

n-Butyltin Compounds

MAK value (2007)	0.004 ml/m³ (ppm) \triangleq 0.02 mg/m³ (as tin)
Peak limitation (2007)	Category I, Excursion Factor 1
Absorption through the skin (2007)	H
Sensitization	–
Carcinogenicity (2007)	Category 4
Prenatal toxicity (2007)	Group C
Germ cell mutagenicity	–
BAT value	–

Mono-*n*-butyltin compounds: $\text{CH}_3(\text{CH}_2)_3\text{SnR}$

Compound	CAS No.	R =	Molecular weight [g/mol]	Synonyms	Solubility in water [mg/l]*	log K_{ow} *
Mono- <i>n</i> -butyltin trichloride (MBTC)	1118-46-3	Cl ₃	282.2	Butyltrichlorostannane; trichlorobutyltin	7.31×10^4 (est)	0.18
Mono- <i>n</i> -butyltin tris(2-ethylhexylmercaptoacetate) [MBT(2-EHMA)]	26864-37-9	($\text{SCH}_2\text{COOCH}_2\text{CH}(\text{C}_2\text{H}_5)\text{C}_4\text{H}_9$) ₃	785.6	Butyltin tris(2-ethylhexylthioglycolate); mono- <i>n</i> -butyltin tris(2-ethylhexylthioacetate)	0.06	12.45
Mono- <i>n</i> -butyltin tris(isooctylmercaptoacetate) [MBT(IOMA)]	25852-70-4	($\text{SCH}_2\text{COO}(\text{CH}_2)_5\text{CH}(\text{CH}_3)_2$) ₃	785.6	Butyltris(isooctyloxy carbonylmethylthio)stannate; Butyltintris(isooctylthioglycolate)	no details	no details
Di-<i>n</i>-butyltin compounds: $(\text{CH}_3(\text{CH}_2)_3)_2\text{SnR}$						
Di- <i>n</i> -butyltin dichloride (DBTC)	683-18-1	Cl ₂	303.8	Di- <i>n</i> -butyltin(IV)di chloride; Dibutylchlorostannate	92	1.56
Di- <i>n</i> -butyltin difluoride (DBTF)	563-25-7	F ₂	270.9	Di- <i>n</i> -butyltin(IV) difluoride; Dibutyldifluorostannane (est)	1.5×10^3 (est)	1.25
Di- <i>n</i> -butyltin diacetate (DBTA)	1067-33-0	(OOCCH ₃) ₂	350.8	Bis(acetyloxy)dibutyltin	6	0.81 (est)
Di- <i>n</i> -butyltin dilaurate (DBTL)	77-58-7	(OOC(CH ₂) ₁₀ CH ₃) ₂	631.6	Bis(dodecanoyloxy) di- <i>n</i> -butylstannane; Bis(lauroyloxy) di(<i>n</i> -butyl)stannane; Dibutylbis[(1-oxododecyl)oxy]stannane;	3	3.12

Compound	CAS No.	R =	Molecular weight [g/mol]	Synonyms	Solubility in water [mg/l]*	log K _{ow} *
Di-n-butyltin maleate (DBTM)	78-04-6	OOCCHCHCOO	347.0	2,2'-Dibutyl-1,3,2-dioxastannepin-4,7-dione; Di-n-butyl(maleate)tin; Di-n-butyl-1,3,2-dioxastannepin-4,7-dione; Dibutyl(maleoyldioxy)tin; Dibutylstannylene maleate	17.4 (est)	3.02 (est)
Di-n-butyltin oxide (DBTO)	818-08-6	O	248.9	Di-n-butylloxostannane; Dibutylloxostannane; Dibutylstannane oxide	0.67 (est)	5.33 (est)
Di-n-butyltin bis(2-ethylhexylmercaptoacetate) [DBT(2-EHMA)]	10584-98-2	(SCH ₂ COOCH ₂ CH(C ₂ H ₅)(C ₄ H ₉) ₂	639.6	Di-n-butyltin di-2-ethylhexylthioglycolate; Di-n-butyltin bis(thioglycolic acid-2-ethylhexylester); Dibutyltin bis(2-ethylhexylthioglycolate); Dibutyltin-S,S'-bis(2-ethylhexylthioglycolate); Dibutyl(di(2-ethylhexyl-oxy-carbonylmethylthio)-stannane; Bis(2-hexylthioglycolate)-dibutyltin	1.54×10 ⁻⁸ (est)	11.43 (est)

Compound	CAS No.	R =	Molecular weight [g/mol]	Synonyms	Solubility in water [mg/l] ^a	log K _{ow} [*]
Di- <i>n</i> -butyltin- <i>S,S'</i> -bis(isooctylthioglycolate) [DBT(IOMA)]	25168-24-5	(SCH ₂ COO(CH ₂) ₅ CH(CH ₃) ₂) ₂	639.6	Di- <i>n</i> -butyl- <i>S,S'</i> -bis(isooctyl-mercapto-acetate)tin; Dibutyltin bis(isooctyl-thioglycolate);	3.33×10 ⁻⁷ (est)	11.4 (est)
Tri- <i>n</i> -butyltin chloride (TBTC)	1461-22-9	Cl	325.5	Chlorotributyltin; Tributylchlorostannate; Tributylchlorotin; Tributylstannium chloride	17	4.76 (est)
Tri- <i>n</i> -butyltin fluoride (TBTF)	1983-10-4	F	309.1	Tributylfluorostannane; Fluorotributylstannane	6	4.39 (est)
Tri- <i>n</i> -butyltin acetate (TBTA)	56-36-0	OOCCH ₃	349.1	Acetoxytributylstannane	65	3.24
Tri- <i>n</i> -butyltin oxide (TBTO)	56-35-9	OSn((CH ₂) ₃ CH ₃) ₃	596.1	Bis(tributyltin)oxide; Hexa- <i>n</i> -butyldistannoxane	19.5	4.05 (est)
Tri- <i>n</i> -butyltin benzoate (TBTB)	4342-36-3	OOC ₆ H ₅	411.2	Tributyltinbenzoate; Benzoyloxy-tributylstannane	0.26 (est)	4.05 (est)
Tri- <i>n</i> -butyltin lineolate (TBTL)	24124-25-2	OOC(CH ₂) ₆ (CH ₂ CHCH ₃) ₂ (CH ₂) ₄ CH ₃	569.5	Tributyl-(1-oxo-9,12-octadecadienyl)-oxystannane	1.98×10 ⁻⁷ (est)	10.67 (est)
Tri- <i>n</i> -butyltin methacrylate (BTM)	2155-70-6	OOC(CH ₃)CH ₂	375.1	Tributyltin methacrylate; Tributyl-(2-methyl-1-oxo-2-propyl)oxystannane	1.27 (est)	4.14 (est)

Compound	CAS No.	R =	Molecular weight [g/mol]	Synonyms	Solubility in water [mg/l]*	log K _{ow} *
Tri-n-butyltin naphthenate (TBTN)	85409-17-2	no details	about 500		no details	no details
Tetra-n-butyltin						
Tetra-n-butyltin (TTBT)	1461-25-2	(CH ₃ (CH ₂) ₃) ₄ Sn	347.2	Tetrabutylstannane	6.4×10 ⁻⁵ (est)	9.37 (est)

* Values from SRC (2007)

1 Toxic Effects and Mode of Action

N-Butyltin compounds are considered to be carcinogenic. In male mice, DBTA causes an increased incidence of hepatocellular adenomas and carcinomas. In male and female rats, TBTO produces significantly increased incidences of benign tumours of the pituitary gland, phaeochromocytomas of the adrenal gland and adenomas of the parathyroid gland.

N-Butyltin compounds are not genotoxic.

To the skin and to the eyes, *n*-butyltin compounds are irritating to corrosive. N-Butyltin compounds are absorbed after inhalation or ingestion and on skin contact, and are found particularly in the kidneys and liver, but also in the spleen, thymus and brain. N-Butyltin compounds can pass the blood-brain barrier and the placenta. In the acid environment of the stomach, the tin/oxygen or tin/sulphur bonds of the *n*-butyltin compounds detach to form the corresponding *n*-butyltin chlorides. In the liver, hydroxylation and dealkylation to mono-*n*-butyltin takes place, as well as its hydrolysis to a hydrocarbon residue and to hydroxytin.

In rats, apathy, nasal discharge, breathing noises, dyspnoea, piloerection and weight loss are observed as signs of intoxication after repeated inhalation of MBTC or TBTO. Inflammatory reactions occur in the entire respiratory tract. In the presence of TBTO, changes in lymphatic organs occur such as thymus atrophy and lymphocytopenia in the thymus-dependent areas of spleen and lymph nodes. After repeated ingestion, the target organs of *n*-butyltin compounds are, in particular, the organs of the lymphatic system, as well as liver, kidneys and brain. The sensitivity to their immunotoxic effects decreases with age. The most sensitive reactions are observed in pups before weaning.

For the sensitization of MBT and DBT(2-EHMA) in guinea pigs, not the *n*-butyltin cation, but the ligand 2-EHMA seems to be responsible. MBT(IOMA) and TBTO are not sensitizing in the maximization test.

A large number of investigations on prenatal and postnatal developmental toxicity exist for *n*-butyltin compounds. Mono-*n*-butyltin compounds and tetra-*n*-butyltin show the lowest efficacy. Di-*n*-butyltin compounds are teratogenic in rats. Tri-*n*-butyltin compounds are embryotoxic in rats, though they do not produce cleft palates until maternally toxic doses are reached. Postnatal developmental toxicity was found to be the most sensitive endpoint in rats after the administration of tri-*n*-butyltin compounds.

2 Mechanism of Action

General biochemical effects

The toxic effects of organotin compounds are determined by the interactions of lipophilic alkyl groups and by the reactivity of alkyltin cations. Absorption from the

gastrointestinal tract as well as penetration into cells and organelles increase with the size and the number of alkyl groups.

The correlation between toxicity and the size of the alkyl residue is complex. In the case of trialkyltin compounds, it was found from in vitro investigations with human HL-60 cell cultures that toxicity increases with the chain length of the methyl- to butyl residues, but decreases again as the chain length increases further (Ade et al. 1996). With trimethyltin, calculations of its electronic properties showed that the greatest reactivity is expected from the central tin cations, though this reactivity decreases as the amount of hydrophobic residues increases. The maximum toxicity seems to be at the butyltin compounds. These findings led to the hypothesis that the lipophilic character of the alkyl groups determines interactions with the membranes as well as absorption by the membranes, but that the reactivity of the central tin cations is responsible for the specific intracellular reactions (Schüürmann and Markert 1998). This hypothesis is also supported by the results from investigations with synthetic lipid membranes: alkyltin chlorides caused an electric depolarization of the membranes, which was correlated with the lipophilicity of the alkyl groups. In the case of trimethyltin, the membrane-depolarizing effect was clearly weaker than with triethyl-, tripropyl- and tributyltin chloride (Zielinska et al. 2000).

From tinorganic chemistry it is known that the electronically positively charged tin of alkyltin halogenides form, on the one hand, adducts with nitrogen and oxygen atoms from donor molecules, but can form covalent bindings with sulphur in thiols on the other hand (Aylett 1979; Haiduc and Zuckerman 1985). Reactions of tributyltin chloride with sulphydryl groups of proteins were demonstrated using haemoglobin as example (Santroni et al. 1997; Taketa et al. 1980). As the carbon in the tin/carbon compound is negatively charged, the cleavage of electrophilic alkyl residues is improbable. Therefore, no direct alkylation of proteins or DNA bases are to be expected.

Specific biochemical effects

The specific biochemical effects of butyltin compounds can be divided into Ca^{2+} -dependent and Ca^{2+} -independent effects which are, however, closely linked up with each other.

Disturbance of Ca^{2+} homeostasis

The Ca^{2+} -dependent effects of organotin compounds are based on an increase in intracellular free Ca^{2+} concentration in different cells including the T cells (Chikahisa and Oyama 1992; Kawanishi et al. 2001; Nakatsu et al. 2006; Oyama et al. 1994; Stridh et al. 1999 a). With thymocytes, tri-n-butyltin induced a Ca^{2+} mobilization both by increasing the Ca^{2+} permeability of the membranes of intracellular organelles and by inhibiting the Ca^{2+} ATPase of the plasma membranes (Oyama et al. 1994).

Via the disturbance of Ca^{2+} homeostasis, tri-*n*-butyltin induced apoptosis in thymocytes (Aw et al. 1990; Raffray and Cohen 1993), spleen cells (Gennari et al. 1997) and PC12 cells among others (Nakatsu et al. 2007). It was found that an intracellular Ca^{2+} increase produced by tri-*n*-butyltin activated caspase-3 (Nakatsu et al. 2006, 2007), which participates in the apoptosis process as central enzyme. In isolated liver mitochondria, tri-*n*-butyltin induced the release of cytochrome c in the presence of an increased Ca^{2+} concentration (Gogvadze et al. 2002). The cytochrome c released into the cytoplasm can in turn induce apoptosis via a signal cascade (Gennari et al. 2002 b). In contrast, tri-*n*-butyltin had no influence on apoptosis in human natural killer cells, as showed from investigations with pro-apoptotic proteins Bax and p53 as well as with anti-apoptotic protein Bcl-2 (Aluoch et al. 2007).

Different signal transmission paths were influenced as a result of the mobilization of intracellular free calcium and the resultant phosphorylation of mitogen-activated protein kinases produced by tri-*n*-butyltin: the MAP kinase JNK in PC12 cells (Nakatsu et al. 2007), the MAP kinases p38, JNK and ERK in human T cells (Yu et al. 2000) as well as the protein kinases p38 and p44/42 in human natural killer cells (Aluoch and Whalen 2005).

The loss of ability of natural killer cells to bind to tumour cells induced by tri-*n*-butyltin was caused by a loss of surface antigens CD16 and CD56 (Whalen et al.) as well as by a decrease in the cytotoxic function of the proteins granzyme B and perforin (Thomas et al. 2004). The inhibition of the cytotoxicity of natural killer cells by tri-*n*-butyltin could be reversed by more than 50% by incubation with interleukin IL2 and IL12, which shows that tri-*n*-butyltin acts through a disturbance of signal transduction (Whalen et al. 2002 b).

Other Ca^{2+} -dependent effects were the depolymerization and disintegration of cytoskeletal and nuclear proteins, such as F-actin (Chow and Orrenius 1994; Galli et al. 1993) and tubulin, induced by tri-*n*-butyltin (Jensen et al. 1989, 1991 a, 1991 b; Tan et al. 1978), as well as inhibition of fMLP-induced actin polymerization and the resultant depolymerization of actin caused by it (Galli et al. 1993). These mechanisms partially explain the cytotoxic effects produced by *n*-butyltin compounds, particularly in thymus and spleen (see also "Immunotoxicity" Section).

In addition, the loss of stainable spindles in V79 hamster fibroblasts caused by di- and tri-*n*-butyltin (Jensen et al. 1991 a), as well as the chromosomal contractions (Jensen et al. 1989) and hyperdiploid cells (Jensen et al. 1991 b) induced in human lymphocytes can be attributed to Ca^{2+} -dependent reactions.

Interaction with proteins and membranes

The pronounced enzyme inhibition of all organotin compounds is Ca^{2+} -independent. It is due to their interaction with proteins, as they produce changes in conformation or enter into coordinative and covalent binding with amino acids. Organotin compounds generally inhibit oxidative phosphorylation (Aldridge and Cremer

1955) and ATP synthesis (Aldridge et al. 1977), whereby mitochondrial degeneration can occur (Yoshizuka et al. 1992). In particular, the trialkyltin compounds disturb mitochondrial function by accumulating within the inner mitochondrial membrane and by forming stable precipitates (Cima et al. 2003) as well as by disturbing Cl^-/OH^- exchange at the membranes, producing structural damage as a result (WHO 1980; Wulf and Byington 1975). Opening of the mitochondrial permeability pores results in a rapid mitochondrial swelling (Cima et al. 2003), capable of producing a bursting of the mitochondria, as shown from the mitochondrial and membrane fragments found in the cytoplasmic vacuoles of hepatocytes in rats after administration of TBTO (Yoshizuka et al. 1992).

In addition, organotin compounds inhibit a number of reactions in xenobiotic metabolism (Rosenberg et al. 1984). The inhibition of important enzymes, such as aromatase (Saitoh et al. 2001), Na^+/K^+ -ATPase (Rao et al. 1987) and glutathione S-transferase (Al-Ghais and Ali 1999) is caused by a reaction of the alkyltin cation with thiol groups.

Apoptosis

The apoptosis caused in vitro and in vivo by *n*-butyltin compounds via the disturbance of Ca^{2+} homeostasis (see also "Disturbance of Ca^{2+} homeostasis") is under discussion as being the underlying mechanism for the immunotoxicity of organotin compounds. Caspases, enzymes that regulate the feedback system of apoptosis, can be both activated (at low concentrations) as well as inhibited (at higher concentrations) by organotin compounds. The former results in apoptosis, the latter induces necrosis (Stridh et al. 1999 b).

Immunotoxicity

The immunotoxicity induced in rats after oral DBTC administration occurring in the form of an atrophy of thymus, spleen and lymph nodes, a reduction in lymphocyte count (Seinen et al. 1977 a) or a delay in transplant rejection reaction as well as a suppressed humoral immune response against sheep erythrocytes (Seinen et al. 1977 b) are not the sequel of an accumulation of organic tin compounds (Penninks and Seinen 1984). Apart from the induction of apoptosis and impairment of the spindle apparatus, the thymus atrophy is probably also based on a selective proliferation inhibition of immature $\text{CD4}^+\text{CD8}^+$ thymoblasts, followed by a reduction of small cortical $\text{CD4}^+\text{CD8}^+$ lymphocytes (Gennari et al. 2002 a). This was confirmed by observations in immature rat thymocytes as well as in rats after DBTC administration with the feed, in which also a proliferation inhibition, but no impairment of the differentiation of thymocytes was demonstrated (Pieters et al. 1994). In rat thymocytes, an inhibition of DNA synthesis and a stimulation of RNA synthesis by DBTC and TBTC was demonstrated in vitro (Gennari et al. 2002 b).

Carcinogenicity

The effects on the disturbance of Ca^{2+} homoeostasis described above are important in the development of the carcinogenicity produced by *n*-butyltin compounds, particularly the loss of binding ability to tumour cells and the loss of cytotoxic function caused by tri-*n*-butyltin in natural killer cells (Aluoch and Whalen 2005; Whalen et al. , 2002 b).

Disturbances of the hormonal feedback regulation systems as a mechanism for tumour formation in endocrine organs should also be discussed. Already after administration of TBTO for six weeks, changed hormone release rates from the pancreas (insulin), pituitary gland (luteinizing hormone, thyroid-stimulating hormone) and thyroid gland (thyroxine) occurred in male rats (Krajnc et al. 1984). As regards the chromaffin cells of the adrenal medulla, it is known that this is where hormones play an indirect part in the formation of hyperplasias and neoplasms. When a partial resection of the pituitary gland was performed in rats with a high spontaneous incidence of adrenal gland tumours, the formation of tumours could be prevented (Tischler et al. 1989).

Impairment of Ca^{2+} homoeostasis can induce proliferations of the chromaffin cells of the adrenal medulla. These proliferations are accompanied by an increased noradrenaline formation (Tischler et al. 1989). Messenger substances regulating the release of catecholamines, are in turn capable of stimulating the proliferation of chromaffin cells in the adrenal medulla (Tischler et al. 1997).

The nephrosis occurring in rats and guinea pigs after long-term administration of TBTO (ACGIH 2001; Wester et al. 1990) may lead to hypocalcaemia, which results in an increased production of parathormone due to the negative feedback regulation. An increase in parathormone formation is accompanied by a proliferation of parathyroid gland cells.

It can therefore be assumed that the disturbance of hormones and Ca^{2+} homoeostasis are responsible for the increased tumour incidences not only in the adrenal medulla and parathyroid glands but also in the pituitary of Wistar rats after long-term administration of TBTO.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

On the basis of workplace-related and personal exposure measurements, a daily absorption of TBTO of 0.83 $\mu\text{g}/\text{kg}$ body weight (0.75 quantile) through the skin and by inhalation was estimated for shipyard employees (no other details on the number of cases or exposure concentration) working with antifouling paints containing tri-*n*-butyltin (BUA 2003).

After oral or intravenous administration of **DBTC** (6 mg/kg body weight), increased tin concentrations were found in the kidneys and liver of rats (about 9 µg/g wet weight). Lower concentrations (about 3 µg/g wet weight) were determined in the pancreas and spleen. No accumulation of tin was found in the thymus. Eight days after a single dose, the organ concentration of tin in the tissues dropped to below 1 µg/g wet weight (Summer et al. 2003).

After administration of a **DBTC** dose of 100 mg/kg feed for one week, the following organ concentrations related to wet weight were measured in pregnant rats: kidneys 4.5, liver 3.2, spleen 1.0, thymus 0.9 and brain 0.5 µg/g tissue (Summer et al. 2003). After oral administration of **DBTC** from days 7 to 17 of gestation to rats, di-*n*-butyltin had been transferred to the embryo or the foetus. When **DBTA** was administered to rats on day 8 of gestation, it was possible to demonstrate the presence of di-*n*-butyltin and mono-*n*-butyltin in the embryo (WHO 2005).

Tri-*n*-butyltin compounds are readily absorbed after inhalation (Beliles 1994), but incompletely absorbed via the oral and dermal route (WHO 1999). After ingestion of different tri-*n*-butyltin compounds, presumably always **TBTC** is absorbed by the gastrointestinal tract after dissociation. In the rat, 20–50% of the administered **TBTO** was absorbed through the gastrointestinal tract, 1–10% through the skin as well as through the lungs (no other details on absorption). **TBTO** is capable of passing the blood-brain barrier and is also transferred to the foetus via the placenta. After a rapid distribution, metabolites in the blood were demonstrable within three hours, particularly in the liver and kidneys. The elimination half-life of **TBTO** metabolites in the mouse was 29 days; in the rat, a biphasic elimination was observed with half-lives of about twelve hours and three days. After oral administration of **TBTO** to the rat for 14 days, steady-state in tissue concentrations was reached after three to four weeks (WHO 1999 BUA 2003). After oral administration of **TBTO** to rats for four weeks, tin concentrations were found in the liver and kidneys which were five to ten times higher than in the brain and fatty tissue (Krajnc et al. 1984). After administration of [¹⁴C]**TBTO** with the drinking water for five to 30 days, mice excreted most of the radioactivity with the faeces. The highest radioactivity was measured in kidneys, liver, spleen and fatty tissue (Evans et al. 1979).

Two studies on dermal penetration *in vitro* are available in which details on absorption after one hour (assumed standard exposure scenario) are also given. After occlusive application of 0.5 mg/cm² **DBTC** on human skin *in vitro* for 24 hours, the penetration rate of tin was 0.463 µg/cm² and hour during the first hour (TSA 2003 a), but only 0.029 µg/cm² and hour when the tin was applied non-occlusively. With **DBT(2-EHMA)** doses of 113 mg/cm², tin fluxes of 0.029 or 0.027 µg/cm² and hour (TSA 2003 b) were measured in the first hour after occlusive and non-occlusive application. The quantities applied did not damage the skin (TSA 2003 a, b). For 0.3 mg/cm² **TBTO**, the average tin flux over eight hours was 0.28 µg/cm² and hour, and that for the tin from 9.62 mg/cm² **TBTM** was 0.01 µg/cm² and hour (RPA 2005). On the rat skin, applying the same quantities as above, higher penetration rates were measured after one hour: 2.78 and 2.39 µg tin/cm² and hour for

DBTC (TSA 2003 a) and 0.196 and 0.109 $\mu\text{g tin}/\text{cm}^2$ and hour for **DBT(2-EHMA)** (TSA 2003 b) for occlusive and non-occlusive application, respectively. The reasons for the partly very different fluxes of the different compounds *in vitro* are not clear. The mean penetration rates determined over eight hours in the TSA studies (2003 a, b) are somewhat lower than those obtained after application for one hour. In these studies, the recovery in percent is relatively low at 50% to 80%. A possible absorption of *n*-butyltin on the glass device used was discussed. Thus, the measured penetration rates could also be higher. In the studies described in RPA (2005), there are no details on whether testing was occlusive, what solvent was used, and how high the recovery was. This limits the comparability of the results.

