Committee for Risk Assessment

RAC

Annex 1

Background document

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

Tributyltin compounds, with the exception of those specified elsewhere in Annex VI

EC number: -

CAS number: -

CLH-O- 0000003769-59-03/A1

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

Adopted

5 December 2013

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

tributyltin compounds,
with the exception of those specified elsewhere in this Annex

EC Number: n.a.

CAS Number: n.a.

Index Number: 050-008-00-3

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CONTENTS

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING 6

1.1 SUBSTANCE 6
1.2 HARMONISED CLASSIFICATION AND LABELLING PROPOSAL 6
1.3 PROPOSED HARMONISED CLASSIFICATION AND LABELLING BASED ON CLP REGULATION AND/OR DSD CRITERIA 7

2 BACKGROUND TO THE CLH PROPOSAL 9

2.1 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING 9
2.2 SHORT SUMMARY OF THE SCIENTIFIC JUSTIFICATION FOR THE CLH PROPOSAL 9
2.3 CURRENT HARMONISED CLASSIFICATION AND LABELLING 9
2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation 9
2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation 9

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL 10

Part B.

SCIENTIFIC EVALUATION OF THE DATA 12

1 IDENTITY OF THE LEAD SUBSTANCES 13

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE 13
1.2 COMPOSITION OF THE SUBSTANCE 15
1.3 PHYSICO-CHEMICAL PROPERTIES OF THE LEAD SUBSTANCE TRIBUTYLTIN OXIDE 16

2 MANUFACTURE AND USES 19

2.1 MANUFACTURE 19
2.2 USES 19

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES 19

4 HUMAN HEALTH HAZARD ASSESSMENT 19

4.1 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 19
4.2 ACUTE TOXICITY 19
4.3 SPECIFIC TARGET ORGAN TOXICITY – SINGLE EXPOSURE (STOT SE) 20
4.4 IRRITATION 20
4.5 CORROSIVITY 20
4.6 SENSITISATION 20
4.7 REPEATED DOSE TOXICITY 20
4.8 SPECIFIC TARGET ORGAN TOXICITY (CLP REGULATION) – REPEATED EXPOSURE (STOT RE) 21
4.9 GERM CELL MUTAGENICITY (MUTAGENICITY) 21
4.10 CARCINOGENICITY 21
4.11 TOXICITY FOR REPRODUCTION 21
4.11.1 Effects on fertility 21
4.11.1.1 Non-human information 21
4.11.1.2 Human information 29
4.11.2 Developmental toxicity 29
4.11.2.1 Non-human information 29
4.11.2.2 Human information 43
4.11.3 Other relevant information 43
4.11.4 Summary and discussion of reproductive toxicity 43
4.11.5 Comparison with criteria 46
4.11.6 Conclusions on classification and labelling 47
4.12 OTHER EFFECTS 48

5 ENVIRONMENTAL HAZARD ASSESSMENT 49
6 OTHER INFORMATION 49
7 REFERENCES 49

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***tributyltin compounds***
EC number:	***n.a.***
CAS number:	***n.a.***
Annex VI Index number:	***050-008-00-3***

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation (2nd ATP to CLP)	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Acute Tox. 3 * Acute Tox. 4 * STOT RE 1 Eye Irrit. 2 Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	T; R25-48/23/25 Xn; R21 Xi; R36/38 N; R50-53
Current proposal for consideration by RAC	**Repr. 1B (H360Fd)**	**R 60/63**
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 3 H301 Acute Tox. 3 H311 STOT RE 1 Eye Irrit. 2 Skin Irrit. 2 **Repr. 1B** Aquatic Acute 1 Aquatic Chronic 1	T; R25-48/23/25-**60/63** Xn; R21 Xi; R36/38 N; R50-53

* Minimum classification for a category

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

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.1.	Acute toxicity - oral	Acute Tox. 3 H301		Acute Tox. 3 * H301	
	Acute toxicity - dermal	Acute Tox. 3 H311		Acute Tox. 4 * H312	
	Acute toxicity - inhalation	none		none	data lacking
3.2.	Skin corrosion / irritation	Skin Irrit. 2; H315: C ≥ 1 %		Skin Irrit. 2; H315: C ≥ 1 %	
3.3.	Serious eye damage / eye irritation	Eye Irrit. 2; H319: C ≥ 1 %		Eye Irrit. 2; H319: C ≥ 1 %	
3.4.	Respiratory sensitisation	none		none	data lacking
3.4.	Skin sensitisation	none		none	data lacking
3.5.	Germ cell mutagenicity	none		none	
3.6.	Carcinogenicity	none		none	
3.7.	Reproductive toxicity	**Repr. 1B**		none	
3.8.	Specific target organ toxicity –single exposure	none		none	
3.9.	Specific target organ toxicity – repeated exposure	STOT RE 1; H372: C ≥ 1 % STOT RE 2; H373: 0.25 % ≤ C < 1 %		STOT RE 1; H372: C ≥ 1 % STOT RE 2; H373: 0.25 % ≤ C < 1 %	
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1; H400 Aquatic Chronic 1; H410 M=10		Aquatic Acute 1; H400 Aquatic Chronic 1; H410 M=10	

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification
* Minimum classification for a category; specific concentration limits for acute toxicity under Directive 67/548/EEC

Labelling: Signal word: Danger

Hazard statements:
H301: Toxic if swallowed.
H311: Toxic in contact with skin.
H315: Causes skin irritation.
H319: Causes serious eye irritation.
H360Fd: May damage fertility.
Suspected of damaging the unborn child.
H372 **: Causes damage to organs through prolonged or repeated exposure; ** Route of exposure cannot be excluded
H410: Very toxic to aquatic life with long lasting effects.

Precautionary statements: **P201, P202, P281, P308 + P313, P405, P501**

Proposed notes assigned to an entry: A1

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Acute toxicity	T; R25: C ≥ 2.5 % Xn; R22: 0.25 % ≤ C < 2.5 % Xn; R21: C ≥ 1 %		T; R25: C ≥ 2.5 % Xn; R22: 0.25 % ≤ C < 2.5 % Xn; R21: C ≥ 1 %	
Acute toxicity – irreversible damage after single exposure	none		none	data lacking
Repeated dose toxicity	T; R48/23/25: C ≥ 1 % Xn; R48/20/22: 0.25 % ≤ C < 1 %		T; R48/23/25: C ≥ 1 % Xn; R48/20/22: 0.25 % ≤ C < 1 %	
Irritation / Corrosion	Xi; R36/38: C ≥ 1 %		Xi; R36/38: C ≥ 1 %	
Sensitisation	none		none	data lacking
Carcinogenicity	none		none	data lacking
Mutagenicity – Genetic toxicity	none		none	data lacking
Toxicity to reproduction – fertility	**Repr. Cat 2; R60**		none	
Toxicity to reproduction – development	**Repr. Cat. 3; R63**		none	
Toxicity to reproduction – breastfed babies. Effects on or via lactation	none		none	data lacking
Environment	N; R50-53: C ≥ 2.5 % N; R51-53: 0.25 % ≤ C < 2.5 % R52-53: 0.025 % ≤ C < 0.25 %		N; R50-53: C ≥ 2.5 % N; R51-53: 0.25 % ≤ C < 2.5 % R52-53: 0.025 % ≤ C < 0.25 %	

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

Labelling:

Indication of danger: T - Toxic, N - Dangerous for the environment

R-phrases:
21- Harmful in contact with skin.
25- Toxic if swallowed.
36/38- Irritating to eyes and skin.
48/23/25- Toxic: danger of serious damage to health by prolonged exposure through inhalation and if swallowed
50/53- Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
60- May impair fertility.
63- Possible risk of harm to the unborn child.

S-phrases: (1/2-)36/37/39-45-60-61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

2.2 Short summary of the scientific justification for the CLH proposal

The German Competent Authority is concerned about the reproductive toxicity of tributyltin compounds under the Annex VI entry "tributyltin compounds, with the exception of those specified elsewhere in this Annex". This entry includes the anionic substituents of tri-n-butyltin compounds such as halides, alkoxylates or carboxylates. As all of them have a common feature of metabolic hydroxylation and dealkylation, the rationale for the assessment of reproductive toxicity is based on the existing toxicity data for bis(tri-n-butyltin) oxide, tri-n-butyltin chloride, and tri-n-butyltin acetate from 27 studies on fertility and developmental toxicity.

2.3 Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

Table 5: Classification to table 3.1 of the EC regulation 1272/2008

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling		Concentration limits
				Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	
050-008-00-3	tributyltin compounds, with the exception of those specified elsewhere in this Annex	-	-	Acute Tox. 3 * STOT RE 1 Acute Tox. 4 * Eye Irrit. 2 Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H301 H372** H312 H319 H315 H400 H410	GHS06 GHS08 GHS09 Dgr	H301 H372** H312 H319 H315 H410	* STOT RE 1; H372: C ≥ 1 % STOT RE 2; H373: 0.25 % ≤ C < 1 % Skin Irrit. 2; C ≥ 1 % Eye Irrit. 2; C ≥ 1 % M=10

* Minimum classification for a category; specific concentration limits for acute toxicity under Directive 67/548/EEC
** Route of exposure cannot be excluded

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

Table 6: Classification according to table 3.2 of the EC regulation 1272/2008

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration limits
050-008-00-3	tributyltin compounds, with the exception of those specified elsewhere in this Annex	-	-	T; R25-48/23/25 Xn; R21 Xi; R36/38 N; R50-53	T; N R: 21-25-36/38-48/23/25-50/53 S: (1/2-)35-36/37/39-45-60-61	T; R25: C ≥ 2.5 % Xn; R22: 0.25 % ≤ C < 2.5 % Xn; R21: C ≥ 1 % T; R48/23/25: C ≥ 1 % Xn; R48/20/22: 0.25 % ≤ C < 1 % Xi; R36/38: C ≥ 1 % N; R50-53: C ≥ 2.5 % N; R51-53: 0.25 % ≤ C < 2.5 % R52-53: 0.025 % ≤ C < 0.25 %

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

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

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

According to Article 37, a competent authority may submit to the Agency a proposal for harmonised classification and labelling of substances and, where appropriate, specific concentration limits or M-factors, or a proposal for a revision thereof.

RAC general comment

In the CLH report, the dossier submitter noted that the current Annex VI entry includes the anionic substituents of tri-n-butyltin (TBT) compounds such as halides, alkoxylates or carboxylates, and that since all of them have a common feature of metabolic hydroxylation and dealkylation, the rationale for the assessment of reproductive toxicity is based on the existing toxicity data for bis(tri-n-butyltin) oxide, tri-n-butyltin chloride, and tri-n-butyltin acetate.

