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Tributyltin compounds (TBT)

Evaluation of health hazards and proposal of health based quality criteria for soil and drinking water

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Title:

Tributyltin compounds (TBT). Evaluation of health hazards and proposal of health based quality criteria for soil and drinking water

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Content

Preface

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to tributyltin componds and a proposal of health based quality criteria for soil and drinking water. This resulted in 2007 in the present report, which was prepared by Anne Kirstine Müller, Elsa Nielsen and Ole Ladefoged, Department of Toxicology and Risk Assessment,
Danish Institute for Food and Veterinary Research, i.e. the present Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark.

The report has been subjected to review and discussion and has been endorsed by a steering committee consisting of representatives from the following Danish authorities:

The Danish Nature Agency,
The Danish Ministry of Food, Agriculture and Fisheries (The Faculty of Agricultural Sciences),
The Danish Veterinary and Food Administration,
The National Board of Health, Denmark,
Danish Regions (former Amternes Videncenter for Jordforurening),
The Danish Environmental Protection Agency.

The Danish Environmental Protection Agency
Copenhagen, December 2013.

1 General description

Organotin compounds consist of mono-, di-, tri- and tetra substituted compounds where the alkyl groups covalently bound to the tin atom can be e.g., methyl, ethyl, butyl and phenyl. The purpose of this evaluation is to give a review of the adverse health effects of tributyltin (TBT) compounds in order to propose a health based quality criterion for TBT in soil and water.

The term TBT covers several TBT-derivates and the chemical difference between the derivates is the nature of the 4th group linked to the tin atom. The following evaluation should ideally include all derivates. However, data only exist for a few of the derivates and TBT-oxide (TBTO) is the only TBT-compound investigated extensively. In the subsequent descriptions of experimental studies, the exact TBT-derivate e.g., TBTO used in the experiment will be specified if possible. In sections with more general considerations of TBT-compounds, the term TBT will be used.

As there are no quantitative data on human toxicity of TBT, the evaluation will focus on animal data and primarily be based on reviews and evaluations by EFSA (2004), ATSDR (2005), US-EPA (1997), WHO (1990) and WHO (1999).

Unless otherwise stated, the doses and concentrations of the TBT-substances are reported as the concentration of the TBT-substance and not as the concentration of tin (Sn). Only few times, has the concentration of TBT been expressed as concentration of Sn e.g., mg Sn/kg. The conversion factor from mg Sn to mg TBT (with no specification of the fourth group) is 2.44. However, the quality criterion for soil and drinking water is expressed as Sn in order to apply the criteria for all TBT compounds.

1.1 Identity and physical / chemical properties

Tributyltin (TBT) compounds are organic derivatives of tetravalent tin characterised by the covalent bonds between three carbon atoms and a tin atom. The general formula is $(n\text{-}C_4H_9)_3Sn\text{-}X$ where X is an anion e.g. Cl, F, OH, CO_3 or a group linked covalently through a heteroatom.

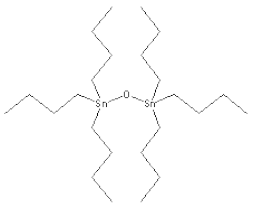
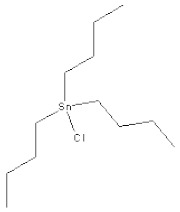
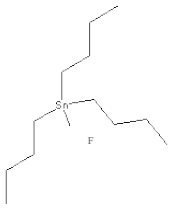
The nature of X influences the physical/chemical properties. In Table 1, identity and physico/chemical properties for some of the important – both industrially as well as toxicological - members of the group TBT-compounds are listed. Other TBT derivates include TBT-benzoate, -linoleate, -naphthenate, -phosphate and -metachrylate.

1.2 Production and use

No information regarding the production of TBT has been found.

TBT compounds have strong biocidal activity toward a range of aquatic organisms including bacteria, fungi, algae, molluscs and crustaceans. They have been used for various purposes e.g. antifouling paints for boats, preservative for wood and textiles, slimicides in industrial processes and on masonry and as molluscicides.

Table 1 Identity and physical/chemical properties of TBT compounds

	Tributyltin oxide	Tributyltin chloride	Tributyltin fluoride
Molecular formula	$C_{24}H_{54}OSn_2$	$C_{12}H_{27}ClSn$	$C_{12}H_{27}FSn$
Structural formula			
Molecular weight	596.11	325.49	309
CAS-no	56-35-9	1461-22-9	1983-10-4
Synonyms	TBTO, Hexabutyl distannoxane	TBTCl, Tibutyl-chloro stannane	TBTF, Tibutyl-fluoro stannane
Description	Liquid, slightly yellow	Liquid, colourless	-
Purity (%)	Typical >96	-	-
Melting point (°C)	<-45	-16	240
Boiling point (°C)	180 (at 2 mmHg)	145-147 (at 5 mmHg)	>350 (extrapolated)
Density (g/cm^3)	1.17 (at 25 °C)	1.2	1.25
Water solubility (mg/l)	4 (at 20 °C)	Insoluble in cold water	-
log$P_{o/w}$	3.19-3.84[2]	-	-
Vapour pressure (mmHg)	7.5 *10^{-6} (at 20 °C)	-	-
References	ATSDR (2005), WHO (1990)	ATSDR (2005), WHO (1990)	WHO (1990)

The extensive use of tri-substituted organotin compounds (OTC) including TBT as antifouling agents for boats which started in the 1960th has now been restricted in many countries due to the adverse effects of these compounds. The European Union (Regulation 782/2003/EC) requires that the application on ships of organotin compounds which act as biocides in antifouling systems are prohibited as from July, 2003 (EFSA 2004). Furthermore, according to the regulation as well as to a convention developed in IMO (International Maritime Organization, United Nations), from January 2008 ships that bear TBT are not allowed. The TBT should either be removed or enclosed.

Organotin compounds, mainly mono- and di- substituted organotins, are also used as stabilizers in e.g. polyvinyl chlorides. Therefore, they can be present in various consumer products e.g. textiles, polyurethane, plastic polymers and silicons. In investigations of organotin-compounds content in different consumer products made by the Danish Environmental Protection Agency mainly dibutyltin- and dioctyltin-compounds were found. However, TBT was also present in some of the products (MST 2001, 2003).

1.3 Environmental fate and occurrence

In general, TBT are released to the environment from anthropogenic sources. Use of antifouling paints, applied as a coat to the immersed sections of boats and floating structures, represents the major source of TBT to the coastal environment. TBT are slowly released from the paint to the water and create thereby an environment that repels the organisms that may attach to the immersed surfaces. The use of TBT for other purposes may also result in their release into the environment.

1.3.1 Air

Release of organotin compounds to air is not significant due to their low vapour pressure and photo degradation (ATSDR 2005). No monitoring data for concentrations of TBT in air have been found.

1.3.2 Water

As a result of its physical and chemical properties nearly all TBT found in natural waters is bound to suspended particles in the water or associated to dissolved organic matter. Under various conditions, different authors have estimated that 10 to 90% of TBTO introduced into water is adsorbed onto particles. Some of the factors on which the adsorption depends are temperature; salinity nature, size and amount of suspended particles and presence of dissolved organic matter in the water. As particles in the water column may settle out, TBT is removed from the water and introduced into the sediment. (WHO 1990).

Degradation of TBT involves breakage of the carbon-tin bond and both abiotic (photolysis and hydrolysis) and biotic degradation can occur. Hydrolysis only occurs under extreme pH conditions and is therefore barely evident under normal conditions in water. Photodegradation of TBT with the formation of DBT derivates has been shown in both field and laboratory experiments. However, the importance of photodegradation in the water varies considerably with varying environmental conditions e.g. turbidity, light and presence of photosensitising substances in the water. (ATSDR 2005, WHO 1990).

Microorganisms, notably bacteria but also microalgae and fungi, are capable of degrading TBT, both under aerobic and anaerobic conditions. The importance of anaerobic degradation is not clear; some consider the anaerobic degradation process as slow, others that it is more rapid and therefore important than aerobic degradation. As with the abiotic degradation, the biotic breakdown is based on the formation of intermediate hydroxylated derivates, progressive oxidative dealkylation founded on the splitting of the carbon-tin bond.
In addition to dealkylation of TBT, methylation of organotin by chemical and/or biological means may also occur. However, the contribution of methylation is not clear. The pathway may result in fully substituted and volatile tin compounds. (ATSDR 2005, WHO 1990).
Methylated butyltin compounds, such as tributylmethyltin, have been found in contaminated harbour sediments and surface waters (Amouroux et al. 2000 & Cooney 1988 – quoted in ATSDR 2005).

As for the other degradation processes, the rate of biodegradation depends on several environmental conditions such as temperature, oxygenation, pH, presence of mineral elements and easily degradable carbon sources and the nature of the microflora. In seawaters, biodegradation is considered most important especially in waters rich in suspended particles, whereas in more clean surface waters photolysis can exceed biodegradation. The half-life of TBT in the seawater varies depending on temperature, pH, turbidity and light; for primary degradation, it is generally estimated to range from 1 day to few weeks. (ATSDR 2005, WHO 1990, Madsen 1998).

In general, the concentration of TBT is higher in water collected close to marinas and areas with a high boat activity compared to more remote areas, close to fishnets and cages treated with TBT and near cooling systems. The concentration is highest in enclosed areas with poor water turnover and the concentration varies

during the season usually with higher concentrations during summer compared to winter. Higher concentrations of TBT have been measured in the surface microlayer than just below the surface.
Reported concentrations of TBT range from <0.5 to 1,800 ng/L in surface and bottom water. Some of these measurements are from the late eighties. TBT concentrations in water have declined since restrictions on the use of TBT have been in place. More recent investigations reported that the range of concentrations in coastal waters and estuaries is 1-10 ng/L and in marinas and major ports is 20-460 ng/L. (WHO 1999).

1.3.3 Sediment

TBT are likely to partition to the sediment and TBT sorption coefficients to sediments can range from 10-10,000. In sediments, TBT is generally persistent and half-lives have been estimated to be several years. TBT are therefore strongly retained in the sediment (ATSDR 2005). Even slow, the degradation processes in sediment are similar to those in water (see previous section).

As for the water, the concentration of TBT is higher in sediment collected within the proximity of marinas and areas with a high boat activity compared to more remote areas. In spite of the restricted use of TBT, the concentration in sediment has remained relatively high. In recent investigations, most sediment samples were below 100 µg/kg although some samples exceeded 1 mg/kg e.g. 10.9 mg/kg in a Swedish port. (WHO 1999).

In the National monitoring program of the aquatic environment (NOVA), concentrations of TBT has been measured in estuaries and coastal water sediments in Denmark from 1998 to 2004 (Ærtebjerg & Andersen 2004). The concentrations of TBT in sediments ranged from 0.5 to 65.8 µg Sn/kg dry weight (dw) (can be converted to 1.2 to 160.6 µg TBT (unspecified)/kg dw) with the highest concentrations found in bays and fjords (MADS 2005).
Seasonal variations have not been found in sediments (ATSDR 2005), but TBT concentration in sediments decrease progressively with increasing depth (WHO 1999).

1.3.4 Soil

No information about release, fate or concentrations of TBT in soil have been found.

1.3.5 Bioaccumulation

TBTO has a moderately high octanol-water partition coefficient ($logP_{o/w} > 3.19$) and bioaccumulation in aquatic organisms occurs and results in high concentrations in some organisms. Bioconcentration factors (BCF) vary considerably between species. BCFs of up to 7,000 have been reported for molluscs and fish from laboratory experiments. Even higher values have been found in field investigations. Higher BCFs for microorganisms (up to 30,000) (Maguire et al. 1984 – quoted in ATSDR 2005) may reflect adsorption rather than uptake. Bioaccumulation of TBT in bivalve molluscs is especially high due to the limited metabolic capacity of these organisms (WHO 1990, ATSDR 2005).

It has been shown that accumulation by mussels was greater if also the phytoplankton used as food was TBT-contaminated. Uptake of TBT from food was more important than uptake directly from water. (Laughlin et al. 1986 – quoted in WHO 1990).

Even though TBTO has a high solubility in fat the accumulation in organisms does not seem to be especially related to this property; reported levels of TBTO are considerably higher in non-adipose tissue e.g., liver and kidney than in e.g., blubber and subcutaneous fat and there does not seem to be transport of TBT via milk to offspring (WHO 1999).

There is no evidence for biomagnification of TBT in marine ecosystems (ATSDR 2005).

1.3.6 Foodstuffs

TBT are mostly found in seafood. In the EU Scientific Cooperation Project (SCOOP) entitled "Assessment of dietary exposure to organotin compounds of the population of EU member states", data on the concentration of organotin compounds, including TBT were collected from 8 European countries (Belgium, France, Greece, Germany, Italy, Norway, The Netherlands and Denmark) covering the period 1995 and 2002. Germany delivered the great majority of data (approximately 86%). The calculated concentrations of TBT in seafood other than fish (including molluscs, crustaceans, echinoderms and cephalopods) were in general higher (14 and 60 µg/kg wet weight (ww), median and mean, respectively) than the concentration in fish (5 and 17 µg/kg ww, median and mean, respectively). Based on fully aggregated data for both fish and seafood other than fish, the calculated concentration median and mean of TBT was 7.0 and 28 µg/kg ww, respectively. (EFSA 2004).

Samples of seafood purchased from markets in 1997 in Sweden, England, France, Singapore, Korea, Australia, Canada and USA revealed average TBT concentrations in bivalve molluscs, pelagic fish, pelagic invertebrates (e.g., crustaceans and cephalopods) and flatfish of 40, 16, 7.4 and 4.6 µg/kg, respectively (Keithly et al. 1999 – quoted in ATSDR 2005).

In the National monitoring program of the aquatic environment (NOVA), concentrations of TBT has been measured in mussels sampled in various estuaries and coastal waters in Denmark from 1998 to 2004. The concentrations ranged from 0.5 to 60 µg Sn/kg dw (can be converted to 1.2 to 146 µg TBT (unspecified)/kg dw). (Ærtebjerg & Andersen 2004).

Recently, the concentrations of various organotin compounds in fish and shellfish have been investigated by the Danish Food and Veterinary Research Institute. In total, 214 samples of 22 different species of fish and shellfish were analysed and, except for one sample in eel with very high concentration (3,264 µg Sn/kg ww), the mean concentration of TBT for each species ranged from below detection limit (0.0982 µg Sn/kg ww) to 12.2 µg Sn/kg ww (can be converted to 0.24 to 29.7 µg TBT (unspecified)/kg ww) and a maximum concentration of 37 µg Sn/kg ww measured in shark. (Hansen 2005).

1.4 Human exposure

Possible sources for human exposure to TBT are food and drinking water, contaminated soil and sediment, TBT-containing consumer products and ambient and indoor air. Of these, diet and especially seafood diet is the most important source of exposure to the general population. (EFSA 2004).

Daily intake of TBT via seafood in Japan in 1991 and 1992 was estimated to be 4.7 and 2.2 µg/day/person, respectively, based on a duplicate portion study. In a market-basket study also in Japan in 1991 and 1992, the daily intake was estimated to be 6.9 and 6.7 µg/day/person, respectively. (WHO 1999).

More recently, TBT intake were estimated in Asia, Europe, USA and Australia based on national diets and analysis of contamination of seafood species purchased in eight cities and it ranged from 0.8 (England) to 2.6 (Korea) µg/day/person (Kiethly et al. 1999 – quoted in EFSA 2004).