A dose of 0.5 ml **TBTO** was applied over a skin surface of 25 cm^2 of two monkeys for seven hours. As the density of the substance was 1.17 g/cm^3 , this is equal to an applied quantity of 0.585 g or 23.4 mg/cm^2 . $8.39 \pm 3.06\%$ of the administered dose was eliminated with the faeces within 16 days, and $1.37 \pm 0.11\%$ with the urine within 13 days, whereby $17.5 \pm 2.2\%$ remained in the stratum corneum. Assuming that the amount of TBTO still present in the stratum corneum is subsequently absorbed, a mean absorption of about 27% can be calculated for TBTO and, from this, an approximate dermal penetration rate of 0.9 mg/cm^2 and hour corresponding to 0.36 $\text{mg tin}/\text{cm}^2$ and hour (Hümpel et al. 1987). Although an irritant effect on the skin that could have increased absorption was not described in this study it is, however, probable since TBTO was highly irritating after application on the back of the hand for two to three hours (see Section 4.3).

After daily administration by gavage of **TBTO** doses of 0, 0.2, 1.0 or 5.0 mg /kg body weight and day for 12 months to 4 male and 4 female beagles the determination of the tin concentration in the urine at different readings revealed that renal elimination was about 10%, 5% or 2.5% of the administered dose. In the fifth week after the start of treatment, steady-state was reached between absorption and elimination (Schering AG 1992).

From studies in rats with repeated oral administration of **TBTO** it was calculated that the steady-state is reached after about four weeks (Hümpel et al. 1987).

In rats, **TTBT** was mainly absorbed in the small intestine. Only a small amount (0.10–0.16%) was dealkylated to the tri-*n*-butyltin cation, which can be eliminated with the urine or the faeces. The TTBT was released into the bile and then metabolized or reabsorbed in the small intestine (Parametrix Inc 2006 j).

3.2 Metabolism

After intraperitoneal administration of **DBTC**, butyl(3-hydroxybutyl)tin dichloride, butyl(4-hydroxybutyl)tin dichloride and MBTC in the form of acid-resistant degradation products were found to be present in male rats. Butyl(3-hydroxybutyl)tin dichloride was mainly found in the kidneys and butyl(4-hydroxybutyl)tin dichloride

in the urine. DBTC and all three metabolites were demonstrated in the brain (Ishizaka et al. 1989).

After oral administration of **TBTC** to male rats, DBTC, MBTC and unchanged TBTC were found in the liver, kidneys, spleen, brain as well as in blood and urine after six and 24 hours. The metabolite butyl(3-hydroxybutyl)tin dichloride was demonstrated in the liver, kidneys, spleen and urine, and butyl(3-carboxypropyl)tin dichloride as the main metabolite in the liver. Small quantities of butyl(3-oxobutyl)tin dichloride and butyl(4-hydroxybutyl)tin dichloride were found in the urine. No tri-*n*-butyltin residues could be determined in any organ. The metabolites butyl(3-carboxypropyl)tin dichloride, butyl(3-oxobutyl)tin dichloride or butyl(4-hydroxybutyl)tin dichloride were administered to the rats by intraperitoneal injection in a further investigation. After one day, the main metabolite, butyl(3-carboxypropyl)tin dichloride, was found with all treatments. This shows that TBTC is mainly dealkylated, but also oxidized (Matsuda et al. 1993). After oral administration of **TBTC** to mice, DBTC (40%) and di-*n*-butyl(3-carboxypropyl)tin chloride (12–26%) were determined as main metabolites in the liver. The other metabolites and unchanged TBTC made up for less than 12% of the entire amount of *n*-butyltin. The formation of TBTC metabolites could be greatly reduced through the inhibition of cytochrome P450 with SKF-525A. After administration of **DBTC**, more than 95% of the dose was demonstrated in the liver in the form of DBTC. Pretreatment with SKF-525A had no influence on DBTC metabolism. These results indicate that, in the mouse liver, cytochrome P450 enzymes play a considerably greater role in the metabolism of TBTC to form DBTC and MBTC than in the metabolism of DBTC to form MBTC (Ueno et al. 1997).

After oral administration of **TBTF** to rats, tri-*n*-butyltin was found in the liver, and the metabolites mono-*n*-butyltin and inorganic tin in the brain (Iwai et al. 1981). In vitro **TBTA** was initially hydroxylated by an isolated rat liver monooxygenase fraction to α -, β -, γ - and δ -hydroxy-tri-*n*-butyltin (Fish et al. 1975; Kimmel et al. 1977). 1-Butanol and di-*n*-butyltin were formed from α -hydroxy-tri-*n*-butyltin, butene and di-*n*-butyltin from β -hydroxy-tri-*n*-butyltin, and γ -keto-tri-*n*-butyltin from γ -hydroxy-tri-*n*-butyltin. The di-*n*-butyltin compounds were further hydroxylated and cleaved to form mono-*n*-butyltin. For mechanistic reasons, it is assumed that the alkyltin binding to the hydrocarbon residue and to hydroxytin is hydrolyzed (Kimmel et al. 1977).

In the case of organic tin compounds, the tin/oxygen compounds dissolve in the acid environment of the stomach with ligands, which are coordinated to the tin ion via oxygen atoms, so that the corresponding alkyltin chlorides as well as their free ligands are formed. In a simulated **DBTL** hydrolysis in 0.07 M HCl at pH <2 and 37°C, the estimated half-life for DBTC formation or laurate cleavage was <0.5 hours. More than 80% of the substance used was hydrolyzed within 0.5 hours (Parametrix Inc 2006 c). In the simulated **DBTM** hydrolysis, the estimated half-life for DBTC formation or maleate cleavage was also less than 0.5 hours. The entire substance was hydrolyzed within 0.5 hours (Parametrix Inc 2006 d). The same investigations with **DBTO** showed an estimated half-life of 3.5 hours for DBTC

formation (Parametrix Inc 2006 e). The use of **DBT(2-EHMA)** revealed a 100% release of the EHMA ligands after one hour (Parametrix Inc 2006 b).

4 Effects in Humans

4.1 Single Exposure

In a case study, five persons were exposed to TBTO, which had been admixed with a latex paint and applied as a paint coat (no other details). Symptoms like nausea, vomiting, headache, sore throat, burning in the nasal mucosa, lacrimation and wheezing breath occurred (BUA 2003).

Acute health impairments such as headache and irritation of the upper respiratory tract were described after short-term exposures above 0.2 mg/m³ (as tin) to organotin compounds (ACGIH 2001).

4.2 Repeated exposure

Personal exposure monitoring in seven PVC-processing plants in Canada and the USA showed that organotin concentrations at the workplace were clearly below 0.1 mg tin/m³ in a total of 102 persons during a normal shift of seven to eight hours. Concentrations of <0.001 to 0.034 mg tin/m³ were measured in 100 cases. A concentration of 0.102 mg tin/m³ was only found once, i.e. during a manual mixing process. Respiratory protection was worn during this activity (Boraiko and Batt 2005).

In the course of annual health screening of exposed employees at an American organotin manufacturer, no differences in clinico-chemical parameters, urinalysis, lung function, ECG and thorax radiography were found compared with new employees or non-exposed persons. Although within the normal range, the erythrocyte count, haematocrit value and haemoglobin content of the exposed persons were, however, significantly lower than in the control collectives. These differences were not observed when the 14 workers of the tri-*n*-butyltin unit were considered exclusively. Altogether, 338 employees were investigated at this factory, of which 44 were directly exposed in organotin-processing areas (Meyer et al. 1987).

4.3 Effects on skin and mucous membranes

Skin

With the exception of **DBTC**, single application of different di-*n*-butyltin compounds (**DBTA**, **DBTM**, **DBTL**, **DBTO**) on the back of the hands was tolerated by volunteers without irritant effects (ACGIH 2001; WHO 1980).

Tri-*n*-butyltin compounds, particularly **TBTA**, caused hyperaemia, followed by folliculitis and pruritus within eight hours. The changes healed spontaneously (ACGIH 2001). **TBTC** had an irritant effect on the skin of volunteers (WHO 1980). After contact with liquids containing **TBTO**, the irritation was reversible and avoidable by timely cleaning of the skin (BUA 2003). Undiluted **TBTO** was found to be extremely irritating in volunteers after two to three hours exposure to the backs of their hands (BUA 1988). In workers coming into contact with 10–11.7% TBTO, a delayed dermatitis was described. Skin irritation still occurred in patch tests using a 0.1% TBTO solution (BUA 1988).

After non-occlusive application for up to eight hours, no increase in skin irritation was found in a study using 1% **TBTO** in model formulations for wood preservatives. It is assumed that TBTO is better tolerated by the skin when in solution than when it is in dispersion (BUA 1988).

Ten hours after skin contact with a liquid containing **TBTO**, lesions developed over the affected areas in one worker (no other details), which subsided within a week during treatment with antibiotics and antihistaminics. The symptoms reoccurred within four hours, after the worker put the clothes on again which had previously been contaminated with TBTO (BUA 2003).

Application of TTBT on the back of the hands caused no irritation in volunteers (WHO 1980).

Eyes

In workers, irritation to the eyes as well as irritation in the upper and lower respiratory tract were reported after contact with **TBTO** in aerosol form (no other details) (BUA 1988).

Exposure to tri-*n*-butyltin compounds, particularly **TBTO**, can produce damage to the mucous membranes. Irritant effects to the eyes and upper respiratory tract were reported in 70% of the affected workers after exposure to 0.19 and 0.29 mg/m³ TBTO (as tin) lasting 32 to 62 minutes (ACGIH 2001).

4.4 Allergenic effect

Skin sensitization

Investigations in workers, who had been exposed to antifouling paints containing **TBTO**, revealed no indication for skin-sensitizing effects in a patch test using TBTO (0.01% in water) (Gammeltoft 1978).

Sensitization of the airways

There are no data available.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

The signs after the single inhalation, ingestion or dermal absorption of *n*-butyltin compounds are similar and usually non-specific: weakness, reduced activity, piloerection, dyspnoea and trembling. Macroscopic findings after oral administration include haemorrhages and inflammation in the gastrointestinal tract, congestions in organs, discoloration of liver, spleen and kidneys as well as peritonitis. After inhalation exposure, haemorrhages in the lung, pulmonary emphysemas and oedemas also develop. Due to the irritant effect, dermal application additionally produces local lesions such as eschar formation, erythemas, deep fissures and local necroses. No systemic effects were found in the surviving animals (Parametrix Inc 2006 a–h).

5.1.1 Inhalation

In male and female rats, 59 mg/m³ was determined as 4-hour LC₅₀ for **DBTC** and 317 mg/m³ for **DBTM** (OECD 2006 b). An LC₅₀ of 22 mg/m³ (no details on exposure period) was given for **DBT(IOMA)** (Parametrix Inc 2006 i). The 1-hour LC₅₀ for **TBTC** was 71000 mg/m³ (Parametrix Inc 2006 f). A 4-hour LC₅₀ of 65 mg/m³ was determined for **TBTO** in male and female rats (Schweinfurth 1985).

5.1.2 Ingestion

The acute oral toxicity of *n*-butyl compounds is shown in Table 1. **MBTC** produced apathy, weight loss, hyperaemia, emphysemas and lung damage (no other details), bloody mucous membrane changes and haemorrhages in the mucosal glands, considerable haemorrhages in the intestine and pancreas, as well as necroses in liver and kidneys (Parametrix Inc 2006 f).

In mice, general weakness and exhaustion, reduced food consumption, reduced reaction to sound and light stimuli as well as shallow breathing occurred within 24 hours as signs of intoxication with **MBT(2-EHMA)**. Gross pathology revealed an enlarged stomach containing bloody content, haemorrhages in intestinal walls and serous membranes, enlargement of the liver and gallbladder and dark discoloured kidneys (Parametrix Inc 2006 g).

Also after di-*n*-butyltin administration, general weakness, lassitude, hypokinesia, lateral position, reduced food consumption, unkempt fur, dyspnoea and diarrhoea (e.g. with **DBTC**; Parametrix Inc 2006 a), exophthalmia (**DBTM**; Parametrix Inc 2006 d and **DBT(2-EHMA)**; Parametrix Inc 2006 b) as well as damage to the liver (**DBTA**; Calley et al. 1967), bile ducts, pancreas (**DBTC**; Barnes and Magee 1958), stomach and intestine (**DBTC**; Parametrix Inc 2006 a) were described in rats, mice or rabbits.

Table 1 Acute oral toxicity of *n*-butyltin compounds

Substance	Species	LD ₅₀ [mg/kg body weight]	LD ₅₀ [mg/kg body weight]	References
Mono-<i>n</i>-butyltin compounds				
MBTC	rat	357–3200	158–1346	WHO 2005
	mouse	> 1240–4000	>522–1682	Parametrix Inc 2006 f
MBT(2-EHMA)	rat	303–334	46–50	Parametrix Inc 2006 g
	mouse	1520	230	Parametrix Inc 2006 g
MBT(IOMA)	rat	485	73	OECD 2006 a
Di-<i>n</i>-butyltin compounds				
DBTC	rat	50–219	20–86	Parametrix Inc 2006 a
DBTA	mouse	110	37	Calley et al. 1967
DBTL	rat	2071	389	Parametrix Inc 2006 c
DBTM	rat	510	174	Parametrix Inc 2006 d
DBTO	rat	172–487	82–232	Parametrix Inc 2006 e
DBT(2-EHMA)	rat	396–4439	73–824	Parametrix Inc 2006 b
DBT(IOMA)	rat	485–3088	90–572	Parametrix Inc 2006 i
Tri-<i>n</i>-butyltin compounds				
TBTC	rat	129–349	47–127	Parametrix Inc 2006 h
	mouse	117	43	Parametrix Inc 2006 h
TBTF	rat	94	36	Schweinfurth 1985
TBTA	rat	50–100	17–34	ACGIH 2001
TBTO	rat	112–234	45–93	BUA 2003
	mouse	84	33	BUA 2003
TBTB	rat	99–203	29–59	Schweinfurth 1985
TBTL	rat	190	40	Schweinfurth 1985
TBTN	rat	224	about 58	Schweinfurth 1985
Tetra-<i>n</i>-butyltin				
TTBT	rat	>2000–6000	>684–2051	Parametrix Inc 2006 j
	mouse	>913–6000	>312–2051	Parametrix Inc 2006 j

As signs of intoxication from **TBTO**, mainly apathy and weight loss as well as irritation of the gastrointestinal tract (BUA 1988, 2003; Schweinfurth 1985) occurred. After single oral administration of **TBTO** (30 or 90 mg/kg body weight), a dose-dependent thymus atrophy in juvenile male Wistar rats was only of short duration. The animals recovered within ten days (BUA 2003). With **TBTA**, **TBTB** and **TBTC**, laboured breathing, apathy, attacks of vertigo, seizures as well as damage to the gastrointestinal tract, the liver and the kidneys occurred in mice

(Pelikan and Cerny 1968 a). Similar symptoms were also observed in rats after administration of **TBTA**. Histopathological investigations revealed congestions of lung, liver, kidneys and brain as well as haemorrhages in the lung and damage to the intestinal mucosa (Attahiru et al. 1991).

In studies on acute toxicity hunched posture, lethargy, ataxia and piloerection were reported after application of **TBT** (Parametrix Inc 2006 j).

To estimate the potency of **TBTC**, **DBTC** and **MBTC**, the activity of ornithine carbamoyl transferase in the serum of mice was investigated. The enzyme was used as a marker for liver damage. The lowest doses at which a significant increase in activity occurred 24 hours after administration were 180 $\mu\text{mol/kg}$ body weight **TBTC** (58.6 mg/kg body weight), 60 $\mu\text{mol/kg}$ body weight **DBTC** (18.2 mg/kg body weight) and 7000 $\mu\text{mol/kg}$ body weight **MBTC** (1975 mg/kg body weight). When the administered doses of *n*-butyltin compounds were equivalent (180 $\mu\text{mol/kg}$ body weight), an increased activity of ornithine carbamoyltransferase occurred with **TBTC** after 24 hours and with **DBTC** after 12 hours; with **MBTC**, no increased activity was observed within 96 hours (Ueno et al. 1994).

In mice, after oral administration of 180 $\mu\text{mol/kg}$ body weight **DBTC** or **TBTC** (54.7 mg **DBTC**/kg body weight; 58.6 mg **TBTC**/kg body weight), it was shown that the hepatotoxicity of **TBTC** could be prevented by a preceding inhibition of cytochrome P450 with SKF-525A for 24 hours. The blocking of cytochrome P450 (see also 3.2) had no influence on the effect of **DBTC**. These results indicate that **DBTC**, the **TBTC** metabolite, is particularly responsible for the toxic effects (Ueno et al. 1997). Male Wistar rats received **MBTC** at single doses of 10 to 180 mg/kg body weight, **DBTC** at 5 to 35 mg/kg body weight or **TBTC** at 5 to 60 mg/kg body weight. The relative weights of thymus and spleen were not significantly reduced by **MBTC**, but significantly by **DBTC** and **TBTC**. The weight losses were most pronounced four days after administration. Dose levels calculated to cause 50% reduction of relative thymus weight were 18 mg **DBTC**/kg body weight or 29 mg **TBTC**/kg body weight. **DBTC** and **TBTC** caused a dose-dependent decrease in lymphocyte count in the thymus cortex, and a considerable reduction in cortex thickness. The greatest increase in the lymphoblast count was accompanied by the maximum of the thymus atrophy. As the effects of **TBTC**, compared with those of **DBTC**, were less pronounced and occurred with a certain delay, it was concluded that the toxic effects are produced by **DBTC** (Snoeijs et al. 1988).

5.1.3 Dermal application

The available data for acute dermal toxicity are summarized in Table 2.

The dermal LD_{50} for **mono- and di-*n*-butyltin compounds** in rats and rabbits is mostly above 2000 mg/kg body weight (Parametrix Inc 2006 b, 2006 e; Summer et al. 2003). Exceptions were the dermal LD_{50} in the mouse for **DBTA** at 108–180 mg kg/body weight (Summer et al. 2003) and in the rat for **DBT(2-EHMA)**, which were between 777 and >1000 mg/kg body weight (Parametrix Inc 2006 b).

Table 2 Acute dermal toxicity of n-butyltin compounds

Substance	Species	LD ₅₀ [mg/kg body weight]	LD ₅₀ [mg/kg body weight]	References
Mono-n-butyltin compounds				
MBT(IOMA)	rat	>2000	>302	Summer et al. 2003
Di-n-butyltin compounds				
DBTF	rat	>2000	>876	Summer et al. 2003
DBTA	mouse	108–180	37–61	Summer et al. 2003
DBTL	rabbit	>2000	>376	Summer et al. 2003
DBTO	rabbit	>2000	>954	Parametrix Inc 2006 e
DBT(2-EHMA)	rat	777– >1000	144– >185	Parametrix Inc 2006 b
DBT(IOMA)	rat	2086–3088	387–573	OECD 2006 b
Tri-n-butyltin compounds				
TBTF	rat	680	261	Sheldon 1975
TBTO	rat	605	241	BUA 1988
	rabbit	11 700	796	BUA 1988

The dermal LD₅₀ for **TBTO** was 605 mg/kg body weight in rats (BUA 1988) and 11700 mg/kg body weight in rabbits (Elsea and Paynter 1958). As signs of intoxication, a loss in body weight, laboured respiration or dyspnoea, weakness of the hind limbs, diarrhoea, unsteadiness, depressed reflexes, prostration and clonic convulsions were described (Elsea and Paynter 1958).

5.1.4 Intravenous, intraperitoneal and intramuscular injection

After single intravenous injection of **DBTC** in doses of 1 mg/kg body weight, a marked decrease in thymus weight particularly after four days was described in rats and mice. This weight loss was reversible within nine days (Penninks and Seinen 1984). Single intravenous injection of **DBTC** in doses of 2.5 mg/kg body weight caused pronounced thymus atrophy in rats (Penninks and Seinen 1982). In mice, **DBTC** caused a decrease in thymus weight and thymocyte count as well as an enlargement of the bile duct diameter and an increase in the activity of alkaline phosphatase four days after intravenous injection of 0, 15 or 20 µmol/kg body weight (0, 5 or 6.8 mg/kg body weight) (Hennighausen et al. 1980). After intravenous administration of **TBTO**, the LD₅₀ was between 5 and 20 mg/kg body weight in rats and 6 mg/kg body weight in mice (BUA 1988).

After intraperitoneal injection, an LD₅₀ of >4000 mg/kg body weight was reported for **TTBT** in Wistar rats (Parametrix Inc 2006 j).

To investigate the development of the nervous system, single **TBTO** doses of 0, 2, 3 or 4 mg/kg body weight were administered to five-day-old rats by intraperitoneal injection. This produced a dose- and time-dependent reduction of proteins, which are involved in neuronal and glial development. The prosencephalon and the cerebellum were most affected, the hippocampus least affected. After TBTO administration, a transient reduction in brain weight was reported at 2 or 3 mg/kg body weight, brain and body weight were reduced at 4 mg/kg body weight TBTO (BUA 2003).

Four hours after intramuscular injection of **TBTO** in doses of 0.5 ml/kg body weight to Wistar rats, swollen mitochondria as well as vacuoles containing degenerated mitochondria and membranes, were observed in the hepatocytes. The fine structure of intrahepatic bile ducts was unchanged. By polarographic analysis, a disturbance of oxidative phosphorylation was found. In the serum, the activities of aspartate and alanine aminotransferase were increased, but not those of alkaline phosphatase and leucine aminopeptidase. The concentration of total bilirubin was unchanged. Four days after injection of TBTO, the hepatocytes had regenerated (Yoshizuka et al. 1992).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

Inhalation of *n*-butyltin compounds produced irritation of the mucous membranes in the respiratory tract as well as a delayed body weight gain. The lung and the lymphatic organs were also found to be target organs.

Groups of 35 male and 35 female CD rats were whole-body exposed to a vapour-aerosol mixture of **MBTC** in concentrations of 0, 1, 10 or 30 mg/m³ (particle size 0.98–1.7 µm) 6 hours per day and 5 days per week for 28 days. Ten animals were killed immediately at the end of exposure, the others after an observation period of two to four weeks. At 30 mg/m³, three males and one female died during exposure. As signs of intoxication, mucoid nasal discharge, rales, lacrimation, salivation, rough coat, abdominal distension (male animals), anogenital staining and fur discoloration were found. The body weights of all males and part of the females were reduced in all treatment groups during the exposure period. The haemoglobin values (in males and females), erythrocyte counts (males) and haematocrit values (females) increased dose-dependently at 1 mg/m³ and above. The haematological findings normalized during the recovery period. Other investigations showed discoloration and amorphous material in the lungs, alveolar oedema, peribronchial accumulation of lymphoid cells, perivascular infiltration of lymphoid cells and an accumulation of alveolar macrophages. No histological examinations of thymus, spleen and lymph nodes were carried out. From this study, a LOAEC (lowest observed

adverse effect concentration) of 1 mg/m³ for MBTC is obtained. A NOAEC (no observed adverse effect concentration) cannot be given (Parametrix Inc 2006 f).

In an inhalation study, ten juvenile male and female Wistar rats per group were exposed 21 to 24 times to **TBTO** vapour in concentrations of 0, 0.03 or 0.16 mg/m³ as well as to a TBTO aerosol of 2.8 mg/m³ on 4 hours per day for 29 to 32 days. At TBTO concentrations of 0.03 or 0.16 mg/m³ no substance-related effects occurred. At 2.8 mg/m³ TBTO, five males and six females died. Apathy, nasal discharge, breathing noises, dyspnoea, piloerection and weight loss were observed as signs of intoxication. A significantly reduced food consumption combined with a delayed body weight gain was found in the males. In the serum, erythrocyte and thrombocyte counts were increased in females. The number of neutrophilic granulocytes was decreased in males. In addition, the concentration of α - and β -globulins decreased and the albumin-globulin ratio increased. Histological examination revealed inflammatory reactions in the entire respiratory tract as well as changes of the lymphatic organs, such as thymus atrophy, decrease of lymphocyte count in the thymus-dependent regions of the spleen and lymph nodes. For TBTO, a NOAEC of 0.16 mg/m³ is obtained from this study (Schering AG 1983).

5.2.2 Ingestion

The target organs for n-butyltin compounds after ingestion are, in particular, the organs of the lymphatic system, the liver, kidneys and brain. With TBTO as example, it was shown that sensitivity to n-butyltin compounds decreases with age. The most sensitive reactions are observed in pups before weaning (Vos et al. 1990).

