irritant - Any test site that does not exhibit signs of dermal irritation (ex., no erythema and/or edema) following application of the test material.
2. Irritant - Any test site that exhibits reversible inflammatory changes (ex., erythema and/or edema) following application of the test material.
3. Corrosive - Any test site that exhibits irreversible damage (ex., necrosis, ulceration, eschar) following application of the test material.

DERMAL IRRITATION GRADING SYSTEM (DRAIZE)

ERYTHEMA

- 0 - No erythema.
- 1 - Very slight erythema (barely perceptible).
- 2 - Well-defined erythema.
- 3 - Moderate to severe erythema.
- 4 - Severe erythema (beet redness) to slight eschar formations (injuries in depth).

EDEMA

- 0 - No edema
- 1 - Very slight edema (barely perceptible).
- 2 - Slight edema (edges of area well defined by definite raising).
- 3 - Moderate edema (raised approximately 1 mm).
- 4 - Severe edema (raised more than 1 mm and extending beyond the area of exposure).

Results and discussion:

Irritation parameter: erythema score.

Mean of 24, 48 and 72 hours: score 4, max. score 4.

Reversibility: not reversible.

Remarks: Same score for all rabbits at all time points.

Irritation parameter: edema score

Mean of 24, 48 and 72 hours: score 3.94, max. score 4.

Reversibility: not reversible.

Remarks: Scores the same for all rabbits at all time points, except for one score of 3 in one rabbit at 48 hours. All other scores were 4.

Irritant/corrosive response data:

Exposure to the test material produced necrosis and blanching with severe edema on 6/6 test sites at the 1 hour scoring interval. The dermal irritation progressed to eschar on 3/6 test sites by the 72 hour scoring interval.

Reliability score 1 (reliable without restriction) was given by the registrant because the study was conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of the relevant results.

[Study 2]

Study reference:

Author not disseminated (1973).

Report date 1973-01-26.

Test type:

Type of method: in vivo.

According to method described in 'Hazardous Substances Regulations' under the U.S. Federal hazardous Substances Labelling Act Sect. 191.11 (February 1965).

Deviations: no data.

Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Name of test material (as cited in study report): TK1137

- Substance type: crystalline powder
- Physical state: solid

Test animals and administration/exposure:

Species: rabbit.

Strain: New Zealand White.

TEST ANIMALS:

- Source: Bred on the premises.
- Age at study initiation: 3 - 4 months.
- Weight at study initiation: mean 2.55 kg (male); mean 2.68 kg (female).
- Housing: individual cages.
- Diet (e.g. ad libitum): commercial irradiated diet (Styles-Oxoid) was fed ad libitum.

- Water (e.g. ad libitum): Sterilised water was available at all times.
- Acclimation period: one week.

ENVIRONMENTAL CONDITIONS:

- Temperature (°C): 20 ± 1 °C.
- Humidity (%): 50 -70.
- Photoperiod (hrs dark / hrs light): 15 / 9.

Type of coverage: occlusive.

Preparation of test site: shaved and abraded.

Vehicle: 50% polyethylene glycol in water.

TEST MATERIAL:

Amount(s) applied (volume or weight with unit): 0.5 g.

Concentration (if solution): moistened with a 50% solution of polyethylene glycol in water.

Duration of treatment / exposure: 24 hours.

Observation period: N/A as animals were destroyed for humane reasons.

Number of animals: Three.

Control animals: no.

TEST SITE:

Area of exposure: 2.5 x 2.5 cm.

Type of wrap if used: square gauze pad covered with aluminium foil secured with adhesive tape.

REMOVAL OF TEST SUBSTANCE:

Washing (if done): no.

Results and discussion:

After 24 hours the skin over the test sites was necrotic, with deep fissuring into the subcutaneous tissue. All animals were destroyed for humane reasons.

No scores are available as the test was ended early for humane reasons.

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

[Study 3]

Study reference:

Affiliated Medical Enterprises Inc. 1971c.

Test type:

Equivalent or similar to Guideline OECD Guideline 404 (Acute Dermal Irritation / Corrosion).

Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Name of test material (as cited in study report): DM-8121.

Test animals and administration/exposure:

Species: rabbit.

Strain: other: New Zealand.

Details on test animals and environmental conditions: NDA.

Type of coverage: no data.

Preparation of test site: shaved and abraded sites

Vehicle: no data.

Amount/concentration applied: 0.5 g of test substance.

Duration of treatment / exposure: 24 hours.

Observation period: 24 and 72 hours.

Number of animals: 6 males.

Control animals: no.

TEST SITE:

Area of exposure: 1" x 1"

Type of wrap if used: Standard patch test plasters

SCORING SYSTEM: Draize (1959)

Results and discussion:

Irritation parameter erythema score:
Mean of 24 and 72 hours: Score 3.
Max. score 3.
Reversibility: not reversible.
Scores were the same for all animals and for intact and abraded skin.

Irritation parameter edema score:
Mean at 24 hours: Score 1.
Max. score 1
Reversibility: fully reversible within 72 hours.

Primary dermal irritation index: 1.75.

[Study 4]

Study reference:

Author not disseminated (1973).

Report date 1973-04-10.

Test type:

Type of method: in vivo.
According to Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics (1959) of the US Association of Food and Drug Officials.
Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Name of test material (as cited in study report): TK 1137.

Test animals and administration/exposure:

Species: rabbit.

Strain: Russian.

Details on test animals and environmental conditions:

TEST ANIMALS

- Diet (e.g. ad libitum): Navag, Gossau, rabbit food available ad libitum.
- Water (e.g. ad libitum): ad libitum.

Test system:

Type of coverage: occlusive.
Preparation of test site: shaved and abraded.
Vehicle: polyethylene glycol.

TEST MATERIAL

Amount(s) applied (volume or weight with unit): 0.5 g.
Concentration (if solution): 50% in PEG.
Duration of treatment / exposure: 24 hours.
Observation period: 72 hours.
Number of animals: 3 male, 3 female.
Control animals: no.

Details on study design

TEST SITE

- Area of exposure: 2.5 x 2.5 cm.
- % coverage: NDA.
- Type of wrap if used: gauze patch covered with plastic film measuring 5 x 5 cm, fixed to body with adhesive tape.

SCORING SYSTEM:

Erythema and Eschar Formation:

- 0 - No erythema
- 1 - Very slight erythema (barely perceptible)
- 2 - Well defined erythema
- 3 - Moderate to severe erythema
- 4 - Severe erythema (beet redness) to slight eschar formation (injuries in depth)

Edema Formation:

- 0 - No edema
- 1 - Very slight edema (barely perceptible)
- 2 - Slight edema (edges of area well defined by definite raising)
- 3 - Moderate edema (raised approximately 1 mm)
- 4 - Severe edema (raised more than 1 mm and extending beyond area of exposure)

Results and discussion:

Irritation parameter: primary dermal irritation index (PDII)

Mean of 24 and 72 hours: Score 0.8.

Reversibility: no data.

The primary irritation index as the measure of the acute skin irritation provoked by the substance is defined as the average of all the ratings found after 24 and 72 hours on the intact and slightly scarified skin. The primary irritation index of the test substance was 0.8.

Other effects:

After 24 hours, the animals showed signs of tremor, slight spasms, asynchronism of the extremities and, additionally after 48 hours, the animals displayed a curved, lateral or ventral position.

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted in accordance with generally accepted scientific principles, possibly with

incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results. Results page missing from paper.

Skin corrosion/irritation - human data

No data.

Skin corrosion/irritation - other data

No data.

3.3.Eye damage/eye irritation

Eye damage/eye irritation - animal data

[Study 1]

Study reference:

Author not disseminated (1973).

Report date 1973-04-11.

Test type:

According to Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics" (1959) of the AFDO.

Equivalent or similar to OECD Guideline 405 (Acute Eye Irritation / Corrosion).

Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Name of test material (as cited in study report): TK 1137.

Test animals:

Species: rabbit.

Strain: Russian.

- Diet (e.g. ad libitum): Navag, Gossau, rabbit food available ad libitum.
- Water (e.g. ad libitum): ad libitum.

Administration/exposure:

Vehicle: unchanged (no vehicle).

TEST MATERIAL

- Amount(s) applied (volume or weight with unit): 0.1 g of test substance placed into left eye of rabbit with spatula.
- Concentration (if solution): N/A.

After application, the rabbits eyelids were held open for a few seconds.

Duration of treatment / exposure: 30 seconds.

Observation period: 7 days.

Number of animals: 3 male, 3 female.

Control animals: other: right eye of animal served as a control.

REMOVAL OF TEST SUBSTANCE

- Washing (if done): yes, with 10 mL luke warm water
- Time after start of exposure: 30 seconds

TOOL USED TO ASSESS SCORE: hand-slit lamp

SCORING SYSTEM:

Cornea:

A. Opacity - degree of density (area most dense taken for reading)

0 - No opacity

1 - Scattered or diffuse area, details of iris clearly visible

2 - Easily discerned translucent areas, details of iris slightly obscured

3 - Opalescent areas, no details of iris visible, size of pupil barely discernible

4 - Opaque, iris invisible

B. Area of cornea involved

1 - One quarter (or less) but not zero

2 - Greater than one quarter, but less than half

3 - Greater than half, but less than three quarters

4 - Greater than three quarters, up to whole area

A x B x 5 Total maximum = 80

Iris:

A. Values

0 - Normal

1 - Folds above normal, congestion, swelling, circum-corneal injection (any or all of these or combination thereof) iris still reacting to light (sluggish reaction is positive)

2 - No reaction to light, hemorrhage, gross destruction (any or all of these)

A x 5

Conjunctivae:

A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

- 0 - Vessels normal
- 1 - Vessels definitely injected above normal
- 2 - More diffuse, deeper crimson red, individual vessels not easily discernible
- 3 - Diffuse beefy red

B. Chemosis

- 0 - No swelling
- 1 - Any swelling above normal (includes nictitating membrane)
- 2 - Obvious swelling with partial eversion of lids
- 3 - Swelling with lids about half closed
- 4 - Swelling with lids about half closed to completely closed

C. Discharge

- 0 - No discharge
- 1 - Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)
- 2 - Discharge with moistening of the lids and hairs just adjacent to lids
- 3 - Discharge with moistening of the lids and hairs, and considerable area around the eye

$(A + B + C) \times 2$ Total maximum = 20

Results and discussion:

Cornea score (mean of all time points): Score 80.

Max. score 80.

Reversibility: not reversible.

Iris score (mean of all time points): Score 10.

Max. score 10.

Reversibility: not reversible.

Conjunctivae score (mean of all time points): Score 12.5.

Max. score 20.

Reversibility: not reversible.

Irritant/corrosive response data:

The results of the eye irritation test are summarised in tables 1 and 2. The total maximum is 80 for the cornea, 10 for the iris and 20 for the conjunctivae. The irritation Index was found to be 80 for the cornea, 10 for the iris and 12.5 for the conjunctivae.

Table 1: Eyes rinsed

Days after applic.	Rabbit 1 (female)			Rabbit 2 (female)			Rabbit 3 (female)		
	Cornea	Iris	Conjunct.	Cornea	Iris	Conjunct.	Cornea	Iris	Conjunct.
1	80	10	14	80	10	14	80	10	14
2	80	10	16	80	10	14	80	10	14
3	80	10	18	80	10	14	Dead within 3 days		
4	80	10	18	80	10	14			
7	Dead within 4 days			80	10	14			

Table 2: Eyes not rinsed

Days after applic.	Rabbit 4 (male)			Rabbit 5 (male)			Rabbit 6 (male)		
	Cornea	Iris	Conjunct.	Cornea	Iris	Conjunct.	Cornea	Iris	Conjunct.
1	80	10	12	80	10	12	80	10	8
2	80	10	8	80	10	14	80	10	10
3	80	10	12	80	10	16	Dead within 3 days		
4	80	10	12	80	10	16			
7	Dead within 5 days			80	10	16			

Other effects:

After 24 hours, the animals showed in addition to the irritation tremor, slight spasms, asynchronism of the extremities and after 48 hours in addition to the above symptoms, curved, lateral or ventral position.

4/6 animals died during the 7-day observation period.

Reliability score 2 (reliable with restrictions) was given by the registrant because the study conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

[Study 2]

Study reference:

Author not disseminated (1971).

Report date 1971-03-14.

Test type:

Type of method: in vivo.

Equivalent or similar to OECD Guideline 405 (Acute Eye Irritation / Corrosion).

Not GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Name of test material (as cited in study report): DM-8121.

Test animals:

Species: rabbit.

Strain: New Zealand.

Administration/exposure:

Details on test animals and environmental conditions: NDA.

Vehicle: unchanged (no vehicle).

Amount/concentration applied: 100 mg.

Duration of treatment / exposure: 2 seconds.

Observation period: 72 hours.

Number of animals: nine males.

Control animals: other: left eye served as a control.

REMOVAL OF TEST SUBSTANCE:

- Washing (if done): luke-warm water

- Time after start of exposure: three animals after two seconds; three animals after four seconds and three animals unwashed

SCORING SYSTEM: Draize (1956)

Results and discussion:

Cornea score (mean of 24, 48 and 72 hours): Score 4.

Max. score 4.

Reversibility: not reversible.

Remarks: Score was the same for all rabbits at all time points.

Iris score (mean of 24, 48 and 72 hours): Score 2.

Max. score 2.

Reversibility: not reversible.

Remarks: scores were the same for all rabbits at all time points.

Conjunctivae score (mean of 24, 48 and 72 hours): Score 3.

Max. score 3.

Reversibility: not reversible.

Remarks: scores were the same for all rabbits at all time points.

Chemosis score (mean of 24, 48 and 72 hours): Score 4.

Max. score 4.

Reversibility: not reversible.

Remarks: scores were the same for all rabbits at all time points.

Irritant/corrosive response data:

The group mean score at 24 hours was 110/110. The group mean scores at 48 and 72 hours were 110/110. The Draize evaluation does not provide for the observation of hemorrhage which was noted in this test. One rabbit from each group was selected for autopsy following the 72 hour reading. Complete enucleation of the eye was noted in each animal examined.

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

[Study 3]

Study reference:

Author not disseminated (1973).

Report date 1973-01-26.

Test type:

Type of method: in vivo.

Equivalent or similar to OECD Guideline 405 (Acute Eye Irritation / Corrosion).

According to procedure in "Hazardous Substances Regulations" under the U.S. Federal Hazardous Substances Labelling Act Sect. 191.12 (February 1965) (with modifications).

Deviations: no data.

GLP compliance: no according to the registrant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Details on test material

- Name of test material (as cited in study report): TK1137.
- Substance type: crystalline powder.
- Physical state: solid.

Test animals:

Species: rabbit.

Strain: New Zealand White.

TEST ANIMALS:

- Source: Bred on the premises.
- Age at study initiation: 2.5 - 3 months.
- Weight at study initiation: mean 2.55 kg (male); mean 2.68 kg (female).
- Housing: caged individually.
- Diet (e.g. ad libitum): commercial irradiated diet (Styles-Oxoid) ad libitum.
- Water (e.g. ad libitum): Sterilised water was available at all times.
- Acclimation period: one week.

ENVIRONMENTAL CONDITIONS:

- Temperature (°C): 20 ± 1 °C.
- Humidity (%): 50 -70.
- Photoperiod (hrs dark / hrs light): 15 / 9 from (light from 8:00 - 23:00).

Administration/exposure:

Vehicle: unchanged (no vehicle).

TEST MATERIAL

Amount(s) applied (volume or weight with unit): 100 mg

Duration of treatment / exposure: Eye held shut for 1 seconds, and then washed out after 30 seconds.

Observation period: The rabbits were examined 1, 6 and 24 hours after application of the test compound.

Number of animals: 3 male, 3 female.

Control animals: other: The right eye served as a control.

REMOVAL OF TEST SUBSTANCE:

- Washing (if done): with warm water.
- Time after start of exposure: 30 seconds.

Results and discussion:

One hour after application of the test material there was partial corneal destruction. The opacity of the cornea was such that the iris was not visible in three animals and partly obscured in one other. Marked chemosis and injection of the conjunctivae was present in all animals. Loss of sensation to touch over the cornea was present in five animals. Conjunctival mucosae of all rabbits was necrotic. At 24 hours moderate to severe necrosis of conjunctivae was present in all animals with severe periorbital oedema. The rabbits were destroyed for humane reasons.

Other effects: Instillation of the test compound into the eye caused pain. After 6 hours all animals appeared lethargic and had severe chemosis with blood stained discharge from the eyes. The cornea was completely opaque in five animals. Phlyctenar occurred on the cornea of one animal. An unpleasant odour was noted from all animals when examined at both 1 and 6 hours.

Scores were not obtained as the animals were killed early for humane reasons.

Reliability score 2 (reliable with restrictions) was given by the registrant because the study conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

Eye damage/eye irritation - human data

No data.

Eye damage/eye irritation - other data

No data.

3.4. Respiratory sensitisation

Respiratory sensitisation - animal data

No data.

Respiratory sensitisation - human data

No data.

Respiratory sensitisation - other data

No data.

3.5.Skin sensitisation

Skin sensitisation - animal data

[Study 1]

Study reference:

Author not disseminated (1973).

Report date 1973-04-11.

Test substance

Test material (EC name): dimethyltin dichloride.

Materials and methods:

Type of method: in vivo.

Type of study: Maurer optimisation test.

According to the experimental procedure was that prescribed by The Food and Drug Administration of the U.S.A. in "Appraisal of the Safety of Chemicals in Food, Drugs and Cosmetics, "1959, p51.

Deviations: no data.

Test animals:

Species: guinea pig.

Strain: Albino, type not specified.

Sex: no data.

Details on test animals and environmental conditions: No data.

Administration/exposure:

Test system: Traditional sensitisation test.
Route of induction exposure: intradermal.
Route of challenge exposure: intradermal.
Vehicle: 1 part PEG to 7.3 parts water
Concentration: 0.1% solution.
No. of animals per dose: 10.

Details on study design (Traditional tests): Prior to each injection of the test sample, the hair from the inter-scapular region was close-clipped using electric clippers. The test material was injected intracutaneously into the clipped inter-scapular region of each guinea-pig. The first injection consisted of 0.05 mL, while the remaining nine were of 0.1 mL each. Ten sensitizing injections were given on alternative days during a three week period.

Challenge controls: After an incubation period of two weeks, a final challenge dose of 0.05mL was injected.

Positive control substance(s): no

Statistics

A comparison was made between the reactions elicited by the challenge dose and the average of readings observed during the sensitizing period. If the value for the challenge reading was substantially higher, the substance under test was considered to have produced sensitization, the degree of sensitisation being proportional to the difference between the two values.

Results and discussion:

From the first 10 induction exposures, the scores were as follows:

Diameter: Average = 0.6, Maximum = 1

Colour: Average = 0.75, Maximum = 2 (in one animal only at one time point; all other scores were 1 or less)

Height: Average = 0.72, Maximum = 1

From the challenge exposure after 2 weeks incubation, no score greater than 1 was seen in any animal for either diameter, colour or height.

Reliability score 3 (not reliable) was given by the registrant due to the extremely low concentration of test substance in the vehicle and the apparent lack of an adjuvant being used, this study cannot be considered as a reliable study to address the skin sensitisation endpoint.

Skin sensitisation - human data

No data.

Skin sensitisation - other data

No data.

3.6. Germ cell mutagenicity

Germ cell mutagenicity - animal data

[Study 1]

Study reference:

Author not disseminated (1990).

Report date 1990-07-24.

Test type:

Type of genotoxicity: gene mutation.

Type of study: bacterial reverse mutation assay (e.g. Ames test).

According to OECD Guideline 472 (Genetic Toxicology: Escherichia coli, Reverse Mutation Assay).

Deviations: no.

GLP compliance: yes.

Test substance:

The test material used in the study is not equivalent to the substance identified in the CLH dossier.

Name of test material (as cited in study report): Dimethyltin Dichloride [CAS No. 753-73-1]:Methyltin Trichloride [CAS No. 993-16-8] (72:28% mixture).

Test material form: crystalline.

Method:

Target gene: Tryptophan.

Species/strain: Species/strain E. coli WP2 uvr A.

Additional strain characteristics other: tryptophan auxotroph.

Metabolic activation: with and without.

Metabolic activation system: S-9 liver fraction from male Sprague-Dawley rats given a single 500-mg/kg intraperitoneal injection of Aroclor 1254.

Test concentrations: 10, 50, 100, 500, 1000, and 5000 µg/plate.

Vehicle:

For test article: Water, deionized.

For positive control substances: Dimethylsulfoxide (DMSO).

Controls:

Negative controls: no.

Solvent / vehicle controls: yes.

True negative controls: no.

Positive controls: yes.

Positive control substance: 1103 at 5 µg/plate.

Negative controls: no.

Solvent / vehicle controls: yes.

True negative controls: no.

Positive controls: yes.

Positive control substance: 1342 2-Anthramine at 20 µg/plate in the presence of metabolic activation will be used to ensure the efficacy of the activation system.

METHOD OF APPLICATION: in agar (plate incorporation).

DURATION:

- Preincubation period: The culture is allowed to sit unshaken for 2 to 4 hours, then gently shaken (100 rpm) for 11 to 14 hours at 37°C.
- Exposure duration: After the top agar has set, the plates are incubated at 37 °C for about 48 hours.
- Expression time (cells in growth medium): The tryptophan-independent revertant colonies are counted following the incubation period; however, if the plates cannot be immediately evaluated, they are refrigerated at 4°C until they can be counted.

NUMBER OF REPLICATIONS: The test article was assayed twice, with three plates per dose level, both with and without a mammalian liver metabolic activation mixture.

NUMBER OF CELLS EVALUATED: 10⁸

DETERMINATION OF CYTOTOXICITY

Toxicity is evidenced by several phenomena: a substantial decrease in the number of revertant colonies on the test plates compared with the controls, the clearing or absence of the background lawn growth, or the formation of pinpoint nonrevertant colonies.

Evaluation criteria:

The tryptophan-independent revertant colonies are counted using an automated colony counter. When accurate counts cannot be obtained (e.g., because of precipitation on the plates), the colonies are counted manually using an electric probe colony counter. The actual numbers of revertant colonies observed and the condition of the background lawn growth are presented in the attached tables. No designation or "-7" indicates a normal background lawn. Toxicity is designated by "-5" for background lawn thinning, "-4" for pinpoint colonies, and "-3" for absence of bacterial growth.

CRITERIA FOR INTERPRETATION:

Positive - A test material is considered a mutagen when it induces a reproducible, dose-related increase in the number of revertants. This increase should occur for at least three consecutive dose levels.

Negative - A test material is considered a nonmutagen when no dose-related increase in the number of revertants is observed in at least two independent experiments.

Inconclusive - When a test material cannot be identified clearly as a mutagen or nonmutagen, the results are classified as inconclusive.

Statistics: The results are a tabulation of the number of colonies appearing on the plates. Mean and standard deviation values were determined for the number of revertants at each dose level.

Results and discussion:

Test results:

Species/strain: *E. coli* WP2 uvr A.
 Metabolic activation: with and without.
 Test system: all strains/cell types tested.
 Genotoxicity: negative.
 Cytotoxicity: yes at 5000 µg/plate.
 Vehicle controls valid: yes.
 Negative controls valid: not examined.
 Positive controls valid: yes.

Additional information on results:

The *E. coli* WP2 (uvrA)/microsome assay was used to evaluate the ability of mixtures of methyltin compounds to induce genetic damage in *E. coli* strain WP2 (uvrA). The assays were performed using the standard plate incorporation procedure, in both the presence and absence of a rat-liver metabolic activation system. The presence of the appropriate genetic

characteristics was verified for the strain used in this study. The results of the controls were acceptable for all assays.

The first assay was conducted in the presence and absence of metabolic activation containing 4% S-9. The doses used were 10, 50, 100, 500, 1000, and 5000 µg/plate. There was no evidence of a dose-related increase in the number of tryptophan revertants. Slightly low revertant counts at the 5000 µg dose may indicate toxicity. Based on these results, the second assay was conducted at the same dose levels, but the percent of S-9 in the metabolic activation mixture was increased to 10% due to the lack of response in the first assay. Note that the portion of the second assay with 10% S-9 was repeated on a separate day due to unacceptable 2-anthramine control values. There was no dose-related increase in the number of tryptophan revertants, and low revertant counts were observed at the 5000 µg dose level, confirming toxicity at this dose.

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of the relevant results. Percentage of DMTC is only 72%. 28% is MMTC.

[Study 2]

Study reference:

Author not disseminated (1990).

Report date 1990-10-25.

Test type:

Type of genotoxicity: chromosome aberration.

Type of study: in vitro mammalian chromosome aberration test.

According to OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test).

Deviations: no.

GLP compliance: yes.

Test substance:

The test material used in the study is not equivalent to the substance identified in the CLH dossier.

Name of test material (as cited in study report): Mixes of Methyltin Compounds.

Substance type: powder.

Physical state: solid.

Storage condition of test material: room temperature in lightproof containers.

Test material form: crystalline.

Method:

Species/strain: lymphocytes.

Details on mammalian cell lines (if applicable): Human peripheral lymphocytes from phytohemagglutinin-M (PHA-M)-stimulated whole blood cultures taken from a 33 year old male blood donor.

Metabolic activation: with and without.

Metabolic activation system: Aroclor 1254-induced rat liver homogenate preparation (S-9).

Test concentrations:

Preliminary: 8, 40, 200, 1000, and 5000 µg/mL.

Test substance in definitive experiment with metabolic activation: 10, 20, 40, 80, 160 µg/mL.

Test substance in definitive experiment without metabolic activation: 2, 4, 8, 16, 32 µg/mL.

Positive control substance in definitive experiment with metabolic activation: 11, 13, 15 µg/mL.

Positive control substance in definitive experiment without metabolic activation: 150, 175, 200 µg/mL.

Vehicle:

Vehicle(s)/solvent(s) used: Sterile deionized water.

Controls:

Negative controls: no.

Solvent / vehicle controls: yes.

True negative controls: no.

Positive controls: yes.

Positive control substance: 1342 Dimethylnitrosamine.

Remarks: positive control for use with metabolic activation.

Negative controls: no

Solvent / vehicle controls: yes.

True negative controls: no.

Positive controls: yes.

Positive control substance: 2072.

Remarks: positive control for use without metabolic activation.

Details on test system and conditions:

DILUTION OF TEST MATERIAL:

Immediately before each assay, the test article was diluted in water to form a series of concentrations that, when diluted in the exposure medium, yielded the appropriate set of test concentrations. The maximum amount of water added to the cultures was 1%.

CYTOTOXICITY STUDIES:

Preliminary cytotoxicity studies were conducted to determine the most appropriate concentrations of the test material to use in the definitive experiment and the optimal cell-fixation time. A series of five concentrations of the test material and a solvent control were used to assess cytotoxicity with duplicate flasks used for each treatment. These studies were conducted both with and without metabolic activation at test material concentrations of 8, 40, 200, 1000, and 5000 µg/mL.

The cytotoxicity studies were designed to assess the effects of the test material on the overall proliferation of the cells (mitotic index, MI) as well as on the kinetics of the cell cycle. The MI was assessed by determining the proportion of cells on a slide that were in metaphase. Cell-cycle kinetics were assessed by determining the number of replications of DNA that occurred after treatment with the test material, using bromodeoxyuridine (BrdU) to differentially label chromatids. In the presence and in the absence of metabolic activation, 48 hours after culture initiation, the cells were exposed to the test article in the metabolic activation mixture or culture medium, respectively, for 5 hours at 37°C. After exposure, the cells were centrifuged, washed two times with PBS and incubated in fresh medium containing 0.01 mM BrdU and 4% PHA-M for 24 hours.

After 22 hours of incubation in the presence of BrdU, colcemid was added to a concentration of 0.2 µg/mL and the cultures were incubated for an additional 2 hours at 37°C. The cells were then harvested, placed on prepared slides, and stained using a fluorescence-plus-Giemsa technique. For each flask, an overall MI was determined based on at least 1000 cells per flask, and 50 cells in metaphase per flask were classified as M1, M2, or M3 cells based on whether they had progressed through one (M1), two (M2), or three (M3) cycles of DNA synthesis during the incubation period after exposure to the test material. A proliferation index (PI) was calculated for each treatment based on the proportions of mitotic cells in their first, second, or third metaphase ($\text{proliferation index} = \#M1 + 2 (\#M2) + 3 (\#M3)/\text{number of metaphase}$).

The MI data were analysed to assess effects of the test material on overall cell proliferation. Cell-cycle data (i.e., the numbers of M1 and M2 cells) and proliferation index were also analysed.

In the absence of any cytotoxicity, the highest concentration of test material to be used in the definitive assay was 5000 µg/mL or the highest soluble concentration, whichever was less. The cell cultures in this case were harvested 18 hours after initiation of exposure to the test material. If the test material had a significant effect on the overall MI but not on cell cycle-kinetics (e.g., lysis of cells), the highest concentration to be used in the definitive assay was within a factor of two of that which resulted in a significant effect on the MI. The cell cultures in this case were harvested 18 hours after initiation of exposure to the test material. If the test material had a significant effect on cell-cycle kinetics (i.e., a significantly reduced number of M2 cells and a corresponding increase in M1 cells compared with the solvent control), the highest concentration used in the definitive assay was within a factor of two of that producing a significant effect on the cell cycle and the harvest time was delayed to 30 hours after initiation of exposure to the test material. Because it was not known whether the test material would induce cell-cycle delay sufficient to preclude analysis of cells for chromosomal aberrations, two sets of duplicate cultures were exposed to the test material; one set (two cultures) being harvested at the earlier time and one set at the later time. The cultures harvested at 18 hours were cytogenetically analysed in preference to the 30-hour cultures if sufficient metaphases were available for analysis. If the harvest time needed to be delayed, extra cultures exposed to the solvent were included and harvested at the later time.

DEFINITIVE STUDIES:

For the definitive assay, the test material was tested with and without MA and duplicate flasks were used for each of five concentrations of the test material in the absence and presence of metabolic activation, as well as the positive and solvent controls. The cells were harvested 18 and 30 hours after initiation of treatment with mixes of methyltin compounds. Because of effects on cell-cycle kinetics, cultures treated with DMN or MMS were harvested 30 hours after initiation of treatment.

For experiments performed without MA, the test positive and solvent control articles were added to Ham's F10 medium and the cells were grown at 37°C for 5 hours. The medium containing the test material was then aspirated, the cells were washed two times with phosphate-buffered saline (PBS) at 37°C, and fresh complete Ham's F10 medium was added. The cultures were then incubated for an additional 10 hours. Colcemid was then added to a final concentration of 0.2 µg/mL.

After 2 hours in colcemid-containing medium, the human lymphocytes were harvested. The cell suspensions were centrifuged and the supernatants discarded. The cells were resuspended in 8 mL of a hypotonic solution of 0.075 M KCl and were incubated at 37°C for 25 minutes. The cells were then suspended three times in a fixative of absolute methanol:glacial acetic acid (3:1) slides were prepared and air-dried. The slides were stained in 3% Giemsa (Gurr R66 in M/15 Sorensen's buffer, pH 6.8) for 10 to 20 minutes, rinsed with deionized water, and passed through xylene; coverslips were mounted with Permount.

CYTOGENETIC ANALYSIS:

Slides prepared from the solvent controls, the positive controls, and three concentrations of the test material were coded by an individual not involved in the microscopic evaluation. In cases where cells were exposed to more than one concentration of a positive control compound, slides from all concentrations were evaluated and slides from the most appropriate concentration (for level of damage) were chosen for coding and microscopic analysis. The slides were decoded only after all slides in each group had been completely analysed.

Slides were evaluated for MI (based on at least 1000 cells/flask) and 50 cells per flask were evaluated for chromosomal aberrations resulting in a total of 100 cells evaluated per experimental point.

For analysis of the slides, score sheets were used to record the quality of the slide, vernier settings, MI and numbers of various categories of chromatid-type and chromosome-type aberrations for each cell scored. SRI's classification of aberrations is based on definitions given by Savage (1975). After analysis was completed and the score sheets were summarised.

Evaluation criteria:

CRITERIA FOR INTERPRETATION:

Positive - A test material is considered to have elicited a positive response in the in vitro human lymphocyte cytogenetic assay if the frequency of cells with structural chromosomal damage is significantly greater ($p < 0.05$) in the test article-treated cells than in the solvent control cells. A positive response will also require a dose-related increase in cells with structural chromosomal abnormalities.

Negative - A test material is considered to have elicited a negative response if neither criteria for a positive response were met.

Inconclusive - The results of this assay are considered inconclusive if there is reason to believe that the concentrations of the test material selected for evaluation were inappropriate or if a statistically significant elevation in chromosomal abnormalities was observed which was not dose related.

Statistics:

Data from the definitive studies in the presence and in the absence of metabolic activation were analysed separately.

The following statistics were calculated for each treatment: MI, the total number of chromosomal aberrations and the frequency of chromosomal aberrations per cell, the frequency of aberrant cells, the number and frequency of aberrations in each category, the number and frequency of cells with structurally aberrant chromosomes, the number and frequency of aneuploid and polyploid cells, and the number and frequency of severely damaged cells (i.e., cells with ≥ 5 chromosome aberrations). The number of aneuploid cells is not reported separately in the tables, because aneuploidy may be caused by cell harvest conditions. Aneuploid cells are included in the total number of aberrant cells and frequency per cell of aberrations. The number of cells with structural chromosomal damage and the total number of structural chromosomal aberrations observed in the test material and positive-control treatment groups were statistically compared with the number of those in the solvent control, using chi-square with a significance level of $P = 0.05$.

A valid chromosomal aberration assay is one in which the chromosomal aberration frequencies in the positive control are significantly ($P < 0.05$) elevated above those in the solvent control and the highest concentration evaluated in the definitive assay was within a factor of two of the level which resulted in a significant effect on the cell cycle or on the mitotic index.

Results and discussion:

Species/strain: lymphocytes.

With metabolic activation:

Test system: all strains/cell types tested.

Genotoxicity: positive $\geq 40 \mu\text{g/mL}$.

Cytotoxicity: yes $\geq 80 \mu\text{g/mL}$.

Vehicle controls valid: yes.

Negative controls valid: not examined.

Positive controls valid: yes.

Species/strain: lymphocytes.

Without metabolic activation:

Test system: all strains/cell types tested.

Genotoxicity: negative.

Cytotoxicity: yes $\geq 16 \mu\text{g/mL}$.

Vehicle controls valid: yes.

Negative controls valid: not examined.

Positive controls valid: yes.

Additional information on results:

CYTOTOXICITY STUDIES:

In the experiment conducted in the absence of metabolic activation, no decrease in MI or retardation of the cell cycle was noted for the $8.0 \mu\text{g/mL}$ dose group. All higher concentrations were cytotoxic (no metaphase cells were recovered). On the basis of these findings, concentrations for the definitive study without metabolic activation of 2, 4, 8, 16 and $32 \mu\text{g/mL}$ were chosen with an 18 hour harvest. Concentrations of 16 and $32 \mu\text{g/mL}$ were chosen for the definitive assay with a 30 hour harvest without metabolic activation.

In the cytotoxicity study conducted in the presence of metabolic activation, no significant decrease in MI or retardation of the cell cycle was noted for 8 or $40 \mu\text{g/mL}$. All higher

concentrations were cytotoxic (no metaphase cells were recovered). Based on these findings, concentrations of 10, 20, 40, 80 and 160 µg/mL were chosen for the definitive study with metabolic activation with an 18 hour harvest. Concentrations of 80 and 160 µg/mL were chosen for the definitive assay with a 30 hour harvest with metabolic activation.

DEFINTIVE STUDIES

The two measures of cytogenetic damage evaluated statistically were the percentage of structurally aberrant cells and the frequency of structural aberrations per cell.

In the absence of metabolic activation, Chi-square analysis revealed no significant differences between the solvent control and treatment groups in the percentage of structurally aberrant cells or in the frequency of structural aberrations per cell. In the presence of metabolic activation, Chi-square analysis revealed significant differences between the solvent control and the 40 and 80 µg/mL treated cells in both the percentage of structurally aberrant cells and in the structural aberration frequency.

The positive control articles in the presence and absence of metabolic activation induced statistically significant increases in chromosomal damage over the solvent control values.

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of the relevant results. Concentration of dimethyltin dichloride in test material is 72.4 %.

[Study 3]

Study reference:

Author not disseminated (1990).

Report date 1990-08-20.

Test type:

Type of genotoxicity: gene mutation.

Type of study: mammalian cell gene mutation assay.

According to OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test).

No deviations.

GLP compliant.

Test substance:

Test material (EC name): dimethyltin dichloride.

Form: crystalline.

Details on test material:

- Name of test material (as cited in study report): Mixes of methyltin compounds (MMC).
- Physical state: solid.
- Storage condition of test material: at room temperature in lightproof container.

Method:

Target gene: HGPRT locus.

Species/strain: Species/strain Chinese hamster Ovary (CHO).

Details on mammalian cell lines (if applicable): The cell line used for the CHO/HGPRT mutation assay was CHO-K1 and was obtained from ATCC. Vials containing the stock cells were stored in a liquid nitrogen freezer. Periodically, a new vial of cells was thawed, and the cells were grown as attached cultures in 75-cm² tissue-culture flasks. All cells were incubated at 37°C in 5% CO₂ at >90% relative humidity.

Metabolic activation: with and without.

Metabolic activation system: Aroclor 1254-induced rat-liver homogenate preparation (S9).

Test concentrations: 0, 49, 61, 77, 96, 120 and 200 µg/mL (without metabolic activation); 0, 61.4, 76.8, 96, 120 and 150 µg/mL (with metabolic activation, replicate 1); 0, 31.4, 39.3, 49.2, 61.4, 76.8 and 96 µg/mL (with metabolic activation, replicate 2).

Vehicle: Sterile purified deionized water.

Controls:

For assay with metabolic activation:

Negative controls: no.

Solvent / vehicle controls: yes.

True negative controls: no.

Positive controls: yes.

Positive control substance: 23.

For assay without metabolic activation:

Negative controls: no.

Solvent / vehicle controls: yes.

True negative controls: no.

Positive controls: yes.

Positive control substance: 2072.

Details on test system and conditions:

METHOD OF APPLICATION: in medium.

DURATION:

- Exposure duration: 4 hours.
- Expression time (cells in growth medium): 7 days.
- Selection time (if incubation with a selection agent): at least 14 days.

SELECTION AGENT (mutation assays): TG.

NUMBER OF REPLICATIONS: Solvent control conducted in triplicate, all other experiments in duplicate. Experiment was then replicated.

DETERMINATION OF CYTOTOXICITY

At the end of the chemical-exposure period, the cells in each culture were detached from the flask with 0.02% trypsin-EDTA and suspended in 10 to 20 mL of F12/5. A Coulter counter was used to determine the number of cells, and an aliquot containing at least $1 \text{ to } 2 \times 10^6$ cells was added to 20 mL of F12/5 in a 75-cm² or larger tissue-culture flask for phenotypic expression (described below). A serial dilution was performed from the cell suspension so that approximately 500 cells were plated into two 100 mm petri dishes in approximately 35 mL of cloning medium per dish. After incubation for at least 14 days, the resulting cell colonies were counted using an automatic colony counter with a standard 50 mm lens. For evaluation purposes, survival was expressed as the cloning efficiency relative to the control cells, calculated by the formula: (average cloning efficiency of treated cells - average cloning efficiency of control cultures) $\times 100$.

DETERMINATION OF MUTANT REQUENCY

The mutant frequency of each culture (the ratio of the number of mutant cells to the number of wild-type cells) was calculated by dividing the number of TGr colonies by 5,000 times the number of unselected (viable) colonies to compensate for the difference in the number of cells cloned initially and the absolute cloning efficiency of the cells.

Evaluation criteria:

Criteria for Acceptability of Mutagenesis Experiments:

Experiments meeting these criteria were evaluated for mutagenic response; experiments that did not meet the criteria were repeated. However, because the mutagenesis assay is a complex biological system, the criteria are used as guidelines and exceptions may be justified.

Negative Controls -The average mutant frequency in an experiment should be less than 60 TGr colonies per 10^6 viable cells.

Positive Controls -The average mutant frequencies should be at least three times greater than the average mutant frequencies of the solvent controls. If the relative cloning efficiency is less than 60%, then the average mutant frequency of the positive control cultures should be fivefold that of the solvent controls.

Criteria for Evaluating Results of Acceptable Mutagenesis Experiments:

Positive - The test results for a particular test article are considered positive if a dose-related increase in the number of mutant colonies occurs and the mutant frequencies of duplicate cultures (with an average initial survival rate of at least 20%) treated with one or more concentrations of the test article are at least three times the average of those from the solvent control cultures.

Negative - The results are considered negative if the test article does not induce a response according to the above criteria.

Equivocal - The results are considered equivocal if the results of the replicate experiments are not consistent.

Statistics: Not performed.

Results and discussion:

Test results:

Species/strain: Chinese hamster Ovary (CHO).

Metabolic activation: with and without.

Test system: all strains/cell types tested.

Genotoxicity: negative.

Cytotoxicity: yes.

Vehicle controls valid: yes.

Negative controls valid: no.

Positive controls valid: yes.

Additional information on results:

Concentrations of the positive control chemicals were 200 µg/mL EMS without S9 and 5 µg/mL MCA with S9. EMS increased the average mutant frequency 5- to 8-fold (to 192-292 mutants per 10^6 cells). With 3-MCA and S9, 3-to 4-fold increases in average mutant frequency (to 78-150 mutants per 10^6 cells) were induced.