Intake of TBT via the seafood diet has also been estimated based on Norwegian consumption data and international concentrations found in fish and other seafood (SCOOP data). Using average consumption rates and median or mean international TBT occurrence levels in seafood, the intake of TBT was estimated to be 0.009 and 0.04 µg/kg b.w./day, respectively. Exposure of high consumers, assessed based on the 95th percentile of the Norwegian consumption rates as well as the median or mean international TBT occurrence levels in seafood, was 0.02 and 0.08 µg/kg b.w./day, respectively. In a worst-case scenario, where high consumers (the 95th percentile of the Norwegian consumption rates) eat highly contaminated seafood (the 95th percentile of TBT occurrence), an exposure of 0.3 µg/kg b.w./day has been estimated (EFSA 2004).

In consumer products, organotin compounds are used as stabilizers in e.g., textiles, polyurethane, plastic polymers and silicons. However, the contribution to human exposure of TBT via consumer products is expected to be relatively low as it is mainly mono- and di- substituted compounds that are used for these purposes.

Information on exposure via drinking water as well as via ambient or indoor air has not been located. However, organotin compounds (unspecified) have been found in household dust in UK (Santillo et al. 2003 – quoted in ATSDR 2005).

2 Toxicokinetics

2.1 Absorption

2.1.1 Inhalation

No quantitative studies were located regarding absorption in humans or animals following inhalation exposure to TBT. However, limited data suggest that absorption of TBT via inhalation is possible (ATSDR 2005).

2.1.2 Oral intake

TBTO (5.54 and 9.88 mg) dissolved in a mixture of cherry brandy (3 ml) and ethanol (7 ml) was given orally to a volunteer. 5.1-5.4 % of the dose was excreted in the urine mainly as DBT. The concentration of metabolites in urine decreased rapidly during the first days following administration. (Uhl 1986 – quoted in EFSA 2004).

Following a single oral administration of ^{113}Sn-labelled TBTO (25 mg/kg b.w.) to rats, the absorption varied from 20-55% depending on the vehicle (Hümpel et al. 1986 –quoted in WHO 1990 and EFSA 2004).

Following a single oral dose of ^{14}C-labelled TBT-acetate to rats, about 20% absorption occurred (Snoeij et al. 1987 – quoted in WHO 1990).

Mice administered a single dose of TBT (unspecified) (180 µmol/kg = 23 mg Sn/kg) excreted approximately 35% of the dose in the urine within 96 hours after dosing (Ueno et al. 1994 – quoted in ATSDR 2005).

2.1.3 Dermal contact

Following dermal application of TBTO to the arm of a volunteer, approximately 0.2% of the dose was excreted in the urine of which 20% was found as TBT (Uhl 1986 – quoted in WHO 1990).

No quantitative studies were located regarding absorption in humans or animals following dermal contact to TBT-compounds.

In WHO (1999), it is mentioned that TBTO is absorbed via the skin of mammals and the absorption range from 1 to 10% (WHO 1999).

2.2 Distribution

2.2.1 Inhalation

No studies were located regarding distribution in humans or animals following inhalation exposure to TBT.

2.2.2 Oral intake

The content of butyltin (monobutultin (MBT), dibutyltin (DBT) and TBT) have been detected in liver samples from humans in Poland (n=9), Japan (n=4) and Denmark (n=18). Total butyl tin burden ranged from 1.1 to 96 µg/kg ww. DBT appears to be the main butyltin compound deposited in human liver and TBT was below detection level (0.3 µg/kg ww in Nielsen & Strand 2002) (Kannan & Falandysz 1997, Takahashi et al. 1999, Nielsen & Strand 2002 - quoted in EFSA 2004).

Levels of total butyltin in blood from humans in U.S.A. (n=32) ranged from not detectable to 101 µg/L (Kannan et al. 1999 – quoted in EFSA 2004).

In rats, high levels of radiolabel were found in the liver and kidney 1 to 3 days following a single administration of ^{113}Sn-labelled TBTO (25 mg/kg b.w., oily solution). In the liver, more than 95% of radiolabel was found as TBTO metabolites. In other organs and tissue (e.g. brain and fatty tissue), the level of radiolabel was lower, but the fraction of unchanged TBTO was higher. (Hümpel et al. 1986 – quoted in WHO 1990 and EFSA 2004).

Daily intragastric administration of ^{113}Sn-labelled TBTO (5mg/kg b.w.) for 14 days to rats resulted in steadily increase of radiolabel in all tissues. Steady state levels were not reached. After 14 days of exposure, the concentration of radiolabel in kidneys and liver corresponded to a 3.5 and 2.8 fold increase, respectively, in relation to a single dose. In other tissues like testicles, at most a 7.4 fold increase was observed. Steady state conditions was not reached during the experiment, but it was estimated that steady state conditions would be reach in rats after 3-4 weeks of daily treatments with accumulation factors of about 10. (Hümpel et al. 1986 – quoted in WHO 1990 and EFSA 2004).

The blood brain barrier is not preventing transfer of TBT into the central nervous system. After a single dose of TBTCl (15 mg/kg b.w.), initially high concentrations were found in the cerebellum but also in the frontal and temporal lobe of rabbits (WHO 1990). After a single dose of TBTCl (40 mg/kg b.w.) to rats, the TBT concentration initially increased in brain. (Iwai et al. 1981 – quoted in EFSA 2004).

Following 5 oral doses of TBT (10 mg/kg) to rats, the tissue:blood concentration ratios of TBT for brain, kidneys and liver were approximately <1, 2-4 and 1-2, respectively (Iwai et al. 1982 – quoted in ATSDR 2005).

In rats exposed to TBTO in the diet for 4 weeks (0.25, 1.4 and 16 mg/kg b.w./day), total tin in kidneys, liver and brain increased with increasing dosage and was similar in females and males. Levels in the brain and adipose tissue were 10-20% of the level in kidneys and liver. (Krajnc et al. 1984 – quoted in ATSDR 2005).

In mouse, following exposure via drinking water to low doses of ^{14}C-labelled TBTO continuously for 30 days, kidney, liver, spleen and fat exhibited the highest accumulation of radioactivity, whereas levels in brain, lung, muscle and especially blood were low. For instance, administration of 17.6 µg TBTO/ml drinking water leads to a concentration of radioactivity in the kidney equivalent to 2.1 µg TBTO/g wet tissue and in the brain to 0.36 µg TBTO/g wet tissue. (Evans et al. 1979 – quoted in WHO 1990 and EFSA 2004).

TBT may cross the placenta to some extent as was shown by the presence of radiolabel in rat foetuses following a single oral dose ^{113}Sn-labelled TBTO (25

mg/kg b.w.) to the mother at day 18 of pregnancy. The concentration in the foetal tissue was comparable to that of the mothers muscle tissue. (Hümpel et al. 1986 – quoted in WHO 1990 and EFSA 2004).

2.2.3 Dermal contact

No studies were located regarding distribution in humans or animals following dermal contact to TBT.

2.3 Metabolism

In vivo, the metabolic pathway of TBT particularly in liver is characterised by the cytochrome P-450 dependent hydroxylation and dealkylation converting TBT to DBT, MBT and inorganic tin. Metabolites are detectable in the liver within 3 hours of TBT-acetat administration (1.2 mg/kg b.w.) to mice. Liberated carbon fragments were further oxidised. $^{14}CO_2$ appeared in the exhaled air in low amounts in addition to butene. (Kimmel et al. 1977 – quoted in EFSA 2004).

In rats, transient elevations in TBT, DBT, MBT and inorganic tin were observed in brain and liver over a 8-day period following a single oral dose of TBTFl (40 mg/kg = 15 mg Sn/kg) indicating that dealkylation had occurred (Iwai et al. 1981 – quoted in ATSDR 2005).

The butyl moieties can also be oxidised at carbon 3 and carbon 4 to yield 3-hydroxybutyl, 3-oxobutyl, 4-hydroxybutyl and 3-carboxy metabolites. Following a oral dose of TBTCl (2 mg/kg b.w.), the principal metabolites found in blood and brain were the simple dealkylation products (di- and monobutyltin) whereas in kidney and liver, hydroxy-, carboxy- and oxo-metabolites were the dominant metabolites (Matsuda et al. 1993 – quoted in ATSDR 2005).

TBT-compounds are substrates for mixed function oxidases. TBT metabolism has been demonstrated *in vitro*. Using hepatic microsomes from various laboratory animals, hydroxylation of the principal carbon-hydrogen bonds (in the alpha and beta position) has been demonstrated. The hydroxylated metabolite is unstable and rapidly splits to form the dibutyl-derivate followed by the formation of 1-butanol and butene. (Casida et al. 1971, Fish et al. 1975, 1976 – quoted in WHO 1990 and EFSA 2004).

Also, microsomes prepared from human liver dealkylate TBT to form di- and monobutyl metabolites (Ohhira et al. 2003 – quoted in ATSDR 2005 and EFSA 2004).

2.4 Elimination

2.4.1 Inhalation

No studies were located regarding excretion in humans or animals following inhalation exposure to TBT.

2.4.2 Oral intake

On cessation of dosing mice with ^{14}C-labelled TBTO in drinking water for 31 days, the examination of the animals for a further 15 days demonstrated loss of radiolabel retained in various tissues. TBTO had disappeared completely from the blood. The loss of radiolabel reached 97% in liver and 73% in kidney. The fat showed relatively high retention with a clearance of only 30%. The principal route of excretion is via the faeces. (Evans et al. 1977 – quoted in WHO 1990 and EFSA 2004).

Following a single oral dose of ^{14}C-labelled TBT-acetate in mice, approximately 16% of the dose was excreted in urine in 5 days, 53% was excreted in the faeces and 22% was exhaled as ^{14}C-labelled CO_2 (Kimmel et al. 1977 – quoted in ATSDR 2005).

In mice orally exposed to ^{113}Sn-labelled TBTO by intraperitoneal injection, data on whole body radioactivity showed an initial rapid decrease that became progressively slower with increasing time. The curve appeared to become asymptotic to that of inorganic tin and TBTO may be converted to inorganic tin in the body. The biological half-life in the mouse was estimated to be 23-29 days. (Brown et al. 1977 – quoted in WHO 1990 and EFSA 2004).

2.4.3 Dermal contact

No studies were located regarding excretion in humans or animals following dermal contact to TBT.

2.5 Mode of action

TBT induces massive cell death and apoptosis in a variety of different cell types in culture at low concentrations. It also induces apoptosis of thymocytes *in vivo*. Several mechanisms seem to play a role in these effects of TBT including elevation of the intracellular calcium concentration, mitochondrial dysfunction and binding of TBT to extrinsic “death receptors”.

Normally intracellular calcium concentration is very low in resting cells, but following TBT treatment, a massive increase of intracellular Ca^{2+} has been demonstrated. The increase may be the result of the opening of various calcium channels in the plasma membrane or the release of intracellular stores; both pathways seem to play a role following TBT treatment. The role of calcium as a critical element in both toxic cell death and apoptosis has been discussed; Ca^{2+} may lead to activation of proteases and caspases (disruption of the cytoskeleton) and phospholipases (impairment of mitochondrial function with a collapse of membrane potential). A multitude of studies have demonstrated the role of calcium in the mechanisms of organotins toxicity. (EFSA 2004).
However, inhibition of TBT-induced increase in Ca^{2+} concentration in human thymocytes achieved by incubating the cells in calcium free medium an additional buffering of the intracellular Ca^{2+} concentration could neither prevent initiation of several apoptotic events (e.g., cell shrinkage and caspase activation) nor membrane blebbing and cell deaths. These data suggest that the rise in Ca^{2+} concentration is not necessarily a component in the early signal transduction pathways in induced apoptosis in human immunokompetent cells. (Krug et al. 2003 – quoted in EFSA 2004).

Mitochondria are highly sensitive to treatment with organotins. TBT inhibits substrate uptake by mitochondria, induce their swelling, inhibits the ATP synthesis and alter ion transport across membranes. Furthermore, it has been demonstrated that the mitochondria plays a central role in the intrinsic apoptotic signalling pathway by the release of small pro-apoptotic proteins e.g., cytochrome c after TBT-treatment; cytochrome c are released from the intermembrane space of the mitochondria but the exact mechanism remains unclear. (EFSA 2004).
The intrinsic apoptotic signalling pathway was demonstrated in rat thymocytes where TBT interfered with both calcium homeostasis and mitochondrial parameters resulting in the generation of reactive oxygen species (ROS), cytochrome c, caspase activation and fragmentation of DNA (Gennari et al. 2000 – quoted in EFSA).
However, an additional apoptotic signalling pathway induced by TBT in human T and B-lymphocytes involving activation of so-called death receptors on cell surfaces has also been described. Two hours following treatment with TBT (1µM) active "death inducing signalling complexes" were found while at this time, the mitochondria remained unaffected. Immunohistochemistry could confirm the binding of TBT to thiol-groups of receptor molecules thereby inducing aggregation and activation. (Krug et al. 2001 – quoted in EFSA 2004).

There is evidence that the above described biochemical mechanisms of cell death and especially the induction of programmed cell death in immunocompetent cells by TBT is a part of the immunosuppressive effect of TBT. Treatment-related increases in phagocytes with engulfed apoptotic thymocytes were observed *in vivo* following administration of TBT (Raffray and Cohen 1993 – quoted in EFSA 2004). Furthermore, increased macrophage clearance activity in thymus following TBT treatment has been noted in many laboratories and decreases in lymphoid organ weights following treatment was associated with a depletion of lymphocytes in the thymus and thymus dependent areas of spleen and lympnodes (EFSA 2004)

The depletion of lymphocytes in the thymus appears to result from not only the induction of apoptosis in mature thymocytes but also from suppression of proliferation of immature thymocytes (Raffrey and Cohen 1993 – quoted in ATSDR 2005). TBT demonstrated a strong anti-proliferation effect *in vitro* in lymphocytes from marine mammals and humans at concentrations below 10^{-6} M Nakata et al. 2002 – quoted in EFSA 2004). The suppression of proliferation of immature thymocytes, which leads to a lower number of circulating functional T-cells, seems also to affect the T-cell-mediated response including antibody formation against foreign antigens and delayed hypersensitivity (Pieters et al. 1994 – quoted in ATSDR 2005).

An additional effect that potentially could enhance the immunosuppressive effect of TBT is the suppression of the cytotoxic activity of natural-killer cells (NK-cells). TBT inhibited the tumour-killing capacity of natural-killer lymphocytes *in vitro* when cells were pre-treated with TBT (200nM) for 1 hour (Whalen et al. 1999 – quoted in EFSA 2004).

3 Human toxicity

3.1 Single dose toxicity

3.1.1 Inhalation

Following acute inhalation exposure to TBTO, irritation of the respiratory tract has been claimed (Anon 1991, Hay & Singer 1991, Shelton 1992 – quoted in USEP 1997; EFSA, 2004).

Few case studies have been reported where inhalation exposure to TBTO mainly in paints resulted in the following symptoms: burning nose and nose bleeding, headache, sore throat, wheezing and coughing, loss of appetite, nausea, vomiting, watery eyes and adenopathy (EFSA 2004).

Seventy % of the workers in a rubber factory using TBTO in the vulcanising process reported irritation of the upper respiratory tract (and eyes) and about 20% also experienced lower chest symptoms, but the pulmonary function was unaffected. The extent of TBTO exposure was not reported. (WHO/FAO 1985 – quoted in WHO 1990).