During public consultation, a member state (NL) raised the issue of the applicability of the present dossier to all "tributyltin compounds, with the exception of those specified elsewhere in this Annex" (stated in the existing entry in Part 3 of Annex VI of the CLP Regulation). In response, the dossier submitter (DS) argued that the tri-n-butyltin compounds which are used in industry (TBTX, X = oxygen, halogen or carboxylate) do not differ substantially in their toxic effects and that the anions attached to the TBT molecule are less relevant to their cellular interactions (see the RCOM for details). The DS also argued that following oral uptake, the TBT compounds dissociate in the gastric juices to form a hydrated TBT cation and the corresponding anion to yield the corresponding TBT chloride. Therefore the TBT species used in the submitted studies are suitable representatives for reproductive toxicity of the whole group of TBT derivatives with the general formula TBTX.

RAC noted that the data in the Dossier only refer to tributyltin chloride (TBT-Cl, EC no 215-958-7), tributyltin acetate (TBT-Ac, EC no 200-269-6) and tributyltin oxide (TBTO, EC no 200-268-0), and the read-across for other compounds depends on the extent to which the other derivatives (which fall within the dossier submitter's proposed Annex VI entry) can decompose to a common active product. As such, TBT does not form salts with organic or inorganic acids, but instead it forms complexes bound by covalent bonds. TBTCl can decompose to hydroxide complexes, TBT-OH and others (PubChem), and in organic fluids it is expected to be stable only at low pH, the TBT-OH conjugates being the predominant forms (Foti et al., 2004; Marine Chemistry 85;157– 167). This is the likely fate of the three compounds included in the report, and it can be inferred that this will be the case for many of the TBT derivatives listed by the DS. However, it is conceivable that a particular TBT derivative may not be decomposable to the hydroxide or other similar complexes, and therefore its bioavailability and toxicity may differ significantly from those considered here. Bearing these considerations in mind, RAC none-the-less considered that the proposed read-across is justified and that there was no need to change the scope of the current Annex VI entry.

In the event that a manufacturer, importer or downstream user of a 'tributyltin compound' covered by this classification considers that the harmonised classification should not apply to their substance, they may submit a proposal (via a member state) for a specific classification for that substance.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

The toxic effects of tributyltin compounds with a non-toxic fourth substituent seem to be mediated by binding of the alkyltin(IV) moieties to N, O, and S donors in living systems with minor relevance of further groups attached (Benya, 1997). Tributyltin compounds, especially tributyltin salts like tri-n-butyltin acetate, can hydrolyze in aqueous media to tri-n-butyltin hydroxide (Appel, 2004). After oral uptake the tributyltin compounds can be converted to tri-n-butyltin chlorides. Bis(tri-n-butyltin) oxide can undergo hydrolytic, nonenzymatic degradation to tri-n-butyltin hydroxide resulting in the same hydrolysation products in the gastro-intestinal tract subjected to further metabolism. Tributyltin compounds like bis(tri-n-butyltin) oxide and tri-n-butyltin chloride have been shown to induce adverse effects on fertility and development following oral administration and can therefore be considered as lead compounds for classification of the whole group.

1 IDENTITY OF THE LEAD SUBSTANCES

1.1 Name and other identifiers of the substance

Table 7: Substance identity tributyltinoxide

EC number:	200-268-0
EC name:	bis(tributyltin) oxide
CAS number (EC inventory):	56-35-9
CAS number:	56-35-9
CAS name:	distannoxane, 1,1,1,3,3,3-hexabutyl-
IUPAC name:	hexabutyldistannoxane
CLP Annex VI Index number:	050-008-00-3 (Group entry)
Molecular formula:	$C_{24}H_{54}OSn_2$
Molecular weight range:	596.1 g/mol

Structural formula:

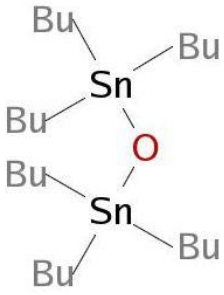


Table 8: Substance identity tributyltin chloride

EC number:	215-958-7
EC name:	tributyltin chloride
CAS number (EC inventory):	1461-22-9
CAS number:	1461-22-9
CAS name:	ttannane, tributylchloro-
IUPAC name:	tributylstannanylium chloride
CLP Annex VI Index number:	050-008-00-3 (Group entry)
Molecular formula:	$C_{14}H_{30}O_2Sn$
Molecular weight range:	349.1 g/mol

Structural formula:

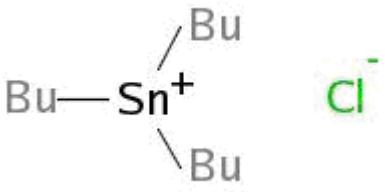
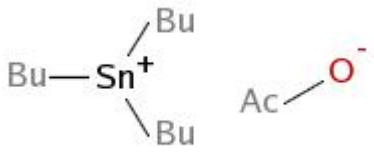


Table 9: Substance identity Tributyltin acetate

EC number:	200-269-6
EC name:	tributyltin acetate
CAS number (EC inventory):	56-36-0
CAS number:	56-36-0
CAS name:	stannane, (acetyloxy)tributyl-
IUPAC name:	tributylstannanylium acetate
CLP Annex VI Index number:	050-008-00-3 (Group entry)
Molecular formula:	$C_{14}H_{30}O_2Sn$
Molecular weight range:	349.1 g/mol

Structural formula:



1.2 Composition of the substance

Table 10: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
bis(tributyltin) oxide EC-No.: 200-268-0			See confidential annex
tributyltin chloride EC-No.: 215-958-7			See confidential annex
tributyltin acetate EC-No.: 200-269-6			Registration dossiers or other information on concentration ranges and on any impurities are not available.

1.3 Physico-chemical properties of the lead substance tributyltin oxide

Table 11: Summary of physico - chemical properties

Property	Value	Reference	Comment	Value	Reference	Comment	Value	Reference	Comment
	bis(tributyltin) oxide, EC-Nr.: 200-268-0			tributyltin chloride, EC-Nr.: 215-958-7			tributyltin acetate, EC-Nr.: 200-269-6		
State of the substance at 20°C and 101,3 kPa	Colourless to slightly yellow liquid with a weak odour	HSDB - Hazardous Substances Data Bank, USA (2010)		Colourless to pale yellow transparent liquid	Migchielsen, M.H.J. 2004, study report		solid		
Melting/ freezing point	-45 °C	SRC PhysProp Database, 2010		< - 20 °C	Butler RE & White DF (2010)	measured	84.7 deg C		
Boiling point	180 °C at 2 mm Hg 210-214 °C at 10 mm Hg 254 °C at 50 mm Hg	Lewis, RJ (2002), Hawley's Condensed Chemical Dictionary, 14th Edition Verschueren, K (2001) Handbook of Environmental Data on Organic Chemicals (4th Edition) Prager, JC (1998) Environmental Contaminant Reference Databook, Volumes 1-3		decompose from approximately 506 K (233°C) at 102.31 kPa	Butler RE & White DF (2010)	measured	322.6±25.0 °C Press: 760 Torr	Calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2012 ACD/Labs)	
Relative density	1.17 g/cm³ at 25 °C	HSDB - Hazardous Substances Data Bank, USA (2010)		1.198 g/ml at 20 °C.	Maier D. (2000)	Measured			
Vapour pressure	much less than 1 mmHg at 20°C	HSDB - Hazardous Substances Data Bank, USA		4.9 x 10 -1 Pa at 25 °C	Atwal SS, Woolley AJ & Tremain SP	Measured	0.0027 mm Hg at 20 deg C	BLUNDEN ,SJ ET AL. (1984)	estimated

		(2010)			(2010)				
Water solubility	71.2 mg/L at 20 °C	Ventur D (1989)		75.8 mg/l at 20 °C	Ventur D (1988)	Measured	65 mg/L	BLUNDEN ,SJ ET AL. (1984)	experiment el
Partition coefficient n-octanol/ water	log P_{OW} 3.84	HSDB - Hazardous Substance s Data Bank, USA (2010)	calculated	2.21 at 23.0 ± 0.5°C.	Butler RE & White DF (2010)	Measured	3.24	MEYLAN, WM & HOWARD, PH (1995)	estimated
Flash point	190°C c.c.	ICSC - Internation al Chemical Safety Cards (2010)							
Flammabili ty	Flammabili ty upon ignition (solids): Testing can be waived, substance is a liquid.. Flammabili ty on contact with water: The study does not need to be conducted because the experience in production or handling shows that the substance does not react with water, e.g. the substance is manufactu red with water or washed with water. Pyrophoric properties: The classificati on procedure needs not to be applied because	BAM 2.2 (2011)	*Data Waiver*						

	the substance is known to be stable into contact with air at room temperature for prolonged periods of time (days).								
Explosive properties	The classification procedure needs not to be applied because there are no chemical groups present in the molecule which are associated with explosive properties.	BAM 2.2 (2011)	*Data Waiver*						
Auto-ignition temperature	data not available								
Oxidising properties	The classification procedure needs not to be applied because there are no chemical groups present in the molecule which are associated with oxidising properties.	BAM 2.2 (2011)	*Data Waiver*						

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Uses

According to the available registration dossiers tributyltin chloride and tributyltin oxide are used as "an intermediate for production of further organotin materials".

Further uses may comprise PVC stabilisers or Catalysts for the production of various consumer products.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Not evaluated in this dossier

4.2 Acute toxicity

In the course of this submission the current minimum classifications for acute oral and dermal toxicity were checked.

Tributyltin compounds are classified according to Directive 67/548/EEC for acute toxicity as T; R25 (toxic if swallowed) and Xn; R21 (harmful in contact with skin). However, the submitter lacks information when and based on which studies this classification had been decided in order to verify the appropriateness of the according minimum classifications. Therefore, the IUCLID datasets from available registration-updates were consulted and differences in the limits of the according reference values of Directive 67/548/EEC and of EC regulation 1272/2008 considered.

Table 12: Comparison of classification criteria for oral and dermal acute toxicity

Exposure route	**Directive 67/548/EEC**	**EC regulation 1272/2008**	
	R25	**Category 3**	**Category 4**
Oral (mg/kg bw)	$25 < LD_{50} \leq 200$	$50 < ATE \leq 300$	$300 < ATE \leq 2000$
	R21	**Category**	**Category**
Dermal (mg/kg bw)	$400 < LD_{50} \leq 2000$	$200 < ATE \leq 1000$	$1000 < ATE \leq 2000$

According to registration updates for tributyltin compounds, e.g. for $TBTCl_2$ (CAS 1461-22-9) and for TBTO (CAS 56-35-9) there is information from oral studies with rats indicating LD_{50} values of 101, resp. of 127 mg/kg bw. These values fit to both, the criteria for labelling with R25 (DSD) as

well as to the criteria for category 3 classification of the CLP regulation. Based on this, it is proposed to change the current minimum classification for acute oral toxicity of tributyltin compounds to the final harmonised classification.