CYTOTOXICITY

The toxicity of test material, as measured by comparing the cloning efficiency of the treated cell cultures with that of the solvent control cultures immediately after the exposure period, was quantitated in each of these experiments. Test concentrations were selected to include at least one concentration that decreased cell survival to less than 20% that of the solvent control cells. In the preliminary cytotoxicity experiment (data not included), the cells were treated with 13 concentrations of test material (from 0.25 to 1000 µg/mL), without and with S9. The relative cloning efficiencies of cultures treated without S9 and with S9 at 125 µg/mL test material were 2% and 29%, respectively. Cloning efficiencies comparable to those of the solvent controls were reached at 31.25 and 62.5 µg/mL with and without S9, respectively. Therefore, concentrations ranging from 49 to 120 µg/mL were chosen for testing without S9, and concentrations from 61.4 to 150 µg/mL with S9 were chosen for the first respective mutagenesis experiments.

The test material was more toxic in the second mutagenesis experiments, especially with S9. In the first experiment without S9 the average cloning efficiency of cells treated with 120 µg/mL was 17% relative to the solvent control cultures. An even more toxic response was observed in the second experiment without S9; 77 µg/mL reduced the average relative cloning efficiency of the cells to 8%.

An increase of toxicity was also observed between the first and second mutagenesis experiments with S9 activation. The average relative cloning efficiency of cells was reduced to 5% in both the first and second experiments after treatment with 96 and 61.4 µg/mL, respectively. A concentration-dependent decrease in the cloning efficiency was observed in both experiments with and without metabolic activation.

MUTAGENICITY

The test material did not induce a mutagenic response (threefold or greater increase in the average frequency of thioguanine-resistant mutant cells) in either of the experiments with or without S9. Mutant frequency values, relative to the average solvent control value, ranged from 0.7 to 1.5 in the first experiment and from 1.0 to 1.6 in the second experiment times that of the average solvent control value for doses evaluated without S9. With S9, increases in mutant frequency ranged from 0.4 to 2.1 in the first experiment and from 0.4 to 1.8 in the second experiment times that of the average solvent control value for doses evaluated with S9. Therefore, the test material did not induce a concentration-dependent increase in mutant frequency either without or with S9 and was evaluated as negative under both activation conditions.

Reliability score 1 (reliable without restriction) was given by the registrant because

the study was conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of the relevant results.

[Study 4]

Study reference:

Author not disseminated (1990).

Report date 1990-07-18.

Test type:

Type of genotoxicity: gene mutation.

Type of study: bacterial reverse mutation assay (e.g. Ames test).

According to OECD Guideline 471 (Bacterial Reverse Mutation Assay).

Deviations: no.

GLP compliance: yes.

Test substance:

The test material used in the study is not equivalent to the substance ID for which the classification is proposed for.

Test material form: crystalline.

Name of test material (as cited in study report): Dimethyltin Dichloride [CAS No. 753-73-1]:Methyltin Trichloride [CAS No. 993-16-8] (72:28% mixture).

Method:

Target gene: Histidine.

Species/strain: *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100.

Additional strain characteristics: histidine auxotrophs.

Metabolic activation: with and without.

Metabolic activation system: Aroclor 1254-induced rat liver S-9.

Species/strain: *S. typhimurium* TA 1538.

Additional strain characteristics: histidine auxotrophs.

Metabolic activation with and without

Metabolic activation system Aroclor 1254-induced rat liver S-9

Test concentrations:

Range finding assay: 10, 50, 100, 500, 1000, and 5000 µg/plate.

First mutagenicity assay: 10, 50, 100, 500, 1000, and 5000 µg/plate.

Second mutagenicity assay: 5, 10, 50, 100, 500, and 1000 µg/plate.

Vehicle:

- Vehicle(s)/solvent(s) used: Deionized water for the test material; DMSO for the positive control substances.

Controls:

Negative controls no.

Solvent / vehicle controls yes.

True negative controls no.

Positive controls: yes.

Positive control substance: 28 hydrochloride. 50 µg/plate for frameshift mutant TA1537.

Negative controls: no.

Solvent / vehicle controls: yes.

True negative controls: no.

Positive controls: yes.

Positive control substance: 2374 5 µg/plate for the base-pair substitution mutants TA1535 and TA100.

Negative controls: no.

Solvent / vehicle controls: yes.

True negative controls: no.

Positive controls: yes.

Positive control substance: 20 50 µg/plate for the frameshift mutants TA1538 and TA98.

Negative controls: no.

Solvent / vehicle controls: yes.

True negative controls: no.

Positive controls: yes.

Positive control substance 1342 2-Anthramine: 2 ug/plate for strains TA1538, TA98, and TA100 and at 4 ug/plate for strains TA1535 and TA1537 in the presence of metabolic activation are used to ensure the efficacy of the activation system.

Details on test system and conditions:

METHOD OF APPLICATION: in agar (plate incorporation).

DURATION:

- Preincubation period: The cultures are allowed to sit unshaken for 2 to 4 hours, then gently shaken (100 rpm) for 11 to 14 hours at 37 °C.

- Exposure duration: After the top agar has set, the plates are incubated at 37°C for about 48 hours.

- Expression time (cells in growth medium): set, the plates are incubated at 37°C for about 48 hours. The histidine-independent revertant colonies are counted following the incubation period; however, if the plates cannot be immediately evaluated, they are refrigerated at 4°C until they can be counted.

NUMBER OF REPLICATIONS: with three plates per dose level, both with and without a mammalian liver metabolic activation.

NUMBER OF CELLS EVALUATED: 10⁸.

DETERMINATION OF CYTOTOXICITY:

Toxicity is evidenced by several phenomena: a substantial decrease in the number of revertant colonies on the test plates compared with the controls, the clearing or absence of the background lawn growth, or the formation of pinpoint nonrevertant colonies.

Evaluation criteria:

The histidine-independent revertant colonies are counted using an automated colony counter. When accurate counts cannot be obtained (e.g., because of precipitation on the plates), the colonies are counted manually using an electric probe colony counter. The actual numbers of revertant colonies observed and the condition of the background lawn growth are presented in the attached tables. No designation or "-7" indicates a normal background lawn. Toxicity is designated by "-5" for background lawn thinning, "-4" for pinpoint colonies, and "-3" for absence of bacterial growth.

CRITERIA FOR INTERPRETATION:

Positive - A test material is considered a mutagen when it induces a reproducible, dose-related increase in the number of revertants in one or more strains. This increase should occur for at least three consecutive dose levels.

Negative - A test material is considered a nonmutagen when no dose-related increase in the number of revertants is observed in at least two independent experiments.

Inconclusive - When a test material cannot be identified clearly as a mutagen or nonmutagen, the results are classified as inconclusive.

Statistics:

The results are a tabulation of the number of colonies appearing on the plates. Mean and standard deviation values were determined for the number of revertants at each dose level.

Results and discussion:

Species/strain: *S. typhimurium* TA 1535, TA 1537, TA 98 and TA 100.

Metabolic activation: with and without.

Test system: all strains/cell types tested.

Genotoxicity: negative.

Cytotoxicity: yes ≥ 1000 $\mu\text{g}/\text{plate}$.

Vehicle controls valid: yes.

Negative controls valid: not examined.

Positive controls valid: yes.

Species/strain: *S. typhimurium* TA 1538.

Metabolic activation: with and without.

Test system: all strains/cell types tested.

Genotoxicity: negative.

Cytotoxicity: yes 1000 $\mu\text{g}/\text{plate}$.

Vehicle controls valid: yes.

Negative controls valid: not examined.

Positive controls valid: yes.

Additional information on results:

The Ames Salmonella/microsome assay was used to evaluate the ability of mixes of methyltin compounds to induce genetic damage in *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100. The assays were performed using the standard plate

incorporation procedure, in both the presence and absence of a rat liver metabolic activation system. The presence of the appropriate genetic characteristics was verified for the strains used in this study. The results of the controls were acceptable for all assays.

We conducted the preliminary assay with tester strain TA100 in the presence and absence of metabolic activation containing 4% S-9. The doses used were 10, 50, 100, 500, 1000, and 5000 µg/plate. Toxicity was indicated with revertant counts below the spontaneous background level at the 1000 µg dose and thinning of the background lawn at 5000 µg, both in the presence and absence of metabolic activation. Based on these results, the first assay was conducted at the same dose levels, with all five tester strains, in the presence and absence of 4% S-9. There was no evidence of a dose-related increase in the number of histidine revertants with any tester strain. Toxicity was noted with all tester strains at 5000 µg as background lawn thinning or complete absence of bacterial growth. Some toxicity was also noted at 1000 µg. The second assay was conducted at dose levels of 5, 10, 50, 100, 500, and 1000 µg/plate, and the .percent of S-9 in the metabolic activation mixture was increased to 10% due to the lack of response in the first assay. Again, there was no dose-related increase in the number of histidine revertants with any tester strain. Toxicity was observed at the 1000 µg dose with some strains, indicated by a decrease in the number of revertant colonies below background levels.

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of the relevant results. Percentage of DMTC is only 72%. 28% is MMTC.

[Study 5]

Study reference:

Reference type: secondary source.

Author: Harper, C., Lladós, F., Diamond, G. & Chappell, L.L..

Year: 2005.

Title: Toxicological Profile for Tin and Tin Compounds.

Bibliographic source: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Primary Source: Jensen et al (1991) *Mutagenesis* 6:409-416.

Test type:

Type of genotoxicity: cytogenicity.

Type of study: spindle inhibition.

No guideline available.

No methodological information available.

GLP compliance: no according to the registrant data.

Test substance:

Dimethyltin dichloride.

Test material form: crystalline.

Method:

Species/strain

Species/strain: Chinese hamster cells.

Metabolic activation: without.

Results and discussion:

Species/strain: Chinese hamster cells.

Metabolic activation: without.

Genotoxicity: no data.

Cytotoxicity: yes.

Vehicle controls valid: no data.

Negative controls valid: no data.

Positive controls valid: no data.

Any other information on results: Spindle inhibition seen.

Reliability score 4 (not assignable) was given by the registrant because of secondary source with extremely limited methodological information. Primary reference not available.

[Study 6]

Study reference:

Author not disseminated (1992).

Test type:

No guideline followed.

Three types of genotoxicity tests were reported:

- 1) Induced mutation frequency (IMF) with *Salmonella typhimurium* TA100.
- 2) Rec assay with *Bacillus subtilis* H17 and M45
- 3) SOS chromotest with *Echerichia coli* PQ37

The IMF test is a modified version of the Ames test and used for substances with a high biotoxicity such as antibiotics.

GLP compliance: no according to the registrant data.

Test substance:

Dimethyltin dichloride.

Test material form: crystalline.

Results and discussion:

Species/strain: *S. typhimurium* TA 100.

Metabolic activation: no data.

Genotoxicity: negative.

Cytotoxicity: no data.

Vehicle controls valid: no data.

Negative controls valid: no data.

Positive controls valid: no data.

Species/strain bacteria, other: *Bacillus subtilis*.

Metabolic activation: no data.

Test system strain/cell type: H17 and M45.

Genotoxicity: positive.

Cytotoxicity: no data.

Vehicle controls valid: no data.

Negative controls valid: no data.

Positive controls valid: no data.

Species/strain *E. coli*, other: PQ37.

Metabolic activation: no data.

Genotoxicity: negative.

Cytotoxicity: no data.

Vehicle controls valid: no data.

Negative controls valid: no data.

Positive controls valid: no data.

Reliability factor 4 (not assignable) was given by the registrant because of a brief article with very little information on methodology or results.

[Study 7]

Study reference:

Reference type: publication.

Author: Hamasaki, T., Sato T., Hagase, H. & Kito H.

Year: 1992.

Title: The genotoxicity of organotin compounds in SOS chromotest and rec-assay.

Bibliographic source: Mutation Research, 280 (1992) 195-203.

Test type:

Type of genotoxicity: DNA damage and/or repair.

Type of study: SOS chromotest and Rec-assay.

Test guideline: no guideline followed.

Principles of method if other than guideline:

The experimental procedures of the SOS chromotest were mainly based on what was established by Quillardet and Hofnung (1985) with minor modifications. The streak method (Kada et al., 1980) was adopted for the Rec-assay.

Quillardet, P., and M. Hofnung (1985) The SOS chromotest, a colorimetric bacterial assay for genotoxins: procedures, *Mutation Res.*, 147, 65-78.

Kada, T., K. Hirano and Y. Shirasu (1980) Screening of environmental chemical mutagens by the rec-assay system with *Bacillus subtilis*, in: A. Hollaender and F.J. de Serres (Eds.), *Chemical Mutagens, Principles and Methods for Their Detection*, Vol. 6, Plenum, New York, pp. 149-173.

GLP compliance: no according to the registrant.

Test substance:

Dimethyltin dichloride.

Test material form: crystalline.

Method:

Species/strain: *E. coli*, other: PQ37

Additional strain characteristics: no data.

Metabolic activation: without.

Species/strain: bacteria, other: *Bacillus subtilis* (H17 Rec + and M45 Rec- strains).

Additional strain characteristics: other: catalase-positive.

Metabolic activation: without.

Vehicle: sterile distilled water.

Controls

Negative controls: no.

Solvent / vehicle controls: yes.

True negative controls: no.

Positive controls: yes.

Positive control substance: 25.

Details on test system and conditions:

SOS CHROMOTEST PROCEDURE:

The experimental procedures of the SOS chromotest were mainly based on what was established by Quillardet and Hofnung (1985), and minor modifications were made. *E. coli* PQ37 tester strain was cultivated in 5 mL of La medium for 15 h at 37°C. The amount of cultivated bacteria was adjusted to 0.3-0.4 of OD600 value with La medium after overnight cultivation. 0.1 mL of the overnight culture was diluted with 5 mL of La medium, and was incubated at 37°C for 2 hours. 2 mL of this culture was diluted with 8 mL of fresh L medium. Then, 100 µl of test sample and 0.5 mL of 0.1 M Na-phosphate buffer (pH 7.4) were added to 1.5 mL of this culture, and incubated at 37°C for 2 hours.

After the reaction, the enzyme activities of β -galactosidase and alkaline phosphatase were measured. For the measurement of β -galactosidase activity, o-nitrophenyl- β -galactoside was used as the substrate and p-nitrophenyl phosphate disodium was used for alkaline phosphatase.

The experiments were performed at the concentration of chemicals at which a decrease of the activity of alkaline phosphatase was observed. When a chemical did not show this decrease, the SOS chromotest was carried out at the highest soluble dose.

There was no metabolic activation with S9 mix. Organotin compounds are dealkylated or dephenylated one by one with liver microsomal monooxygenase systems in mammals (Kimmel et al., 1977; Wada et al., 1982). The metabolites of organotin compounds with S9 mix must be contained in tested chemicals.

REC-ASSAY PROCEDURE:

The procedure adopted was the streak method (Kada et al., 1980). Two strains of *Bacillus subtilis* H17 Rec⁺ and M45 Rec⁻ were grown overnight (16 h, 37°C) in B-2 broth (meat extract 10 g, polypeptone 10 g, NaCl 5 g, water 1 liter, pH 7.0). Each culture was streaked radially from the center of a petri dish with a 0.1-mL pipette on the dry surface of B-2 broth agar such that two streaks did not intersect. A sterile filter paper disk (diameter 15 mm) was placed on the starting point of the streaks, and 50 µl of each tested chemical solution (10-10,000 µg/50 µl) was dropped on the paper disk. The plate was incubated at 37°C for 24 h. Each length of growth inhibition was measured.

Evaluation criteria:**SOS CHROMOTEST PROCEDURE:**

When a significant increase in β -galactosidase units of the tested chemical compared to that of the control was recognised by t-test ($p < 0.05$) and an enhancement of I(C) according to the increase of treated amount was shown, the chemical was recognised as an SOS inducer.

REC-ASSAY PROCEDURE:

When inhibition of cellular growth by a tested chemical is more pronounced with Rec⁻ than with Rec⁺, it is ascertained that this chemical damages cellular DNA.

Results and discussion:

Species/strain: *E. coli*, other: PQ37.

Metabolic activation: without.

Test system: all strains/cell types tested.

Genotoxicity: negative.

Cytotoxicity: no data.
Vehicle controls valid: yes.
Negative controls valid: not applicable.
Positive controls valid: yes.

Species/strain: bacteria, other: *Bacillus subtilis*.
Metabolic activation: without.
Test system strain/cell type: H17 Rec+ and M45 Rec-.
Genotoxicity: positive.
Cytotoxicity: no data.
Vehicle controls valid: yes.
Negative controls valid: not applicable.
Positive controls valid: yes.
Additional information on results:
Dimethyltin dichloride did not cause produce DNA damage in the SOS chromotest procedure.
Dimethyltin dichloride did however show DNA damage in the Rec-assay.

Reliability factor 3 (not reliable) was given by the registrant because there is insufficient information in the methodology and results to accurately assess the genotoxic potential of the chemical.

[Study 8]

Study reference:

Reference type publication

Author: Dopp, E., Hartmann, L.M., von Recklinghausen, U., Florea, A.M., Rabieh, S., Shokouhi, B., Hirner, A.V., Obe, G. & Rettenmeier, A.W.

Year: 2007.

Title: The cyto- and genotoxicity of organotin compounds is dependent on the cellular uptake capability.

Bibliographic source: Toxicology 232: 226–234.

Materials and methods

Type of genotoxicity: investigations into induction of micronuclei, chromosome aberrations and sister chromatid exchanges.

Principles of method if other than guideline: cytokinesis blocked micronucleus assay (CBMN), nuclear division index (NDI), chromosome aberrations (CA) and sister chromatid exchanges (SCE) were examined by looking at the effects of dimethyltin dichloride on CHO-9 cells.

GLP compliance: no according to the registrant.

Test materials

Dimethyltin dichloride.

Test material form: crystalline.

Method

Species/strain: Chinese hamster Ovary (CHO).

No data on additional strain characteristics.

Metabolic activation: without.

Test concentrations: 0.5 μ M – 1.0mM.

Details on test system and conditions:

CYTOTOXICITY TEST:

CHO-9 cells were treated at 1 μ M – 1 mM for 1 hour and 24 hour exposure in triplicate. Cell viability was evaluated immediately after exposure. Treated and untreated cells were harvested by trypsin treatment and cell counting was performed following trypan blue staining. The cell suspension was mixed with an equivalent volume of 0.4% trypan blue solution, incubated at 37 °C for 4 min and subsequently evaluated under the light microscope. Cell viability is expressed as percentage of surviving cells compared to the total number of cells. The decrease of cell viability is referenced to the negative control. A substance is considered to be cytotoxic if the cell viability in the exposed sample is decreased more than 50% of that observed in the negative control.

CYTOKINESIS BLOCKED MICRONUCLEUS ASSAY (CBMN) AND NUCLEAR DIVISION INDEX (NDI):

For MN analysis, 2×10^5 CHO-9 cells were seeded in each well of Quadriperm-dishes and cultured overnight. Then the tin compounds were applied for 1 hours and 24 hours at different concentrations (5 μ M - 1 mM). Subsequently, cells were washed twice with PBS and incubated for an additional 24 hours with cytochalasin B (3 μ g/ml) to block the cytokinesis (recovery time). Cells were fixed with Carnoy's solution (methanol:acetic acid, 3:1) for 5 min and stained with May-Grünwald and 6% Giemsa solution. The frequency of MN formation was expressed as number of binucleated (bn) cells with MN. Two thousand binucleated cells were evaluated in each case, and all experiments were performed at least in duplicate.

Using the cytokinesis-blocked MN assay, the extent and progression of nuclear division was measured by analysing the NDI. After exposure of cells to non-cytotoxic concentrations of tin compounds for 1 hour and 24 hours, cells were incubated with cytochalasin B (3 μ g/ml) for additional periods of 14, 28 and 35 hours. The frequency of mononucleated (MI), binucleated (MII) and multinucleated (MIII + MIV) cells was determined and the NDI calculated according to Eastmond and Tucker (1989): $NDI = (MI + 2MII + 3MIII + 4MIV) / N$

A minimum of 1000 cells/ slide was analysed.

CHROMOSOME ABERRATIONS (CA) AND SISTER CHROMATID EXCHANGES (SCE):

CHO-9 cells were exposed at final concentrations in the range of 10 μ M–1 mM for 1 hour. After incubation, cells were washed twice with McCoy's medium and 5ml culture medium was

added. For the evaluation of chromosomal alterations, seen as CA and SCE, CHO-9 cells were labelled with 20 μM BrdU. Thereafter, cells were cultivated for additional 16 hours (one cell cycle) for the analysis of CA and for 32 hours (two cell cycles) for the analysis of SCE. Cell growth was stopped by treatment of CHO-9 cells with 0.08 $\mu\text{g/ml}$ colcemid solution for 2 hours. Metaphases were stained as described by Hill and Wolff (1982). A minimum of 100 uniformly stained first post-treatment metaphases were analysed for CA and 100 differentially stained second post-treatment metaphases were analysed for SCE. All experiments were performed at least in duplicate.

ANALYSIS OF INTRACELLULAR TIN CONCENTRATION:

CHO-9 cells (5×10^6) were exposed to a concentration range of 0.5 M–1.0 mM for 1 hour. After incubation, cells were washed with PBS and subsequently resuspended in 10 ml fresh culture medium. Following counting of cells, the cell suspension was centrifuged for 5 min at $300 \times g$ and the pellet was resuspended in 10 ml distilled water/sample for 30 min to lyse the cells. The cell suspension was controlled microscopically for the absence of intact cells. Cell-free extract was produced by centrifuging the cell suspension (whole-cell extract) at $300 \times g$ for 20 min. All uptake experiments were performed in duplicate. Total tin concentration in the cell extracts was determined by inductively coupled plasma-mass spectrometry (ICP-MS). The ICP-MS was operated at 1260W rf-power, with argon flows of 15 L/min (plasma gas), 0.98 L/min (carrier gas) and 0.9 L/min (auxiliary gas). Solutions (up to 1 in 100 dilutions) were delivered at 0.3 ml/min to a Babington nebuliser and routed through a double-pass Scott-type spray chamber maintained at 2 °C. The signals 118Sn (1000 ms) and 115In (1000 ms) were monitored. Quantitation was performed by external calibration with a standard solution and validated by analysing CRM SERO 201605.

ELECTROPORATION:

Electroporation according to Eppendorf Soft Pulse technology was carried out in hypoosmolar buffer (250 mOsmol/kg), in which the cells absorb water shortly before the pulse and then swell as a result of the treatment. A number of effects, including a decreased optimal pulse voltage, ensure that the plasma membrane is permeated by this treatment. The tolerance of CHO-9 cells to hypoosmolar conditions was tested in preliminary experiments. The survival rate of the cells was >90%.

For measurement of the intracellular tin concentration after electroporation, approximately 1×10^6 CHO-9 cells were prepared in basal medium + 0.5% FCS and centrifuged. A solution of DMT (50 μM) was prepared in hypoosmotic buffer and added to the cells for 30 min. Cells were then transferred to electroporation cuvettes with 4mm gap. Samples were treated with electrical impulses of 530V for 40 μs and all experiments were performed in triplicate. The cell suspension was allowed to stand for 10 min at room temperature was then centrifuged (5 min, $190 \times g$) and washed twice in fresh culture medium. Samples of whole-cell and cell-free extract were prepared as described above.

For micronucleus assay after electroporation a suspension of cells (0.5×10^6) was prepared in medium for electroporation (basal medium + 0.5% FCS) and centrifuged. The tested DMT was dissolved in hypoosmotic buffer and added to the cells to obtain a final volume of 800 μl . The resulting cell suspension was then transferred to electroporation cuvettes. After 30 min

hypoosmotic exposure, electroporation was performed at 530V for 40 μ s. Following centrifugation, the cell pellet was washed twice in culture medium and resuspended in 5ml fresh medium containing cytochalasin B (3 μ g/ml). After an incubation period of 24 h (recovery time), the CBMN-assay was carried out. The experiments were set up as follows:

- (a) unexposed control,
- (b) unexposed control and electroporation,
- (c) exposure to tin compounds and
- (d) exposure to tin compounds with electroporation.

All experiments were performed in duplicate and measurements were repeated three times.

Statistics: The Chi-square-test was used for statistical analysis of the results of the micronucleus assay and the chromosome aberration test, and the two tailed Student's t-test for evaluating the data from the cytotoxicity test, the sister chromatid exchange test and the NDI.

Results and discussions

Additional information on results:

DMT showed a concentration-dependent decrease in cell viability after an exposure time of 1 hour. DMT was cytotoxic (decrease of cell viability > 50%) at concentrations \geq 300 μ M. DMT was cytotoxic at the lowest concentration tested (1 μ M).

DMT significantly increased the number of MN after 1 hour at \geq 500 μ M and 1 mM, respectively and after 24 h at 100 μ M. However, the significant increase in MN formation after DMT exposure was obtained at cytotoxic concentrations.

No significant cell cycle delay was found for concentrations of 5 and 25 μ M DMT.

Analysis of chromosome aberrations and sister chromatid exchanges in CHO-9 cells following treatment with different concentrations (10 μ M–1 mM) for 1 h indicated that DMT induced a significant increase in percentage of CA and SCE at a cytotoxic concentration of 1mM (Table 1).

To assess membrane permeability, CHO-9 cells were incubated with these species at different concentrations (Table 2) for 1 and 24 h. Since there was no significant difference in uptake of tin over exposure periods of 1 and 24 hours, a 1 hour exposure time was used for all experiments. No significant difference of uptake was observed between whole-cell extract and cell-free (membrane removed) extract. A concentration dependency was observed, which reached a maximum at the highest applied tin concentration.

If the intracellular tin concentrations are calculated as percentage of substrate associated with the cells, there is no increase in intracellular tin substrate concentrations with increased extracellular tin concentrations. Lower DMT concentrations (0.5 μ M) are better taken up by the cells than extremely high concentrations (1 mM). It appears that the uptake is inhibited and/or the efflux is increased at higher concentrations in the treatment solution (Table 2).

After forced uptake of 50 μM DMT by electroporation, the number of induced MN was significantly increased in all cases (Table 3).

Table 1: Results of chromosome aberration test and sister chromatid exchanges in CHO cells

DMTC dosage	Number of cells analysed	Chromosomal aberrations (%)	Sister chromatid exchanges (%)
10 μM	300	0.70	9.20
100 μM	477	3.00	9.58
1 mM	846	7.31**	16.11**

Chi-square test: **p < 0.01.

Experiments were performed in triplicates. Data are expressed as means (S.D. < 20%).

Table 2: Cellular uptake of DMTC by CHO-9 cells after 1 hour exposure

Conc. of tin in exposure medium (μM)	Detected tin concentrations \pm S.D.			
	Whole-cell extract		Cell-free (membrane removed) extract	
	ng/ 10^6 cells	Tin associated with cell (%)	ng/ 10^6 cells	Tin associated with cell (%)
Control	Nd	-	0.19 \pm 0.16	-
0.5	0.43 \pm 0.14	0.37	0.41 \pm 0.12	0.35
1	0.29 \pm 0.18	0.13	0.29 \pm 0.20	0.12
10	1.18 \pm 0.15	0.11	0.95 \pm 0.17	0.10
100	7.38 \pm 1.54	0.12	6.45 \pm 0.60	0.11
500	149.48 \pm 6.20	0.12	142.69 \pm 28.00	0.12
1000	405.00 \pm 21.20	0.17	381.50 \pm 60.18	0.15

Table 3: Uptake by CHO-9 cells under forced uptake conditions (electroporation) and under hypoosmotic conditions

	Detected tin concentrations \pm S.D.			
	Whole-cell extract		Cell-free (membrane removed) extract	
	ng/ 10^6 cells	Tin substrate (%)	ng/ 10^6 cells	Tin substrate (%)
Control	Nd	-	0.37 \pm 0.20	-
Control + E	Nd	-	0.37 \pm 0.20	-
Exposure	9.37 \pm 0.34	0.02 \pm 0	8.82 \pm 1.05	0.01 \pm 0
Exposure + E	9.06 \pm 0.19	0.02 \pm 0	8.66 \pm 0.74	0.01 \pm 0

Reliability score 2 (reliable with restrictions) was given by the registrant, because the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which did not affect the quality of the relevant results.

[Study 9]

Study reference:

Reference type: publication.

Author: Jensen, K.G., Andersen, O. & Ronne, M.

Year: 1991.

Title: Organotin compounds induce aneuploidy in human peripheral lymphocytes in vitro

Bibliographic source Mutation Research 246: 109-112.

Test type:

Type of genotoxicity: Cytogenicity.

Type of study: frequency of aneuploidy.

Test guideline: no guideline followed.

GLP compliance: no according to the registrant.

Test substance: Dimethyltin dichloride.

Test material form: crystalline.

Method:

Species/strain: human lymphocytes.

Details on mammalian cell lines (if applicable): Blood taken from healthy 21 year old non-smoking female with no previous medical record, and no history of exposure to organotin compounds.

Additional strain characteristics: no data.

Metabolic activation: without.

Test concentrations: 10^{-3} to 10^{-9} M.

Details on test system and conditions:

METHOD OF APPLICATION: colloidal suspension in growth medium.

DURATION:

- Preincubation period: in air at 37.5°C for a total of 72 hours.

- Exposure duration: 48 hours.

The concentrations in the growth medium of the solvents employed did not exceed 0.2%. In each experiment, the control cultures matched the test cultures with regard to exposure time and concentrations of the solvent used. Colcemid (0.2 µg/mL final concentration) was added 2 hours before harvest.

After hypotonic treatment with 0.075 M KCl and fixation with methanol-acetic acid (3:1), air-dried slides were prepared, stained in Giemsa (3%) and mounted with Eukitt. For each dose, approximately 100 metaphases selected at random were photographed and chromosomes (centromeres) were counted directly on high magnifications of the negatives using an enlargement apparatus. All experiments were scored blind.

Evaluation criteria:

Selection criteria for photographing metaphases:

- (1) no other metaphase found in close proximity (2 metaphase diameter);
- (2) asymmetrical metaphases with very dispersed chromosomes across the slide were rejected;
- (3) chromosome counting was based on the presence of centromeres;
- (4) inconclusive metaphases with indistinct chromosomes were rejected;
- (5) only well-spread metaphases without obstructing debris were accepted for scoring.

Statistics

Individual values at each dose were compared with pooled controls, using a 2 x 2 X 2 test, after rejection of the zero hypothesis that the highest control \neq the lowest control.

Results and discussion:

Species/strain lymphocytes: human.

Metabolic activation: without.

Test system: all strains/cell types tested.

Genotoxicity: negative.

Cytotoxicity: no data.

Vehicle controls valid: not examined.

Negative controls valid: not examined.

Positive controls valid: not examined.

Additional information on results: Hypodiploid.

Table 1: Chromosome Counts of Metaphase Plates

Conc. of DMTC (M)	Chromosome counts							Poly-diploid	Total cells counted	% Hypo-diploid	% Diploid	% Hyper-diploid
	≤ 43	44	45	46	47	48	≥ 49					
0	3	2		103	2	1			111	4.5	92.8	2.7
0	3		2	97	2				104	4.8	93.3	1.9
0	2	2	9	76	2	1			92	14.1	82.6	3.3
0	3	1	5	78	2				89	10.1	87.6	2.2
3×10^{-4}	*	*	*	*	*	*	*	*	*	*	*	*
10^{-4}	2	2	8	82	2	1	1		98	12.2	83.7	4.1
10^{-5}		2	10	86	2	1			101	11.9	85.1	2.9
10^{-6}	2	3	6	84	1	1			97	11.3	86.6	2.1

[Study 10]

Study reference:

Reference type: publication.

Author: Hamasaki, T., Sato T., Hagase, H. & Kito H.

Year: 1993.

Title: The mutagenicity of organotin compounds as environmental pollutants.

Bibliographic source: Mutation Research, 300 (1993) 265-271.

Test type:

Type of genotoxicity: gene mutation.

Type of study: bacterial reverse mutation assay (e.g. Ames test).

Test guideline: equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay).

Deviations: yes.

Principles of method if other than guideline: Nohmi T., M. Matsui and M. Isidate Jr. (1986) Method for the induced-mutation frequency test using bacterial tester strains, Toxicol. Forum, 9, 189-198.

GLP compliance: no according to the registrant.

Test substance:

Test substance: Dimethyltin dichloride.

Test material form: crystalline.

Method:

Target gene: Histidine.

Species/strain: *S. typhimurium* TA 98.

Additional strain characteristics: no data.

Metabolic activation: without.

Species/strain: *S. typhimurium* TA 100.

Additional strain characteristics: no data.

Metabolic activation: without.

Test concentrations: 0.1 - 10 µg/tube.

Vehicle: sterile water.

Controls:

Negative controls: yes.

Solvent / vehicle controls: yes.

True negative controls: no.

Positive controls: yes.

Positive control substance: 25.

Details on test system and conditions:

METHOD OF APPLICATION: in agar (plate incorporation);

DURATION:

- Preincubation period: 15 h at 37°C
- Exposure duration: 48 h 37°C

Statistics:

t-test.

Results and discussion:

Species/strain *S. typhimurium* TA 98:

Metabolic activation: without.

Test system: all strains/cell types tested.

Genotoxicity: negative.

Cytotoxicity: no data.

Vehicle controls valid: no data.

Negative controls valid: no data.

Positive controls valid: no data.

Species/strain *S. typhimurium* TA 100:

Metabolic activation: without.

Test system: all strains/cell types tested.

Genotoxicity: positive In the presence of cytotoxicity.

Cytotoxicity: no data.

Vehicle controls valid: no data.

Negative controls valid: no data.

Positive controls valid: no data.

Additional information on results: Dimethyltin dichloride showed mutagenicity in the range of 0.5-5 µg/tube, and the surviving ratio of tested strains was 64-72% in that range. The induced mutation frequency (IMF) value of dimethyltin dichloride was 3.6×10^{-7} at 1 µg/tube.

Reliability score 3 (not reliable) was given by the registrant because the study is well reported, but deviations from guideline lower the reliability of the study. Only two tester strains used. Limited detail in methodology and results.

Germ cell mutagenicity - human data

Not available.

Germ cell mutagenicity - in vitro data

[Study 1]

Study reference:

Author not disseminated (1993).

Report date 1993-07-11.

Test type:

Type of genotoxicity: DNA damage and/or repair

Type of study: unscheduled DNA synthesis.

Test guideline: equivalent or similar to Guideline OECD Guideline 486 (Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo).

Deviations from the Protocol: Extra animals were dosed in the 16 hour high dose groups of both definitive UDS assays to avoid loss of data because of animal death. This circumstance did not affect the outcome of the study.

Circumstances that may have affected the outcome of the study:

The temperature and humidity in the rooms housing the animals was not within the recommended range (64.4-78.8°F, and 40-70% humidity). The temperature of the room housing the rats during the range-finding assays fluctuated between 58 and 79°F. This circumstance did not affect the outcome of the study because the dose levels for the second definitive UDS assay, which provided the UDS data in this report, were based on the pattern of animal deaths that occurred in the first definitive UDS assay, not the range-finding assays. The temperature and humidity in the room housing the rats used during the first and second definitive assays was approximately 64.5 to 71°F with 40 to 55% humidity, and 67 to 73°F with 56 to 69% humidity, respectively. It is not believed that these conditions affected the outcome of the study.

The humidity of the room housing the animals dropped as low as 32% and sharp spikes reaching higher than 70% occurred while the animal room was being washed down. It is not believed that these circumstances affected the outcome of the study.

GLP compliance: yes.

Test substance

Name of test material (as cited in study report): Mixture of methyltin chloride compounds.

Test material form: crystalline.

Physical state: solid.

Storage condition of test material: ≤25°C.

Description of test design:

Test animals:

Species: rat

Strain: Fischer 344.

Sex: male.

Source: Charles River Laboratories, 401 South New Hope Road, Raleigh, North Carolina, 27610, USA.

Age at study initiation: approximately 9 weeks.

Weight at study initiation: 161.9-199.6 (range-finding study); 195.8-228.3 g (first definitive UDS assay); 175.1-208.2 g (second definitive UDS assay).

Assigned to test groups randomly: yes.

Fasting period before study: NDA.

Housing: no more than 3 per cage in polycarbonate cages containing hardwood-chip bedding.

Diet (e.g. ad libitum): Purina Certified Rodent Chow No. 5002 ad libitum.

Water (e.g. ad libitum): Deionized, UV-exposed tap water was provided ad libitum.

Acclimation period: ca. 10 days.

ENVIRONMENTAL CONDITIONS:

Temperature (°F): 58 – 78.

Humidity (%): 32 to 89.

Photoperiod (hrs dark / hrs light): 12/12.

IN-LIFE DATES: From: 26th April 1993 To: 14th June 1993.

Administration / exposure:

Route of administration: oral: gavage.

Vehicle(s): Water.

Details on exposure: The route of dosing and the method of test article administration were chosen to maximize exposure of the liver to the test article and controls. All test article dosing solutions were prepared within 1 hour before dosing.

Duration of treatment / exposure: Single dose.

Frequency of treatment: Once.

Post exposure period: 7 days (range finding study); 2 or 16 hours (UDS assays).

Doses / concentrations:

Actual ingested: First range finding study: 25, 50, 100, 200 and 400 mg/kg.

Actual ingested: Second range finding study: 600 and 800 mg/kg.

Actual ingested: First definitive UDS assay: 90, 175 and 350 mg/kg.

Actual ingested: Second definitive UDS assay: 50, 110 and 225 mg/kg.

No. of animals per sex per dose:

First range finding assay: 3 males at each dose.

Second range finding assay: 3 males at each dose.

First definitive UDS assay (dosed 2 hours prior to sacrifice): 3 males at each dose, except negative control with 0.

First definitive UDS assay (dosed 16 hours prior to sacrifice): 3 males at each dose, except highest dosage group with 4 and positive control with 0.

Second definitive UDS assay (dosed 2 hours prior to sacrifice): 3 males at each dose, except negative control with 0.

Second definitive UDS assay (dosed 16 hours prior to sacrifice): 3 males at each dose, except highest dosage group with 4 and positive control with 0.

Control animals: yes.

Positive control(s): Dimethylnitrosamine.

- Route of administration: oral.

- Doses / concentrations: 10 mg/kg bw.

Examinations:

Tissues and cell types examined: Primary cell cultures were obtained from livers of treated male rats. Livers were perfused in situ with a collagenase solution. Isolated hepatocytes were combed out of the perfused livers, and cell concentrations were calculated from a hemocytometer count. Viable cells per millilitre were determined by the trypan blue exclusion method, and approximately $2.5\text{--}5.0 \times 10^5$ cells were inoculated into six-well culture dishes (containing coverslips) in Williams medium E (WE, pH 6.8) supplemented with 2 mM L-glutamine, 50 µg/mL gentamicin sulfate, and 10% fetal bovine serum. After 1.5 to 2.0 hours of incubation in a humidified atmosphere at 37°C, 5% CO₂, the cultures were washed to remove nonviable cells (those not attached to the coverslips). All washes and subsequent culturing were performed in serum-free medium. Cultures were incubated in WE containing 10 µCi/mL 3H-methylthymidine (specific activity, approximately 80 Ci/mmol) for 4 hours at 37°C, 5% CO₂, followed by 14 to 18 hours in WE containing 0.25 mmol unlabeled thymidine.

Details of tissue and slide preparation:

CRITERIA FOR DOSE SELECTION:

Death data from both range-finding assays were analysed together to estimate the LD₅₀ at 443 mg/kg bw methyltin chlorides. The LD calculated by an SRI generated program on the VAX-3100 (LD₅₀) that uses a point estimate of the linear interpolation of the log doses. The dose levels of methyltin chloride in the first definitive in vivo-in vitro hepatocyte DNA repair (UDS) assay were set at 90, 175 and 350 mg/kg bw on the basis of the results of the dose range-finding assays (approximately 20, 40 and 80% of the estimated LD₅₀).

TREATMENT AND SAMPLING TIMES:

Animals were dosed once, either 2 or 16 hours prior to sacrifice.

DETAILS OF SLIDE PREPARATION:

The cultures were washed with culture medium, swelled in 1% sodium citrate, fixed in 1:3 glacial acetic acid:ethanol, and washed with deionized water. The coverslips were mounted on slides, dipped in Kodak NTB-2 emulsion, and exposed at -20°C for approximately 7 days before development. Cells were stained with 1% methyl green pyronin Y.

METHOD OF ANALYSIS:

Measurement of UDS:

Quantitative autoradiographic grain counting was accomplished by use of an ARTEK Model 880 or 980 colony counter interfaced with a Zeiss Universal microscope via an ARTEK TV camera. Data were fed directly into a VAX 3100 computer via an ARTEK BCD-RS232 Omni-Interface using an SRI-generated data collection program (ARTEK). Thirty morphologically unaltered cells on a randomly selected area of the slide were counted. The highest count

from two nuclear-sized areas over the most heavily labelled cytoplasmic areas adjacent to the nucleus was subtracted from the nuclear count to give the net grains/nucleus (NG). The percentage of cells in repair (% IR) indicates the extent of the response throughout the liver (cells in repair are those showing at least 5 NG). For each dose, a minimum of 3 slides were scored for each animal. The data were summarised by SRI-generated programs on the VAX 3100 and the average NG and % IR were calculated for each dose group.

Evaluation criteria:

CRITERIA FOR A VALID ASSAY

Slide Evaluation: Slides were evaluated under a light microscope after the autoradiographic procedures. At that point, the groups were evaluated for UDS and unscorable groups (if any) were determined.

Unscorable Slide Criteria: Unscorable slides may result for any of the following reasons: (1) animal death after dose with test material, (2) poor or no cell attachment, or (3) pyknotic cells or other obvious morphologic changes.