Studies of the effects of n-butyltin compounds on the immune function showed that the histopathological effects of TBTO on the lymphatic organs of the rat are accompanied by disturbances in the function of the immune system. Infection models with bacteria and parasites, such as *Listeria monocytogenes* or *Trichinella spiralis*, were found to be particularly sensitive (Verdier et al. 1991; Vos et al. 1990). In a comparative study, in which TBTO was administered with the food to weaned and one-year-old rats for four to six months, resistance after infection by *Trichinella* larvae was more clearly suppressed in the younger animals. For this effect, the authors gave a NOAEL (no observed adverse effect level) of about 0.05 mg/kg body weight and day (weaned animals) or about 0.25 mg/kg body weight and day (adult animals). After exposure for 16 months, the activity of natural killer cells of the spleen and peritoneum was suppressed at about 0.05 mg/kg body weight and day. This effect did not occur after treatment for 4.5 months (Vos et al. 1990).

Results from studies on the effects of n-butyltin compounds after repeated oral administration are shown in Table 4.

Table 3 Effects of *n*-butyltin compounds after repeated oral administration

Species, strain, number per group	Exposure	Findings	References
MBTC			
Rat, Wistar, 10 ♂, ♀	13 weeks, 0, 300, 1500, 7500 mg MBTC/kg feed (about 0, 20, 100, 529 mg/kg body weight and day)	about 100 mg/kg body weight: NOAEL about 529 mg/kg body weight: relative liver weight increased; serum: activity of ALT increased, AST increased, γ -GT increased, bile acids decreased, triglycerides decreased, phospholipids decreased, potassium decreased, prothrombin time increased, δ : number of reticulocytes de- creased, leukocytes and lymphocytes increased, ϕ : mean erythrocyte volume decreased; no abnormal findings in histopathological examina- tions	Parametrix Inc 2006 f
DBTC			
Rat, Wistar (juvenile) 10 ♂, ♀	14 days, 0, 50, 150 mg DBTC/kg feed (about 0, 5, 15 mg/kg body weight and day)	about 5 mg/kg body weight and above : dose-dependent relative weight of thymus, spleen and lymph nodes decreased (with decreased body weights); lymphocyte content in lymphatic organs decreased, especially in thymus cortex decreased, humoral immunity (antibody formation against sheep erythrocytes) decreased about 15 mg/kg body weight: cellular immunity (rejection reaction against implanted skin) reduced; relative liver weight increased; liver: pro- liferation of epithelial cells in bile ducts, pericholangitis, periportal fibro- sis; kidneys normal; no further organs investigated	Seinen et al. 1977 a
Rat, Wistar, 10 ♂	14 days, 0, 50, 100 mg DBTC/kg feed (about 0, 2.5, 5 mg/kg body weight and day)	about 2.5 mg/kg body weight and above: body weights significantly decreased, relative thymus and spleen weights significantly decreased about 5 mg/kg body weight: relative liver weights significantly increased, mortality: 2/10; lymphocyte depletion in lymphatic organs, particularly in the thymus cortex and spleen; liver: proliferation of bile duct epithelial cells and pericholangitis; kidneys normal; no further organs investigated	Penninks and Seinen 1982

Table 3 (Continued)

Species, strain, number per group	Exposure	Findings	References
Mouse, Swiss (juvenile)10 ♂	14 days, 0, 50, 150 mg DBTC/kg feed (about 0, 7.5, 22.5 mg/kg body weight and day)	about 22.5 mg/kg body weight: NOAEL for effects on body weight, weight of thymus, spleen and lymph nodes; no further investigations	Seinen et al. 1977 a
Rat, CFE, 16 ♂, ♀	90 days, 0, 10, 20, 40, 80 mg DBTC/ kg feed (about 0, 0.5, 1.0, 2.0, 4.0 mg/kg body weight and day)	about 2 mg/kg body weight: NOAEL about 4 mg/kg body weight: body weight gain in ♀ significantly reduced; serum: haemoglobin content significantly decreased, erythrocyte, reticu- locyte, lymphocyte count and activities of AST and ALT not significantly changed; absolute weight of left kidney in ♂ significantly decreased, abso- lute weight of the other organs unchanged (including spleen weight – thymus weight not measured); ♂ mild hypochromiaemia; no conspicuous gross pathology findings (no other details)	Gaunt et al. 1968
DBTA			
Rat, Fischer- 344, controls: 20 ♂, ♀, DBTA: 50 ♂, ♀	78 weeks, 0, 6.65, 13.3 mg DBTA/kg feed (about 0, 6.65, 13.3 mg/ kg body weight and day) 26 weeks recovery period	at 6.65 mg/kg body weight and above: mortality in ♂ increased, body weight gains of ♂ reduced 13.3 mg/kg body weight: mortality in ♀ increased, body weight gains of ♀ reduced; no macroscopic and microscopic abnormalities; however, study is invalid due to the loss of animals such as missing animals, canni- balism or autolysis	NCI 1978
Mouse, B6C3F1, (no other details)	78 weeks, 0, 76, 152 mg DBTA/kg feed (about 0, 11.4, 22.8 mg/kg body weight and day) 14 weeks recovery period	11.4 mg/kg body weight: mortality in ♀ increased, body weight gains re- duced; no macroscopic and microscopic abnormalities; however, study is invalid due to the loss of animals such as missing animals, cannibalism or autolysis	NCI 1978

Table 3 (Continued)

Species, strain, number per group	Exposure	Findings	References
DBTL			
Rat , (no other details)	3 days , 0, 40, 80 mg DBTL/kg body weight and day	40 mg/kg body weight: mortality 20% 80 mg/kg body weight: mortality 25%; brain: noradrenaline, dopamine, serotonin decreased, locomotor activity decreased, disturbed learning ability; no histological investigations	Alam et al. 1988
Rat , (no other details) (juvenile, 20 ♂, ♀	3 days , 0, 20, 80 mg DBTL/kg body weight and day	20 mg/kg body weight: mortality: ♂ 10%, ♀ 15%; body weights dose-dependently decreased; lethargy, listlessness, weakness, locomotor and amphetamine-induced activity dose-dependently decreased, decrease more pronounced than in adult animals 40 mg/kg body weight: mortality: ♂ 20%, ♀ 25%, weakness of hind limbs decreased; no histological investigations	Alam et al. 1993
Rat , (no other details) (adult), 20 ♂, ♀	3 days , 0, 20, 40 mg DBTL/kg body weight and day	20 mg/kg body weight: mortality: ♂ 10%, ♀ 20%, locomotor and amphetamine-induced activity dose-dependently decreased 40 mg/kg body weight: mortality: ♂ 25%, ♀ 30%; no histopathological examinations	Alam et al. 1993
Rat , (no other details)	3 days , 0, 40, 80 mg DBTL/kg body weight and day	at 40 mg/kg body weight and above: brain: diacylglycerin and phosphoinositides decreased; no histopathological examinations	Subramoniam et al. 1991
Rat , (no other details) ♂	15 days , 0, 17.5 mg DBTL/kg body weight and day	17.5 mg/kg body weight: lethargy, mortality 20% body weight gains reduced; no effect on organ weights; brain: activities of succinic dehydrogenase, adenosine triphosphatase, acetylcholinesterase and monoamine oxidase unchanged; liver: activities of glucose-6-phosphatase, aminopyr-in-N-demethylase, benzphetamine-N-demethylase, aniline hydroxylase and benzo(a)pyrene hydroxylase significantly decreased; cytochrome-P450 content decreased, significant influence on heme metabolism in hepatocytes; duration of barbiturate-induced sleep increased; no histopathological examinations performed	Mushtaq et al. 1981

Table 3 (Continued)

Species, strain, number per group	Exposure	Findings	References
Rat, Holtzmann (no other details)	13 weeks , up to 400 mg DBTL/kg feed (up to about 20 mg/kg body weight and day)	about 20 mg/kg body weight: congested and haemorrhagic lungs, haemorrhagic submaxillary lymph nodes (no other details)	Parametrix Inc 2006 c
TBTC			
Rat, Wistar, 10 ♂	14 days , 0, 15, 50, 100 mg TBTC/kg feed (about 0, 0.75, 2.5, 5 mg/kg body weight and day)	about 0.75 mg/kg body weight: NOAEL about 2.5 mg/kg body weight and above: absolute and relative thymus weights significantly decreased, relative liver weights significantly and dose-dependently increased, relative spleen weights significantly decreased; lymphocyte count in thymus cortex reduced 5 mg/kg body weight: body weight gains significantly reduced, food consumption significantly reduced, brain weights significantly decreased	Snøeij et al. 1985
Rat, Wistar, 4–8 ♂	28 days , 0, 0.5, 25 mg TBTC/kg feed (about 0, 0.025, 1.25 mg/kg body weight and day)	about 0.025 mg/kg body weight: NOAEL about 1.25 mg/kg body weight: one week after start of administration: food consumption significant reduced, body weight gains significantly reduced, relative liver weights significantly increased; thymus: thymocyte count decreased, number of epithelial cells increased, decrease in size of cortex, size of medulla increased; 4 weeks after start of administration: absolute and relative thymus weights significantly decreased; histological examination: no substance-induced changes in spleen, liver, kidneys; haemorrhagic and partially atrophied lymph nodes	Bressa et al. 1991
TBTO			
Rat, Sprague Dawley (juvenile) 50 ♂	3 days , 0, 37.5, 75 mg TBTO/kg body weight and day	37.5 mg/kg body weight and above: mortality: 6/50; dopamine, noradrenaline and serotonin in the brain decreased, Mg ²⁺ - and Na ⁺ /K ⁺ -ATPase decreased; hyperaemia; punctiform haemorrhages in vacuole-containing myelinated nerve fibres, chromatolysis or complete necrosis of the	BUA 2003

Table 3 (Continued)

Species, strain, number per group	Exposure	Findings	References
Mouse, Swiss (adult), 10 ♂	4 days, 0, 232, 696 mg TBTO/kg feed (about 0, 11.6, 34.8 mg/ kg body weight and day)	neurons, degenerative changes or complete disappearance of Purkinje cells in the cerebellum; other organs not examined 75 mg/kg body weight: mortality: 15 animals about 11.6 mg/kg body weight and above: body weight decreased at about 34.8 mg/kg body weight and above: leukocyte- and lymphocyte counts decreased, erythrocyte count increased, haemoglobin increased, haematocrit value increased; no histopathological examination	Ishaaya et al. 1976
Mouse, Swiss (juvenile)10 (♂)	7 days, 0, 77 mg TBTO/kg feed (about 0, 7.7 mg/kg body weight and day)	about 7.7 mg/kg body weight: body weights decreased, spleen weights decreased	Ishaaya et al. 1976
Rat, Wistar, 10 ♂, ♀	10–11 days, 0, 1, 25 mg TBTO/kg body weight and day	1 mg/kg body weight: NOAEL 25 mg/kg body weight: mortality: 2 ♂/ ♀; microcytic anaemia, chronic inflammation of the bile duct, lymphotoxicity	Schweinfurth 1987
Rat, Wistar, 5♂, ♀	28 days, 0, 4, 100, 500 mg TBTO/kg feed (about 0, 0.2, 5, 25 mg/ kg body weight and day)	about 0.2 mg/kg body weight: NOAEL about 5 mg/kg body weight and above: food consumption reduced, body weights decreased, ♂ absolute thymus weights decreased about 25 mg/kg body weight and above: high mortality; apathy; weight loss; lymph node weights decreased; lymphocyte content in lymphatic organs decreased	Schweinfurth 1987
Rat, Wistar, 4–8 ♂	28 days, 0, 0.5, 25 mg TBTO/kg feed (about 0, 0.025, 1.25 mg/kg body weight and day)	about 0.025 mg/kg body weight: NOAEL about 1.25 mg/kg body weight: one week after start of administration: food consumption significantly reduced, body weight gains significantly reduced, relative liver weight significantly increased; thymus cortex: size decreased, lymphocyte count decreased, epithelial cells increased, thymus medulla: size	Bressa et al. 1991

Table 3 (Continued)

Species, strain, number per group	Exposure	Findings	References
Rat, Sprague Dawley (weanlings), 10 ♂, ♀	28 days, up to 50 mg TBTO/kg feed (about 5 mg/kg body weight and day)	increased; 4 weeks after start of administration severity of all symptoms increased, thymus size significantly decreased, tin concentration highest in liver and kidneys - higher than with TBTC; TBTO more immunotoxic than TBTC up to about 5 mg/kg body weight: clinical laboratory parameters including differential blood count unchanged; immune reaction to sheep erythrocytes (plaque formation), delayed-type response to bovine serum albumin unchanged about 5 mg/kg body weight: ♂ body weights decreased, food and water consumption reduced; thymus: weight decreased, cortex thickness decreased, cell count decreased; resistance to <i>Listeria monocytogenes</i> infection impaired	Verdier et al. 1991
Rat, Wistar, 10 ♂, ♀	4 weeks, 0, 5, 20, 80, 320 mg TBTO/kg feed (about 0, 0.25, 1.0, 4, 16 mg/kg body weight and day)	about 0.25 mg/kg body weight and above: rosettes in mesenterial lymph nodes, iron accumulation in the spleen decreased about 1 mg/kg body weight and above: ♂ thymus weights decreased; AST and ALT activities increased about 4 mg/kg body weight: food and water consumption reduced; microcytic anaemia, lymphocyte content in lymphatic organs decreased; serum: IgG decreased, IgM increased about 16 mg/kg body weight: number of neutrophilic granulocytes increased; liver necroses with inflammatory reaction; bile duct hyperplasia; serum glucose and liver glycogen concentration decreased	Krajnc et al. 1984
Rat, Holtzman, 10 ♂	30 days, 0, 32, 100, 320 mg TBTO/kg feed (about 0, 1.6, 5, 16 mg/kg body weight and day)	about 1.6 mg/kg body weight and above: body weight gains reduced about 16 mg/kg body weight: food consumption reduced; mortality: 6/10; gross pathology without conspicuous findings	Elsea and Paynter 1958

Table 3 (Continued)

Species, strain, number per group	Exposure	Findings	References
Rat, Wistar (wean-lings) je 9–10 ♂	6 weeks, 0, 20, 80 mg TBTO /kg feed (about 0, 2, 8 mg/kg body weight and day)	about 2 mg/kg body weight and above: thymus-dependent immune response and non-specific immune resistance significantly impaired, serum: IgE titer decreased, IgM and IgG titers unchanged, function of macrophages decreased about 8 mg/kg body weight: function of natural killer cells decreased	Vos et al. 1984
Rat, Wistar, 10 ♂	6 weeks, 0, 20, 80 mg TBTO /kg feed (about 0, 1, 4 mg/kg body weight and day)	about 1 mg/kg body weight and above: serum: insulin decreased about 4 mg/kg body weight: serum: thyroxine decreased, thyroid stimulating hormone (TSH) decreased, luteinizing hormone (LH) increased, follicle stimulating hormone and corticosterone unchanged; immunocytochemistry in pituitary gland: number and staining intensity of TSH-producing cells decreased, number of LH-producing cells increased	Krajnc et al. 1984
Rat, Wistar, 6 ♂	6 weeks, 0, 20, 80 mg TBTO /kg feed (about 0, 1, 4 mg/kg body weight and day)	about 1 mg/kg body weight: activity of natural killer cells in the lung decreased (effect not very clearly pronounced) about 4 mg/kg body weight: body and spleen weights (slightly) decreased, thymus weights (markedly) decreased	van Loveren et al. 1990
Rat, Wistar, 20 ♂, ♀	13 weeks, 0, 4, 20, 100 mg TBTO /kg feed (about 0; 0.2, 1.0, 5 mg/ kg body weight and day)	about 0.2 mg/kg body weight: NOAEL about 1 mg/kg body weight: clotting-time ♂ increased; food consumption ♀ increased with normal body weights about 4 mg/kg feed: food consumption reduced, body weight gains reduced; serum: activity of alkaline phosphatase increased, albumin in ♀ increased, γ-globulin in ♀ decreased; weight of thymus, lymph nodes and thyroid decreased, adrenal weights of ♂ increased	Schweinfurth 1987

Table 3 (Continued)

Species, strain, number per group	Exposure	Findings	References
Rat, Wistar (adult), 5–12 ♂	5 months, 0, 0.5, 5, 50 mg TBTO/kg feed (about 0.025, 0.25, 2.5 mg/kg body weight and day)	about 0.25 mg/kg body weight: NOAEL about 2.5 mg/kg body weight: resistance to <i>Listeria monocytogenes</i> in the spleen impaired, reaction to infection with <i>Trichinella spiralis</i> de- creased, (IgE response, number of larvae in the muscle and elimination of mature stages) unchanged up to about 2.5 mg/kg body weight: body weights, spleen weights; activity of natural killer cells in the spleen	Vos et al. 1990
Rat, Wistar (wean- lings) 5–12 ♂	4–6 or 15–17 months, 0, 0.5, 5, 50 mg TBTO/kg feed (about 0; 0.05, 0.5, 5 mg/kg body weight and day)	about 0.05 mg/kg body weight and above: activity of natural killer cells from spleen and peritoneum: unchanged after 4.5 months, after 16 months significantly decreased (not dose-dependently) about 0.5 mg/kg body weight and above: reaction to infection with <i>Tri- chinella spiralis</i> decreased (IgE response, number of larvae in the muscle), ratio between T- and B-lymphocytes in the mesenterial lymph nodes de- creased about 5 mg/kg body weight: thymus weights (after 4.5 months) de- creased; clearance of <i>Listeria monocytogenes</i> in spleen decreased unchanged up to about 5 mg/kg body weight: body weights, spleen weights; antibody formation versus sheep erythrocytes, delayed-type reac- tion to ovalbumin and tuberculin, IgM- and IgG formation versus ovalbu- min and <i>Trichinella spiralis</i> , response of thymus- and spleen cellsto mito- gens: phytohaemagglutinin, concavalin A, Pokeweed mitogen, <i>Escherichia coli</i> lipopolysaccharide	Vos et al. 1990
Dogs, Beagle, 4 ♂, ♀	12 months, 0, 0.2, 1.0 or 5.0 mg TBTO/ kg body weight and day	0.2 mg/kg body weight: NOAEL at 1.0 mg/kg body weight and above: local changes: reddening, swelling and eschar formation on the skin as sequel of extensive recovery periods due to bad general condition; serum: alkaline phosphatase increased,	Schering AG 1992

Table 3 (Continued)

Species, strain, number per group	Exposure	Findings	References
		from week 13 tendency to IgG decreased, IgA clearly decreased, IgM decreased, atrophy in the cortical and paracortical regions of the iliac and mesenteric lymph nodes; atrophy of Peyer's patches in the ileum; effect on IgA level correlates with morphological changes in gut-associated lymphoid tissue (GALT), GALT system in dogs (contrary to humans) main source of serum IgA	
		5.0 mg/kg body weight: body weights decreased, food and water consumption reduced, emaciation, dehydration; atactic gait, apathy; preterm killing of 2 ♂ and 3 ♀ in moribund condition; haematocrit value and haemoglobin content slightly decreased, atrophy of bone marrow, considerable thymus atrophy, spleen atrophy, thymus and spleen weight decreased; liver: single cell degeneration, fatty change or ballooning of hepatocytes, activities of alkaline phosphatase, ALT and γ -GT increased, shift of serum proteins from albumin to globulin, fibrinogen level increased	
Rat, Wistar, 60 ♂, ♀	2 years, 0, 0.5, 5, 50 mg TBTO/kg feed (about 0, 0.025, 0.25 or 2.5 mg/kg body weight and day)	about 0.25 mg/kg body weight: NOAEL about 2.5 mg/kg body weight: mortality increased, body weight gains reduced, emaciation, apathy, ataxia; anaemia, lymphocytopenia, thrombocytosis, haemoglobin and haematocrit value decreased, activities of AST, ALT and alkaline phosphatase increased, kidney function impaired, serum IgM and serum IgA concentrations increased, but IgG titer ♀ decreased; no hormonal changes; weight of adrenal glands, pituitary gland, liver, kidneys increased, thyroid gland, thymus weight ♀ decreased, ovaries and spleen weight ♀ increased, heart weight ♂ increased; thyroid follicle cell height decreased; kidney: function impaired, vacuolation and pigmentation of the proximal tubular epithelium, nephrosis	Wester et al. 1990

Table 3 (Continued)

Species, strain, number per group	Exposure	Findings	References
Mouse, CD1, 50 ♂, ♀	18 months, 0, 5, 25, 50 mg TBTO/kg feed (about 0; 0.8, 4.2, 8.5 mg/kg body weight and day)	about 0.8 mg/kg body weight and above: mortality increased; liver: enlarged and slightly discoloured; incidence of glomerular or interstitial amyloidosis in the kidneys of ♀ increased about 8.5 mg/kg body weight: food consumption reduced; absolute and relative liver weights of ♀ increased	BUA 2003
TBTN			
Rat, Wistar, 10 ♂, ♀	28–32 days, 0, 2, 8, 40, 200 mg TBTN/kg feed (about 0; 0.1, 0.4, 2.0, 10 mg/kg body weight and day)	0.4 mg/kg body weight: serum-Na ⁺ decreased (not dose-dependently) 2 mg/kg body weight and above: relative weights of thymus, lymph nodes, kidneys decreased; thymolysis; TSH decreased 10 mg/kg body weight: food consumption reduced, body weight gains reduced; ALT increased, T ₃ and LH decreased; erythrocyte infiltration with rosette formation in mesenterial lymph nodes, haemosiderin in spleen decreased, absolute and relative weights of prostate and pancreas decreased	Schering AG 1988
TTBT			
Rat, Wistar, 12 ♂, ♀	♂: 33 days, ♀ start 2 weeks before mat- ing to PND 4–5, 0, 100, 300, 2000, 10000 mg TTBT/kg feed (about 0, 6.5, 19, 119, 421 mg/kg body weight and day)	about 6.5 mg/kg body weight: NOAEL for effects on spleen and thymus about 19 mg/kg body weight: relative and absolute spleen weights significantly decreased, thymus atrophy dose-dependent, lymphocyte deficiency in lymphatic organs about 119 mg/kg body weight: body weight gains significantly reduced, food consumption significantly reduced; thrombocyte count increased, prothrombin time decreased, activity of γ-GT increased, cholesterol, triglycerides and phospholipids increased; haemosiderin deposit and tissue degeneration in lymph nodes about 421 mg TTBT/kg body weight: aggravation of all effects, especially body weights decreased	ORTEP 2004

Mono-n-butyltin compounds

In rats, repeated administration of **MBTC** caused liver damage and changes of the haematopoietic system (Parametrix Inc 2006 f). In this investigation, however, thymus and lymph nodes were not histologically investigated, and the thymus weight was also not determined.

Di-n-butyltin compounds

Already at doses of about 2.5 mg/kg body weight and day and above, administration of **DBTC** for 14 days produced a significant decrease in relative thymus and spleen weights, and a reduction of the lymphocyte count in the lymphatic organs, particularly in the thymus, in Wistar rats. In addition, a significant retardation in body weight gain occurred (Penninks and Seinen 1982; Seinen et al. 1977 b). These effects of DBTC were not found in mice (Seinen et al. 1977 a). In a study with **DBTC** in CFE rats lasting 90 days, a NOAEL of about 20 mg/kg body weight and day was obtained for the decrease in body weight gain and the serum haemoglobin content (Gaunt et al. 1968). At and above around 20 mg/kg body weight and day, **DBTL** caused 20% mortality (Alam et al. 1993), changes in enzyme activities in the brain and liver, and a reduction in neurotransmitters in the brain (Alam et al. 1988; Mushtaq et al. 1981; Subramoniam et al. 1991). After treatment of Holtzman rats with DBTL doses of about 20 mg/kg body weight and day, congested and haemorrhagic lungs as well as haemorrhagic submaxillary lymph nodes were found in (Parametrix Inc 2006 c). Therefore, no NOAEL can be given for the effects on the thymus. The LOAEL (lowest observed adverse effect level) was at 2.5 mg/kg body weight and day DBTC.

Tri-n-butyltin compounds

In a 28-day study in Wistar rats, administration of **TBTC** at about 1.25 mg/kg body weight and day produced, apart from a significant decrease in food consumption and body weight gain, lymph node and thymus changes as well as a reduction of relative thymus weights and an increase in relative liver weight. From this study, a NOAEL of about 0.025 mg/kg body weight and day was obtained (Bressa et al. 1991). After 14 days administration of **TBTC** to Wistar rats, the relative spleen weight was reduced at and above about 2.5 mg/kg body weight and day, and also the brain weight at about 5 mg/kg body weight and day (Snoeijs et al. 1985).