3.1.2 Oral intake

No data have been found.

3.1.3 Dermal contact

In case-studies, where dermal exposure to TBTO occur through contact between TBTO containing paints and human skin, the following symptoms have been reported: itchiness, skin burning, swelling and blisters and dermatitis. The symptoms appear 5-10 hours following the exposure (EFSA 2004).

Human data summarised by Boyer (1989 – quoted in EFSA 2004) also suggest that TBTO is a potent non-allergenic dermal irritant.

3.2 Repeated dose toxicity

No data have been found.

3.3 Toxicity to reproduction

No data have been found.

3.4 Mutagenic and genotoxic effects

No data have been found.

3.5 Carcinogenic effects

No data have been found.

4 Animal toxicity

4.1 Single dose toxicity

4.1.1 Inhalation

After a single 4-hour exposure of rats to aerosols of TBTO, the LC_{50}-value was estimated to 77 mg/m^3 (total particles) and 65 mg/m^3 (particles<10µm). The rats showed signs of irritation such as nasal discharge, lung oedema and congestion. (Schweinfurth & Gunzel 1987 – quoted in WHO 1990 and ATSDR 2005).

When rats (10/sex/group) were exposed to atmospheres containing almost saturated vapours of TBTO, TBT-benzoate or TBT-naphthenate (concentrations not stated) once for 7 hours, no deaths occurred during exposure or in the 14-day observation period. Only minor clinical signs such as slight nasal discharge were noted occasionally. (Schweinfurth 1985 – quoted in WHO 1990).

In mice exposed to an aerosol of TBTO in olive oil (50 to 400 mg/m^3) for 1 hour (or seven 1-hour periods on successive days), significant increases in exploratory behaviour was observed in the lowest doses while higher doses reduced exploratory behaviour (Truhaut et al. 1979 – quoted in WHO 1990).

Guinea pigs exposed to aerosols of TBTO for 1 hour in concentrations of 0.2 mg/L (males, all) or 1 mg/L (females, 12 out of 15) died. In these animals, a general congestion of the lungs was observed. Exposure to 0.17 mg/L caused no deaths during the 7-day observation period though nasal irritation was observed. (Anger et al. 1976 – quoted in WHO 1990).

4.1.2 Oral intake

Oral LD_{50} values have been determined in mouse and rat for various TBT compounds (e.g., oxide, fluoride, chloride, acetate, benzoate, oleate, linoleate, abietate, naphthenate, laurate). For the various compounds, the LD_{50} values are comparable. In rat, reported oral LD_{50} values of TBT compounds range from 94 to 234 mg/kg b.w. In mouse, the values were between 44 and 230 mg/kg b.w. (WHO 1990, EFSA 2004).

Toxicological effects of acute exposure are characteristically delayed for a couple of days and beside a lethal effect, they may include alterations in blood lipid levels, effects on the endocrine system, damage of liver and spleen and transient deficits in the brain development (as summarised in WHO 1990).

After a single treatment with TBTCl (6.3, 12.5, 25 and 50 mg/kg b.w.), diurnal activity on day 1-4 was higher in high-dose groups compared to controls. Spontaneous motor activity during the dark phase was significantly decreased but returned to normal 4 days after dosing. At 25 mg/kg b.w., also the acquisition of conditioned avoidance responses was significantly impaired (Ema et al. 1991 – quoted in ATSDR 2005 and EFSA 2004).

4.1.3 Dermal contact

The acute dermal LD_{50} value in rabbits is reported to be approximately 9,000 mg/kg b.w. for TBT (unspecified) (WHO 1999) and 11,700 mg/kg b.w. for TBTO (Elsea & Paynter 1958 – quoted in ATSDR 2005).

For rats, an LD_{50} value for TBT (unspecified) of 605 mg/kg has been reported (Smith 1978 – quoted in ATSDR 2005).

4.2 Irritation

4.2.1 Skin irritation

In rats, TBTO preparations (Lastanax T (20% TBTO in a medium of water, alcohols and n-alkyl-polyethylene oxide) or Lastanax P (15% TBTO in the same medium but also containing bis-(5-chloro-2-hydroxyphenyl)) applied to shaved skin in water dilutions of 1-100% induced both clinical and histological effects on the skin. The effect was seen in all treated rats even with the lowest dilution (1% of a 15% TBTO preparation (Lastanax) with severity increasing with increasing exposure dose. One to two days following application, there was reddening and development of oedema. On day 3, haemorrhagic eschars developed, which later joined to form larger areas of ulceration. All signs had disappeared after 35-50 days depending on the exposure concentration. (Pelikan & Cerny 1969 – quoted in WHO 1990 and ATSDR 2005).

TBTCl, in absolute ethanol (67 nmol/cm^2), was applied to a shaved area of the dorsal skin of male rats. Two hours later, microscopic changes in the skin were observed. Eight hours later separation of dermis and epidermis had occurred and fluid collected in this separation and within 12 to 24 hours, there was widespread epidermal necrosis and formation of vesicles. (Middleton & Pratt 1977 – quoted in WHO 1990).

TBT (unspecified) produced focal epidermal necrosis and dermal inflammation at levels as low as 33 nmol/cm^2, fairly extensive epidermal necrosis and dermal inflammation at 67 nmol/cm^2, and almost total epidermal necrosis at 167 nmol/cm^2 (Middleton & Pratt 1978 – quoted in WHO 1990).

In rabbits, TBTO is a severe irritant to the skin whereas TBTFl did only produce minimal skin irritation (Sheldon 1975 – quoted in ATSDR 2005).

4.2.2 Eye irritation

Necrotic changes in the cornea of rabbit's eye were reported following treatment with TBTO (1% solution of Lastanax T (20% TBTO) or Lastanax P (15% TBTO)). The effect appeared 3 hours after application, symptoms worsened the next 2-5 days and recovery was incomplete 100 days later. At higher concentrations (10% solutions, equal to 4.6 and 6.1 mg/kg b.w. for the 2 preparations), both treated rabbits died 11 and 12 days following application (Pelikan 1969 – quoted in WHO 1990).

TBTO and TBTFl was reported to be potent irritants to rabbit's eye (Sheldon 1975 – quoted in ATSDR 2005).

4.2.3 Respiratory irritation

Following acute inhalation exposure to TBTO, rats showed signs of irritation such as nasal discharge, lung oedema and congestion (Schweinfurth & Gunzel 1987 – quoted in WHO 1990 and ATSDR 2005).

4.3 Sensitisation

No sensitising action of TBTO (1% (intradermal phase) and 5% (topical phase) was demonstrated in guinea pigs (n=20) using the Magnusson-Kligman Maximization test with challenge concentrations of 0.25% and 0.1% (Poitou et al.1978 – quoted in WHO 1990 and WHO 1999). WHO (1999) pointed out that it is not clear whether these challenge concentrations represent maximum non-irritant concentrations or what positive control substances that were used to demonstrate the sensitivity of the assay.

In a mouse ear swelling test, TBTO induced contact sensitisation when applied to mice for 3 days and challenged with it 3 days later. The lowest concentration tested was 0.25% by volume and it triggered a positive response. (Stringer et al. 1991 – quoted in ATSDR 2005).

4.4 Repeated dose toxicity

4.4.1 Inhalation

Rats were exposed to TBTO in "nose-only" chambers for 4 hours to concentrations of 0, 0.03 (vapour), 0.16 (vapour) or 2.8 (aerosol) mg/m^3, 5 days per week for a total of 21-24 treatments. At the highest concentration, severe toxic effects were produced. Mortality (5/10 in males and 6/10 in females), inflammatory reactions (not further specified) in the respiratory tract and histological changes (not further specified) in the lymphatic organs were observed. No local or systemic effects were observed at lower concentrations (evaluated endpoints were not specified). (Schweinfurth & Gunzel 1987 – quoted in US-EPA 1997 and WHO 1999).

Rats were exposed to TBTCl in a 95-day inhalation study at nominal concentrations of 4 and 6 mg/m^3. In the final month of exposure, the rats showed minor irritation in eye and nose and at termination, there were inflammatory changes in the respiratory tract of exposed animals. There was an initial increase in relative liver weight, but a significant reduction over the whole experimental period. Fatty degeneration was observed in liver at necroscopy. (Gohlke et al. 1969 – quoted in WHO 1990 and ATSDR 2005).

4.4.2 Oral intake

Several repeated oral dose toxicity studies have been performed. For an overview of the studies, see Table 2. A short description of each study is presented as well.

<u>Rats, 28-day dietary study, TBTO:</u> (Verdier et al. 1991 – quoted from US-EPA 1997 and WHO 1999).
TBTO was administered in the diet at concentrations of 0, 0.5, 2, 5 and 50 mg/kg diet.
Effects observed at the highest dose in males included slightly and inconsistently reduced body weight gain accompanied by slightly reduced food and water

Table 2. Repeated dose toxicity studies on oral intake of TBT

Species / strain Substance / purity	Duration/ Dose levels (mg/kg b.w./day)	Effects	NOAEL (mg/kg b.w./ day)	LOAEL (mg/kg b.w./ day)	Reference
Sprague-Dawley rat, weanlings 10/sex/group TBTO (96.5%)	28-days, diet 0, 0.05, 0.2, 0.5, 5	5:↓w thymus, ↓host defence (Listeria)	0.5	5 (US-EPA 1997, WHO 1999)	Verdier et al.(1991)
Wistar rat 4-8 ♂/group TBTO (pure / commercial product (80%))	1 to 4 weeks, diet pure: 0, 0.4, 1.4 commercial: 0, 0.3, 1.7	1 week: 1.4: ↑w liver, hist thymus/spleen 4 weeks: 1.4: ↓bwg, ↓fc, ↓w thymus ≥0.4: hist lympnodes		0.4 (US-EPA 1997, WHO 1999, EFSA 2004)	Bressa et al. (1991)
SPF-derived Wistar rat, weanlings 4 weeks: 10/sex/group 6 weeks: 4-10 ♂/group TBTO (95.3%)	4 to 6-weeks, diet 4 weeks: 0, 0.5, 2, 8, 32 6 weeks: 0, 2, 8 and 0, 8, 32 (WHO 1990)	4 weeks: 32:↓bwg, ↓fc ≥8: ↓w thymus, ≥2: hist spleen/liver/thymus ≥0.5 hist lympnodes 6 weeks: 8: ↓ thyroxin/TSH, ↓w thyroid, hist. pituitary/thyroid, ↑LH act. ≥2↓host defense (Listeria, Trichinella), ↓dth, ↓insulin, ↓ haematocrit	-	0.5 (US-EPA 1997, WHO 1999)	Krajnc et al. (1984) Voss et al. (1984)
Wistar rat 5 ♂/group TBTO (95.3%)	6 weeks + 15, 17 or 20 day post infection (p.i.), diet 0, 2, 8	≥2: ↓host defence (cytomegalovirus)		2 (US-EPA 1997, WHO 1999)	Garssen et al. (1995)
Wistar rat, weanling Number not specified TBTO (95.3%)	6 weeks, diet 0, 2, 8	8: ↓bw, ↓w spleen/thymus	2	8 (US-EPA 1997, WHO 1999)	Van Loveren et al. (1990)
F344 rat, weanlings 8/sex/group TBTO (96%)	18 weeks, diet 0, 16	16: ↓bwg, ↓w thymus, hist bile duct		16 (US-EPA 1997, WHO 1999)	Carthew et al. (1992)

Species / strain Substance / purity	Duration/ Dose levels (mg/kg b.w./day)	Effects	NOAEL (mg/kg b.w./ day)	LOAEL (mg/kg b.w./ day)	Reference
Wistar rat 5-12 ♂/group TBTO (95.3%)	5 months, diet 0, 0.025, 0.25, 2.5	2.5: ↓w thymus, ↓host defence (Listeria and Trichinella)	0.25	2.5 (US-EPA 1997, WHO 1999)	Voss et al. (1990)
Wistar rat, SPF derived Riv:TOX General effects: 12-18 ♂/group Immunotox: 9-12 ♂/group TBTO (95.3%)	General effects: 4.5 months Immunotox: 4.5-6 month, 15-17 month diet 0, 0.025, 0.25, 2.5	2.5: ↓w thymus, ↓mesenteric lymph node T-cells, ↓host defence (Listeria) ≥0.25: ↑mesenteric lymph node B-cells, ↓host defence (Trichinella)	0.025	0.25 (US-EPA 1997, WHO 1999, EFSA 2004, ATSDR 2005)	Voss et al. (1990)
Wistar rat 60/sex/group TBTO (purity not specified)	104 weeks, diet 0/0, 0.019/0.025, 0.19/0.25, 2.1/2.5, ♂/♀	2.1/2.5: ↓survival, ↓bw, ↑w liver/kidney/adrenal gland/heart, ↓w thyroid, hist liver/spleen/thyroid, ↑serum imunoglobulins ≥0.19/0.25: ↓freeT$_4$:total T$_4$ ratio, ↓lymphocytes, ↑thrombocytes	0.19 (WHO 1999) 0.25 (ATSDR 2005) 0.025 (EFSA 2004, Wester et al. 1990)	2.1 (WHO 1999) 2.5 (ATSDR 2005) 0.25 (EFSA 2004, Wester et al. 1990)	Wester et al. (1987, 1988, 1990)
CD-1 mouse 50/sex/group TBTO (97.1%)	18 months, diet 0, 0.75, 3.75, 7.5	7.5: ↓fc, ↑w liver ≥0.75: ↓survival	-	0.75 (WHO 1999)	Daly (1992)
Cynomolgus monkey 3-4 /group TBTO (96%)	22 weeks (6 days/ week), diet 0, 0.14	0.14: ↓ leucocytes	-	0.14 (WHO 1999)	Karrer et al. (1992)

↓: reduced
↑: increased
♂ / ♀: male / female
fc: food consumption
bw: body weight
bwg: body weight gain
hist: histological changes
w: weight
NK: natural killer cells
dth: delayed-type hypersensitivity

consumption (not quantified), decreased absolute liver weight (not quantified) and 30% decrease in relative thymus weight. Splenic clearance of *Listeria monocytogenes* was moderately but significantly suppressed at the high-dose group. Splenic plaque-forming cell response was significantly increased in the two highest dose groups; however, these values were within the range of historical controls.

Rats, 1 to 4-week dietary study, TBTO: (Bressa et al. 1991 – quoted in US-EPA 1997).
Pure TBTO and commercial TBTO were administered in the diet at concentrations of 0, 5 or 25 mg/kg diet. Half of the rats in control and high-dose group were treated for 1 week; the remaining rats were treated for 4 weeks.
In the high dose-group after 1-week treatment, the relative liver weight increased significantly and histological changes indicative of atrophy and lymphocyte depletion in the thymus cortex as well as a decrease in thymus-dependent lymphocytes in spleen were observed. After 4 weeks treatment, body weight gain, food consumption and relative and absolute thymus weights were significantly reduced. However, no signs of thymic histopathology were observed. In all treated groups both with pure and commercial TBTO, lymph nodes were markedly hemorrhagic and partially atrophic.