DATA ANALYSIS AND INTERPRETATION:

Criteria for a Valid Assay: The UDS data generated were considered acceptable if the vehicle-control data were within historical ranges (-1 mean NG, $\leq 10\%$ IR) and if positive controls had significant elevations in NG and % IR.

Statistics:

When results did not clearly establish a positive or negative response, the presence of a dose response, the frequency distribution of cellular responses, increases in the % IR, the nuclear and cytoplasmic grain counts, and the reproducibility of data among animals were all considered. The following decision tree was used for classifying the response of the test material:

1. If there was no absolute increase in the nuclear counts of test groups over vehicle controls, the test material was considered "negative."

2. If there was no absolute increase in the nuclear counts and all dose groups had less than 0 NG but the % IR of individual dose groups was elevated, the net grains of cells in repair (NGIR, the average NO value of all cells with ≥ 5 NG) was evaluated.

- a. If the NGIR was ≤ 10 , the test material was considered "negative."

- b. If the NGIR was ≥ 10 , this value suggests that a small subpopulation of cells may have been preferentially affected by the test material but that most cells did not respond; therefore, the test article was considered "equivocal."

3. If there were increases in nuclear counts, NG and % IR for individual animals in one or more dose groups (dose-related or nondose-related) but the response was not observed in all animals in the dose group, the results of the test were considered "inconclusive."

4. If other unusual biological effects occurred that confounded the interpretation of the data to a point where a clear determination could not be rendered, the results of the test were considered "inconclusive".

Results and discussion:

Sex: male.

Genotoxicity: negative.

Toxicity: yes.

Vehicle controls valid: not examined.

Negative controls valid: yes.

Positive controls valid other: One rat from the positive control group yielded unscorable slides because of poor cell attachment and pyknotic nuclei and nuclei not associated with cytoplasm.

Additional information on results:

RANGE-FINDING ASSAYS:

In the first range-finding assay one rat in the 400 mg/kg dose group died on the second day after dosing. Clinical signs of rats dosed with methyltin chloride included rough fur, diarrhea, weakness, humped back, difficulty breathing, bloody nose, lacklustre eyes, and blood around eyes. The two surviving rats in the high-dose group had extreme weight loss (rats weighed 52 and 66% of their Day 0 weight when they were sacrificed on Day 7). All of the rats in the second range-finding assay died before their scheduled sacrifice. Clinical signs of rats dosed with 600 or 800 mg/kg bw methyltin chloride included rough fur, weakness, diarrhea, lacklustre eyes, humped back, hypoactive, and blood around eyes. The LD50 of methyltin chloride was estimated at approximately 443 mg/kg bw by summarising results of both range-finding assays together.

UDS ASSAY:

Dose levels for the first definitive in vivo-in vitro hepatocyte DNA repair (UDS) assay were set at 90, 175 and 350 mg/kg bw (approximately 20, 40 and 80% of the LD50). Three rats from the 16 hour 350 mg/kg methyltin chloride dose group were found dead on the morning after dosing. All rats from the 16 hour 175 mg/kg dose-group had rough fur on the morning after dosing. One rat from the 2 hour 90 mg/kg dose-group and two rats from the 2 hour 350 mg/kg dose-group had diarrhea. The surviving rat from the 16 hour 350 mg/kg dose-group had rough fur, humped back, diarrhea, labored breathing, and was hypoactive. The first definitive UDS assay was terminated after the first eight test animals produced insufficient viable cells for evaluation of UDS probably because of a technical error in the preparation of the cell culture medium.

A second definitive UDS assay was conducted using a new batch of cell culture medium. The LD50 was adjusted downward to approximately 278 mg/kg bw methyltin chloride based on the pattern of death observed in the first definitive assay. Dose levels for the second UDS assay were set at 50, 110 and 225 mg/kg bw (approximately 20, 40, and 80% of the adjusted LD50). One rat in the 16 hour 110 mg/kg dose-group was found the morning after

dosing. The remaining rats in the 16 hour 110 and 225 mg/kg dose-groups had rough fur at the time of sacrifice. One rat from the 16 hour 225 mg/kg dose-group had diarrhea, and another rat from the high-dose group had a sore on its right eye at the time of sacrifice. Three rats from the 2 hour 225 mg/kg dose-group had rough fur and diarrhea at the time of sacrifice. Only three of the five rats dosed in the 16 hour high-dose group were evaluated for UDS. One rat from the positive control group yielded inscorable slides because of poor cell attachment and pyknotic nuclei and nuclei not associated with cytoplasm. Slides obtained from rats treated with the negative control 16 hour before sacrifice or 50, 110 or 225 mg/kg bw methyltin chlorides either 2 or 16 hour before sacrifice yielded slides with ≤ 6.1 mean net grains per nucleus (NG) and ≤ 2 percentage of cells in repair (% IR). In contrast, rats treated with 10 mg/kg bw DMN 2 hours before sacrifice yielded slides with 28.4 NG and 93% IR. These results indicate that the test material did not induce unscheduled DNA synthesis under the conditions of this study.

Table 1: Summary of Definitive UDS Assay

Treatment	Dose (mg/kg)	Time (hr)	n	NG (mean \pm S.E.)	% IR (mean \pm S.E.)	NGIR (mean \pm S.E.)
Control/water	---	16	3	-6.8 \pm 0.7	1 \pm 0	8.0 \pm 1.6
Methyltin chloride	50	2	3	-7.4 \pm 1.0	2 \pm 1	6.6 \pm 0.8
	50	16	3	-9.0 \pm 0.4	0 \pm 0	--- \pm ---
	110	2	3	-7.7 \pm 0.5	2 \pm 1	7.2 \pm 0.8
	110	16	2	-6.5 \pm 0.4	1 \pm 1	6.4 \pm 0.0
	225	2	3	-8.9 \pm 0.2	0 \pm 0	--- \pm ---
	225	16	3	-6.1 \pm 0.4	1 \pm 0	9.1 \pm 2.7
Dimethylnitrosamine	10	2	2	28.4 \pm 6.0	93 \pm 2	30.8 \pm 5.9

Standard errors (S.E.) represent variation among animals

n = Number of animals

NG = Net grains/nucleus

% IR = Percentage of cells in repair (with at least 5 NG)

NGIR = Average net grains/nucleus of cells in repair

Reliability score 2 (reliable with restrictions) was given by the registrant because of the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

[Study 2]

Study reference:

Reference type: review article or handbook.

Author: Summer K.H., Klein, D. & Greim, H.

Year: 2003.

Title: Ecological and Toxicological Aspects of Mono and Disubstituted Methyl-, Butyl-, Octyl-, and Dodecyltin Compounds - Update 2002.

Bibliographic source : Organotin Environmental Programme (ORTEP) Association. Primary source: SRI International (1991) Measurement of Micronuclei in Bone Marrow Erythrocytes of Swiss-Webster Mice following Treatment with Mixes of Methyltin Compounds. Study No.: 7692-C10-90.

Test type:

Type of genotoxicity: chromosome aberration.

Type of study: micronucleus assay.

Principles of method if other than guideline: No methodological information.

GLP compliance: no according to the registrant data.

Test substance

Name of test material (as cited in study report): monomethyltin trichloride and dimethyltin dichloride.

Test material form: crystalline.

Description of test design:

Species: mouse.

Strain: Swiss Webster.

Sex: male/female.

Route of administration: oral: gavage.

Doses / concentrations:

Actual ingested: 100, 200, 400 mg/kg bw in water, i.g.

No. of animals per sex per dose: Fifteen.

Control animals: no data.

Tissues and cell types examined: Micronucleus (counts at 24, 48, 72 h).

Results and discussion:

Sex: no data.

Genotoxicity: negative.

Toxicity: no data.

Vehicle controls valid: no data.

Negative controls valid: no data.

Positive controls valid: no data.

Additional information on results: No induction of micronuclei. Mortality 6/30 and 11/30 at 200 and 400 mg/kg bw, respectively.

Reliability score 4 (not assignable) was given by the registrant because the study is reported in a review article with no methodological information.

Germ cell mutagenicity - other data

No data.

3.7.Carcinogenicity

Carcinogenicity - animal data

[Study 1]

Study reference:

Reference type: review article or handbook.

Author: Summer K.H., Klein, D. & Greim, H.

Year: 2003.

Title: Ecological and Toxicological Aspects of Mono and Disubstituted Methyl-, Butyl-, Octyl-, and Dodecyltin Compounds - Update 2002.

Bibliographic source : Mosinger M., Centre D'Exploitations et de Recherches Medicales, Marseille (1975). Final Report Advastab TM 181 FS (Cincinnati-Milacron).

Test type:

Principles of method: No methodological information available.

GLP compliance: no according to the registrant data.

Test substance:

Name of test material (as cited in study report): dimethyltin bis(2-ethylhexylthioglycolate) + monomethyltin tris(2-ethylhexylthioglycolate).

Test material form: crystalline.

Test animals

Species: rat.

Sex: male/female.

Administration / exposure

Route of administration: oral (feed).

Duration of treatment / exposure: Two years.

Doses / concentrations: 100 ppm.

No. of animals per sex per dose: Twenty.

Results and discussion:

Details on results: dimethyltin dichloride: methyltin trichloride (72:28% mixture).

No malignant tumours were reported in in animals dosed with 100 ppm dimethyltin bis(2-ethylhexylthioglycolate) + monomethyltin tris(2-ethylhexylthioglycolate) for two years as compared to 8/164 in control animals.

Reliability score 4 (not assignable) was given by the registrant because of a secondary source with no methodological information and read across from dimethyltin bis(2-ethylhexylthioglycolate) + monomethyltin tris(2-ethylhexylthioglycolate).

Carcinogenicity - human data

Not available.

Carcinogenicity - In vitro data

Not available.

Carcinogenicity - other data

Not available.

3.8.Reproductive toxicity

Reproductive toxicity - animal data

[Study 1]

Study reference:

Reference type: publication.

Author: Ehman, K.D., Phillips, P.M., McDaniel, K.L., barone Jr, S. & Moser, V.C.

Year: 2007.

Title: Evaluation of developmental neurotoxicity of organotins via drinking water in rats: Dimethyl tin.

Bibliographic source: Neurotoxicology and Teratology 29: 622–633.

Test type:

Limit test: no.

Test guideline: equivalent or similar to U.S. Environmental Protection Agency, Developmental Neurotoxicity Study, Health Effects Test Guidelines, OPPTS 870.6300, EPA 712-C-98- 239.

Deviations: no data.

Principles of method:

The primary objective of the current study was to examine and characterize the potential developmental neurotoxicity of DMT. The neurotoxicological effects of DMT exposure were characterized in a rat model using two separate dosing paradigms, the first to replicate the approach used earlier by Noland et al. and the second to use a more standard exposure paradigm as described in the US EPA Developmental Neurotoxicity Test Guidelines. In both studies, behavioural assays were conducted beginning at PND 11, and continued throughout development and into adulthood. Moreover, specific brain regions were analysed for apoptotic cell death and neuropathology.

E.A. Noland, D.H. Taylor, R.J. Bull, Monomethyl and trimethyltin compounds induce learning deficiencies in young rats, *Neurobehav. Toxicol. Teratol.* 4 (1982) 539–544.

GLP compliance: no according to the registrant data.

Test substance:

Test material: dimethyltin dichloride.

Test material form: crystalline.

Test animals:

Species: rat.

Strain: Sprague-Dawley.

Details on test animals and environmental conditions:

EXPERIMENT 1

One hundred and twenty nulliparous Sprague–Dawley (CD) female rats (Charles River, Raleigh, NC, USA), 53 days old, were housed in AAALAC-International accredited, temperature- and humidity-controlled rooms (16–21 °C and 40–70%, respectively) on Beta-Chip bedding with food (Purina Rodent Chow 5001) and filtered tap water ad libitum, and maintained on a reverse 12-h light:dark cycle (lights on at 1200). Females were housed in pairs until mating. Two weeks prior to DMT exposure, estrus cycles were monitored through daily vaginal smears. Females (30/dose) were then rearranged and paired according to synchronized estrus cycles (over 4 days).

EXPERIMENT 2

Eighty-seven (n=21 control, n=22 per DMT dose groups (3, 15, and 74 ppm) timed-pregnant Sprague–Dawley (CD) female rats (Charles River, Raleigh, NC, USA), were received at gestational day (GD) 2 (sperm-positive considered gestational day 0). Upon arrival, all females were housed individually as in Experiment 1, except for the light cycle (lights on at

0600) and the temperature (19–21 °C). The total experiment was replicated over ten cohorts of dams, with treatment counterbalanced across groups, such that births occurred two to four consecutive days each week.

Administration / exposure

Route of administration: oral (drinking water).

Vehicle: water.

Details on exposure:

PREPARATION OF DOSING SOLUTIONS:

Dimethyl tin dichloride was dissolved in distilled/deionized water at concentrations of 0, 3, 15 or 74 mg/l (0, 1.6, 8.1, or 40 ppm Sn). Rats received the DMT or water in polypropylene/polyethylene water bottles containing double ballbearing sipper tubes. A concentrated stock solution (740 mg/l) was prepared every two weeks and stored at –20 °C; dilutions were made from this stock. Water bottles were changed and weighed twice weekly, and rats were always weighed at the same time.

Analytical verification of doses or concentrations: yes.

Details on analytical verification of doses or concentrations:

Analyses were conducted to verify the concentration and speciation of the methyl tins in the DMT solutions. The concentrated stock solution and solutions of the low and high test concentrations were sampled daily for 5 days from water bottles maintained under conditions of the animal exposure. Ion chromatography with inductively coupled plasma mass spectrometry was used to determine levels of MMT, DMT and TMT. Total tin levels were measured using inductively coupled plasma optical emission spectrometry. The limit of detection was 1 ng Sn/mL for DMT and TMT and 10 ng Sn/mL for MMT.

Total tin analysis revealed that the low concentration, as prepared, was about 10% higher than the nominal value, whereas the high concentration was only about 2% higher. The concentration of DMT did not decrease over days and neither MMT nor TMT were detected in the samples.

Details on mating procedure:

EXPERIMENT 1

After two weeks of DMT exposure, the females were bred by placing two receptive females (i.e. late-stage proestrus) with a breeder male near the end of the light cycle, and removed the next day at lights-on. Following this cohabitation period, females were individually housed, and maintained on the DMT solutions with food (Purina Formulab Chow 5008) throughout gestation and lactation. Assignment of treatment was counterbalanced across cohorts.

EXPERIMENT 2

Timed-pregnant animals were used.

Duration of treatment / exposure

Experiment 1:

Doisng was performed before mating (2 weeks), throught gestation and lactation (PND21).

Experiment 2

DMTC exposure occured from gestational day 6 to day 21 of lactation.

Frequency of treatment: daily.

Doses / concentrations: 0, 3, 15 and 74 mg/l (0, 1.6, 8.1 and 40 ppm Sn).

Basis: nominal in water.

No. of animals per sex per dose:

Experiment 1: 30 females per dose.

Experiment 2: 22 females per dose group, 21 for control group.

Control animals: yes.

Further details on study design:

EXPERIMENT 1

After two weeks of DMT exposure, the females were bred by placing two receptive females (i.e. late-stage proestrus) with a breeder male near the end of the light cycle, and removed the next day at lights-on. Following this cohabitation period, females were individually housed, and maintained on the DMT solutions with food (Purina Formulab Chow 5008) throughout gestation and lactation. Assignment of treatment was counterbalanced across cohorts.

The day of birth was designated postnatal day (PND) 0, and litters (n=13 control, 9 at 3, 74 ppm, and 10 at 15 ppm) were culled to 8 males on PND 1. In a few cases, female pups were kept to maintain equal litter sizes. On PND 21, remaining offspring were weaned and the littermates separated and housed individually on Beta-Chip bedding and provided food (Purina Rodent Chow 5001) and filtered tap water. Only male offspring, one from each litter, were tested in the different neurobehavioral tasks; each pup was evaluated in only one test. Offspring were weighed at least weekly throughout.

EXPERIMENT 2

Using the same concentrations as in Experiment 1, DMT exposure began at GD 6 and continued through gestation and lactation. Litters (n=21 control, n=22 at 3, 15 ppm, and n=20 at 74 ppm), were culled to 4 males and 4 females on PND 4. On PND 21, the offspring were weaned and the littermates separated and housed individually on Beta-Chip bedding and provided food (Purina Rodent Chow 5001) and filtered tap water ad libitum. 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.

Dams were weighed on the days that bottles were changed, and also on specific gestational

and lactational days. All pups were weighed on specific postnatal days, and in addition, pups used for each of the neurobehavioral tests were weighed on the day of testing.

Examinations

Maternal examinations: Clinical signs, food consumption and body weight were recorded.

Fetal examinations:

RUNWAY LEARNING TEST

The runway learning test is an appetitive learning paradigm in which a food-deprived PND 11 rat pup was trained to negotiate a runway for a dry suckling reward from its anesthetized mother in the goal box. Briefly, the apparatus was a Plexiglas runway with a goal box at the end, which was maintained at 37 °C through the use of water-circulating heating pads. The pups learned to traverse the runway to reach the anesthetized dam and latency was recorded. Acquisition consisted of 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 for either (R) or to be immediately placed in the holding cage. Extinction (blocked access to the dam) immediately followed acquisition. The specific parameters for each experiment are outlined below.

Experiment 1:

Dams were anesthetized using 2 mL/kg i.p. Chloropent® equivalent. The dams were dosed approximately 15 min before testing began. Pups (n=11 control, 9 at 3 and 74 ppm, and 10 at 15 ppm) were food-deprived for 10 h prior to testing, and tested during their dark cycle. Acquisition consisted of 25 alternating (R) and (N) trials, with a maximum of 120 s for each trial and an inter-trial interval of 15 s in a holding cage. 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. All pups were tested until they met criterion, regardless of the number of trials required.

Experiment 2:

Dams were anesthetized using Nembutal sodium solution (0.65 mL i.p.; 50 mg/mL). A different training schedule was used in Experiment 2. In this paradigm, 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 (R) trials, followed by a 2 min retention interval in the holding cage. There were then 25 acquisition trials in which (R) and (N) trials alternated in blocks of 5 trials, beginning and ending with 5(R) trials, with an 8-second inter-trial interval. The maximum time allowed for each trial was 100 s. Extinction trials began on the 26th trial, and the criterion to extinction was one trial with a 100-second latency. A maximum of 10 extinction trials were run.

MOTOR ACTIVITY

Motor activity data were collected using automated figure-eight chambers. Photocell interruptions (counts) were recorded over 5-min intervals of the 30-minute test session. In Experiment 1, motor activity was assessed in males at PNDs 13, 17 and 21 (n=14 control, n=9 at ppm, n=10 at 15 ppm, and n=9 at 74 ppm). In Experiment 2, only PND 17 male and

female offspring (one male and one female from each litter; n=21 control and 15 ppm, n=20 at 3 ppm, and n=17, 74 ppm) were tested.

SPONTANEOUS ALTERATION (Experiment 2 only)

Spontaneous alternation was measured on PND 25 using a Plexiglass T-shaped apparatus. Although others have not reported adult levels of alternation (85–90%) until one month of age or older, pilot studies in our laboratory indicated that in our two-choice alternation task, using a freechoice, continuous exploration protocol, pups achieved 80% alternation by PND 25. Thus, in the present study, PND 25 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. The rat was then allowed to explore freely between only the two 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. A minimum of six arm entries was required to calculate alternations to avoid erroneous data due to low activity. Percent alternation was calculated as the number of opposite arm entries divided by the total arm entries less one (to subtract the first entry). Both males and females were tested at 10/dose.

MORRIS WATER MAZE (Experiments 1 and 2)

Spatial memory was evaluated using a Morris water maze. Rats were tested as adolescents/young adults (beginning about 7 weeks old in Experiment 1, 12 weeks old in Experiment 2). Each test trial was videotaped and the image digitized for computer analysis using maze-tracking software. Dependent 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.

For spatial training, rats learned the fixed position of the platform during 2 trials a day with an inter-trial interval of 5 min, for 9 days. The starting position was semi-randomly varied (all four starting positions were used before one was repeated, but the order itself never repeated) every 2 days. The maximum trial time was 60 s, after which time the observer guided the rat to the platform. On the 10th day, a probe trial was conducted in which the platform was removed and the subject's tendency to search in the correct quadrant was measured over 60 s. Dependent variables were the Gallagher proximity score, and percent total time within each quadrant. A visible probe trial was also conducted using a raised platform of a contrasting colour to confirm that the tested animals were not visually impaired.

In Experiment 1, only males were tested in the water maze (n=11 control, 7 at 3 ppm, 9 at 15 ppm, and 11 at 74 ppm) whereas both males and females (one from each litter) were tested in Experiment 2 (n=10/sex/dose).

NEUROPATHOLOGY (Experiments 1 and 2)

Experiment 1:

For neuropathological evaluations, male rats (n=6–8/dose at PND1, n=5–8/dose at PND 12, n=5–9/dose at PND22, and n=5/dose at adult age, 80–90 days old) were deeply anesthetized with pentobarbital and perfused via the left ventricle with buffered 4%

formaldehyde:0.1% glutaraldehyde. Sagittal blocks of tissue were embedded in paraffin and sectioned to include all major structural landmarks of the brain in each section (e.g., olfactory bulb, striatum, cerebral cortex, hippocampus, thalamus, hypothalamus, brainstem, cerebellum). Twenty-four sections of brain from each rat, from each age, at each dose, were stained with hematoxylin and eosin.

Brains from all control and high-dose rats at all ages were evaluated by a certified pathologist. Step-down assessments, i.e., evaluation of the lower dose groups, were only conducted in the adult rats due to the remarkable findings in the high-dose group. Scoring of the severity of observed changes was conducted with the pathologist blind to the dosage group.

Experiment 2:

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

BRAIN WEIGHTS (Experiments 1 and 2)

Male rats were decapitated under CO₂-induced anesthesia at PND1, 12, 22, and as adults (Experiment 1; n=4–11/dose/age) or PND12, 22, and as adults (Experiment 2; n=7–9/dose/age). From PND12 on, all subjects came from different litters. Brains were quickly removed and weighed.

APOPTOSIS ASSESSMENT

Apoptosis was quantified using a Cell Death ELISA procedure. Modifications to the procedure in the kit have been described previously; the kit has been adapted for use with intact tissue and validated with both fresh and frozen brain tissue. Moreover, the results have been corroborated qualitatively by agarose gel and TUNEL data. In short, the enzyme-linked immunosorbent assay (ELISA) uses antibodies to bind fragmented DNA characteristic of apoptotic cell death. The bound fragments (i.e. nucleosomes) are then quantified photometrically.

Experiment 1:

After weighing, brains were dissected free-hand into the following regions: brainstem, neocortex, hippocampus and cerebellum. Each region was immediately frozen in 2-methyl butane on dry ice for 30 s and stored at –70 °C [30]. ELISA assays were conducted only on tissues collected at PND22 (n=3–7/dose/region) and as adults (n=2–4/dose/region).

Experiment 2:

Tissues were collected from male rats only as described for Experiment 1 on PND12, 22, and as adults, with n=6–8/dose/region.

Statistics:

Where data were collected from littermates, they were analysed using litter as the unit (e.g., sex or multiple observations nested within litter). Continuous data (e.g., body weight, water intake, activity counts, etc.) were analysed using a general linear model ANOVA. Extinction in the runway test was analysed with a survival model for censored data and count data (e.g.,

number of pups learning, pregnancy rate) were compared using Fisher's exact test. In the second runway study, within-subject trend analyses (SAS) of the slopes of each 5-trial block were used to evaluate whether the latencies were increasing or decreasing (significantly different from a slope of zero) over the 5 trials, or whether there was no change (slope of zero). Whenever the same rat was used in repeated tests (e.g., repeated motor activity testing, body weights over time, within-session activity), the analyses included time (or interval) as a repeated factor; however, if no interaction between time/interval and treatment was revealed, data were collapsed across time. Likewise, if there was a significant interaction between sex and dose (or sex, dose, and age/time), only then were males and females analysed separately to evaluate dose (and/or age/time) effects. Where sex interactions were not significant, the data were combined. Dunnett's t-test was used to compare dose groups with the control. In all cases, resulting twotailed probability values ≤ 0.05 were considered significant.

Results and discussion:

Details on maternal toxic effects:

MATERNAL FLUID INTAKE AND WEIGHTS:

Experiment 1:

Overall, water consumption increased among all dose groups throughout gestation and lactation. In the first two weeks of exposure (prematuring), consumption was significantly decreased in all treated 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 $F(18, 180)=1.98$, $p=0.013$). Although the high-concentration consumption appeared lower throughout the rest of exposure, these differences were not significantly different from control (dose $F(3, 26)=2.56$, $p=0.076$).

Only the high concentration altered maternal weight gain, which was significantly lower than control throughout exposure (time-by-dose $F(18, 180)=3.58$, $p<0.0001$).

Experiment 2:

From the beginning of exposure to the end of gestation, fluid intake was significantly lower in the 15 and 74 ppm dose groups (time-by-dose $F(30, 270)=3.61$, $p<0.0001$). Additionally, the 3 ppm dose group showed decreased consumption during the second week of exposure. Intake returned to control levels in all but the high-concentration group during lactation. Corresponding reductions in body weight were not evident until lactation, at which time the high-concentration body weight was significantly lower than controls (time-by-dose $F(9, 117)=2.96$, $p=0.003$).

REPRODUCTIVE PARAMETERS:

Experiment 1:

The overall pregnancy success rate was very low (47 of 120 rats, 39%). There was no treatment effect on the number of pregnancies: $n=10$ control, 14 at 3 and 15 ppm, and 9 at 74 ppm. All births occurred within the 24 hour time frame typically observed in this laboratory.

Experiment 2:

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

Developmental effects: yes.

Details on developmental effects:

OFFSPRING NUMBER AND GROWTH:

Experiment 1:

The total numbers of live pups per litter were (mean±SEM): control, 14.4±1.0; 3 ppm, 13.3±0.7; 15 ppm, 15.0±0.9; 74 ppm, 12.8±0.8. Although there was a trend (dose $F(3, 43)=2.35$, $p=0.085$) towards fewer males in the treated groups, there was no significant treatment-by-sex interaction. 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 PND1, three litters (one control, two at 74 ppm) lost three to four more pups each. Average pup weight per litter (males only) was not significantly altered by DMT exposure (data not shown). Pups weighed at the time of testing for the runway, motor activity and water maze showed no differences from control.

Experiment 2:

There were no differences in the number of pups per litter (control, 12.9±0.4; 3 ppm, 12.6±0.4; 15 ppm, 13.2±0.4; 74 ppm, 12.1±0.6), or the sex ratio within the litters (data not shown). 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 changes among the pups during the lactation period showed a significant dose-by-sex interaction ($F(3, 69)=3.0$, $p=0.037$). Step-down analyses of males and females revealed that males in the high-concentration group weighed significantly less than controls throughout lactation, and the decrease in females in the same group reached significance only at PND 17 and 21. In contrast, there were no treatment effects on body weight measured weekly after weaning, or at the times of behavioural testing. Thus, the high concentration suppressed growth during lactation in the second, but not the first, study.

RUNWAY TESTING

Experiment 1:

Using a criterion of having at least one latency less than the maximum time of 120 s, several pups in each treatment group failed to learn the task; however, there was no treatment-related difference in the incidence of non-learners (control, 3 of 12; 3 ppm, 1 of 9; 15 ppm, 0 of 10; and 74 ppm, 1 of 9). Latency was quite variable, and analysis of latency across time for those that learned the task showed no significant treatment-related differences (data not shown). In the extinction phase, the median number of trials to reach the criterion (two consecutive trials of 100 s) for each dose group was: control, 18; 3 ppm, 13; 15 ppm, 15.5; and 74 ppm, 24. Although higher in the high-dose group, there were no significant differences in trials to extinction.

Experiment 2:

Using the massed-alternation paradigm, the percentage of pups that failed to learn (same criterion as above) 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. This difference, however, did not reach statistical significance. Only the "learners" were included in the data analysis for acquisition and extinction.

Statistical analyses (ANOVA) did not reveal treatment effects using the measure of latency due to the high between-subject variability. Analysis of the within-subject slopes (linear trend analysis) for each 5-trial block was much less variable. Learning, as defined by decreasing latencies during the reinforced blocks, gives a negative slope, whereas extinction, or increasing latencies (or no change in latency) during non-reinforced blocks, gives either a positive or zero slope. Such a pattern shows the contingency control over the behaviour. Both the control and low-dose groups showed significant decreasing slopes (p 's < 0.04 for all) for each of the set of R-trials, and the slopes during N-trials were not different from zero. In contrast, the 15 ppm group did not show decreased latencies on any of the R-trials, and the only significant slope change (p =0.006) was in the first N-trial (increasing latencies). The high-dose group showed a decreasing slope (p =0.008) only during the last block of R-trials. Thus, learning was not apparent during any R-trial blocks in the 15 ppm group, and was only achieved at the last set of trials in the 74 ppm group.

A number of pups did not extinguish the behaviour in 10 trials, and therefore did not meet the criterion for extinction: control, 4 of 17; 3 ppm, 2 of 15; 15 ppm, 3 of 14; and 74 ppm, 1 of 13. Furthermore, some pups extinguished almost immediately, and the median number of trials to criterion were: control, 2; 3 ppm, 6; 15 ppm, 4; and 74 ppm, 1. These differences were not significantly different across treatment groups.

MOTOR ACTIVITY:

Experiment 1:

Total motor activity counts during 30 min 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 there were no treatment-related differences. Analysis of the within-session activity (in 5-min intervals) showed that habituation (significant change in activity across intervals) was not evident until PND 21 in all treatment groups.

Experiment 2:

There were no group differences in PND 17 motor activity (either total activity counts or habituation) and no interactions with sex. Total counts for each group (sexes combined, mean \pm SEM) were: control, 115.4 \pm 7.8; 3 ppm, 114.2 \pm 5.6; 15 ppm, 121.5 \pm 9.2; and 74 ppm, 119.0 \pm 8.8). Unlike in Experiment 1, habituation was evident in all treatment groups at PND 17.

SPONTANEOUS ALTERATION (Experiment 2 only)

All pups showed a high number of alterations over the 5-minute test period. The overall dose effect did not reach significance (dose $F(3,36)$ =2.48, p =0.077). Male and female control pups averaged 10.5 arm visits during the session, with the other treatment groups ranging from 6.7 \pm 1.6 (males, 15 ppm) to 11.7 \pm 0.7 (males, 74 ppm). The percent of alternations showed no significant difference across groups or gender (mean \pm SEM): control, 84.5 \pm 2.9; 3 ppm, 84.3 \pm 2.5; 15 ppm, 86.1 \pm 2.4; and 74 ppm, 78.7 \pm 2.6.

MORRIS WATER MAZE:

Experiment 1:

All groups eventually learned; however, the middle dose group showed significantly longer latencies during the first week of training (dose $F(3, 31)$ =3.57, p =0.025). Analyses of the spatial distribution of swimming indicated that, 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). This represented a less efficient search strategy, since the platform was located in

the middle zone, and may have influenced the longer latencies observed in this treatment group. The high dose group showed no differences on any of these parameters.

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).

Experiment 2:

As in Experiment 1, the middle dose group had significantly higher latencies to learn the platform position (dose $F(3, 71)=3.1$, $p=0.032$). This was seen in both sexes in the second week, as evidenced by the lack of a treatment-by-sex interaction. There were, however, differences between the sexes in terms of the spatial pattern of swimming (first week, middle zone block-by-trial-by-dose-by-sex $F(12, 284)=2.03$, $p=0.022$; outer zone block-by-trial-by-dose-by-sex $F(12, 284)=1.87$, $p=0.038$). In the first week of training, females spent less time in the middle zone, and more time in the outer zone. On days 2 and 3, this pattern was significant in all dose groups. 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.

These tracings are from individual rats whose latencies approximated the averages from each dose group on day 7 of training. In both cases, the 15 ppm rats spent more time searching in the 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. The time in the quadrants differed (dose-by-quadrant $F(3, 71)=4.22$, $p=0.008$), with the low dose group showing significantly less time in the correct quadrant, and the middle dose group showing a similar trend (mean % time in correct quadrant \pm SE: control, $48.3 \pm 2.2\%$; 3 ppm, 39.7 ± 3.0 ; 15 ppm, $40.6 \pm 2.2\%$; 74 ppm, $48.6 \pm 2.2\%$).

There were no differences across dose groups in swim speed or latency to find the visible platform.

NEUROPATHOLOGY:

Experiment 1:

Histopathological alterations in the brain of offspring of dams exposed to DMT were noted in the cerebral cortex of rats sacrificed at PND22 and as adults. Three of five (60%) adult offspring at 74 ppm and one of five (20%) PND22 rats at 74 ppm had slight/mild vacuolation of the neuropil of the gray matter of the cerebral cortex. Step-down evaluations of the lower dose groups 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 PND1 or 12, or in the offspring at The lower doses at PND22. 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 appearance of these lesions was very similar to that observed in rats exposed in a similar paradigm to MMT. 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).

Experiment 2:

One male offspring at 74 ppm had a single neuron in the midbrain with central chromatolysis. There were no axonal lesions or increases in glial cells associated with the chromatolytic neuron, and no other rats at this dose level had any microscopic lesions. Thus,

the significance of this finding in a single neuron in a single treated rat remains undetermined.

BRAIN WEIGHT:

Experiment 1:

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 highdose groups when collapsed across the ages; overall, 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.

Experiment 2:

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%.

APOPTOSIS:

Experiment 1:

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, which were then determined to be significant decreases only at PND22 in the cerebellum (15 and 74 ppm) and cortex (all doses). These changes, however, 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).

Experiment 2:

A dose-by-age interaction ($F(6, 60)=11.90$, $p<0.0001$) for the brainstem data presented no treatment effect at PND12, with significant increases relative to control at PND22, and decreases in adults. These significant effects were seen in the mid and high dose groups. These changes may represent a shift in the normal decrease observed in control rats. In addition, cerebellar data revealed a small but significant increase (dose-by-age $F(6, 60)=3.36$, $p=0.006$) in DNA fragmentation at PND12, 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).

Table 1: DMTC intake (mg/kg/day) calculated from average fluid consumption and body weights for each phase of Experiments 1 and 2

	Concentration (ppm)	Intake (ml/d)	Weight (g)	DMTC (mg/kg/d)
<i>Experiment 1</i>				
Pre-mating	0	27.6	266.1	-
	3	24.1a	255.0	0.28
	15	20.4a	263.3	1.16
	74	14.7a	248.6a	4.38
Gestation	0	37.5	332.4	-
	3	34.7	321.0	0.32
	15	34.1	331.4	1.54
	74	29.6b	307.2a	7.13
Lactation	0	61.9	339.5a	-
	3	58.0	336.2	0.52
	15	58.3	342.7	2.55
	74	50.3	304.2a	12.2

<i>Experiment 2</i>				
Gestation	0	36.8	301.0	-
	3	32.1b	296.3	0.33
	15	30.1a	295.8	1.53
	74	28.2a	287.6	7.26
Lactation	0	62.8	324.3	-
	3	60.9	318.3	0.57
	15	56.7	318.3	2.67
	74	47.6a	297.1a	11.9

aIndicates data significantly different from control throughout the period measured.

bIndicates data significantly different from control during part of the period measured.

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

[Study 2] etc.

Study reference:

Reference type: publication.

Author: Noda, T.

Year: 2001.

Title: Maternal and Fetal Toxicity of Dimethyltin in Rats.

Bibliographic source: Journal of Health Science, 47(6) 544-551.

Materials and methods

Test guideline: Equivalent or similar to OECD TG 414 (Prenatal Developmental Toxicity Study.)

Deviations: yes.

Principles of method: The method followed the basic general outline of OECD Guideline 414, however there were several deviations.

The most notable of these were:

- Dosing occurred GD7 – 17.
- Group size was below that recommended in the guideline (Study I, 5 groups of 10; Study II, 8 groups of 8 – 11).

GLP compliance: no according to the registrant data.

Test materials

Identity of test material: Dimethyltin dichloride.

Test material form: crystalline.

Test animals

Species: rat.

Strain: Wistar.

Details on test animals and environmental conditions:

TEST ANIMALS:

- Source: CLEA Japan Inc. (Tokyo, Japan).
- Age at study initiation: 3 months.
- Housing: Animals were individually housed.
- Diet (e.g. ad libitum): ad libitum. Fed commercial laboratory chow (NMF; Oriental Yeast Co., Ltd. Tokyo, Japan).
- Water (e.g. ad libitum): ad libitum tap water.

ENVIRONMENTAL CONDITIONS:

- Temperature (°C): $23 \pm 2^{\circ}\text{C}$.
- Humidity (%): $60 \pm 20\%$ relative.
- Photoperiod (hrs dark / hrs light): Constant day/night cycle (lights on from 7:00 to 19:00 hr).

Administration / exposure

Route of administration: oral (gavage).

Vehicle: saline

Analytical verification of doses or concentrations: no.

Details on mating procedure: A female rat was paired overnight with a male of the same age and the day on which sperm was observed in the vaginal smears was designated as day 0 of gestation.

Duration of treatment / exposure: Two teratological studies were performed.

In the first (Study I), DMTC was administered to pregnant animals during the organogenetic phase of gestation (days 7-17 of gestation). DMTC was administered to pregnant females at 0, 5, 10, 15 or 20 mg/kg/day on days 7-17 of gestation. The volume of vehicle was held constant at 2 mL/kg based on maternal body weight.

In the second teratological study (Study II), DMTC was administered to pregnant animals at 0, 20 or 40 mg/kg/day for two to three consecutive days at one of four different periods of gestation (gestational days 7-9, 10-12, 13-15 and 16-17). The group treated with DMTC at 20 mg/kg/day on days 16-17 was not tested. Control animals were administered an appropriate volume of vehicle without DMTC on days 10-12 of gestation.

Duration of test: 20 days.

Doses / concentrations:

Basis actual ingested Study I: 0, 5, 10, 15, 20 mg/kg/day.

Basis actual ingested Study II: 0, 20, 40 mg/kg/day.

No. of animals per sex per dose:

Study I: Mated females were randomly assigned to five groups of ten rats each.

Study II: Mated females were randomly assigned to eight groups of 8-11 rats each.

Control animals: yes, concurrent vehicle.

Further details on study design: All procedures were substantially the same in the two teratological studies. Animals were randomly assigned to test groups.

Examinations

Maternal examinations:

Maternal body weight and food intake were measured daily. Pregnant females were observed daily for clinical signs of toxicity and were sacrificed by overdose of ether anesthesia on day 20 of gestation. Maternal thymus and brain weights, and the gravid uteri weights (only in study II) were also recorded.

Ovaries and uterine content:

The position and the number of living fetuses and implantation loss in the uterus, and the number of corpora lutea, were recorded. Uteri with total resorption were isolated and stained with 10% ammonium sulfide to determine the total number of implantations.

Fetal examinations:

The living fetuses were examined for their sex and external malformations, and were then weighed. Half of the living fetuses in each litter were fixed with 95% ethanol and processed for staining of the skeleton by the alizarin red S dye method. These preparations were examined for skeletal abnormalities. The other half of each litter was fixed in Bouin's solution and examined for visceral abnormalities according to the method of Wilson.

Statistics:

Data on the number of dams with living fetuses and with total resorption and the number of malformed fetuses were analysed by Fisher's exact test. Other data were analysed by Dunnett's multiple comparison method) in a parametric or non-parametric manner. The litter was used as the statistical unit for calculation of fetal values.

Results and discussions

Details on maternal toxic effects:

STUDY I

Oral treatment of pregnant rats with DMTC during the organogenetic phase of gestation (days 7-17 of gestation) caused reduction in the maternal body weight gain in a dose dependent manner. Significantly reduced body weight gain was observed in pregnant rats treated with DMTC at 15 or 20 mg/kg/day in late gestation. Maternal food in-take was significantly reduced by DMTC-treatment at 20 mg/kg/day, but no other statistically significant differences in maternal food intake were present.

Oral administration of DMTC at 20 mg/kg/day resulted in the death of two pregnant rats, one died on day 18 of gestation and another died on day 19 of gestation. These deaths were considered to be DMTC treatment related because the animals exhibited severe clinical signs of toxicity (piloerection, ataxia, perinasal and periocular staining, vaginal bleeding, tremor and convulsion) for about four days prior to their deaths. No gross pathological changes were noted upon necropsy in the organs of the dead dams. No other mortalities were observed in either the control or DMTC-treatment groups.

Perinasal and periocular staining, piloerection and ataxia were observed in all the pregnant rats administered DMTC at 20 mg/kg/day mainly after day 15 of gestation. In addition to these clinical signs of toxicity, vaginal bleeding, tremor and convulsion were observed in three pregnant animals other than two dead pregnant rats in the late stage of gestation. No clinical signs of toxicity were observed in the other groups. Maternal thymus weights were reduced in a dose-dependent manner with significance at 15 and 20 mg/kg/day on day 20 of gestation. Maternal brain weight was unaffected in any group.

Total resorption was observed in one of eight living pregnant rats at 20 mg/kg/day. This

same rat also exhibited vaginal bleeding, tremor and convulsion in the late stage of gestation. Mean body weight in living fetuses of both sexes decreased in a dose-dependent manner with significance at 15 and 20 mg/ kg/day. No significant differences were noted in the number of corpora lutea, implants and living fetuses, and the incidence of post-implantation loss and sex ratio.