A NOAEL of 1 mg/kg body weight and day was obtained from different subacute studies with administration of **TBTO** for up to eleven days (Schweinfurth 1987). Body weight gains were delayed at about 7.7–11.6 mg/kg body weight and day and above (Ishaaya et al. 1976), with mortality, microcytic anaemia, chronic inflammation of the bile ducts and lymphotoxicity occurring at 25 mg/kg body weight and day (Schweinfurth 1987) and brain damage being found at 37.5 mg/kg body weight and day and above (BUA 2003).

In rats, four to six weeks exposure to **TBTO** produced changes in lymph nodes and spleen at and above about 0.25 mg/kg body weight and day, thymus and liver

disorders (Krajnc et al. 1984) as well as changed hormone release rates (Krajnc et al. 1984) at and above about 1 mg/kg body weight and day, delayed body weight gains at and above about 1.25 mg/kg body weight and day (Bressa et al. 1991) and immunotoxicity at about 5 mg/kg body weight and day (Verdier et al. 1991). Mortality occurred at about 16 mg/kg body weight and day (Elsea and Paynter 1958). The changes caused by TBTO as described by Krajnc et al. (1984) at 0.25 mg/kg body weight and day did not occur when exposure to this dose was over an extended period (see below).

From the studies with subchronic and chronic administration of TBTO to rats (Schweinfurth 1987) and dogs (Schering AG 1992), a NOAEL of about 0.2 or 0.25 mg/kg body weight and day was derived (Wester et al. 1990). In rats, impairment of their resistance to infection was found at about 0.5 mg/kg body weight and day and above (Vos et al. 1990); in dogs, the general condition worsened at 1.0 mg/kg body weight and day and above, with changes in liver, lymph nodes and immune system also occurring (Schering AG 1992). With TBTO at and above about 2.5 mg/kg body weight and day in rats, mortality, weight loss, apathy, ataxia, anaemia, lymphocytopenia, thrombocytosis, reduced haemoglobin content and haematocrit value, liver and kidney damage as well as organ weight changes were found (Wester et al. 1990). Already at the lowest dose of about 0.8 mg/kg body weight, mortality, liver and kidney damage occurred in mice (BUA 2003).

In a 4-week study with rats, **TBTN** caused a significant decrease in the blood sodium concentration at 0.4 mg/kg body weight and day and a decrease in thymus, lymph node and kidney weights at 2 mg/kg body weight and day and above, as well as damage to liver, lymph nodes and spleen at 10 mg/kg body weight and day and above (Schering AG 1988). The NOAEL for these effects was 0.1 mg/kg body weight and day.

Tetra-n-butyltin

In a combined subchronic toxicity and reproduction toxicity study with **TTBT**, a NOAEL of about 6.5 mg/kg body weight and day was found for the effects on spleen and thymus after 33 days of exposure. At about 119 mg/kg body weight and day, there occurred a decrease in body weight gain, damage to the lymph nodes and liver, and an effect on blood clotting (ORTEP 2004). It may be concluded from these data that tetra-n-butyltin has a lower systemic toxicity than di- and tributyltin.

5.2.3 Dermal application

After 50-day dermal application of **TBTO** in doses of 0.10 or 40 mg/kg body weight and day on the shaved skin of ten guinea pigs, swellings, degeneration and damage of the kidney tubular epithelium were found at 10 mg/kg body weight and day and above. The glomeruli were not affected. Increased amounts of sodium, chloride, phosphate, glucose and amino acids were eliminated with the urine. The concentra-

tion of amino acids, phosphate and vitamin D3 (calcitrol) in the serum was reduced (Mori et al. 1984).

5.2.4 Intravenous injection

Five intravenous injections of **DBTC** with doses of 0, 1, 2 or 4 mg/kg body weight produced a significantly reduced body weight at 4 mg/kg body weight and above, and a significantly and dose-dependently reduced thymus weight at 1 mg/kg body weight and above (Seinen et al. 1977 b).

5.3 Effects on skin and mucous membranes

5.3.1 Skin

Undiluted **MBTC** was corrosive to the rabbit skin after 30 minutes (Parametrix Inc 2006 f).

In an occlusive patch test lasting four hours, **MBTC** was found to be corrosive to the abraded rabbit skin. The animals showed severe erythemas, slight oedemas, necroses, eschar formation and skin damage (Parametrix Inc 2006 f).

Studies carried out according to OECD Test Guideline 404 showed that **MBT(2-EHMA)** and **MBT(IOMA)** caused slight erythemas but no oedemas in rabbits (OECD 2006 a).

After non-occlusive exposure for four hours, **DBTC** was found to be severely irritating to the rabbit skin. Semi-occlusive administration of **DBTC** in petrolatum to Wistar rats produced severe skin irritation already after exposure for five minutes (Parametrix Inc 2006 a). Dermal application of 67 nmol/cm² **DBTC** to rats caused slight damage to the skin of the back (no other details), while application of 335 nmol/cm², after some delay, caused cellular necroses (ACGIH 2001). In the rabbit after occlusive application for 24 hours, **DBTM** (50% in polyethylene glycol) was found to be moderately irritating to the skin (Parametrix Inc 2006 d), and **DBTO** was slightly irritating to irritating in rabbits in a semi-occlusive 4-hour test (Parametrix Inc 2006 e). Slight irritation to corrosion were described in rabbits for **DBT(2-EHMA)** after semi-occlusive administration for four hours. In a further study with rabbits, **DBT(2-EHMA)** was moderately irritating to the skin after occlusive application for 24 hours (Parametrix Inc 2006 b). **DBT(IOMA)** was found to be corrosive in rabbits after (semi-occlusive) application for four hours and after (occlusive) application for 72 hours (Parametrix Inc 2006 i). On the other hand, in another study with rabbits, it was stated that **DBT(IOMA)** was slightly irritating after semi-occlusive treatment for four hours (Parametrix Inc 2006 i).

Dermal application of 67 nmol/cm² **TBTC** induced tissue necroses on the skin of the back of rats (ACGIH 2001). In solid form, **TBTF** only had a slight irritant effect on the rabbit skin. On the other hand, in a paint formulation, **TBTF** was severely

irritating (Sheldon 1975). Undiluted **TBTO** was found to be severely irritating to the rabbit skin (no other details) (BUA 1988). 0.25% solutions of **TBTO** produced haemorrhages, slight oedemas and erythemas on the rat skin (Pelikan and Cerny 1968 b).

There are no data available for **TTBT**.

5.3.2 Eyes

In studies carried out according to OECD Test Guideline 405, **MBT(2-EHMA)** (Parametrix Inc 2006 f) and **MBT(IOMA)** were not irritating to the conjunctiva of male rabbits (OECD 2006 a).

To the rabbit eye, undiluted **DBTC** was found to be severely irritating (Parametrix Inc 2006 a), **DBTM** (Parametrix Inc 2006 d) and **DBTO** (Parametrix Inc 2006 e) to be irritating, and **DBTL** (Parametrix Inc 2006 c), **DBT(2-EHMA)** (Parametrix Inc 2006 b) and **DBT(IOMA)** (Parametrix Inc 2006 i) to be slightly irritating.

Either undiluted or in an antifouling paint, **TBTF** was severely irritating to the rabbit eye (Sheldon 1975). The effect of **TBTO**, either undiluted or in an antifouling paint, was severely irritating to the rabbit eye (BUA 1988). In 0.15% to 0.2% aqueous solutions, **TBTO** caused ulcerating inflammation of the eyelids and cornea, opacity and necrosis of the cornea, and chemosis and necrosis of the conjunctiva. The effects were more pronounced with 1.5% and 2% solutions, producing destruction of the eyes and death in two rabbits (Pelikan 1969).

A slight irritant effect to the eye is reported for **TTBT** (ECB 2000).

5.4 Allergenic effects

In studies carried out according to OECD Test Guideline 406, **MBT(2-EHMA)** (Parametrix Inc 2006 f), but not **MBT(IOMA)** (OECD 2006 a), was sensitizing in guinea pigs.

In two maximization tests with guinea pigs performed according to OECD Test Guideline 406, **DBT(2-EHMA)** (Parametrix Inc 2006 b) was assessed as sensitizing. **DBT(IOMA)** was found to be sensitizing in one maximization test, but it was not in another (Parametrix Inc 2006 i).

In a maximization test with guinea pigs involving two antifouling paints containing **TBTO**, there was no indication of a skin-sensitizing effect (BUA 1988). With mice, on the other hand, **TBTO** induced contact allergy (BUA 2003).

There are no data available on **TTBT**.

The results of these investigations suggest that it is not the alkyltin cation which is responsible for a sensitizing potential of the n-butyltin compounds, but the organic ligands. In the above studies with mono-n-butyl and di-n-butyl compounds,

always the 2-EHMA ligands were found to be sensitizing in the maximization test with guinea pigs.

5.5 Reproductive toxicity

5.5.1 Fertility

Studies on the effects of *n*-butyltin compounds on fertility are shown in Table 4.

Table 4 Generation studies and studies on the fertility of *n*-butyltin compounds

Species, strain, number per group	Exposure	Findings	References
MBTC			
Rat, Wistar, 10 ♂, ♀	OECD Screening Test 421 0, 300, 1500, 7500 mg MBTC/kg feed, about 0, 20, 100, 530 mg MBTC/kg body weight and day; exposure: ♂ start 10 weeks before and up to end of mating, ♀ start 2 weeks before mating and up to PND 4–6; investigation PND 4	about 100 mg/kg body weight: NOAEL for systemic toxicity from 13-week study about 530 mg/kg body weight: NOAEL for fertility, NOAEL for postnatal developmental toxicity	Parametrix Inc 2006 f
MTB(2-EHMA)			
Rat, Sprague Dawley, 12 ♂, ♀	OECD Screening Test 422 0, 10, 50, 150 mg MTB(2-EHMA)/kg and day; exposure: ♂ and ♀ start 15 days before mating; investigation PND 4	50 mg/kg body weight: NOAEL for fertility, NOAEL for systemic toxicity, NOAEL for postnatal developmental toxicity 150 mg/kg body weight: F ₀ : mortality increased, body weight gains reduced, food consumption reduced, liver and kidney weights increased, vacuole formation in hepatocytes increased; mucus production in the cervical and vaginal epithelium of ♀ increased; F ₁ : number of surviving animals decreased, body weight gains reduced	Parametrix Inc 2006 g

Table 4 (Continued)

Species, strain, number per group	Exposure	Findings	References
DBTC			
Rat, Wistar, 12 ♂, ♀	OECD Screening Test 421 0, 5, 30, 200 mg DBTC/kg feed, ♂: about 0, 0.4, 2.0, 12 mg DBTC/kg body weight and day; ♀: about 0, 0.4, 2.0, 11 mg DBTC/kg and day; exposure: ♂ start 10 weeks before and up to end of mating, ♀ start 2 weeks before mating up to PND 4–6; investigation PND 4–6	about 0.4 mg/kg body weight: NOAEL for systemic toxicity about 2 mg/kg body weight: NOAEL for fertility, NOAEL for postnatal developmental toxicity; F ₀ : thymus atrophy in ♀, body weight gains reduced about 11 mg/kg body weight: F ₀ : gestation index decreased ♀, body weight gains reduced, thymus atrophy; F ₁ : postimplantation losses increased, number of live offspring decreased, surviving animals to PND 4 decreased, body weight gains reduced, runts	Parametrix Inc 2006 a
Rat, Wistar, 10 ♀	4 days, 0, 4, 8, 16 mg DBTC/kg body weight; exposure: F ₀ : start PND 0–3, investigation PND 5	16 mg/kg body weight: implantations decreased, serum progesterone level at PND 4 decreased, progesterone administration on PND 0–4 protected against implantation loss	Harazono and Ema 2003
TBTO			
Mouse, ICR, 6 ♂, 5 weeks old	4 weeks, 0, 0., 2, 10 mg TBTO/kg body weight, 2× per week, oral	0.4 mg/kg body weight: NOAEL for effects on spermatozoa 2 mg/kg body weight and above: sperm density ("sperm head count") in testes homogenate decreased, tin concentration in testes increased	Kumasaka et al. 2002
Rat, Sprague Dawley, 30 ♂, ♀	2-generation study 0, 0.5, 5.0, 50 mg TBTO/kg feed, ♂: about 0, 0.02, 0.29, 2.95 mg/kg body weight and day, ♀: about 0, 0.03, 0.34, 3.43 mg/kg body weight and day; exposure: F ₀ : start 10	about 0.3 mg/kg body weight: NOAEL for systemic toxicity in F ₀ , F ₁ and F ₂ ; NOAEL for postnatal developmental toxicity about 3 mg/kg body weight: NOAEL for fertility; body weight gains reduced (F ₀ , F ₁ pups PND 14, 21; F ₁ parents, F ₂ pups PND 7, 14, 21), absolute and relative thymus weights decreased (F ₀ ♀, F ₁ ♀, ♂); no abnormal findings at histopathology of all relevant organs; only weights of thymus, lymph	BUA 1988

Table 4 (Continued)

Species, strain, number per group	Exposure	Findings	References
	weeks before and up to end of mating; F ₁ : start of mating to PND 145, investigations to PND 211; F ₂ : investigations to PND 21	nodes and spleen determined; no investigation of sperm, oestrous cycle or external sex characteristics of offspring	
TBTC			
Rat, Wistar, 30 ♂, ♀	2-generation study 0, 5, 25, 125 mg TBTC/kg feed, about 0; 0.4, 2, 10 mg/kg body weight and day; exposure: F ₀ : start at mating; F ₁ : start of mating to PND 92; investigations to PND 119; F ₂ : investigations to PND 91	about 0.4 mg/kg body weight: NOAEL for fertility and postnatal developmental toxicity about 2 mg/kg body weight and above: ♂: spermatid count decreased (F ₂) about 10 mg/kg body weight: ♀: birth weights and postnatal body weight gains reduced (F ₁ , F ₂), anogenital distance increased (F ₁ , F ₂), delayed opening of vagina (F ₁ , F ₂), delayed oestrous cycle (F ₁ , F ₂), relative ovarian weights decreased (F ₁), relative uterus weights increased (F ₂); ♂: birth weights and postnatal body weight gains reduced (F ₁ , F ₂), spermatid count decreased (F ₁ , F ₂), sperm count decreased (F ₂), relative prostate weights decreased (F ₁ , F ₂), testosterone concentration increased (F ₁ , F ₂), 17β-oestradiol concentration decreased (F ₂)	Ogata et al. 2001; Omura et al. 2001
TTBT			
Rat, Wistar, 12 ♂, ♀	OECD Screening Test 422 0, 100, 300, 2000 mg TTBT/kg feed, about 0; 6.5, 19, 119 mg TTBT/kg body weight and day; exposure: ♂ 33 days (no other details), ♀ start 2 weeks before mating to PND 4–5; investigation PND 4–5	about 6.5 mg/kg body weight: NOAEL for systemic toxicity about 19 mg/kg body weight: NOAEL for developmental toxicity; spleen weights of ♂ decreased, thymus: weight decreased and atrophy about 119 mg/kg body weight: body weight gains reduced, food consumption reduced, postimplantation losses increased, number of live offspring decreased, surviving animals to PND 4 decreased, foetal weights decreased, runts	ORTEP 2004

PND = postnatal day

Mono-n-butyltin compounds

In OECD Screening Test 421, no treatment-related changes were observed in Wistar rats with **MBTC** up to 530 mg/kg body weight and day (Parametrix Inc 2006 f). Accordingly, the NOAEL for fertility was 530 mg/kg body weight and day for MBTC. In a 13-week study conducted in parallel, the systemic NOAEL was about 100 mg/kg body weight and day due to increased liver enzyme activities in the serum and increased relative liver weights at 530 mg/kg (see Section 5.2.2) (Parametrix Inc 2006 f).

In the OECD Screening Test 422 with administration of **MTB(2-EHMA)** to Sprague Dawley rats, increased mortality and, in the surviving rats, decreased body weight gain and food consumption, increased liver and kidneys weights and increased vacuole formation in the hepatocytes occurred at 150 mg/kg body weight and day. At this dose, two female animals died on day 21 of gestation, one animal was killed on gestation day 22 due to maternal dystocia, and another animal on the first day of lactation owing to the death of all pups. Increased mucus formation in cervical and vaginal epithelia of the females was found postpartum. In this study a NOAEL of 50 mg/kg body weight and day was found for the systemic toxicity of MTB(2-EHMA) (Parametrix Inc 2006 g). The NOAEL for fertility was also 50 mg/kg body weight and day, as it is not possible to exclude an effect on the fertility of the females due to maternal dystocia and pup mortality on the first day after birth at 150 mg/kg.

Di-n-butyltin compounds

In the OECD Screening Test 421 with female Wistar rats, **DBTC** at about 11 mg/kg body weight and day produced a significant reduction in the gestation index and increased postimplantation losses as well as pronounced foetal toxicity with increased postnatal mortality. The NOAEL for fertility was about 2 mg/kg body weight and day. The NOAEL for systemic toxicity, such as reduced body weight gain or thymus atrophy, was about 0.4 mg/kg body weight and day (Parametrix Inc 2006 a).

Tri-n-butyltin compounds

In a four-week study with **TBTO** on testicular toxicity in ICR mice, the sperm density in the testis homogenate was decreased and the tin concentration in the testes increased at 2 mg/kg body weight and above. The NOAEL for the effects on sperms was 0.4 mg/kg body weight and day in this study (Kumasaka et al. 2002).

In a two-generation study with Sprague Dawley rats, no effects on fertility were observed with **TBTO** up to the highest dose of about 3 mg/kg body weight and day. At this dose, reduced body weight gains were found in the F_0 , F_1 and F_2 generations, and the absolute and relative thymus weights were decreased in F_0 females and in females and males of the F_1 generation. In the F_0 , F_1 and F_2 generations the NOAEL for systemic toxicity was about 0.3 mg/kg body weight and day, and that for fertility

was 3 mg/kg body weight and day (BUA 1988). In this study, neither the sperms nor the oestrous cycle were investigated.

In a two-generation study with **TBTC** in Wistar rats, the highest dose of about 10 mg/kg body weight and day caused reduced birth weights and reduced postnatal body weight gains in the male and female F_1 and F_2 offspring. In addition, in the female offspring, the anogenital distance was increased, vaginal opening delayed, the total number and percentage of normal oestrus cycles reduced, the relative ovarian weight decreased, and the relative uterus weight increased (Ogata et al. 2001). At this dose, in the male offspring, reduced sperm and spermatid counts, decreased relative prostate weights, increased testosterone concentrations and decreased 17β oestradiol concentrations in the F_2 offspring were determined. The authors suspected an inhibition of aromatase (Omura et al. 2001). As the spermatid count was also significantly reduced in the male F_2 offspring at 2 mg/kg body weight and day, the NOAEL for fertility and also for the postnatal developmental toxicity is at the lowest dose of 0.4 mg/kg body weight and day, as the study design allows no differentiation between direct toxicity and reproductive toxicity to the sperms. Other effects on male and female offspring were observed at both low doses which, however, the Commission did not assess as relevant. Thus, absolute testis and epididymis weights were slightly, but significantly decreased in the adult male F_1 animals at 0.4 mg/kg body weight and day and above. However, the decrease was not dose-dependent, and there was no decrease in the F_2 generation despite extended exposure duration. Already from the low dose upward, the anogenital distance was dose-dependently slightly increased in the female F_1 and F_2 offspring on the first and fourth day after birth, but was only found to be statistically significant for the F_1 generation on the first day after birth. These findings are not assessed as relevant, as the increases in anogenital distance were only slight in the low and middle dose groups and were within the range of biological variation, and other effects, such as changes in vaginal opening times or the oestrous cycle, were not observed.

Tetra-*n*-butyltin

In the OECD Screening Test 422 in female Wistar rats, **TTBT** in doses of about 119 mg/kg body weight and day increased postimplantation loss, caused a reduced number of live offspring and an increased postnatal mortality, though no impairment of fertility. In view of the reduced spleen and thymus weights as well as thymus atrophy at about 19 mg/kg body weight and day the NOAEL for the systemic toxicity of **TTBT** was thus about 6.5 mg/kg body weight and day (ORTEP 2004).

Summary

Based on the 2-generation study with **TBTC** in rats (Ogata et al. 2001; Omura et al. 2001) and the mechanistic study with **TBTO** in mice (Kumasaka et al. 2002), the NOAEL for fertility with tri-*n*-butyltin compounds is assessed at 0.4 mg/kg body weight and day. The 2-generation study with **TBTO** (BUA 1988), as well as the

OECD screening tests with mono-n-butyltin and di-n-butyltin compounds can, in assessing fertility, only be used to a limited extent, as the relevant endpoints, such as sperm parameters, time of vaginal opening, or the oestrous cycle of the offspring, were not determined.

5.5.2 Developmental toxicity

Prenatal developmental toxicity

Studies on the effect of n-butyltin compounds on prenatal development are given in Table 5.

Table 5 Studies on the prenatal developmental toxicity of n-butyltin compounds

Species, strain, number per group	Exposure	Findings	References
MBTC			
Rat, Wistar, 13–14 ♀	GD 7–17, 0, 50, 100, 200, 400 mg MBTC/kg body weight and day, gavage, investigation on GD 20	400 mg/kg body weight: dams: NOAEL; no significant effects on body weight gains, food consumption or absolute thymus weights; <i>foetuses</i> : NOAEL	Noda et al. 1992
Rat, Wistar, 16 ♀	GD 0–3 or 4–7, 0, 56, 226, 903 mg MBTC/kg body weight and day, gavage, investigation on GD 20	903 mg/kg body weight: dams: body weight gains reduced; <i>foetuses</i> : foetal weights decreased (♀)	Ema and Harazono 2001
Rat, Wistar, 6–11 ♀	GD 7–8, 0, 1000, 1500, 2000 mg MBTC/kg body weight and day, gavage, investigation on GD 20	1000 mg/kg body weight: dams: body weight gains decreased 1500 mg/kg body weight: dams: mortality (5/11); <i>foetuses</i> : number of live foetuses/litter decreased. foetal weights decreased 2000 mg/kg body weight: dams: mortality (6/6)	Ema et al. 1995 a
DBTC			
Rat, Wistar, 10–12 ♀	GD 7–15, 0, 2.5, 5, 7.5, 10 mg DBTC/kg body weight and day, gavage, investigation on GD 20	2.5 mg/kg body weight: <i>foetuses</i> : NOAEL 5 mg/kg body weight and above: dams: NOAEL; body weight gains slightly reduced; <i>foetuses</i> : foetal weights decreased, number of malformations increased (craniofacial region, skeletal system)	Ema et al. 1991

Table 5 (Continued)

Species, strain, number per group	Exposure	Findings	References
		7.5 mg/kg body weight and above: <u>dams</u> : mortality, body weight gains reduced, food consumption reduced, abortions; <u>foetuses</u> : postimplantation loss increased, number of live foetuses/litter decreased 10 mg/kg body weight: <u>dams</u> : mortality (75%)	
Rat, Wistar, 25♀	GD 6–15, 0, 1, 2.5, 5, 10 mg DBTC/kg body weight and day, gavage, investigation on GD 20	5 mg/kg body weight: <u>dams</u> : NOAEL; <u>foetuses</u> : NOAEL (one foetus with oedema) 10 mg/kg body weight: <u>dams</u> : body weight gains reduced, food consumption reduced, thymus weights decreased, no mortality; <u>foetuses</u> : number of malformations increased (4 foetuses from 3 litters: oedemas, ankyloglossia, hydrocephalus, anophthalmia, diaphragmatic hernia, mandibular defects, skeletal abnormalities)	Farr et al. 2001
Rat, Wistar, 16–19 ♀	GD 0–3 or 4–7, 0, 4, 8, 15 mg DBTC/kg body weight and day, gavage, investigation on GD 20	4 mg/kg body weight and above: <u>dams</u> : NOAEL for body weight gains; food consumption reduced (GD 0–3); <u>foetuses</u> : postimplantation loss increased (GD 4–7), foetal weights decreased (GD 4–7) 8 mg/kg body weight and above: <u>dams</u> : body weight gains reduced (GD 0–3; 4–7), gestation rate decreased (GD 0–3), number of implantations decreased (GD 0–3), preimplantation loss increased (GD 0–3); <u>foetuses</u> : postimplantation loss increased (GD 0–3), resorptions and number of dead foetuses increased (GD 4–7), number of live foetuses decreased (GD 4–7) 15 mg/kg body weight: <u>foetuses</u> : number of complete resorptions increased <i>investigations only covered external abnormalities</i>	Ema and Harazono 2001
Rat, Wistar, 6–10 ♀	GD 7–8, 0, 10, 15 mg DBTC/kg body weight and day, gavage, investigation on GD 20	10 mg/kg body weight and above: <u>dams</u> : body weight gains <i>reduced</i> ; <u>foetuses</u> : postimplantation loss increased, number of live foetuses/litter decreased, foetal weights decreased, malformations increased (exencephaly, encephalocele, cleft jaw, cleft lip and cleft palate, ankyloglossia, cleft tongue, om-	Ema et al. 1995 a