Rats, 4 to 6-week dietary study, TBTO: (Krajnc et al. 1984, Voss et al. 1984 – quoted in WHO 1990, US-EPA 1997 and EFSA 2004).
TBTO was administered in the diet at concentrations of 0, 5, 20, 80 and 320 mg/kg diet for 4 weeks (10/sex/group), 0, 20 and 80 mg/kg diet for 6 weeks (8-10 males/group), and 0, 80 and 320 for 3 days to 6 weeks (4-8 males/group).
The 4-week study evaluated clinical signs, food and water consumption, haematology, serum chemistry, organ weights and histopathology. The main objective of the 6-week study was evaluation of immune- and endocrine function.
In the 4-week study, rats exhibited ptosis or enophthalmia and slight ataxia at the highest dose (no evidence of brain oedema). Decreased body weight gain and food consumption were also observed at the highest dose. The relative thymus weight was significantly reduced at the two highest doses. There were evidence of haemorrhage in lymph nodes in all dose-groups and other histopathological changes (atrophy in thymix cordex, spleen and centrilobular hepatocytes) occurred at the two highest doses.
Alterations in haematological and biochemical parameters were significant from 20 mg/kg diet and up (increased alanine aminotransferase activity), from 80 mg/kg diet and up (reduced blood haemoglobulin, haematocrit, IgG levels, leukocyt counts and increased IgM level) and in high-dose group (decreased serum glucose level, liver glycogen and increased aspartate aminotransferase activity).
In the 6-week study, immunity was significantly suppressed at the lowest dose tested (20 mg/kg diet); there was decrease in delayed-type hypersensitivity reactions to ovalbumin, a decrease in resistance to *Trichinella spirallis*, a suppression of response to thymocytes to stimulation of PHA, suppression of antibody response and impaired splenic clearance of *L. monocytogenes*. Haematocrit levels were also reduced. Other immunological parameters were also affected but only in the high-dose group.
Effects on the endocrine system were observed in the 6-week study at doses from 2 mg/kg b.w./day in form of decreased serum insulin levels. At doses of 8 mg/kg b.w./day, decreased thyroid activity assessed by thyroxin and TSH levels and corresponding histology of the thyroid (flattened epithelial lining in thyroid follicles) and pituitary, decreased absolute and relative thyroid weight as well as an increase for LH producing pituitary cells was observed.

Rats, 6-week dietary study, TBTO: (Garssen et al. 1995 – quoted in US-EPA 1997).
TBTO was administered in the diet at concentrations of 0, 20 or 80 mg/kg diet. After 6 weeks of treatment, rats were inoculated (i.p.) with 10^5 plaque-forming units of cytomegalovirus. Exposure to TBTO continued during the infection period (15, 17 or 20 days post infection). Virus titers were determined in the salivary gland, lungs and spleen by plaque assay in 5 rats per sampling time per dose. There was significant increase in virus titers at both doses in salivary glands (day 15 and 17), at lowest dose in lungs (day 15) and at highest dose in spleen (day 17).

Rats, 6-week dietary study, TBTO: (Van Loveren et al. 1990 – quoted in US-EPA 1997).
TBTO was administed in the diet at concentrations of 0, 20 or 80 mg/kg diet. At the high-dose, there was a depression of body weight, spleen weight and thymus weight. There was a significant overall trend for a decrease in natural killer cell activity with increasing exposure, measured on lymphoid cell suspension from lung and using a 4-hour release assay with ^{15}Cr-labelled YAC lymphoma target cells.

Rats, 18-week dietary study, TBTO: (Carthew et al. 1992 - quoted in US-EPA 1997, ATSDR 2005).
TBTO was administered in the diet at concentrations of 0 or 150 mg/kg diet. After 6 weeks of exposure, groups of rats were intranasally infected with pneumonia virus of mice (PVM) or intranasally infected with *Mycoplasma pulmonis* followed 1 week later by infection with PWM. Four rats/group were sacrificed 4 and 12 weeks following infection. TBTO exposure was maintained throughout the infection period.
No statistical significant changes in extent or persistence of PVM-induced lung lesions indicative of chronic infection or in susceptibility to secondary mycoplasma pneumonia were observed. However, body weight gain and relative weight of thymus was decreased in treated group and there was an increased incidence of cholangitis with severe biliary retention due to obstruction of the extrahepatic bile duct.

Rats, 5-month dietary study, TBTO: (Vos et al. 1990 – quoted in US-EPA 1997 and WHO 1999).
TBTO was administered in the diet at concentrations of 0, 0.5, 5 and 50 mg/kg diet. For evaluation of body and organ weights 12 males/group, for resistance to *T. spiralis* 5-12 males/group, and for resistance to *L. monocytogenes* 6 males/group were examined. Compound-related effects were seen in the high-dose group only and consisted of significantly decreased thymus weight, impaired resistance to *T. spiralis* (significant increased number of larvae in muscle and increased recovery of adult worms from the small intestine) and to *L. monocytogenes* (significant increased spleenic bacterial count).

Rats, 4-6- and 15-17-month dietary study, TBTO: (Vos et al. 1990 – quoted in US-EPA 1997, WHO 1999, ATSDR 2005 and EFSA 2004).
TBTO was administered in the diet at concentrations of 0, 0.5, 5 and 50 mg/kg diet. General effects as body weight and weight of spleen and thymus were measured in groups of 18, 12 and 12 males, respectively, following exposure to 4.5 month. Immunological function studies were conducted on 9-12 males per dose following 4-6 month or 15-17 month of exposure. Antigen-specific functional assays evaluated were IgM and IgG responses to sheep red blood cell (after 16 months exposure); IgM and IgG responses to ovalbumin and delayed-type hypersensitivity (24, 48 and 72 hour) responses to ovalbumin and *Mycobacterium tuberculosis* (after 6 and 15 months exposure); resistance to oral infection by *Trichinella spiralis* larvae (after 5.5 sand 16.5 months exposure). Non specific resistance was

assessed by splenic clearance after i.v. injected *Listeria monocytogenes* bacteria (after 5 and 17 months exposure); natural cell mediated cytotoxicity of spleen cells (after 4.5 and 16 months exposure) and peritoneal cells (after 4.5 months exposure) using a 4-hour ^{51}Cr-release assay. Non-specific endpoints included numbers of viable nucleated thymus and spleen cells and responses of thymus and spleen cells to T-cell and B-cell mitogens (after 4.5 and 16 months exposure) as well as numbers of viable nucleated lymph node cells with cell surface marker analysis (after 6 and 18 months exposure).
There were no major differences in the effects between the animals exposed 4-6 or 15-17 months. Thymus weight was significantly reduced in the high dose-group. Statistical significant changes occurred in the percentage of mesenteric lymph node T-lymphocytes (a decrease in high-dose group) and B-lymphocytes (an increase in mid- and high-dose group). The absolute number of T- and B-lymphocytes per lymph node was not significant altered. Low-dose group was not tested in this assay. *In vivo* clearance of injected *L. monocytogenes* was impaired in the high-dose group (significant increase in number of viable bacteria per spleen). Resistance to *T. spiralis* was suppressed in rats exposed to the mid- and high-dose group (significant reduced IgE titers, increased number of larvae in muscle and moderately reduced inflammatory reaction around cysts in parasitised musculature).
The activity of natural killer cells isolated from the spleen was suppressed at all doses only after 16 month of exposure; however, there was no clear treatment-related progress and the authors consider these data equivocal.

Rat, 106-week dietary study, TBTO: (Wester et al. 1990, Wester et al. 1987, 1988 - quoted in WHO 1999, ATSDR 2005, US-EPA 1997 and EFSA 2004).
TBTO were administered in the diet at concentrations of 0, 0.5, 5 and 50 mg/kg diet. Investigations included clinical observations, body weight and food consumption analysis, evaluation of incidence of neoplastic lesions, haematology, urinalysis, clinical chemistry and endocrinology.
In males, feed consumption was increased in all treated groups. In the high-dose group, increased mortality occurred after approximately week 90 (males) or week 96 (females) and terminal body weights were significantly reduced. Significant changes in absolute organ weights (relative organ weights were not reported) were also observed in the high-dose group; in both sexes liver and kidney weight increased, in females the weight of the thyroid decreased and in males the weight of the heart and the adrenals increased. In the high-dose group, non-neoplastic histological changes occurred in liver (bile duct changes), spleen (decreased haemosiderin content) and thyroid (decreased follicular epithelial cell height). Haematological changes (anaemia, lymphocytopenia and thrombocytosis) were noted mainly in the high dose group. However, in females there was a significant increase in thrombocyte count in the high-dose group after 12 months and in the mid-and high-dose group after 24 months. There was also a significant decrease in lymphocyte count in the mid- and high-dose group after 12 months (females only) and in the high-dose group after 24 months (both males and females).
Serum immunoglobulin levels significantly increased in the high-dose group (*t*-test) and in the mid-dose group a slight but significant (ANOVA, $p<0.05$) increase in the concentration of IgM was also observed. No significant changes in the serum levels of several hormones were observed; however there was a significant decrease in the free thyroxine:total thyroxine ratio for both sexes in the mid- and high-dose group. In the mid-dose group the decrease was transient; it occurred only following 12 month and not following 24 month of exposure. According to the authors, a NOAEL of 0.025 mg/kg b.w. can be established based on the marginal haematological, immunological and hormonological changes.

Mice, 18-month dietary study, TBTO: (Daly 1992 – quoted in US-EPA 1997 and WHO 1999).
TBTO were administered in the diet at concentrations of 0, 5, 25 and 50 mg/kg diet. Statistical significant decreases in survival occurred in treated mice of both sexes (e.g., 59, 48, 40 and 27% in the control to high-dose group in females). Other treatment related effects included significantly decreased food consumption and increased absolute and relative liver weight in females in the highest dose group.

Monkey, 22-week dietary study, TBTO: (Karrer et al. 1992 – quoted in EFSA 2004 and ATSDR 2005).
TBTO was dissolved in vegetable oil and added to Tween 80-augmented pear juice (no information on TBTO concentration in juice), which the monkeys drank.
Total leukocyte count was lower in the treated group compared to the control group during weeks 8-10 and 16-20. In the interim period, the leukocyte count returned to control values. No changes in differential count, serum immunoglobulin levels and other parameters (not further specified) were observed.

4.4.2.1 Neurotoxicity

TBTO given orally to male Sprague Dawley rats (37.5 or 75 mg/kg b.w.) for 3 consecutive days increased the mortality rates and in the remaining animals, aggression and seizures were seen. There was a complete absence of Purkinje cells in the cerebellum at day 7 following treatment. The activity of total brain ATPaseNa^+/Ka^+ ATPase and Mg^{2+} ATPase was suppressed (Elsabbagh et al. 2002 - quoted in EFSA 2004).

A daily dose of 2.5 mg TBTBr/kg b.w. for 6 days induced slight tremors and weakness in Sprague-Dawley rats (Yallapragada et al. 1991 – quoted in ATSDR 2005).

After oral administration of TBTOAc (100 mg/kg diet to male rats and lethal dose to female rats) in 3 months, induction of interstitial oedema of the white matter of the central nervous system was reported (Barnes and Stoner 1958, 1959 – quoted in EFSA 2004).

4.4.3 Dermal contact

An ethanolic solution of TBTO were painted daily on the shaved dorsal skin of guinea-pigs for 50 days at doses equivalent to 10 and 40 mg/kg b.w. An increased loss of sodium, chloride, phosphate, glucose and amino acids in the urine and a concomitant loss of electrolytes in serum were observed. At termination of the study, histological lesions of the kidney tubules (swelling, degeneration and destruction of tubular epithelium) were observed. (Mori et al. 1984 – quoted in WHO 1990).

4.5 Toxicity to reproduction

4.5.1 Inhalation

Rats were exposed to a mixture of TBT-bromide (81.2%) with other compounds such as DBT-dibromide at a concentration of 2 mg Sn/m^3 for acute- and intermediate-duration exposure. Pregnancy rates were markedly reduced after 4 weeks to 3 months of exposure, but returned to near normal when exposure was

discontinued. No histopathological changes were seen in males. In females atrophy of the glandular uterus was observed as early as 14 days of exposure. All effects were reversed during a recovery period. (Iwamoto 1960 – quoted in ATSDR 2005).

No studies regarding developmental effects following inhalation exposure were located.

4.5.2 Oral intake

Several studies concerning effects on reproduction and development following oral exposure have been performed. For an overview of the studies, see Table 3. A short description of each study is presented as well.

Rat, exposed day 6-9 of gestation by gavage, TBTO: (Schroeder et al. 1981 – quoted from US-EPA 1997 and EFSA 2004).
TBTO in corn oil were administered to mated females by gavage. The dams were sacrificed at day 20.
Clinical signs (staining of the fur in the ano-genital area) and decreased body weight gain was seen at mid- and high dose at day 6-20. Adjusted weight gain (excluding uterus) was 5.5, 22.2 and 69.4% lower than controls at the low-, mid- and high-dose, respectively.
Indications of developmental toxicity were observed in all dose groups. Effects included increased incidence of minor anomalies of the axial skeleton (asymmetric sternebrae, rudimentary ribs, 14^{th} rib pair) as well as of other skeletal abnormalities (cervical unilateral and bilateral ribs, un-ossified caudal vertebrae) and malformations (cleft palate). Lack of statistical analysis and litter incidences complicates the evaluation of these data but the percentages of foetuses with at least one skeletal abnormality were increased at mid- and high dose.
Other effects occurred at the high-dose including significant decrease in the percentage of foetuses to implants, increased percentage of resorptions and decreased fetal weight

Rat, exposed day 6-20 of gestation by gavage, TBTO: (Crofton et al. 1989 – quoted in WHO 1990, US-EPA 1997 and EFSA 2004).
TBTO in corn oil were administered by gavage. The examination of postnatal toxicity including e.g. survival, body and brain weight and motor activity was only investigated in the groups receiving 0, 2.5, 5 and 10 mg/kg b.w. day.
Maternal toxicity included vaginal bleeding in the two highest dose groups and significant reduction in body weight gain in the three highest dose groups and body weight in the top dose group.
Litter size and pup body weight was significantly reduced at the three highest doses where also pup survival (day 1-3) was reduced. Postnatal survival (day 21) and postnatal body weight gain (up to day 19) were reduced in the highest dose examined (10 mg/kg b.w./day) and a significant delay in vaginal opening was also observed in this group. At 10 mg/kg b.w./day, motor activity was significantly reduced on postnatal day 47 and 62 and weight of brain, cerebellum and hippocampus (measured on postnatal day 110) was significantly reduced. No malformations were observed.

Rat, *in utero* exposure study, exposure by gavage, TBTO: (Smialowicz et al. 1989 – quoted in US-EPA 1997).
The rats were dosed 3 times/week for a total of 10 doses. Reductions in mitogen responses were observed at the two highest doses in both adults and pre-weanlings. In the high-dose group, suppression of the mixed lymphocyte reaction in both age groups and natural killer cell activity in pre-weanlings was observed.