Table 1. Effects of DMTC on Maternal Thymus and Brain Weights on Day 20 of Gestation

	Saline 2 mL/kg	DMTC (mg/kg)			
		5	10	15	20
Body wt. (g)	333 ± 26.7	334 ± 21.9	321 ± 21.0	315 ± 9.2	252 ± 41.1**
Thymus wt. (mg)	169 ± 37.5	190 ± 32.6	168 ± 26.8	151 ± 13.6*	45 ± 21.7**
Brain wt. (g)	1.75 ± 0.02	1.72 ± 0.05	1.71 ± 0.07	1.76 ± 0.04	1.68 ± 0.04

Pregnant rats were treated orally with DMTC on days 7-17 of gestation. These weights were recorded on day 20 of gestation. Values are mean ± S.D. *Significantly different from control, $p < 0.05$. **Significantly different from control, $p < 0.01$.

STUDY II

Oral gavage administration of DMTC at 40 mg/ kg/day to pregnant rats on days 10-12 of gestation caused significant reductions of maternal body weight gain on days 13, 16 and 17 of gestation and reduction of food intake on the consecutive days of gestation after day 12 of gestation. No significant reduction in maternal body weight gain was observed in other treatment period groups, in spite of the reduced food intake caused by the treatment. There were no significant differences in general behaviour among the groups including controls.

Maternal thymus weights and adjusted body weight gain were reduced significantly in the group treated with DMTC at 20 mg/kg/day on days 10-12 of gestation and in every treatment period groups at 40 mg/kg/day. Gravid uterus and maternal brain weights were unaffected at either dose level in any treatment period group. Total resorption was observed in one of 10 dams in the group treated with DMTC at 40 mg/kg/day on days 7-9 of gestation. In this group, mean fetal body weight of the females was reduced. No significant differences were noted in the number of corpora lutea, implants and living fetuses and incidence of postimplantation loss, sex ratio and male fetal body weight at either dose level in any treatment period group.

Table 2. Effects of DMTC Exposed to Pregnant Rats on Two or Three Consecutive Days at One of Four Different Treatment Periods of Gestation, on Maternal Brain, Thymus, Gravid Uterus Weights and Adjusted Body Weight Gain at the Day 20 of Gestation

	Saline days 10-12 2 mL/kg	DMTC (mg/kg)						
		days 7-9		days 10-12		days 13-15		days 16-17
		20	40	20	40	20	40	40
Body wt. (g)	338 ± 19.7	330 ± 23.2	330 ± 47.4	325 ± 23.1	312 ± 22.7	331 ± 12.7	325 ± 14.8	340 ± 23.4
Brain wt. (g)	1.79 ± 0.08	1.79 ± 0.04	1.80 ± 0.05	1.79 ± 0.04	1.76 ± 0.06	1.75 ± 0.06	1.79 ± 0.05	1.80 ± 0.05
Thymus wt. (mg)	253 ± 45.1							
Body weight gain (g)	112 ± 21.6	220 ± 22.7	208 ± 37.6*	207 ± 39.9*	190 ± 36.9**	217 ± 15.3	182 ± 32.7**	172 ± 45.1**
Gravid uterus wt. (g)	65 ± 20.0	110 ± 12.5	110 ± 13.1	107 ± 18.0	95 ± 17.8	111 ± 9.0	103 ± 12.4	102 ± 22.6
Adjusted body weight gain ^a (g)	47 ± 7.0				66 ± 19.7			
		69 ± 10.2	75 ± 11.7	72 ± 11.1		69 ± 4.5	71 ± 8.6	71 ± 15.6
		40 ± 7.4	35 ± 6.0**	35 ± 9.6*	30 ± 9.2**	42 ± 6.5	33 ± 6.6**	31 ± 13.3**

Pregnant rats were sacrificed on day 20 of gestation. Values are the mean ± S.D. of 8-11 animals per group.
^a(Body weight gain from day 0 to 20 of gestation) - (gravid uterus weight). *Significantly different from control, $p < 0.05$. **Significantly different from control, $p < 0.01$.

Details on embryotoxic / teratogenic effects:

STUDY I

The incidence of external malformations increased in fetuses from dams exposed to DMTC at 20 mg/kg/day from days 7-17 of gestation. There were 21 fetuses with cleft palate 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. There were two fetuses with omphalocele from one dam exposed to DMTC at 15/mg/day, but the incidence was not statistically significant. No other external malformations were observed in either the control or DMTC-treatment groups.

No significant difference was observed in the incidence of skeletal malformations and skeletal variations. No significant difference was observed in the incidence of visceral malformations, however the number of visceral variation, dilation of the renal pelvis, was significantly increased at 20 mg/kg/day.

	Saline 2 ml/kg	DMTC (mg/kg)			
		5	10	15	20
No. of females inseminated	10	10	10	10	10
No. of pregnant females	10	10	10	9	10
No. of dams with living fetuses	10	10	10	9	7
No. of dams with total resorption	0	0	0	0	1
No. of dead dams	0	0	0	0	2
No. of corpora lutea ^{a)}	15.8±1.32	15.5±1.18	15.9±1.91	16.0±1.73	15.9±2.47
No. of implants ^{a)}	15.4±1.43	14.4±0.84	15.1±1.37	14.0±2.06	14.8±2.12
Incidence of postimplantation loss %	4.69	4.12	6.86	2.96	19.35
Early stage	2.63	4.12	6.86	2.96	6.86
Late stage	2.05	0.00	0.00	0.00	12.49
No. of living fetuses ^{a)}	14.6±1.90	13.8±1.03	14.1±1.79	13.7±2.06	11.9±5.22
Sex ratio (M/F)	70/76	69/69	79/62	68/55	46/49
Body weight of living fetuses ^{a,b)} g					
Male	3.5±0.20	3.4±0.22	3.2±0.25	2.9±0.16*	2.2±0.46**
Female	3.3±0.16	3.2±0.20	3.2±0.27	2.8±0.15*	2.1±0.41**

Rats were treated orally on GD 7-17 with DMTC. Cesarean sections were performed on GD20. The litter was used as the statistical unit for the calculation of foetal values. a) values are mean ± SD. b) values are means of litter means within each group. * statistically significantly different from control $p < 0.05$. ** statistically significantly different from control $p < 0.01$.

	Saline	DMTC (mg/kg)			
	2 ml/kg	5	10	15	20
External observations					
No. of fetuses examined	146	138	141	123	95
Incidence of fetuses with malformations %	0	0	0	2.5(1)	22.5(5)**
No. of fetuses with malformations	0	0	0	2(1)	21(5)**
Cleft palate	0	0	0	0	21(5)**
General edema	0	0	0	0	2(2)
Pes varus	0	0	0	0	1(1)
Omphalocele	0	0	0	2(1)	0
Skeletal observations					
No. of fetuses examined	78	73	79	64	50
Incidence of fetuses with malformations %	0	0	3.3(1)	0	0
No. of fetuses with malformations	0	0	2(1)	0	0
Fused ribs	0	0	1(1)	0	0
Fused cervical vertebral arches	0	0	2(1)	0	0
No. of fetuses with variations					
Cervical ribs	2(2)	3(3)	3(3)	6(5)	3(3)
Splitting of 1st cervical vertebral arches	0	1(1)	0	1(1)	0
Rudimentary lumbar ribs	6(1)	0	2(1)	0	1(1)
Splitting of ossification centers of thoracic vertebral bodies	0	0	2(1)	1(1)	4(4)
Visceral observations					
No. of fetuses examined	68	65	62	59	45
Incidence of fetuses with malformations %	0	0	4.8(3)	1.6(1)	2.0(1)
No. of fetuses with malformations	0	0	3(3)	1(1)	1(1)
Ventricular septal defect	0	0	1(1)	1(1)	1(1)
Dilatation of lateral ventricle	0	0	2(2)	0	0
No. of fetuses with variations					
Dilatation of the renal pelvis	0	0	1(1)	4(4)	5(4)*
Thymic remnant in the neck	0	0	2(1)	2(2)	1(1)
Kinked ureter	0	0	1(1)	1(1)	0

Rats were treated orally on GD 7-17 with DMTC. Cesarean sections were performed on GD20. () No of conceived mother with case. * statistically significantly different from control $p < 0.05$. ** statistically significantly different from control $p < 0.01$.

STUDY II

The incidence of external, skeletal and visceral malformations did not significantly increase at either close in any treatment period group. No cleft palate were found in any fetus treated with DMTC at 20 or 40 mg/kg/day on two to three consecutive days at one of four different treatment periods of gestation. The numbers of fetuses with skeletal variation and visceral variation increased significantly at 40 mg/kg/day in some treatment periods. Numbers of fetuses with cervical ribs increased in the groups treated with DMTC on days 7-9 or 13-15 of gestation. Numbers of fetuses with splitting of first cervical vertebral arches and with kinked ureter increased in the group treated with DMTC on days 7-9 and 16-17 of gestation, respectively.

	Saline	DMTC (mg/kg)							
	days 10-12	days 7-9		days 10-12		days 13-15		days 16-17	
	2 ml/kg	20	40	20	40	20	40	40	
No. of pregnant females	10	10	10	10	11	9	8	10	
No. of dams with living fetuses	10	10	9	10	11	9	8	10	
No. of dams with total resorption	0	0	1	0	0	0	0	0	
No. of dead dams	0	0	0	0	0	0	0	0	
External observations									
No. of fetuses examined	127	136	139	141	145	118	113	142	
Incidence of fetuses with malformations %	0.0	0.0	0.6(1)	0.0	0.0	0.0	0.9(1)	0.0	
No. of fetuses with malformations	0	0	1(1)	0	0	0	1(1)	0	
Peaked mandible	0	0	1(1)	0	0	0	0	0	
Vestigial tail	0	0	0	0	0	0	1(1)	0	
Skeletal observations									
No. of fetuses examined	69	72	73	74	79	64	61	76	
Incidence of fetuses with malformations %	0.0	0.0	2.6(2)	0.0	0.0	0.0	1.6(1)	0.0	
No. of fetuses with malformations	0	0	2(2)	0	0	0	1(1)	0	
Absence of thoracic vertebral arches	0	0	1(1)	0	0	0	0	0	
Fused mandible	0	0	1(1)	0	0	0	0	0	
Agenesis of the sacro and coccygeal vertebrae	0	0	0	0	0	0	1(1)	0	
No. of fetuses with variations									
Cervical rib	0	0	5(4)*	1(1)	2(2)	1(1)	4(4)*	2(2)	
Splitting of 1st cervical vertebral arches	0	0	6(4)*	0	1(1)	0	1(1)	0	
Variation in number of lumbar vertebrae	0	0	0	0	0	0	1(1)	0	
Rudimentary lumbar rib	1(1)	2(2)	4(2)	4(3)	3(1)	2(2)	5(3)	2(2)	
Visceral observations									
No. of fetuses examined	58	64	66	67	66	54	52	66	
Incidence of fetuses with malformations %	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
No. of fetuses with variations									
Thymic remnant in the neck	2(1)	1(1)	6(3)	1(1)	7(5)	1(1)	1(1)	0	
Dilatation of renal pelvis	1(1)	2(1)	5(4)	3(3)	1(1)	1(1)	1(1)	4(2)	
Kinked ureter	0	4(2)	3(2)	2(1)	1(1)	0	2(2)	6(3)*	

Cesarean sections were performed on GD20. () No of conceived mother with case. * statistically significantly different from control $p < 0.05$.

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted to GLP in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

[Study 3]

Study reference:

Reference type: publication.

Author: Noda, T. & Morita, S.

Year: 1994.

Title: Teratogenicity Study of Dimethyltin Dichloride in Rats.

Bibliographic source: The Japanese Society of Toxicology: J. Toxicol. Sci. 19:366. E-22.

Materials and methods

Limit test: no.

Principles of method if other than guideline: A teratogenicity study using pregnant wistar rats.

GLP compliance: no according to the registrant.

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across): yes.

Test material form: crystalline.

Test animals

Species: rat.

Strain: Wistar.

Details on test animals and environmental conditions: Pregnant Wistar rats.

Administration / exposure

Duration of treatment / exposure: 11 days (days 7 to 17 of gestation).

Duration of test: 17 days.

Doses / concentrations: 0, 5, 10, 15 and 20 mg/kg bw.

No. of animals per sex per dose: 10 females per dose.

Control animals: yes.

Results and discussions

Vaginal bleeding, convulsion, decreased body weight gain and dead dams (2/10) were observed at 20 mg/kg in the late stage of gestation. On day 20 of gestation, incidence of fetuses with cleft plate was increased significantly at 20 mg/kg bw.

In order to clarify the teratogenic potential of DMTC, the compound was given at 20 or 40 mg/kg for 2 or 3 consecutive days at four different periods of gestation (days 7-9, 10-12, 13-15 or 16-17). No maternal toxicity and no fetus with cleft plate was observed in any DMTC-treated group.

Reliability score 4 (not assignable) was given by the registrant because the study source was a brief summary rather than full article and because of limited methodology and results. It cannot be excluded that Noda and Morita (1994) and Noda (2001) refer to the same study.

Reproductive toxicity - human data

Not available.

Reproductive toxicity - other data

[Study 1]

Study reference:

Reference type: publication.

Author: Jenkins, S.M., et al.

Year: 2004.

Title: Structure-activity comparison of organotin species: dibutyltin is a developmental neurotoxicant in vitro and in vivo.

Bibliographic source: Developmental Brain Research 151 (2004) 1-12.

Materials and methods

Test type: in vitro.

Limit test: no.

Test guideline: no guideline available.

Principles of method if other than guideline: In vitro examination of neurotoxic potential, by looking at neurite outgrowth and cell viability.

GLP compliance: no according to the registrant (not reported).

Test materials

Identity of test material: Dimethyltin dichloride.

Test material form: crystalline.

Administration / exposure

Route of administration: in vitro.

Examinations

Statistics:

Neurite outgrowth and cytotoxicity were examined using a split-plot design with subsampling. The main plots were the plates, subplots were the wells, and subsampling occurred with the sampling of two images per well. Each well was considered the minimum unit of measure (n) and subsampling of a well was considered a replicate and averaged. Analysis of variance (ANOVA) was used on raw data to determine if differences between the groups were significant. Following the identification of significant differences, Tukey's studentized range post hoc test was employed. Statistically significant differences were reported when p values were ≤ 0.05 . ANOVA followed by Tukey's studentized range post hoc test were also used for assessment of cell viability and DNA fragmentation. Statistical analyses were performed using SAS.

Results and discussions

Addition of DMT to primed PC12 cells inhibited neurite outgrowth and decreased cell viability in a concentration dependent manner. Quantitation revealed that total neurite outgrowth was significantly inhibited by 7.0 μM (43% decrease), 10 μM (52% decrease), 20 μM (64% decrease), and 50 μM (93% decrease) DMT. No significant effect was seen at concentrations below 7.0 μM . As shown above, the absence of NGF decreased neurite outgrowth. DMT prevented neurite branching in a concentration-dependent manner with significant inhibition observed at 7.0 μM (46% decrease), 10 μM (50% decrease), 15 μM (58% decrease), 20 μM (62% decrease), and 50 μM (93% decrease). Removal of NGF also decreased the number of branch points. Segment length was significantly decreased by 20 μM (28% decrease) and 50 μM (32% decrease) DMT. No significant effects were detected on segment length at DMT concentrations of 15 μM or less. The percentage of cells staining positively for trypan blue increased from 7.0% in control cells to 20%, 19%, 24%, and 47% in the presence of 10, 15, 20, and 50 μM DMT, respectively.

Reliability score 4 (not assignable) was given by the registrant because the study is an in vitro study on a range of organotin substances and not sufficient to determine neurotoxicity on its own.

[Study 2]

Reference

Reference type: publication.

Author: DeWitt, J.C., et al.

Year: 2007.

Title: Immune function is not impaired in Sprague–Dawley rats exposed to dimethyltin dichloride (DMTC) during development or adulthood.

Bibliographic source: Toxicology 232 (2007) 303–310.

Materials and methods

Test type: subacute and developmental.

Test guideline: no guideline followed.

Principles of method if other than guideline: Repeated dose drinking water immunotoxicity study, looking at the effects of adult or developmental exposure to dimethyltin dichloride-containing drinking water on two types of adaptive immune responses and on one aspect of innate immunity in rats.

GLP compliance: no according to the registrant (not reported).

Test materials

Identity of test material: dimethyltin dichloride.

Test material form: crystalline.

Test animals

Species: rat.

Strain: Sprague-Dawley.

Sex: male/female.

Details on test animals and environmental conditions:

ADULT STUDY:

TEST ANIMALS

- Source: Charles River Laboratories (Raleigh, NC).
- Age at study initiation: 6 - 7 weeks old
- Housing: animals were housed individually in polycarbonate cages with pine shavings
- Diet (e.g. ad libitum): ad libitum (PMI 5001, PMI Nutrition International, Richmond, IN)
- Water (e.g. ad libitum): ad libitum
- Acclimation period: at least 10 days before dosing began.

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 22.3 ± 1.1 °C
- Humidity (%): 50 ± 10 %

- Air changes (per hr):
- Photoperiod (hrs dark / hrs light): 12 h light (06:00 h) to dark (18:00 h) cycle

DEVELOPMENTAL STUDY:

TEST ANIMALS

- Source: Charles River Laboratories (Raleigh, NC).
- Age at study initiation: 9–10-weeks old, nulliparous
- Housing: Dams were housed individually in polycarbonate cages with alpha cellulose bedding (ALPHA-dri, Shepherd Specialty Papers, Watertown, TN)
- Diet (e.g. ad libitum): ad libitum (PMI 5001, PMI Nutrition International, Richmond, IN)
- Water (e.g. ad libitum): ad libitum
- Acclimation period: at least 10 days before dosing began.
- Other: Dams arrived on gestational day (GD) two, using the definition of GD1 as the day following overnight mating. After weaning, pups from each litter were separated by sex, housed four/sex/cage on hardwood chip bedding, and given access to both food (PMI 5001, PMI Nutrition International, Richmond, IN) and water ad libitum.

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 22.3 ± 1.1 °C
- Humidity (%): $50 \pm 10\%$
- Air changes (per hr):
- Photoperiod (hrs dark / hrs light): 12 h light (06:00 h) to dark (18:00 h) cycle

All procedures employed in both studies were approved in advance by the Institutional Animal Care and Use Committee of the National Health and Environmental Effects Research Laboratory, US EPA.

Administration / exposure

Route of administration: oral: drinking water.

Vehicle: water.

Details on exposure:

Dosing solutions were prepared twice weekly in 25 L polycarbonate carboys and then transferred into individually labelled plastic drinking water bottles. For the 40 mg DMTC/L solution, the appropriate mass of DMTC was added to an appropriate volume of deionized water and mixed for 10–20 min with a 1.5 in. propeller blade affixed to a Ryobi (Anderson, SC) 10 inch drill press. The 20 mg DMTC/L dosing solution was made by diluting a portion of the 40 mg/L solution with deionized water.

Analytical verification of doses or concentrations: yes.

Details on analytical verification of doses or concentrations: The concentration of DMTC/L of dosing solution was confirmed by ICP/AEC (EPA Method 200.7 Rev 4.4).

Duration of treatment / exposure:

Adult: 28 days

Developmental: Dosing of the dams was via drinking water from GD6, 1 day prior to the beginning of fetal hematopoiesis, and continued until pups were weaned on postnatal day 22 (PND22, or 22 days after birth).

Frequency of treatment: ad libitum.

Doses / concentrations:

20 and 40 mg/L (nominal in water).

1.7 and 3.4 mg DMTC/kg bw (actual ingested adult male/female rats)

2.4 and 4.6 mg DMTC/kg bw (actual ingested pregnant female rats)

3.6 and 6.9 mg DMTC/kg bw (actual ingested females from parturition through weaning of pups)

No. of animals per sex per dose:

Adult: 24 animals/sex/dose.

Developmental: 8–9 dams/dose.

Control animals: yes.

Examinations

Observations and clinical examinations performed and frequency:

ADULTS:

Animals were weighed twice/week during the dosing period and just prior to sacrifice. Dosing water was changed and water consumption was monitored twice/week. Immune function was evaluated in separate groups of animals beginning the day after exposure ended.

DEVELOPMENTAL:

Dams were weighed twice/week during the dosing period; pups were weighed twice/week from PND7 through weaning, once/week from weaning through PND37, and then just prior to sacrifice.

On PND2, pups from each dam were removed, sexed, counted, and weighed individually. Four males and four females from each litter were randomly selected and returned to their biological mothers, unless a given dam did not deliver at least four male and four female pups. In those cases, extra pups from different dams within the same dose group were randomly selected and used to adjust litters to four males and females. One litter in the 20 mg DMTC/L dose group and four litters in the 40 mg DMTC/L dose group were backfilled in this way; although these dams delivered an average number of pups, they were either short

1–2 male or 1–2 female pups. However, none of these litters were used for immunotoxicity evaluation. On PND27, pups from each litter were separated by sex, housed four/sex/cage, and were randomly assigned a pup number (1–4), indicated by an ear punch. Immune function was evaluated in separate groups of 6–9-week-old non-littermates from full litters (four males + four females from the same dam); individuals from separate litters were randomly selected for each experimental endpoint and housed two or three/cage until sacrifice.

Humoral immunity examinations:

ANTIBODY RESPONSES:

Eight (adult) or six (developmental) animals/sex/group were used to measure the IgM and IgG T cell-dependent antibody responses to SRBC. Animals were immunized by intravenous injection of 2.0×10^8 SRBCs in 0.5 mL of sterile saline and were bled 5 days later by tail vein transection. Blood was held at room temperature for 30 mins and centrifuged at 4 °C to separate serum; serum was frozen at –80 °C until analysis of SRBC-specific IgM. Two weeks after primary immunization, a booster immunization of SRBCs (2.0×10^8) was administered intravenously to the same animals. Blood from decapitated animals was collected 5 days after the booster immunization and processed as described above for later analysis of SRBC-specific IgG. The relative serum titers of SRBC-specific IgM and IgG antibodies were measured by ELISA. Titers represent the mean dilution at which a 0.5 optical density was achieved. All ELISA procedures were optimized for differences in SRBC membrane preparations and each new lot of secondary antibody.

Specific cell-mediated immunity:

DELAYED-TYPE HYPERSENSITIVITY (DTH) REACTION:

Eight (adult) or six (developmental) animals/sex/group were used to measure the DTH responses to purified (Fraction V) bovine serum albumin (BSA; Sigma). BSA (2 mg/mL in sterile saline) was emulsified in Freund's complete adjuvant (CFA; Difco, Detroit, MI) at a 1:1 ratio. Animals anesthetized with isoflurane were sensitized by injecting 0.1 mL of BSACFA subcutaneously into the caudal tail fold. Seven days later animals were anesthetized with isoflurane and challenged by injecting 0.1 mL of heat aggregated BSA into the right rear footpad. The left rear footpad was injected with the same volume of saline and served as the injection control. BSA was aggregated by heating 10 mg BSA/mL of sterile saline to 75 °C for 1 h and removing excess saline by centrifuging for 10 min at $650 \times g$. After 24 h, footpad thickness (triplicate measurements) was determined in anesthetized animals with an electronic caliper designed and built in the model shop at the US EPA (Research Triangle Park, NC). The device applies very light, even, and reproducible pressure on the footpad for each measurement, thus increasing the accuracy of measurements. Standards of known thickness were measured before and after experimental measurements. Swelling was calculated by subtracting the mean saline-injected, left footpad thickness from the mean BSA-injected right footpad thickness.

Non-specific cell-mediated immunity:

NATURAL KILLER (NK) CELL ACTIVITY:

Eight (adult) or six (developmental) animals/sex/group were used to measure NK cell activity as described by Smialowicz et al. (1991). Briefly, terminal BW was recorded, the spleen and thymus were removed, and the weights of both organs were recorded. Splenocyte single cell suspensions were prepared and cultured with ⁵¹Cr-labelled murine YAC-1 lymphoma target cells (100 Ci/1×10⁶ cells) in round-bottomed microtiter plates (Costar, Corning Incorporated, Corning, NY) at effector to target cell ratios of 25:1, 50:1 and 100:1. After a 4 h incubation at 37 °C, microtiter plates were centrifuged (290 × g) and 25 µl of supernatant were transferred to a 96-well LumaPlate (Packard, Meriden, CT), covered, and read on a TopCount NXT (Packard) microplate scintillation counter. The data are presented as the average adjusted percent cytotoxicity for three replicates [((mean counts per minute in supernatant–spontaneous release of ⁵¹Cr)/(mean releasable counts from target cells–spontaneous release of ⁵¹Cr))×100].

Statistics:

All data are presented as mean ± standard error of the mean (S.E.M.). Statistical analyses were carried out with the SAS System (SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA) was used to analyze dose within each gender and dose × gender interactions and when appropriate, linear regressions were used to determine dose–response. When ANOVA indicated a statistically significant relationship, individual post hoc comparisons were made using Tukey’s test and the least squares means t-test with a Tukey’s adjustment for controlling the familywise error rate. A repeated measures ANOVA was used to analyze BW changes over time and dose. Statistical significance was determined using an α of 0.05.

Results and discussions

Clinical signs and mortality: not examined.

Body weight and weight gain: no effects.

Food consumption and compound intake (if feeding study): not examined.

Food efficiency: not examined.

Ophthalmoscopic examination: not examined.

Haematology: not examined.

Clinical chemistry: not examined.

Urinalysis: not examined.

Gross pathology: not examined.

Cell viabilities: not examined.

Humoral immunity examinations: no effects.

Specific cell-mediated immunity: no effects.

Non-specific cell-mediated immunity: no effects.

Other functional activity assays: not examined.

Other findings: not examined.

Details on results:

BODY WEIGHT:

Adult study:

Body weights did not vary by dose for either sex during any part of the dosing period nor when terminal weights were collected

Developmental study:

As with the adult study, although water consumption was decreased in the dose groups, body weights did not vary by dose during gestation or after parturition (data not shown).

Body weights of both male and female offspring from dams dosed with 40 mg DMTC/L were about 10% greater relative to body weights from control animals from PND7 through PND37 ($p < 0.05$; data not shown). Body weights did not vary by dose for either sex when terminal weights were collected at PND44, 58, or 77. Pup mortality was limited to the 40 mg DMTC/L group; two pups from one litter died between PND14 and 17 and one pup from a second litter died on PND14.

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study):

Adult study:

Water consumption by both males and females was reduced by approximately 30% in both the 20 and 40 mg DMTC/L dose groups relative to controls ($p < 0.05$)

Developmental study:

From GD6 through GD21, water consumption by dams in both DMTC dose groups was reduced by approximately 20% relative to controls ($p < 0.05$; data not shown). Immediately after birth and through PND9, water consumption did not vary by dose. From PND9 through PND19, consumption in the DMTC dose groups was again reduced by approximately 20% relative to controls ($p < 0.05$; data not shown), but was back to control levels by the day of weaning, at PND22.

IMMUNE FUNCTIONS:

Adult study:

IgM (Fig. 1A) and IgG (Fig. 1B) antibody responses to sheep red blood cells (SRBCs), delayed-type hypersensitivity responses to bovine serum albumin (Fig. 1C), and natural killer

cell cytotoxicity (Table 1) did not vary by dose for either sex. Spleen and thymus weights (collected and weighed from animals during the natural killer cell assay) did not vary by dose for either sex. In addition, no sex-related differences existed in responses for any of the endpoints.

Developmental study:

IgM (Fig. 2A) and IgG (Fig. 2B) antibody responses to SRBCs, delayed-type hypersensitivity responses to bovine serum albumin (Fig. 2C), and natural killer cell cytotoxicity (Table 1) did not vary by dose for either sex. Spleen and thymus weights (collected and weighed from animals during the natural killer cell assay) did not vary by dose for either sex. In addition, no sex related differences existed in responses for any of the endpoints.

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

3.9. Specific target organ toxicity (single exposure)

Specific target organ toxicity (single exposure) - animal data

See acute toxicity.

Specific target organ toxicity (single exposure) - human data

See acute toxicity.

Specific target organ toxicity (single exposure) - other data

[Study 1]

Reference type: publication.

Author: Ariyoshi, T., et al.

Year: 1991.

Title: Increase of DT-diaphorase Activity and Atrophy of Thymus by Organotin Compounds.

Bibliographic source: Bull. Environ. Contam. Toxicol. (1991) 46: 100-107.

Materials and methods

Test type: acute.

Limit test: no.

Test guideline

Qualifier: no guideline followed.

Principles of method if other than guideline: Immunotoxicity study looking at DT-diaphorase activity and thymus atrophy. Male Wistar rats were dosed via subcutaneous injection.

GLP compliance: no according to the registrant (not reported).

Test materials

Identity of test material: Dimethyltin dichloride.

Test material form: crystalline.

Test animals

Species: rat.

Strain: Wistar.

Sex: male.

Details on test animals and environmental conditions: Animals received normal rat chow and water ad libitum.

Administration / exposure

Route of administration: subcutaneous.

Vehicle: corn oil.

Details on exposure: Rats were generally given a single subcutaneous injection with organotin compounds which dissolved in corn oil at 1 mL per dose per kg of body weight.

Analytical verification of doses or concentrations: no data.

Duration of treatment / exposure: Single exposure.

Frequency of treatment: Single exposure.

Doses / concentrations (actual injected): 11 mg/kg bw (50 µmoles/kg bw).

No. of animals per sex per dose: 4 male rats per dose.

Control animals: no.

Further details on study design: After treatment with organotin animals were killed by decapitation, and thymus and spleen were dissected out, washed with saline, blotted, and

weighed. The liver was perfused in situ with a cold 0.9% saline and then removed, washed, blotted and weighed.

Examinations

Heme oxygenase activity was calculated from the amount of bilirubin formed by using an extinction coefficient of $40 \text{ mM}^{-1}\text{cm}^{-1}$ between 464 and 530 nm as described by Maines and Kappas (1976). Cytochrome P-450 was estimated by the method of Omura and Sato (1964) using an extinction coefficient of $91 \text{ mM}^{-1}\text{cm}^{-1}$ between the absorbance spectra at 450 and 490 nm following carbon monoxide bubbling. DT-diaphorase activity was measured spectrophotometrically by the reduction of DCPIP using procedure of Ernster (1967). P-Aminophenol formed by aniline hydroxylase was determined by the method of Imai et al. (1966), and formaldehyde formed by aminopyrine N-demethylase was determined according to Nash (1953). 7-Ethoxycoumarin O-deethylase (7-EC) activity was measured by recording the fluorescence increase due to the formation of 7-hydroxycoumarin as reported by Ullrich and Weber (1972). Protein concentration was determined according to Lowry et al. (1951) using bovine serum albumin as a standard.

Results and discussions

NOAEL (male) ≥ 11 based on test mat.

Basis for NOAEL: losses in cytochrome P-450 and enzyme activity.

Results of examinations

Dimethyltin dichloride showed induced heme oxygenase, and that activity reached peak values at 48 hours after treatment, and then gradually decreased. Dimethyltin dichloride produced no significant losses in cytochrome P-450 content at any time point.

DT-diaphorase activity and thymus atrophy were examined at 24, 48 and 96 hours after treatment of rats with organotins. Dimethyltin dichloride did not significantly change the enzyme activity at any time after injection.

Results obtained from dimethyltin dichloride treatment showed that there was a good reciprocal correlation between DT-diaphorase activity and thymus weight ($r=0.73$, $n=12$).

Reliability factor 4 (not assignable) was given by the registrant because of brief methodology and results and because only single dose was used.

3.10. Specific target organ toxicity (repeated exposure)

Specific target organ toxicity (repeated exposure) - animal data

[Study 1]

Reference

Rohm and Haas (1999).

Materials and methods

Test type: subchronic.

Limit test: no.

Test guideline: equivalent or similar to OECD TG 408 (Repeated Dose 90-Day Oral Toxicity in Rodents).

GLP compliance: yes.

Test materials

Test material form: crystalline.

Details on test material:

- Name of test material (as cited in study report): methyltin chlorides (mixture of dimethyltin dichloride/methyltin trichloride).
- Substance type: powder.
- Physical state: solid.
- Storage condition of test material: room temperature and out of direct sunlight.

Test animals

Species: rat.

Strain: Sprague-Dawley.

Sex: male/female.

Details on test animals and environmental conditions:

TEST ANIMALS

- Source: Charles River Canada Inc., St. Constant, Quebec
- Age at study initiation: 65-68 days of age
- Weight at study initiation: 308 to 414 g (males); 181 to 268 g (females).
- Housing: All rats were housed individually in stainless-steel mesh-bottomed cages equipped with a water bottle.
- Diet (e.g. ad libitum): A standard certified commercial pelleted laboratory diet (PMI Certified Rodent Chow 5002) was provided through a feeding box ad libitum.
- Water (e.g. ad libitum): Water (with test article incorporated, where appropriate) which had been further treated by reverse osmosis and ultraviolet sterilization was provided ad libitum.
- Acclimation period: approx. 4 weeks.

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 22 ± 3°C.
- Humidity (%): 30 – 70.
- Photoperiod (hrs dark / hrs light): 12/12.

IN-LIFE DATES: From: 10th July 1996 To: 26th October 1996.

Administration / exposure

Route of administration: oral (drinking water).

Vehicle: unchanged (no vehicle).

Details on oral exposure:

PREPARATION OF DOSING SOLUTIONS:

A solution of the test material in drinking water was prepared daily (rather than weekly as indicated in the protocol) for each group by direct dilution. Appropriate amounts of the test material were added to drinking water and mixed until dissolved.

Analytical verification of doses or concentrations: yes.

ANALYSIS OF TEST MATERIAL:

An appropriate sample of test material was analysed using GC/MS prior to and at the end of the treatment period. The purity of the test material was assessed against analytical standards of certified purity. Confirmation of purity was established by comparison of the retention times of the reference standards and the major components of the test material. Characterisation was performed by inspecting the MS of each of the eluted compounds and checking for characteristic mass-to-charge ratio.

ANALYSIS OF DOSE PREPARATION:

Homogeneity of the treated drinking water was evaluated prior to treatment initiation.

Stability of the test material in drinking water was evaluated prior to the commencement of treatment by analysis of samples obtained immediately after preparation and after storage for one day at room temperature.

The accuracy of mixing of the treated drinking water was checked during the treatment period by analysis of a sample taken from each group after preparation as follows during the study:

Group 1 - Days 0, 8, 19, 47 and 91

Group 2 - Days 0, 1, 6, 12, 19, 47 and 91

Group 3 - Days 0, 1, 6, 7, 12, 19, 47 and 91

Group 4 - Days 0, 1, 8, 12 and 19

Concentrations were considered acceptable when the mean of duplicate analysis (and

subsequent triplicate analysis if deemed necessary) of a sample was $\pm 20\%$ of the nominal concentration.

Duration of treatment / exposure: Up to 90 days.

Frequency of treatment: Daily.

Doses/concentrations (nominal in water): 0, 25, 75, 200 (reduced to 150 during 5th week of treatment and terminated during 6th treatment week) ppm

No. of animals per sex per dose: 15 per sex per dose in main study; 15 per sex per dose in neurotoxicity study.

Control animals: yes.

Details on study design: Approximately 4 weeks before treatment commenced, all animals were weighed and 30 males and 30 females were randomly assigned to each of the 4 groups using a computer-based randomisation procedure which ensured the homogeneity of group means and variances for body weight. Males and females were randomised separately. The animals within each group were split into several replicates of approximately equal numbers of animals, and treatment initiation and evaluations were staggered over 3 days.

Positive control: no.

Examinations:

CLINICAL OBSERVATIONS:

All animals were checked twice daily for mortality and signs of ill health or reaction to treatment. In addition, a physical examination of each animal was conducted at least once weekly.

BODY WEIGHT:

The animals were individually weighed on a weekly basis. Neurotoxicity component animals were also weighed on each day of behavioural testing. Fasted body weights were recorded prior to terminal sacrifice.

FOOD CONSUMPTION:

Individual food consumption was determined weekly for all animals.

WATER CONSUMPTION:

Individual water consumption was determined daily for all animals.

OPHTHALMOSCOPIC EXAMINATION:

Funduscopy (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations were performed on all animals during the prestudy period and on the main study animals during the 13th week of treatment.

HAEMATOLOGY:

Prior to the commencement of treatment, hematological examinations were performed on

the 10 male and 10 female health screen animals. These same investigations were performed for the main study animals after 4 weeks of treatment (Day 29) on 5/sex/group (except Group 4 females), preterminals (where possible) prior to sacrifice, and after 13 weeks of treatment (Day 92) on 10/sex/group. Food was removed overnight from animals to be sampled. For all animals, blood samples were obtained from the abdominal aorta immediately following sodium pentobarbital anesthesia.

Parameters examined:

hematocrit; hemoglobin; red blood cell count; red cell distribution width; blood cell morphology; platelet count; mean platelet volume; white blood cell counts (total and differential); Wintrobe's constants (calculated); prothrombin time; activated partial thromboplastin time; reticulocyte count.

CLINICAL CHEMISTRY:

Prior to the commencement of treatment, blood chemistry examinations were performed on the 10 male and 10 female health screen animals. These same investigations were performed for the main study animals after 4 weeks of treatment (Day 29) on 5/sex/group (except Group 4 females), preterminals (where possible) prior to sacrifice, and after 13 weeks of treatment (Day 92) on 10/sex/group. Food was removed overnight from animals to be sampled. For all animals, blood samples were obtained from the abdominal aorta immediately following sodium pentobarbital anesthesia.

Parameters examined:

blood urea nitrogen; total protein; albumin; globulin; A/G ratio; alkaline phosphatase; alanine aminotransferase; aspartate aminotransferase; glucose; sodium; potassium; calcium; chloride; creatinine; total bilirubin; phosphorus.

URINALYSIS:

Prior to the commencement of treatment, urinalysis examinations were performed on the 10 male and 10 female health screen animals. These same investigations were performed for the main study animals after 4 weeks of treatment (Day 29) on 5/sex/group (except Group 4 females), preterminals (where possible) prior to sacrifice, and after 13 weeks of treatment (Day 92) on 10/sex/group. Urine samples were collected from individual animals placed in metabolism cages overnight, during which time they were deprived of food and water.

Parameters examined:

colour and appearance; pH; glucose; ketones; urobilinogen; blood; nitrite; volume; specific gravity; protein; bilirubin; microscopy of centrifuged deposit.

NEUROBEHAVIOURAL EXAMINATION:

Functional Observed Battery:

All neurotoxicity component animals (15/sex/group) were tested prior to the initiation of treatment (prestudy) and once during the 4th, 8th and 13th weeks of treatment. In addition, neurotoxicity component recovery animals (10/sex/group) were tested approximately 2 weeks after treatment cessation. Testing was performed by the same trained technicians who were unaware of the animals treatment.

Observations in Home Cage:

body position; tremors, twitches, convulsions; bizarre/stereotypic behaviour.

Removal from Home Cage:

ease of removal; vocalization.

Observations in Arena:

rearing; ataxic, hypotonic and impaired gait; overall gait incapacity; bizarre/stereotypic behaviour; palpebral closure; tremors, twitches, convulsions; piloerection; respiratory rate/pattern; locomotor activity level arousal; grooming; defecation; urination; olfactory response.

Handling Observations:

lacrimation; pupil size; salivation; urinary staining; diarrhea; body tone; extensor thrust; corneal reflex; pinna reflex; toe and tail pinch; visual placing.

On Surface:

auricular startle; air righting reflex.

On Top of Box:

positional passivity.

Grip Strength:

Calibration:

Before the start of testing and following completion of testing on each day, the Chatillon strain gauges were checked using calibration weights and the readings recorded.

Forelimb:

The dial on the gauge was set to "0". The rat was held by the body and/or tail and allowed to grip the mesh, and then was pulled slowly and steadily until it released its grip. Maximum strain was recorded two times alternating with hindlimb grip testing.

Hindlimb:

The dial on the gauge was set to "0". The rat was allowed to set its hindpaws against the mesh and was pulled backward by the base of the tail until it released its grip. The maximum strain was recorded two times alternating with forelimb grip testing.

Kindlimb splay:

Landing foot spread was measured using inking of the hind feet. Hindlimb splay was recorded twice. The ink was wiped off the feet after testing using a paper towel dampened with water.

Body temperature:

The rectal probe was gently inserted, the reading allowed to stabilize and the temperature then recorded.

The functional observational battery was performed with equipment built for this purpose. The arena was a 2' square of plexiglass placed on a raised platform. The tests were conducted in the room housing the animals, where temperature, humidity and photoperiod are monitored. Odors in the room were minimised by maintaining adequate air changes and cleaning of equipment, as necessary.

Motor Activity:

The neurotoxicity component animals (15/sex/group) were transferred (following FOB evaluations) to a testing room where activity levels were measured individually in figure 8 enclosures. Animals were tested prior to treatment (prestudy), and once during the 4th, 8th and 13th weeks of treatment. In addition, neurotoxicity component recovery animals (10/sex/group) were tested approximately 2 weeks after treatment cessation. Animals from the control and treated groups were balanced across enclosures, where possible, using a preassigned distribution. The sessions were of 1 hour duration and activity counts were recorded by a microcomputer in 6 successive 10 minute intervals.

In the testing room, temperature and humidity were monitored, and a background sound level of approximately 70 dBA and an illumination of approximately 800-1200 Lux maintained throughout testing. Light levels in the testing room were measured before the start of testing and following completion of testing on each day. The sound level was recorded on a continuous basis throughout testing on each day.

In addition to the "diagnostic" function in the system, a check of each beam was made by manually "breaking" each beam a predetermined number of times and verifying that the "breaks" were properly recorded. These checks were made at least prior to the start of testing and at the completion of testing on each day.