Table 5 (Continued)

Species, strain, number per group	Exposure	Findings	References
		phalocele, club foot, defect of mandible, fusion and absence of cervical and thoracic vertebral arches, vertebral arches and bodies, fusion of sternbrae, anophthalmia and microphthalmia)	
Rat, Wistar, 10 ♀	GD8, 0, 80 µmol/kg body weight; 24 mg DBTC /kg body weight, 28 mg DBTA /kg body weight, 28 mg DBTM /kg body weight, 20 mg DBTO /kg body weight, 50 mg DBTL /kg body weight, gavage, investigation on GD 20	80 µmol/kg body weight: dams: NOAEL for body weight gains and food consumption; <u>foetuses</u> : malformations increased (cleft jaw, exencephaly, cranial hypoplasia, fused ribs) and variations increased with all DBT compounds	Noda et al. 1993
Rat, Wistar, 20 ♀	GD 7–9, 10–12 or, 13–15 or 6, 7, 8 or 9, 0, 20, 40 mg DBTC /kg body weight and day, gavage, investigation on GD 20	induction of teratogenic effects GD 7–9, most effective day GD 8	Ema et al. 1992
Rat, Wistar, 11–13 ♀	GD 13–17, 0, 165, 330 µmol DBTC /kg body weight and day (0, 50, 100 mg/kg body weight and day), gavage, investigation on GD 20	50 mg/kg body weight and above: dams: body weight gains reduced, mortality increased; <u>foetuses</u> : body weights decreased	Ema et al. 1996
TBTO			
Mouse, Swiss, 8 ♀	GD 6–15, 0, 5, 20, 40 mg TBTO /kg body	5 mg/kg body weight and above: dams: placenta weights increased, absolute spleen weights decreased	Baroncelli et. al. 1990

Table 5 (Continued)

Species, strain, number per group	Exposure	Findings	References
	weight and day, gavage, investigation on GD 17	20 mg/kg body weight and above: <u>dams</u> : body weight gains reduced 40 mg/kg body weight: <u>dams</u> : piloerection, lethargy, hunched posture, vaginal bleeding, complete resorptions, body weights decreased during the first 4 days: <u>foetuses</u> : resorptions increased, number of live foetuses decreased, foetal weights decreased <i>investigations covered external abnormalities only</i>	
Mouse, NMRI, 40 ♀	GD 6–17, 0, 0.5, 2, 5, 14, 27 mg TBTO/kg body weight and day, gavage, investigation on GD 18	14 mg/kg body weight: <u>dams</u> , <u>foetuses</u> : NOAEL 27 mg/kg body weight: <u>dams</u> : mortality increased (3/40), salivation, apathy, absolute and relative thymus weights decreased; <u>foetuses</u> : foetal weights decreased, skeletal abnormalities [including cleft palates 11.4%, micrognathia 5%, fused basis of os occipitalis 3%] increased	Faqi et al. 1997
Mouse, NMRI, 6–20 ♀; 118 controls	GD 6–15, 0, 1, 4, 6, 12, 23, 35 mg TBTO/kg body weight and day, gavage, investigation on GD 18	6 mg/kg body weight: <u>dams</u> , <u>foetuses</u> : NOAEL 12 mg/kg body weight: <u>dams</u> : body weight gains reduced; <u>foetuses</u> : cleft palates 7% (controls 0.7%) 23 mg/kg body weight: <u>foetuses</u> : skeletal abnormalities and variations increased 35 mg/kg body weight: <u>dams</u> : mortality increased (1/6); <u>foetuses</u> : total number of resorptions (1/5). resorptions increased, live foetuses decreased, foetal weights decreased, cleft palates 48%, variations increased	Davis et al. 1987
Rat, Sprague Dawley, je 24 ♀	GD 6–19, 0.5.9, 18 mg TBTO/kg body weight and day, gavage, investigation on GD 20	5 mg/kg body weight: <u>dams</u> : NOAEL (body weight gains slightly reduced); <u>foetuses</u> : variations increased (asymmetric sternum, rudimentary structures, 14 th rib pair) 9 mg/kg body weight: <u>dams</u> : body weight gains reduced, 18 mg/kg body weight: <u>foetuses</u> : resorptions increased, number of foetuses/implantation sites decreased, foetal weights decreased, malformations (sternum malformations, cleft palates)	US EPA 1997

Table 5 (Continued)

Species, strain, number per group	Exposure	Findings	References
Rat, Sprague Dawley, 12 ♀	GD 0–19 or GD 8–19, 0, 0.25, 2.5, 10, 20 mg TBTC/kg body weight and day, gavage, investigation on GD 20	10 mg/kg body weight and above: <u>foetuses</u> : number of Sertoli cells decreased 20 mg/kg body weight: <u>foetuses</u> : number of gonocytes decreased light and electron microscopic evaluation of foetal testes and ovaries as well as gene expression	Kishta et al. 2007
Rabbit, White New Zealand, 20 ♀	GD 6–18, 0,0.2, ,2.5 mg TBTO/kg body weight and day, gavage, investigation on GD 29	1 mg/kg body weight: <u>dams, foetuses</u> : NOAEL 2.5 mg/kg body weight: <u>dams</u> : body weights decreased, abortions increased (7/20; controls 3/20); <u>foetuses</u> : foetal weights decreased (not significantly)	WHO 1990
TBTC			
Rat, Sprague Dawley, 12–25 ♀	GD 0–19, 0, 0.25, 2.5, 10, 20 mg TBTC/kg body weight and day, gavage, investigation on GD 20	0.25 mg/kg body weight: <u>foetuses</u> : anogenital distance increased ♂ 2.5 mg/kg body weight: <u>dams</u> : NOAEL 10 mg/kg body weight: <u>dams</u> : thyroxine and triiodothyronine concentration decreased; <u>foetuses</u> : variations increased (unfused ossification centres, such as ster-noschisis) 20 mg/kg body weight: <u>dams</u> : gestation rate decreased. body weight gains reduced, <u>foetuses</u> : postimplantation loss increased, litter size decreased, foetal weights decreased, malformations increased (cleft sternum 2/23)	Adeeko et al. 2003
Rat, Wistar, 10–12 ♀	GD 7–15, 0, 5, 9, 15, 25 mg TBTC/kg body weight and day, gavage, investigation on GD 20	5 mg/kg body weight and above: <u>dams</u> : food consumption reduced; <u>foetuses</u> : foetal weights (♀) decreased, delayed ossification of the sternum 9 mg/kg body weight and above: <u>dams</u> : body weight gains reduced; <u>foetuses</u> : resorptions increased. number of dead foetuses increased 25 mg/kg body weight: <u>dams</u> : mortality (7/10), sedation, diarrhoea, salivation; <u>foetuses</u> : no live foetuses	Itami et al. 1990

Table 5 (Continued)

Species, strain, number per group	Exposure	Findings	References
Rat, Wistar, 10–14♀	GD 0–7, 0, 8, 12, 16 mg TBTC/kg body weight and day, ga- vage, investigation on GD 20	controls: all animals pregnant 8 mg/kg body weight and above: dams: food consumption reduced, body weight gains reduced, non-pregnant animals 18% 12 mg/kg body weight and above: dams: toxic, non-pregnant animals 71%; <u>foetuses:</u> foetal weights decreased, delayed ossifica- tion 16 mg/kg body weight: <u>dams:</u> non-preg- nant animals 77%	Harazono et al. 1996
Rat, Wistar, 11–14 ♀	GD 7–8, 0, 40, 80 mg TBTC/kg body weight and day, gavage, investiga- tion on GD 20	40 mg/kg body weight and above: <u>dams:</u> body weight gains decreased; <u>foetuses:</u> post- implantation losses increased, foetal weights decreased 80 mg/kg body weight: <u>foetuses:</u> resorp- tions increased. number of live foetuses de- creased, malformations increased (cleft pa- lates)	Ema et al. 1995 a
Rat, Wistar, 11–140 ♀	GD 13–15, 165, 330 µmol TBTC/kg body weight and day (0, 54, 107 mg/kg body weight and day), gavage, inves- tigation on GD 20	54 mg/kg body weight and above: <u>dams:</u> body weight gains reduced; <u>foetuses:</u> malfor- mations increased (cleft palates) 107 mg/kg body weight: <u>foetuses:</u> foetal weights decreased	Ema et al. 1996
Rat, Wistar, 11–14 ♀	GD 7–9, 10–12 or 13–15, 0, 25, 50, 100 mg TBTC/kg body weight and day, ga- vage, investigation on GD 20	25 mg/kg body weight and above: <u>dams:</u> body weight gains reduced; <u>foetuses:</u> total number of resorptions, live foetuses de- creased, postimplantation losses GD 7–9, number of cleft palates increased (GD 13–15) 100 mg/kg body weight: <u>foetuses:</u> foetal weights decreased, postimplantation losses increased, cleft palates increased (GD 10– 12), foetal weights decreased (GD 13–15)	Ema et al. 1995 b
Rat, Wistar, 10–12 ♀	single GD 7–15, 0, 100, 200 mg TBTC/kg body weight and day ga- vage, investigation on GD 20	200 mg/kg body weight: <u>dams:</u> body weight gains reduced; <u>foetuses:</u> total number of re- sorptions (GD 7–9), postimplantation losses and number of live foetuses decreased (GD 7–11), foetal weights decreased (GD 7–15), external malformations (cleft palates, GD 7–14, especially GD 11–14) increased	Ema et al. 1997

Table 5 (Continued)

Species, strain, number per group	Exposure	Findings	References
Rat, Wistar, 12–16 ♀	GD 0–3 or 4–7, 0;8; 16, 33, 65 mg TBTC/kg body weight and day, ga- vage, investigation on GD 20	8 mg/kg body weight and above: dams: food consumption reduced 16 mg/kg body weight and above: dams: body weight gains reduced (GD 4–7), <u>foe-</u> <u>tuses:</u> postimplantation losses increased (GD 4–7), foetal weights decreased 33 mg/kg body weight: foetuses: postim- plantation losses increased (GD 0–3), num- ber of live foetuses decreased (GD 4–7), no increased variations or malformations	Harazono et al. 1998
TBTA			
Rat, Wistar, 10–14 ♀	GD 7–17, 0, 1, 2, 4, 8, 16 mg TBTA/kg body weight and day, ga- vage, investigation on GD 20	2 mg/kg body weight: dams: NOAEL 4 mg/kg body weight and above: dams: thymus weights <i>decreased</i> 16 mg/kg body weight: dams: salivation, food consumption reduced, body weight gains reduced, gestation rate decreased (10/ 14 pregnant); <u>foetuses:</u> total number of re- sorptions (5/10 litters), live foetuses de- creased, number of cleft palates increased (6/27), skeletal variations increased	Noda et al. 1991
TTBT			
Rat, Wistar, 10– 13, ♀	GD 13–15, 0, 330, 660, 1320, 2640, 5280 µmol TTBT/kg body weight (0, 115, 229, 458, 917, 1833 mg TTBT/kg body weight and day), gavage, investiga- tion on GD 20	114 mg/kg body weight: dams: NOAEL 229 mg/kg body weight and above: dams: body weight gains reduced 917 mg/kg body weight and above: foe- tuses: number of malformations increased, cleft palates in 23/138 foetuses in 3/12 lit- ters; controls: 0/133 foetuses in 0/11 litters 1833 mg/kg body weight: foetuses: cleft pa- lates in 13/115 foetuses in 6/9 litters, ac- cording to authors not statistically signifi- cant until this dose is reached	Ema et al. 1996

GD = gestation day

Mono-n-butyltin compounds

In a valid developmental toxicity study with MBTC in Wistar rats, no significant maternal or developmental toxicity was observed after oral administration of 400 mg/kg body weight and day from days 7 to 17 of gestation (Noda et al. 1992).

In the OECD Screening Test 421 (see Table 5), which is not sufficient for a final evaluation of prenatal developmental toxicity, no indications of developmental toxicity were found for **MBTC** up to a dose of 530 mg/kg body weight and day (Parametrix Inc 2006 f). Administration of MBTC at 903 mg/kg body weight and day from days 0 to 3 of gestation, or from days 4 to 7 of gestation (Ema and Harazono 2001), caused decreased foetal weights with simultaneously reduced maternal body weight gains. Mortality of dams and foetuses was increased after administration of 1500 and 2000 mg/kg body weight and day on days 7 and 8 of gestation (Ema et al. 1995 a). No increase in malformations was observed. Therefore, for MBTC, the NOAEL for maternal toxicity and developmental toxicity is 400 mg/kg body weight and day.

Di-n-butyltin compounds

In two developmental toxicity studies with Wistar rats, **DBTC** was administered from days 6 to 15 of gestation (Farr et al. 2001) or days 7 to 15 of gestation (Ema et al. 1991). Foetotoxicity and malformations were observed at 5 mg/kg body weight and day and above (Ema et al. 1991), or at 10 mg/kg body weight and day (Ema et al. 1995 a; Farr et al. 2001). Increased mortality occurred at 7.5 mg/kg body weight and day (Ema et al. 1991). For DBTC, the NOAEL is 5 mg/kg body weight and day for maternal toxicity, and 2.5 mg/kg body weight and day for developmental toxicity (Ema et al. 1991).

In addition to **DBTC**, **DBTO**, **DBTA**, **DBTM** and **DBTL** were also found to be teratogenic at 180 µmol/kg body weight and day in mechanistic studies with Wistar rats (24 mg/kg body weight DBTC, 28 mg/kg body weight DBTA, 28 mg/kg body weight DBTM, 20 mg/kg body weight DBTO and 50 mg/kg body weight DBTL) (Noda et al. 1993). It was discussed that the di-n-butyl group is responsible for the teratogenic potential (Noda et al. 1993). Exencephaly, encephalocele, cleft jaw, cleft lip and cleft palates, ankyloglossia, cleft tongue, omphalocele, club foot, defect of the mandible, fusion and absence of cervical and thoracic vertebral arches and bodies, fusion of sternbrae, microphthalmia or anophthalmia were listed as teratogenic findings (Ema et al. 1995 a). The induction of teratogenic effects by **DBTC** took place between days 7 and 9 of gestation (Ema et al. 1992, 1995 a; Noda et al. 1993). Treatment from the beginning of gestation up to day 3 of gestation produced pre- and postimplantation losses (7.6 mg/kg body weight), administration from days 4 to 7 of gestation caused reduced foetal weights (3.8 mg/kg body weight) and increased embryo and foetal mortality (7.6 mg/kg body weight and day). Higher doses during late organogenesis, i.e. between days 13 and 17 of gestation (50 mg/kg body weight and day), decreased foetal body weights only (Ema et al. 1996).

Tri-n-butyltin compounds

Administration of **TBTO** to Swiss mice on days 6 to 15 of gestation (Baroncelli et al. 1990) or to NMRI mice on days 6 to 15 of gestation (Davis et al. 1987) or 6 to 17 (Faqi et al. 1997) caused an increase in cleft palates at doses (12 mg/kg body weight

and day) causing slight maternal toxicity (reduced body weight gains) (Davis et al. 1987). Reduced foetal weights as well as other abnormalities and variations were observed at doses of 27 mg/kg body weight and day (Faqi et al. 1997) or 35 mg/kg body weight and day, which induced marked toxicity in the dams (Davis et al. 1987). The number of resorptions or complete resorptions increased at 35 mg/kg body weight and day (Davis et al. 1987) and 40 mg/kg body weight and day (Baroncelli et al. 1990). The NOAEL for maternal toxicity is below 5 mg/kg body weight and day for TBTO in mice owing to a reduction in spleen weights (Baroncelli et al. 1990) and the NOAEL for developmental toxicity is 6 mg/kg body weight and day (Davis et al. 1987).

After administration of 5 mg/kg body weight and day and above to rats, a maternally non-toxic or slightly toxic dose, tri-n-butyltin compounds (mostly **TBTC** was investigated) produced in a few studies increased variations (US EPA 1997) as well as delayed ossifications (Itami et al. 1990). In other investigations, increased variations or delayed ossification were not observed until 10 mg/kg (Adeeko et al. 2003) or 12 mg/kg body weight and day and above (Harazono et al. 1996) was reached. Malformations, mostly in the form of cleft palates, were found at maternally toxic doses of 16 mg/kg body weight and day (Noda et al. 1991), 18 mg/kg body weight and day (US EPA 1997) or 20 mg/kg body weight and day (Adeeko et al. 2003) and above. The increase in anogenital distance observed only in male foetuses on day 20 of gestation at the lowest TBTC dose of 0.25 mg/kg body weight and day and above in the study by Adeeko et al. (2003) seems to be of little relevance, as this was not dose-dependent.

It can be seen from mechanistic studies that cleft palates were induced, particularly on days 13, 14 or 15 of gestation (Ema et al. 1995 b, 1997). Exposures on preceding days were embryotoxic, with increased postimplantation losses and resorptions (Ema et al. 1995 b, 1997; Harazono et al. 1998). Exposures at later readings showed reduced foetal weights (Ema et al. 1995 b, 1996). The NOAEL for maternal and developmental toxicity of tri-n-butyltin compounds in rats is 2.5 mg/kg body weight and day (Adeeko et al. 2003). In a 1987 study with rabbits quoted from WHO, TBTO already produced marked toxicity in the dams and abortions at 2.5 mg/kg body weight and day. In the foetuses, however, only the body weights were slightly reduced (WHO 1990). In the rabbit, therefore, the NOAEL for maternal and developmental toxicity of TBTO is 1 mg/kg body weight and day.

Tetra-n-butyltin

At a maternally toxic dose of 917 mg/kg body weight and day, **TTBT** produced an increase in malformations in the form of cleft palates, which were not considered by the authors to be statistically significant until reaching 1833 mg/kg body weight and day (Ema et al. 1996). In the OECD Screening Test 422 (see Table 4), no externally visible malformations were observed in rats up to the highest dose of 119 mg/kg body weight and day (ORTEP 2004).

Postnatal developmental toxicity

Studies on the effects of *n*-butyltin compounds on postnatal development are shown in Table 6.

Mono-*n*-butyltin compounds

In the OECD Screening Test 421 (see Table 4) with **MBTC** in Wistar rats, no treatment-related changes in the parents or in the offspring investigated up to day 4 after birth were observed up to the highest dose of 530 mg/kg feed (Parametrix Inc 2006 f).

In the OECD Screening Test 422 (see Table 4) with **MTB(2-EHMA)** in Sprague Dawley rats, maternal toxicity (including increased mortality, reduced body weight gains and food consumption among others) occurred at 150 mg/kg body weight and day. In the offspring, mortality was increased and body weight gains reduced up to day 4 after birth. In this study, the NOAEL for postnatal developmental toxicity and for systemic toxicity was found to be 50 mg/kg body weight and day (Parametrix Inc 2006 g).

Di-*n*-butyltin compounds

In the OECD Screening Test 421 (see Table 4) with **DBTC** in Wistar rats, 11 mg/kg body weight and day and above caused an increase in postimplantation losses as well as pronounced foetotoxicity with increased mortality up to day 4 after birth. For this endpoint, the NOAEL is 2 mg/kg body weight and day (Parametrix Inc 2006 a).

Tri-*n*-butyltin compounds

In Swiss mice, the administration of **TBTO** at the lowest dose of 5 mg/kg body weight and day and above on days 6 to 15 of gestation caused decreased maternal body weight gains, as well as a transient reduction in cell volume and haemoglobin content of the erythrocytes and a slightly increased leukocyte count in the newborn pups on the first day after birth. In the offspring, the body weight gains were reduced and the mortality increased at 10 mg/kg body weight and day and above (Baroncelli et al. 1995; Karrer et al. 1995). As the effects on erythrocytes and leukocytes were not dose-dependent, and also no longer occurred on days 7, 14 or 21 after birth, they were not considered to be relevant. Thus, the NOAEL for the postnatal toxicity of TBTO in mice is 5 mg/kg body weight and day.

A study on the postnatal developmental toxicity on the immune system of mice after prenatal exposure to 0.1 mg/kg body weight and day is only available in the form of an abstract. Here, a suppression of hypersensitivity, an inhibition of antibody reactions, a changed proliferation of thymocytes and spleen cells, as well as an increased number of white blood cells were reported (Buckiova et al. 1992). As the individual data are not described, and only one dose of **TBTO** was administered,

Table 6 Studies on the postnatal developmental toxicity of n-butyltin compounds

Species, strain, number per group	Exposure	Findings	References
TBTO			
Mouse, Swiss, 8–36 ♀	GD 6–15, 0, 5, 10, 20, 30 mg TBTO/kg body weight and day, ga- vage, investigation PND 7, 14, 21	5 mg/kg body weight: <u>dams</u> : body weight gains reduced; <u>offspring</u> : NOAEL, transient haematological findings in the newborn pups (PND 1) without clear dose-dependency (cell volume, MCV decreased, haemoglobin content of erythrocytes decreased, MCH changed, number of leukocytes slightly increased) 10 mg/kg body weight and above: <u>dams</u> : body weight gains reduced, neglected care of offspring, cannibalism; <u>offspring</u> : body weight gains to PND 7 decreased, postnatal surviving animals to PND 7 decreased 20 mg/kg body weight and above: <u>offspring</u> : number of live pups decreased, birth weights decreased, thymus weights to PND 21 unchanged 30 mg/kg body weight: <u>dams</u> : vaginal bleeding (1 animal GD 12)	Baroncelli et al. 1995; Karrer et al. 1995
Mouse, ICR, (no other details)	GD 4–17 or 11–17, 0; 0.1 mg TBTO/kg body weight and day, ga- vage, investigation weeks 4 and 8 after birth	0.1 mg/kg body weight: <u>offspring</u> : delayed type suppression of the hypersensitivity to sheep erythrocytes, inhibition of antibody reactions to ovalbumin and lipopolysaccharide, changed proliferation of thymocytes and spleen cells, increased number of white blood cells. <i>Study only available as abstract, description of data absent</i>	Buckiova et. al. 1992
Rat, Long Evans, 15–18 ♀	GD 6–20, 0, 2.5, 5, 10, 12, 16 mg TBTO/kg body weight and day, gavage, inves- tigation up to PND 110	5 mg/kg body weight: <u>dams, offspring</u> : NOAEL 10 mg/kg body weight and above: <u>dams</u> : body weight gains reduced, <u>offspring</u> : litter size decreased, birth weights decreased, body weight gains reduced, surviving animals decreased, delayed vaginal opening (♀) and reduced motor activity PND 14, 47, 62 (corresponding to lower body weights), absolute weights of brain, cerebellum and hippocampus decreased PND 110 (not related to the reduced body weights)	Crofton et al. 1989

Table 6 (Continued)

Species, strain, number per group	Exposure	Findings	References
		<p>at 12 mg/kg body weight and above: dams: vaginal bleeding; <u>offspring</u>: surviving animals decreased, cleft palates increased</p> <p>16 mg/kg body weight: <u>dams</u>: body weight reduction</p>	
TBTC			
Rat, Sprague Dawley, 16 ♀, off- spring 12 ♂, ♀	GD 8-PND 30, 60 or 90, 0, 0.025, 0.25, 2.5 mg TBTC /kg body weight and day, gavage, inves- tigation PND 30, 60, 90	<p>0.025 mg/kg body weight and above: <u>off- spring</u>: NOAEL, up to PND 21: no n-butyltin in the stomach contents</p> <p>0.25 mg/kg body weight and above: <u>off- spring</u>: up to PND 21: no n-butyltin in the stomach contents; relative spleen weights decreased (PND 30 ♂), relative thymus weights decreased (PND 60 ♀)</p> <p>2.5 mg/kg body weight: <u>dams</u>: no effects; <u>offspring</u>: relative liver weights decreased (PND 60 ♀, PND 90 ♂)</p>	Cooke et al. 2004
Rat, Sprague Dawley, 10 ♀, offspring 10 ♂ or ♀	GD 8-PND 30, 60 oder 90, 0, 0.025, 0.25, 2.5 mg TBTC /kg body weight and day, gavage, inves- tigation PND 30 (♂,♀); 60(♀); 90 ♂	<p>0.025 mg/kg body weight and above: <u>off- spring</u>: lymphocytopenia and atrophy of lymph nodes increased (PND 30 ♂); IgM increased, spleen atrophy (PND 60 ♂); activity of natural killer cells increased (PND 90 ♂)</p> <p>0.25 mg/kg body weight and above: <u>off- spring</u>: thymusatrophy increased (PND 30 ♂, ♀), number of CD4⁺8⁺ (immature T-lymphocytes) increased and decreased resistance to <i>Listeria monocytogenes</i> (PND 60 ♀); IgA decreased, IgG increased and immune response of the delayed type to oxalone increased (PND 90 ♂)</p> <p>2.5 mg/kg body weight: <u>offspring</u>: number of natural killer cells decreased (PND 30 ♂,♀); IgG2_α decreased, IgM increased (PND 90 ♂)</p>	Tryphonas et al. 2004

GD = gestation day

this study cannot be considered for the assessment of postnatal developmental toxicity.