Table 3. Reproductive toxicity studies on oral intake of TBT

Species / strain Substance / purity	Duration / Dose levels (mg/kg b.w./day)	Effects	NOAEL (mg/kg b.w./ day)	LOAEL (mg/kg b.w./ day)	Reference
CD Sprague-Dawley rat 24 ♀/group TBTO (96.9%)	Day 6-9 of gestation, gavage 0, 5, 9,18	18:↑resorptions, ↓foetuses/implants, ↓ fetal bw ≥9: ↓bwg M ≥ 5: ↓adjusted bwg M, ↑ minor skeleton abnomalities	-	Parental tox.: 5 (EFSA 2004), 9 (US-EPA 1997) Repro/develop, tox.: 5 (EFSA 2004, US-EPA 1997)	Schroeder et al. (1981)
Long Ewans rat 15-18 ♀/group TBTO (97%)	Day 6-20 of gestation, gavage 0, 2.5, 5, 10, 12, 16 (postnatal analysis: 0, 2.5, 5, 10)	16: ↓bw M ≥ 12:vaginal bleeding ≥ 10:↓bwg M, ↓litter size, ↓ bw pup 10: delayed vaginal opening, ↓ motor activity, ↓ w brain/cerebellum/hippocampus	Parental tox.: 5 Repro/develop tox.: 5	Parental tox.: 10 Repro/develop tox.: 10 (US-EPA 1997, EFSA 2004)	Crofton et al. (1989)
Fisher rat, adults and pre-weanlings Number not specified TBTO	3 weeks (3 times/week), gavage 0/0, 2.5/5, 5/10, 10/20, pre-weanling/adult	Adult: 20: ↓lymp. reaction ≥10:↓mitogen response pre-weanling: 10: ↓lymp. reaction, ↓NK act. ≥5: ↓mitogen response	2.5	5 (US-EPA 1997, WHO 1999)	Smialowicz et al. (1989)
Crl:CD(SD)BR rat 30/sex/group TBTO (97.1%)	2 generation study, 10-weeks prior mating of P0 until mating of F1 (15 weeks), diet P0: 0/0, 0.02/0.03, 0.29/0.34, 2.95/3.43, ♂/♀ F1: 0/0, 0.03/0.04, 0.36/0.44, 3.98/4.42, ♂/♀	P0: 2.95/3.43: ↓ w thymus (not sign) F1: 3.98/4.42: ↓ bwg, ↓ w thymus F1/F2: 3.43/4.42: ↓ bw pup	Parental tox.: 0.29 Repro/develop tox.: 0.34 (US-EPA 1997, EFSA 2004)	Parental tox.: 2.95 Repro/develop, tox.: 3.43 (US-EPA 1997, EFSA 2004)	Schroeder (1990)
Wistar rat P0: 11-16 dams/group TBTCl (purity not given)	Day 0-3 or 4-7 of gestation, gavage 0, 8.1, 16.3, 32.5 and 65.1	Day 0-3: 16.3:↑ post implantation losses ≥8.1:↑pregnancy failure, ↓w foetuses Day 4-7: 65.1:↑ pregnancy failure ≥16.3:↑ post implantation losses, ↓ live foetuses/litter, ↓ fetal bw	-	8.1	Harazono et al. (1998)
CD rat 9/16 dams/group,	Dams: gestation day 8 - weaning, gavage	2.5: ↓w liver/thymus/spleen, ↓ serum thyroxine ♂, ↓serum triglycerides/creatin/Mg ♀, ↑ NK/IgM	0.025 (EFSA 2004, ATSDR 2005)	0.25 (EFSA 2004, ATSDR 2005)	Cooke et al. (2004) Tryphonas et al. (2004)

Species / strain Substance / purity	Duration / Dose levels (mg/kg b.w./day)	Effects	NOAEL (mg/kg b.w./ day)	LOAEL (mg/kg b.w./ day)	Reference
12/22 pups/group; (Cooke et al. 2004) 10 dams/group, 10 pups/group (Tryphonas et al. 2004) TBTCl	Pup: weaning – post natal day 30 (♂+♀), 60 (♀) or 90 (♂) gavage 0, 0.025, 0.25, 2.5	≥0.25: ↓amylase ♂, ↑ IgG/T lymp. =0.25: ↓host defence (Listeria) ≥0.025: ↓fc/bwg♀, ↑fc/bwg♂, ↑NK act. =0.025: ↓w liver ♀, ↑ IgM,			
Wistar rat P0:10/sex/group F1:10/sex/group TBTCl (purity not given)	2-generation study, from pregnancy (day 0) of P0 until weaning of F2, diet 0, 0.25, 1.25, 6.25	F1: 6.25: ↓ no. of pups/bw pups(d1)/ bwg of pups ♀: ↑ ag dist. (d4), delayed eye opening ↓ w ovarian, delayed vaginal opening, altered oestrus cyclicity ♂: ↓ w epididymis/ventral prostate, ↓ spermatid counts, hist testis, ↑ testosterone, ↓17betaestradiol ≥0.25: ♀↑ ag dist. (d1) ♂: ↓ w testis F2: 6.25: ↓ no. of pups/bw pups (d1)/bwg of pups ♀: ↑ ag dist. (d1 & 4), delayed eye opening, delayed vaginal opening, ↑ w uterus, altered oestrus cyclicity ♂: ↓ w testis/epididymis, ↓ sperm counts, hist testis, ↑ testosterone,↓17betaestradiol	-	Repro tox: 0.25 (EFSA 2004)	Ogata et al. (2001) Omura et al. (2001)
NMRI mice 6-118 ♀/group TBTO (purity not given)	Day 6-15 of gestation, gavage 0, 1.2, 3.5, 5.8, 11.7, 23.4, 35	35:↑resorption ≥23.4:↓ fetal bw, ↑ minor skeleton abnormalities ≥11.7:↓bwg M, ↑ freq. of cleft palate	Parental tox.: 5.8 Repro/develop tox.: 5.8	Parental tox.: 11.7 Repro/develop tox.: 11.7 (US-EPA 1997, EFSA 2004)	Davis et al. (1987)
Swiss albino mice 8 ♀/group TBTO (>96%)	Day 6-15 of gestation, gavage 0, 5, 20, 40	40: ↓ bwg/bw M, ↓ fetal bw, ↑ resorptions ≥5: ↓ w spleen M, ↑ w placenta, ↓ fetal/placental w ratio	Parental tox.: 20 Repro/develop tox.: 20 (US-EPA 1997)	Parental tox.: 40 Repro/develop tox.: 40 (US-EPA 1997)	Baroncelli et al. (1990)
Swiss mice 8 pups/group TBTO (purity not	Day 6-15 on gestation, gavage 0, 5, 10, 20, 30 (blood analysis: 0, 5, 10, 20)	≥20: ↓litter size, ↓ bw pup ≥10: ↓ bwg pup ≥5: ↓ bwg M, ↑ resorption, ↑ early parturitions, altered maternal behaviour, ↑ mean corpuscular volume in neonates	Parental tox.: - Repro/develop tox.: 20 (US-EPA 1997)	Parental tox.: 5 Repro/develop tox.: - (US-EPA 1997)	Karrer et al. (1995) Baroncelli et al. (1995)

Species / strain Substance / purity	Duration / Dose levels (mg/kg b.w./day)	Effects	NOAEL (mg/kg b.w./ day)	LOAEL (mg/kg b.w./ day)	Reference
given)					
ICR mouse Number not specified TBTO	Gestation day 4-17 or 11-17, gavage 0, 0.1	0.1: ↓primary antibody response, ↑ leukocytes, ↓dth	-	0.1 (US-EPA 1997, WHO 1999)	Buckiova et al. (1992)
ICR mice Number not specified TBTO (purity not given)	4 weeks, 2 times/week up to 10	10: ↓sperm count, hist testis	-	-	Kumasaka et al. (2002)
New Zealand white rabbits 20 ♀/group TBTO (purity not given)	Gestation day 6-18, by gavage 0, 0.2, 1, 2.5	2.5: ↓bw M, ↓ fetal bw (not significant)	Parental tox.: 1 Repro/develop tox.: 1	Parental tox.: 2.5 Repro/develop tox.: 2.5 (WHO 1990)	Nemec (1987)

↓: reduced
↑: increased
♂ / ♀: male / female
bw: body weight
bwg: body weight gain
hist: histopathological changes
w: weight
NK: natural killer cells
Lymp.: lymphocytes
dth: delayed-type hypersensitivity
Ag dist.: Anogenital distance
M: maternal
d: day
act:

Rat, 2-generation dietary study, TBTO: (Schroeder 1990 – quoted in US-EPA 1997 and EFSA 2004).
TBTO were administered in the diet at concentrations of 0, 0.5, 5.0 and 50 mg/kg diet. P0 males and females were exposed 10 weeks prior to mating and with exposure of females during gestation and lactation. Groups of 30 offsprings of each sex were fed the parental diets for 15 weeks, and were mated to produce the F2 generation.
Body weight gain was significantly reduced in the high-dose F1 generation at the beginning of the pre-mating growth period. Absolute and relative thymus weights were slightly lower than control values in the high-dose F0 generation and significantly lower than controls in the high-dose F1 generation. No histological changes in thymus were observed.
Compound-related reproductive and developmental effects were significantly decreased pup body weight during lactation in both generations at the high dose.

Rat, exposed day 0-3 or 4-7 of gestation by gavage, TBTCl: (Harazono et al. 1998 – quoted in EFSA 2004).
TBTCl dissolved in olive oil were orally administered, on days 0-3 (only up to 32.5 mg/kg; 12-16 dams/group) or 4-7 (11-13 dams/group) of gestation. Pregnancy outcome was determined at day 20.
There was no apparent maternal toxicity. When dosed on day 0-3, there was a dose-related increase in pregnancy failure at all doses and an increase in incidence of post-implantation losses at the two highest doses. When dosed on day 4-7, there was a significant increase in pregnancy failure at top dose. There was a marked and dose-related increase in incidence of post-implantation losses as well as a dose-related reduction in litter size at the three highest doses. No increase in incidence of fetal malformations was seen but the fetal body weight was reduced at all doses (day 1-3) and at the three highest (day 4- 7).

Rats, exposed from gestation day 8 until weaning, by gavage, TBTCl: (Cooke et al. 2004. Tryphonas et al. 2004).
TBTCl in olive oil were administrated by gavage to dams from gestation day 8 until weaning. Post weaning, randomly selected pups were gavaged daily, with the same dose as their mothers and sacrificed at post-natal day (PND) 30 (males and females), PND 60 (females) or PND 90 (males). Two litters from each dose group were sacrificed at day 7 and the organotin concentration in the stomach contents was assessed.
Stomach contents of the suckling pups contained undetectable levels of TBT. DBT was only detectable in the highest dose-group, indicating negligible transfer to pups via lactation. TBTCl had no effect on dams body weights, food consumption, litter size, sex ratio or survival of pups to weaning. However, all doses of TBTCl significantly affected parameters of the growth profile of the pups (mean body weights, average slope, curvature) and the ratio of weekly food consumption to weekly body weight gain indicated enhanced food conversion to body mass in females but a decreased conversion in males. In males at the highest dose-group, significantly reduced serum thyroxine levels were evident. Liver weight decreased significantly at 0.025 and 2.5 mg/kg b.w./day, but not in the intermediate dose group at PND 60 in females and at top dose in males on PND 90. No histopathological changes in liver were seen, but some markers (serum glycerides, creatine, magnesium (females, top dose) and amylase (males, >0.25 mg/kg b.w./day)) were significantly reduced. At the top dose, significant decreases in spleen and thymus weight were observed.
Detailed examination of immune function revealed significant increase in mean percent and absolute natural killer (NK) cell numbers at the top dose (in both sexes), and a significant non-linear dose-response increase in NK cell activity at all dose levels (significant for trend in both females and males, in males all treatment

groups significant different from control, in females only the high-dose). Furthermore, significant increases in serum immunoglobulin levels were observed. In females, IgM levels were significantly increased at the low- and high-dose; Levels at the mid dose was also higher but not significant. In males, IgG levels were increased at the mid- and high dose and IgM levels at the high dose. A significant increase in mean percentage of CD4(+)8(+) (immature)T lymphocytes at the middle- and high-dose in females was observed as well as significant increased mean number of L. monocytogenes colony-forming bacteria in the spleens of females on day 2 and 3 of post-infection at the mid dose. In both males and females, an increase in mean number of L. monocytogenes colony-forming bacteria was observed at all doses (significant for trend). Increased delayed-type hypersensivity response to oxazolone in the low and middle doses and a decrease in the high dose (only significant for trend) was also observed (Tryphonas et al. 2004).

Rat, 2-generation dietary study, TBTCl: (Ogata et al. 2001, Omura et al. 2001 – quoted in EFSA 2004).
Rats were fed 0, 5, 25 and 125 TBTCl/kg diet from pregnancy of P0 females (day = 0) until weaning of F1. Treatment of F1 continued throughout mating (PND 92), pregnancy, lactation and up to weaning of the F2. F2 males and females were killed on postnatal day 119 and 91, respectively.
At the top dose, decreased number of live pups, live birth index, body weight of pups and pup weight gain up to postnatal day 14 were seen in both F1 and F2 were observed.
A dose- related and significant increase in ano-genital distance was found in all treated female groups of postnatal day 1 in the F1 and in the top dose in F2. At postnatal day 4 it was seen at the top-dose only for both F1 and F2. Furthermore, a delay in eye opening (1 day) and vaginal opening (1 week) and a significant reduction in the number of animal with normal oestrous cyclicity was observed in top dose groups in both F1 and F2. Ovarian relative weight was significantly reduced in F1 females and uterine weight significantly increased in F2 females in top-dose groups. In the females no histopathological changes were seen and no changes in serum oestradiol or testosterone concentration in females were detected. In F1 males, a dose-related decrease of testis weight was seen in all groups and at the top dose of F2. At the top dose decreases of epidydimal weight and ventral prostate weight as well as of homogenisation-resistant spermatid counts and sperm counts were also observed in both F1 and F2. Slight histopathological changes (e.g. vacuolisation of the seminiferous epithelium, spermatid retention and delayed spermiation) were observed in the testis in the high-dose groups. The serum 17betaestradiol concentration in males was decreased at top doses, whereas concentration of testosterone was increased in males at top dose in F1 and F2.

Mouse, exposed day 6-15 of gestation by gavage, TBTO: (Davis et al. 1987 – quoted in WHO 1990, US-EPA 1997 and EFSA 2004).
TBTO in olive oil were administered by gavage. Dams were sacrificed on gestation day 18.
Slight maternal toxicity, indicated by reduced bodyweight gain, was observed at 11.7 mg/kg b.w./day and higher. Fetal effects included a dose-related increase in the frequency of cleft palate (significant from 11.7 mg/kg b.w./day and higher) and in the two highest dose groups; reduced average foetal body weight, increased number of foetuses with minor skeletal abnormalities and skeletal variations. Increased resorption rates were observed following treatment with the highest dose.

Mouse, exposed on gestation day 6-15 by gavage, TBTO: (Baroncelli et al. 1990 – quoted in US-EPA 1997).

TBTO in vegetable oil was administrated by gavage. The dams were sacrificed on gestation day 17.
Maternal toxicity was seen at the high dose including decreased body weight gain and body weight, piloerection, lethargy, hunched posture and vaginal bleeding. A dose related and significant decrease in the relative spleen weight was observed in all dose groups, but no histological or other pertinent endpoints were evaluated to elucidate these changes. Indications of developmental toxicity occurred in the high-dose group including increased resorption and reduced fetal body weights. A significant and dose-related increase in placenta weight and decrease in foetal/placenta weight ratio were observed at all doses.