Labelling with R21 implies available information from dermal studies with rats or rabbits with a dermal LD_{50} value as indicated in the table above. In the accessible registration updates, however, nothing but a note on a dermal study with rabbits (without any reference) indicating a LD_{50} of 500 mg/kg bw is available. Based on this information it is proposed to change the current minimum classification for acute dermal toxicity of tributyltin compounds to category 3 for final harmonised classification.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

The current acute toxicity classification for TBT is Acute Tox. 3* (H301) and Acute Tox. 4* (H312), with the asterisk (*) denoting a minimum classification. Following re-assessment of the available data, the DS proposed Acute Tox. 3 (H301) and R25 (under DSD) based on oral LD_{50} values of 101 and 127 mg/kg in rats. The DS also proposed Acute Tox. 3 (H311) and R21 (DSD) based on a dermal LD_{50} value of 500 mg/kg in rabbits, but also commented that this was based on a note in a registration update (without any reference).

Comments received during public consultation

No comments were received on acute toxicity during public consultation

Assessment and comparison with the classification criteria

The oral LD_{50} values were within the range 50 to 300 mg/kg, therefore classification as Acute Tox. 3 (H301) as proposed by the DS is warranted (R25 under DSD). However, RAC considerd that there was insufficient evidence to change the classification for acute dermal toxicity and therefore the current minimum classification as Acute Tox. 4* (H312) should be maintained.

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

Not evaluated in this dossier

4.4 Irritation

Not evaluated in this dossier

4.5 Corrosivity

Not evaluated in this dossier

4.6 Sensitisation

Not evaluated in this dossier

4.7 Repeated dose toxicity

Not evaluated in this dossier

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

Not evaluated in this dossier

4.9 Germ cell mutagenicity (Mutagenicity)

Not evaluated in this dossier

4.10 Carcinogenicity

Not evaluated in this dossier

4.11 Toxicity for reproduction

4.11.1 Effects on fertility

4.11.1.1 Non-human information

The studies used for hazard assessment are considered reliable with restrictions unless stated otherwise in the study description. Where a guideline was followed it is explicitly stated. Otherwise, there is no or no information on guideline compliance. The information on reproductive toxicity from the registration dossiers was considered for the assessment. No studies in addition to the publicly available information were provided by the registrants.

Type of study:	**Two-generation reproduction study (OECD 416)**
Reference:	Schroeder, 1990; cited from EPA, 1997
Animal species & strain:	Rat (Sprague Dawley) 30m/30f per group in P0 and F1 generation
Test substance:	Bis (tri-n-butyltin) oxide (TBTO), purity 97.1 %
Doses, vehicle, duration:	Diet 0, 0.5, 5.0, 50 ppm TBTO highest dosage according to mean daily intake of: P0 m: 2.95 mg/kg bw P0 f: 3.43 mg/kg bw F1 m: 3.98 mg/kg bw F1 f: 4.42 mg/kg bw F0 animals: 10 weeks prior to mating, during cohabitation with exposure of females continuing during gestation and lactation F1 animals: 15 weeks prior to mating and during cohabitation with exposure of females continuing during gestation and up to weaning
Result:	No treatment-related effects on food consumption or gross or histopathology in either sex or generation **0.5 - 50 ppm (about 0.03 to 3 mg/kg/d)** no changes in clinical observations no effects on mating, pregnancy and fertility rates in either generation no changes in duration of mating, gestation and parturition, no changes in maternal behaviour, no changes in gross pathology, histopathology and

	numbers of implantations no effect on number of pups, litter size and pup survival in either generation in comparison to controls **50 ppm (about 3 mg/kg/d)** reduced body weight gain ($p<0.5$) in F1 parental animals (m: 19 %, f: 15 %), decreased absolute, resp. relative thymus weights in parental animals (F0 m: 8 %, resp. 8 %, F0 f: 13 %, resp. 17%; F1 m: 38 %, resp. 31%, F1 f: 28 %, resp. 26 %; $p<0.01$), decreased pup body weight gain during lactation (days 7, 14 and 21: F1 pups 10, 14 and 17%, F2 pups 14, 17 and 20 %)
Type of study:	**Female fertility**
Reference:	Harazono et al., 1996
Animal species and strain:	Rat (Jcl:Wistar)
Test substance:	Tributyltin chloride (TBTCl), purity 96 %
Doses, vehicle, duration:	oral (gavage) vehicle: olive oil 8.1, 12.2, 16.3 mg /kg/day TBTCl mated females treated from g.d. 0-7 sacrifice on g. d. 20
Result:	control (olive oil) 10/10 pregnant, pregnancy failure*): 0 % *) pregnancy failure evidenced by absence of implantation sites **8.1 mg/kg/d** decreased maternal food consumption (64 % of the controls) decreased maternal body weight gain (10% of the controls) no significant change in adjusted maternal weight gain compared to controls 2/11 non-pregnant, pregnancy failure*): 18 % **12.2 mg/kg/d** decreased mat. food consumption (33 % of the controls) mat. body weight loss (of 9 %) no significant change in adjusted maternal weight gain compared to controls 10/14 non-pregnant, pregnancy failure*): 71 % lower live fetal body weights correlating to delayed ossification (reduced numbers of ossified sternebrae and sacrococcygeal vertebrae) **16.3 mg/kg/d** decreased mat. food consumption (27 % of the controls) mat. body weight loss (of 12 %) no significant change in adjusted maternal weight gain compared to controls 10/13 non-pregnant, pregnancy failure*): 77 % ∑: clinical signs (sluggishness, bloody stain around nose and eyes, diarrhea) and decreases in body weight – yet not in adjusted maternal weight gain - and food consumption during the administration period pregnancy failure in females with positive matings in a dose-dependent manner; however, for treated females achieving pregnancy the numbers of corpora lutea, implantations and live fetuses per litter were comparable to the

	control group higher (statistically not significant, not dose-related) percentages of pre-implantation loss/litter in treated groups in comparison to control group no fetuses with external, skeletal and internal malformations in treated or control groups
Type of study:	**Female fertility**
Reference:	Harazono et al., 1998a
Animal species and strain:	Rat (Jcl:Wistar)
Test substance:	Tributyltin chloride (TBTCl), purity 96 %
Doses, vehicle, duration:	oral (gavage) vehicle: olive oil 8.1, 16.3, 32.5 mg/kg/d TBTCl from g.d. 0-3 8.1, 16.3, 32.5, 65.1 mg/kg/d from g.d. 4-7 mated females treated from g.d.0-3 or g.d.4-7 sacrifice on g.d. 20
Result:	**treatment g.d. 0-3:** control 12/12 pregnant **8.1 mg/kg/d** decreased maternal food consumption (28% of the controls) mat. body weight loss (of 6.4%) no significant change in adjusted maternal weight gain compared to controls 2/13 non-pregnant, pregnancy failure*): 15.4 % **16.3 mg/kg/d** decreased maternal food consumption (21% of the controls) mat. body weight loss (of 7.8%) no significant change in adjusted maternal weight gain compared to controls 10/16 non-pregnant, pregnancy failure*): 62.5 % lower live fetal body weights correlating to delayed ossification (reduced numbers of ossified sternebrae and sacrococcygeal vertebrae) **32.5 mg/kg/d:** decreased maternal food consumption (19 % of the controls) mat. body weight loss (of 9%) no significant change in adjusted maternal weight gain compared to controls 14/16 non-pregnant, pregnancy failure*): 87.5 % lower live fetal body weights correlating to delayed ossification (reduced numbers of ossified sternebrae and sacrococcygeal vertebrae) ∑: g.d. 0-3: pregnancy failure in females with positive matings in a dose-dependent manner; for treated females achieving pregnancy the numbers of corpora lutea, implantations and live fetuses per litter were comparable to the control group higher (statistically not significant, not dose-related) percentages of pre-implantation loss/litter in treated groups in comparison to the control group **treatment g.d. 4-7:** control (olive oil)

12/12 pregnant
postimplantation loss/litter: 6.0 %

8.1 mg/kg/d:
decreased maternal food consumption (67 % of the controls)
11/11 pregnant
postimplantation loss/litter: 5.6 %

16.3 mg/kg/d:
decreased maternal food consumption (41 % of the controls)
mat. body weight loss (of 4.6 %)
no significant change in adjusted maternal weight gain compared to controls
2/12 non-pregnant, pregnancy failure*): 16.7 %
postimplantation loss/litter: 26.5 %

32.5 mg/kg/d:
decreased maternal food consumption (41 % of controls)
mat. body weight loss (of 4.2 %)
no significant change in adjusted maternal weight gain compared to controls
1/13 non-pregnant, pregnancy failure*): 7.7 %
1/12 dams with complete resorptions
stat. sign. decreased number of live fetuses/litter (10.2 vs 14.2 in controls)
postimplantation loss/litter: 32.4 %

65.1 mg/kg/d:
decreased maternal food consumption (33 % of controls)
mat. body weight loss (of 6.5 %)
no significant change in adjusted maternal weight gain compared to controls
5/13 non-pregnant, pregnancy failure*): 35.5 %
1/8 dams with complete resorptions
stat. sign. decreased number of live fetuses/litter (7.1 vs 14.2 in controls)
postimplantation loss/litter: 52.5 %

*) pregnancy failure evidenced by absence of implantation sites

∑: both treatment periods: clinical signs (sluggishness, chromodacryorrhea around nose and eyes, diarrhea) increased with increasing doses
no fetuses with external, skeletal and internal malformations in treated or control groups
indications of lower live fetal body weights correlating to delayed ossification (reduced numbers of ossified sternebrae and sacrococcygeal vertebrae)
TBTCl in this study revealed to be systemically toxic to females and to female reproduction in all treatment groups;
implantation failure was the most remarkable effect on reproduction, when TBTCl was administered on days 0-3;
postimplantation embryolethality was the most remarkable effect, when TBTCl was administered on days 4-7

Type of study:	**Female fertility**
Reference:	Harazono et al., 1998b
Animal species and strain:	Rat (Jcl:Wistar)