Administration of a **TBTO** dose of 16 mg/kg body weight and day to Long Evans rats on days 6 to 20 of gestation, showed reduced maternal body weight gains at 10 mg/kg body weight and day and above. In the offspring, litter size, birth weights

and body weight gains were decreased and mortality was increased. The reduced body weights were not accounted for in the assessment of effects on motor activity and on absolute brain weights (Crofton et al. 1989). However, it is to the decreased body weights in particular that the effects can plausibly be attributed. As the dose was also within the lethal range for pups, no neurotoxic effect can be derived from these findings. The NOAEL for postnatal toxicity in this study is 5 mg/kg body weight and day.

TBTC was administered to female Sprague Dawley rats from gestation day 8 onwards, their offspring receiving further doses up to the age of 90 days. No effects on the newborn pups were reported. Postnatal mortality was not significantly increased. The relative spleen and thymus weights were significantly decreased in the pups at 0.25 mg/kg body weight and day, and decreased relative liver weights were found at 2.5 mg/kg body weight and day (Cooke et al. 2004). For **TBTC**, the NOAEL for toxic effects in the pups, such as reduced thymus weight, was 0.025 mg/kg body weight and day. No significant effects on the offspring at birth or during lactation could be seen in this study.

Sprague Dawley rats exposed to **TBTC** in utero at 0.025, 0.25 or 2.5 mg/kg body weight and day from day 8 and up to postnatal days 30, 60 or 90 showed a reduction in lymphocyte count and atrophy of the lymph nodes as well as spleen atrophy at 0.025 mg/kg body weight and day and above, and thymus atrophy at 0.25 mg/kg body weight and day and above. As changes in immune cells, increased activities of natural killer cells were observed with **TBTC** at doses of 0.025 mg/kg body weight and day and above, and an increased number of immature T-lymphocytes at 0.25 mg/kg body weight and above. In the serum, the immunoglobulin content was changed at 0.025 mg/kg body weight and day and above (Tryphonas et al. 2004). The LOAEL for the effects on lymph nodes and spleen of pups was 0.025 mg/kg body weight and day.

Neither of these two studies are suitable for assessing the embryotoxic effect of **TBTC** at the workplace, as the effects were found in exposed pups.

In a two-generation study in rats (see Table 4) with administration of **TBTO** in doses of about 3 mg/kg body weight and day, the body weight gains of the pups during lactation as well as the absolute and relative thymus weights of the adults were decreased. The NOAEL was 0.3 mg/kg body weight and day (BUA 1988).

In a further two-generation study administration of **TBTC** to Wistar rats caused reduced birth weights and reduced postnatal body weight gains at the highest dose of about 10 mg/kg body weight and day (see Table 4). At this dose, the effects on the female offspring were a clearly increased anogenital distance in the newborn pups, delayed vaginal opening in the adults, shortened and irregular oestrus cycles, and decreased relative ovarian and uterus weights. In this dose group, decreased relative prostate weights, decreased spermatid and sperm counts, increased testosterone and decreased 17 β -estradiol concentrations were also determined in the male adult offspring. At 2 mg/kg body weight and day, the spermatid count was significantly decreased in the male offspring (Ogata et al. 2001). The NOAEL for the effects of **TBTC** on male offspring was accordingly 0.4 mg/kg body weight and

day, the NOAEL for effects on female offspring 2 mg/kg body weight and day. As the effects in the females were in part already recognizable at birth, the damage could, possibly, already have been induced prenatally. From this study, a NOAEL of 0.4 mg/kg body weight and day is derived for postnatal toxicity.

Tetra-*n*-butyltin

In the OECD Screening Test 422 (see Table 4) in Wistar rats, postimplantation losses were increased, the number of live offspring decreased and the postnatal mortality of the offspring increased after administration of **TTBT** at about 119 mg/kg body weight and day. Thus, for **TTBT**, the NOAEL was about 19 mg/kg body weight and day for developmental toxicity and about 6.5 mg/kg body weight and day for systemic toxicity, such as reduced spleen and thymus weights and thymus changes (ORTEP 2004).

5.6 Genotoxicity

5.6.1 In vitro

Table 7 shows the available data from in vitro genotoxicity studies. *n*-Butyltin compounds were not found to be mutagenic in bacterial test systems. The studies by Hamasaki et al. (1993), in which **MBTC**, **MBTO**, **DBTC** and **TBTC** were described as being positive in *Salmonella typhimurium* TA100, form an exception. In this case, however, the number of spontaneous mutations obtained was less than double. Other studies, some carried out with the same strain, were negative.

Further positive findings, using the so-called rec-assay, were reported for **MBTO**, **DBTC** and **TBTC** by the same group of authors (Hamasaki et al. 1992). This test measures the preferential killing of recombination-negative bacteria versus recombination-positive bacteria, i.e. no mutations. It is considered applicable as a screening indicator test for genotoxic chemicals in bacteria. However, these findings were not confirmed by another group of authors who had investigated **TBTO** over a wide range of endpoints in a comprehensive study (Davis et al. 1987).

In the so-called fluctuation test with *Salmonella typhimurium* TA100, **TBTO** produced no positive results until reaching the toxic range, and no clear dose-dependency could be recognized (Davis et al. 1987).

In the SOS-Chromotest, **MBTC**, **MBTO** and **DBTC** were found to be positive (Hamasaki et al. 1992). It is known that inhibitors of DNA synthesis which do not damage DNA are positive in this test.

In yeast cells, no gene mutations and no mitotic gene conversions were induced by *n*-butyltin compounds (Davis et al. 1987).

In mammalian cells, **MBTC**, **DBTC** and **TBTO** caused no mutations in the HPRT test or in the mouse lymphoma test. The study by Li et al. (1982), in which **DBTC** was positive basically in the cytotoxic range, forms an exception.

Table 7 Genotoxicity of *n*-butyltin compounds in vitro

Endpoint	Test system	Substance	Concentration range [µg/plate] ^{a)}	Effective concentration ^{b)}	Cytotoxicity ^{c)1)}	Results	References
Rec-assay	<i>Bacillus subtilis</i> M45(rec-), H17(rec+)	MBTO	10–10 000	100		+ –S9 +S9	Hamasaki et al. 1992
	<i>Bacillus subtilis</i> M45(rec-), H17(rec+)	DBTC	10–10 000	2000		+	Hamasaki et al. 1992
	<i>Bacillus subtilis</i> M45(rec-), H17(rec+)	TBTC	10–10 000	10		+	Hamasaki et al. 1992
	<i>Bacillus subtilis</i> M45(rec-), H17(rec+)	TBTO	58 500	–	58500	–	Davis et al. 1987
	<i>Bacillus subtilis</i> M45(rec-), H17(rec+)	TTTCT	10–10 000	–		–	Hamasaki et al. 1992
	<i>Saccharomyces cerevisiae</i> D4	DBTC	up to 100	–		–	Parametrix Inc 2006 h
Mitotic gene conversion	<i>Saccharomyces cerevisiae</i> D4	TBTO	0.003–0.5 µg/ml			–	Davis et al. 1987
	<i>Saccharomyces cerevisiae</i> D4	TBTO	0.0001–0.01 µl/plate	–	0.01 µl/plate	–	Reimann and Lang 1987
	<i>Saccharomyces cerevisiae</i> D4	TBTO	10–10 000	5000		+	Hamasaki et al. 1992
SOS- Chromotest	<i>Escherichia coli</i> PQ37	MBTC	10–10 000	5000		+	Hamasaki et al. 1992
	<i>Escherichia coli</i> PQ37	DBTC	0.1–5.0	0.1		+	Hamasaki et al. 1992
	<i>Escherichia coli</i> PQ37	TBTC	bis 1000	–		–	Hamasaki et al. 1992
	<i>Escherichia coli</i> PQ37	TBTC	10–10 000	–		–	Parametrix Inc 2006 h
	<i>Escherichia coli</i> PQ37	TBTC	10–10 000	–		–	Hamasaki et al. 1992

Table 7 (Continued)

Endpoint	Test system	Substance	Concentration range [$\mu\text{g}/\text{plate}$] ^{a)}	Effective concentration ^{b)}	Cytotoxicity ^{b)}	Results	References
Gene mutation	<i>Escherichia coli</i> PQ37	TTBT	10–10 000	–	–	–S9 +S9	Hamasaki et al. 1992
	<i>Salmonella typhimurium</i> TA98, MBTC		62–5000		185–>5000		Parametrix Inc 2006 f
	TA100, TA1535, TA1537; <i>Escherichia coli</i> WP2 uvr A						
	<i>Salmonella typhimurium</i> TA100	MBTC	1–100	50	>100	+	Hamasaki et al. 1993
	<i>Salmonella typhimurium</i> TA100	MBTO	1–100	100	≥ 100	+	Hamasaki et al. 1993
	<i>Salmonella typhimurium</i> TA98, MBT(2-EHMA)		up to 5000			–	Parametrix Inc 2006 g
	TA100, TA1535, TA1537; <i>E. coli</i> WP2 uvr A						
	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	DBTC	0.5–1000			–	Parametrix Inc 2006 a
	<i>Salmonella typhimurium</i> TA100	DBTC	0.1–10	10	≥ 10	+	Hamasaki et al. 1993
	<i>Salmonella typhimurium</i> TA 97, TA98, TA100, TA1535	DBTA	33–3333	–		–	Zieger et al. 1987
	<i>Salmonella typhimurium</i> TA 97, TA98, TA100, TA1535	DBTL	1–166	–		–	Zieger et al. 1987

Table 7 (Continued)

Endpoint	Test system	Substance	Concentration range [μg/plate] ^{a)}	Effective concentration ^{a)}	Cytotoxicity ^{a)}	Results	References
						-S9 +S9	
	<i>Salmonella typhimurium</i> TA98, TA100, TA1537; <i>E. coli</i> WP2 uvr A	DBTM	0.3–62		21–62	–	Parametrix Inc 2006 d
	<i>Salmonella typhimurium</i> TA1535	DBTM	0.3–62	–	≥7	–	Parametrix Inc 2006 d
	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537; <i>E. coli</i> WP2 uvr A	DBTO	1.25–62		21–62	–	Parametrix Inc 2006 e
	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537; <i>E. coli</i> WP2 uvr A	DBT(2- EHMA)	6.2–5000		≥2500	–	Parametrix Inc 2006 b
	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537	DBT (IOMA)	25–5000		no details	–	Parametrix Inc 2006 h
	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537	DBT (IOMA)	625–5000		≥2500	–	Parametrix Inc 2006 i
	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537; <i>E. coli</i> WP2 uvr A	TBTC	0.01–1	0.05	0.1	+	Hamasaki et al. 1993
	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA1537; <i>E. coli</i> WP2 uvr A	TBTC	0.1–21	–	7–21	–	Parametrix Inc 2006 h
	<i>Salmonella typhimurium</i> TA 97, TA98, TA100, TA 1530, TA1535	TBTO	2.5–500	–		–	Davis et al. 1987

Table 7 (Continued)

Endpoint	Test system	Substance	Concentration range [µg/plate] ^{a)}	Effective concentration ^{a)}	Cytotoxicity ^{a)}	Results	References
Sister chromatid exchange	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA 1537, TA 1537	TBTO	0.0001–0.16 µl/plate	–	≥0.0025	–	Reimann and Lang 1987
	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA 1537, TA 1537	TBTO	0.0001–0.16 µl/plate	–	≥0.02	–	Reimann and Lang 1987
	<i>Salmonella typhimurium</i> TA100 ^{f)}	TBTO	0.05–5.0 µM	0.5	≥0.5	+	Davis et al. 1987
	<i>Schizosaccharomyces pombe</i> P1	TBTO	0.003–0.5	0.5	0.5	–	Davis et al. 1987
	<i>Klebsiella pneumoniae</i> ^{f)}	TBTO	19.7–1970 µM	–	–	–	Davis et al. 1987
	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA 1537; <i>E. coli</i> WP2 uvr A	TTBT	5.0–5000	–	–	–	Parametrix Inc 2006 j
	<i>Salmonella typhimurium</i> TA98, TA100, TA1535, TA 1537, TA1538	TTBT	5.0–5000	–	–	–	Parametrix Inc 2006 j
	<i>Salmonella typhimurium</i> TA98, TA100	TTBT	0.1–100	–	–	–	Hamasaki et al. 1993
	CHO cells	TBTO	0.0005–0.5	–	–	–	Davis et al. 1987
	V79 cells	MBTC	62.5–500 µg/ml	–	–	–	Parametrix Inc 2006 f
Chromosome aberrations							

Table 7 (Continued)

Endpoint	Test system	Substance	Concentration range [μg/plate] ^{a)}	Effective concentration ^{a)}	Cytotoxicity ^{a)1)}	Results	References
	human lymphocytes	DBTC	up to 7.5 μg/ml			-59 +59	
	human lymphocytes	TBTO	0.005–0.1 μg/ml	–	0.1 μg/ml	+	Parametrix Inc 2006 a
	CHO cells	TBTO	0.01–1.0 μg/ml 0.8–8.4	– 8.4	1.0 μg/ml 8.4	– +	Reimann and Lang 1987 Davis et al. 1987
Gene mutation HPRT	V79 cells	MBTC	up to 5000	–		–	Parametrix Inc 2006 f
	V79 cells	DBTC	up to 0.0005 μl/ml	–		–	Parametrix Inc 2006 a
	V79 cells	DBTC	up to 0.00006 μl/ml	–		–	Parametrix Inc 2006 a
	CHO cells	DBTC	0.165–0.99 μM	0.33 μM	0.66 μM	+	Li et al. 1982
	V79 cells	TBTO	0.02–0.22 μM	–		–	Davis et al. 1987
			0.02–4.12 μM	–		–	
TK ⁺ /–	L5178Y mouse lymphoma cells	TBTO	0.02–0.15 μM	–		–	Davis et al. 1987

^{a)} Unless indicated otherwise, figures relate to [μg/plate], ^{f)} Fluctuation test, ¹⁾ Information only given if cited in publication

TBTO induced chromosome aberrations in CHO cells, though only at highly toxic concentrations, at which no colony formation was observed any more (Davis et al. 1987). It is not possible to discuss the positive findings obtained with **DBTC**, i.e. the induction of chromosome aberrations in lymphocytes (Parametrix Inc 2006 a), as the original study could not be obtained. In a well-documented study with **TBTO**, no chromosome aberrations in lymphocytes were induced (Reimann and Lang 1987).

In total, the in vitro investigations show that positive test results only occur at cytotoxic concentrations, or that positive investigations results by other authors could not be confirmed.

5.6.2 In vivo

Soma cells

A micronucleus test was carried out according to OECD Test Guideline 474 after administration of single oral MBTC doses of 0, 10, 50 or 250 mg/kg body weight to ICR mice. At 250 mg/kg body weight, the number of micronuclei in 1000 polychromatic erythrocytes was significantly increased (1.3 as compared with 0.6 in the controls, no cytotoxicity) in the bone marrow after 48 hours. No difference in the number of micronucleus-containing erythrocytes was observed in treated and untreated animals after 24 and 72 hours. No biological significance was attributed to the findings after 48 hours (no other details; Parametrix Inc 2006f). In a micronucleus test with ICR mice (according to OECD Test Guideline 474), no increased number of micronuclei was found in the bone marrow cells after administration of single oral **MBT(2-EHMA)** doses of 175, 350 or 700 mg/kg body weight (male animals) or 225, 450 or 900 mg/kg body weight (female animals), (Parametrix Inc 2006 g).

In a micronucleus test with **TBTC**, carried out according to OECD Test Guideline 474, groups of five male Swiss mice were given doses of 0, 75, 150 or 300 mg/kg body weight by gavage. At 150 mg/kg body weight, piloerection and blepharospasms were observed in one animal. At the highest dose, lethargy, blepharospasms and piloerection occurred. Three animals died before the end of the study. A significant increase in the incidence of polychromatic erythrocytes with micronuclei occurred only at the highest dose after 48 hours. However, this was not assessed as a positive result, as the number of cells with micronuclei was unexpectedly low in the control group, and the cytotoxicity in the bone marrow cells was significantly increased in the animals of the high-dose group (ORTEP 2003 a).

After administration of single **TBTO** doses of 0, 30 or 60 mg/kg body weight to male and female BALB/c mice, an increased frequency of micronuclei in polychromatic erythrocytes of the bone marrow was determined only in the males after 48 hours at 60 mg/kg body weight. No cytotoxicity in the bone marrow cells occurred (Davis et al. 1987). This positive result was not confirmed in a subsequent re-evaluation of the samples (Schering AG 1986).

Single TBTO doses of 0, 31.25, 62.5, 125 or 250 mg/kg body weight were administered by gavage to NMRI mice. The bone marrow was investigated after 24, 48 and 72 hours. At 250 mg/kg body weight, mortality was so high that the bone marrow cells of the surviving animals could not be assessed. At 125 mg/kg body weight one of five male and three of five females died. At 62.5 mg/kg body weight and above, a significant reduction in the number of polychromatic erythrocytes and thus cytotoxicity was found in the bone marrow cells only after 48 hours. No significant increase in the number of micronuclei was demonstrated at any dose (Reimann and Lang 1987).

The frequency of mitomycin C-induced micronuclei in peripheral reticulocytes of the mouse was increased by approximately 50% after administration of 50 mg/kg body weight **TBTO**. In this study, TBTO itself induced no micronuclei (BUA 2003).

No increased number of micronuclei and no cytotoxicity in bone marrow cells was found in male and female NMRI mice after 24, 48 and 72 hours when they were given **TBTN** doses of 0, 50, 158 or 500 mg/kg body weight (Reimann and Lang 1987).

In a micronucleus test performed in male Swiss mice according to OECD Test Guideline 474, with administration of single oral doses of 0, 500, 1000 or 2000 mg/kg body weight, **TTBT** proved to be negative. No cytotoxicity in bone marrow cells was found (ORTEP 2003 b).

Altogether, the investigations on the induction of micronuclei by MBTC in the bone marrow were negative. In one micronucleus test with mice, positive findings were obtained for DBTC at concentrations inducing frank and myelotoxicity; results were negative in one other test. In one study, TBTO induced micronuclei at one concentration and in male mice only; however, subsequent re-evaluation did not confirm the result. Another micronucleus test performed with TBTO was negative. TBTN and TTBT produced no increased number of micronuclei in the bone marrow of mice.

Germ cells

DBTA (Woodruff et al. 1985) and **TBTO** (Davis et al. 1987) caused no X-chromosome-linked recessive lethal mutations in *Drosophila melanogaster*.

To summarize, the available data on genotoxicity in vitro and in vivo indicate no direct genotoxic mechanism of action with n-butyltin compounds. Their genotoxicity is thus assessed to be negative.

5.7 Carcinogenicity

5.7.1 Short-term studies

In a two-stage cell transformation assay using 3-methylcholanthrene as initiator, **TBTC** and **DBTC** promoted the morphological transformation of C3H/10T1/2 cells and induced the expression of proliferin. The effective concentrations of TBTC were in the range of 20 to 75 nM and those of DBTC at 80 nM. Beside proliferin, **DBTC** induced a number of proto-oncogenes (*fos*, *jun*) and related mRNA species (*c-myc*, *egrl* and *odc* among others) (Parfett and Pilon 1993; Parfett et al. 2000).

5.7.2 Long-term studies

DBTA was administered to 50 male and 50 female Fischer 344 rats in doses of 0, 6.65 or 13.3 mg/kg (about 0, 6.65 or 13.3 mg/kg body weight and day) with the feed for 78 weeks. Thereafter, the animals received normal feed for another 26 weeks. Twenty animals each were used as controls. B6C3F1 mice were given DBTA in concentrations of 0, 76 or 152 mg/kg feed (about 0, 11.4 or 22.8 mg/kg body weight and day) for 78 weeks. The recovery period was 14 weeks. A dose-dependent and statistically significant increase in mortality (no figures cited) occurred in the male rats and the female mice. No other signs of intoxication were described.

However, the statement that no macroscopic and microscopic abnormalities occurred is not meaningful, as it was not possible to relate the results to the total number of animals. Losses due to missed animals, cannibalism or autolysis were cited.

Nevertheless, an increased incidence of hepatocellular adenomas and carcinomas (see Table 8) was found in the highest dose group (22.8 mg/kg body weight and day) of the investigated B6C3F1 mice. Although male and female B6C3F1 mice showed the same type of tumour and the formation of these tumours was consid-

Table 8 Tumour incidences in B6C3F1 mice after ingestion of DBTA for 78 weeks (calculated from NCI 1978)

		DBTA [mg/kg body weight and day]		
		0	11.4	22.8
Sex		Incidence (%)		
Hepatocellular adenomas Hepatocellular carcinomas	♂	2/19 (11%)	11/49 (22%)	15/49 (31%)*
	♀	1/20 (5%)	4/47 (9%)	12/43 (28%)**

* $p = 0.08$, ** $p < 0.05$

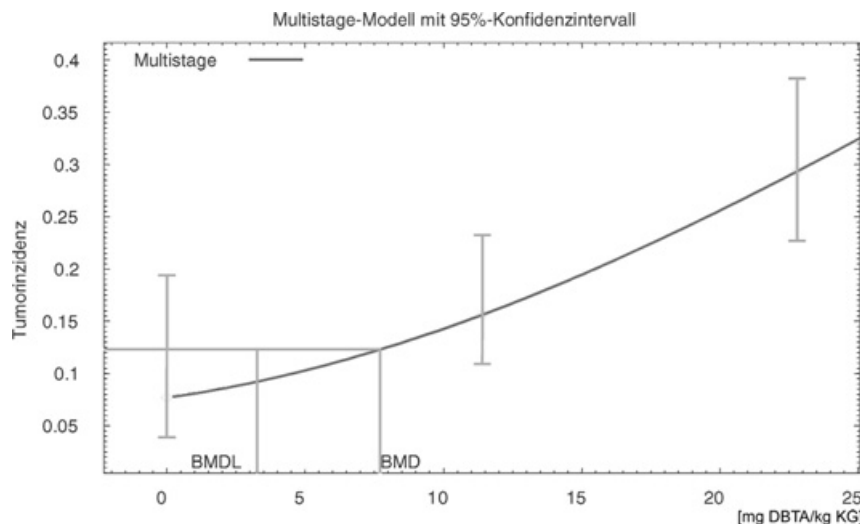


Figure 1 Calculation of the benchmark dose (BMD) and/or the lower confidence limit (BMDL) for a 5% increase in liver tumour incidence after 78 weeks of DBTA ingestion. The BMDL 95 for a 5% incidence increase (extra risk) of liver tumours caused by DBTA in male and female mice is 3.2 mg/kg body weight = 1 mg tin/kg body weight, calculated with the multistage model. Other models (Gamma, Log Logistic) yield very similar values.

ered by the authors to be substance-related, they assessed the carcinogenic potential as showing “no conclusive evidence”.

To test how far the doses at which liver tumours were induced in B6C3F1 mice are relevant to workplace conditions, a benchmark calculation was performed. For an increase in tumour incidence by 5%, a BMDL (lower 95% confidence limit) of 3.2 mg/kg body weight (1 mg tin/kg body weight) is obtained from the carcinogenicity study with DBTA (Figure 1).

In the high-dose female rats, a loss of uterine tissue samples limited the validity of the study, particularly as an increased incidence of adenocarcinomas in the uterus had occurred in the low-dose group (3/49 vs 0/20 in the control animals) (NCI 1978). As the tumours in the male and female mice were of the same type, and there was a statistically significant increase in hepatocellular adenomas and carcinomas in the females, this study indicates a carcinogenic potential for DBTA.