Mouse, exposed on gestation day 6-15 by gavage, TBTO: (Baroncelli et al. 1995, Karrer et al. 1995 – quoted in US-EPA 1997).
Pregnant mice were treated with TBTO on gestation day 6-15. At birth litters were normalized to 8 pups and evaluation of pup growth rate and behavioural observations of dams were conducted. Maternal body weight gain was reduced and the number of resorptions significantly increased in all dose groups. Furthermore, the incidence of early parturitions increased in all dose-groups. In the two highest dose-groups litter size and pup weight was reduced. Postnatal death rate and growth rate of treated pups were affected by altered maternal behaviour (e.g. lack of cage building, absence of parental care, infanticidal events) (Baroncelli et al. 1995 – quoted in US-EPA 1997).
The effect of *in utero* TBTO exposure on haematological parameters in neonates, pups and dams from the three lowest dose-groups was investigated in a companion study. No effects of TBTO were seen in blood composition or in spleen or thymus weight at any dose in dams or pups. In neonates, the mean corpuscular volume was significantly higher at all doses than in control group, but the increase was not dose-related and the effect was not seen in pups at any time (Karrer et al. 1995 – quoted in US-EPA 1997).

Mouse, exposed on day 4-17 or 11-17 of gestation in by gavage, TBTO: (Buckiova et al. 19992 – quoted in US-EPA 1997 and WHO 1999).
TBTO in Tween 80:ethanol:saline (1:2:97) were administered by gavage. Humoral and cell-mediated immune responses in offspring were evaluated 4 and 8 weeks after birth.
Effects in offspring included suppressed primary antibody response to sheep red blood cells, ovalalbumin and lipopolysaccharide and increased number of leucocytes as well as suppressed delayed-type hypersensivity to sheep red blood cells. Unspecified changes in polyclonal proliferative responses of thymocytes and spleenocytes were also observed. The severity of these effects were higher in the mice exposed on gestation day 11-17 compared to those exposed on gestation day 4-17. According to WHO (1999), the full publication of this study is not available.

Mouse, males, 4 weeks-study, TBTO: (Kumasaka et al. 2002 – quoted in ATSDR 2005).
Treatment of male mice with TBTO 2 times/week in 4 weeks significantly reduced sperm count. Light microscopy showed disorganized seminiferous tubules with vacuolisation of Sertoli cells and some loss of germ cells

Rabbit, exposed on gestation day 6-18 by gavage, TBTO: (Nemec 1987 – quoted in WHO 1990).
TBTO in corn oil were administrated by gavage. At day 29 of gestation the experiment were terminated. In the high dose group, there was a significant decrease in body weight of dam in the period of dosing and a slight but non-significant decrease in mean fetal weight at study termination. There were no treatment related differences in type or frequency of fetal malformations.

4.5.2.1 Developmental neurotoxicity

After oral administration of TBTCl (1 or 5 mg/kg b.w.) to rats at day 6-20 of gestation, mothers as well as offsprings showed clear evidence of alterations of behaviour (e.g. hyperactivity, dysfunction of spatial learning performance). Neurohistological changes were not investigated. (Gardlund et al. 1991 – quoted in EFSA 2004).

4.5.3 Dermal contact

No data have been found.

4.6 Mutagenic and genotoxic effects

4.6.1 *In vitro* studies

TBTO was not mutagenic in the rec assay in *Bacillus Subtilis*, it did not induce reverse mutations in *Klebsiella pneumoniae* or induce point mutations in *Salmonella typhimurium* TA1530, TA1535, TA1538, TA97, TA98 or TA100 in the presence or absence of liver activation system (S9). However, TBTO was mutagenic in *S. typhimurium* TA100 in a fluctuation test in the presence of S9 at cytotoxic concentrations. (Davis et al. 1987 – quoted in WHO 1990, WHO 1999 and ATSDR 2005).
Hamasaki et al. (1993 – quoted in ATSDR 2005) also found that TBTO induced mutations in *S. typhiurium* TA100 (in absence of S9) whereas Reiman & Lang (1987 - quoted in WHO 1990) found that TBTO had no ability to produce point mutations in *S. typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100.

TBTO did not induce mutations in *Schizosaccharomyces pombe* or mitotic gene conversion in *Saccharomyces cerevisae*. Furthermore, TBTO did not induce mutations in V79 Chinese hamster cells or mouse lymphoma cells. (Davis et al. 1987 – quoted in WHO 1990, WHO 1999 and ATSDR 2005).

The clastogenic potential of TBTO has been studied in two types of mammalian cells. TBTO did not induce sister chromatid exchange in Chinese hamster ovary cell in the presence or absence of S9. However, structural chromosomal aberrations (endoreduplicated and polyploid cells) were observed in Chinese hamster ovary cells. The aberrations were detected only at the first sampling time (8 hours) and only at the highest concentration tested where also cytotoxicity was observed. (Davis et al. 1987 – quoted in WHO 1990, WHO 1999 and ATSDR 2005).
In human lympocytes no increase in chromosomal aberrations were found (Reimann & Lang 1987 – quoted in WHO 1990).

4.6.2 *In vivo* studies

TBTO did not induce recessive lethal mutations in adult male *Drosophila melanogaster* by either feeding or injection (Davis et al. 1987 – quoted by WHO 1990, WHO 1999 and ATSDR 2005).

An increase in number of cells with micronuclei was observed in polychromatic erythrocytes (PCE) of male BALBc mice 48 hours but not 30 hours following a single oral dose of TBTO (60 mg/kg b.w.). A lower dose (30 mg/kg b.w.) showed

no effect. (Davis et al. 1987 – quoted in WHO 1990, WHO 1999 and ATSDR 2005). Reanalysis of the slides from the high dose group failed to confirm the increase in micronucleated PCEs at the last sampling time (WHO 1990).

In other micronucleus studies, TBTO did not increase the number of PCE in mice at 31.25 and 62.5 mg/kg b.w. (Reimann & Lang, 1987 – quoted in WHO 1990) or at 125 mg/kg b.w. (Schweinfurth & Gunzel 1987 – quoted in ATSDR 2005).

TBTO, together with triphenyltin chloride (TPTCl), has been showed to be a co-clastogen in mice. Oral administration of 50 mg TBTO and 100 mg TPTCL/kg b.w. enhanced the number of mytomycin C-induced micronucleated peripheral reticulocytes approximately 50%. No effect were seen when TBTO (100 mg/kg b.w.) was given separately. (Yamada & Sasaki 1993 – quoted in WHO 1999 and ATSDR 2005).

4.7 Carcinogenic effects

4.7.1 Inhalation

No studies were located regarding cancer effects in animals following inhalation exposure to TBT compounds.

4.7.2 Oral intake

Groups of 60 male and 60 female rats (Wistar) were exposed to dietary TBTO for 2 years. The ingested doses were approximately 0.019, 0.19 or 2.1 mg/kg b.w./day in males and 0.025, 0.25 or 2.5 mg/kg b.w./day in females. At termination in high dose groups survival was decreased (males: 40 versus 60% in controls, females: 54 versus 74% in controls) and body weight was reduced. Increased incidence of benign pituitary tumours were observed in both sexes from the low- and high-dose groups, but not in the mid-dose groups (Table 3). Other endocrine-related tumours such as pheochromocytomas in the adrenal medulla and parathyroid adenomas were found only at high dose and regarding parathyroid adenomas it was only found in males. (Wester et al. 1990 – quoted in EFSA 2004, ATSDR 2005 and US-EPA 1997). According to the authors these tumours normally occur in this rat strain with high and variable incidence. Reported background occurrence of these tumours is listed in Table 4.

Table 4. Neoplastic lesions in rats

Conc. of TBTO (mg/kg diet)	Pituitary Tumours[a]		Pheochromo-cytomas[a]		Parathyroid adenomas[b]	
	female	male	female	male	female	male
0	22/50	34/50	3/50	16/50	0/64	0/39
0.025	32/50*	39/50*	3/50	13/50	0/44	2/50
0.25	22/50	29/50	3/50	14/50	1/40	1/51
2.5	35/50**	43/50***	34/50***	33/50***	1/44	6/43**
Reported background occurence[c]	32% and 55%	34% and 66%	10% and 12%	26% and 44%	-	-

(Wester et al.1990)
[a]Values marked wit asterisks differ significantly (Peto, one tailed) from control values (*P<0.05;**P<0.01;***P<0.0001)
[b]Values marked wit asterisks differ significantly (chi-square test) from control values (**P<0.01)
[c](Kroes et al. 1981 and Wester et al. 1985 – quoted in US-EPA 1997).

Groups of 50 male and 50 female mice (CD-1) were exposed to dietary TBTO (purity 97.1%) at doses of 0, 0.75, 3.75 and 7.5 mg/kg b.w./day for 18 months. Survival was significantly reduced in treated animals, and there was an increase in common spontaneous non-neoplasic lesions, particularly glomarular/interstitial amyloidosis of the kidney. There was no statistical increase in the incidence of any tumour or groups of tumours. (Daly 1992 – quoted in US-EPA, 1997, WHO 1999, EFSA 2004).

4.7.3 Dermal contact

In a limited evaluation of carcinogenicity following dermal exposure, TBTFl (15 mg of 5% or 10% TBTFl in propylene glycol) was applied to the shaved backs of male white mice 3 times/week during 6 month. Hyperplastic changes were observed in the 5% group, but not in the 10% group. Carcinogenic effects were not observed (Sheldon 1975 – quoted in ATSDR 2005).

5 Regulations

5.1 Ambient air

Denmark (C-value):	0.0005 mg/m^3 (TBTO, tributyltinnaphethenat) (MST 2002)
WHO:	-
US-EPA :	-

5.2 Drinking water

Denmark:	-
WHO:	2 µg TBTO/L (WHO 1996) The guideline value was derived on the following basis: The most sensitive end-point appears to be immunotoxicity, with a lowest NOAEL of 0.025 mg/kg of body weight per day in a 17-month feeding study in rats related to the suppression of resistance to the nematode *Trichinella spiralis* (Vos et al. 1990). The significance to humans of this finding is not completely clear, but this NOAEL is consistent, within an order of magnitude, with other NOAELs for long-term toxicity. A TDI of 0.25 µg/kg of body weight was calculated by applying an uncertainty factor of 100 (for inter- and intraspecies variation) to the NOAEL of 0.025 mg/kg of body weight per day. The guideline value for TBTO is 2 µg/litre (rounded figure) based on an allocation of 20% of the TDI to drinking water.
US-EPA:	-

5.3 Soil

Denmark:	-	
The Netherlands:	Target value:	0.00002 mg/kg dw
	Target value (salt waters):	0.000007 mg/kg dw
	Intervention value:	2.5 mg/kg dw

Note: The intervention value applies to the total concentration of observed an-organic compounds. The target value stated indicates the NR level. When testing for quality, the target value for the sum of anorganic compounds of 0.001 mg/kg dw is used. (NL 1999).

5.4 Sediment

The Netherlands:	Target value:	0.0001 mg/kg dw
	Intervention value:	2.5 mg/kg dw
	Note: The intervention value applies to the total concentration of observed anorganic compounds. The target value stated indicates the NR level. When testing for quality, the target value for the sum of anorganic compounds of 0.001 mg/kg dw is used. (NL 1999).	

5.5 Occupational Exposure Limits

Denmark:	0.002 ppm (0.05 mg/m^3) for tri-n-butyltin compounds (AT 2005)
ACGIH:	0.1 mg/m^3 (ACGIH 2003 – quoted in ATSDR 2005)
Germany:	0.05 mg/m^3 for tributyltin compounds (TBTO, TBTCl, TBTF, TBT-benzoate, TBT-linoleate, TBT-metachrylate and TBT-naphtenate) (MAK 2005)

5.6 Classification

Tribytultin-compounds are classified for acute toxic effects (Xn; R21 - harmful in contact with skin; T;R25 - toxic if swallowed), for effects following repeated exposure (T;R48/23/25 - toxic: danger of serious damage to health by prolonged exposure through inhalation and if swallowed), for irritative effects (Xi; R36/38 - irritating to eyes and skin) and for toxicity to the environment (N; R50/53 - very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment) (MM 2002).

5.7 IARC

-

5.8 US-EPA

US-EPA has assigned TBTO to group D weight-of-evidence classification: not classifiable as to human carcinogenicity (under the 1986 cancer guidelines) or to the “cannot be determined” category (under the 1996 proposed cancer guidelines) (IRIS 2003).

US-EPA derived an oral Reference Dose (RfD) of 0.0003 mg/kg/day for TBTO using a benchmark dose analysis of immunological effects in rats in the 18-month dietary study by Voss et al. (1990). A 10% relative change was chosen as the benchmark response (BMR). (IRIS 2003).

The health effects data for tributyltin oxide (TBTO) were determined to be inadequate for derivation of an inhalation RfC as it lacks the minimum criteria for derivation of an RfC (i.e., a 90-day inhalation bioassay) (IRIS 2003).

5.9 ATSDR

ATSDR derived an intermediate-duration oral MRL of 0.0003 mg/kg/day for TBTO based on a NOAEL of 0.025 mg/kg/day for immunological effects in rats in a 4.5-6 month dietary study in rats made by Voss et al. (1990) and an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability) (ATSDR 2005).

5.10 EFSA

EFSA established a group Tolerable Daily Intake (TDI) of 0.25 µg/kg b.w./day for TBT, DBT, TPT and DOT. This value is based on a NOAEL for TBTO of 0.025 mg/kg/day for immunological effects in rats in chronic dietary studies (Voss et al. 1990, Wester et al. 1988, 1999) and applying an uncertainty factor of 100 (EFSA 2004). The effects of these compounds were considered as additive as they exert their immunotoxicological effects by similar mode of action and potency. The TDI is 0.1 µg/kg b.w./day when expressed as Sn content. (EFSA 2004).

5.11 WHO

WHO calculated a TDI of 0.25 µg/kg b.w./day for TBT. The value is based on a NOAEL for TBTO of 0.025 mg/kg/day for immunological effects observed in rats in a chronic dietary study made by Voss et al. (1990) and applying an uncertainty factor of 100 (for intra- and interspecies variation) (WHO 1996).
The guideline value for TBTO in drinking water is 2 µg/litre (rounded figure) based on an allocation of 20% of the TDI to drinking water and a daily intake of 2 litres of drinking water for an adult weighing 70 kg.

6 Summary and evaluation

6.1 Description

The term tributyltin (TBT) covers several TBT-derivates and the chemical difference between the derivates is the nature of the 4th group linked to the tin atom e.g. Cl, F, OH or CO_3. Tributyltin oxide (TBTO) is the TBT-compound most intensively investigated.
TBT-compounds have been used for various biocidal purposes e.g. antifouling paints for boats, preservative for wood and textiles, slimicides in industrial processes and on masonry and as molluscicides.
TBTO is a slightly yellow liquid. The solubility in water is 4 mg/L and the $LogP_{o/w}$ range from 3.2-3.9. The reported vapour pressure is low ($7.5*10^{-6}$ mmHg).

6.2 Environment

TBT-compounds have been found in water, sediment and biota. Levels are elevated in areas close to pleasure boating activity, commercial harbours cooling systems and fish net and cages treated with TBT.

As a result of its physical and chemical properties a great part (10 to 90%) of the TBT released to natural waters will bind to suspended particles in the water or associate to dissolved organic matter. As particles in the water column may settle out, TBT is removed from the water and introduced into the sediment.

In the water, both abiotic and biotic degradation of TBT occur. However, the importance of both degradation processes varies considerably with varying environmental conditions. The half-live of TBT in the seawater is generally estimated to range from 1 day to few weeks.
In recent investigations the reported range of concentrations in coastal waters and estuaries was 1-10 ng/L and in marinas and major ports 20-460 ng/L.