Sacrifice and pathology

GROSS PATHOLOGY:

Prior to commencement of treatment, the 10 male and 10 female rats designated for health screen purposes were anesthetized by intraperitoneal injection of sodium pentobarbital (35 mg/kg or as required). A blood sample was collected from the abdominal aorta after which the animal was euthanised by exsanguination from the abdominal aorta and then subjected to necropsy. Abnormal tissue samples were retained in neutral buffered 10% formalin.

In order to avoid autolytic change, a complete necropsy was conducted immediately on any animal euthanised during the study or at its conclusion (except those perfused). For animals found dead during the study, the necropsy was completed as soon as possible after death. Prior to necropsy, the carcass was stored in a refrigerator at circa 4°C.

All animals found dead or euthanised for humane reasons during the study were subjected to necropsy and tissue samples were preserved. Animals euthanised for humane reasons were killed by an intraperitoneal injection of sodium pentobarbital (35 mg/kg or as required) followed by exsanguination from the abdominal aorta.

After 4 or 13 weeks of treatment, the main study animals were anesthetized by an

intraperitoneal injection of sodium pentobarbital (35 mg/kg or as required). A blood sample was collected from the abdominal aorta. The animals were then euthanised by exsanguination from the abdominal aorta and subjected to necropsy. A similar proportion of animals from each treatment group and/or sex, as appropriate, were euthanised on any one day unless otherwise specified.

Following completion of the recovery period, all neurotoxicity component recovery animals not perfused were anesthetized by an intraperitoneal injection of sodium pentobarbital (35 mg/kg or as required). The animals were then euthanised by exsanguination from the abdominal aorta and subjected to necropsy. Abnormal tissue samples were retained in neutral buffered 10% formalin.

For each animal, necropsy consisted of an external examination, including identification of all clinically recorded lesions, as well as a detailed internal examination.

All animals were fasted before scheduled euthanasia.

ORGAN WEIGHTS:

For each main study animal euthanised after 4 or 13 weeks of treatment, the following organs were dissected free of fat and weighed:

adrenals; brain; heart; kidneys; liver; ovaries; pituitary; spleen; testes; thymus

Paired organs were weighed together. Organ weight ratios relative to body weight were calculated.

TISSUE PRESERVATION:

On completion of the necropsy of each main study animal euthanised after 4 or 13 weeks of treatment (and preterminals), the following tissues and organs were retained. Neutral buffered 10% formalin was used for fixation and preservation unless otherwise indicated.

abnormal tissues; animal identification++; adrenals; aorta (thoracic); bone and marrow (femur and sternum); brain (cerebrum, cerebellum and medulla oblongata); cecum; colon; duodenum; epididymides*; esophagus; eyes*; heart; ileum; jejunum; kidneys; larynx; liver (sample of 2 lobes); lungs (sample of 2 lobes)**; lymph nodes (cervical/mandibular and mesenteric); mammary gland (inguinal+); nasal cavity and sinuses; optic nerves*+; ovaries; pancreas; pharynx; pituitary; prostate; rectum; salivary gland; sciatic nerve; seminal vesicles; skeletal muscle; skin; spinal cord (cervical, thoracic and lumbar); spleen; stomach; testes*; thymus; thyroid lobes (and parathyroids+); tongue; trachea; urinary bladder; uterus; vagina

*Fixed in Zenker's fluid (sacrificed animals only).

** Lungs were infused with neutral buffered 10% formalin (sacrificed animals only).

+ Only examined histopathologically when present in routine sections of eyes (optic nerves), thyroid lobes (parathyroids) or mammary gland (inguinal).

++Retained but not processed.

For each sacrificed animal, three femoral bone marrow smears were prepared, one of which was stained with May-Grünwald-Giemsa. All smears were retained for possible future examination.

HISTOPATHOLOGY:

Histopathological examination was performed on the aforementioned tissues following embedding in paraffin wax, sectioning and staining with hematoxylin and eosin as follows:

- a) All animals in the control and mid dose groups.
- b) Lungs, liver, kidneys, thymus, spleen, lymph nodes, brain and spinal cord (selected regions) and any gross lesions for animals in the low dose group.

Other examinations

TISSUE PRESERVATION - NEUROTOXICITY COMPONENT

After 13 weeks of treatment and at the end of the recovery period, 5 rats/sex/group from Group 1-3 were deeply anesthetized by intraperitoneal injection of sodium pentobarbital (approximately 35 mg/kg or as required). When anesthesia was deep, the thorax was opened. A 16-gauge needle was inserted into the left ventricle and the right atrium was opened. Perfusion with lactated Ringer's solution containing heparin (1000 IU/liter) and sodium nitrite (0.02 g/L) was initiated and continued until the auricular effluent was essentially free of blood. The perfusion fluid was then changed to a mixture of 3% glutaraldehyde, 3% paraformaldehyde, 0.05% calcium chloride and 0.1% picric acid in 0.1 M cacodylate buffer (pH 7.3 to 7.5). Six males and five females from Group 4 were perfused during Week 5 or 6.

NEUROPATHOLOGY - NEUROTOXICITY COMPONENT

On completion of the perfusion, the calvarium and the dorsal vertebral column were exposed by removing the skin and underlying muscle. The skin on the lateral surface of the hindlimbs was also removed. The skin and tissues on the ventral surface of the abdomen and thorax were removed and discarded. The thoracic and abdominal organs were removed as two groups of tissues and placed in a separate tissue bag in neutral buffered 10% formalin. The remaining carcass containing the brain, spinal cord and limbs was placed in another tissue bag containing neutral buffered 10% formalin.

The following tissues listed were sampled for all animals. Tissues from the control, mid dose and high dose animals were processed for neuropathological evaluation. Tissues of brain and spinal cord from the low dose group were also processed for neuropathological examination. The remaining trimmed tissues from the low dose group were kept in neutral buffered 10% formalin. The remaining carcasses from all animals were retained. The nervous system tissues of animals were retained. The nervous system tissues of animals in all groups were grossly examined at the time of sampling and any pathology observed was recorded and reported.

TISSUES FOR PARAFFIN EMBEDDING, SECTIONING AND STAINING

Brain (6 levels) - forebrain (through the septum), center of the cerebrum (through the hypothalamus), midbrain, cerebellum and pons, midcerebellum and medulla oblongata, and

medulla oblongata.

Spinal cord - cervical, thoracic, lumbar (cross-section)

Skeletal muscle - gastrocnemius (longitudinal and cross-sections)

Grossly abnormal central nervous system tissues.

Note: Brain weight (excluding olfactory bulbs), length and maximum coronal width were recorded prior to trimming.

The tissues listed above for the control, low (limited tissues), mid and high dose animals were prepared for examination by embedding in paraffin wax and sectioning at 6 microns. The skeletal muscle was stained with hematoxylin and eosin. Adjacent sections of brain and spinal cord were stained with hematoxylin and eosin, Klüver Barrera, Holmes and PTAH (or alternate) stains and then examined by light microscopy.

TISSUES FOR EPOXY EMBEDDING, SECTIONING AND STAINING

Peripheral Nervous System (pNs):

Sciatic nerve (mid-thigh region) (cross-section),

Sciatic nerve (at sciatic notch) (longitudinal and cross-sections) ,

Sural nerve (at knee) (cross-section),

Tibial nerve (at knee) (longitudinal and cross-sections).

Central Nervous System (CNS):

Gasserian ganglion - left (cross-section),

Lumbar dorsal root ganglion (L4) (cross-section),

Lumbar dorsal root (L4) (cross-section),

Lumbar ventral root (L4) (longitudinal and cross-sections),

Cervical dorsal root ganglion (C5) (cross-section),

Cervical dorsal root (C5) (cross-section),

Cervical ventral root (C5) (cross-section).

Grossly abnormal central or peripheral nervous system tissues.

For the control, mid dose and high dose group animals, the tissues listed above were rinsed in 0.1 M sodium cacodylate buffer and placed in 2% osmium tetroxide for 2 hours. Each piece of tissue was then rinsed in buffer and stained in a 1% aqueous solution of uranyl acetate for 2 hours. The tissues were then rinsed in distilled water, dehydrated in ascending concentrations of ethyl alcohol and embedded in a mixture of Jembed and Araldite. Epoxy sections (0.5 µm) were obtained with a glass knife, stained with borate-buffered 1% toluidine blue, cover slipped and examined by light microscopy.

The tibial branches to the calf musculature were retained for possible future examination.

Statistics:

See 'Any other information on materials and methods incl. tables'.

Results and discussions

NOAEL (male) < 1.6 other: mg/kg bw/day based on test mat. Basis for effect level /

Remarks: No mortality and reduced food (males only) and water intake and neuropathological lesions. Vacuolisation of white matter in the brain and spinal cord was also observed for animals in the 25 ppm group.

NOAEL (female) < 2.2 other: mg/kg bw/day based on test mat. Basis for effect level /

Remarks: No mortality and reduced food (males only) and water intake and neuropathological lesions. Vacuolisation of white matter in the brain and spinal cord was also observed for animals in the 25 ppm group.

Results of examinations

Clinical signs and mortality: yes.

Body weight and weight gain: yes.

Food consumption and compound intake (if feeding study): yes not a feeding study.

Food efficiency: not examined.

Water consumption and compound intake (if drinking water study): yes.

Ophthalmoscopic examination: no effects.

Haematology: no effects.

Clinical chemistry: yes.

Urinalysis: yes.

Neurobehaviour: yes.

Organ weights: yes.

Gross pathology: yes.

Histopathology (non-neoplastic): yes.

Histopathology (neoplastic): not examined.

Details on results

CLINICAL SIGNS AND MORTALITY:

A number of 200 ppm animals (7 males and 21 females) either died or were sacrificed (due to poor condition) between Days 18 and 34 of the treatment period. The remaining 200 ppm animals were sacrificed by Day 36 of the study as their survival to study completion was doubtful. One 75 ppm male (No. 3025) was found dead on Day 41. No other deaths occurred.

Clear treatment-related clinical signs were noted for the 200 ppm group. Findings for animals found dead and sacrificed were tremors, convulsions, aggression/hypersensitivity/difficulty (when handled), appearance of dehydration/thin/weak, cold to touch, lying on side, and decreased home-cage activity levels. An increased incidence of fur staining at the head, body and limbs was also noted. Few abnormal clinical signs were noted for the 75 ppm group. Findings included tremors, hypersensitivity (difficulty when handled) and a thin dehydrated body condition for the male that died, a thin and dehydrated appearance at the beginning of the treatment period for another male and abnormal gait/behaviour at the beginning of the treatment period for a third male. One female from this group had findings including hypersensitivity, convulsions and reduced activity towards the end of treatment period. No abnormal clinical signs were clearly evident for the 25 ppm group.

BODY WEIGHT AND WEIGHT GAIN

The body weights of the 200 ppm group (males and females) were significantly ($P < 0.05$ or $P < 0.01$) lower than the control group for all intervals measured following treatment initiation. For the last 2 or 3 body weight occasions prior to termination of this group, these animals showed a weight loss with several animals having large body weight reductions. Body weights were significantly ($P < 0.05$ or $P < 0.01$) reduced for 75 ppm males on most occasions when compared to the control group. No significant differences were noted between the control and 75 ppm females or 25 ppm group.

FOOD CONSUMPTION

Food intake values were significantly ($P < 0.01$ or $P < 0.001$) lower for the 200 ppm group (males and females) during all intervals measured after treatment start. Food consumption values for the 75 ppm males were also significantly ($P < 0.05$ or $P < 0.01$) reduced on most occasions when compared to the control group. Occasional significant ($P < 0.05$ or $P < 0.01$) reductions in food consumption were noted for the 75 ppm females and 25 ppm males when compared to the control group. No significant differences were noted between the control and 25 ppm females.

WATER CONSUMPTION

Water consumption was significantly ($P < 0.05$, $P < 0.01$ or $P < 0.001$) decreased for males and females in all treated groups on most daily assessments during the study. Following treatment termination, water consumption values were generally comparable between the control and treated groups.

OPHTHALMOSCOPIC EXAMINATION

There were no ocular changes attributed to treatment with the test material.

HAEMATOLOGY

No significant differences were detected between the control and treated groups for hematological parameters.

CLINICAL CHEMISTRY

On Day 29, males in the 200 ppm group showed significant ($P < 0.05$ or $P < 0.01$) increases in BUN, creatinine and phosphorus. Potassium levels were significantly ($P < 0.01$) decreased for

200 ppm males. In addition, slight increases in the mean AST and ALT levels were observed for 200 ppm males. Females in the 75 ppm group showed a significant ($P<0.05$) decrease in total protein, and a decrease in albumin was observed for the 25 and 75 ppm group females. This latter finding (decreased total protein/albumin) was most probably attributable to intergroup variation as the values were comparable to those collected for health screen animals prior to treatment initiation and/or they were within historical control ranges. Many of the 200 ppm animals that were sacrificed preterminally showed marked changes in various blood biochemical parameters including increases in BUN, creatinine, AST, ALT and phosphorus. At the Day 92 assessment, the biochemical parameters were essentially the same between the control and treated groups.

URINALYSIS

Urinalysis data was generally comparable between the control and treated groups for the Day 29 and 92 assessments. However, males in the 200 ppm group did show an elevated urine pH at the Day 29 assessment.

NEUROBEHAVIOUR

Functional Observational Battery (FOB):

Males:

At the Week 4 assessment, one 200 ppm male showed slight ataxia resulting in a slight overall gait incapacity, slight tremors at the limbs and clonus of the jaws (chomping). One 75 ppm male had a severe ataxia including falling over which resulted in a severe overall incapacity. This animal also showed bizarre behaviour in the form of unusual hindlimb movements. Several 200 ppm males did show a rapid response to the toe pinch test.

At the Week 8 assessment, the same 75 ppm male was observed with a swaying movement in the home cage and continued to exhibit ataxic gait. The number of fecal boli (defecation) was significantly ($P<0.05$) increased for the 75 ppm group but the interpretation of this finding is unclear.

At the Week 13 assessment, the same 75 ppm male showed side to side body shaking together with the ataxia noted earlier. Defecation was again significantly ($P<0.01$) increased for the 75 ppm group. At the recovery assessment, this animal continued to show the same findings.

A significantly ($P<0.01$) easier removal from the home cage for the 25 ppm males at the prestudy assessment was due to intergroup variation.

Females:

At the Week 4 assessment, three females in the 200 ppm group showed slight or moderate tremors at the limbs or head. Clonic convulsions were also observed for these animals. Two animals had a hunched posture and one of these animals, a severe overall gait incapacity attributed to a severe ataxia was noted as was red liquid material at the urogenital region. The incidence of a rearing body position in the home cage as well as the average number of rearing incidents in the arena were significantly ($P<0.01$) reduced for the 200 ppm group. In addition, hindlimb grip strength was significantly ($P<0.05$) decreased and body temperature

was significantly ($P<0.01$) reduced for the 200 ppm females. The 75 ppm group showed a significantly ($P<0.05$) lower body temperature.

At the Week 8 assessment, 75 ppm females showed a significant ($P<0.01$) decrease in rearing incidents in the arena.

At the Week 13 assessment, 75 ppm females showed a significantly ($P<0.001$) lower number of rearing incidents in the arena. The body temperature was also significantly ($P<0.01$) reduced for 75 ppm females. Defecation was significantly ($P<0.01$) increased for 75 ppm females.

At the recovery assessment, the number of rearing incidents in the arena was significantly ($P<0.05$) reduced for the 75 ppm group.

Motor Activity:

No significant differences were detected between the control and treated males on any occasion. Females in the 75 ppm group showed significant ($P<0.01$ or $P<0.001$) reductions in total activity counts at the Week 4, 8 and 13 assessments. In addition, at the Week 13 assessment, the linear constructed variable was also significantly ($P<0.01$) different between the control and 75 ppm group.

ORGAN WEIGHTS

Non-Perfused Animals:

Several statistically significant ($P<0.05$ or $P<0.01$) differences in absolute weights were noted for various organs between control and treated groups. At the interim sacrifice, 200 ppm males showed decreases in spleen, heart, pituitary and thymus weights while 75 ppm females had reduced heart weight, and 25 ppm females had reduced heart and liver weights. At the terminal evaluation, 75 ppm males showed decreases for heart and thymus weights, and the kidney weights were increased for the 25 and 75 ppm females. At the recovery period, 25 ppm males showed an increase in heart weight.

Most of these differences were no longer statistically significant once the organ weights were expressed relative to body weight. When this was done, however, the thymus weight continued to be reduced for 200 ppm males at the interim sacrifice and 75 ppm males at the terminal sacrifice, 25 and 75 ppm females continued to have increased kidney weights at the terminal evaluation, and the 25 ppm males had increased heart weight at the recovery assessment. In addition, the relative (to body weight) kidney, brain and adrenals weights of the 200 ppm males and spleen weight of 25 ppm males were increased at the interim evaluation, as was the relative kidney weight of the 75 ppm males and the relative heart weight of the 25 ppm males at the recovery evaluation.

Perfused Animals:

The brain weight of the 75 ppm males was significantly ($P<0.05$) increased at the terminal evaluation. The significance of this finding is unclear as no significant difference was detected for the 75 ppm females, and the brain weight of the 75 ppm males at the end of the recovery period was comparable to that of the control group.

GROSS PATHOLOGY**Non-Perfused Animals:**

Preterminal animals in the 200 ppm group showed various findings. Several animals had a small thymus and/or spleen, fluid was noted in the brain on occasion, and other findings included emaciated carcass, dilatation of stomach/intestine, discolored digestive material, dark/depressed areas in the stomach, and dark areas of the lungs.

Perfused Animals:

Dilatation of the brain ventricles was noted for some preterminal animals in the 200 ppm group. At the terminal evaluation, brain ventricular dilatation was seen for one male in each of the 25 and 75 ppm groups and for 2 females in the 75 ppm group and 1 control group female

HISTOPATHOLOGY: NON-NEOPLASTIC**Non-Perfused Animals:**

At the interim evaluation, no obvious treatment-related histopathological findings were noted. Terminal evaluations indicated clear changes in brain tissue characterised by neuronal necrosis and vacuolization of white matter, together with possible treatment-related lymphoid atrophy of the thymus, for animals in the 75 ppm group. Vacuolization was also noted in brain tissue for 25 ppm animals.

Perfused Animals:

Preterminal 200 ppm animals showed treatment-related lesions in the brain and spinal cord in the form of neuronal necrosis and vacuolization of white matter. Axonal degeneration of spinal cord tissue was also seen as was degeneration of peripheral nerves but the significance of this latter finding is unclear. At the terminal evaluation, neuronal necrosis and vacuolization of white matter in brain and spinal cord tissue were seen for animals in the 75 ppm group. Vacuolization of white matter in the brain and spinal cord was also observed for animals in the 25 ppm group.

OTHER:

The range (average) of achieved intakes for the males were 1.2 to 2.2 (1.6), 4.6 to 6.2 (5.2) and 13.1 to 17.4 (15.5) mg/kg/day for the 25, 75 and 200 ppm groups, respectively. The range (average) achieved intakes for the females were 1.9 to 2.8 (2.2), 6.3 to 7.1 (6.7) and 17.4 to 20.5 (19.4) mg/kg/day for the 25, 75 and 200 ppm groups, respectively.

Reliability score 1 (reliable without restriction) was given by the registrant because the study was conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of the relevant results.

[Study 2]**Study reference:**

Elf Atochem NA (1996).

Materials and methods

Test type: subchronic.

Test guideline: according to OECD TG 408 (Repeated Dose 90-Day Oral Toxicity in Rodents).

Deviations: yes the part on neurobehavioural testing and neuropathology, which had been drafted according to OECD Guideline for the Testing of Chemicals 424 (adopted 21 July 1997).

Some deviations from the study protocol occurred. None of these were felt to affect the validity of the study.

- All animals were weighed twice instead of once prior to the start of the study;
- Male animals were housed in macrolon cages type 4 and females animals were housed in macrolon cages type 3.5, instead of in macrolon cages type 3;
- The spinal ganglion L4 and Gasserian ganglion were dissected completely, instead of in parts and the Gasserian ganglion was dissected longitudinally instead of transversally;
- M.H.K. Bos-Kuijpers, DVM, PhD and M.V.W. Wijnands, DVM were responsible for pathology instead of R.A. Woutersen PhD;
- The relative humidity in the animal room was generally higher than 70% for daily periods of 2 h to 24 h, throughout the study;
- Erroneously, the animals of the satellite 15 ppm dose-group and the satellite controls were also ophthalmoscopically examined towards the end of the study;
- The fixed tissues obtained from the animals from the satellite 200 ppm dose group (group E) were stored at 2-10°C in monthly-refreshed phosphate-buffer (pH 7.2-7.4; containing 0.02% sodium azide) prior to processing for microscopy;
- Instead of ranking the morphological status of the cerebrum at each of the 4 protocol-levels separately, i.e. 1) telencephalon, 2) di-encephalon, 3) transition di- and mesencephalon and 4) mesencephalon) the results of the 4 levels were taken together and presented in the tables and appendices as 'cerebrum' to increase the precision of the ranking system on localisation, intensity and severity of the brain lesions;
- The dorsal and ventral roots at L4 were dissected, embedded and examined microscopically as indicated in the protocol. If present, the roots included in the spinal cord sections (at lumbar, thoracic and cervical levels) were examined microscopically as well. Primarily, the results of the roots attached to the lumbar spinal cord were used to complete the neuropathology data, if necessary. If not present in the section, the results of the roots attached to the thoracic or cervical spinal cord were used;
- One animal of the high-dose (200 ppm) group (no. E141) was found dead on day 27. Protocol tissues of this animal were fixed by immersion-fixation and were embedded. Sections were cut of the blocs. However, the results of the microscopical examination are not included in the report. They are, however, kept as raw data;
- In addition to the protocol, clinical signs were observed for the animals of the neuropathology subgroups, prior to perfusion fixation;
- Measurements or microscopic examinations could not always be performed on all animals specified in the protocol; the reasons are given in the Appendices.

— The Department of Pesticides and Industrial Toxicology was renamed Department of Residue Analysis.

GLP compliance: yes (incl. certificate).

Test materials

Test material form: crystalline.

Details on test material:

- Name of test material: MMTTC/DMDTC (30/70)
- Storage condition of test material: 2-10°C

Test animals

Species: rat.

Strain: Wistar.

Sex: male/female.

Details on test animals and environmental conditions:

TEST ANIMALS

- Source: Charles River Deutschland, Sulzfeld, Germany
- Age at study initiation: approximately 6 weeks old; pre-test neurobehavioural testing was to be conducted on animals of 5 weeks of age.
- Weight at study initiation: 149.8 g to 191.5 g (mean 174.3 g) for males and from 123.4 g to 154.2 g (mean 137.7 g) for females.
- Housing: under conventional conditions in one room, in macrolon cages, type 3.5 (males) or type 4 (females) with sterilized wood shavings (Woody Clean, Type 3/4) as bedding material, five (main groups) or three (satellite groups) rats per cage, separated by sex. In the last week of the treatment period, the rats of the main groups were placed in stainless-steel metabolism cages (one rat per cage) for 16 hours to collect urine.
- Diet (e.g. ad libitum): Rat & Mouse No. 3 Breeding Diet, RM3 ad libitum.
- Water (e.g. ad libitum): tap water, ad libitum.
- Acclimation period: 12 days.

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 22 ± 3°C.
- Humidity (%): At least 30%. The relative humidity exceeded 70% on most days of the study during 2 to 24 hours per day, to a maximum of 95%.
- Air changes (per hr): 10.
- Photoperiod (hrs dark / hrs light): 12 hours light and 12 hours darkness.

IN-LIFE DATES: From: 7th June 1999 To: 20th September 1999.

Administration / exposure

Route of administration: oral (feed).

Vehicle: unchanged (no vehicle).

Details on oral exposure:

DIET PREPARATION:

The test substance was incorporated in the basal diet by mixing in a mechanical blender. Four batches of test diets were prepared, viz. on 31 May, 28 June, 20 July and 20 August 1999. A 10,000 ppm premix was prepared first. The high-dose (200 ppm) diet was prepared by diluting the premix with the appropriate amount of RM3 diet. Subsequently, the 6 and 15 ppm diets were prepared by diluting the high-dose diet with the appropriate amount of RM3 diet. The 1 ppm diet was prepared by diluting the 15 ppm diet with the appropriate amount of RM3 diet. The controls received the unsupplemented RM3 diet. Directly after preparation the diets were stored in a freezer (<-18°C) until use.

Analytical verification of doses or concentrations: yes.

Details on analytical verification of doses or concentrations:

The diets were extracted with a methanol/water solution. The organic tin chlorides were ethylated with sodium tetraethyl borate (NaBEt₄). The methyl ethyl tin compounds were subsequently extracted with hexane. The concentrations of MMTTC and DMDTC were determined using a Carlo Erba/Fisons QMD-1000 GC/MS under the following conditions:

Column: fused silica 60 m x 0.32 mm; 0.25 µm DB-1.

Column temperature: After 3 minutes at 50°C at a rate of 5°/min. to 140°C; then at a rate of 10°/min. to 260°C; 10 minutes at 260°C.

Carrier: Helium (70 kPa).

Injection volume: 1 µl.

Injection temperature: 250°C.

Injection method: splitless .

Ionisation: Electron Impact, 70 eV.

Mass range: 100 - 250 amu.

Mass fragments used DMTDC low concentrations: m/z = 179.

for quantification: DMTDC high concentrations: m/z = 193.

MMTTC low concentrations: m/z = 193.

MMTTC high concentrations: m/z = 205.

TMTMC: m/z = 165.

Internal standard (naphthalene) m/z = 102.

Homogeneous distribution of the test substance in rat feed was demonstrated by using the same diet preparation protocol and concentrations that were used in the present study. Moreover, the stability of the test substance in rat feed under simulated experimental conditions (7 days at room temperature in an open container) and after 5 weeks in a freezer was demonstrated.

Duration of treatment / exposure: 90 days.

Frequency of treatment: Daily.

Doses/concentrations:

1, 6, 15 and 200 ppm (nominal in diet)

No. of animals per sex per dose:

10 per sex per dose group (main study),

6 per sex per dose group (satellite groups).

Control animals: yes, plain diet.

Details on study design:

The study comprised five main groups of 10 males and 10 females each and five satellite groups (neuropathology study) of 6 males and 6 females each. One control group receiving unsupplemented basal diet and four test groups receiving diets containing different levels of the test substance for 13 weeks. These groups are intended to provide information on the subchronic oral toxicity of the test substance and to establish a no-observed-adverse-effect level (NOAEL).

Examinations

CLINICAL OBSERVATIONS:

Each animal was observed daily in the morning hours by cage-side observations and, if necessary, handled to detect signs of toxicity. On working days, all cages were checked again in the afternoon for dead or moribund animals to minimise loss of animals from the study. On Saturdays, Sundays and public holidays only one check per day was carried out. All abnormalities, signs of ill health or reactions to treatment were recorded. Any animal showing signs of severe debility or intoxication, particularly if death appeared imminent, was killed by exsanguination or by whole body perfusion fixation (satellite animals used for neuropathology), under ether anaesthesia to prevent loss of tissues by cannibalism or autolytic degeneration.

BODY WEIGHT:

The body weight of each animal was recorded twice during the acclimatization period, at initiation of treatment and once weekly thereafter. In addition, the animals were weighed on the day of scheduled necropsy (terminal body weight, for calculation of the organ to body weight ratios).

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):

Food consumption was measured per cage over weekly periods by weighing the feeders. The results were expressed in g per animal per day.

The intake of the test substance per kg body weight per day was calculated from the nominal concentration of the test substance in the diet, the food consumption and the body weight (mean of the body weights measured at the start and at the end of each week).

FOOD EFFICIENCY:

The efficiency of food utilization was calculated and expressed in g weight gain per g food consumed.

WATER CONSUMPTION:

Water intake was measured per cage by weighing the bottles. The results were expressed in g per animal per day. The consumption was measured daily during four days in week 1, 6 and 12 for rats of the main groups only.

OPHTHALMOSCOPIC EXAMINATION:

Ophthalmoscopic observations were made prior to the start of the study in all rats of the main groups and towards the end of the treatment period in all rats of the main and satellite control group and the main and satellite 15 ppm dose-group. Eye examinations were carried out using an ophthalmoscope after induction of mydriasis by a solution of atropine sulphate.

HAEMATOLOGY:

At necropsy at the end of treatment, blood samples were taken from the abdominal aorta of all surviving rats of the main groups whilst under ether anaesthesia. K2-EDTA was used as anticoagulant.

The following parameters were examined: haemoglobin, packed cell volume, red blood cell count, reticulocytes, total white blood cell count, differential white blood cell count, prothrombin time and thrombocyte count.

The following parameters were calculated: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

CLINICAL CHEMISTRY:

Clinical chemistry determinations were made on all surviving rats of the main groups. Fasting glucose was determined shortly before the end of the treatment period (on nominal day 88) in blood collected from the tip of the tail after deprivation of food for 16 hours. The collection of fasting blood was combined with the collection of urine (see next section). At necropsy at the end of treatment, blood was collected from the abdominal aorta in heparinized plastic tubes and plasma was prepared by centrifugation.

The following measurements were made in the plasma: alkaline phosphatase activity (ALP), aspartate aminotransferase activity (ASAT), alanine aminotransferase activity (ALAT), gamma glutamyl transferase activity (GGT), total protein, albumin, albumin/globulin ratio, urea, creatinine, bilirubin (total), cholesterol (total), triglycerides, phospholipids, calcium (Ca), sodium (Na), potassium (K), chloride (Cl) and inorganic phosphate.

URINALYSIS:

In the urine samples collected from the rats in the renal concentration test, the following determinations were carried out in individual samples: appearance, glucose, pH, occult blood, ketones, protein, bilirubin, urobilinogen and microscopy of the sediment.

RENAL CONCENTRATION TEST:

On nominal days 87 and 88, all surviving rats of the main groups were deprived of water for 24 hours and of food during the last 16 hours of this period. During the last 16 hours of deprivation, the rats were kept in metabolism cages (one rat per cage) and urine was collected. The concentrating ability of the kidneys was investigated by measuring the urinary volume and density of the individual samples.

NEUROBEHAVIOURAL EXAMINATION:

Before the start of the study and in weeks 1, 4, 8 and 13, spontaneous motor activity measurements and Functional Observational Battery (FOB) tests were performed in four animals of both sexes of each main group (two animals with the lowest identification numbers from each cage) and in all animals of each satellite group.

Sacrifice and pathology**GROSS PATHOLOGY:**

Based on the severe clinical signs and mortality observed in the high-dose (200 ppm) group, it was decided to sacrifice the main and satellite high-dose groups after approximately one month of treatment. The surviving animals of the main and satellite high-dose groups were considered moribund and were killed by exsanguination from the abdominal aorta under ether anaesthesia or by whole body perfusion fixation, under ether anaesthesia, respectively.

After completion of the 13 week treatment period, all surviving animals of the main groups were killed in such a sequence that the average time of killing was approximately the same for each group. The animals were killed by exsanguination from the abdominal aorta under ether anaesthesia and then examined macroscopically for pathological changes. A thorough autopsy was also performed on all animals that died intercurrently or that had to be killed because they were moribund.

The following organs of all surviving animals of the main groups were weighed: adrenals, ovaries, brain, spleen, epididymides, testes, heart, thymus, kidneys, thyroid (with parathyroids), liver and uterus.

Samples of the following tissues and organs of all animals of the main groups were preserved in a neutral aqueous phosphate-buffered 4 per cent solution of formaldehyde (10% solution of formalin): adrenals, aorta, * axillary lymph nodes, brain (brain stem, cerebrum and cerebellum), caecum, colon, epididymides, * eyes, * exorbital lachrymal glands, * femur with joint, heart, kidneys, liver, lungs, mammary glands (females), *mandibular (cervical) lymph nodes, mesenteric lymph nodes, nerve-peripheral (sciatic nerve), oesophagus, ovaries, pancreas, parathyroids, parotid salivary glands, pituitary, prostate, rectum, * seminal vesicles with *coagulating glands, * skeletal muscle (thigh), skin (flank), small intestines (duodenum, ileum, jejunum), spinal cord (three levels), spleen, sternum with bone marrow, stomach (glandular, non-glandular), sublingual salivary glands, submaxillary salivary glands, testes, thymus, thyroid, trachea/bronchii, urinary bladder, uterus (with cervix), * vagina and all gross lesions.

* The tissues marked with an asterisk were preserved but not processed for histopathological examination, unless histopathological examination was considered necessary on the basis of the results of gross observations.

HISTOPATHOLOGY:

The tissues required for microscopic examination were embedded in paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin. Histopathological examination was performed on all tissues and organs listed above of all animals of the main control group and the main high-dose (200 ppm) group and of all animals that died during the study. Based on the clinical findings in the high-dose animals, the premature termination of the high-dose group and after consultation with the sponsor, it was decided to extend the histopathology to the main 15 ppm dose-group. Gross lesions were examined microscopically in all rats of all main groups.

Other examinations

NEUROPATHOLOGY:

The animals of the satellite high-dose (200 ppm) group were sacrificed on 7, 8 and 9 July 1999 by whole body perfusion fixation, under ether anaesthesia. Based on the neurotoxic findings in the high-dose animals, the premature termination of the high-dose group and after consultation with the sponsor, it was decided to extend the neuropathology to the satellite 15 ppm dose-group.

The surviving animals of the satellite groups were killed on 13 to 17 September 1999. They were subjected to whole body perfusion fixation, under ether anaesthesia, with buffered glutaraldehyde/formaldehyde mixture (pH 7.2 - 7.4; 12 minutes; pressure: 130 mm Hg), preceded by saline solution (approximately 30 seconds; pressure: 130 mm Hg). This was done in such a sequence that the average time of killing was approximately the same for each group. After perfusion, relevant tissues were taken from the skull (the brain), the spinal column (spinal cord with roots and spinal ganglia) and the left hind limb (the sciatic nerve, the tibial nerve, the sural nerve and the plantar nerve). These tissues were stored overnight in the fixative. The remaining tissues and the intact right hind limb were also stored in the fixative. Subsequently, the tissue were rinsed in phosphate-buffer (pH 7.2-7.4) for a period of at least 48 hours at 2-10°C.

For embedding in paraffin, the following tissues from the satellite control and the 15 and 200 ppm groups were further dissected:

- transversal slabs from the cerebrum:
- telencephalon
- diencephalon
- transition zone diencephalon/mesencephalon
- transversal slab from mesencephalon (pons cerebri)
- transversal slab from cerebellum and medulla oblongata
- transversal slab from medulla oblongata

These tissues were dehydrated in ethanol and embedded in paraffin. Sections (2-5 µm thick) were cut from these blocks and sections were stained with either haematoxylin and eosin

(H&E). Sections were examined by light microscopy.

For embedding in epoxy resin, the following tissues from the satellite control and the 15 and 200 ppm groups were further dissected:

- transversal slabs from spinal cord:
- the cervical spinal cord
- the thoracic spinal cord
- the lumbar spinal cord
- the dorsal spinal nerve root at the level of L4
- the ventral spinal nerve root at the level of L4
- the spinal ganglion at the level of L4
- Gasserian ganglion

- transversal slabs from nerve tissue from left hind limb:
- the sciatic nerve
- the tibial nerve
- the sural nerve
- the plantar nerve

These tissues were post-fixed with osmiumtetroxide and were dehydrated in a series of increasing ethanol solutions, followed by propylene oxide before embedding in epoxy resin.

Semi-thin sections were cut from these blocks. The semi-thin sections were stained with toluidine blue and were examined light-microscopically. All epoxy-resin embedded tissues of the perfusion-fixed animals of the satellite control group and the satellite 15 and 200 ppm groups were examined.

The fixed tissues obtained from the animals from the satellite 200 ppm dose group were stored at 2-10°C in monthly-refreshed phosphate-buffer (pH 7.2- 7.4; containing 0.02% sodium azide) prior to processing for microscopy. The fixed tissues obtained from the animals from the satellite 1 and 6 ppm dose groups were stored at 2-10°C in monthly-refreshed phosphate-buffer (pH 7.2-7.4; containing 0.02% sodium azide; awaiting possible processing for neuropathology) and were archived in buffered formalin after completion of the study. During storage, the fixed tissues in phosphate-buffer were visually inspected weekly for contamination.

Statistics:

The statistical procedures used in the evaluation of data were as follows:

- body weight: one-way analysis of covariance (covariate: body weight on day 0) followed by Dunnett's multiple comparison tests;
- water intake: one-way analysis of variance (Anova) followed by least significant difference tests;
- food consumption/efficiency, red blood cell and coagulation variables, total white blood cell counts, absolute differential white blood cell counts, clinical chemistry values, urinary volume and density, and organ weights: one-way Anova followed by Dunnett's multiple comparison

tests;

- relative differential white blood cell counts, and semi-quantitative urinary determinations: Kruskal-Wallis non-parametric Anova followed by Mann-Whitney U-tests;
- histopathological changes: Fisher's exact probability test.

Probability values of $p < 0.05$ were considered significant.

Results and discussions

NOAEL (males) 0.98 mg/kg bw/day (actual dose received) based on test mat. Basis for effect level / Remarks: Neurological effects.

NOAEL (females) 1.02 mg/kg bw/day (actual dose received) based on test mat. Basis for effect level / Remarks: Neurological effects.

Results of examinations

Clinical signs and mortality: yes.

Body weight and weight gain: yes.

Food consumption and compound intake (if feeding study): yes.

Food efficiency: yes.

Water consumption and compound intake (if drinking water study): no effects.

Ophthalmoscopic examination: no effects.

Haematology: no effects.

Clinical chemistry: no effects.

Urinalysis: no effects.

Neurobehaviour: yes.

Organ weights: no effects.

Gross pathology: yes.

Histopathology (non-neoplastic): yes.

Histopathology (neoplastic): not examined.

Details on results:

CLINICAL SIGNS AND MORTALITY:

Three females of the high-dose (200 ppm) group died towards the end of the first month of treatment. Almost simultaneously, most of the females and a number of males of the high-dose group started to show severe neurological signs, including tremors and convulsions.

Based on the death of three females and the severe nature of the clinical signs, all animals of the high-dose group were considered moribund and killed for humane reasons.

The daily observations did not reveal treatment-related clinical signs among the animals of the other groups. The few signs that were observed in these animals are common findings in Wistar rats and were randomly distributed among the remaining groups.

BODY WEIGHT AND WEIGHT GAIN:

Generally, from day 7 to day 28 the body weight of the animals of the high-dose (200 ppm) group was lower than that of the controls, except on day 14 for male rats. On days 7 and 28 the difference reached the level of statistical significance for male rats.

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):

Food consumption was statistically significantly decreased in high-dose (200 ppm) animals on day 7 (males and females) and increased on day 28 (females). Food consumption was similar in the other groups throughout the study.

Due to the well-known decrease in food consumption per kg body weight with increasing age of rats, the intake of test substance per kg body weight decreased in the course of the study. The mean intakes of MMTTC/DMTDC in the groups receiving 1, 6, 15 or 200 mg per kg diet 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, respectively.

FOOD EFFICIENCY:

Food conversion efficiency values tended to be decreased in high-dose animals on days 21 (males) and 28 (males and females). This decrease was statistically significant in high-dose males on day 21. Food conversion efficiency values were similar in the other groups throughout the study.

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study):

Water consumption was generally similar among the groups throughout the study. The water consumption was statistically significantly decreased in females dosed with 6 ppm or with 200 ppm on day 1 only.

NEUROBEHAVIOUR:

In the first week of administration of the test substance, no effects of treatment were observed. In week 4 of treatment, increased activity was observed in females of the high-dose (200 ppm) group, together with increased rearing in the open field. Also convulsive activity was observed in a number of females from this group. These effects were not observed in males at this test time point. In males, increased landing footsplay was measured in the 200 ppm group. These effects were considered treatment-related and toxicologically relevant. Shortly after week 4, the high-dose (200 ppm) animals were excluded from the study, so no behavioural observations were recorded at later test time points for this group.

In weeks 8 and 13, no toxicologically relevant effects were observed.

OPHTHALMOSCOPIC EXAMINATION:

The ophthalmoscopic examination of rats of the 15 ppm dose-group and the controls in week 13 of the study did not reveal any treatment-related ocular changes.

HAEMATOLOGY:

No statistically significant changes in red blood cell and coagulation variables or total and differential white blood cell counts were observed.

CLINICAL CHEMISTRY:

Clinical chemistry values showed statistically significantly increased alanine amino transferase and aspartate amino transferase activities in males of the 1 ppm dose-group. In the absence of similar changes at higher dose -levels, the increases at the 1 ppm level are considered fortuitous findings.

No other statistically significant changes in clinical chemistry values were observed between treated rats and controls.

URINALYSIS AND RENAL CONCENTRATION:

The urinary density was statistically significantly increased in females of the 6 ppm dose-group. In the absence of a similar change at the next higher dose-level, this is considered a fortuitous finding. No other statistically significant changes were observed in urinary volume or density, semi-quantitative urinary observations or microscopy of the sediment.

GROSS PATHOLOGY:

Some high-dose animals demonstrated gross skin changes, which, at least in part, were probably related to treatment with the test material. Apart from that, no treatment-related gross changes were observed at autopsy. No treatment-related changes were observed in absolute or relative organ weights.