Test substance:	Tributyltin chloride (TBTCl), purity 96 %
Doses, vehicle, duration:	oral (gavage) 16.3 mg/kg/d TBTCl mated females treated from g.d. 0-7 sacrifice on g.d. 20 parallel to the TBTCl-treated group (I) a feed-restricted group (II) and a control group (III) were run
Result:	**TBTCl treated group (16.3 mg /kg/d) (I):** food consumption during days 0-8: 17 ± 21 g body weight gain during days 0-8: –37 ± 21 g 11/13 non-pregnant, pregnancy failure: 85 % preimplantation loss/litter: 9.4 ± 4.4 postimplantation loss/litter: 3.4 ± 4.7* No. of live fetuses/litter: 14.0 ± 0.0* strongly decreased live fetal body weight*§ **feed restricted group (II):** food consumption during days 0-8: 5 g body weight gain days 0-8: –43 ± 5 g 3/15 non-pregnant, pregnancy failure: 20 % preimplantation loss/litter: 9.9 ± 7.2 postimplantation loss/litter: 46.5 ± 20.8§ No of live fetuses/litter: 6.9 + 3.0§ decreased live fetal body weight* **(III) control group (olive oil):** food consumption during days 0-8: 90 ± 11 g body weight gain days 0-8: 15 ± 6 g 11/11 pregnant, pregnancy failure: 0 % No of preimplantation loss/litter: 0.6 ± 0.9 No of postimplantation loss/litter: 1.5 ± 0.8 No of live fetuses/litter: 12.9 ± 1.8 * stat. sig. ($p<0.01$) diff. from group (II) § stat. sig. ($p<0.01$) diff. from group (III) ∑: rate of pregnancy failure in the TBTCl-treated group was significantly higher than that in the control and feed-restricted groups, while that in the feed-restricted group was not significantly different from the control. A higher incidence of post-implantation loss and reduced numbers of live fetuses/litter was noted in the feed-restricted group. Thus it appears that severely reduced feed intake and/or weight loss during early pregnancy may not necessarily interfere with implantation, but rather cause postimplantation loss.
Type of study:	**Two-generation study as claimed by the authors** not conform to guidelines for two-generation studies for the following reasons: original study design as well as the small and varying animal numbers/dose groups were not guideline compliant, the numbers of pups, which had been evaluated for different parameters were small and arbitrary (8 to 10 per group for female pups) or varied considerably (7 to 18 per group for male pups) across investigations on F1 and F2 offspring and were even inconsistent within segments of the study (F1), whole study carried out in

	three blocks with adding-up of results with findings on progeny reported separately for either males or females
Reference:	Omura et al., 2001; Omura et al., 2004; Ogata et al., 2001
Animal species and strain:	Rat (Kud:Wistar) female sex: across 2 generations, male sex: across 1 generation
Test substance:	Tributyltin chloride (TBTCl); purity > 95 %
Doses, vehicle, duration:	0, 5, 25, 125 ppm calculated to yield a mean daily intake of 0.4, 2.0, 10.0 mg/kg bw sperm positive females (P0) (n=10-12 per group) with no pre-treatment were exposed from day of copulation during gestation and lactation until weaning (PND 22) when their litters were culled to 4 pups/sex/group; offspring (F1 males/females) exposed from weaning until sacrifice on PND 119 (males) or PND 148 (females); F1 males/females (n=13-18 per group) mated on PND 92 to produce the next generation F2 males) offspring (F2 males/females) exposed from weaning until sacrifice on PND 91 (males) or PND 92 (females)
Result:	**5 ppm:** no adverse effects on fertility observed **25 ppm:** offspring body weight stat. sig. lower on PND 14 and 21 in F1 males; decreased abs organ weights of testis in F1, decreased abs organ weights of ventral prostate in F2; decreased homogenization-resistant spermatid counts in F2 **125 ppm:** no differences in maternal food consumption in P0 and F1 dams, maternal body weight gain reduced (34 to 27 % less than controls) during gestation in P0 and F1 dams; sign. decreased No. of pups/litter (13.3 vs 16.1 in controls for P0 and 11.4 vs 14.8 in controls for F1) and percentage of live pups in offspring of P0 (88.9% vs 96.4% in controls) and F1 (91.1% vs 99.2% in controls) decreased pup body weight in F1 and F2 male/female offspring on PND 1 (18 % less than controls); no apparent gross malformations; offspring body weight stat. sig. lower on PND 1, 4, 14 and 21 in F1 males and in F2 males; significantly lower postweaning body weight up to PND 91 in F1 and F2, delay in time of eye opening in F1 male and F2 male/female offspring (0.6 to 1.2 days), delay in vaginal opening (6 days) in F1 and F 2 no difference to controls in testes descent decreased abs organ weights of testis, epididymis and ventral prostate in F1 and F2 males (rel. organ weights not provided) at sacrifice; homogenization-resistant spermatid counts reduced to 80 % of controls in F1 and F2, no effects observed on sperm motility and morphology; decreased rel. ovarian weight in F1 at sacrifice with no changes in histopathology; percentage of normal estrous cycling reduced in F1 and F2 no investigations on thymus had been performed
Type of study:	**Male fertility**

	two repeat experiments with focus on: testicular organ weight testicular sperm head count with either histopathology of testes (1st trial) or determination of total tin concentration in testes (2nd trial)
Reference:	Kumasaka et al., 2002
Animal species and strain:	5 week old ICR mice 6 animals per group
Test substance:	Bis tributyltin oxide (TBTO), no information on purity
Doses, vehicle, duration:	oral (gavage) 0.4, 2.0, 10.0 mg/kg bw twice a week (Tuesdays and Fridays repeat administration to juvenile male mice for a period of 4 weeks) vehicle: 0.2 % ethanol in water
Result:	no data on thymus
	no effects on body weight gain or organ weights (liver, kidney, spleen, testes) observed in either trial
	0.4 mg/kg: no effects on testicular sperm head count
	2 mg/kg: testicular sperm head count stat. sig. ($p<0.05$) reduced to 70 % of the control no histopathological changes in testes observed
	10 mg/kg: testicular sperm head count stat. sig. ($p<0.01$) reduced to 60 % of the control
	several seminiferous tubules failed to organise, in which vacuolisation of Sertoli cells appeared, moreover, loss of germ cells and giant cells were observed in some seminiferous tubules
	total tin concentration in testis stat. sig. ($p<0.01$) increased in comparison to control
Type of study:	**Male fertility**
	Two repeat experiments with focus on: testicular development sperm parameters with histopathology of epididymis and evaluation of spermatozoa
	not conform to guidelines or GLP with small animal numbers/dose group and uncontinuous treatment with low doses
Reference:	Chen et al., 2008
Animal species and strain:	21 days old KM mice 8 animals per group
Test substance:	Tributyltin chloride (TBTCl), purity greater than 97%
Doses, vehicle, duration:	oral (gavage) 0.5, 5 and 50 µg/kg bw once every 3 days for 30 days vehicle: 0.1 % ethanol in 0.85% sodium chloride in water

Result:	no data on thymus no effects on body weight, no abnormalities in clinical signs or gross findings no significant alteration of testosterone levels in testes compared to control **0.5 µg/kg:** sperm count and viability in left epididymis reduced in comparison to control **5 µg/kg:** relative testes weights reduced compared to control sperm count and viability in left epididymis reduced in comparison to control sperm abnormality in left epididymis increased in comparison to control **50 µg/kg:** absolute testes weights slightly reduced compared to control relative testes weights slightly reduced compared to control sperm count in left epididymis reduced 3-fold in comparison to control sperm viability in left epididymis reduced in comparison to control sperm abnormality in left epididymis doubled in comparison to control limited relevance of the study due to low doses and small animal numbers/dose group, which question the statistical significance of the observed effects
Type of study:	Male fertility experiments with focus on: sperm parameters epididymal function with histopathology of epididymis and evaluation of spermatozoa not conform to guidelines or GLP with small animal numbers/dose group and uncontinuous treatment with low doses
Reference:	Yan et al., 2009
Animal species and strain:	21 days old KM mice 6 animals per group
Test substance:	Tributyltin chloride (TBTCl), purity > 97%
Doses, vehicle, duration:	oral (gavage) 0.5, 5 and 50 µg/kg bw once every 3 days for 45 days vehicle: 0.1 % ethanol in 0.85% sodium chloride in water
Result:	no data on thymus no effects on body weight, no abnormalities in clinical signs or gross findings relative epididymis weights in treated animals reduced without significant difference compared to the control no obvious histological damage observed in caput, corpus and cauda epididymis after exposure **0.5 µg/kg:** abnormal sperm in left epididymis slightly increased compared to control **5 µg/kg:**

abnormal sperm in left epididymis increased compared to control

50 µg/kg:
sperm viability in left epididymis reduced in comparison to control
sperm counts in left epididymis decreased 2.5-fold in comparison to control
sperm abnormality in left epididymis increased in comparison to control
abnormal sperm in left epididymis increased compared to control

limited relevance of the study due to low doses and small animal numbers/dose group, which question the statistical significance of the observed effects

4.11.1.2 Human information

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Type of study:	**Prenatal developmental toxicity**
Reference:	Davis et al., 1987
Animal species and strain:	NMRI-mice
Test substance:	Bis (tri-n-butyltin) oxide (TBTO), no information on purity
Doses, vehicle, duration:	oral (gavage) vehicle: olive oil g.d. 6-15 pregnant animals terminated on g.d. 18 0, 1.2, 3.5, 5.8, 11.7, 23.4, 35 mg/kg/d with 100, 10, 9, 20, 18, 10 and 6 pregnant dams/dose
Result:	<u>Maternal effects:</u> no information on clinical observations weight reduction (not quantified) 1 out of 6 pregnant dams died in the 35 mg/kg/d group <u>Developmental effects:</u> effects on conceptus: **1.2–35 mg/kg/d:** no changes in number of implantations/litter **1.2–23.4 mg/kg/d:** no changes in number of resorptions/litter no changes in number of living fetuses/litter **1.2–11.7 mg/kg/d:** no changes in average fetal body weight **11.7 mg/kg/d:** 7 % cleft palates (0.7 % in control) **23.4 mg/kg/d:** slightly reduced fetal body weight (not quantified) 24 % cleft palates (0.7 % in controls) increased frequency of variations (irregular ossification centres of sternebrae 41 % vs. 6 % in controls; fused basis of the os occipitalis 27 % vs. 0.4 % in controls)

	35 mg/kg/d: 1out of 5 litters completely resorbed increased number of resorptions/litter (7.5 versus 1.2 in controls) reduced number of living fetuses/litter (5 versus 11.5 in controls reduced average fetal body weight (20 % less than controls) 48 % cleft palates (0.7 % in control) increased frequency of variations (irregular ossification centres of sternebrae 38 % versus 6 % in controls; fused basis of the os occipitalis 29 % versus 0.4 % in controls) In an accompanying experiment, no embryonic damage (assessed using light and electron microscopy) was found in mice 26 and 48 hours after treatment with a single gavage dose of 30 or 110 mg/kg body weight on g.d. 10
Type of study:	**Prenatal developmental toxicity**
Reference:	Faqi et al., 1997
Animal species and strain:	NMRI mice 40 mated dams/group
Test substance:	Bis (tri-n-butyltin) oxide (TBTO), purity 95.3 %
Doses, vehicle, duration:	oral (gavage) vehicle: peanut oil 0.5, 1.5, 4.5, 13.5, or 27 mg/kg/d g.d. 6 to 17 pregnant animals terminated at g.d.18
Result:	Maternal effects **0.5–27 mg/kg/d:** pregnancy rates did not differ significantly among groups no differences among groups in relative and absolute maternal organ weights (thymus, spleen, liver, kidney) no differences among groups in maternal weight gain (actual and/or adjusted) **27 mg/kg/d:** clinical signs of salivation and apathy; 3 out of 40 animals died Developmental effects effects on conceptus: **0.5–27 mg/kg/d:** litters with complete resorptions in all groups (data not presented) except at 0.5 mg/kg/d number of implantations/litter similar across groups percentage of resorptions/implantation sites similar across groups number of viable fetuses/litter similar across groups **27 mg/kg/d:** fetal body weight stat. sign. ($p<0.05$) reduced no visceral anomalies skeletal anomalies in fetuses (no litter based data): cleft palate (11.4 % vs. 0.8 % in controls), bent radius (1.2 % vs. 0.0 % in controls), short mandible (5.0 % vs. 0.0 % in controls), occipital/basioccipital fusion (3.0 % vs. 0.0 % in controls)
Type of study:	**Embryotoxicity**
Reference:	Baroncelli et al., 1990
Animal species and	Swiss albino mice