TBT are likely to partition to the sediment and TBT sorption coefficients to sediments can range from 10-10,000. In sediments, TBT is generally persistent and half-lives have been estimated to be several years. Therefore, in spite of the restricted use of TBT, the concentration in sediment has remained relatively high. In recent investigations most sediment samples were below 100 µg/kg although some samples exceeded 1 mg/kg.
In Denmark, concentrations of TBT measured in estuaries and coastal water sediments from 1998 to 2004 ranged from 1.2 to 160.6 µg/kg dw.

No information about release, fate or concentration of TBT in soil and air has been found. However, release to air is not expected due to the low vapour pressure of TBT.

TBTO has a moderately high octanol-water partition coefficient and bioaccumulation in aquatic organisms occurs. Bioconcentration factors (BCF) of up to 7,000 have been reported for molluscs and fish (laboratory experiments). Bioaccumulation of TBT in bivalve molluscs is especially high due to the limited

metabolic capacity of these organisms. There is no evidence for biomagnification of TBT in marine ecosystems.

In foodstuff, TBT is predominantly present in seafood. Concentrations of TBT in seafood collected in 8 European countries in 1995 to 2002 were 7.0 and 28 µg/kg ww (median and mean, respectively). In samples collected in 1997 in 8 different cities throughout the world, the average TBT concentration in bivalve molluscs, pelagic fish, pelagic invertebrates (e.g. crustaceans and cephalopods) and flatfish was 40, 16, 7.4 and 4.6 µg/kg, respectively. In Denmark, TBT concentrations in mussels sampled from 1998 to 2004 ranged from 1.2 to 146 µg/kg d. In another danish survey, mean concentrations of TBT in various seafood ranged from below 0.24 to 29.7 µg TBT (unspecified)/kg ww).

6.3 Human exposure

The general human population is predominantly exposed to TBT via the diet especially the seafood diet.
In Japan in 1991/1992, the daily intake of TBT via seafood has been estimated to range from 2.2 to 6.9 µg/day/person depending on study-design. More recently, TBT intake estimated based on data from 8 cities throughout the world ranged from 0.8 (England) to 2.6 (Korea) µg/day/person. In a recent study based on Norwegian seafood consumption data and international concentrations found in seafood, the daily intake of TBT was estimated to be in the same order; 0.009 to 0.08 µg/kg b.w./day (equivalent to 0.63-5.6 µg/day/person assuming a body weight of 70 kg) using either average or 95th percentile consumption rates and either median or mean international TBT occurrence levels in seafood.

6.4 Toxicokinetics

TBT is slowly and incomplete absorbed via the gastrointestinal tract (20-55%) and via the skin of mammals (1 to 10%). No quantitative information regarding absorption via the respiratory tract was located.

Following absorption, TBT are rapidly and widely distributed among tissue. In a number of animal species, it is distributed especially to liver and kidney and to a lesser extent to spleen, fat, lung and muscle. It can be transferred across the blood - brain barrier as well as via the placenta to the foetus.

TBT metabolism is rapid. TBT-metabolites are detectable in the blood and liver within 3 hours of administration. The metabolic pathway of TBT particularly in liver is characterised by the cytochrome P-450 dependent hydroxylation and de-alkylation converting TBT to DBT and butanol. The butyl moieties can also be oxidised at carbon 3 and carbon 4 to yield different hydroxy-, carboxy- and oxo-metabolites. Liver contains a relatively high fraction of TBT-metabolites, whereas e.g. brain and fatty tissue contain a higher fraction of unchanged TBT. In several investigations, levels of TBT in human liver samples were below detection limit.

The rate of loss of TBT is tissue specific; highest clearance was seen in blood and liver followed by kidney, then fatty tissue and complete retention in lung. The principal route of excretion is via the faeces rather than via the urine. Biological half-life (whole body radioactivity) in mouse was estimated to be 23-29 days.

6.5 Mode of action

TBT induces cell death and apoptosis *in vitro* as well as *in vivo*. Several mechanisms seem to play a role in these effects of TBT: elevation of the intracellular calcium concentration which may lead to activation of proteases and caspases (disruption of the cytoskeleton) and phospholipases (impairment of mitochondrial function with a collapse of membrane potential); inhibition of substrate uptake and ATP synthesis by mitochondria, induction of their swelling and alteration of the ion transport across membranes; and binding of TBT to extrinsic "death receptors".

There is evidence that these biochemical mechanisms of cell death and especially the induction of programmed cell death is a part of the immunosuppressive effect of TBT, but also suppression of cell proliferation in immature thymocytes by TBT is thought to play a role.

6.6 Human toxicity

There are case reports regarding acute human inhalation and dermal exposure to TBT mainly through exposure to TBTO-containing paints. The reported symptoms are indicative of an irritating potential of TBTO; both in the respiratory tract, the eyes and the skin.

6.7 Animal toxicity

6.7.1 Single dose toxicity

LC_{50}-values (4 hours) in rats of 65 and 77 mg/m^3 have been reported for TBTO aerosols. Guinea pigs exposed to either 200 (males) or 1,000 (females) mg TBTO/m^3 for 4 hours died. Exposure of rats to almost saturated vapour of various TBT-compounds produced no effects.

Acute oral LD_{50}-values for various TBT-compounds are comparable. In rat, oral LD_{50} values of TBT-compounds range from 94 to 234 mg/kg b.w., in mouse between 44 and 230 mg/kg b.w.

Acute dermal LD_{50}-values of 9,000 (unspecified) and 11,700 (TBTO) mg/kg b.w. have been reported for rabbits. For rats, the reported LD_{50} value for TBT (unspecified) was 605 mg/kg.

Toxicological effects of acute exposure are characteristically delayed for a couple of days and beside lethal effect they may include alterations in blood lipid levels, effects on the endocrine system, damage of liver and spleen and transient deficits in the brain development.

TBT-compounds are potent skin irritants; acute studies have demonstrated the skin irritating potential of TBTO and TBT-chloride (TBTCl) when applied to rat skin and of TBTO when applied to rabbit skin. The compounds are also potent irritants to rabbit eye with necrotic corneal injuries reported.

With regard to sensitisation TBTO showed no potential as such in a Magnusson-Kligman Maximization test in guinea pigs. In an ear swelling test in mice 0.25% (v/v) TBTO triggered a positive response.

6.7.2 Repeated dose toxicity

Exposure to TBTO aerosol (2.8 mg/m^3) 5 days per week for a total of 21-24 treatments produced high mortality, inflammatory reactions in the respiratory tract and histological changes in the lymphatic organs in rats. Exposure to the highest attainable vapour concentration (0.16 mg/m^3) produced no effects.

Exposure of rats to TBTCl in a 95-days inhalation study (4 and 6 mg/m^3) showed minor irritation in eye and nose and at termination there were inflammatory changes in the respiratory tract of exposed animals. Changes in liver weight and at necroscopy fatty degeneration in liver were observed.

A number of oral studies with TBTO have been performed mainly in rats but also in mice and monkey where the durations of exposure range from few weeks up to 2 years (see Table 2).

General toxic effects including reduced survival (in rats at 2.1 mg/kg b.w./day for 104 weeks, in mice from 0.75 mg/kg b.w./day for 18-month), decreased body weight (in rats at 2.1 mg/kg b.w./day for 104 weeks) or body weight gain (in rats from 1.4 mg/kg b.w./day for 4 week) as well as increased weight of liver (in rats at 2.1 mg/kg b.w./day for 104 weeks and 1.4 mg/kg b.w./day for 1 week; in mice at 7.5 mg/kg b.w./day for 18-month) and kidney (in rats at 2.1 mg/kg b.w./day for 104 weeks). Histological changes were also observed in the liver of rats (bile duct changes at 2.1 mg/kg b.w./day for 104 weeks; atrophy in centrilobular hepatocytes from 2 mg/kg b.w./day for 4 weeks).

Toxic response of the endocrine system was observed in rats as a decrease in thyroid weight together with histological changes (at 2.1 mg/kg b.w./day for 104 weeks (females only) and at 8 mg/kg b.w./day for 6 weeks). In the 6-week study, also serum levels of insulin were decreased (from 2 mg/kg b.w./day) as well as serum levels of thyroxine and TSH (at 8 mg/kg b.w./day). In the 104-week study, no significant changes in serum levels of hormones were observed, but a decrease in the free thyroxine:total thyroxine ratio (from 0.19 mg/kg b.w./day) was observed. However, at this dose (0.19 mg/kg b.w./day) the decrease in FT4:T4 was transient.

Effects in the immune system were also observed in rats following oral exposure to TBTO. Decreased weight of the thymus has been demonstrated in several studies e.g. in animals exposed to 2.5 mg/kg b.w./day for 4-6 month or to 1.4 mg/kg b.w./day for 4 weeks. A decrease in the weight of the spleen has also been reported (at 8 mg/kg b.w./day for 6 weeks) together with histopathological changes in the spleen (at 2.1 mg/kg b.w./day for 104 weeks) and in the thymus and the spleen (from 2 mg/kg b.w./day for 4 weeks and 1.4 mg/kg b.w./day for 1 week). Evidence of haemorrhage in lymph nodes in rats exposed from 0.4 or 0.5 mg/kg b.w./day for 4 weeks was reported in 2 studies. In other isolated studies effects on white blood cells in form of increased number of leucocytes (in monkeys, 1.4 mg/kg b.w./day), decreased number of lymphocytes (in rats, 0.19 mg/kg b.w./day for 104 weeks) or changes in the percentage of mesenteric lymph node T-lymphocytes (from 0.25 mg/kg b.w./day for 4-6 month) were observed.
In 5 studies and at relatively low doses (e.g. from 2 mg/kg b.w./day for 6 weeks and 0.25 mg/kg b.w./day for 4-6 month) decreased resistance to either cytomegalovirus, *Listeria* or *Trichinella* was reported. Furthermore, in the 104-week dietary study with rats, an increase in serum immunoglobulin (2.1 mg/kg b.w./day) was reported.

Regarding neurotoxicity, slight neurological effects were seen at relatively high doses; Rats exhibited ptosis or enophthalmia and slight ataxia following 4-week exposure to 30 mg/kg/day TBTO with no evidence of brain oedema.
Aggression and seizures were seen in rats exposed to lethal doses of TBTO for 3 days. There was a complete absence of Purkinje cells in the cerebellum day 7 following treatment and the activity of total brain ATPaseNa^{+}/Ka^{+} ATPase and Mg^{2+} ATPase was suppressed.
Oral administration of TBTOAc (100 mg/kg diet to male rats and lethal dose to female rats) in 3 months, induced interstitial oedema of the white matter of the central nervous system.

Following daily dermal exposure (50 days) to TBTO on the shaved dorsal skin of guinea-pigs at doses equivalent to 10 and 40 mg/kg b.w. histological lesions of the kidney tubules (swelling, degeneration and destruction of tubular epithelium) were observed.

6.7.3 Toxicity to reproduction

Only one study regarding reproductive performance following inhalation exposure was located.
Pregnancy rates were markedly reduced in rats after 4 weeks to 3 months of inhalation exposure to a mixture of TBT-bromide (81.2%) with other compounds at concentration of 2 mg Sn/m^2. No histopathological changes were seen in males whereas in females atrophy of the glandular uterus was observed. The effects were reversible. No studies regarding developmental effects following inhalation exposure were located.

A number of reproductive and developmental studies with oral exposure to TBTO and TBTCl in rats, mice and rabbits have been performed with exposure durations from a few days during gestation and up to 2-generations (see Table 3).

Reduced weight of testis was observed in rats (from 0.25 mg TBTCl/kg b.w./day, 2-generations study). Other effects of the male reproductive system in rat in form of reduced weight of epididymis and ventral prostate, reduced sperm or spermatid counts, histopathological observations in testis and alterations of level of several hormones were observed at higher doses.

Observed effects on the female reproductive system in rat included an increased ano-genital distance (from 0.25 mg TBTCl /kg b.w./day, 2-generations study) and at higher doses also decreased ovarian and uterus weight, delayed vaginal opening and altered oestrus cyclicity were observed.
Reduced litter size, increased post implantation loss or increased percentages of resorptions was reported for rats (from 6.25 mg TBTCl/kg b.w./day (2-generations) and from 10 mg TBTO/kg b.w./day (gestation day 6-20)) and mice (from 5 mg TBTO/kg b.w./day (gestation day 6-15)). At these doses maternal toxicity (decreased body weight or body weight gain) was also observed.

Developmental effects in form of minor skeleton abnormalities, increased frequency of cleft palate and decreased body weight of either pups or foetuses or body weight gain of pups were observed in rats (from 3.43 mg TBTO/kg b.w./day (2-generations) and from 8.1 mg TBTCl/kg b.w./day (gestation day (GD) 0-3)) and in mice (from 10 mg TBTO/kg b.w./day (GD 6-15)). At these doses maternal toxicity (decreased body weight or body weight gain) was also observed.

No reproductive or developmental studies following dermal exposure to TBT-compounds have been located.

As depression of the immunological response seems to be one of the critical effects of TBT-compounds three studies (2 in rats and 1 in mice) have focused on the potential of TBTO to exert developmental immunotoxicity.

In an *in utero* exposure study in rat reductions in mitogen responses were observed (from 5 mg/kg b.w./day) as well as suppression of the mixed lymphocyte reaction and natural killer cell activity in pre-weanlings (from 10 mg/kg b.w./day).

In a comprehensive *in utero* as well as postnatal exposure study of TBTCl in rats all doses of TBTCl (from 0.025 mg/kg b.w./day) significantly affected parameters of the growth profile of the pups as well as the ratio of weekly food consumption to weekly body weight gain. Furthermore decreased liver weight was observed in low and high dose (0.025 and 2.5 mg/kg b.w./day). No histopathological changes in the liver were seen, but some markers (serum triglycerides, creatine, magnesium, amylase) were reduced. At the high dose, serum thyroxine levels were also reduced.
Effects on the immune function included decreases in spleen and thymus weight (2.5 mg/kg b.w./day), increased natural killer cell numbers (2.5 mg/kg b.w./day) and activity (from 0.025 mg/kg b.w./day, non linear dose-response) as well as increased mean percentage of CD4(+)8(+) (immature)T lymphocytes (from 0.25 mg/kg b.w./day). Serum immunoglobulin (IgG or IgM) levels were increased (IgM: from 0.025 mg/kg b.w./day, IgG: from 0.25 mg/kg b.w./day) and host defence against *L. monocytogenes* was suppressed (0.25 mg/kg b.w./day). Several parameters (e.g. number of *L. monocytogenes* in spleen and delayed-type hypersensitivity) were increased at all doses. However, the increase was only significant for trend; not in pair wise comparisons with control.

In mice, effects in offspring following *in utero* exposure included suppressed primary antibody response to sheep red blood cells, increased number of leucocytes as well as suppressed delayed-type hypersensivity to sheep red blood cells (from 0.1 mg/kg b.w./day). However, the full publication of this study is not available.

Regarding developmental neurotoxicity, offspring of rats showed evidence of alterations of behaviour after oral administration of TBTCl (from 1 mg/kg b.w.) at day 6-20 of gestation. Neurohistological changes were not investigated in the study.
In another study at relatively high concentrations (10 mg/kg b.w./day), brain weight and motor activity was significantly reduced.