HISTOPATHOLOGY (NON-NEOPLASTIC):

At microscopic examination treatment-related histopathological changes were observed in the brain, the kidneys and the thymus of the animals treated with 200 ppm of the test substance. The histopathological changes in the brain comprised cell death, especially in the Ammon's horn, the dentate gyrus and the piriform cortex, and submeningeal oedema. In females, the extensiveness of the cell death was slightly higher than in males. The kidneys demonstrated treatment-related tubular dilatation in the cortical area. The tubules involved were the last parts of the distal tubules and the first part of the collecting tubules. In general, the tubular dilatation was not accompanied by degenerative or inflammatory changes. In one control and some treated females very slight to slight nephrocalcinosis in the kidneys was found. Nephrocalcinosis is a common finding in female rats. The distribution of 1 in the control group, 0 in the 1 ppm group, 4 in the 15 ppm group and 4 in the 200 ppm group was not considered to be an indication of a relationship with treatment but a fortuitous finding. Two control and six 15 ppm females showed minimal mineralisation in the ovaries. The difference in incidence in these groups was not statistically significant. Mineralisation in ovaries of 200 ppm females was not observed. However, those animals were exposed to the test substance for only one month. Speculations with respect to a possible incidence of this change after 3 months exposure cannot be founded. Therefore, the mineralisation in the

ovaries was not considered to be treatment-related. In females treated with 200 ppm an increased incidence of corticomedullary haemorrhage in the thymus was observed. Most of the high-dose females also showed cortical lymphoid depletion in the thymus. This was not considered a direct toxic effect of the test substance, but an indirect effect caused by stress, due to the severe effects of the test material. All females and males treated with 200 ppm showed decreased accumulation of brown pigment in the spleen. Accumulation of brown pigment is a normal phenomenon in rats, which gradually increases with age. The 200 ppm animals were killed at an early stage of the study, at which they had not yet accumulated pigment in the spleen. Therefore, the decreased splenic pigment was not an effect of the treatment, but was the consequence of the difference in age between these animals and those of the other treatment groups and the controls, that were killed two months later. The aforementioned treatment-related histopathological changes were not observed in dose-group treated with 15 ppm. It was concluded that, based on the microscopic examination, 15 ppm was the no observed adverse effect level under the given conditions. Therefore, the animals of the lower dose groups were not examined microscopically.

NEUROPATHOLOGY:

Clinical signs:

One animal of the high-dose (200 ppm) subgroup for neuropathology was found dead on day 27. The other animals of this high-dose subgroup were killed moribund on day 31.

Convulsions, blepharospasm and tremors were observed in these animals prior to whole body perfusion fixation. One animal showed hypothermia and four animals showed a hunched posture.

Inspection of the remaining high-dose animals, the control animals and animals of the 15 ppm subgroup, assigned for neuropathological examination did not reveal treatment-related changes prior to fixation.

Macroscopic observations:

No treatment-related gross changes were observed at necropsy.

Microscopic observations:

Microscopic examination of the cerebrum of the rats of the high dose (200 ppm) group showed pronounced neuronal death in a number of areas. This effect was most pronounced in female rats. Predominantly affected in both sexes were the hippocampal pyramidal neurons in the CA2/CA1 area and the CA4 neurons, embraced by the polymorphous layer of the dentate gyrus. Strikingly, neuronal death was not observed in the hippocampal CA3 area. The dying neurons typically showed karyorhexis and karyolysis and intense eosinophilic cytoplasm. Little or no glial reaction was observed. Occasionally, the surrounding neuropil showed very slight edema. Also the granular neurons of the dentate gyrus were locally affected (mainly in female rats), although the extent of damage was far less compared to the CA 1/2 and CA4 pyramidal cells. In addition, neuronal death was observed in the (pre- / para-)subiculum. Other predominantly lesioned areas in the cerebrum were the piriform and entorhinal cortices and the amygdala. The adjacent perirhinal cortex showed dying neurons as well.

In the fore-brain (telencephalon) the tenia tecta and adjacent area of the olfactory nuclei were affected, as was the frontal part of the piriform cortex.

In general, the morphological status of the neocortex was good, particularly in the male rats. In female rats, the results of the 4 brain levels, taken together, indicated that incidentally a single or just a few dying neurons were observed in the orbital cortex (one animal), the insular, retrosplenial and temporal cortices (five animals), the parietal cortex (three animals), the occipital cortex (two animals) and frontal cortex (one animal). These dying neurons, which showed a pathological appearance as described for those in the predominantly lesioned areas, were located mainly in those parts of these cortical regions neighbouring one of the severely affected predilection areas. In the male rats single cell necrosis of in the neocortex was observed only in the cingulate cortex of one animal and in the orbital, cingulate and parietal cortices of another animal.

One animal showed a slight dilatation of the lateral ventricle, a phenomenon which was observed to a lesser extent in another animal.

Microscopic examination of other protocol organs for neuropathology revealed neuronal micro-vacuolization in both the spinal ganglion (L4) and Gasserian ganglion in the control and in both dose groups. Neuronal microvacuolization may be induced by processes like hypoxia or ischemia but also by certain toxins. In this study, neuronal micro-vacuolization was observed in the lumbar spinal ganglion in 4/5 females and 6/6 males of the high-dose (200 ppm) group, in 6/6 females and 6/6 males of the 15 ppm group, in 6/6 females and 6/6 males of the control group, and in the Gasserian ganglion in 2/4 females and 5/6 males of the high-dose group, in 4/6 females and 3/6 males of the 15 ppm group, in 4/5 females and 4/6 males of the control group. In short, no dose-response relationship was found and, therefore, the observed neuronal micro-vacuolization was not considered to be treatment-related.

Incidentally, degenerating axons (1 to 3) were observed in peripheral nerves, and dorsal and ventral roots of animals of the high-dose (200 ppm) group, but in animals of the other groups as well. This incidental axonal degeneration was not considered treatment-related, because the finding was within the range of the normal background pathology for rats of this strain and age.

Also in the spinal cord (3 levels) degenerating axons were present. In addition, small numbers of apparent 'swollen axons' (i.e. the axon is retracted from the relatively thinned and widened myelin sheet), were observed in animals of all groups. These swollen axons were sparsely distributed over the white matter with minor preference for the ventral part of the spinal cord. In the lumbar spinal cord their number was slightly higher in the high-dose (200 ppm) group (in 4/5 females and 4/6 males of the 200 ppm group, in 1/6 females and 0/6 males of the 15 ppm group, in 1/6 females and 2/6 males of the control group). If at all treatment-related, these effects were very mild.

Summarizing, the results of the neuropathology showed treatment-related neuronal death in

a number of predilection areas in the tel-and mesencephalon. Predominantly lesioned areas in the mesencephalon were the hippocampal region in broader sense (i.e. including subiculum and dentate gyrus), the piriform, entorhinal and perirhinal cortices and amygdala. In the telencephalon, neuronal death was present in the olfactory nuclei and tenia tecta. Neuronal death was observed in both sexes but was most pronounced in female rats and was, for both sexes, restricted to animals of the high-dose (200 ppm) group.

In the spinal cord, incidental swollen axons were observed in the white matter and their number was slightly increased in animals of the high-dose (200 ppm) group. This effect, if at all treatment related, was very mild. No treatment -related effects were observed in the peripheral nerves, spinal roots and spinal ganglia.

It was concluded that treatment of rats with 200 ppm MMTTC/DMTDC (30/70) induced marked neural lesions in a number of areas in the cerebrum, whereas no neuropathology was found in the 15 ppm MMTTC/DMTDC (30/70) group and in the control group.

The achieved concentrations in the present study were close to the nominal levels and were all in accordance with the criteria for content of the test substance (the acceptable maximum mean concentration).

Reliability score 2 (reliable with restrictions) **was** given by the registrant because the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

[Study 3]

Data source

Reference type: study report.

Author not disseminated: 1997.

Report date: 1997-08-26.

Materials and methods

Test type: 7 day palatability study/

Limit test: no.

Test guideline: no guideline available.

Principles of method if other than guideline: The objective of this study was to evaluate the palatability of a methyltin chloride mixture when administered via the drinking water to the female Sprague-Dawley rat for 7 days.

GLP compliance: yes.

Test materials

Test material: Dimethyltin dichloride.

Test material form: crystalline.

Details on test material:

- Name of test material (as cited in study report): methyltin chlorides (mixture of dimethyltin dichloride/methyltin trichloride).
- Substance type: powder.
- Physical state: solid.
- Storage condition of test material: room temperature and out of direct sunlight.

Test animals

Species: rat.

Strain: Sprague-Dawley

Sex: female.

Details on test animals and environmental conditions:

TEST ANIMALS

- Source: Charles River Canada Inc., St. Constant, Quebec.
- Age at study initiation: 48-49 days of age.
- Weight at study initiation: 165 to 188 g.
- Housing: All animals were housed individually in stainless-steel mesh-bottomed cages equipped with a water bottle.
- Diet (e.g. ad libitum): standard certified commercial pelleted laboratory diet (PMI Certified Rodent Chow 5002) ad libitum.
- Water (e.g. ad libitum): Water (with test article incorporated, where appropriate) which had been further treated by reverse osmosis and ultraviolet sterilization was provided ad libitum.
- Acclimation period: ca. 1 week.

ENVIRONMENTAL CONDITIONS

- Temperature (°C): 22 ± 3 .
- Humidity (%): 50 ± 20 %.
- Photoperiod (hrs dark / hrs light): 12 hours light and 12 hours dark.

IN-LIFE DATES: 21st August 1995 From: To: 28th August 1995.

Administration / exposure

Route of administration (oral): drinking water.

Vehicle: water.

Details on oral exposure:

PREPARATION OF DOSING SOLUTIONS:

A solution of the test material in drinking water was prepared once for each group by direct dilution. Appropriate amounts of the test material were added to drinking water and mixed using a stir plate until dissolved. The solutions were then divided into aliquots for each days use and stored refrigerated in glass containers until required.

Analytical verification of doses or concentrations: yes.

Details on analytical verification of doses or concentrations:

Homogeneity of the test material in the drinking water for each group was evaluated prior to commencement of treatment by analysis of appropriate samples obtained from the top, middle and bottom of the mixing jar.

The accuracy of mixing of the treated drinking water was checked during the treatment period by analysis of a sample taken immediately after preparation of the drinking water mixture. Stability of the test material in drinking water was evaluated by analysing a sample from each groups solution at the end of the study.

Duration of treatment / exposure: 7 days.

Frequency of treatment: when drinking water.

Doses/concentrations (nominal in water): 0, 250 and 500 ppm.

No. of animals per sex per dose: 5 females per dose.

Control animals: yes.

Details on study design: Three days before treatment commenced, all animals were weighed and 5 females were randomly assigned to each of the 3 groups using a computer-based randomization procedure which ensured the homogeneity of group means and variances for body weight.

Positive control: no.

Examinations

Observations and examinations performed and frequency:

CLINICAL EXAMINATIONS:

All animals were observed at least twice daily for mortality and signs of ill health or reaction to treatment. Mortalities and observed clinical signs were individually recorded.

BODY WEIGHT:

The animals were weighed at least once during the acclimation period and twice weekly during the treatment period including the day of terminal sacrifice.

FOOD CONSUMPTION:

Food consumption was measured daily commencing two days prior to treatment start.

WATER CONSUMPTION:

Water consumption was measured daily commencing two days prior to treatment start.

Sacrifice and pathology: Not examined.

Statistics: Group variances for quantitative data were compared using Bartlett's test. When the differences between group variances were not significant ($P > 0.001$), a one-way analysis of variance (ANOVA) was performed. If significant differences ($P < 0.05$) were indicated by the ANOVA, Dunnett's test was used to compare the control and treated groups. When the differences between group variances were significant ($P < 0.001$) by Bartlett's test, the Kruskal-Wallis test was then performed. Where significant differences ($P < 0.05$) between the groups were indicated by the Kruskal-Wallis test, the values for the control and treated groups were compared using Dunn's test. Significant differences were declared at the 0.05, 0.01 or 0.001 levels, where appropriate.

Results and discussions

Clinical signs and mortality: yes.

Body weight and weight gain: yes.

Food consumption and compound intake (if feeding study): yes.

Food efficiency: not examined.

Water consumption and compound intake (if drinking water study): yes.

Ophthalmoscopic examination: not examined.

Haematology: not examined.

Clinical chemistry: not examined.

Urinalysis: not examined.

Neurobehaviour: not examined.

Organ weights: not examined.

Gross pathology: not examined.

Histopathology (non-neoplastic): not examined.

Histopathology (neoplastic): not examined.

Details on results:

CLINICAL SIGNS AND MORTALITY:

No animals died and none were sacrificed prior to completion of the study. All treated animals showed signs of dehydration commencing on Day 4 of the study. No other clinical

signs were seen.

BODY WEIGHT AND WEIGHT GAIN:

A dose-related decrease in body weight was observed for treated animals when compared to the control group with significant ($P<0.05$ or $P<0.01$) reductions occurring on Day 4 for the 250 and 500 ppm groups and on Day 8 for the 500 ppm group. Losses in body weight occurred for both treated groups from Days 1 to 4 but from Days 4-8 both showed body weight gains. Overall for the treatment period, the mean body weight change for the control, 250 and 500 ppm groups was 15.2, 4.2 and -13.2 g, respectively.

FOOD CONSUMPTION:

Food intake values were significantly ($P<0.05$ or $P<0.01$) lower for the 500 ppm group on the following occasions: Days 3-4, 5-6, 6-7 and 7-8 and were slightly reduced from Days 4-5. Slight reductions when compared to the control group were observed for the 250 ppm group for these same intervals. Overall for the treatment period, the mean food intake for the control, 250 and 500 ppm groups was 120.4, 106.2 and 90.8 g, respectively.

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study):

Water consumption was significantly ($P<0.05$ or $P<0.01$) decreased for the 250 and 500 ppm groups for the following occasions: Days 3-4, 4-5, 5-6 and 7-8 and slight reductions were noted for both treated groups from Days 6-7. Overall for the treatment period, the mean water consumption for the control, 250 and 500 ppm groups was 189.0 (n=4), 119.5 (n=4) and 84.4 (n=5) g, respectively. This corresponded to achieved intakes of the methyltin chloride mixture of 24 and 35 mg/kg bw/day for the 250 and 500 ppm groups, respectively.

No NOEL or NOAEL is determined as this is a palatability study.

Reliability score 2 (reliable with restrictions) WAS given by the registrant because the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

[Study 4]

Data source

Reference type: study report.

Author not disseminated (1997).

Report date: 1997-08-28.

Materials and methods

Test type: subacute.

Limit test: yes.

Test guideline: no guideline followed.

Principles of method if other than guideline: The objective of this study was to evaluate the toxicity of a methyltin chloride mixture when administered via the drinking water to male and female Sprague-Dawley rats for 14 days.

GLP compliance: yes.

Test materials

Identity of test material: Dimethyltin dichloride.

Test material form: crystalline.

Details on test material:

- Name of test material (as cited in study report): methyltin chlorides (mixture of dimethyltin dichloride/methyltin trichloride).
- Substance type: powder.
- Physical state: solid.
- Storage condition of test material: room temperature and out of direct sunlight.

Test animals

Species: rat.

Strain: Sprague-Dawley.

Sex: male/female.

Details on test animals and environmental conditions:

TEST ANIMALS:

- Source: Charles River Canada Inc., St. Constant, Quebec.
- Age at study initiation: 50-51 days of age.
- Weight at study initiation: 234 to 258 g (males); 173 to 193 g (females).
- Fasting period before study: No, fasted overnight prior to sacrifice.
- Housing: All animals were housed individually in stainless-steel mesh-bottomed cages equipped with a water bottle.
- Diet (e.g. ad libitum): A standard certified commercial pelleted laboratory diet (PMI Certified Rodent Chow 5002) ad libitum.
- Water (e.g. ad libitum): Water (with test article incorporated, where appropriate) which had been further treated by reverse osmosis and ultraviolet sterilization was provided ad libitum.
- Acclimation period: ca. 1 week.

ENVIRONMENTAL CONDITIONS:

- Temperature (°C): 22 ± 3.
- Humidity (%): 50 ± 20 %.
- Photoperiod (hrs dark / hrs light): 12 hours light and 12 hours dark.

IN-LIFE DATES: From: December 14, 1995 To: December 28, 1995.

Administration / exposure

Route of administration: oral (drinking water).

Vehicle: water.

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations:

The accuracy of mixing of the treated drinking water was checked during the treatment period by analysis of a sample taken immediately after preparation of each batch of drinking water mixture.

Although not stated in the protocol, stability of the test material in drinking water was evaluated by analysing a sample from the first weekly preparation for each group that was taken at the end of the week.

Duration of treatment / exposure: 14 days.

Frequency of treatment: when drinking water.

Doses/concentrations (nominal in water): 0, 25, 75, 150 and 200 ppm.

No. of animals per sex per dose: 5 animals per sex per dose.

Control animals: yes.

Details on study design:

Three days before treatment commenced, all animals were weighed and 5 males and 5 females were randomly assigned to each of the 5 groups using a computer-based randomisation procedure which ensured the homogeneity of group means and variances for body weight. Males and females were randomised separately.

Examinations

Observations and examinations performed and frequency:

CLINICAL OBSERVATIONS:

All animals were checked twice daily for mortality and signs of ill health or reaction to treatment. In addition, a physical examination of each animal was conducted once weekly. Abnormal clinical signs were individually recorded.

BODY WEIGHT:

The animals were weighed at least once during the acclimation period and twice weekly

during the treatment period as well as on the day of terminal sacrifice.

FOOD CONSUMPTION:

Food consumption was measured once prior to treatment initiation and twice weekly during the treatment period.

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study):

Water consumption was measured daily commencing two days prior to treatment start.

Sacrifice and pathology:

GROSS PATHOLOGY:

On completion of the treatment period, all animals were euthanised by CO₂ asphyxiation followed by exsanguination from the abdominal aorta and then subjected to necropsy. Abnormal tissues were retained in neutral buffered 10% formalin. All animals were fasted overnight prior to scheduled sacrifice.

ORGAN WEIGHTS:

The following organs were dissected free of fat and weighed for each animal: kidneys; liver; spleen; thymus. Paired organs were weighed together and organ weight ratio's relative to body weight were calculated.

Statistics:

Group variances for quantitative data were compared using Bartlett's test. When the differences between group variances were not significant ($P > 0.001$), a one-way analysis of variance (ANOVA) was performed. If significant differences ($P < 0.05$) were indicated by the ANOVA, Dunnett's test was used to compare the control and treated groups. When the differences between group variances were significant ($P < 0.001$) by Bartlett's test, the Kruskal-Wallis test was then performed. Where significant differences ($P < 0.05$) between the groups were indicated by the Kruskal-Wallis test, the values for the control and treated groups were compared using Dunn's test. Significant differences were declared at the 0.05, 0.01 or 0.001 levels, where appropriate.

Results and discussions

Clinical signs and mortality: no effects.

Body weight and weight gain: no effects.

Food consumption and compound intake (if feeding study): yes.

Food efficiency: not examined.

Water consumption and compound intake (if drinking water study): yes.

Ophthalmoscopic examination: no effects.

Haematology: no effects.

Clinical chemistry: no effects.

Urinalysis: no effects.

Neurobehaviour: no effects.

Organ weights: yes.

Gross pathology: no effects.

Histopathology (non-neoplastic): not examined.

Histopathology (neoplastic): not examined.

Details on results:

CLINICAL SIGNS AND MORTALITY:

No animals died and none were sacrificed prior to completion of the study. No abnormal clinical signs related to treatment were observed.

BODY WEIGHT AND WEIGHT GAIN:

No significant differences in body weight were seen. However, the average body weight for males in the 200 ppm group and females in all treated groups was slightly lower than the control group at the end of the study.

FOOD CONSUMPTION:

Food intake values were significantly ($P < 0.05$ or $P < 0.01$) lower for the 200 ppm group females from Days 1-5 and 8-12. On the other occasions during the treatment period, the food intake values were slightly lower for the 200 ppm females than those of the control group. A slight reduction was also noted for females in the other (lower) treatment groups on most occasions following treatment initiation and for males in the 200 ppm group.

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study):

Water consumption was either slightly or significantly ($P < 0.05$ or $P < 0.01$) decreased for males and females in all treated groups on most daily assessments during the study. Overall for the treatment period, the total mean water consumption for the control, 25, 75, 150 and 200 ppm groups was 568, 464, 440, 461 and 449 g, respectively, for males, and 433, 378, 339, 359 and 377 g, respectively, for females.

GROSS PATHOLOGY:

No gross pathological lesions were noted that were considered treatment-related.

ORGAN WEIGHTS:

No significant differences in absolute organ weights were noted between the control and treated groups. However, the relative (to body weight) kidney weight of females in the 200 ppm group was significantly ($P < 0.01$) increased.

No NOEL or NOAEL determined.

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

[Study 5]

Study reference:

Reference type: study report.

Author not disseminated (1978).

Report date: 1978-11-20.

Materials and methods

Test type: subchronic.

Limit test: yes.

Test guideline: equivalent or similar to OECD TG 408 (Repeated Dose 90-Day Oral Toxicity in Rodents).

GLP compliance: no according to the registrant.

Test materials

Read across from 78/22 Mono/Di-methyltin dichloride which has a concentration of DMTC which is too low for this study to be relied upon.

Test material form: crystalline.

Details on test material:

- Name of test material (as cited in study report): 1175-114.
- Physical state: solid.

Test animals

Species: rat.

Strain: Wistar.

Sex: male/female.

Details on test animals and environmental conditions:

TEST ANIMALS:

- Source: Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands.
- Age at study initiation: newly weaned.
- Weight at study initiation: NDA.
- Fasting period before study: NDA.
- Housing: All rats were kept under conventional conditions, five to a cage, in suspended stainless steel cages, fitted with wire-mesh.
- Diet (e.g. ad libitum): All rats had free access to food, except when determinations of blood glucose and blood urea nitrogen and urine examinations were being conducted. The rats were fed powdered stock diet of the following percentage composition:
 yellow maize 29.7 brewer's yeast 3
 whole wheat 36 grass meal 3
 soya bean oil meal 11 soya bean oil 3
 meat scraps 4 vitamin preparations 0.4
 fish meal 7 trace mineralized salt 0.5
 dried whey 2 steamed bone meal 0.4
- Water (e.g. ad libitum): All rats had free access to tap water, except when determinations of blood glucose and blood urea nitrogen and urine examinations were being conducted.
- Acclimation period: 7 days.

ENVIRONMENTAL CONDITIONS:

- Temperature (°C): 24 ± 1 .
- Humidity (%): 45 ± 5 .
- Photoperiod (hrs dark / hrs light): 12/12.

IN-LIFE DATES: From: 10th October 1977 To: January 1978.

Administration / exposure

Route of administration: oral (feed).

Vehicle: unchanged (no vehicle).

Details on oral exposure:

PREPARATION OF DOSING SOLUTIONS:

Test material was added to the diet to provide the desired concentrations for the test groups. Homogeneity was achieved by mixing for 2 minutes in a mechanical blender (Stephan). The diet containing 500 ppm was prepared first; the other two test diets were obtained by further diluting the 500 ppm diet with stock diet. All diets were freshly prepared once a fortnight and stored at ambient temperature. The levels of the test material in the diets were checked by tin analyses of diet samples taken from the first and from the last batch of diets immediately after preparation.

Analytical verification of doses or concentrations: yes.

Details on analytical verification of doses or concentrations: The figures show that the levels of tin found in the 3 test diets were close to the calculated levels except for a too low value obtained with the high-dose diet in the first series of samples analysed.

Duration of treatment / exposure: 13 weeks.

Frequency of treatment: Daily.

Doses/concentrations (nominal in diet): 0, 20, 100 or 500 ppm.

No. of animals per sex per dose: 10 per sex per dose.

Control animals: yes.

Positive control: No.

Examinations

CLINICAL OBSERVATIONS:

The general condition and behaviour of all animals were checked regularly; all signs of ill-health were recorded.

BODY WEIGHT:

The individual body weights of all rats were recorded initially and then weekly.

FOOD CONSUMPTION:

The food consumption of each group was measured over weekly periods from weeks 0-12.

FOOD EFFICIENCY:

The efficiency of food utilization was calculated during the period of rapid growth (weeks 0-4) and was expressed as grams weight gain per gram food consumed.

HAEMATOLOGY:

Samples of blood were collected from the tip of the tail of all rats of each sex and group at week 12; all blood samples were analysed as follows:

1. Haemoglobin concentration (Hb), by cyanmethemoglobin method using Zap Oglobine of Coulter Electronics.
2. Packed cell volume (PCV), as microhaematocrit.
3. Red blood cell count by Coulter Counter Model ZF.
4. White blood cell count by Coulter Counter Model ZF.
5. Differential white blood cell count by direct visual count of smear after Pappenheim staining. Counts are recorded under the following categories: L = lymphocytes; N = neutrophils; M = monocytes and E = eosinophils.

CLINICAL CHEMISTRY:

At autopsy, at week 13, blood samples were collected in heparinized plastic tubes from the aorta of all rats whilst under ether anaesthesia. The blood samples were centrifuged at 2,000 rpm for 15 minutes using Sure-Sep from General Diagnostics for good separation of the

plasma. The following measurements were made by a Coulter KEM-O-LAB using the Coulter test methodology.

1. Plasma glutamic-oxalacetic transaminase (GOT)
2. Plasma glutamic-pyruvic transaminase (CPT)
3. Plasma alkaline phosphatase (AP)
4. Total plasma protein (TP)
5. Plasma albumin

In blood samples collected from the tip of the tail after overnight fasting of the rats in week 13, the following measurements were made:

1. Fasting blood glucose by Technicon Auto-Analyzer, method N-9a; and
2. Blood urea nitrogen (BUN), by the automated phenazone/diacetyl monoxime technique of Ceriotti and Spandrio (1965).

URINALYSIS:

Individual urine samples were collected from 10 rats/sex/ group during the last 16 hours of a 24 hour period of deprivation of food and water at week 12. The following measurements were made:

- a. Specific gravity by refractometer;
- b. Volume by calibrated tube.

The following semi-quantitative tests in pooled samples from 10 rats/ sex/group were made:

1. pH
2. Protein
3. Sugar
4. Occult blood
5. Ketones

all using Labstix from Ames Laboratories

Deposits were examined for: erythrocytes, leucocytes, epithelial cells, amorph crystals, phosphate crystals, casts, bacteria and sperm cells.

The following grading system was used: - = negative; \pm = minimal; + = slight; ++ = moderate; +++ = high; ++++ = very high.

Sacrifice and pathology

GROSS PATHOLOGY:

In week 14, all rats were anaesthetized by ether, exsanguinated by cannulating the aorta and examined grossly for pathological changes.

The following organs were weighed:

heart testicles
kidneys ovaries
liver thymus
spleen thyroid
brain adrenals

Samples of these organs and of a wide range of other organs from all rats were fixed in a 4 per cent neutralized formaldehyde solution. Organs and tissues which were to be studied

microscopically were processed through paraffin, sectioned at 5 µm and stained with haematoxylin and eosin.

HISTOPATHOLOGY:

Detailed microscopical examinations were carried out on all male and female rats of the top dose group and on all control rats. The following organs, together with those which had been weighed, were examined:

axillary lymph nodes coagulating glands
gastro-intestinal tract (six levels) epididymis
aorta uterus
pancreas lungs
mesenteric lymph nodes salivary glands (2)
urinary bladder seminal vesicles
prostate mammary glands

Water content of the brain:

After weighing the organ, appropriate samples of the brain (hemispheres) of 5 rats/sex/group were taken for determination of the water content by drying at 105°C till constant weight (24 hours).

Statistics

Body weights, haematological indices, biochemical blood values and urinary findings were analysed statistically by the Wilcoxon test; the relative organ weights were evaluated by the Student t test.

Results and discussions

Clinical signs and mortality: yes.

Body weight and weight gain: yes.

Food consumption and compound intake (if feeding study): yes.

Food efficiency: no effects.

Water consumption and compound intake (if drinking water study): not examined.

Ophthalmoscopic examination: not examined.

Haematology: no effects.

Clinical chemistry: no effects.

Urinalysis: yes.

Neurobehaviour: not examined.

Organ weights: yes.

Gross pathology: no effects.

Histopathology (non-neoplastic): yes.

Histopathology (neoplastic): not examined.

Details on results:

CLINICAL SIGNS AND MORTALITY:

During the course of the experiment there were no deaths or signs of intoxication. Only a few rats exhibited signs of ill health apparently not related to treatment. In the 500 ppm group 2 females showed sniffing during week 12 and 13, while in the 100 ppm group alopecia was observed in 3 males during the 4th week.

BODY WEIGHT, WEIGHT GAIN AND FOOD CONSUMPTION:

Mean body weights were decreased only at 500 ppm in both sexes. This phenomenon was in agreement with the lower food intake in this group. Growth and food intake was not materially affected by lower dosage levels.

FOOD EFFICIENCY:

Food efficiency figures showed no treatment-related changes amongst the various groups

HAEMATOLOGY:

There were no changes in the haematological indices which could be related to the feeding of the test substance.

CLINICAL CHEMISTRY:

There were no outstanding differences in blood and plasma analyses between the test groups and controls in levels of sugar, urea and proteins or in the activities of transaminases and alkaline phosphatase.

URINALYSIS:

There were no changes in the composition of the urine which could be attributed to the test substance, except for an increase in the amount of phosphate crystals at 500 ppm in females. Specific gravity was markedly decreased and volume was slightly increased at 500 ppm in both sexes.

ORGAN WEIGHTS:

The relative weight of the kidneys was moderately increased at 500 ppm in females only. In females the kidney weights were also relatively high at the 20 and 100 ppm levels, but there was no evidence of a dose-related response at these two dietary levels.

GROSS PATHOLOGY:

Gross examination at autopsy did not show any obvious adverse effects.

HISTOPATHOLOGY (NON-NEOPLASTIC):

Histopathological changes that could be ascribed to treatment were observed in the urinary

bladder and in the kidneys. Hyperplasia of the epithelium of the urinary bladder (varying from moderate to slight) was observed in most of the males and females of the highest dose group, in a number of males and females of the mid-dose group (100 ppm) and in two females of the lowest dose group (20 ppm). The renal changes occurred in four males and in one female of the highest dose group and consisted of slightly enlarged pale nuclei and foamy cytoplasm of the epithelial cells of the proximal tubules in the intercortico-medullary region. The other pathological changes listed were about equally distributed among the control group and the different test groups or occurred only in a single animal. Therefore, none of these abnormalities, which are common findings in the strain of rats used, are ascribed to the feeding of the test material.

OTHER FINDINGS

The water content of the brain was comparable in all groups in both sexes.

Reliability score 3 (not reliable) was given by the registrant because the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results.

Specific target organ toxicity (repeated exposure) - human data

[Study 1]

Study reference:

Reference type: secondary source.

Author: Harper, C., Lladós, F., Diamond, G. & Chappell, L.L.

Year: 2005.

Title: Toxicological Profile for Tin and Tin Compounds.

Bibliographic source: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Primary Source: Fortemps et al (1978) Trimethyltin poisoning. Report of two cases. Int Arch Occup Environ Health 41: 6.

Materials and methods

Study type: poisoning incident.

Endpoint addressed: neurotoxicity.

GLP compliance: no according to the registrant.

Test materials

Identity of test material: Dimethyltin dichloride and trimethyltin chloride vapours.

Test material form: crystalline.

Method

Type of population: occupational.

Route of exposure: inhalation.

Reason of exposure: unintentional, occupational.

Exposure assessment: no data.

Results and discussions

Two chemists who had been intermittently exposed to vapours of dimethyltin dichloride and trimethyltin chloride for about 3 months abruptly developed a status of mental confusion with generalized epileptic seizures. Before the acute episode, the subjects had complained of headaches, pain in various organs, and psychological disturbances such as memory defects, vigilance loss, insomnia, anorexia, and disorientation. Both patients recovered completely following removal from exposure.

Reliability score 4 (not assignable) was given by the registrant because of the secondary source with very little information.

[Study 2]

Reference

Reference type: secondary source.

Author: Harper, C., Lladós, F., Diamond, G. & Chappell, L.L.

Year: 2005.

Title: Toxicological Profile for Tin and Tin Compounds.

Bibliographic source: U.S. Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry. Primary Source: Rey C. et. al. (1984) Methyltin intoxication in six men: Toxicologic and clinical aspects. Vet Hum Toxicol 26: 121-122.

Materials and methods

Study type: poisoning incident.

Endpoint addressed: neurotoxicity.

Test guideline: no guideline followed.

Principles of method if other than guideline: Case study of workers exposure.

GLP compliance: no according to the registrant.

Test materials

Details on test material: Mixture of half dimethyltin and half trimethyltin chloride vapour.

Test material form: crystalline.

Method

Type of population: occupational.

Subjects: 6 workers (male and female).

Ethical approval: no data.

Route of exposure: inhalation.

Exposure assessment: no data.

Details on exposure: Maximum exposure was a total of 1.5 hours over a 3 day working period. No estimates of exposure levels were given.

Results and discussions

Six chemical workers exposed to methyltins primarily by inhalation experienced headache, tinnitus, deafness, impaired memory, disorientation, aggressiveness, psychotic and other severe neuropsychiatric behaviour, syncope and loss of consciousness as symptoms of exposure; one subject died. The two surviving workers with the highest urinary tin levels exhibited fixed neurological effects which were not resolved more than six years after exposure. The remaining three survivors returned to work, but had memory loss, which persisted for six months.

Reliability score 4 (not assignable) was given by the registrant because of secondary source containing minimal information and because the primary source was not available.

Specific target organ toxicity (repeated exposure) - other data

[Study 1]

Study reference:

Reference type: publication.

Author: Seinen, W., et al.

Year: 1977.

Title: Toxicity of Organotin Compounds. II. Comparative in Vivo and in Vitro Studies with Various Organotin and Organolead Compounds in Different Animal Species with Special Emphasis on Lymphocyte Cytotoxicity.

Bibliographic source: Toxicology and Applied Pharmacology 42, 197-212 (1977).

Materials and methods

Limit test: no.

Test guideline: no guideline available.

Principles of method if other than guideline: Literature paper investigating the relationship between the chemical structure of organotin compounds and thymolytic activity. Data considered in the review include: oral treatment studies, intravenous treatment studies and in vitro experiments. An intravenous treatment study and in vitro experiment included the assessment of dimethyltin dichloride.

GLP compliance: no according to the registrant (not reported).

Test materials

Identity of test material: dimethyltin dichloride.

Test material form: crystalline.

Details on test material: Not less than 98% pure Dimethyltin dichloride.

Test animals

Species: rat.

Strain: no data.

Sex: female.

Administration / exposure

Route of administration: intravenous.

Vehicle: water.

Details on exposure: Dimethyltin dichloride was dissolved in saline solution before being injected into the tail vein.

Analytical verification of doses or concentrations: no data.

Duration of treatment / exposure: Animals were killed 2 days after the last injection.

Frequency of treatment: 5 doses.

Doses / concentrations (actual injected): 0, 12, 24, 48 mg/kg bw/day.

No. of animals per sex per dose: Groups of 5 - 8 female rats were used per dose.

Control animals: yes.

Examinations

Sacrifice and pathology: The liver, kidneys, spleen and thymus were weighed and processed for histopathological examination.

Cell viabilities: In some experiments the total number of thymus lymphocytes and their viability were determined. (No further detail provided.)

Statistics: Data analyses for significance of differences using Students t-Test.

Results and discussions

NOAEL (female) 24 mg/kg bw/day based on test mat. Basis for effect level / Remarks: No adverse effects seen.

LOAEL (female) 48 mg/kg bw/day based on test mat. Basis for effect level / Remarks: mortality.

Results of examinations

Intravenous Experiment: Dimethyltin dichloride did not affect the thymus. Even at the highest dose, no effect was seen on thymus weight or any other organ weights. No effects were seen on any of the lymphoid organs. However, a single injection of 48 mg/kg bw caused 3 of the 8 animals to die within 2 days. Furthermore, iv injection of dimethyltin dichloride did not affect the lymphoid organs. No liver or bile duct changes were found. Other treatment-related histopathological changes were not noted.

In vitro experiment: No effect in the cell count or viability was observed at any dimethyltin dichloride concentration tested.

Reliability score 4 (not assignable) was given by the registrant because the study source was a literature paper with limited methodology and results for dimethyltin dichloride.

[Study 2]

Study reference:

Reference type: secondary source.

Author: Dobson, S., Howe, P.D. & Floyd, P.

Year: 2006.

Title: Mono- and disubstituted methyltin, butyltin, and octyltin compounds.

Bibliographic source: Concise International Chemical Assessment Document 73, World Health Organisation Primary source: Arakawa, Y. & Wada, O. (1993) Biological properties of alkyltin compounds. In: Sigel, H., Sigel, A., eds. Metal ions in biological systems Vol. 9. pp. 101–136.

Materials and methods

Test type: subacute.

Limit test: no.

Test guideline: no guideline available.

Principles of method if other than guideline: No methodological information available.

GLP compliance: no according to the registrant data.

Test materials

Identity of test material: dimethyltin dichloride.

Test material form: crystalline.

Test animals

Species: rat.

Administration / exposure

Analytical verification of doses or concentrations: no data.

Doses / concentrations: 5 mg/kg bw/day. Basis nominal conc.

Results and discussions

NOAEL (no data on sex) \geq 5 mg/kg bw/day based on test mat. Basis for effect level /
Remarks: Thymus weight.

Reliability score 4 (not assignable) was given by the registrant because the study was available via a secondary source with no methodological information.

3.11. Aspiration hazard

No data available.

4. ENVIRONMENTAL HAZARDS

4.1. Hazardous to the aquatic environment

4.1.1 Ready *biodegradability (screening studies)*

Study 1

Purpose flag: key study; **Study result type:** experimental result;

Study period: 14th May 2002 to 20th June 2002;

Reliability score 1 (reliable without restriction) was given by the registrant because the study was conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which did not affect the quality of the relevant results, according to the registrant.

Reference type: study report; **Year:** 2003; **Report date:** 2003-06-22;

Materials and methods

Test type: ready biodegradability;

Guideline: OECD Guideline 301 F (Ready Biodegradability: Manometric Respirometry Test);

Deviations yes (additional NaNO₃ was added to the mineral medium to prevent nitrogen limitation)

Guideline EU Method C.4-D (Determination of the "Ready" Biodegradability - Manometric Respirometry Test); **Deviations** yes (additional NaNO₃ was added to the mineral medium to prevent nitrogen limitation)

GLP compliance: yes

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline

Details on test material

- Name of test material (as cited in study report): dichlorodimethylstannane
- Physical state: solid

Oxygen conditions: aerobic

Inoculum or test system: activated sludge, domestic, non-adapted

Details on inoculum:

CHARACTERISATION OF THE BACTERIAL INOCULUM

A sample of activated sludge was taken from an oxidation ditch situated in the municipality of Hazerswoude, the Netherlands, on May 14, 2002. The oxidation ditch is used to treat domestic waste water. The activated sludge was aerated until use. Before the start of the test, the dry weight of the sludge was determined to be 4.4 g/l. In order to get a concentration of solids of approximately 30 mg/l, 2.05 mL sludge was added to 300 mL of mineral medium.

MINERAL MEDIUM

The mineral medium was prepared from concentrated stock solutions in ultrapure water, and was aerated vigorously before use.

Composition of the nutrient stock solutions of the mineral medium:

Nutrient stock solutions

a) KH_2PO_4 (potassium dihydrogen phosphate): 8.5 g
 K_2HPO_4 (dipotassium hydrogen phosphate): 21.8 g
 $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ (disodium monohydrogen phosphate heptahydrate): 33.4 g
 NH_4Cl (ammonium chloride): 1.5 g
 NaNO_3 (sodium nitrate): 25.0 g
 Dissolved in and made up to 1000 mL with ultrapure water.
 The pH of this solution was set to 7.4 ± 0.1 .

b) Nutrient stock solution b per 1000 mL ultrapure water:
 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (magnesium sulphate heptahydrate): 22.5 g
 Dissolved in and made up to 1000 mL with ultrapure water.

c) Nutrient stock solution c per 1000 mL ultrapure water:
 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (calcium chloride dihydrate): 36.4 g
 Dissolved in and made up to 1000 mL with ultrapure water.

d) Nutrient stock solution d per 100 mL ultrapure water:
 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (iron(III) chloride hexahydrate)
 Dissolved in and made up to 100 mL with ultrapure water: 0.025 g
 This solution was prepared immediately before use.

One mL of each of the nutrient stock solutions was added per litre of aerated ultrapure water.

Duration of test (contact time): 35 d

Initial conc: 111 mg/L

Based on: ThOD/L

Parameter followed for biodegradation estimation

O₂ consumption

Details on study design

TEST SUBSTANCE CONCENTRATIONS

The determination was performed with a single concentration of dichlorodimethylstannane, i.e. 111 mg/l, corresponding to a ThODNH₃ of 56.6 mg O₂/l, or 17.0 mg O₂/flask.

PREPARATION OF THE BIODEGRADATION TEST FLASKS

The final test concentrations of dichlorodimethylstannane was prepared by dissolving 0.8325 g of the test substance in 25 mL ultrapure water (Stock I). An aliquot of 1 mL of Stock I was added to the test flasks that were subsequently filled with 300 mL mineral medium. This resulted in the final concentration of 111 mg per litre. This series was completed with an inoculum blank, containing mineral medium only.

PREPARATION OF THE ACTIVITY CONTROL

The final test concentration of sodium acetate was prepared by dissolving 2.993 g of the reference substance in 100 mL of ultrapure water. From this stock solution one mL was added to 300 mL mineral medium resulting in a final nominal concentration of 100 mg/l sodium acetate.

PREPARATION OF THE TOXICITY CONTROL

The test substance was added to the test media as described for the biodegradation test flasks. One mL of the reference stock solution was subsequently added.