strain:	8 pregnant dams/group
Test substance:	Bis (tri-n-butyltin) oxide (TBTO), purity > 96 %
Doses, vehicle, duration:	oral (gavage) vehicle: semisynthetic vegetable oil g.d. 6 – 15 pregnant animals terminated at g.d 17 0, 5, 20, 40 mg/kg/d
Result:	Maternal effects: **5–40 mg/kg/d:** no mortalities; no effects on brain, liver and kidney weights; spleen weight dose-dependent and stat. sig. reduced; placental weight dose-dependent and stat. sig. increased (+8.1, +18.1 and +24.0%, respectively) **5 mg/kg/d:** no effect on body weight gain **20 mg/kg/d:** reduced bw gain (+73 % bw gain vs. +87 % in controls) **40 mg/kg/d**: piloerection, lethargy, hunched posture vaginal bleeding on g.d. 8 and 9 (in 3 dams with total litter resorption) weight loss during the first 4 days of exposure reduced bw gain (+43.5 % bwg in those still pregnant vs. +87% in controls) Developmental effects: effects on conceptus: visceral and skeletal examinations not performed **5–40 mg/kg/d:** no changes in number of implantations/litter **5, 20 mg/kg/d:** no changes in number of living fetuses/litter no effects on number of resorptions/litter no observation of fetal external malformations **40 mg/kg/d:** 5 litters completely resorbed (0 in controls) reduced number of living fetuses/litter (6.3 versus 12.0 in controls) increased number of resorptions/litter (10.1 versus 0.2 in controls) of the 3 dams still pregnant several had 12-13-day-old embryos reduced mean fetal weight (80 % of controls)
Type of study:	**Developmental toxicity**
Reference:	Baroncelli et al., 1995
Animal species and strain:	Swiss albino mice
Test substance:	Bis (tri-n-butyltin) oxide (TBTO), puritiy > 96 %
Doses, vehicle, duration:	oral (gavage) vehicle: semi synthetic vegetable oil g.d. 6 – 15 dams were allowed to litter litters were normalised at birth to 8 pups offspring terminated at p.n.d. 7, 14 or 21 0, 5, 10, 20, 30 mg/kg bw/d with 17, 26, 25, 36 and 8 dams/dose
Result:	Maternal effects **5-30 mg/kg/d:** no mortalities; no clinical signs;

	stat. sign. (p < 0.01-0.001) and dose-dependently reduced weight gain from g.d. 6 to p.n.d. 1 (of 3.8, 3.8, 3.5, and 2.5 g versus 5.3 g in controls), dose-related increase in early or late deliveries (g.d. 18 and 20, respectively) **10, 20 mg/kg/d:** reduced nest-building activity **> 10 mg/kg/d:** stat. sign. (p <0.01) reduced weight gain during g.d. 6-18 **> 20 mg/kg/d:** reduced weight gain during nursing; altered nursing behaviour **30 mg/kg/d:** vaginal bleeding of 1 dam on g.d. 12 <u>Developmental effects</u> effects on conceptus: visceral and skeletal examinations not performed **5-30 mg/kg/d:** stat. sign. (p<0.05) decreased ratio of pups/implantation sites (96.8 % in control, 90.4, 88.4, 80.6, 88.5%) no observable malformations among pups **10 mg/kg/d:** postnatal survival decreased on pnd 7 (66 % vs 95 % in controls, p< 0.01) postnatal pup body weight gain decreased on pnd 7 (p<0.01) **20 mg/kg/d:** number of pups/litter decreased (10.8 versus 12.2 in controls) percentage of live pups decreased on p.n.d. 1 (69 % versus 99 % in controls) pup body weight decreased on pnd 1 (1.43 g vs 1.61 g in controls, p< 0.01) postnatal survival decreased on pnd 7 (52 % versus 95 % in controls, p< 0.01) postnatal pup body weight gain decreased on pnd 7 (p<0.002) one cleft palate in 20 mg/kg group **30 mg/kg/d:** number of pups/litter decreased (9.9 vs 12.2 in controls, p<0.05) percentage of live pups decreased on p.n.d. 1 (54 % versus 99 %in controls) pup body weight on p.n.d. 1 decreased (1.22 g vs 1.61 g in controls, p< 0.01) postnatal survival decreased on p.n.d. 7 (64 % vs 95 % in controls, p< 0.01) **remark:** a high percentage of dams (8.3% and 14% in the 20 and 30 mg/kg dose groups, respectively) cannibalised their entire litter on the day of parturition postnatal death rate and growth rate of treated pups were affected by altered maternal behavior pups, apparently viable and with normal weight, were often found scattered throughout the cage with signs of wounds and the percentage of dams that had not build a nest increased in the 10, 20, and 30 mg/kg dose groups total absence of parental care was noted in many litters, and many infanticidal events were reported.
Type of study:	**Two-Generation study (OECD 416)**
Reference:	Schroeder, 1981; cited from EPA, 1997
Animal species and strain:	Sprague-Dawley rats 24 mated females/group
Test substance:	Bis (tri-n-butyltin) oxide (TBTO), purity 97.1 %
Doses, vehicle, duration:	oral (gavage) vehicle: corn oil g.d. 6 -19 dams sacrificed on g.d. 20 0, 5, 9, 18 mg/kg bw/d

Result:	Maternal effects **5 and 9 mg/kg/d:** adjusted weight gain (excluding uterus) 5.5 and 22.2 % lower than in controls **> 9 mg/kg/d:** staining of the fur (anogenital region) **18 mg/kg:** adjusted weight gain (excluding uterus) 69.4 % lower than in controls ($p<0.01$) actual weight gain (g.d. 6-20) 26 % lower than in controls ($p<0.01$) Developmental effects effects on conceptus: **≥ 5 mg/kg/d:** increased incidences of ossification variations in exposed fetuses (asymmetric sternebrae, rudimentary structures, 14th rib pair) with percentages of fetuses with at least 1 skeletal ossification variation significantly ($p<0.01$) increased in the mid and the high dose group **18 mg/kg/d:** 13.2 % resorptions (5.3 % in control) lower ratio of fetuses/implants of 86.8 % versus 94.7% in controls decreased fetal weight (16 % lower than in controls)
Type of study:	**Teratology and Behavior 1st study**
Reference:	Crofton et al., 1989
Animal species and strain:	Long-Evans rats 18 dams/group
Test substance:	Bis (tri-n-butyltin) oxide (TBTO), purity 97 %
Doses, vehicle, duration:	Oral (gavage) vehicle: corn oil g.d 6-20 dams allowed to litter offspring evaluated on pnd 1 and 3 0, 12, 16 mg/kg bw/d
Result:	Maternal effects **Controls:** 15 out of 18 pregnant **12 mg/kg/d:** 1 out of 18 died; 12 out of 18 pregnant 60 % of dams with vaginal bleeding on g.d. 14-16 body weight gain (g.d. 6-20) 62 % reduced **16 mg/kg/d:** 1 out of 18 died; 6 out of 18 pregnant; 1 rat only littered body weight loss (g.d. 6-20) of –13 ± 1 g 75 % of dams with vaginal bleeding on g.d. 14-16 Developmental effects offspring observations: visceral and skeletal examinations not performed **12 mg/kg/d:** litter size on p.n.d. 1 reduced 73 % compared to control pup viability further reduced on p.n.d. 3 (litter size 12 % of control) pup weight on p.n.d. 1 reduced to 45 % of controls 2/71 born dead with cleft palate, 6/71 born dead with attached placenta **16 mg/kg/d:** litter size on p.n.d. 1 reduced 96 % compared to control pup weight on p.n.d. 1 reduced 45 % compared to controls

	no pups survived to p.n.d. 3 5 pups born alive without malformations
Type of study:	**Teratology and Behavior 2nd study**
Reference:	Crofton et al., 1989
Animal species and strain:	Long-Evans rats 15-16 dams/group
Test substance:	Bis (tri-n-butyltin) oxide (TBTO), purity 97 %
Doses, vehicle, duration:	oral (gavage) vehicle: corn oil, g.d. 6-20 dams allowed to litter offspring evaluated on p.n.d. 1 and 3 for litter size body weight and external malformations followed by evaluation of postnatal growth and behaviour (motor activity with figure-eight maze; acoustic startle response) up to p.n.d. 110 0, 2.5, 5.0, 10 mg/kg/bw
Result:	Maternal effects **Controls:** 9 out of 15 pregnant **2.5 mg/kg/d:** 12 out of 16 pregnant **5.0 mg/kg/d:** 10 out of 16 pregnant **10 mg/kg/d:** 1 out 16 died; 7 out of 15 pregnant 20 % lower weight gain than controls Developmental effects offspring observations: motor activity: preweaning activity decreased in all dose groups (stat. sign. on p.n.d. 14 only) acoustic startle response: no persistent effects **2.5 and 5 mg/kg/d:** no pups with external malformations no effects on landmarks of sexual development (testes descent, vaginal opening) **10 mg/kg/d:** no pups with external malformations, reduced litter size on p.n.d. 1 and 3 (50 and 63 % in comparison to controls) reduced pup weight on p.n.d. 1 and 3 (68 and 66 % in comparison to controls) body weight remained stat. sig. ($p < 0.05$) reduced up to p.n.d. 110 no effects on age of testes descen post weaning activity reduced on p.n.d. 47 and 62 brain wt at p.n.d. 110: stat. sign. ($p < 0.05$) reduced to 1.66 g in comparison to 1.84 g in controls
Type of study:	**Teratology and Behavior 3rd study**
Reference:	Crofton et al., 1989
Animal species and strain:	Long-Evans rats offspring postnatal exposure
Test substance:	Bis (tri-n-butyltin) oxide (TBTO), purity 97 %