6.7.4 Mutagenic and genotoxic effects

TBTO was not mutagenic in *in vitro* test with *Bacillus Subtilis* and *Klebsiella pneumoniae*. In the vast majority of tests and strains of *Salmonella typhimurium* TBTO did not induce point mutations. However, TBTO was mutagenic in *S. typhimurium* TA100 in a fluctuation test in the presence of S9 at cytotoxic concentrations and in another study in absence of S9. TBTO did not induce mutations in yeast, insects or Chinese hamster and mouse lymphoma cells.

TBTO did not cause sister chromatid exchange in Chinese hamster ovary cell or chromosomal aberrations in human lympocytes. However, structural chromosomal aberrations (endoreduplicated and polyploid cells) were observed in Chinese

hamster ovary cells. The aberrations were detected only at the first sampling time and only at the highest concentration tested where also cytotoxicity was observed.

In two of three *in vivo* micronucleus tests TBTO did not increase the incidence of micronucleated cells in mice. In the third study, an increase in micronucleated PCEs were observed at the highest dose tested at the last sampling time. However, reanalysis of the slides from the high dose group failed to confirm this increase.

TBTO, together with triphenyltin chloride (TPTCl), has been showed to be a co-clastogen in mice.

6.7.5 Carcinogenic effects

No studies were located regarding cancer effects in animals following inhalation exposure to TBT-compounds.

Two oral carcinogenicity studies with TBTO in two different animal species have been located.
In a 2-year dietary study with rats a significant increase in the incidence of benign pituitary tumours were observed in both sexes in the low (0.019/0.025 mg/kg b.w./day) and high (2.1/2.5 mg/kg b.w./day)-dose group, but not in the mid-dose group. Other endocrine-related tumours such as pheochromocytomas in the adrenal medulla and parathyroid adenomas (only males) were found only at the high dose group.
In an 18-month dietary study with mice, there was an increase in common spontaneous non-neoplasic lesions, particularly glomerular/interstitial amyloidosis of the kidney. There was no statistical increase in the incidence of any tumour or groups of tumours.

In one limited evaluation of carcinogenicity following dermal exposure TBT-flouride induced hyperplastic skin changes when applied to mice skin in 5% solution, but not in 10% solution. Carcinogenic effects were not observed in this study.

6.8 Evaluation

TBT is slowly and incomplete absorbed via the gastrointestinal tract (20-55%) and via the skin of mammals (1 to 10%). TBT are rapidly and widely distributed among tissue and it can be transferred across the blood - brain barrier as well as via the placenta to the foetus. TBT metabolism is rapid and the principal route of excretion is via the faeces rather than via the urine.

TBT-compounds are moderately to highly acute toxic to laboratory animals with oral LD_{50} values of TBT-compounds ranging from 94 to 234 mg/kg b.w. in rats and 44 and 230 mg/kg b.w. in mice.

TBT-compounds are potent skin irritants in rat and rabbit and also potent irritants to rabbit eye. With regard to sensitisation, two different studies gives contradictory results; one test in Guinea pigs showed no sensitisation potential of TBTO whereas in an ear-swelling test in mice 0.25% (v/v) TBTO triggered a positive response. Based on the available data no conclusion with regard to sensitisation can be taken.

A number of repeated oral dose studies mainly in rats but also in mice and monkey with exposure durations from few weeks up to 2 years have been performed (see Table 2). The only TBT-compound tested in these studies was TBTO.
Several general toxic effects were seen at almost similar doses (in rats, from 1.4 mg/kg b.w./day for 1 or 4 weeks or from 2.1 mg/kg b.w./day for 104 weeks, in mice from 0.75 or 7.5 mg/kg b.w./day for 18-month) including decreased survival, decreased body weight or body weight gain as well as increased weight and histopathology of liver.
Toxic response of the endocrine system in form of decreased weight and histopathology of thyroid, decreased weight of adrenal glands as well as decreased serum levels of insulin, thyroxine and TSH was observed in 2 studies of rats at similar dose levels (2.1 mg/kg b.w./day for 104 weeks – females only, 2 or 8 mg/kg b.w./day for 6 weeks). In the 104 week study, effect on endocrine function in form of a decrease in the free thyroxine:total thyroxine ratio was observed at a lower dose (0.19 mg/kg b.w./day for 104 weeks). However, at this dose the decrease in FT4:T4 was transient.
Effects on the immune system in form of either decreased weight or histopathology of thymus or decreased weight or histopathology of spleen were observed in 8 different studies with rats at similar dose levels (e.g. at 2.1 mg/kg b.w./day for 104 weeks, 2.5 mg/kg b.w./day for 4-6 month, 1.4 mg/kg b.w./day for 1 or 4 weeks and 2 mg/kg b.w./day for 1 week). At a similar dose level increase in serum immunoglobulin was also observed in one study (2.1 mg/kg b.w./day for 104 weeks). At lower doses in isolated studies, evidence of haemorrhage in lymph nodes (in rats, from 0.4 or 0.5 mg/kg b.w./day for 4 weeks), increased number of leucocytes (in monkeys, 0.14 mg/kg b.w./day), decreased number of lymphocytes (in rats, 0.19 mg/kg b.w./day for 104 weeks) or changes in the percentage of mesenteric lymph node T-lymphocytes (from 0.25 mg/kg b.w./day for 4-6 month) were also reported. In 5 studies decreased resistance to either cytomegalovirus, *Listeria* or *Trichinella* was reported (e.g. from 2 mg/kg b.w./day for 6 weeks and 0.25 mg/kg b.w./day for 4-6 month). The immunosuppressive effect of TBT can be explained by several biochemical mechanisms e.g. the induction of apoptosis in immuno competent cells.
Although observed changes in immunological as well as hormonal parameters in the 104-weeks repeated oral dose toxicity study in rats with TBTO were marginal and sometimes transient, a NOAEL of 0.019 mg/kg b.w./day can be established for the immune- and endocrine system. As supporting evidence, an almost similar NOAEL (0.025 mg/kg b.w./day) can be established in the 4-6 months study in rats for immune response (e.g. host defence against *Trichinella*).

Trimethyltin and triethyltin are well known potent neurotoxic compounds. TBT-compounds in contrast cause no severe neurological signs or histopathological changes in brain tissue of rats. Although transfer of TBT across the blood:brain barrier into the central nervous system occur, existing studies does not convincingly show that TBT is neurotoxic at non-lethal doses. There are some indications of neurobehavioral effects following exposure (including *in utero* exposure) to TBT-compounds with the lowest effect level being 1 mg TBTCl/ kg b.w./day in a neuro-developmental study. However, the potential for neurotoxicity has not been completely investigated with focused studies.

Two oral carcinogenicity studies with TBTO in two different animal species have been located. The cancer bioassay in mice showed no increase in the incidence of any tumour or groups of tumours.
The bioassay in rats showed a significant increase in the incidence of benign pituitary tumours at the lowest (0.019/0.025 mg/kg b.w./day) and highest (2.5 mg/kg b.w./day) dose tested. Other endocrine- related tumours were found only at the high dose group where toxicity was induced in various organs. The observed

tumours were considered to have high and variable spontaneous incidence in the rat strain used (Wistar). Furthermore, the effect was not dose-related, as it did not occur in the mid-dose group and the significance of the increase in incidence of these tumours is therefore uncertain. No mechanism of tumour induction has been established. The genotoxicity of TBTO has been investigated in multiple *in vitro* and *in vivo* tests with vide variety of genetic endpoints. Negative results were obtained in the vast majority of these test and the few positive findings were mainly at cytotoxic concentrations. The weight of evidence shows that TBTO has no genotoxic potential. Therefore, if TBT induce tumour formation, it may probably be triggered by hormonal and/or immunological actions and may only be relevant at cytotoxic doses. It is therefore expected that a NOAEL established based on changes in hormonal and/or immunological parameters will also protect against a potential induction of tumour formation by TBT.

A number of reproductive and developmental studies following oral exposure to TBTO and TBTCl in rats, mice and rabbits with exposure durations from a few days during gestation and up to 2-generations have been performed (see Table 3). In a recent 2-generation study in rats, effects on the male reproductive system in offspring in form of reduced weight of testis in all doses tested (from 0.25 mg TBTCl/kg b.w./day) and at higher doses reduced weight of epididymis and ventral prostate, reduced sperm or spermatid counts, histopathological observations in testis and alterations of level of several hormones. In the same study, observed effects on the female reproductive system in offspring included an increased ano-genital distance (also from 0.25 mg TBTCl /kg b.w./day) and at higher doses also decreased ovarian and uterus weight, delayed vaginal opening and altered oestrus cyclicity.
Other reproductive effects observed following TBT exposure during pregnancy was reduced litter size, increased post implantation loss or increased percentages of resorptions in rats and mice. These effects as well as developmental effects in form of minor skeleton abnormalities, increased frequency of cleft palate and decreased body weight of either pups or foetuses or body weight gain of pups were mainly seen at doses were also maternal toxicity was observed.
However, in a recent and comprehensive *in utero* as well as postnatal exposure study of TBTCl in rats general toxicological parameters (the growth profile of the pups the ratio of weekly food consumption to weekly body weight gain as well as liver weight) were affected at the lowest dose (from 0.025 mg/kg b.w./day). However, the decrease in liver weight did not show dose-dependence and no histological changes in liver were seen. Some markers indicating hepatotoxicity were affected significantly mainly at the high-dose (2.5 mg/kg b.w./day).
The main objective of the study however, was to investigate the immunotoxic potential of TBTCl in rats and the effects seen at the lowest dose tested (0.025 mg/kg b.w./day) included increased natural killer cell activity (non-linear dose response) and serum IgM level. The increased IgM level were only significant different from control in the low- and high-dose group; however in the mid-dose group the level were also elevated. Immunoglobulin levels (IgM and IgG) were also increased in males. This increase was only significant at mid- and high doses, but the levels were also elevated at low dose. Several parameters (e.g. number of L. monocytogenes in spleen and delayed-type hypersensitivity) were also increased at the lowest dose. However, the increase was only significant for trend; not in pair wise comparisons. Effects observed at higher doses were increased mean percentage of CD4(+)8(+) (immature) T lymphocytes, suppressed host defence against *L. monocytogenes* increased number of natural killer cells and decreased spleen and thymus weight
Significant effects on the immune system at the lowest dose tested (0.025 mg/kg b.w./day) were not as frequently observed as in the mid- and high dose group, and the effects did not show dose-dependence. However, significant effects were seen

at the low dose. Furthermore, in the overall picture the effects seen at the low dose is supported by significant effects seen at higher doses as well as of not-significant changes in immunological parameters at the low dose; e.g. IgM in females were not the only elevated serum immunoglobulin level as elevated IgM as well as IgG levels were also observed in the low dose group of males. Therefore, based on changes in immunological parameters (increased natural killer cell activity and serum IgM level) a LOAEL of 0.025 mg/kg b.w./day can be established. This LOAEL also covers the more general effects seen in rats on pup growth parameters and liver weight as well as the depression of functionel immune response in mice offspring following *in utero* exposure to TBTO (from 0.1 mg/kg b.w./day). No NOAEL for developmental effects can be established.

6.8.1 Critical effect and NOAEL

Although the observation with regard endocrine toxicity (NOAEL: 0.019 mg/kg b.w./day) give some support for concern, the critical endpoint for establishing health based quality criteria for TBT in soil and drinking water is considered to be their immunotoxic action with the suppression of thymus dependent immunity. These effects observed mainly in rats but also in mice, are considered to have relevance for humans as *in vitro* studies with human lymphocytes indicated sensitivity to TBT.

A NOAEL cannot be established as effects in the immune system were seen at the lowest dose tested in the *in utero* and postnatal exposure study with TBTCl made by Tryphonas et al. (2004). Based on this study, a LOAEL of 0.025 mg/kg b.w./day can be identified.

The immunosuppressive effect was seen for both TBTO and TBTCl. TBTCl is the substance for which a LOAEL is established, however this has not necessarily anything to do with the potency of TBTCl versus the potency of TBTO. It is probably more likely due to differences in study designs (doses, measured endpoints etc.) between TBTCl-studies and TBTO studies.
Furthermore, it is reasonable to assume that similar effects can be expected for other TBT-compounds due to the activation of apoptotic mechanisms in immuno competent cells. Based on similar mode of action, the effects of the different TBT compounds can be considered additive.

7 TDI and quality criteria

7.1 TDI

The TDI is calculated based on a LOAEL of 0.025 mg/kg b.w./day observed for effects of TBTCl in the immune system (Tryphonas et al. 2004):

$$TDI = \frac{LOAEL}{UF_I * UF_{II} * UF_{III}} = \frac{0.025 \text{ mg/kg b.w./day}}{10 * 10 * 3} = 0.08 \text{ µg/kg b.w./day}$$

The uncertainty factor UF_I accounting for interspecies variability is set to 10 assuming that humans are more sensitive than animals. The UF_{II} accounting for intraspecies variability is set to 10 reflecting the range in biological sensitivity within the human population. The UF_{III} is set to 3 because of using a LOAEL instead of a NOAEL.

Based on the TBTCl molecular mass, this TDI is equivalent to 0.03 µg/kg b.w./day when expressed as Sn, 0.074 µg/kg b.w./day when expressed as TBTO and 0.072 µg/kg b.w./day when expressed as unspecified TBT-compound.

7.2 Allocation

Possible sources for human exposure to TBT are food and drinking water, contaminated soil and sediment, ambient and indoor air and TBT containing consumer products.

The contribution to human exposure of TBT via consumer products is expected to be relatively low and no information exist regarding the exposure via air and drinking water.

The intake via the diet and especially the seafood diet is the most important source of exposure to the general population. According to exposure estimates from Norway, the intake of TBT especially for high seafood consumers (0.02 µg/kg b.w./day or 0.08 µg/kg b.w./day) is at the same level as the calculated TDI (0.08 µg/kg b.w./day).

Therefore, only 50% of the TDI is allocated to ingestion of soil and only 10% to ingestion of drinking water.

7.3 Quality criterion in soil

The quality criterion for TBT in soil QC_{soil} is calculated based on the TDI of 0.03 µg Sn/kg b.w. per day and assuming a daily ingestion of 0.2 g soil ($ingestion_{soil}$) for a child weighing 13 kg (w_{child}):

$$QC_{soil} = \frac{TDI * X * w_{child}}{ingestion_{soil}} = \frac{30 * 10^{-6} \text{ mg Sn/kg day} * 0.5 * 13 \text{ kg}}{0.0002 \text{ kg/day}}$$

$$= 1 \text{ mg Sn/kg soil (rounded figure)}$$

7.3.1 Quality criteria in soil

Quality criterion: 1 mg Sn/kg soil - for the sum of TBT compounds.

7.4 Quality criterion in drinking water

The quality criterion in drinking water QC_{dw} is calculated based on the TDI of 0.03 µg Sn/kg b.w. per day and assuming a daily ingestion of 0.03 L/kg b.w. of drinking water ($ingestion_{dw}$) for children 1-10 years old:

$$QC_{dw} = \frac{TDI * Y}{ingestion_{dw}} = \frac{0.03 \text{ µg Sn/kg day} * 0.1}{0.03 \text{ L/kg b.w./day}}$$

$$= 0.1 \text{ µg Sn/l}$$

7.4.1 Quality criterion in drinking water

Quality criterion: 0.1 µg Sn/l - for the sum of TBT compounds.

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Tributyltin compounds (TBT)

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to tributyltin compounds (TBT). This resulted in 2007 in the present report which includes estimation of a quality criterion for the mentioned compounds in soil and drinking water.

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