TEST SERIES

The following test series were prepared:

Test substance:

Concentrations : 0, 111 mg/l

Number of replicate flasks : 3

Oxygen concentration determined : every 4 hours

Inoculum activity control:

Concentration of the reference substance : 100 mg/l

Number of replicate flasks : 2

Oxygen concentration determined : every 4 hours

Toxicity control:

Concentration of reference substance : 111 mg/l

Concentration of test substance : 100 mg/l

Number of replicate flasks per time point : 2

Oxygen concentration determined : every 4 hours

pH measurement:

After addition of the test substance and mineral medium, the pH was measured in each treatment before inoculation, except for the blanks. If necessary, the pH was adjusted to 7.4 ± 0.2 with 0.1 M HCl or 0.4 M NaOH solution. The pH was also measured in the test media at the end of the test.

Inoculation, Incubation and measurements:

After the measurement of the pH and its adjustment, the filled flasks were inoculated with 2.05 mL of the diluted sludge. This resulted in a concentration of 30 mg (dry weight)/l mineral medium. The flasks were closed and placed in the incubator of the Manometric Respirator (Micro-Oxymax). When all test flasks were placed in the incubator and connected to the Micro-Oxymax, the incubator was closed and the oxygen and temperature measurements were started.

Operation of the Micro-Oxymax:

The Micro-Oxymax measures the percentage oxygen in the air of the respective flasks and calculates, based on the earlier measurement, the resulting oxygen consumption in a certain time period. Based on these values the oxygen consumption per flask can be derived.

The measurements stopped for short periods of time and the software had to be restarted. The cumulative oxygen consumption has been corrected for these situations. Negative and high values of oxygen consumption were most likely caused by leakages of the system. These have been corrected, when considered too large.

Termination of the experiment:

After 28 days of incubation it was decided to extend the test with two weeks because the oxygen consumption had not reached the plateau phase. The actual termination was after 35 days. After termination of the test, the pH was measured in each flask.

Reference substance: acetic acid, sodium salt

Results and discussions

% Degradation of test substance

%Degr. 3; **Parameter:** O₂ consumption; **Sampling time:** 35 d

Details on results

Dichlorodimethylstannane was slightly (3%) biodegraded after 35 days of incubation. Dichlorodimethylstannane was considered not to have exceeded the pass level of 60% ThODNH₃ removal within 28 days, classifying it as not readily biodegradable

Test conditions

The pH of the medium in the test flasks decreased to 7.3-7.4 after 35 days of incubation. In the inoculum activity and the toxicity controls the pH at the end of the test was 8.0-8.2. The higher pH in these flasks is due to the higher CO₂ production.

The pH value was within the limits of the validity criteria. The average temperature in the flasks was 23 + 0.5 (sd) °C during the test (range 19.6-24.0°C), which is higher than planned in the protocol, but within the limits prescribed by the guidelines. Therefore the temperature was considered acceptable.

Inoculum activity and toxicity control tests

The mean oxygen consumption values of the inoculum blanks, the inoculum activity control (sodium acetate), and the toxicity control (sodium acetate and test substance) at day 14 (336 hours), 28 (671 hours) and the end of the test (830 hours) are given in Table 1.

Table 1: Results of the inoculum activity and toxicity control tests: mean values of the cumulative oxygen consumption (mg O₂/flask) and biodegradation as percentage of the ThOD_{NH3} (mean values) after 14, 28 and 35 days.

Time (days)	Inoculum blank mg O ₂ /flask	Inoculum activity control ⁽¹⁾		Toxicity control ⁽²⁾	
		mg O ₂ /flask ⁽³⁾	Biodegradation ThOD %	mg O ₂ /flask (3)	Biodegradation ThOD %
14 (336 hours)	2.4	19.0	82	21.0	50
28 (671 hours)	3.2	21.0	87	23.8	55
35 (830 hours)	3.5	21.4	88	24.3	56

⁽¹⁾100 mg/l sodium acetate

⁽²⁾100 mg/l sodium acetate + 111 mg/l dichlorodimethylstannane

⁽³⁾Not corrected for blank

The reference test was sufficiently degraded within 14 days of incubation (82%). The cumulative oxygen consumption in the toxicity control (sodium acetate and test substance) was 21.0 mg O₂/flask after 14 days, which was slightly higher than that of the inoculum activity control with sodium acetate only (19.0 mg O₂/flask). This indicated that the test substance did not inhibit the degradation of sodium acetate at the concentration tested. Based on the combined ThOD of both substances, a biodegradation degree >25% was reached, which, according to the guidelines, means that it is considered not toxic to the inoculum.

Biodegradation tests

Table 2 Shows the relevant mean Biochemical Oxygen Demand (BOD) values and the percentage biodegradation of dichlorodimethylstannane at a concentrations of 111 mg/l, calculated from the ThOD_{NH3} (0.51 mg O₂/mg) of the test substance.

Table 2: Biodegradation of dichlorodimethylstannane (111 mg/l) expressed as the BOD (mg O₂/mg) and as percentage of its ThOD_{NH3} (mean values).

Time (days)	BOD (mg O ₂ /mg)	% Biodegradation ThOD _{NH3}
14 (336 hours)	0.02	3
28 (671 hours)	0.01	3
35 (830 hours)	0.02	3

Dichlorodimethylstannane was slightly (3%) biodegraded after 35 days of incubation. Dichlorodimethylstannane was considered not to have exceeded the pass level of 60% ThOD_{NH3} removal within 28 days, classifying it as not readily biodegradable.

4.1.2 BOD₅/COD

Not available.

4.1.3 Aquatic simulation tests

Not available.

4.1.4 Other degradability studies

Hydrolysis

Purpose flag: key study; **Study result type:** experimental result

Study period: 20 May 2003 - 4 July 2003;

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted in compliance with GLP and with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which do not affect the quality of the relevant results. However, the analytical method used involved derivatization. This method measures the amount of the alkyltin moiety present, but cannot identify the other ligands attached to the tin. Currently, there is no analytical method available that can quantify the actual named substance, i.e., the entire organotin compound with its associated ligands

Reference type: study report; **Year** 2004; **Report date** 2004-06-17

Materials and methods

Test guideline: Qualifier according to; **Guideline:** OECD Guideline 111 (Hydrolysis as a Function of pH); **Deviations:** no

Principles of method if other than guideline

A number of deviations from the original study protocol were made. None of these deviations were felt to have affected the validity of the study.

1) As of 1 November 2003, the analytical departments have merged to two departments. Therefore, the current name of the department of the study director, i.e. the Analytical Sciences Department, represented by M.W.H. den Reijer, is listed in this report.

2) For each test substance, 5 x 100 g was received from the sponsor instead of 3 x 100 g for each test substance, as was listed in the protocol.

3) Extracts of calibration solutions were stored in the dark at 2- 10 °C for up to two weeks instead of ≤ 1 week, in order to allow simultaneous GC-MS analysis of extracts of $t = 0$ and $t = 5$ days hydrolysis solutions, in all cases. Derivatised organotin compounds were found to be stable in hexane extracts at 2 - 10 °C for at least 2 weeks.

4) In order to check the derivatisation and extraction of the hydrolysis solutions, QC samples were freshly prepared on each day of analysis of hydrolysis solutions. The results of QC samples should be between 80 % and 120 % of the prepared concentration, in order to approve the results of the hydrolysis solutions analysed together with these QC samples. Preparation, analysis and criterion of QC samples had not been included in the protocol.

5) The derivatisation and extraction procedure of the hydrolysis solutions (and the validation samples) was adapted from the procedure described in the protocol, to allow for the transfer of the hydrolysis solution from the vial in which the incubation had taken place, to a Greiner tube for performance of the derivatisation and extraction procedure. The incubation vials used have a relatively small volume of headspace above the hydrolysis solution (10 ml), whereas larger Greiner tubes (50 ml) are necessary for the performance of the derivatisation step. Therefore, any organotin compounds remaining in the incubation vial were also derivatised and extracted, as well as the hydrolysis solution itself, which was transferred to the Greiner tube. Consequently, the derivatisation and extraction procedure of the calibration solutions (and the QC samples) was adapted accordingly.

6) The total amount of STEB used was increased from 0.5 mL of a 20 % (m/V) solution of STEB in THF to 1 mL of a 20 % (m/V) solution of STEB in THF (0.75 mL + 0.25 mL for hydrolysis solutions), to compensate for any effects of the ions in the buffer solution on the STEB derivatisation reaction, ensuring that there was an excess of STEB.

7) After phase separation, approximately 3 mL of the hexane layer was removed and washed with approximately 3 mL of 2 mol/l HCl, instead of approximately 4 mL of the hexane layer washed with approximately 4 mL of 2 mol HCl. Approximately 3 mL of hexane layer was sufficient for the analyses.

8) Due to the relatively high concentrations of test substance in the hydrolysis solutions, all hexane extracts were diluted 50 times with hexane (containing naphthalene, approximately 0.1 mg/l), prior to GC-MS analysis.

9) The mass range of the MS detector was extended from 60 - 400 amu to 60 - 600 amu, to be able to detect any mass fragments with high m/z values.

10) For clarity, in the quality criteria the detector response is defined as Q-value (ratio peak area test substance / peak area internal standard) instead of peak area.

11) The pH value of one of each set of duplicate hydrolysis solutions was measured, instead of all hydrolysis solutions. Measurement of the pH value of one hydrolysis solution per test condition per time point was sufficient.

12) At the request of the sponsor, the study title was changed into 'Abiotic degradation of Trichloromethylstannane (CAS # 993-16-8) and Dichlorodimethylstannane (CAS # 753-73-1) according to OECD Test guideline 111 - a preliminary test (Tier 1)'.

GLP compliance: yes

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline; **Radiolabelling:** no

Details on test material

- Name of test material (as cited in study report): Dichlorodimethylstannane
- Storage: in the dark at < - 18°C

Analytical monitoring: yes

Details on sampling

- Sampling intervals for the parent/transformation products: The concentration of DMTC was determined at pH 4.0, 7.0, 9.0 and in Milli-Q water at t = 0 and t = 5 days, plus one intermediate point in time (t = 1 day). At each pH, the percent hydrolysis of DMTC after 5 days was determined.
- Sampling intervals/times for pH measurements: The pH was measured at t = 0, t = 1 and t = 5 days.
- Sampling intervals/times for sterility check: Sterility check was performed at the end of the test.
- Sample storage conditions before analysis: Placed in an amber coloured vial and at stored at 2 - 10 °C.

Buffers

Buffer solutions were prepared as follows:

- pH 4.0
0.1 mol/L monopotassium citrate + 0.1 mol/L NaOH
A solution of 0.1 mol/L monopotassium citrate was prepared containing 11.50 g

monopotassium citrate in 500 mL Milli-Q water. To this solution, 90 mL of a 0.1 mol/L NaOH solution (prepared by dissolving 3.99 g NaOH in 1L Milli-Q water) was added and the volume was brought to 1L. The pH of this buffer solution was 3.98.

-pH 7.0

0.1 mol/L monopotassium phosphate + 0.1 mol/L NaOH

A solution of 0.1 mol/L monopotassium phosphate was prepared containing 6.84 g KH_2PO_4 in 500 mL Milli-Q water. To this solution, 295 mL of a 0.1 mol/L NaOH solution (prepared by dissolving 3.99 g NaOH in 1L Milli-Q water) was added and the volume was brought to 1L. The pH of this buffer solution was 6.99.

-pH 9.0

0.1 mol/L boric acid (in 0.1 mol/L KCl) + 0.1 mol/L NaOH

A solution of 0.1 mol/L boric acid in 0.1 mol/L KCl was prepared containing 3.09 g boric acid and 3.75 g KCl in 500 mL Milli-Q water. To this solution, 215 mL of a 0.1 mol/L NaOH solution (prepared by dissolving 3.99 g NaOH in 1L Milli-Q water) was added and the volume was brought to 1L. The pH of this buffer solution was 9.03.

All buffer solutions and Milli-Q water, used for preparation of the hydrolysis solutions, were sterilised by filtration over a 0.45 μm filter and nitrogen was bubbled through the buffer solutions and Milli-Q water for approximately 5 minutes.

Details on test conditions

The following internal analytical standards were used: Monoheptyltin trichloride (MHT); Diheptyltin dichloride (DHT); Tripropyltin chloride (TPT); Tetrapropyltin (TPPT). They were stored in the dark at $< -18^\circ\text{C}$. Stock solutions of DMTC in methanol with a concentration of approximately 10 mg/mL were prepared. Furthermore an internal standard solution was prepared containing all four internal standards in methanol. Stock solutions and internal standard solution were stored in the dark at $2-10^\circ\text{C}$.

PREPARATION OF HYDROLYSIS SOLUTIONS

For each test substance, a sufficient number of hydrolysis solutions per test condition (at least 12) were prepared by addition of 100 μL of a stock solution of the test substance in methanol (approximately 10 mg/mL, see above) to a vial. Subsequently, 10.0 mL of sterilised buffer solution pH 4.0, pH 7.0 or pH 9.0, or 10.0 mL of sterilised Milli-Q water, was added. Each vial was closed with a crimpcap closure. For DMTC, the initial concentration of the hydrolysis solutions was 102.1 mg/L. This initial concentration was <0.01 mol/L.

HYDROLYSIS

The preliminary tests (Tier 1) of the abiotic degradation of DMTC were carried out as described below. This procedure was based on OECD Guideline for Testing of Chemicals 111, Proposal for updating guideline 111 (Hydrolysis as a function of pH). In addition, the hydrolysis of DMTC was carried out in Milli-Q water.

-The hydrolysis tests were carried out at three pH values: 4.0, 7.0 and 9.0. The tests were also carried out in Milli-Q water. The pH value of one of each set of duplicate hydrolysis

solutions was determined with calibrated pH electrodes after cooling down to room temperature.

-The hydrolysis tests were carried out at 50 ± 1 °C using a thermostatically controlled enclosure (stove). The temperature of the stove was registered every hour during the hydrolysis tests.

-All hydrolysis tests were carried out in the dark to avoid photolytic interference.

-In order to exclude the occurrence of biodegradation of the test substance, glassware was sterilised at 120 °C for approximately 30 minutes before use. All buffer solutions and Milli-Q water were sterilised by filtration over a 0.45 µm filter. In order to check the effectiveness of these measures, a test for sterility was carried out at the end of the hydrolysis tests.

Hydrolysis solutions (pH 4.0, 7.0 and 9.0, and Milli-Q water) were incubated on a TSBA plate at 37 °C for at least three days.

-Oxygen was excluded by bubbling nitrogen through the buffer solutions and Milli-Q water for approximately 5 minutes, before preparation of the hydrolysis solutions.

-The concentration of DMTC in the hydrolysis solutions was determined in duplicate at $t = 0$ and $t = 5$ days for each pH value and in Milli-Q water, using the analytical method described. As a control measure, the concentration of DMTC in the hydrolysis solutions was also determined in duplicate at one intermediate point in time ($t = 1$ day).

-For each pH value at which the hydrolysis test was carried out, the percent hydrolysis after 5 days at 50 °C was calculated from the concentrations of DMTC measured at $t = 0$ and $t = 5$ days. The percent hydrolysis in Milli-Q water after 5 days at 50 °C was also calculated. If less than 10 % decrease in DMTC (i.e. Me₂SnEt₂ in GC-MS chromatogram) was observed after 5 days, the test substances were considered hydrolytically stable.

Duration of test: 5 d; **pH:** 4.0; **Temp:** 50 °C; **Initial conc. measured:** 103.2 mg/L

5 d; **pH:** 7.0; **Temp:** 50 °C; **Initial conc. measured:** 100.7 mg/L

5 d; **pH:** 9.0; **Temp:** 50 °C; **Initial conc. measured:** 94 mg/L

5 d; **pH:** Milli-Q Water; **Temp:** 50 °C; **Initial conc. measured:** 108.5 mg/L; **Number of replicates:** 2

Positive controls: no; **Negative controls:** no

Statistical methods: Mean concentrations of hydrolysis vessels calculated.

Results and discussion

Preliminary study

The percentage hydrolysis of the test substance DMTC (analysed after derivatisation to the corresponding ethyl compounds) was less than 10 % after incubation in buffer solutions pH 4.0, pH 7.0 and pH 9.0 and in Milli-Q water, for 5 days at 50 °C. Therefore, DMTC was considered hydrolytically stable and no further testing was performed.

Test performance - Remarks

QUALITY CRITERIA

Validation:

For the test substance, the recovery was between 70 % and 110 %. The blank buffer solutions and Milli-Q water did not show any peaks for DMTC (i.e. concentrations were < 1 mg/L).

All calibration graphs used for the determination of the concentration of DMTC in validation samples and in hydrolysis solutions were linear, i.e. the correlation coefficient r obtained after linear regression of the calibration graphs constructed from the organotin calibration solutions (Q-value (ratio peak area test substance / peak area internal standard) vs. concentration) was at least 0.99 (calibration graphs of DMTC in Milli-Q water: $r > 0.998$).

All QC samples were between 80 % and 120 % of the prepared concentration

Transformation products: no

Details on hydrolysis and appearance of transformation product(s)

RESULTS OF THE HYDROLYSIS TESTS

During the hydrolysis tests, the pH of all incubated hydrolysis solutions remained within ± 0.1 of the initial pH values. The pH of the hydrolysis solutions of DMTC in Milli-Q water (prepared concentration 102.1 mg/L) was approximately 3.5.

The sterility tests performed at the end of the hydrolysis tests did not show any bacterial activity. Biodegradation of the test substances in the hydrolysis solutions did not take place.

The percentage hydrolysis of DMTC was less than 10 % after incubation in buffer solutions pH 4.0, pH 7.0 and pH 9.0 and in Milli-Q water, for 5 days at 50 °C.

It should be noted that the analytical method applied involves derivatisation of the test substance with STEB to the corresponding ethyl compounds (dimethyltin diethyl). As a consequence, the alkyltin moieties of DMTC were monitored in the hydrolysis tests, but not the chloride ligands. Therefore, the alkyltin moiety, DMT is hydrolytically stable ($t_{1/2} > 1$ year at 25°C).

Total recovery of test substance (in %)

%Recovery: 95.4; **pH:** 4; **Temp.** 50 °C; **Duration** 5 d

%Recovery: 102.9; **pH:** 7; **Temp:** 50 °C; **Duration:** 5 d;

%Recovery 102.5; **pH:** 9; **Temp:** 50 °C; **Duration:** 5 d;

Details on results

Analysis of the validity and quality criteria solutions showed the method of analysis to be suitable and the test system to be acceptable.

Any other information on results incl. tables

Table 1. Results of the preliminary hydrolysis test of DMTC in buffer solutions at pH 4.0, 7.0 and 9.0 and in Milli-Q water at 50 °C

Hydrolysis solutions	Measured concentration of DMTC in hydrolysis solutions (mg/L)*			Relative difference between concentration at t = 5 days and t = 0 (%)
	t = 0	t = 1 day	t = 5 days	
pH 4.0	104.0	99.6	95.1	-7.6
	102.4	101.5	95.7	
	Mean: 103.2	Mean: 100.5	Mean: 95.4	
pH 7.0	98.8	100.0	104.9	+2.2
	102.6	109.9	101.0	
	Mean: 100.7	Mean: 105.0	Mean: 102.9	
pH 9.0	93.2	89.5	102.7	+9.1
	94.7	91.8	102.4	
	Mean: 94.0	Mean: 90.6	Mean: 102.5	
Milli-Q water	108.3	84.3	101.0	-6.2
	108.8	98.0	102.7	
	Mean: 108.5	Mean: 91.1	Mean: 101.8	

All calculations in this table were carried out with non-rounded numbers. However, rounded values are reported.

*The GC-MS analysis of the extracts of all hydrolysis solutions (t = 0, t = 1 day and t = 5 days) was performed simultaneously per test condition (i.e. buffer solutions pH 4.0, pH 7.0 and pH 9.0 and Milli-Q water), except for the extracts of the following hydrolysis solutions: buffer solution pH 4.0 at t = 1 day, buffer solution pH 7.0 at t = 5 days and Milli-Q water at t = 0. The extracts of these hydrolysis solutions were reinjected into the GC-MS using the same calibration graph as for the extracts of the other hydrolysis solutions, due to chromatographic errors in the first analysis of these extracts.

Phototransformation

Purpose flag: supporting study; **Study result type:** no data; **Study period:** not reported;

Reliability score 4 (not assignable) was given by the registrant because it was a secondary source with no methodological information.

Reference type: secondary source; **Author** Dobson, S., Howe, P.D. & Floyd, P.; **Year** 2006;

Title Mono- and disubstituted methyltin, butyltin, and octyltin compounds

Bibliographic source Concise International Chemical Assessment Document 73

Materials and methods

Principles of method if other than guideline: Methodological details not reported

GLP compliance: no according to the registrant data

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline

Results and discussions

Any other information on results incl. tables

Photodegradation constant was reported to be $1.8 \times 10^{-12} \text{cm}^3/\text{molecule/second}$ (25 °C).

4.1.5 Bioaccumulation test on fish**Not available****4.1.6 Bioaccumulation test with other organisms****Bioaccumulation study 1****Purpose flag:** supporting study; **Study result type:** experimental result; **Study period:** not reported;

Reliability score 4 (not assignable) was given by the registrant because it was a review article with no methodological information. Original source not available.

Reference type: review article or handbook; **Author:** Hall, L.W. & Pinkey, A.E.; **Year:** 1985; **Title** Acute and Sublethal Effects of Organotin Compounds on Aquatic Biota: An Interperative Literature Evaluation**Bibliographic source** CRC Critical Reviews in Toxicology. Volume 14, Issue 2. Primary source: Ishii, T. (1982) Tin in Marine Algae. Bull. Jpn. Soc. Sci. Fish., 48, 1609**Materials and methods****Test guideline:** no guideline followed; **GLP compliance:** no according to the registrant**Identity of test material same as for substance defined in section 1 (if not read-across):** yes**Test material form:** crystalline; **Radiolabelling:** no data; **Vehicle:** no data;**Test organisms (species):** other: Alga: natural community**Route of exposure:** aqueous**Test type:** static; **Water media type:** saltwater; **Total exposure / uptake duration:** 5 d;**Test temperature:** 15 ± 2 °C; **Nominal and measured concentrations:** 0.05 mg/L**Results and discussions****Bioaccumulation factor: Conc. in environment / dose:** 0.05**Type:** BCF; **Value:** 1.6-5.9 dimensionless; **Basis:** no data

Bioaccumulation study 2

Purpose flag: supporting study; **Study result type:** experimental result

Study period: not reported;

Reliability score 4 (not assignable) was given by the registrant because of limited methodological information available. No guidelines followed.

Reference type: publication; **Author:** Hadjispyrou, S., Kungolos, A. & Anagnostopoulos, A.; **Year:** 2001; **Title:** Toxicity, Bioaccumulation, and Interactive Effects of Organotin, Cadmium, and Chromium on *Artemia franciscana*

Bibliographic source Ecotoxicology and Environmental Safety 49, 179-186. Environmental Research, Section B

Materials and methods

Test guideline: no guideline followed

Principles of method if other than guideline: Bioaccumulation study using *Artemia franciscana*.

GLP compliance: no according to the registrant

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline; **Radiolabelling:** no; **Vehicle:** no

Test organisms (species): other aquatic crustacea: *Artemia franciscana*

Details on test organisms

TEST ORGANISM

- Common name: brine shrimp
- Source: cysts bought from Creasal, Belgium
- Age at study initiation (mean and range, SD): less than 24 hours

Route of exposure: aqueous; **Test type:** static; **Water media type:** saltwater

Total exposure / uptake duration: 24 h

Test temperature: 25 ± 1 °C; **Salinity:** 35 ppt

Nominal and measured concentrations: 10 and 100 mg/L Sn in water.

Details on test conditions

TEST SYSTEM

- Test vessel: glass beakers
- Material, size, headspace, fill volume: 100 mL test solution

- No. of organisms per vessel: 1000 neonates

TEST MEDIUM / WATER PARAMETERS

- Source/preparation of dilution water: 26.4 g/l NaCl, 0.84 g/l KCl, 1.26 g/l CaCl₂, 2.15 g/l MgCl₂, 2.72 g/l MgSO₄, 0.17 g/l NaHCO₃ and 0.03 g/l H₃BO₃ dissolved in distilled water.

Details on estimation of bioconcentration

The average weight of one Artemia neonate was found to be 3 µg. Assuming that the amount of tin in each solution that was measured by ICP-AES was absorbed by 1000 organisms, and having estimated the average weight of Artemia neonates, the quantity of tin absorbed by each animal and the bioconcentration factor were determined.

Results and discussions

Bioaccumulation factor

Conc. in environment / dose 10 mg Sn/L, **Type** BCF, **Value** 50 dimensionless

Basis no data

Conc. in environment / dose 100 mg Sn/L, **Type** BCF, **Value** 6 dimensionless

Basis: no data

Bioaccumulation study 3

Purpose flag: supporting study; **Study result type** (Q)SAR

Study period: not reported;

Reliability score 3 (not reliable) was given by the registrant because it was a secondary source of information from a review dossier. Original source was not available and there was very limited methodological information according to the registrant. QSAR method was not fully validated for substances containing a metal.

Reference type: review article or handbook; **Year** 2006; **Report date** 2006-07-23

Materials and methods

Test guideline: no guideline followed

Principles of method if other than guideline

Current versions of EPIWIN, developed by Syracuse Research Corporation, have not been validated for estimating endpoints for chemicals that contain metals in their molecular structure; therefore, the estimated values derived from the EPIWIN models should be used with caution.

GLP compliance: no according to the registrant

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline; **Radiolabelling:** no; **Vehicle:** no

Results and discussions

Bioaccumulation factor

Type: BCF; **Value** 3.16 L/kg; **Basis:** no data

Type other: log BCF; **Value:** 0.5; **Basis** no data

4.1.7 Short-term toxicity to fish

Fish acute toxicity – Study 1

Purpose flag: key study; **Study result type:** experimental result

Study period: 14 October 2002 - 6 December 2002;

Reliability score 1 (reliable without restriction) was given by the registrant because the study was conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which did not affect the quality of the relevant results, according to the registrant.

Reference type study report; **Report date** 2003-09-14

Guideline OECD Guideline 203 (Fish, Acute Toxicity Test); **Deviations** yes see below

Guideline EU Method C.1 (Acute Toxicity for Fish); **Deviations** yes see below

Principles of method if other than guideline

Deviations from the protocol

- At the request of the sponsor the test aquaria were not aerated during the first three hours of the test. This was to check whether a decrease in oxygen level occurred and aeration was necessary.
- The test substance was stored in a refrigerator until 23 August 2001 and not in a freezer.
- At the start of the test the temperature was 25.1 °C, which is just above the intended temperature of 24 ± 1 °C.
- Some extra measurements of pH, oxygen concentration and temperature were carried out.
- All samples for chemical analysis were taken in duplicate, except the controls, which were taken as single samples.
- The samples taken as reserve were stored in a freezer until chemical analysis. From 1 November 2002, the Department of Environmental Toxicology has been discontinued; the

remaining ecotoxicological activities were finalised by the Project Organisation Ecotoxicology.

- Drs H.H. Ernrnen replaced Dr W. Wouters as management from February 11, 2003.
- At the request of the sponsor the title of the study was changed from "Semistatic acute toxicity test with dichlorodimethylstannane and the zebra fish *Brachydanio rerio* " into "Dichlorodimethylstannane (CAS # 753-73-1): Semistatic acute toxicity test with the zebra fish *Brachydanio rerio*"

There were no further deviations from the protocol.

The deviations have not affected the results of the study.

GLP compliance: yes (incl. certificate)

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline

Details on test material:

- Name of test material (as cited in study report): dichlorodimethylstannane
- Physical state: solid
- Storage condition of test material: <-18 °C

Analytical monitoring: yes

Details on sampling

The actual concentrations of dichlorodimethylstannane in the exposure media were determined by chemical analysis. Two sets of samples of the newly prepared (clean) exposure medium were taken in duplicate together with two (single) sets of the control at t = 0h and t = 48h. Two sets of the same (spent) media were sampled again at t = 48h and at t = 96h. All samples were aliquots of exactly 10 ml; they were taken in plastic Corning tubes. One set of samples was transferred to the analytical laboratories immediately after sampling. All other samples were stored in a freezer.

Vehicle: no

Details on test solutions:

Concentrations for the final test were determined in a pre-test programme. Two final tests were carried out; the first test had to be repeated due to a mistake in the dosing. In the second final test one concentration of dichlorodimethylstannane (100 mg/l) was tested. The test substance was dissolved directly in DSWL-E.

Test organisms (species): *Brachydanio rerio* (new name: *Danio rerio*)

TEST ORGANISM

- Common name: zebra fish
- Source: Atlanta, Hellevoetsluis, the Netherlands
- Length at study initiation (length definition, mean, range and SD): 2.3 ± 0.19 cm

- Weight at study initiation (mean and range, SD): 0.11 ± 0.03 g
- Feeding during test: none

Test type: semi-static; **Water media type:** freshwater; **Limit test:** yes

Total exposure duration: 96 h; **Post exposure observation period:** None

Hardness: 209 mg/L; **Test temperature:** 24.0 - 25.1 °C; **pH:** 6.4 - 8.2

Dissolved oxygen: 7.2 - 8.6 mg O₂/l; **Nominal and measured concentrations:**

0 and 100 mg/L;

TEST SYSTEM

- Test vessel: 3.2 litre all-glass aquaria
- Type (delete if not applicable): closed
- Material, size, headspace, fill volume: 3 litre exposure medium
- Aeration: slightly from t = 3 h
- Renewal rate of test solution (frequency/flow rate): at t = 48 hours
- No. of organisms per vessel: 10
- No. of vessels per concentration (replicates): 1
- No. of vessels per control (replicates): 1

TEST MEDIUM / WATER PARAMETERS

- Source/preparation of dilution water: groundwater obtained from a locality near Linschoten, The Netherlands, with the following salts added to create the synthetic medium DSWL-E: Na⁺ 1.22 mmol/l, K⁺ 0.20 mmol/l, Ca²⁺ 1.38 mmol/l, Mg²⁺ 0.71 mmol/l, Cl⁻ 2.81 mmol/l and sulphate 0.65 mmol/l.
- Total organic carbon: 1.91 mg/L

OTHER TEST CONDITIONS

- Photoperiod: 16 hours light/8 hours dark

EFFECT PARAMETERS MEASURED (with observation intervals if applicable): The dead and living animals were counted and if applicable the dead ones removed after 3, 24, 48, 72 and 96 hours. At the same time the condition (swimming behaviour, colour or any other visually observable morphological or behavioural criterion) of the survivors was compared with that of the control animals.

Reference substance (positive control): no

Duration 96 h : **Endpoint** LC50; **Effect conc.** > 100 mg/L;

Nominal/Measured: nominal; **Conc. based on** test mat.; **Basis for effect:** mortality

Duration: 96 h; **Endpoint:** NOEC; **Effect conc.** >= 100 mg/L;

Nominal/Measured: nominal; **Conc. based on** test mat.; **Basis for effect** other: mortality and condition

Details on results: There was no mortality in the control medium at the end of the test. At 100 mg/l there were no adverse effects observed with respect to survival or condition.

Reported statistics and error estimates: Not required.

Validity criteria: There was no mortality in the control.

The dissolved oxygen concentration was above 5.1 mg/l ($\geq 60\%$ of the air saturation value at 24 °C).

The test substance concentration was satisfactorily maintained above 80% of the nominal concentration.

The test fulfilled the validity criteria as required in the guideline.

The results of the test were (in mg/l):

Parameter	Effect	Concentration
3 h LC50	mortality	>100
24 h LC50	mortality	>100
48 h LC50	mortality	>100
72 h LC50	mortality	>100
96 h LC50	mortality	>100
96 h LC100	mortality	>100
96 h NOEC	mortality	≥ 100
96 h NOEC	condition	≥ 100

Fish acute toxicity – Study 2

Purpose flag: supporting study; **Study result type:** experimental result

Study period: not reported;

Reliability score 4 (not assignable) was given by the registrant since the documentation was insufficient for assessment. Test was conducted for 96 hours; however, results were reported for 48 hours only. No data were provided to assess exposure conditions (e.g., pH, DO conc., etc.). Test organism response data by concentration not provided. Response data for controls not provided. Final concentration of vehicle not provided. No information on the stability of exposure concentrations.

Reference type publication

Author Nagase, H., Hamasaki, T., Sato, T., Kito, H., Yoshioka, Y. & Ose, Y.

Year 1991

Title Structure-activity relationships for organotin compounds on the red killifish *Oryzias latipes*

Bibliographic source Applied Organometallic Chemistry 5, 91-97

Test guideline: according to OECD Guideline 203 (Fish, Acute Toxicity Test)

Deviations yes, Only conducted for 48 hours; **GLP compliance:** no according to the registrant data

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline; **Analytical monitoring:** no; **Vehicle:** no

Details on test solutions: Dissolved directly in water

Test organisms (species): *Oryzias latipes*

TEST ORGANISM

- Common name: Red killifish

ACCLIMATION

- Acclimation period: at least 7 days
- Acclimation conditions: In dechlorinated tap water. Same as test conditions

Test type: semi-static; **Water media type:** freshwater; **Limit test:** no

Total exposure duration: 96 h; **Test temperature:** 20 ± 1 °C

Nominal and measured concentrations: At least five concentrations (details not provided).

TEST SYSTEM

- Test vessel: 2 litre vessel
- No. of organisms per vessel: 10

OTHER TEST CONDITIONS

- Photoperiod: 16 hours light/8 hours dark

TEST CONCENTRATIONS

- Spacing factor for test concentrations: 1.8

EFFECT PARAMETERS MEASURED (with observation intervals if applicable): mortality

Reference substance (positive control): no

Results and discussions

Effect concentrations

Duration : 48 h; **Endpoint:** LC50; **Effect conc.:** 6 mg/L; **Nominal/Measured:** nominal;
Conc. based on: test mat.; **Basis for effect:** mortality

Remarks (e.g. 95% CL) Reported as 0.0273 mmol/l

Fish acute toxicity – Study 3

Purpose flag supporting study; **Study result type** experimental result; **Study period** not reported;

Reliability score 4 (not assignable) was given by the registrant because it was a review article with no methodological information.

Reference type review article or handbook

Author Summer, K.H., Klein, D. & Greim, H.; **Year** 2003

Title Ecological and Toxicological Aspects of Mono and Disubstituted Methyl-, Butyl-, Octyl-, and Dodecyltin Compounds - Update 2002

Bibliographic source Organotin Environmental Programme (ORTEP) Association, The Hague, The Netherlands. Result taken from Danil'chenko O.P. (1977). The Sensitivity of Fish Embryos to the Effect of Toxicants. J. Ichthyol. 17, 455-463

Materials and methods

Principles of method if other than guideline

No methodological information available.

GLP compliance: no according to the registrant data

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline; **Analytical monitoring:** no data; **Vehicle:** no data

Test organisms (species): other: Misgurnus fossilis (?); **Test type:** no data

Water media type: no data; **Total exposure duration:** **Remarks** no data

Results and discussions

Endpoint other: maximum acceptable concentration for development

Effect conc. 100 1000 mg/L

Nominal/Measured: no data; **Conc. based on** no data; **Basis for effect** no data

Remarks (e.g. 95% CL) no duration specified;

4.1.8 Short-term toxicity to aquatic invertebrates

Invertebrates acute toxicity – Study 1

Purpose flag: key study; **Study result type:** experimental result

Study period: 24th September 2002 - 18th October 2002

Reliability score 1 (reliable without restriction) was given by the registrant because the study was conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which did not affect the quality of the relevant results.

Reference type study report; **Year:** 2003 **Report date:** 2003-09-14

Materials and methods

Test guideline:

Qualifier according to **Guideline:** OECD Guideline 202 (Daphnia sp. Acute Immobilisation Test); **Deviations:** no;

Qualifier according to **Guideline** EU Method C.2 (Acute Toxicity for Daphnia); **Deviations** no

GLP compliance: yes (incl. certificate)

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline

Details on test material

- Name of test material (as cited in study report): dichlorodimethylstannane
- Physical state: solid
- Storage condition of test material: <-18 °C

Analytical monitoring: yes

Details on sampling

The actual concentrations of dichlorodimethylstannane in the exposure media were determined by chemical analysis. Samples of all exposure media were taken in duplicate at

the start of the test ($t = 0\text{h}$) and at the end of the test ($t = 48\text{h}$). Aliquots of 100 mL from the control, 1.8, 3.2 and 5.6 mg/l solutions or 10 mL from the 10, 18, 32 and 56 mg/l solutions were taken in plastic Coming tubes.

One replicate of each $t = 0\text{h}$ sample (all newly prepared solutions) and one replicate sample of the spent solutions containing nominal 3.2, 18 and 56 mg/l, taken at $t = 48\text{h}$ (end of the test) were transferred to the analytical laboratories immediately after sampling. All other samples were stored as reserve in a freezer.

Vehicle: no

Test organisms (species): *Daphnia magna*

Details on test organisms

The test organism was cultured in the laboratory under standard conditions in the dilution water, used for the test. The quality of the daphnia was checked by testing with $\text{K}_2\text{Cr}_2\text{O}_7$, the 24h and 48h EC50 value were within the ranges of 0.7 - 1.7 mg/l and 0.6 - 1.5 mg/l respectively. Every week a number of cultures is started with ca. 150 daphnids of the same age (about one day) in ca. 4 litres of dilution water. The cultures are fed daily with ca. 4×10^9 algal cells (*Chlorella*) and ca. 0.13 g of yeast per 4 litre. The medium is completely replaced at least once a week; at the same time all young born are removed. The cultures are kept at $20 \pm 1^\circ\text{C}$ under a 16h light and 8h dark regime with transition periods of ca. 30 minutes. After 4 weeks the cultures are discarded.

Test type: static; **Water media type:** freshwater; **Limit test:** no

Total exposure duration: 48 h; **Post exposure observation period:** None

Hardness: 210 mg/L CaCO_3 ; **Test temperature:** 19.5°C ; **pH:** 6.9 - 8.2

Dissolved oxygen: 8.7 - 9.0 mg/L; **Nominal and measured concentrations**

Nominal: 1.8, 3.2, 5.6, 10, 18, 32 and 56 mg/L.

Details on test conditions

TEST SYSTEM

The test was performed in 150 mL all-glass beakers, each containing 100 mL of exposure medium. Four beakers containing 5 daphnids each were used for each exposure medium. The exposure media were not aerated or replaced. The daphnids were not fed during the test.

TEST MEDIUM / WATER PARAMETERS

- Source/preparation of dilution water: groundwater obtained from a locality near Linschoten, The Netherlands, with the following salts added to create the synthetic medium DSWL-E:
 Na^+ 1.22 mmol/l, K^+ 0.20 mmol/l, Ca^{2+} 1.38 mmol/l, Mg^{2+} 0.71 mmol/l, Cl^- 2.81 mmol/l and sulphate 0.65 mmol/l.
- Total organic carbon: 1.91 mg/L

OTHER TEST CONDITIONS

- Photoperiod: 16h light/8h dark regime

EFFECT PARAMETERS MEASURED (with observation intervals if applicable) :

The mobile and, if applicable the immobile animals, were counted after 24 hours and at the end of the test (animals not able to swim within 15 seconds after gentle agitation of the test containers). At the same time the immobile animals were removed and the condition (swimming behaviour, colour or any other visually observable morphological or behavioural criterion) of the mobile animals was compared with that of the control animals. The condition of the daphnids from the exposure media of 5.6 mg/l and higher was also checked under a microscope at the end of the test.

TEST CONCENTRATIONS

- Spacing factor for test concentrations: ca. 1.8
- Range finding study
- Results used to determine the conditions for the definitive study: yes

Reference substance (positive control): no

Results and discussions**Effect concentrations**

Duration: 48 h; **Endpoint:** EC50; **Effect conc:** 17 mg/L; **Nominal/Measured:** nominal; **Conc. based on:** test mat.; **Basis for effect:** mobility; **Remarks (e.g. 95% CL):** 95% C.I. 12-24 mg/l

Duration: 48 h; **Endpoint:** EC100; **Effect conc.** > 56 mg/L; **Nominal/Measured:** nominal; **Conc. based on:** test mat.; **Basis for effect:** mobility;

Duration: 48 h; **Endpoint:** NOEC; **Effect conc.:** 3.2 mg/L; **Nominal/Measured:** nominal; **Conc. based on:** test mat.; **Basis for effect:** mobility

Duration: 48 h; **Endpoint:** NOEC; **Effect conc:** 3.2 mg/L; **Nominal/Measured:** nominal; **Conc. based on:** test mat.; **Basis for effect:** other: condition; **Results with reference substance:** N/A

Analysis of study samples

There was almost no difference between the nominal and measured concentrations (see Table 1).

Table 1: Concentration Analysis

Sampling time (h)	Nominal concentration (mg/l)	Measured concentration (mg/l)
0	0	< 0.04

0	1.8	1.75
0	3.2	3.62
0	5.6	5.74
0	10	12.1
0	18	18.3
0	32	34.6
0	56	55.5
48	0	< 0.04
48	3.2	3.78
48	18	20.0
48	56	59.0

Immobilization and other observations on the test animals

There were no immobile animals in the control medium at the end of the test. At 24 and 48 hours, all animals at 1.8 and 3.2 mg/l were mobile and in good condition. At t = 24h, one animal at 18 mg/l and 6 animals at 56 mg/l were dead. At t = 48h, death occurred in animals at concentrations of 5.6 mg/l and higher. At both 24h and 48h time points, non-lethal effects, with respect to condition, were observed at concentrations of 5.6 mg/l and higher. At 48h, partial immobility was observed at concentrations of 5.6, 10, 18, 32 and 56 mg/l (see Table 2).