A significantly increased incidence of benign tumours of the pituitary (male and female animals), pheochromocytomas of the adrenals (male and female animals) and adenomas of the parathyroid glands (male animals) was found in male and female Wistar rats of the high-dose group after treatment with **TBTO** in concentrations of 0, 0.5, 5 or 50 mg/kg feed (about 0, 0.025, 0.25 or 2.5 mg/kg body weight and day) for two years. In addition, the very rare anaplastic carcinoma of the exocrine pancreas was found in a number of female animals. The tumour incidences are listed in Table 9, and the systemic effects in Table 3 (Wester et al. 1990).

Table 9 Tumour incidences in Wistar rats after two-year ingestion of TBTO (Wester 1990)

		TBTO [mg/kg body weight and day]			
		0	0.025	0.25	2.5
Sex		Incidence (%)			
Tumours of the pituitary	♂	34/50 (68%)	39/50 (78%)	29/50 (58%)	43/50 (86%)*
	♀	22/50 (44%)	32/50 (64%)	22/50 (44%)	35/50 (70%)**
Pheochromocytomas of the adrenals	♂	16/50 (32%)	13/50 (26%)	14/50 (28%)	33/50 (66%)**
	♀	3/50 (6%)	3/50 (6%)	3/50 (6%)	34/50 (68%***)
Adenomas of the parathyroid gland	♂	0/39 (0%)	2/50 (4%)	1/51 (2%)	6/43 (12%)*
	♀	0/64 (0%)	0/44 (0%)	1/40 (2%)	1/44 (2%)
Anaplastic carcinoma of the exocrine pancreas	♀	0/50 (0%)	1/50 (2%)	0/50 (0%)	2/50 (4%)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

In a further study, no significant differences in tumour incidences between treated and untreated animals was observed (see also Section 5.2.2) in 50 male and 50 female CD1 mice receiving 97.1% **TBTO** in concentrations of 0, 5, 25 or 50 mg/kg (about 0, 0.7, 3.7 or 7.7 mg/kg body weight and day in males and about 0, 0.9, 4.8 or 9.2 mg/kg body weight and day in females) with their feed for 18 months (WHO 1999).

5.8 Other effects

Independently of the species investigated, the most important T-cell-mediated immune functions, including the thymus-dependent humoral immune response, were suppressed at 0.1 $\mu\text{mol/l}$ and above as sequels of the thymolytic or lymphotoxic effects of **di- and tri-*n*-butyltin compounds**. In addition, the ability to survive, proliferate and differentiate was also decreased in B-lymphocytes at 0.1 μM and above (de Santiago and Aguilar-Santelises 1999).

At concentrations of 0.1 $\mu\text{g/ml}$ and above, **DBTC** inhibited the replication of lymphocytes from thymus and spleen of the rat in vitro after stimulation with phytohaemagglutinin or concanavalin A (Penninks and Seinen 1982). In addition, it reduced the function of human natural killer cells after incubation for 24 hours at 0.5 $\mu\text{mol/l}$ and above. In this experiment, **MBTC** had an inhibiting effect at 5.0 $\mu\text{mol/l}$, **TBTC** already at 0.2 $\mu\text{mol/l}$ (Whalen et al. 1999).

On the basis of observations that environmental contamination with tri-n-butyltin caused masculinization and infertility ("imposex") in marine gastropods (BUA 2003), **TBTC** was described as being a competitive inhibitor of microsomal aromatase in the human placenta (IC_{50} 6.2 μ M). On the other hand, **DBTC** showed only a slight inhibitory effect, and **MBTC** was inactive (Cooke 2002; Heidrich et al. 2001). In an investigation with human placental choriocarcinoma cells **TBTC** caused an increase in aromatase activity (Nakanishi et al. 2002).

In humans, effects on aromatase, an enzyme that metabolizes androgens to oestrogens, are not to be expected, as tri-n-butyltin compounds ingested or absorbed through the skin do not accumulate in the body, so that the concentrations are too low to produce any endocrine effect (Römer et al. 2002).

In MCF-7 cells (human oestrogen-dependent breast cancer cells), **TBTC** (10 nM) and **DBTC** (500 nM) inhibited cell proliferation induced either by 17 β -oestradiol or testosterone. The inhibition of testosterone-induced cell proliferation could be reduced by further addition of testosterone, and could not be influenced by flutamide. From this, it was concluded that the TBTC effect is not mediated via oestrogen receptors (Nielsen and Rasmussen 2004)

6 Manifesto

n-Butyltin compounds are not genotoxic.

Two studies are available in which a carcinogenic potential of n-butyltin compounds is described. In one study, orally administered DBTA induced a statistically significant increase in hepatocellular adenomas and carcinomas in male B6C3F1 mice (NCI 1978). In the second study, in Wistar rats, a significantly increased incidence of benign tumours of the pituitary (males and females), pheochromocytomas of the adrenals (males and females) and adenomas of the parathyroid gland (males) as well as a very rare anaplastic carcinoma of the exocrine pancreas (females) was found after administration of TBTO with the feed (Wester et al. 1990).

Hormone disturbances and disruption of Ca^{2+} homeostasis (see Section 2 "Mechanism of Action") are considered to be the inducers of tumours in the pituitary, adrenal medulla and parathyroid glands found in Wistar rats after TBTO administration. This mechanism of action does not, however, apply to the increased occurrence of hepatocellular adenomas and carcinomas in male B6C3F1 mice. High-dose effects are here assumed to be the probable cause.

In summary the n-butyltin compounds are considered to be substances with a carcinogenic potential, whereby non-genotoxic mechanisms of action are in the foreground. For this reason, n-butyltin compounds are classified in Carcinogen Category 4.

No suitable data in humans are available to establish a MAK value. From a 4-week inhalation study with rats with 4-hour exposure to TBTO per day (Schering AG 1983), a NOAEC of 0.16 mg/m³ (vapour) is obtained. The next higher concen-

tration investigated was 2.8 mg/m³ (aerosol). At this concentration, mortality, inflammations of the respiratory tract, thymus atrophy and lymphocyte depletion in the thymus-dependent regions of spleen and lymph nodes occurred. Due to the large interval between the NOAEC and LOAEC, the NOAEC could also be higher. However, the daily exposure period of four hours was unusually short. Therefore the derivation of the MAK value is based on the experimental NOAEC. No time-dependent decrease of the NOAEL for the effects on the rat thymus was found in oral studies, in which TBTO was administered for 28 days, 13 weeks or two years. Therefore, an aggravation of systemic effects by longer exposure durations is not assumed. In addition, in a 12-month study in the dog, the same NOAEL was found as for rats. Consequently, a MAK value of 0.05 mg/m³, corresponding to 0.002 ml/m³, is derived for TBTO, taking the usual difference of 2 for a NOAEC based on animal experiments and the so-called "preferred value approach" into account. The MAK value expressed as tin is 0.02 mg/m³ or 0.004 ml/m³. Similar systemic effects are also found with the other n-butyltin compounds after oral administration. Thus, not the ligands are responsible for the effects observed, but the tin cation. The tin-related MAK value derived above is therefore also established for all n-butyltin compounds.

After short-term exposure to organotin compounds above 0.2 mg/m³ (as tin), irritation of the upper respiratory tract was described (ACGIH 2001). The NOEL for this irritant effect is, however, not known. Thus, Peak limitation category I and an excursion factor of 1 have been established for n-butyltin compounds.

To test how far the doses at which liver tumours were induced in B6C3F1 mice are relevant to workplace conditions, a benchmark calculation was performed. For an increase in tumour incidence by 5%, a BMDL (95% lower confidence limit) of 3.2 mg/kg body weight (1 mg tin/kg body weight) is obtained from the carcinogenicity study with DBTA (see Figure 1 in Section 5.7.2). Assuming a respiratory volume of 10 m³ inhaled over eight hours and a body weight of 70 kg with complete retention of the tin, a dose of 1 mg/kg body weight and day in humans corresponds to a tin concentration in the air of 7 mg/m³. The difference to the MAK value of 0.02 mg/m³ is thus sufficient, as tumour induction is not based on a genotoxic mechanism.

The *in vitro* studies on genotoxicity showed that positive test results occurred only at cytotoxic concentrations, or could not be confirmed by other authors. In animal studies, the tests on the induction of micronuclei in bone marrow cells were negative [MBTC, MBT(2-EHMA), DBTC, TBTC, TBTO, TBTN, TTBT], or were not reproducible in various studies (DBTC), or initially positive results could not be confirmed in a re-evaluation (TBTO). To summarize, the available data on genotoxicity indicate an indirect genotoxic mechanism of action. No classification in a germ cell mutagen category for n-butyltin compounds is possible.

A large number of investigations on prenatal and postnatal developmental toxicity of n-butyltin compounds exist. Mono-n-butyltin compounds did not produce reduced foetal weights in rats until reaching maternally toxic doses above 900 mg/kg body weight and day. The NOAEL for maternal and developmental toxicity was

400 mg/kg body weight and day. In rats, di-n-butyltin compounds were found to be teratogenic at maternally toxic doses of 5 mg/kg body weight and day and above. The NOAEL for this was 2.5 mg/kg body weight and day. Tri-n-butyltin compounds were found to be embryotoxic in rats at doses of 5 mg/kg body weight and day and above, though not producing cleft palates until attaining toxic doses of 16 mg/kg body weight and day. The NOAEL for maternal and developmental toxicity was 2.5 mg/kg body weight and day. In the rabbit, the NOAEL for prenatal developmental toxicity was 1 mg/kg body weight and day for TBTO. The most sensitive endpoint was, however, postnatal developmental toxicity after administration of tri-n-butyltin compounds. For TBTO, a NOAEL of 0.3 mg/kg body weight and day was obtained for postnatal toxicity in a two-generation study with Sprague Dawley rats, and a NOAEL of 0.4 mg/kg body weight and day in a further study. Assuming a respiratory volume of 10 m³ inhaled over eight hours and a body weight of 70 kg, a dose of 0.3 mg/kg body weight and day corresponds to a TBTO concentration in the air of 2.1 mg/m³ in humans. In relation to tin, this means 0.84 mg/m³. The difference to a MAK value of 0.02 mg/m³ is thus sufficiently great. The difference to the teratogenic effects of DBTC (at 5 mg/kg body weight and day, corresponding to 35 mg/m³ DBTC, and thus to a tin concentration of 13.67 mg/m³) is also sufficient. n-Butyltin compounds are therefore classified in pregnancy group C. No data are available on the dermal absorption of n-butyltin compounds in humans. With a MAK value for tin of 0.02 mg/m³, taking into account both local and systemic effects, 0.2 mg tin are taken up by inhalation per working day assuming 100% absorption. In studies with experimental animals, dermal penetration of TBTO was much higher than that found in vitro, whereby the irritant effect presumably accelerated penetration. Even under the assumption that the amount of the substance penetrating the human skin determined in vitro is more representative, and no irritant effect was induced, the tin absorbed after exposure to TBTO over a skin surface of 2000 cm² for one hour is so high at 0.56 mg that the dermally absorbed amount exceeds the amount absorbed by inhalation when the MAK value is observed. Similar conditions apply in the case of DBTC. Therefore, n-butyltin compounds are designated with an "H".

Data on sensitization in humans are not available. The available animal investigations are not sufficient to confirm a contact or respiratory sensitization for n-butyltin compounds. Therefore, n-butyltin compounds are not designated with "Sa" or "Sh". However, for n-butyltin compounds whose organic ligands have already been designated with "Sa" or "Sh", these designations also apply.

References

- ACGIH (American Conference of Governmental and Industrial Hygienists) (2001) Tin, organic compounds. Documentation of the threshold limit values and biological exposure indices, ACGIH, Cincinnati, OH, USA

- Ade T, Zaucke F, Krug HF (1996) The structure of organometals determines cytotoxicity and alteration of calcium homeostasis in HL-60 cells. *Fresenius J Anal Chem* 354: 609–614
- Adeeko A, Li D, Forsyth DS, Casey V, Cooke GM, Barthelemy J, Cyr DG, Trasler JM, Robaire B, Haies BF (2003) Effects of in utero tributyltin chloride exposure in the rat on pregnancy outcome. *Toxicol Sci* 74: 407–415
- Alam MS, Husain R, Srivastava SP, Seth PK (1988) Age and sex related behavioral changes induced by dibutyltin dilaurate in rats. *Bull Environ Contam Toxicol* 50: 371–377
- Alam MS, Husain R, Seth PK, Srivastava SP (1993) Influence of di-butyltin dilaurate on brain neurotransmitter system and behavior in rats. *Arch Toxicol* 61: 286–292
- Aldridge WN, Cremer JE (1955) The biochemistry of organo-tin compounds. *Biochem J* 61: 406–418
- Aldridge WN, Casida JE, Fish RH, Kimmel EC, Street BW (1977) Action on mitochondria and toxicity of metabolites of tri-*n*-butyltin derivatives. *Biochem Pharmacol* 26: 1997–2000
- Al-Ghais SM, Ali B (1999) Inhibition of glutathion S-transferase catalysed xenobiotic detoxification by organotin compounds in tropical fish tissues. *Bull Environ Contam Toxicol* 62: 207–213
- Aluoch A, Whalen M (2005) Tributyltin-induced effects on MAP kinases p38 and p44/42 in human natural killer cells. *Toxicology* 209: 263–277
- Aluoch A, Odman-Ghazi SO, Whalen MM (2007) Pattern of MAP kinase p44/42 and JNK activation by non-lethal doses of tributyltin in human natural killer cells. *Arch Toxicol* 81: 271–277
- Atochem North America Inc (1991) Initial Submission. Dibutyl tin chloride – assessment of clastogenic action on bone marrow erythrocytes in the micronucleus test. Report No 91/0357, Auftragsinstitut: Life Science Research Ltd, unpublished
- Attahiru US, Iyaniwura TT, Adaodi AO, Bonire JJ (1991) Acute toxicity studies of tri-*n*-butyltin and triphenyltin acetates in rats. *Vet Hum Toxicol* 33: 554–556
- Aw TY, Nicotera P, Manzo L, Orrenius S (1990) Tributyltin stimulates apoptosis in rat thymocytes. *Arch Biochem Biophys* 283: 46–50
- Aylett BJ (1979) The main group elements. In: Coates GE, Ayett BJ, Green MLH, Mingos DMP, Wade K (Eds.) *Organometallic Compounds*. 4th Edition, Vol. 1, Chapman and Hall, New York
- Barnes JM, Magee PN (1958) The biliary and hepatic lesions produced experimentally by dibutyltin salts. *J Pathol Bacteriol* 75: 267–279
- Baroncelli S, Karrer D, Turillazzi PG (1990) Embryotoxic evaluation of bis(tri-*n*-butyltin)oxide (TBTO) in mice. *Toxicol Lett* 50: 257–262
- Baroncelli S, Karrer D, Turillazzi PG (1995) Oral bis(tri-*n*-butyltin) oxide in pregnant mice. I. Potential influence of maternal behavior on postnatal mortality. *J Toxicol Environ Health* 46: 355–367
- Beliles RP (1994) The metals. In: Clayton GD, Clayton FE (Eds.) *Patty's industrial hygiene and toxicology*, Vol. II, Part C, John Wiley & Sons, New York, 2270–2275
- Boraiko C, Batt J (2005) Evaluation of employee exposure to organic tin compounds used as stabilizers at PVC processing facilities. *J Occup Environ Hyg* 2: 73–76
- Bressa G, Hinton RH, Price SC, Isbir M, Ahmed RS, Grasso P (1991) Immunotoxicity of tri-*n*-butyltin oxide (TBTO) and tri-*n*-butyltin chloride (TBTC) in the rat. *J Appl Toxicol* 11: 397–402
- BUA (1988) Tributylzinnoxid, BUA Bericht 36 (Tributyltin oxide, BUA / Federal Environment Agency, Report 36) (German), VCH, Weinheim

- BUA (2003) Tributylzinnoxid, BUA Bericht 238 (Ergänzungsberichte IX) (Tributyltin oxide, BUA / Federal Environment Agency, Report 36, Supplementary Reports IX) (German), Hirzel, Stuttgart
- Buckiova D, Dostal M, Hofmannova V (1992) Embryotoxicity of organotin. *Reprod Toxicol* 6: 178–179
- Calley DJ, Guess WL, Autian J (1967) Hepatotoxicity of a series of organotin esters. *J Pharm Sci* 56: 240–243
- Chow SK, Orrenius S (1994) Rapid cytoskeleton modification in thymocytes induced by immunotoxicant tributyltin. *Toxicol Appl Pharmacol* 127: 19–26
- Cima F, Craig PJ, Harrington C (2003) Organotin compounds in the environment. In: Organometallic compounds in the environment. PJ Craig Ed., John Wiley & Sons Ltd, West Sussex, UK
- Chikahisa L, Oyama Y (1992) Tri-n-butyltin increases intracellular Ca^{2+} in mouse thymocytes: a flow-cytometric study using fluorescent dyes for membrane potential and intracellular Ca^{2+} . *Pharmacol Toxicol* 71: 190–195.
- Cooke GM (2002) Effects of organotin on human aromatase activity *in vitro*. *Toxicol Lett* 126: 121–130
- Cooke GM, Tryphonas H, Pulido O, Caldwell D, Bondy GS, Forsyth D (2004) Oral (gavage), in utero and postnatal exposure of Sprague Dawley rats to low doses of tributyltin chloride. Part I: Toxicology, histopathology and clinical chemistry. *Food Chem Toxicol* 42: 211–220
- Crofton KM, Dean KE, Boncek VM, Rosen MB, Sheets LP, Chernoff N, Reiter LW (1989) Prenatal or postnatal exposure to bis(tri-n-butyltin)oxide in the rat: postnatal evaluation of teratology and behavior. *Toxicol Appl Pharmacol* 97: 113–123
- Davis A, Barale R, Brun G, Forster R, Gunther T, Hautefeuille H, van der Heijden CA, Knaap AG, Krowke R, Kuroki T, Loprieno N, Malaveille C, Merker HK, Monaco M, Mosesso P, Neubert D, Norppa H, Sorsa M, Vogel E, Voogd CE, Umeda M, Bartsch H (1987) Evaluation of the genetic and embryotoxic effects of bis(tri-n-butyltin)oxide (TBTO), a broad-spectrum pesticide, in multiple *in vivo* and *in vitro* short-term tests. *Mutat Res* 188: 65–95
- ECB (European Chemicals Bureau) (2000) Tetrabutyltin. IUCILID dataset, 18.02.2000, ECB, Ispra, Italien
- Elsea JR, Paynter OE (1958) Toxicological studies on bis(tri-n-butyltin) oxide. *AMA Arch Ind Health* 18: 214–217
- Ema M, Harazono A (2000) Adverse effects of dibutyltin dichloride on initiation and maintenance of rat pregnancy. *Reprod Toxicol* 14: 451–456
- Ema M, Harazono A (2001) Toxic effects of butyltin trichloride during early pregnancy in rats. *Toxicol Lett* 125: 99–106
- Ema M, Itami T, Kawasaki H (1991) Teratogenicity of di-n-butyltin dichloride in rats. *Toxicol Lett* 58: 347–356
- Ema M, Itami T, Kawasaki H (1992) Susceptible period for the teratogenicity of di-n-butyltin dichloride in rats. *Toxicol* 73: 81–92
- Ema M, Kurosaka R, Amano H, Ogawa Y (1995 a) Comparative developmental toxicity of butyltin trichloride, dibutyltin dichloride and tributyltin chloride in rats. *J Appl Toxicol* 15: 297–302
- Ema M, Kurosaka R, Amano H, Ogawa Y (1995 b) Further evaluation of the developmental toxicity of tributyltin chloride in rats. *Toxicology* 96: 195–201
- Ema M, Kurosaka R, Amano H, Ogawa Y (1995 c) Comparative developmental toxicity of butyltin trichloride, dibutyltin dichloride and tributyltin chloride in rats. *J Appl Toxicol* 15: 297–302

- Ema M, Kurosaka R, Amano H, Ogawa Y (1996) Comparative developmental toxicity of di-, tri-, and tetrabutyltin compounds after administration during late organogenesis in rats. *J Appl Toxicol* 16: 71–76
- Ema M, Harazono A, Miyawaki E, Ogawa Y (1997) Effect of the day of administration on the developmental toxicity of tributyltin chloride in rats. *Arch Environ Contam Toxicol* 33: 90–96
- Evans WH, Cardarelli NF, Smith DJ (1979) Accumulation and excretion of [^{1-14}C]bis(tri-*n*-butyltin) oxide in mice. *J Toxicol Environ Health* 5: 871–877
- Faqi AS, Schweinfurth H, Chahoud I (1997) Determination of the no-effect dose of bis(tri-*n*-butyltin)oxide (TBTO) for maternal toxicity and teratogenicity in mice. *Congenit Anom* 37: 251–258
- Farr CH, Reinisch K, Holson JF, Neubert D (2001) Potential teratogenicity of di-*n*-butyltin dichloride and other dibutyltin compounds. *Teratog Carcinog Mutagen* 21: 405–415
- Fish RH, Kimmel EC, Casida JE (1975) Bioorganotin chemistry: biological oxidation of tributyltin derivatives. *J Organometallic Chem* 93, C1–C4
- Galli CL, Viviani B, Marinovich M (1993) Cell cultures: a tool for the study of mechanisms of toxicity. *Toxicol In Vitro* 7: 559–568
- Gammeltoft M (1978) Tributyltin oxide is not allergenic. *Contact Dermatitis* 4: 238
- Gaunt IF, Colley J, Grasso P, Creasey M, Gangolli SD (1968) Acute and short-term toxicity studies on di-*n*-butyltin dichloride in rats. *Food Cosmet Toxicol* 6: 599–608
- Gennari A, Potters M, Seinen W, Pieters R (1997) Organotin-induced apoptosis as observed in vitro is not relevant for induction of thymus atrophy at antiproliferative doses. *Toxicol Appl Pharmacol* 147: 259–266
- Gennari A, Bol M, Seinen W, Penninks A, Pieters R (2002 a) Organotin-induced apoptosis occurs in small CD4(+)CD(+) thymocytes and is accompanied by an increase in RNA synthesis. *Toxicology* 175: 191–200
- Gennari A, Bleumink R, Viviani B, Galli CL, Marinovich M, Pieters R, Corsini E (2002 b) Identification by DNA microarray of nur77 as a gene induced by di-*n*-butyltin dichloride: its role in organotin-induced apoptosis. *Toxicol Appl Pharmacol* 181: 27–31
- Gogvadze V, Stridh H, Orrenius S, Cotgreave I (2002) Tributyltin causes cytochrome c release from isolated mitochondria by two discrete mechanisms. *Biochem Biophys Res Commun* 292: 904–908
- Haiduc I, Zuckerman JJ (1985) Basic organometallic chemistry. Walter de Gruyter, Berlin
- Hamasaki T, Sato T, Nagase H, Kito H (1992) The genotoxicity of organotin compounds in SOS chromotest and rec-assay. *Mutat Res* 280: 195–203
- Hamasaki T, Sato T, Nagase H, Kito H (1993) The mutagenicity of organotin compounds as environmental pollutants. *Mutat Res* 300: 265–271
- Harazono A, Ema M (2003) Suppression of decidual cell response induced by dibutyltin dichloride in pseudopregnant rats: as a cause of early embryonic loss. *Reprod Toxicol* 17: 393–399
- Harazono A, Ema M, Ogawa Y (1996) Pre-implantation embryonic loss induced by tributyltin chloride in rats *Toxicology Letters* 89: 185–190
- Harazono A, Ema M, Ogawa Y (1998) Evaluation of early embryonic loss induced by tributyltin chloride in rats: phase- and dose-dependent antifertility effects. *Arch Environ Contam Toxicol* 34: 94–99
- Heidrich DD, Steckelbroeck S, Klingmüller (2001) Inhibition of human cytochrome P450 aromatase activity by butyltins. *Steroids* 66: 763–769
- Hennighausen G, Lange P, Merkord J (1980) The relationship between the length of the alkyl chain of dialkyltin compounds and their effects on thymus and bile ducts in mice. *Arch Toxicol Suppl* 4: 175–178