Doses, vehicle, duration:	oral (gavage) vehicle: corn oil single dose on pnd 5 to 1 male and 1 female pup/litter evaluation of postnatal growth and behaviour up to pnd 64 0, 40, 50, 60 mg/kg/bw
Result:	Developmental effects offspring observations: observations on postnatal development following postnatal exposure only: motor activity: no effects on either preweaning or post weaning activity at any dosage acoustic startle response: no persistent effects **40 mg/kg/d:** stat. sign. ($p < 0.05$) lower body weight gain (25 % lower than controls on p.n.d. 10) remaining decreased up to p.n.d. 30 and recovery by p.n.d. 62 **50 mg/kg/d:** 14 % mortality stat. sign. ($p < 0.05$) lower body weight gain (25 % lower than controls on pnd 10) remaining decreased up to p.n.d 30 and recovery by p.n.d. 62 **60 mg/kg/d:** 32 % mortality stat. sign. ($p < 0.05$) lower body weight gain (25 % lower than controls on p.n.d. 10) remaining decreased up to p.n.d. 30, no recovery up to p.n.d. 62 brain wt at p.n.d. 64: stat. sign ($p < 0.05$) reduced to 1.64 g in comparison to 1.74 g in controls
Type of study:	**Prenatal developmental toxicity**
Reference:	Nemec et al., 1987; cited from WHO/EHC 116
Animal species and strain:	New Zealand white rabbits 20 inseminated females/group
Test substance:	Bis (tri-n-butyltin) oxide (TBTO), non information on purity
Doses, vehicle, duration:	oral (gavage) vehicle: corn oil g.d. 6-18 pregnant animals terminated on g.d. 29 0, 0.2, 1, 2.5 mg/kg/bw
Result:	Maternal effects **Controls:** 3 animals with abortions **0.2 mg/kg:** no clinical signs; 1 animal with abortion **1 mg/kg:** 1 out of 20 animals died; no clinical signs; 1 animal with abortion **2.5 mg/kg:** no clinical signs; stat. sign. mean body weight loss during g.d. 6-18 (detailed data not available); 7 animals with abortions (increased occurrence of abortions was considered to be a secondary effect of maternal toxicity by authors) Developmenal effects effects on conceptus: **0.2, 1 mg/kg:** no effect on survival or growth of fetuses **2.5 mg/kg:** slight decrease in mean fetal weight (statistically non-significant)

	no differences in types or frequency of fetal malformations related to treatment
Type of study:	**Prenatal developmental toxicity**
Reference:	Noda et al., 1991
Animal species and strain:	Wistar rats 10-14 mated females/group
Test substance:	Tri-n-butyltin acetate, no information on purity
Doses, vehicle, duration:	oral (gavage) vehicle: olive oil g.d. 7-17 dams sacrificed at g.d. 20 0, 1, 2, 4, 8, 16 mg/kg/d
Result:	Maternal effects **1 and 2 mg/kg/d:** no stat. sign. effect on thymus weight **4 mg/kg/d:** decreased thymus weight (to 76 % of the control) **8 mg/kg/d:** decreased thymus weight (to 47 % of the control) **16 mg/kg/d:** clinical signs (salivation, emaciation) decreased food intake during treatment period stat. sign. decreased body weight on g.d. 20 (234 ± 25.2 g vs 292 ± 10.1 g in controls, $p<0.01$) decreased thymus weight (to 28 % of the control) Developmental effects effects on the conceptus: **< 8 mg/kg/d:** no embryotoxic and fetotoxic effects were observed **8 mg/kg/d:** stat. not sign. increase of fetuses with variations **16 mg/kg dose group:** 10/14 inseminated females were pregnant 5/10 pregnant dams with complete resorptions significantly increased ratio of early stage (42% vs 3.7% in controls) and late stage (20.1% vs 0% in controls) resorbed fetuses significantly decreased mean number of live fetuses (5.2 vs 12.9 in controls) significantly decreased mean fetal weights (2.05 g vs 3.0 g in controls) 6/27 fetuses with cleft palate increased ratio in skeletal variations (8/15 fetuses with cervical ribs, 9/15 fetuses with rudimentary lumbar ribs)
Type of study:	**Prenatal developmental toxicity**
Reference:	Itami et al,. 1990
Animal species and strain:	Wistar rats 10-12 inseminated females/group
Test substance:	Tributyltin chloride (TBTCl), purity 96 %
Doses, vehicle, duration:	oral (gavage) vehicle: olive oil

	g.d. 7-15 dams sacrificed on g.d. 20 0, 5, 9, 15, 25 mg/kg/d
Result:	Maternal effects **5 mg/kg/d**: decreased food consumption (g.d. 7-15) of 138 ± 9 g vs 167 ± 9 g in controls **9 mg/kg/d**: decreased food consumption (g.d. 7-15) of 131 ± 8 g vs 167 ± 9 g in controls decreased weight gain (g.d. 7-15) of 35 ± 6 g vs 49 ± 2 g in controls **15 mg/kg/d**: decreased food consumption (g.d. 7-20) of 99 ± 19 g vs 167 ± 9 g in controls decreased weight gain (g.d. 7-15) of 10 ± 14 g vs 49 ± 2 g in controls **25 mg/kg/d**: 75 % of the dams died clinical signs: sedation, diarrhoea, salivation decreased food consumption (g.d. 7-20) of 80 ± 5 g vs 132 ± 5 g in controls body weight loss of –25 ± 3 g (g.d. 7-15), ↓ weight gain (g.d. 15-20) Developmental effects effects on the conceptus: no changes in number of corpora lutea/litter and number of implantations/litter between control and treated groups no fetal external, skeletal and internal malformations were observed in any of the dose groups and no changes between groups in the incidence of skeletal variations stat. sign. increases in placental weight were observed in all treated groups **5 mg/kg/d:** decreased fetal (f) body weight of 3.50 ± 0.08 g vs 3.75 ± 0.06 g in controls **9 mg/kg/d:** decreased fetal (f) body weight of 3.38 ± 0.12 vs 3. 75 ± 0.06 g in controls **25 mg/kg/d:** no live fetuses
Type of study:	**Prenatal developmental toxicity**
Reference:	Ema et al., 1995a Ema and Harazono, 2001
Animal species and strain:	Wistar rats 11-14 pregnant females/dose group
Test substance:	Tributyltin chloride (TBTCl), purity 96 %
Doses, vehicle, duration:	oral (gavage) vehicle: olive oil g.d.7-9: 25, 50 mg/kg bw/d or g.d.10-12: 50, 100 mg/kg bw/d or g.d.13-15: 25, 50, 100 mg/kg bw/d dams sacrificed on g.d. 20
Result:	Maternal effects **treatment g.d. 7-9** at both dose levels: no differences in fetal incidences of external, skeletal and

	internal malformations **25 mg/kg bw/d:** maternal weight loss (g.d. 7-9: –10 ± 7 g) 5/13 with complete resorptions number of live fetuses/litter: 7.2 vs 13.1 in controls % post-implantation loss/litter: 49.8 vs 9.4 in controls **50 mg/kg bw/d:** maternal weight loss (g.d. 7-9: –17 ± 6 g) 2/14 with complete resorptions number of live fetuses/litter: 1.2 vs 13.1 in controls % post-implantation loss/litter: 90.4 vs 9.4 in controls **treatment g.d. 10-12** **50 mg/kg bw/d:** maternal weight loss (g.d. 10-12: –16 ± 6 g) no other changes observed no differences in fetal incidences of external, skeletal and internal malformations **100 mg/kg bw/d:** maternal weight loss (g.d. 10-12: –19 ± 7 g) 2/11 with complete resorptions number of live fetuses/litter: 7.5 vs 13.1 in controls % post-implantation loss/litter: 46.4 vs 9.4 in controls 11/82 (6/9 litters) with cleft palate decreased body weight of live fetuses **treatment g.d. 13-15** **25 mg/kg bw/d:** maternal weight loss (g.d. 13-15: –9 ± 6 g) 18/127 (5/11 litters) with cleft palate **50 mg/kg bw/d:** maternal weight loss (g.d. 13-15: –6 ± 9 g) 15/138 (6/11 litters) with cleft palate **100 mg/kg bw/d:** maternal weight loss (g.d. 13-15: –8 ± 6 g) decreased body weight of live fetuses 32/133 (7/11 litters) with cleft palate <u>Developmental effects</u> at any dose and any treatment regimen: no differences in number of implantation sites/litter
Type of study:	**Prenatal developmental toxicity**
Reference:	Ema et al., 1995b
Animal species and strain:	Wistar rats 11, resp. 14 pregnant females/group
Test substance:	Tributyltin chloride (TBTCl), no information on purity
Doses, vehicle, duration:	oral (gavage) vehicle: olive oil g.d.7-8 sacrifice on g.d. 20 0, 40, 80 mg/kg/d
Result:	<u>Maternal effects</u> **40 mg/kg/d:** maternal body weight loss (g.d. 7-9: –8 ± 7 g)

	2/11 with complete resorptions postimplantation loss: 44% vs 11.8% in controls number of live fetuses/litter: 7.6 vs 13.5 in controls **80 mg/kg/d:** maternal body weight loss (g.d. 7-9: –15 ± 8 g) 9/14 with complete resorptions postimplantation loss: 68.5% vs 11.8% in controls number of live fetuses/litter: 4.9 vs 13.5 in controls <u>Developmental effects</u> at any dose: no differences in number of implantation sites/litter, no significantly increased incidence of malformed fetuses observed
Type of study:	**Prenatal developmental toxicity**
Reference:	Ema et al. (1996)
Animal species and strain:	Wistar rats 10, resp. 11 pregnant females/group
Test substance:	Tributyltin chloride (TBTCl), no information on purity
Doses, vehicle, duration:	oral (gavage) vehicle: olive oil g.d. 13-15 sacrifice on g.d. 20 0, 165(54), 330(107) µmol(mg)/kg/d
Result:	<u>Maternal effects</u> **165 µmol/kg/d:** maternal body weight loss (g.d. 13-16: –13 ± 10 g) postimplantation loss: 7.5% vs 11.8% in controls number of live fetuses/litter: 12.4 vs 12.1 in controls 30/124 fetuses (8/10 litters) with cleft palate **330 µmol/kg/d:** 1/11 dams died maternal body weight loss (g.d. 13-16: –12 ± 4 g) postimplantation loss: 19.3% vs 13.4% in controls number of live fetuses/litter: 10.9 vs 12.1 in controls 42/109 (6/10 litters) with cleft palate <u>Developmental effects</u> at any dose: no differences in number of implantation sites/litter, no significantly increased incidence for any other skeletal or for internal malformations observed
Type of study:	**Prenatal developmental toxicity**
Reference:	Ema et al., 1997
Animal species and strain:	Wistar rats 10-12 pregnant females/group
Test substance:	Tributyltin chloride (TBTCl), purity 96 %
Doses, vehicle, duration:	oral (gavage)