Table 2: Number of mobile animals in the control and exposure media of dichlorodimethylstannane

Time (h)	Concentration of the test substance (mg/l)							
	0	1.8	3.2	5.6	10	18	32	56
0	20	20	20	20	20	20	20	20
24	20	20	20	20	20	19	20	14

48	20	20	20	13	8	14	11	1
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The results of the test were (95% confidence interval in parentheses):

Parameter (h = hours)	Effect	Concentration (in mg/L)
24h EC50	mobility	> 56
48h EC50	mobility	17 (12 - 24)
48h EC100	mobility	> 56
48h NOEC	mobility	3.2
48h NOEC	condition	3.2

Invertebrates acute toxicity – Study 2

Purpose flag: supporting study; **Study result type:** experimental result; **Study period:** not reported;

Reliability score 4 (not assignable) was given by the registrant. It was a guideline study but without detailed documentation; test was conducted for less than 48 hours. Actual test concentrations and control response were not reported.

Reference type publication; **Author** Vighi, M. & Calamari, D.; **Year** 1985

Title QSARs For Organotin Compounds on Daphnia Magna

Bibliographic source Chemosphere, Vol. 14 No.11/12, pp 1925-1932

Materials and methods

Test guideline: **Qualifier** according to

Guideline: other guideline: Proposed OECD Acute Immobilization Test (1981)

Deviations: no data; **GLP compliance:** no according to the registrant

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline; **Analytical monitoring:** no data; **Vehicle:** no

Test organisms (species): Daphnia magna

Details on test organisms: Less than 24 hours old.

Test type: no data; **Water media type:** freshwater; **Limit test:** no

Total exposure duration: 24 h; **Test conditions:** **Hardness:** 200 mg/L CaCO₃

Test temperature: a 20 °C; **pH:** 7.5; **Dissolved oxygen:** > 70% oxygen saturation at end of test

Details on test conditions: 20 animals, five for each tank.

Reference substance (positive control): no

Results and discussions

Effect concentrations

Duration: 24 h; **Endpoint:** EC₅₀; **Effect conc:** 88 mg/L; **Nominal/Measured:** nominal; **Conc. based on:** test mat.; **Basis for effect:** mortality

Reported statistics and error estimates

The EC₅₀ and slopes were obtained after plotting mortality data on log-probability paper, according to the method of Litchfield and Wilcoxon (1949).

Invertebrates acute toxicity – Study 3

Purpose flag: supporting study; **Study result type:** experimental result

Study period: not reported;

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which did not affect the quality of the relevant results.

Reference type: publication; **Author** Kungolos, A., Hadjispyrou, S., Petala, M., Tsiridis, V., Samaras, P. & Sakellaropoulos; **Year** 2004

Title Toxic properties of metals and organotin compounds and their interactions on Daphnia magna and Vibrio fischeri

Bibliographic source Water, Air, and Soil Pollution: Focus 4: 101–110

Title ASSESSMENT OF TOXICITY AND BIOACCUMULATION OF ORGANOTIN COMPOUNDS

Bibliographic source Proceedings of the 7th International Conference on Environmental Science and Technology, pp. 499 – 505, Sept. 2001

Materials and methods

Principles of method if other than guideline

The toxicity tests on *D. magna* were carried out using a commercial toxicity test (Daphtoxkit F magna, 1996) provided by Microbiotest Inc., Belgium. Experiments with Daphtoxkit F are based on the immobilization of the crustacean *D. magna* due to the action of toxicants (International Standards Organisation, 1982).

GLP compliance: no according to the registrant data

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline; **Analytical monitoring:** no; **Vehicle:** no

Test organisms (species): *Daphnia magna*

Details on test organisms

The ephippia of *D. magna* were hatched from dormant eggs in three days under continuous illumination of 6000 lux at 20 °C. Neonates (younger than 24 hours) were exposed to the samples for 24 hours at a temperature of 20°C in darkness.

Test type: static; **Water media type:** freshwater; **Limit test:** no

Total exposure duration: 24 h; **Hardness:** NDA; **Test temperature:** 20 °C; **pH:** NDA

Dissolved oxygen: NDA; **Salinity:** N/A; **Nominal and measured concentrations:** NDA;

Details on test conditions

Neonates (younger than 24 hours) were exposed to the samples for 24 hours at a temperature of 20 °C in darkness. Twenty neonates were used for each concentration or combination of concentrations examined in a series of 4 well; each well containing 10 mL toxicant solution and five neonates. The solutions of the tested substances were prepared using a standard fresh water medium (International Standards Organisation, 1982).

Reference substance (positive control): no

Results and discussions

Effect concentrations

Duration: 24 h; **Endpoint:** EC50; **Effect conc. :** 19.27 mg/L; **Nominal/Measured:** nominal; **Conc. based on:** element Sn; **Basis for effect:** mortality; **Remarks (e.g. 95% CL):** (95% CL of 12.20 - 30.36)

Reported statistics and error estimates

The effective concentrations causing 50% immobilization to *D. magna* was calculated according to Probit model (EPA, 1993).

Invertebrates acute toxicity – Study 4

Purpose flag: supporting study; **Study result type:** experimental result;

Study period: not reported;

Reliability score 2 (reliable with restrictions) was given by the registrant because the study was conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which did not affect the quality of the relevant results.

Reference type: publication; **Author** Hadjispyrou, S., Kungolos, A. & Anagnostopoulos, A.; **Year** 2001

Title Toxicity, Bioaccumulation, and Interactive Effects of Organotin, Cadmium, and Chromium on *Artemia franciscana*

Bibliographic source Ecotoxicology and Environmental Safety 49: 179-186. Environmental Research, Section B

Title ASSESSMENT OF TOXICITY AND BIOACCUMULATION OF ORGANOTIN COMPOUNDS

Bibliographic source Proceedings of the 7th International Conference on Environmental Science and Technology, pp. 499 – 505, Sept. 2001

Materials and methods

Test guideline: no guideline followed;

Principles of method if other than guideline: The acute toxicity of dimethyltin dichloride to *Artemia franciscana* was examined in a 24 hour salt-water study.

GLP compliance: no according to the registrant data; **Identity of test material same as for substance defined in section 1 (if not read-across):** yes; **Test material form:** crystalline

Analytical monitoring: no data; **Vehicle:** no;

Test organisms (species): other: *Artemia franciscana*;

Details on test organisms

TEST ORGANISM

- Common name: brine shrimp
- Strain: *Artemia franciscana*
- Source: Creasel, Belgium
- Age at study initiation (mean and range, SD): < 24 hours
- Weight at study initiation (mean and range, SD): NDA

- Length at study initiation (length definition, mean, range and SD): NDA
- Valve height at study initiation, for shell deposition study (mean and range, SD): N/A
- Peripheral shell growth removed prior to test initiation: N/A
- Method of breeding: The Artemia neonates were hatched with the help of a Novital 504 incubator. Cyst hatching was initiated 48 hours prior to the start of the toxicity test. The Artemia cysts were transferred into Petri dishes together with 12 mL of standard seawater. The hatching Petri dish was exposed to a light source (1000-4000 lx) for one hour and then incubated in darkness for 24 hours at 25 ± 1 °C. After 24 hours, the larvae were transferred with a micropipet to a new hatching Petri dish containing fresh standard seawater. The Petri dish was incubated for another 24 hours, in the dark, at 25 ± 1 °C, to allow the larvae to molt to the second or third instar stage. Before the start of the experiment, the organisms were transferred into rinsing cuvettes, in the appropriate test solution, thus minimizing dilution of the test solution during transfer.
- Feeding during test: NDA

Test type: static; **Water media type:** saltwater; **Limit test:** no;

Total exposure duration: 24 h; **Post exposure observation period:** None

Hardness: NDA; **Test temperature:** 25 ± 1 °C; **pH:** NDA; **Dissolved oxygen:** NDA

Salinity: 35 ppt;

Nominal and measured concentrations

Range finder: 0.01, 0.1, 1, 10 and 100 mg/L

Main test: 70, 75, 79, 81, 83, 85, 90 and 95 mg/L Sn

Details on test conditions

TEST SYSTEM

- Test vessel: multiwell plate
- Type (delete if not applicable): closed
- Material, size, headspace, fill volume: 5 mL solution
- Aeration: N/A
- Type of flow-through (e.g. peristaltic or proportional diluter): N/A
- Renewal rate of test solution (frequency/flow rate): N/A
- No. of organisms per vessel: 10
- No. of vessels per concentration (replicates): 2
- No. of vessels per control (replicates): NDA
- No. of vessels per vehicle control (replicates): NDA
- Biomass loading rate: NDA

TEST MEDIUM / WATER PARAMETERS

Standard seawater with a salinity of 35 ppt, prepared in the laboratory, was used as a hatching medium. Standard seawater was prepared by dissolving the following substances in distilled and deionized water: NaCl, 26.4 g/L; KCl, 0.84 g/L; CaCl₂, 1.26 g/L; MgCl₂, 2.15 g/L; MgSO₄, 2.72 g/L; NaHCO₃, 0.17 g/L; and H₃BO₃, 0.03 g/L.

OTHER TEST CONDITIONS

- Adjustment of pH: None
- Photoperiod: conducted in dark
- Light intensity: N/A

EFFECT PARAMETERS MEASURED (with observation intervals if applicable): mortality at end of test

TEST CONCENTRATIONS

- Spacing factor for test concentrations: 1.05 - 1.1
- Justification for using less concentrations than requested by guideline: N/A

Reference substance (positive control): no

Results and discussions

Effect concentrations

Duration: 24 h; **Endpoint:** LC50; **Effect conc.:** 80.7 mg/L; **Nominal/Measured:** nominal; **Conc. based on:** element Sn; **Basis for effect:** mortality; **Remarks (e.g. 95% CL)** 95% CL (78.0 - 82.3);

Reported statistics and error estimates

The LC50 determination was done by means of a specific computer BASIC program.

Invertebrates acute toxicity – Study 5

Purpose flag: supporting study; **Study result type:** no data; **Study period:** not reported;

Reliability score 4 (not assignable) was given by the registrant because it was a review paper quoting a result only. No methodological information available.

Reference type: review article or handbook; **Author:** Eisler, R; **Year:** 1989

Title: Tin Hazards to Fish, Wildlife and Invertebrates: A Synoptic Review

Bibliographic source Biological Report 85 (1.15). Contaminant Hazard Reviews Report No. 15.

Materials and methods

Principles of method if other than guideline

No methodological information available.

GLP compliance: no according to the registrant data

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline

Analytical monitoring: no data; **Vehicle:** no data;

Test organisms (species):

other aquatic crustacea: *Rithropanopeus harrisi*

Total exposure duration: 14 d

Reference substance (positive control): no data

Results and discussions

Effect concentrations

Duration: 14 d; **Endpoint:** LC50; **Effect conc. :** > 10 <= 20 mg/L

Nominal/Measured: no data; **Conc. based on:** test mat.; **Basis for effect:** mortality

Invertebrates acute toxicity – Study 6

Purpose flag: supporting study; **Study result type:** no data; **Study period:** not reported;

Reliability score 4 (not assignable) was given by the registrant because the value was taken from a review paper, with no methodological information.

Reference type: review article or handbook; **Author** Summer K.H., Klein, D. & Greim, H.; **Year** 2003

Title Ecological and Toxicological Aspects of Mono and Disubstituted Methyl-, Butyl-, Octyl-, and Dodecyltin Compounds - Update 2002

Bibliographic source Organotin Environmental Programme (ORTEP) Association, The Hague, The Netherlands. Primary source: Sun, H.W., Huang, G.L., Dai, S.G. & Chen, T.Y. (1997) Toxicol Environ Chem 60: 75-85

Materials and methods

Principles of method if other than guideline: No methodological information available.

GLP compliance: no according to the registrant

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline; **Analytical monitoring:** no data; **Vehicle:** no data;

Test organisms (species): other: *Brachionus plicatilis*

Test type: no data; **Water media type:** no data; **Total exposure duration:** 24 h

Reference substance (positive control): no data

Results and discussions

Effect concentrations

Duration 24 h; **Endpoint** LC50; **Effect conc.** 74 mg/L; **Nominal/Measured** no data; **Conc. based on** no data;

4.1.9 Algal growth inhibition tests

Toxicity to aquatic algae – study 1

Purpose flag: key study; **Study result type:** experimental result; **Study period** 25 September 2002 - 18 October 2002;

Reliability score 1 (reliable without restriction) was given by the registrant because the study was conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which did not affect the quality of the relevant results.

Reference type: study report; **Year:** 2003; **Report date** 2003-09-08

Materials and methods

Test guideline: according to OECD Guideline 201 (Alga, Growth Inhibition Test)

Deviations: yes 150 mg/l NaHCO₃, not 50 mg/l as specified in the OECD Guideline

Test guideline: according to EU Method C.3 (Algal Inhibition test); **Deviations:** no

GLP compliance: yes (incl. certificate)

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline

Details on test material

- Name of test material (as cited in study report): dichlorodimethylstannane
- Physical state: solid
- Storage condition of test material: <18 °C

Analytical monitoring: yes

Details on sampling

At the start of the growth inhibition test, duplicate samples were taken from all media (without algae) containing all nominal test substance concentrations tested, together with a control. At the end of the test, duplicate samples were taken from the media containing the same test substance concentrations with algae. The sample volume of the control and the two lowest test substance concentrations was 100 ml. The sample volume of the three highest test substance concentrations was 10 ml. One of the duplicate sample series at the start and at the end of the test was analysed.

Vehicle: no

Details on test solutions

The following solutions of the test substance were made:

- For the range-finding test, 56.3 mg test substance was dissolved in 500 mL algal medium. Dilutions were then prepared in medium so as to yield the final test substance concentration series.
- For the growth inhibition test, 112.6 mg test substance was dissolved in 1000 mL algal medium. Dilutions were then prepared in medium so as to yield the final test substance concentration series.

TEST MEDIUM

The medium was prepared from concentrated stock solutions in ultra pure water. It was sterilized by micropore filtration and contained 150 mg/l NaHCO₃ (not 50 mg/l as specified in the OECD Guideline, this in order to improve the buffer capacity of the medium). Furthermore, the medium contained Fe-citrate, because the growth of the algae can become erratic in the absence of complexed iron.

Test organisms (species): *Scenedesmus subspicatus* (new name: *Desmodesmus subspicatus*)

Details on test organisms

The algal culture was supplied by the Collection of Algal Cultures, Institute for Plant Physiology, University of Gottingen, Nikolausberger Weg 18, D-37073 Gottingen, Germany.

Test type: static; **Water media type:** freshwater; **Limit test:** no

Total exposure duration: 72 h; **Hardness:** 24.2 mg/l CaCO₃; **Test temperature**

22.6 °C - 24.9 °C; **pH:** 6.3 - 8.1

Nominal and measured concentrations:

Nominal: 0, 1.1, 3.6, 11, 36 and 111 mg/l.

Geometric mean: 0, 1.07, 3.53, 10.79, 35.41 and 109.41 mg/l.

Details on test conditions

RANGE-FINDING TEST AND GENERAL CONDITIONS

Suitable numbers of 200 mL conical test flasks were covered with silicone sponge caps, autoclaved and coded.

A suspension of algae in the algal medium containing 3×10^5 cells/mL was prepared by dilution of a pre-culture containing 3.8×10^6 cells/mL. One mL of this algal suspension was added to 100 mL of the appropriate solutions of the test substance in the test flasks, yielding a nominal inoculum cell density of 0.3×10^4 cells/mL. The nominal test substance concentration series tested was 0, 0.11, 1.1, 11 and 111 mg/l after addition of the algal suspension.

The test was carried out in duplicate with four controls containing algae only, and a single background series containing test substance without algae. All flasks were incubated at 23 ± 2 °C and shaken (at approximately 100 rpm) in a Gallenkamp orbital shaker. The light intensity radiated by the fluorescent lamps was within the standard range of 60-120 $\mu\text{mol/s/m}^2$ (measured with a Bottemanne Weather Instruments Photosynthetic Radiometer RA200 Q). After 3 days of incubation algal densities (cells/mL) and algal biovolume ($\mu\text{m}^3/\text{mL}$) were determined with the electronic particle counter (Coulter Multisizer IIe). Measured values were corrected for the background values in the appropriate blanks.

GROWTH INHIBITION TEST

A suspension of algae in the algal medium containing 0.3×10^6 cells/mL was prepared by dilution of a pre-culture containing 1.7×10^6 cells/mL. One mL of this algal suspension was added to 100 mL of the appropriate solutions of the test substance in the test flasks, yielding a nominal inoculum cell density, of 0.3×10^4 cells/mL. The nominal test substance concentration series was 0, 1.1, 3.6, 11, 36 and 111 mg/l after addition of the algal suspension.

The test was carried out in triplicate with six controls containing algae only and a single background concentration series containing test substance without algae. The pH of the test media was influenced by the addition of the test substance, but was not adjusted to pH 8.

The initial algal cell density was calculated from the measured density in the preculture used for the inoculation, divided by the appropriate dilution factor. Algal densities (cells/mL) and algal biovolume ($\mu\text{m}^3/\text{mL}$) were determined after 26, 50.5 and 71.5 hours. Measured values were corrected for the background values in the appropriate blanks. The mean values were used for further calculations.

The pH was measured at the start (medium without algae) and after 71.5 hours in selected cultures. The morphology of the algae was examined visually with the aid of a microscope at the start and end of the test. The light intensity at two different culture positions was measured at the start of the test.

Reference substance (positive control): no

Results and discussions

Effect concentrations

Duration: 72 h; **Endpoint:** NOEC; **Effect conc:** 1.1 mg/L; **Nominal/Measured:** meas. (geom. mean); **Conc. based on:** test mat.; **Basis for effect:** growth rate

Duration: 72 h; **Endpoint:** EC10; **Effect conc:** 5.7 mg/L; **Nominal/Measured:** meas. (geom. mean); **Conc. based on:** test mat.; **Basis for effect:** growth rate;

Duration: 72 h; **Endpoint:** EC50; **Effect conc:** 37; **Nominal/Measured:** meas. (geom. mean); **Conc. based on:** test mat.; **Basis for effect:** growth rate; **Remarks (e.g. 95% CL):** 95% CL of 30 - 46

Duration 72 h; **Endpoint:** EC90; **Effect conc:** > 110 mg/L; **Nominal/Measured:** meas. (geom. mean); **Conc. based on:** test mat.; **Basis for effect:** growth rate

Duration 72 h; **Endpoint:** EC10; **Effect conc.** 2.9 mg/L; **Nominal/Measured:** meas. (geom. mean); **Conc. based on:** test mat.; **Basis for effect:** biomass; **Remarks (e.g. 95% CL):** 95% CL of 1.1 - 3.5;

Duration: 72 h; **Endpoint** EC50 **Effect conc.** 11.8 mg/L **Nominal/Measured** meas. (geom. mean) **Conc. based on** test mat. **Basis for effect** biomass **Remarks (e.g. 95% CL)** 95% CL of 11 - 35

Duration 72 h **Endpoint** EC90 **Effect conc.** 47 mg/L **Nominal/Measured** meas. (geom. mean) **Conc. based on** test mat. **Basis for effect** biomass **Remarks (e.g. 95% CL)** 95% CL of 35 - 109

Details on results

The range-finding test revealed that inhibiting effects could be expected at test substance concentrations >1.1 mg/l.

The algal growth was completely inhibited at the highest test substance concentration. Although the low test medium pH value of 6.3 may have contributed to this effect, it is not considered to be of importance for the assessment of the inherent toxicity of DMTC to the algae.

The average specific growth rate (μ) and percentage inhibition was determined according to the OECD Guideline 201. A statistically significant difference on the growth rate was found at the lowest tested concentration, an effect not expected from the results of the range finding test. However, further inspection of the results indicates only a relatively small inhibition of the growth and mainly at the end of the test where the greatest variation in cell density may be expected (average inhibition was 4.9 % and 5.8 % at 1.1 and 3.6 mg/l, respectively). Combined with the results of the range-finding test, it was assessed that the NOEC was 1.1 mg/l. No significant NEC could be calculated for the reason mentioned above.

Microscopic inspection of the morphology of algal cells in the pre-culture at the start of the

test revealed normal cells. At the end of the test no abnormal cells were observed in the cultures containing different concentrations of dichlorodimethylstannane. At a test substance concentration of 35 mg/l a large number of bacteria were observed. At the highest test substance concentration only a few algal cells were observed.

Reported statistics and error estimates

CALCULATION OF THE EC50 VALUES

The EC values with respect to the growth rate during and exponential growth (ErC values), were calculated by means of a parametric model assuming a constant error per measurement. The parametric model allows curve fitting with different assumptions about the growth pattern (exponential or logistic) and the nature of the effect of the test substance (growth rate, yield or viability of the inoculum). The best fitting model is chosen on the basis of the variance and the AIC (Akaike Information Criterion). This calculation method is based on the assumptions made in the OECD Guideline 201. It has been used in ring tests of algal growth inhibition test Guidelines. These ring tests have demonstrated that ErC50 values calculated by this method are identical to those calculated by the method given in the Guidelines.

EC values with respect to the area under the growth curve (EbC values) were calculated by the method given in the OECD Guideline. The values were calculated by linear interpolation of a plot of the percentage reduction in growth (IA) against the log concentration of the test substance.

DETERMINATION OF THE NOEC AND NEC VALUES

The NOEC (no-observed-effect concentration) was determined as the highest concentration at which no (statistically) significant inhibition was observed. Statistical significance was determined with a one tailed t-test ($\alpha=5\%$).

In addition, model calculations were carried out using the DEBtox software package according to the Dynamic Energy Budgets Theory developed by Kooijman and Bedaux. Model parameters for population growth and their asymptotic standard deviation and correlation coefficients were estimated. The NEC (no-effect concentration), was calculated from the Profile Ln Likelihood function. This method offers a completely different basis for calculating No Effect and offers complementary information to the traditional statistical NOEC or EC10/20 approach.

Any other information on results incl. tables

Table 1: Individual growth rates and % inhibition

Nominal	Average	% Inhibition
0	1.22	2.1
0	1.27	-1.5
0	1.25	-0.3
0	1.23	1.3
0	1.25	-0.1

0	1.27	-1.5
1.1	1.16	7.0
1.1	1.23	1.5
1.1	1.17	6.2
3.6	1.21	2.9
3.6	1.18	5.2
3.6	1.13	9.2
11	1.02	18.7
11	0.98	21.5
11	0.97	22.2
36	0.51	58.8
36	0.61	51.4
36	0.57	54.1
111	0.17	86.4
111	0.15	88.3
111	0.16	87.5

Toxicity to aquatic algae – study 2

Purpose flag: supporting study; **Study result type:** experimental result; **Study period:** not reported;

Reliability score 4 (not assignable) was given by the registrant. The study was conducted using ethanol solvent. Not written in sufficient detail to fully assess the reliability. According to the registrant.

Reference type: publication; **Author:** Huang, G., Dai, S. & Sun, H.; **Year:** 1996;

Title Toxic Effects of Organotin Species on Algae

Bibliographic source Applied Organometallic Chemistry, Vol. 10, 377-387

Materials and methods

Test guideline: no guideline followed

Principles of method if other than guideline

Acute toxicity of dimethyltin dichloride to aquatic algae using *Scenedesmus obliquus* and *Platyrmonas* sp. Inhibition of growth rate was examined.

GLP compliance: no according to the registrant

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline; **Analytical monitoring:** no; **Vehicle:** yes

Details on test solutions

- Chemical name of vehicle (organic solvent, emulsifier or dispersant): absolute ethanol
- The maximum ethanol concentration in the test media was not more than 3%. An ethanol control test indicates that under this concentration ethanol has no deleterious effect on the growth of the two algae.

Test organisms (species): other: *Scenedesmus obliquus* and *Platymonas* sp.

Details on test organisms

S. obliquus was cultured in Aquatic-4, and the nutrition medium for *Platymonas* was artificial seawater (salinity 18 ‰) fortified with trace metals and nutrient salts. The pH values of the nutrition media were 7.6 and 7.8 for *S. obliquus* and *Platymonas* sp., respectively.

Test type: no data

Total exposure duration: 96 h; **Post exposure observation period:** None

Test temperature: 25 ± 1 °C; **pH:** NDA; **Dissolved oxygen:** NDA;

Details on test conditions

The experiment was conducted in a 250 mL Erlenmeyer flask that was sealed with a bacterium-eliminated ventilating film. The medium in the flask was sterilised by pasteurization at 120 °C and allowed to cool to room temperature. Algae stock solution, in the logarithmic stage of growth, was transferred into the nutrient medium immediately after the organotin standard solution had been mixed well in it. The volumes of test medium were 100 mL for *S. obliquus* and 60 mL for *Platymonas* sp. Six concentrations of toxicant were used, and each concentration as well as the untreated control was tested several times. Each compound was tested one to three times. Algae were cultured for 96 hours at 25±1 °C under cycles of 12 hours of light (4000 lux) and 12 hours of darkness. Each day the test media were shaken for 5 min on a shaker (100 rpm) at 3 hour intervals. The cells were enumerated every morning.

Typical poisoning symptoms of the algae were viewed and recorded using microphotography. *Platymonas* contaminated with TBT was observed using an electron microscope.

Reference substance (positive control): no

Results and discussions**Effect concentrations**

S. obliquus: **Duration** 96 h **Endpoint** EC50 **Effect conc.** 805.84 ng/L **Nominal/Measured** no data **Conc. based on** element Sn **Basis for effect** growth rate **Remarks (e.g. 95% CL)**

Platymonas sp.: **Duration** 96 h **Endpoint** EC50 **Effect conc.** 988.69 ng/L **Nominal/Measured** no data **Conc. based on** element Sn **Basis for effect** growth rate **Remarks (e.g. 95% CL)**

Toxicity to aquatic algae – study 3

Purpose flag: supporting study; **Study result type:** experimental result; **Study period:** not reported;

Reliability score 4 (not assignable) was given by the registrant. It was a non-standard test method with some information lacking. The reliability of the data was based on dose response and structure-activity relationships, outlined and discussed by the authors. The study was conducted using a solvent. No analytical confirmation of exposure concentrations. Purity of test material was not reported.

Reference type: publication; **Author:** Wong, P.T.S., Chau, Y.K., Kramar O. & Bengert, G.A.; **Year:** 1982; **Title:** Structure-toxicity Relationship of Tin Compounds on Algae

Bibliographic source Can. J. Fish, Aquat. Sci. 39: 483-488

Materials and methods

Test guideline: no guideline followed; **GLP compliance:** no according to the registrant

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline; **Analytical monitoring:** no

Test organisms (species): other: *Scenedesmus quadricauda*, *Ankistrodesmus falcatus* and *Anabaena flos-aquae*

Details on test organisms

Three axenic algal cultures were used: *Scenedesmus quadricauda* (culture collection No. 11, Dr P. Healey, Freshwater Institute, Winnipeg, Man.), *Ankistrodesmus falcatus* var. *acicularis* and *Anabaena flos-aquae* (Ontario Ministry of Natural Resources, P.O. Box 213, Rexdale, Ont.). Each was grown in 100 mL of a modified CHU-10 medium at 20 °C on a rotary shaker (100 rpm) under conditions of 18 hours of light (5000 lx) and 6 hours darkness, No chelator (ethylene-diamineteraacetic acid) was added to the medium to avoid a high degree of chelation of the metals. Only the major nutrients, nitrate and phosphate, and some bicarbonate to buffer the medium to pH 8, were present. When the cells reached the logarithmic phase of growth (about 1 week), they were used as inoculum.

Water media type: freshwater;

Details on test conditions

Primary productivity was measured by the amount of ¹⁴C-carbonate taken up by algae over a 4h period. One millilire of the culture (ca. 7×10^5 cells/mL) was added to 13.9 mL of CHU-10 medium containing various tin compounds at 0-50 mg as tin/L in a 25 mL Elenmeyer flask.

After 24 hours incubation at 20 °C under conditions described above, a 0.1 mL aliquot of 7.4×10^4 Bq/mL ¹⁴C-sodium carbonate was added to each flask. The flasks were tightly capped with rubber stoppers wrapped in aluminium foil. A similar set of flasks were incubated in the dark. After a further 4h incubation, the cells were fixed with 0.05 mL of neutralised formalin. They were filtered through a 0.45 µm membrane filter and rinsed rapidly with 10 mL fresh CHU-10 medium to remove extracellular ¹⁴C-sodium carbonate. Filters containing radioactive labelled cells were dissolved in 10 mL PCS scintillation counting fluor. Radioactivity taken up by algae in the dark (<5% of total) was subtracted from the total radioactive counts. Radioactivity in the control was taken as 100%. The concentration causing a 50% reduction in primary productivity (median inhibition of concentration, IC₅₀) was estimated.

Reference substance (positive control): no

Results and discussions

Effect concentrations

S. quadracuata: **Endpoint** IC₅₀ **Effect conc.** 4.1 mg/L **Nominal/Measured** no data **Conc. based on** test mat. **Basis for effect** growth rate **Remarks (e.g. 95% CL)**

A. falcatus: **Endpoint** IC₅₀ **Effect conc.** 21 mg/L **Nominal/Measured** no data **Conc. based on** test mat. **Basis for effect** growth rate **Remarks (e.g. 95% CL)**

A. flos-aquae: **Endpoint** IC₅₀ **Effect conc.** > 5 mg/L **Nominal/Measured** no data **Conc. based on** test mat. **Basis for effect** growth rate **Remarks (e.g. 95% CL)**

Toxicity to aquatic algae – study 4

Purpose flag: supporting study; **Study result type:** experimental result;

Study period: 26 January 1996 - 12 March 1996;

Reliability score 1 (reliable without restriction) was given by the registrant because the study was conducted in compliance with agreed protocols, with no or minor deviations from standard test guidelines and/or minor methodological deficiencies, which did not affect the quality of the relevant results.

Reference type: study report; **Year:** 1996; **Report date:** 1996-03-12;

Materials and methods

Test guideline: according to OECD Guideline 201 (Alga, Growth Inhibition Test);
Deviations: no

Test guideline: according to EPA OTS 797.1050 (Algal Toxicity, Tiers I and II); **Deviations:** no

GLP compliance: yes

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline; **Analytical monitoring:** no; **Vehicle:** no;

Details on test solutions

A 10 mg/L stock solution was formulated by the addition of 1.3 mL of the 1,887 ppm solution of test substance to sterile dilution water in a class A volumetric flask and adjusting the final water volume to 250 mL. Test media were prepared at 0.098, 0.49, 0.98, 4.9, and 9.8 mg/L by combining an appropriate volume of the 10 mg/L stock solution and dilution water.

Water used for culture and acclimation of algae and for toxicity testing was sterile enriched media (U.S. EPA, 1978) with a pH of 8.1. Test media from a control vessel was analysed for particulate matter in dilution water at the start of the test and in a control vessel at the end of the test. Particulate matter measured in dilution water at the start of the test was 58 mg/L and particulate matter measured in water from a control vessel at the end of the test was 106 mg/L. The 96 hour value results, at least in part, from the presence of algae.

Test organisms (species): *Skeletonema costatum*

Details on test organisms

Algae used for the test was originally procured from the Culture Collection of Algae at the University of Texas at Austin on January 10, 1996. The culture was transferred to sterile enriched media identical to media used for this test and maintained at test conditions for at least 14 days before the definitive test. The identity of the algae was verified using an appropriate taxonomic key. The inoculum used for the test was from a single source that had been growing for 7 days prior to testing.

Test type: static; **Water media type:** saltwater; **Limit test:** no; **Total exposure duration:** 96 h

Post exposure observation period: None

Hardness: NDA; **Test temperature:** 20.3 - 20.5 °C; **pH:** 8.0 - 9.9; **Dissolved oxygen:** NDA; **Salinity:** NDA

Nominal and measured concentrations: Nominal: 0.098, 0.49, 0.98, 4.9 and 9.8 mg/L

Details on test conditions

The test was conducted at a target temperature of $20 \pm 1^\circ\text{C}$, with five concentrations of test substance and a dilution water control. Algae were distributed among two replicates of each treatment at the rate of approximately 10,000 cells/mL (this differs from the TSCA test guidelines requirement of 77,000 cells/mL). The test was performed in 250 mL glass Erlenmeyer flasks that contained 50 mL of test solution.

Test vessels were randomly arranged on a rotary shaker adjusted to 100 rpm in an incubator during the test. A 16 hour light and 8 hour dark photoperiod was automatically maintained . with cool-white fluorescent lights that provided a light intensity of 49-50 $\mu\text{Ein}/\text{m}^2\text{sec}$ (no transition period was provided between dark and light and the light intensity, photoperiod, and rotation rate of the shaker differ from the TSCA guideline). Test vessels were capped with inverted glass beakers.

The number of algal cells/mL in each test vessel and the occurrence of relative size differences, unusual cell shapes, colours, flocculations, adherence of cells to test containers, or aggregation of cells was determined visually by means of direct microscopic examination with a haemocytometer. Cell counts were made and recorded at the beginning of the test and after 72 and 96 hours. At the conclusion of the test a 0.5 mL subsample of test media from each flask containing the highest concentration of Dimethyltin dichloride was combined with 100 mL of fresh media to determine whether algicidal or algistatic effects had occurred. This flask was incubated under test conditions for 72 hours and examined for algae.

Temperature of the incubator was measured and recorded daily and the temperature in a beaker of water incubated among the test vessels was recorded continuously. The pH (Beckman model pHI 12 meter; instrument number 144 pHT5) was determined in each test vessel at the beginning and end of the test.

Reference substance (positive control): no

Results and discussions

Effect concentrations

Duration 72 h **Endpoint** EC50 **Effect conc.** > 9.8 mg/L **Nominal/Measured** nominal **Conc. based on** test mat. **Basis for effect** cell number

Duration 72 h **Endpoint** NOEC **Effect conc.** 4.9 mg/L **Nominal/Measured** nominal **Conc. based on** test mat. **Basis for effect** cell number

Duration 96 h **Endpoint** EC50 **Effect conc.** > 9.8 mg/L **Nominal/Measured** nominal **Conc. based on** test mat. **Basis for effect** cell number

Duration 96 h **Endpoint** NOEC **Effect conc.** 0.98 mg/L **Nominal/Measured** nominal **Conc. based on** test mat. **Basis for effect** cell number

Duration 72 h **Endpoint** EC50 **Effect conc.** > 9.8 mg/L **Nominal/Measured** nominal **Conc. based on** test mat. **Basis for effect** growth rate

Duration 72 h **Endpoint** NOEC **Effect conc.** 4.9 mg/L **Nominal/Measured** nominal **Conc. based on** test mat. **Basis for effect** growth rate

Duration 96 h **Endpoint** EC50 **Effect conc.** > 9.8 mg/L **Nominal/Measured** nominal **Conc. based on** test mat. **Basis for effect** growth rate

Duration 96 h **Endpoint** NOEC **Effect conc.** 4.9 mg/L **Nominal/Measured** nominal **Conc. based on** test mat. **Basis for effect** growth rate

Details on results

No insoluble material was noted in test vessels containing dimethyltin dichloride. The pH of test media at the start of the test was slightly decreased by the test substance. The algal population grew well during the test, resulting in an average of 1,119,000 cells/mL in the control.

No effects (size differences, unusual cell shapes, colours, flocculations, adherence of cells to test containers, or aggregation of cells) were noted. After 72 hours, examination of media from the flask containing a 0.5 mL subsample of test media from the flasks containing the highest concentration of Dimethyltin dichloride (9.8 mg/L) and 100 mL of fresh media contained 1,016,000 algal cells/ml, indicating that the effect of the test substance at this concentration was algistatic.

Reported statistics and error estimates

The average specific growth rate was calculated as the natural log of the number of cells/mL at 72 and 96 hours minus the natural log of the number of cells/mL at 0 hours divided by the exposure period. The percent change from the control was calculated by subtracting the treatment average specific growth rate from the control average specific growth rate, dividing the difference by the average specific growth rate in the control, and multiplying that value by 100. The EC50 and NOEC values were calculated using nominal concentrations of test substance, when warranted. The values were computed twice, once using the average number of cells/mL at each concentration expressed as a percent of the control and a second time using the average specific growth rate expressed as the percent change from the control.

The binomial/nonlinear interpolation method (Stephan, 1983) was used to calculate EC50 values. The slope of the 96 hour dose response curve (cell density) was computed using the probit method. The NOEC is the highest concentration of test substance that allowed cell growth equal to at least 90% of the control growth.

Toxicity to aquatic algae – study 5

Purpose flag: supporting study; **Study result type:** experimental result; **Study period** not reported;

Reliability score 4 (not assignable) was given by the registrant. A non-standard method was used, sufficiently documented. The study was conducted using a solvent. Not clear whether a control solvent was included. No information on stability of test material in solution. No analytical confirmation of exposure concentrations. Purity of test material not reported.

Reference type: publication; **Author** Huang, G., Bai, Z., Dai, S. & Xie, Q.; **Year:** 1993; **Title** Accumulation and toxic effect of organometallic compounds on algae **Bibliographic source:** Applied Organometallic Chemistry, Vol. 7, 373-380

Materials and methods**Test guideline:** no guideline followed**Principles of method if other than guideline:** Method according to Rand and Petrocellic: Rand, G.M. and Petrocellic, S.R. Fundamentals of Aquatic Toxicity, HPC, New York, 1984, pp 1-55, 526-555**GLP compliance:** no according to the registrant**Identity of test material same as for substance defined in section 1 (if not read-across):** yes**Test material form:** crystalline; **Analytical monitoring:** no; **Vehicle:** no**Test organisms (species):** other: *Scenedesmus obliquus***Test type:** no data; **Water media type:** freshwater; **Limit test:** no**Total exposure duration:** 96 h;**Test temperature:** 25 ± 1 °C;**Nominal and measured concentrations:** Nominal: 0, 5.0, 10, 50, 100, 500, 1000, 2000, 3000, 4000 and 5000 µg/L.**Details on test conditions**

The nutrition media used was Aquatic-4 medium.

After inoculation, each culture was incubated in 100 mL of nutrition medium at (25 ± 1) °C under conditions of 12h of light (4000 lx) and 12h of darkness.

Results and discussions

Effect concentrations**Duration** 96 h; **Endpoint** EC50; **Effect conc.** 1118.4 µg/L; **Nominal/Measured** nominal; **Conc. based on** test mat.; **Basis for effect** growth rate;**Any other information on results incl. tables***Table 1: Cell number ($\times 10^4$ /ml) vs time for various concentrations of DMTC, with *S. obliquus*.*

No.	Concentration of DMTC (µg/l)	Time (h)				
		0	24	48	72	96
0	0	44	150	400	785	785
1	5.0	42	140	390	750	750
2	10	41	127	350	650	650

3	50	38	125	326	600	615
4	100	37	124	300	520	525
5	500	36	114	245	490	495
6	1000	35	105	131	480	480
7	2000	29	107	114	450	445
8	3000	29	100	105	365	360
9	4000	25	96	100	300	295
10	5000	24	54	93	185	170

Toxicity to aquatic algae – study 6

Purpose flag: supporting study; **Study result type:** no data; **Study period:** not reported;

Reliability score 4 (not assignable) was given by the registrant because a full study report was not available. Only limited details from secondary source.

Reference type: secondary source; **Author** Dobson, S., Howe, P.D. & Floyd, P.; **Year:** 2006; **Title:** Mono- and disubstituted methyltin, butyltin, and octyltin compounds

Bibliographic source Concise International Chemical Assessment Document 73, World Health Organisation. Primary source: Walsh, G.E., McLaughlin, L.L. & Lores, E.M. (1985) Chemosphere, 14(3–4): 383–392

Materials and methods

Principles of method if other than guideline: No methodological information.

GLP compliance: no according to the registrant data

Identity of test material same as for substance defined in section 1 (if not read-across): yes

Test material form: crystalline

Test organisms (species): other: *Skeletonema costatum* and *Thalassiosira pseudonana*

Water media type: saltwater

Total exposure duration: 72 h; **Reference substance (positive control):** no data

Results and discussions

Effect concentrations: **Duration** 72 h; **Endpoint** : EC50; **Effect conc.** > 0.93 mg/L

Nominal/Measured: no data; **Conc. based on:** test mat.; **Basis for effect:** growth rate; **Remarks (e.g. 95% CL):** *Skeletonema costatum* and *Thalassiosira pseudonana*

Details on results

Same result reported for both species tested.

Registrant's summary and conclusion

Validity criteria fulfilled: no data

Conclusions

The 72 hour growth rate EC50 of dimethyltin dichloride to marine diatom (*Skeletonema costatum* and *Thalassiosira pseudonana*) is > 0.93 mg/L.

Executive summary

In a literature source with limited detail, the 72 hour growth rate EC50 of dimethyltin dichloride to marine diatom (*Skeletonema costatum* and *Thalassiosira pseudonana*) is > 0.93 mg/L.

4.1.10 Lemna sp. growth inhibition test

Not available.

4.1.11 Fish early-life stage (FELS) toxicity test

Not available.

4.1.12 Fish short-term toxicity test on embryo and sac-fry stages

Not available.

4.1.13 Aquatic Toxicity – Fish, juvenile growth test

Not available.

4.1.14 Chronic toxicity to aquatic invertebrates

Not available.

4.1.15 Chronic toxicity to algae or aquatic plants

Not available.

4.1.16 Acute and/or chronic toxicity to other aquatic organisms

Not available.