- Hümpel M, Kühne G, Täuber U, Schulze PE (1987) Studies on the kinetics of bis(tris-n-butyl-¹¹³tin) oxide. In: Toxicology and analytics of the tributyltins – the present status. ORTEP Association, Vlissingen-Oost, NL, 122–136
- Ishaaya I, Engel JL, Casida JE (1976) Dietary triorganotins affect lymphatic tissues and blood composition of mice. *Pestic Biochem Physiol* 6: 270–279
- Ishizaka T, Suzuki T, Saito Y (1989) Metabolism of dibutyltin dichloride in rats. *J Agric Food Chem* 37: 1096–1101
- Itami T, Ema M, Amano H, Murai T, Kawasaki H (1990) Teratogenic evaluation of tributyltin chloride in rats following oral exposure. *Drug Chem Toxicol* 13: 283–295
- Iwai H, Wada O, Arakawa Y (1981) Determination of tri-, di-, and monobutyltin and inorganic tin in biological materials and some aspects of their metabolism in rats. *J Anal Toxicol* 5: 300–306
- Jensen KG, Andersen O, Rønne M (1989) Spindle-inhibiting effects of organotin compounds. II. Induction of chromosomal supercontraction by di- and tri-alkyl and -aryl compounds. *Appl Organometal Chem* 3: 225–229
- Jensen KG, Önfelt A, Wallin M, Lidums V, Andersen O (1991 a) Effects of organotin compounds on mitosis, spindle structure, toxicity and *in vitro* microtubule assembly. *Mutagenesis* 6: 409–416
- Jensen KG, Andersen O, Rønne M (1991 b) Organotin compounds induce aneuploidy in human peripheral lymphocytes *in vitro*. *Mutat Res* 246: 109–112
- Karrer D, Baroncelli S, Turillazzi PG (1995) Oral bis(tri-n-butyltin) oxide in pregnant mice. II. Alterations in hematological parameters. *J Toxicol Environ Health* 46: 369–377
- Kawanishi T, Kiuchi T, Asoh H, Shibayama R, Kawai H, Ohata H, Momose K, Hayakawa T (2001) Effect of tributyltin chloride on the release of calcium ion from intracellular calcium stores in rat hepatocytes. *Biochem Pharmacol* 62: 863–872
- Kimmel EC, Fish RH, Casida JE (1977) Bioorganotin chemistry. Metabolism of organotin compounds in microsomal monooxygenase systems and in mammals. *J Agric Food Chem* 25: 1–9
- Krajnc EI, Wester PW, Loeber JG, van Leeuwen FXR, Vos JG, Vaessen HAMG, van der Heijden CA (1984) Toxicity of bis(tri-n-butyltin)oxide in the rat. I. Short-term effects on general parameters and on the endocrine and lymphoid systems. *Toxicol Appl Pharmacol* 75: 363–386
- Kishta O, Adeeko A, Li D, Luu T, Brawer JR, Morales C, Hermo L, Robaire B, Haies BF, Barthelmy J, Cyr DG, Trasler JM (2007) In utero exposure to tributyltin chloride differentially alters male and female fetal gonad morphology and gene expression profiles in the Sprague-Dawley rat. *Reprod Toxicol* 23: 1–11
- Kumasaka K, Miyazawa M, Fujimaki T, Tao H, Ramaswamy BR, Nakazawa H, Makino T, Satoh S (2002) Toxicity of the tributyltin compound on the testis in premature mice. *J Reprod Dev* 48: 591–597
- Li AP, Dahl AR, Hill JO (1982) In vitro cytotoxicity and genotoxicity of dibutyltin dichloride and dibutylgermanium dichloride. *Toxicol Appl Pharmacol* 64: 482–485
- van Loveren H, Krajnc EI, Rombout PJA, Blommaert FA, Vos JG (1990) Effects of ozone, hexachlorobenzene, and bis(tri-n-butyltin)oxide on natural killer activity in the rat lung. *Toxicol Appl Pharmacol* 102: 21–33
- Matsuda R, Suzuki T, Saito Y (1993) Metabolism of tri-n-butyltin chloride in male rats. *J Agric Food Chem* 41: 489–495
- Meyer CR, Buncher CR, Gioscia R, Dees J (1987) Oceans '87 Proceedings, Vol. 4: 1432, International Organotin Symposium, Halifax, Nova Scotia, Canada, Sept. 28–Oct. 1, 1987, The IEEE Service Center, Piscataway, NJ, and The Marine Technology Society, Washington, DC, USA

- Mori Y, Iesato K, Ueda S, Mori T, Iwasaki I, Ohnishi K, Seino Y, Wakashin Y, Wakashin M, Okuda K (1984) Renal tubular disturbances induced by tributyl-tin oxide in guinea pigs: a secondary Fanconi syndrome. *Clin Nephrol* 21: 118–125
- Mushtaq M, Mukhtar H, Datta KK, Tandon SG, Seth PK (1981) Toxicological studies of a leachable stabilizer di-*n*-butyltin dilaurate (DBTL): effects on hepatic drug metabolizing enzyme activities. *Drug Chem Toxicol* 4: 75–88
- Nakanishi T, Kohroki J, Suzuki S, Ishizaki J, Hiromori Y, Takasuga S, Itoh N, Watanabe Y, Utoguchi N, Tanaka K (2002) Trialkyltin compounds enhance human CG secretion and aromatase activity in human placental choriocarcinoma cells. *J Clin Endocrinol Metab* 87: 2830–2837
- Nakatsu Y, Kotake Y, Ohta S (2006) Tributyltin-induced cell death is mediated by calpain in PC12 cells. *Neurotoxicology* 27: 587–593
- Nakatsu Y, Kotake Y, Ohta S (2007) Concentration dependence of the mechanism of tributyltin-induced apoptosis. *Toxicol Sci* 97: 438–447
- NCI (National Cancer Institute) (1978) Bioassay of dibutyltin diacetate for possible carcinogenicity. NCI-CG-TR-183, Bethesda MD, USA
- Nielsen JB, Rasmussen TH (2004) Antiproliferative effect of butyltin in MCF-7 cells. *Environ Res* 96: 305–310
- Noda T, Morita S, Baba A (1993) Teratogenic effects of various di-*n*-butyltins with different anions and butyl(3-hydroxybutyl)tin dilaurate in rats. *Toxicology* 85: 149–160
- Noda T, Morita S, Yamano T, Shimizu M, Nakamura T, Saitoh M, Yamada A (1991) Teratogenicity study of tri-*n*-butyltin acetate in rats by oral administration. *Toxicol Lett* 55: 109–115
- Noda T, Yamano T, Shimizu M, Saitoh M, Nakamura T, Yamada A, Morita S (1992) Comparative teratogenicity of di-*n*-butyltin diacetate with *n*-butyltrichloride in rats. *Arch Environ Contam Toxicol* 23: 216–222
- OECD (Organisation of Economic Co-operation and Development) (2006 a) Monobutyltin chloride and selected esters. OECD SIDS Initial Assessment Report, Final draft, OECD, Paris, FR
- OECD (Organisation of Economic Co-operation and Development) (2006 b) Dibutyltin dichloride and selected thioesters and catalysts. OECD SIDS Initial Assessment Report, Final draft, OECD, Paris, FR
- Ogata R, Omura M, Shimasaki Y, Kubo K, Oshima Y, Aou S, Inoue N (2001) Two-generation reproductive toxicity study of tributyltin chloride in female rats. *J Toxicol Environ Health* 63: 127–144
- Omura M, Ogata R, Kubo K, Shimasaki K, Aou S, Oshima Y, Tanaka A, Hirata M, Makita Y, Inoue N (2001) Two-generation reproductive toxicity study of tributyltin chloride in male rats. *Toxicol Sci* 64: 224–232
- ORTEP (Organotin Environmental Programme) (2003 a) Tributylchlorostannane [CAS No. 1461-22-9]: micronucleus test in bone marrow cells of mice. TNO Report V 4404/02, 16. July 2003, unpublished, Zeist, NL
- ORTEP (Organotin Environmental Programme) (2003 b) Tetrabutylstannane [CAS No. 1461-25-2]: micronucleus test in bone marrow cells of mice. TNO Report V 4404/04, 2. April 2003, unpublished, Zeist, NL
- ORTEP (Organotin Environmental Programme) (2004) Tetrabutylstannane [CAS No. 1461-25-2]: Combined oral repeated dose toxicity with the reproduction/developmental toxicity screening test in rats. Volumes I and II, TNO Report V 4904, 29. January 2004, unpublished, Zeist, NL

- Oyama Y, Ueha T, Hayashi A, Chikahisa L (1994) Effect of tri-n-butyltin on intracellular Ca^{2+} concentration of mouse thymocytes under Ca^{2+} -free condition. *Eur J Pharmacol Environ Toxicol* 270: 137–142
- Parametrix Inc (2006 a) Dibutyltin dichloride. IUCLID data set, 13.10.2000, update 24.07.2006, prepared for the Organotin Environmental Programme (ORTEP), Bellevue, WA, USA
- Parametrix Inc (2006 b) 2-Ethylhexyl-4,4-dibutyl-10-ethyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate. IUCLID data set, 13.10.2000, update 24.07.2006, prepared for the Organotin Environmental Programme (ORTEP), Bellevue, WA, USA
- Parametrix Inc (2006 c) Dibutyltin dilaurate. IUCLID dataset, 13.10.2000, update 24.07.2006, prepared for the Organotin Environmental Programme (ORTEP), Bellevue, WA, USA
- Parametrix Inc (2006 d) Dibutyltin maleate. IUCLID data set, 13.10.2000, update 24.07.2006, prepared for the Organotin Environmental Programme (ORTEP), Bellevue, WA, USA
- Parametrix Inc (2006 e) Dibutyltin oxide. IUCLID data set, 13.10.2000, update 24.07.2006, prepared for the Organotin Environmental Programme (ORTEP), Bellevue, WA, USA
- Parametrix Inc (2006 f) n-Butyltin trichloride. IUCLID data set 13.10.2000, update IUCLID 24.07.2006, prepared for the Organotin Environmental Programme (ORTEP), Bellevue, WA, USA
- Parametrix Inc (2006 g) 2-Ethylhexyl-4-butyl-10-ethyl-4-[[2-[(2-ethylhexyl)oxy]-2-oxoethyl]thio]-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate. IUCLID data set, 13.10.2000, update 24.07.2006, prepared for the Organotin Environmental Programme (ORTEP), Bellevue, WA, USA
- Parametrix Inc (2006 h) Tributyltin chloride. IUCLID data set, 13.10.2000, update 24.07.2006, prepared for the Organotin Environmental Programme (ORTEP), Bellevue, WA, USA
- Parametrix Inc (2006 i) Diisooctyl-2,2'-[[dibutylstannylene]bis(thio)] diacetate. IUCLID data set, 13.10.2000, update 24.07.2006, prepared for the Organotin Environmental Programme (ORTEP), Bellevue, WA, USA
- Parametrix Inc (2006 j) Tetrabutyltin. IUCLID data set, 13.10.2000, update 21.08.2006, prepared for the Organotin Environmental Programme (ORTEP), Bellevue, WA, USA
- Parfett CL, Pilon R (1993) Tri-n-butyltin chloride promotes morphological transformation and induces proliferin expression in C3H10T1/2 cells. *Cancer Lett* 71: 167–176
- Parfett CL, Marquardt T, Pilon R (2000) Promotion of morphological transformation by di-n-butyl chloride in C3H/10T1/2 cells: prediction by prior expression of tumour promoter-responsive genes. *Food Chem Toxicol* 38: 339–349
- Pelikan Z (1969) Effects of bis(tri-n-butyltin) oxide on the eyes of rabbits. *Br J Ind Med* 26: 165–170
- Pelikan Z, Cerny (1968 a) Die toxische Wirkung von Tri-n-butylzinn-Verbindungen auf weiße Mäuse (The toxic effects of tri-n-butyltin compounds on white mice) (German). *Arch Toxicol* 23: 283–292
- Pelikan Z, Cerny E (1968 b) The effect of low doses of bis-(tri-n-butyl-tin)-oxide on the skin of rats. *Berufsdermatosen* 16: 340–349
- Penninks AH, Seinen W (1982) Comparative toxicity of alkyltin and estertin stabilizers. *Food Chem Toxicol* 20: 909–916
- Penninks AH, Seinen W (1984) Mechanisms of dialkyltin immunopathology. *Vet Q* 6: 209–215
- Pieters RHH, Bol M, Ariens T, Punt P, Seinen W, Bloksma N, Penninks AH (1994) Selective inhibition of immature CD4- CD8+ thymocyte proliferation, but not differentiation, by the thymus atrophy-inducing compound di-n-butyltin dichloride. *Immunology* 81: 261–277
- Raffray M, Cohen GM (1991) Bis(tri-n-butyltin) oxide induces programmed cell death (apoptosis) in immature rat thymocytes. *Arch Toxicol* 65: 135–139

- Raffray M, Cohen GM (1993) Thymocyte apoptosis as a mechanism for tributyltin-induced thymic atrophy *in vivo*. *Toxicology* 67: 231–236
- Rao KSP, Chetty SC, Desai D (1987) Effects of tricyclohexylhydroxytin on the kinetics of adenosine triphosphatase system and protection by thiol reagents. *J Biochem Toxicol* 2: 125–140
- Reimann R, Lang R (1987) Mutagenicity studies with tributyltin compounds. In: Toxicology and analytics of the tributyltins – the present status. ORTEP Association, Vlissingen-Oost, NL, 66–90
- Römer HC, Golka K, Degen GH (2002) Tributylzinn-Verbindungen: endokrin wirksame Schadstoffe beim Menschen? (Tributyltin compounds: Endocrine disruptors in humans) In: Bolt HM, Griefahn B, Heuer H (Hrsg/Publ.) Arbeitsphysiologie heute (Occupational Physiology Today) (German), Band/Volume 4, IfADo, Dortmund
- Rosenberg DW, Anderson KE, Kappas A (1984) The potent induction of intestinal heme oxygenase by the organotin compound bis(tri-*n*-butyltin) oxide. *Biochem Biophys Res Commun* 119: 1022–1027
- RPA (Risk & Policy Analysts) (2005) Risk assessment on targeted consumer applications of certain organotin compounds. Final report prepared for the European Commission, Norfolk, UK
- Saitoh M, Yanase T, Morinaga H, Tanabe M, Mu YM, Nishi Y, Nomura M, Okabe T, Goto K, Takayanagi R, Nawata H (2001) Tributyltin or triphenyltin inhibits aromatase activity in the human granulosa-like tumor cell line KGN. *Biochem Biophys Res Commun* 289: 198–204
- de Santiago A, Aguilar-Santelises M (1999) Organotin compounds decrease *in vitro* survival, proliferation and differentiation of normal human B lymphocytes. *Hum Exp Toxicol* 18: 619–624
- Santroni A, Fedeli D, Gabbianelli R, Zolese G, Falcioni G (1997) Effect of organotin compounds on trout hemoglobins. *Biochem Biophys Res Commun* 238: 301–304
- Schering AG (1983) Repeated dose inhalation study of ZK 21.955 in the rat for 29–32 days (21–24 exposures). Pharma Research Report, IC 1/83, Schering AG, Berlin, 17.1.1983, unpublished
- Schering AG (1986) Re-evaluation of a “mouse micronucleus test on TBTO”; a confidential report of the Institute of Occupational Health, Helsinki, Finland, dated Sept. 30, 1984. Pharma Research Report, IC 7/86, Schering AG, Berlin, 21.11.1986, unpublished
- Schering AG (1988) ZK 22.688 (Tributyltin naphthenate). Systemic toxicity study in rats with daily oral (dietary) administration over 28–32 days. Pharma Research Report, IC 35/88, Schering AG, Berlin, 11.11.1988, unveröffentlicht
- Schering AG (1992) Bis(tri-*n*-butyltin)oxide (TBTO; ZK21.955): Twelve-month chronic oral toxicity study in beagle dogs. Research Report, IC 7/91, Schering AG, Berlin, 27. August 1992
- Schüürmann G, Marken B (Eds) Ecotoxicology – ecotoxicological fundamentals, chemical exposure and biological effects. John Wiley & Sons Inc., New York, USA, 665–749
- Schweinfurth H (1985) Toxicology of tributyltin compounds. Tin and its uses. No 143: 9–12, Schering AG, Berlin
- Schweinfurth H (1987) The tributyltin compounds – a review of their toxicity. In: Toxicology and analytics of the tributyltins – the present Status. ORTEP Association, Vlissingen-Oost, NL, 14–34
- Seinen W, Vos JG, van Spanje I, Snoek M, Brands R, Hooykaas H (1977 a) 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. *Toxicol Appl Pharmacol* 42: 197–212
- Seinen W, Vos JG, van Krieken R, Penninks A, Brands R, Hooykaas H (1977 b) Toxicity of organotin compounds. III. Suppression of thymus-dependent immunity in rats by di-*n*-butyltin-dichloride and di-*n*-octyltin-dichloride. *Toxicol Appl Pharmacol* 42: 213–224.

- Sheldon AW (1975) Effect of organotin anti-fouling coatings on man and his environment. *J Paint Technol* 47: 54–58
- Snoeijs NJ, van Iersel AAJ, Penninks AH, Seinen W (1985) Toxicity of triorganotin compounds: comparative in vivo studies with a series of trialkyltin compounds and triphenyltin chloride in male rats. *Toxicol Appl Pharmacol* 81: 274–286
- Snoeijs NJ, Penninks AH, Seinen W (1988) Dibutyltin and tributyltin compounds induce thymus atrophy in rats due to a selective action on thymic lymphoblasts. *Int J Immunopharmacol* 10: 891–899
- SRC (Syracuse Research Corporation) (2007)
<http://www.syrres.com/esc/physdemo.htm>
- Stridh H, Gilgiotti D, Orrenius S, Cotgreave I (1999 a) The role of calcium in pre- and postmitochondrial events in tributyltin-induced T-cell apoptosis. *Biochem Biophys Res Commun* 266: 460–465
- Stridh H, Orrenius S, Hampton MB (1999 b) Caspase involvement in the induction of apoptosis by three environmental toxicants tributyltin and triphenyltin. *Toxicol Appl Pharmacol* 156: 141–146
- Subramoniam A, Husain R, Seth PK (1991) Reduction of phosphoinositides and diacylglycerol levels in repeatedly dibutyltin-dilaurate-treated rat brain. *Toxicol Lett* 57: 245–250
- Summer KH, Klein D, Greim H (2003) Ecological and toxicological aspects of mono- and disubstituted methyl-, butyl-, octyl-, and dodecyltin compounds – Update 2002 – Organotin Environmental Programme (ORTEP) Association, Bellevue, WA, USA
- Taketa F, Siebenlist K, Kasten-Jolly J, Palosaari N (1980) Interaction of triethyltin with cat hemoglobin: identification of binding sites and effects on hemoglobin function. *Arch Biochem Biophys* 203: 466–472
- Tan LP, Ng ML, Kumar Das VG (1978) The effect of trialkyltin compounds on tubulin Polymerisation. *J Neurochem* 31: 1035–1041
- Thomas LD, Shah H, Green SA, Brakhurst AD, Whalen MM (2004) Tributyltin exposure causes decreased granzyme B and perforin levels in human natural killer cells. *Toxicology* 200: 221–233
- Tischler AS, Ruzicka LA, Donahue SR, DeLellis RA (1989) Chromaffin cell proliferation in the adult rat adrenal medulla. *Int J Dev Neurosci* 7: 439–448
- Tischler AS, Powers JF, Shahsavari M, Ziar J, Tsokas P, Downing J, McClain RM (1997) Comparative studies of chromaffin cell proliferation in the adrenal medulla of rats and mice. *Fundam Appl Toxicol* 35: 216–220
- Tryphonas H, Cooke G, Caldwell D, Bondy D, Parenteau M, Hayward S, Pulido O (2004) Oral (gavage), in utero and post-natal exposure of Sprague-Dawley rats to low doses of tributyltin chloride: Part II: effects on the immune system. *Food Chem Toxicol* 42: 221–235
- TSA (Tin Stabilizer Association) (2003 a) Dibutyltin dichloride: in vitro absorption through human and rat epidermis. Central Toxicology Laboratory, CTL/JV1698/Regulatory/Report, 9 January 2003, Philadelphia, PA, USA, unpublished
- TSA (Tin Stabilizer Association) (2003 b) Dibutyltin bis(2-ethylhexylmercaptoacetate): in vitro absorption through human and rat epidermis. Central Toxicology Laboratory, CTL/JV1699/Regulatory/Report, 8 January 2003, Philadelphia, PA, USA, unpublished
- Ueno S, Susa N, Furukawa Y, Sugiyama M (1994) Comparison of hepatotoxicity caused by mono-, di- and tributyltin compounds in mice. *Arch Toxicol* 69: 30–34
- Ueno S, Suzuki T, Susa N, Furukawa Y, Sugiyama M (1997) Effect of SKF-525A on liver metabolism and hepatotoxicity of tri- and dibutyltin compounds in mice. *Arch Toxicol* 71: 513–518

- US EPA (US Environmental Protection Agency) (1997) Tributyltin oxide (TBTO). Washington DC, USA,
<http://www.epa.gov/iris/subst/0349.htm>
- Verdier F, Virat M, Schweinfurth H, Descotes J (1991) Immunotoxicity of bis(tri-*n*-butyltin) oxide in the rat. *J Toxicol Environ Health* 32: 307–317
- Vos JG, De Klerk A, Krajnc EI, Kruizinga W, van Ommen B, Rozing J (1984) Toxicity of bis(tri-*n*-butyltin)oxide in the rat. *Toxicol Appl Pharmacol* 75: 387–408
- Vos JG, De Klerk A, Krajnc EI, van Loveren H, Rozing J (1990) Immunotoxicity of bis(tri-*n*-butyltin) oxide in the rat: Effects on thymus-dependent immunity and on nonspecific resistance following long-term exposure in young versus aged rats. *Toxicol Appl Pharmacol* 105: 144–155
- Wester PW, Krajnc EI, van Leeuwen FXR, Loeber JG, van der Heijden CA, Vaessen HAMG, Helleman PW (1990) Chronic toxicity and carcinogenicity of bis(tri-*n*-butyltin)oxide (TBTO) in the rat. *Food Chem Toxicol* 28: 179–196
- Whalen MM, Loganathan BG, Kannan K (1999) Immunotoxicity of environmentally relevant concentrations of butyltins on human natural killer cells *in vitro*. *Environ Res* 81: 108–116
- Whalen MM, Ghazi S, Loganathan BG, Hatcher F (2002 a) Expression of CD16, CD18 and CD56 in tributyltin-exposed human natural killer cells. *Chem Biol Interact* 139: 159–176
- Whalen MM, Williams TB, Green SA, Loganathan BG (2002 b) Interleukins 2 and 12 produce recovery of cytotoxic function in tributyltin-exposed human natural killer cells. *Environ Res A* 88: 199–209
- WHO (World Health Organization) (1980) Tin and organic compounds: a preliminary review. IPCS – Environmental health criteria No. 15, WHO, Geneva, CH
- WHO (World Health Organization) (1990) Tributyltin Compounds. IPCS – Environmental health criteria No. 116, WHO, Geneva, CH
- WHO (World Health Organization) (1999) Tributyltin oxide. IPCS – Concise international chemical assessment document No. 14, WHO, Geneva, CH
- WHO (World Health Organization) (2005) Mono- and disubstituted methyltin, butyltin, and octyltin compounds. IPCS – Concise international chemical assessment document No. 73, WHO, Geneva, CH
- Woodruff RC, Mason JM, Valencia R, Zimmering S (1985) Chemical mutagenesis testing in *Drosophila*. V. Results of 53 coded compounds tested for the National Toxicology Program. *Environ Mutagen* 7: 677–702
- Wulf RG, Byington KH (1975) On the structure-activity relationships and mechanism of organotin induced, nonenergy dependent swelling of liver mitochondria. *Arch Biochem Biophys* 167: 176–185
- Yoshizuka M, Hara K, Haramaki N, Yokoyama M, Mori N, Doi Y, Kawahara A, Fujimoto S (1992) Studies on the hepatotoxicity induced by bis (tributyltin) oxide. *Arch Toxicol* 66: 182–187
- Yu ZP, Matsuoka M, Wispiyono B, Iryo Y, Igisu H (2000) Activation of mitogen activated protein kinases by tributyltin in CCRF-CEM cells: role of intracellular Ca^{2+} . *Toxicol Appl Pharmacol* 168: 200–207
- Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K, Speck W (1987) Salmonella Mutagenicity Tests: III. Results from the Testing of 255 Chemicals. *Environ Mutagen* 9: 1–110
- Zielinska D, Radecka H, Radecki J (2000) Contribution of membrane surface charge in the interaction of lead and tin derivatives with model lipid membrane. *Chemosphere* 40: 327–330

completed 27.06.2007