	vehicle: olive oil 100 mg/kg on g.d. 7 or 8 or 9 or 200 mg/kg on either g.d. 7, 8, 9, 10, 11, 12, 13, 14 or 15 sacrifice on g.d. 20
Result:	Maternal effects maternal body weight loss (of up to 15 g) during the 2-3 days following administration in all treated groups adjusted net weight gain (maternal weight excluding the gravid uterus) significantly lower at 200 mg/kg on day 8, 11 and onwards (42 ± 13 g in controls vs 20 ± 11 g or loss of –12 ± 13 g in treated groups), 3-9 dams in either treatment group with complete resorptions after tretament at days 7, 8 or 9 50-93% implantation loss in either treatment group after treatment at days 7,8 or 9 10-40% implantation loss after treatment at days 11, 12, 13, 14, or 15 Developmenal effects: decreased fetal body weights in all treatment groups external malformations in fetuses of rats given TBTCl on day 7 at 100 and 200 mg/kg and on days 8-14 at 200 mg/kg were observed all externally malformed fetuses (except five at 100 mg/kg on day 7 and 1 at 100 mg/kg at day 8) had cleft palate no significant increase in the incidence of fetuses with skeletal and internal malformations was found
Type of study:	**Prenatal developmental toxicity**
Reference:	Adeeko et al., 2003
Animal species and strain:	Sprague Dawley rats 12 inseminated females/dose group, control group n=25
Test substance:	Tributyltin chloride (TBTCl), no information on purity
Doses, vehicle, duration:	oral (gavage) vehicle: olive oil administration g.d. 0-19 or g.d. 8-19 sacrifice on g.d. 20 0; 0.25; 2.5; 10; 20 mg/kg/d
Result:	fetal visceral and skeletal evaluations were performed on 2/sex/litter from the control, 2.5, 10 and 20 mg/kg/d dose groups (g.d. 0-19) and the 10 mg/kg/d dose group (g.d. 8-19) Maternal effects **treatment g.d. 0-19** **20 mg/kg bw/d:** 9/13 females pregnant (vs 23/25 in controls) reduced dams body weight gain (86.5 g vs 116 g in controls) increased post-implantation loss (2.4% vs 0.5% in controls) decreased litter size (11.5 vs 14.2 in controls)

	reduced fetal bw (2.1 g vs 3.2 g in controls) no such effects observed in the lower dose groups **treatment g.d. 8-19** **20 mg/kg bw/d:** 11/12 females pregnant (vs 23/25 in the control) reduced dams body weight gain (95 g vs 116 g in controls) no such effects observed in the lower dose groups <u>Developmental effects</u> no indications for external malformations in any segment of the study some indication for an increase in the incidence of skeletal variations (ossification of sternebrae)
Type of study:	**Developmental toxicity**
Reference:	Cooke et al., 2004
Animal species and strain:	Sprague Dawley rats 16 dams/dose group 12 randomly selected pups/dose group
Test substance:	Tributyltin chloride (TBTCl), purity 98.8 %
Doses, vehicle, duration:	oral (gavage) vehicle: olive oil dams treated from g.d. 8 until birth and throughout lactation dams sacrificed postweaning pups treated from weaning onwards and sacrificed p.n.d 30 (males and females), p.n.d. 60 (females only) and p.n.d. 90 (males only) 0; 0.025; 0.25; 2.5 mg/kg bw/d
Result:	<u>Maternal effects</u> no effects on body weight or food consumption of dams all gave birth at expected time no significant differences in litter size, sex ratio or pup survival at weaning all 8 dams selected for histopathology exhibited mild multifocal chronic interstitial nephritis <u>Developmental effects</u> growth profiles of pups (mean body weights, average slope, curvature) and ratio of weekly food consumption/weekly body weight gain affected in the exposed groups (no further details provided) no effects on pup brain or kidney weights pup liver weights tended to decrease with increasing dose and were stat. sign. ($p<0.05$) lower at the 2.5 mg/kg dose group in 60 day old females (–20%) and 90 day old males (–15%) pup spleen weights tended to decrease with increasing dose and were stat. sign. ($p<0.05$) lower (–20%) at the 2.5 mg/kg dose group in 60 day old females and 30 day old males pup thymus weights tended to decrease with increasing dose and being stat. sign. lower for females at day 60 (0.25 and 2.5 mg/kg/d) and males at day 30 (2.5 mg/kg/d)

Type of study:	**Postnatal sexual development/male pubertal assay**
Reference:	Grote et al., 2004
Animal species and strain:	Wistar rats 15 juvenile males/group
Test substance:	Tributyltin (test substance not further characterised)
Doses, vehicle, duration:	oral (gavage) 23 day-old pups treated daily for 30 days 0 (no further information on vehicle) 5, 15 mg/kg bw/d
Result:	**0.5 mg/kg bw/d:** no effects observed **15 mg/kg bw/d:** decreased body weight gain during treatment (140 ± 37 g vs 163 ± 8 g in controls) rel. and abs. thymus weight decreased abs. spleen weight decreased delay in sexual maturation (delay in completion of preputial separation) rel. and abs. epididymal weight decreased rel. and abs. prostate weight decreased rel. and abs. seminal vesicle weight decreased no change in testes weight

Type of study:	**Developmental toxicity**
Reference:	Cooke et al., 2008
Animal species and strain:	Sprague Dawley rats 12 pregnant females per dose group
Test substance:	Tributyltin chloride (TBTCl), purity 98.8 %
Doses, vehicle, duration:	oral (gavage) vehicle: olive oil single administration on g.d. 8 sacrifice on g. d. 20, p.n.d. 6 and p.n.d. 12 0, 0.25, 2.5, 10 mg/kg bw/d
Result:	<u>Maternal effects</u> **until g.d. 20:** **10 mg/kg bw**: sign. lower body weight compared with control **postnatally:** **0.25 mg/kg bw**: increased body weight **2.5 mg/kg bw**: no effect on dams' body weights **10 mg/kg bw:** sign. lower body weight compared with control ($p<0.05$) <u>Developmental effects</u> **≤ 2.5 mg/kg bw**: pups body weights not sign. different from controls **10 mg/kg bw/d:** sign. reduced pup weight (male and female) on p.n.d. 6 and p.n.d. 12

	sign. reduced liver weight in female pups.
Type of study:	**Developmental neurotoxicity**
Reference:	Asakawa et al., 2010
Animal species and strain:	Wistar rats 6 pregnant females per dose group
Test substance:	Tributyltin chloride (TBTCl), no information on purity
Doses, vehicle, duration:	oral (diet) exposure in F_1 rats in utero until 3 weeks after delivery and/or from 9 to 15 weeks of age (n = 10/group) 0, 125 ppm (estimated for each daily body weight and food intake by the time of delivery 8.13 ± 0.13 mg/kg body weight)
Result:	<u>Maternal effects</u> percentage of live F_1 rats among the number of implantations sign. reduced compared to control <u>Developmental effects</u> body weight of female F_1 rats exposed via the placenta and their dams' milk sign. lower than in those only treated from 9 to 15 weeks of age and in the control on p.n.d. 63 and 105 impaired locomotor activity and inhibited exploratory behaviors neurotoxic effects greater with exposure via the placenta and dams' milk than via food

4.11.2.2 Human information

4.11.3 Other relevant information

4.11.4 Summary and discussion of reproductive toxicity

Read across considerations on reproductive toxicity

For the assessment of the reproductive toxicity of tributyltin (TBT) compounds results from studies with tributyltin salts - e.g. with TBTCl and TBT acetate as well as with TBTO are considered relevant. Tributyltin compounds, especially tributyltin salts like tri-n-butyltin acetate, can hydrolyze in aqueous media to tri-n-butyltin hydroxide (Appel, 2004). After oral uptake the tributyltin compounds can be converted to tri-n-butyltin chloride in the stomach. TBTO can undergo hydrolytic, nonenzymatic degradation to tri-n-butyltin hydroxide resulting in the same hydrolysation products in the gastro-intestinal tract subjected to further metabolism. The relatively weak Sn-C bond can be cleaved by hydrolysis alone (Benya, 1997), e.g. after oral ingestion of TBT compounds in the gut system, or by metabolic enzymes to form dibutyltin derivatives as common first metabolites.

Tributyltin compounds are substrates for mixed function oxidases, with several in vitro studies with liver microsomal preparations having demonstrated the formation of carbon-hydroxylated dibutyltin (DBT) derivatives subsequently followed by formation of 1-butanol and butene. Also in vivo, the process of biotransformation particularly in liver is characterised by progressive Cytochrome P450 dependent hydroxylation and rapid dealkylation of the unstable hydroxymetabolites leading to DBT derivatives, monobutyltin (MBT) compounds and finally inorganic tin. The formation of MBT from

DBT derivatives includes perhaps both nonenzymatic dealkylation and Cytochrome P450 dependent hydroxylation reactions, but the rate of debutylation is low (Appel, 2004; BUA, 2003).

As DBT derivatives appear to be important in vivo metabolites of TBT compounds, it is thus reasonable to consider the toxic properties of DBT compounds and in particular properties for adverse impairment of reproduction and development, when evaluating the toxic profile of TBT compounds. So far, two dibutyl compounds amongst them $DBTCl_2$ have already been classified as Repr. Cat 1B, H360FD (Cat. 2, R60/61, Regulation (EC) No 1272/2008 Annex VI Table 3.2).

With regard to the immunotoxic properties of the butyltin compounds, it appears that primarily quantitative differences are of relevance for TBT and DBT compounds.

A comparative assessment in Wistar rats revealed TBTCl to be about 40 % less active than $DBTCl_2$ in reducing relative thymus weight. Also, the delay in the effects of TBTCl compared to $DBTCl_2$ suggested that TBT-induced thymus atrophy might be induced by its DBT metabolites and with a lower activity of TBTCl itself. A single oral (gavage) dose as low as 5 mg $DBTCl_2$ per kg body weight was effective in initiating reductions in relative thymus weight, whereas for TBTCl a single dose of 10 mg per kg body weight was similarly effective. The dose levels calculated to cause a 50 % reduction of relative thymus weight amounted to 18 mg $DBTCl_2$/kg bw and 29 mg TBTCl/kg bwt (Snoeij et al., 1988).

Fertility

In a guideline compliant two-generation feeding study with rats (Schroeder, 1990) the highest tested dietary concentration of 50 ppm TBTO (according to a mean daily intake of 3.0 to 4.4 mg/kg bw) was effective on body weight gain (reduced) and on thymus organ weight (reduced) in the parental animals and revealed effects on postnatal development in terms of postnatal growth retardation evidenced by decreased pup body weight gain during lactation. No effects on male/female fertility and reproduction were revealed in this study for dietary concentrations up to and including 50 ppm, however, dose levels higher than 3.0 to 4.4 mg/kg bw had not been tested.

Clear indications for impairment of female fertility, however, were revealed from several studies with TBTCl administered (Harazono et al., 1996; Harazono et al., 1998a). Orally applied dosages (gavage) of > 8.1 mg/kg bw/day during the early gestational period led to apparent pregnancy failure in rats, which resulted from implantation failure evidenced from absence of implantation sites. These effects occurred in presence of marked maternal toxicity (in terms of reduced maternal food consumption and of body weight impairment). Results from additional studies with feed restricted pregnant rats did not explain pregnancy failure of TBTCl treated females as a secondary effect due to food deprivation and/or body weight loss during early pregnancy (Harazono et al., 1998b).

Comparable effects on fertility and implantation failure, respectively, and early embryonic loss in impregnated females is well known to result from treatment of pregnant rats with DBTCl. Whereas DBTCl was shown to impair normal functions of the pregnant uterus as well as homeostasis of progesterone (Ema et al., 2003; Harazono and Ema, 2003), indicating specific disturbance of the preimplantational environment, no such investigations are available for TBT compounds. However, further indirect evidence of fertility impairment is also derived from a study with dietary exposure of TBTCl to pregnant rats and their subsequently mated offspring (Ogata et al., 2001), during which - similarly to the study with TBTO (Schroeder, 1990) - no effects were observed at the lower dose range (25 ppm according to about 2 mg/kg bw/d), but reduced numbers of pups/litter in both of the generations were observed at a daily intake of about 10 mg/kg (125 ppm).

In addition, indications of spermatotoxic potential of TBTO had been revealed. In a study with juvenile ICR mice with repeat oral (gavage) administration of TBTO twice a week dosages of > 2 mg/kg/d for four weeks resulted in significantly reduced sperm head count and of 10 mg/kg/d resulted in failure of seminiferous tubules to organise as well as in vacuolisation of Sertoli cells (Kumasaka et al., 2002). Adverse effects concerning sperm parameters were also observed in low dose studies with exposure of young KM mice showing dose-dependently reduced sperm counts and viability and increased percentages of abnormal sperm after exposure to TBTCl (Chen et al., 2008; Yan et al., 2009). Albeit, these studies are of limited relevance due to low doses and small animal numbers/dose group, which question the statistical significance of the observed effects. Additionally, studies lack guideline compliance and the substance was administered uncontinuously. Furthermore, the adverse effects mentioned were not observed in valid studies even at higher doses. Further, in a rat study reductions in homogenization-resistent spermatid counts were revealed after exposure of weanlings to TBTCl with daily dosages of > 2 mg/kg/d (Omura et al. 2001).

In summary, although effects on the thymus may be expected to be prevalent at the effective dosages, the effects on female fertility and the spermatotoxic effects are not considered to be induced secondary to systemic toxicity. Accordingly, TBT compounds are proposed to be *classified as Repr. 1B, H360F (Repr. Cat. 2, R60,* according to Directive 67/548/EEC*).*

Developmental toxicity

Investigations focussing on impairment of development after pre-/postnatal exposure are available from in vivo studies with TBTO and tributyltin salts (TBT acetate, TBTCl), respectively. Three different species (rabbit, rat, and mouse) were treated by oral (gavage) route of application.

All in vivo studies have shown effects on pre- and postnatal development concomitant with significant maternal toxicity as indicated by maternal death, maternal weight loss and/or reduction of maternal weight gain. In comparison to mice and rats pregnant rabbits (Nemec et al., 1987) were the most sensitive species concerning maternally toxic effects (already induced at 1-2.5 mg TBTO/kg bw/d).

The studies with rats and mice revealed embryo-/fetal lethality (evidenced from increased resorptions, litters with complete resorptions) induced at about 18 mg TBTO/kg bw/d in rats (Schroeder, 1981) and at 35 mg TBTO/kg bw/d in mice (Davis et al., 1987) and fetotoxic effects (reduced numbers of living fetuses/litter, reduced fetal body weight) after intrauterine exposure of the conceptus as well as impairment of postnatal viability and development (evidenced from reduced offspring survival and reduced offspring weight gain) after pre- or postnatal exposure.

Impairment of postnatal development in terms of growth retardation was also observed in offspring of the two-generation study in rats after dietary exposure to about 3 mg TBTO/kg bw/d (Schroeder, 1981). Toxic effects towards the developing immune system (in terms of decreases in spleen and in thymus organ weights) were observed in rats resulting from intrauterine and postnatal oral exposure to about 2.5 mg TBTCl/kg bw/d (Cooke et al., 2004). Neurotoxic effects were observed following intrauterine and/or postnatal oral exposure with TBTCl as well (Asakawa et al., 2010).

Studies including external or skeletal evaluations revealed induction of structural abnormalities in mice and in rats, however, not in rabbits. Two studies with TBTO in NMRI-mice revealed increased fetal incidences (litter incidences not provided) of cleft palate and of occipital/basioccipital fusion at 11.7 and 27 mg TBTO/kg bw/d, respectively (Davis et al., 1987, Faqi et al., 1997). Effective dosages were associated with significant maternal toxicity as indicated by clinical signs, maternal

weight reduction and maternal death. Induction of cleft palates was also observed in the rat resulting from prenatal exposure to TBT acetate (Noda et al., 1991) or TBTCl (Ema et al., 1995a) at dose levels with significant maternal toxicity (clinical signs, emaciation and maternal weight loss).

Indications for a teratogenic potential of TBTO were also obtained from studies with in vitro systems. Limb buds derived from 11-12 day old mouse embryos or from 13 day old rat embryos were cultured for 3-6 days in TBTO containing medium. Cell proliferation, differentiation as well as development of the bones were inhibited by low concentrations already (mouse 50 nM, rat 40 nM) of TBTO (Barrach and Neubert, 1986; Krowke et al., 1986; Yonemoto et al., 1993).

Comparable effects on prenatal development and increases in resorptions, respectively, as well as induction of structural abnormalities of the skull, are also known to result from oral (gavage) treatment of pregnant rats with DBTCl. Similarly to DBTCl the developmentally toxic effects of the TBT compounds were also observed in a small dosing segment close to maternal lethality.

In summary, from the available data base the potential of TBT compounds related prenatally induced developmentally toxic effects is characterised to comprise embryo/fetal lethality, fetal growth retardation and induction of structural abnormalities (e.g. cleft palate in the rat and skull abnormalities in mice). Taking into account, that these effects were only induced at dosages that were associated with maternal deaths and/or significant maternal weight impairment, it is proposed to *classify TBT compounds as Repr. 1B, H360d (Repr. Cat 3/R63,* according to Directive 67/548/EEC*).*

4.11.5 Comparison with criteria

Rationale for classification Repr. 1B, H360Fd:

Classification in Repr. 1A is not appropriate as it should be based on human data and no human data on reproductive toxicity are available.

Overall, based on animal studies:

- Female fertility in rats was impaired in fertility studies with TBTCl. Implantation failure was the most remarkable effect on reproduction and could not be explained as a secondary effect due to food deprivation and/or maternal body weight loss.
- Spermatotoxicity of TBTO in mice resulted in significantly reduced sperm head counts, failure of seminiferous tubules organisation, and in vacuolisation of Sertoli cells. Rats showed reductions in homogenization-resistent spermatid counts after exposure to TBTCl in absence of other toxic effects.

It is concluded that the data in this report provide clear evidence of adverse effects on male and female sexual function and fertility. There is no mechanistic evidence that these effects are not relevant for humans. The studies available on TBT compounds are considered reliable.

There is evidence from experimental animals of significant toxic effects on development in the offspring:

- Studies with rats and mice induced embryo-/fetal lethality, fetal growth retardation, and structural abnormalities as well as impairment of postnatal viability and development following pre- or postnatal exposure with TBTO or TBT salts.

All effects on pre- and postnatal development were shown concomitant with significant maternal toxicity as indicated by maternal death, maternal weight loss and/or reduction of maternal weight gain. Maternal mortality is not considered to be excessive (greater than 10%) and irreversible

effects of developmental toxicity are not solely produced as a secondary consequence of maternal toxicity.

Classification **Repr. 1B –H360Fd** is therefore warranted (Repr. Cat. 2; R60, Repr. Cat. 3; R63 according to Directive 67/548/EEC). As no data are available for reproductive toxicity by inhalation or dermal route, it is proposed not to specify the route of exposure in the hazard statement.

4.11.6 Conclusions on classification and labelling

Classification **Repr. 1B –H360Fd** is proposed (Repr. Cat. 2; R60, Repr. Cat. 3; R63 according to Directive 67/548/EEC) with no specific route of exposure added.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

The proposed classification is based exclusively on animal studies, mainly in rodents. Fertility effects were found in both males and females. In female rats, implantation failure at relatively high doses of TBTCl (30-60 mg/kg/d) was the most significant effect on reproduction and could not be explained as a secondary effect resulting from food deprivation and/or maternal body weight loss. TBTO (50 mg/kg/d) resulted in significantly reduced sperm head counts, failure of seminiferous tubule organisation, and in vacuolisation of Sertoli cells in male mice. Rats showed reductions in homogenisation-resistant spermatid counts after exposure to TBTCl in the absence of other toxic effects.

There is evidence of significant toxic effects on development in the offspring in rats and mice. Studies with rats and mice showed that TBTO or TBT salts induced embryo-/foetal lethality, foetal growth retardation, and structural abnormalities as well as impairment of postnatal viability and development following pre- or postnatal exposure. All effects on pre- and postnatal development occur concurrently with significant maternal toxicity (maternal death, maternal weight loss and/or reduction in maternal weight gain). However, maternal mortality was less than 10% and was not considered to be excessive; irreversible effects on developmental toxicity were not considered to be a secondary consequence of maternal toxicity.

Comments received during public consultation

Comments were received from four Member States. Three MS agreed with the proposed classification. One MS (NL) put forward the issue of the read across of toxicological data to other TBT compounds.

Assessment and comparison with the classification criteria

No human data were provided, therefore Repr. 1A is not appropriate.

The CLH report provided convincing data for adverse effects on fertility (especially in females) occurring with only limited other toxic effects in rats and mice. This corresponds to Repr. 1B (H360F) based on the CLP criteria and Repr. Cat 2 (R60) under the DSD.

The effects on the development of offspring were somewhat masked by the moderate to severe maternal toxicity observed in all the developmental toxicity studies summarised in the CLH report.

Whereas some of the observed effects in offspring (low weight, resorptions) could be linked to maternal toxicity, RAC considers that at least some of the serious adverse effects on foetuses (e.g. cleft palate), seen in multiple studies in both rats and mice, are not secondary to maternal toxicity. Spontaneous cleft palates are rare in rats, suggesting a specific MoA for this effect.

Cleft palates are mentioned in the RAC opinion for Dioctyltin bis(2-Ethylhexyl mercaptoacetate) (http://echa.europa.eu/documents/10162/5266b444-9e22-4051-86ec-e0c59a95649b), which concluded that classification of the substance as Repr. 1B (H360D) according to the CLP Regulation was appropriate. A potential metabolite of TBT, dibutyltin dichloride also has a harmonised classification as Repr. 1B (H360FD). Although these considerations represent only indirect support for the Repr. 1B classification, they do suggest that developmental defects, including cleft palates, are intrinsic, adverse effects specifically associated with some organotin compounds.

RAC therefore considers that these effects warrant classification as Repr. 1B – H360D for developmental toxicity (Repr. Cat. 2 (R61) under the DSD). No data suggest that the observed effects may not be relevant to humans.

Combining the toxicological data for both development and fertility, the RAC considered that the appropriate resulting classification was Repr. 1B (H360FD) under the CLP Regulation and Repr. Cat. 2; R60-61 according to DSD.

4.12 Other effects

Not relevant for this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier.

6 OTHER INFORMATION

No other information.

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