CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Dibutyltin dilaurate

EC Number: **201-039-8**

CAS Number: **77-58-7**

Index Number: **-**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Dibutyltin dilaurate***
EC number:	***201-039-8***
CAS number:	***77-58-7***
Annex VI Index number:	**-**
Degree of purity:	> 95%
Impurities:	Not relevant for classification of this substance

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	-	-
Current proposal for consideration by RAC	Muta. 2 – H341 Repr. 1B – H360FD Stot RE 1 – H372 (immune system)	N/A
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Muta. 2 – H341 Repr. 1B – H360FD Stot RE 1 – H372 (immune system)	N/A

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.1.	Acute toxicity - oral	none		none	not evaluated
	Acute toxicity - dermal	none		none	not evaluated
	Acute toxicity - inhalation	none		none	not evaluated
3.2.	Skin corrosion / irritation	none		none	not evaluated
3.3.	Serious eye damage / eye irritation	none		none	not evaluated
3.4.	Respiratory sensitisation	none		none	data lacking
3.4.	Skin sensitisation	none		none	not evaluated
3.5.	Germ cell mutagenicity	Muta. 2		none	
3.6.	Carcinogenicity	none		none	data lacking
3.7.	Reproductive toxicity	Repr. 1B		none	
3.8.	Specific target organ toxicity –single exposure	none		none	not evaluated
3.9.	Specific target organ toxicity – repeated exposure	STOT RE 1 (immune system)		none	
3.10.	Aspiration hazard	none		none	not evaluated
4.1.	Hazardous to the aquatic environment	none		none	not evaluated
5.1.	Hazardous to the ozone layer	none		none	not evaluated

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Signal word:	Danger
Pictogram code:	GHS08
Hazard statements:	H341
	H360FD
	H372
Precautionary statements:	Not harmonized

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

In 2006 the European Chemicals Bureau's Technical Committee on Classification and Labelling (TC C&L) recommended that DBTDL should be included in the 31st ATP with the following classification:

Repr. Cat 2; R60-61
Muta. Cat. 3; R68
Xn; R22
Xi; R38
T; R48/25

However, this classification has not been included in the legislation.

2.2 Short summary of the scientific justification for the CLH proposal

The published information on the toxicity of dibutyltin dilaurate (DBTDL) is limited. However, there is sufficient data to support that DBTDL is toxicologically similar to other dibutyltin (DBT) compounds and DBTDL can be regarded as a precursor to dibutyltin dichloride (DBTC) through the oral route since DBTDL hydrolyses into DBTC in the stomach.

Further, in Noda et al., 1993 the developmental effects of several di-n-butyltin compounds were examined and developmental effects of DBTDL could be compared with the effects of DBTC. The effects observed for the other di-n-butyltin compounds (di-n-butyl diacetate, -dichloride, -maleate and -oxide) were similar to the developmental effects of DBTDL.

DBTC has a harmonized classification for several hazard classes; Acute Tox. 3 (H301), Acute Tox. 4 (H312), Skin Corr. 1B (H314), Acute Tox. 2 (H330), Muta. 2 (H341), Repr. 1B (H360FD), STOT RE1 (H372), Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410). A weight of evidence approach using data for DBTC to classify DBTDL is used in this CLH proposal. DBTC studies were also taken into account for the classification of DBTDL by TC C&L in 2006.

Only the hazard classes Muta and Repr, together with STOT RE, has been evaluated in this dossier.

In this CLH-report all relevant studies included in the REACH registration dossiers for DBTDL and DBTC has been considered, in addition to other scientific publications.

2.3 Current harmonised classification and labelling

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

DBTDL is currently not listed in Annex VI in the CLP regulation.

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

None.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Table 4: The following classifications have been notified by Industry to the C&L inventory (1140 notifications, 20.06.2014)

Hazard classes:	**H-statements:**	**Notifications relevant for this dossier:**
Acute Tox. 3/Acute Tox. 4	H301/H302	-
Acute Tox. 4	H312	-
Skin Corr. 1C/ Skin Irrit. 2	H314/H315	-
Skin Sens. 1	H317	-
Eye Dam. 1 /Eye Irrit. 2	H318/H319	-
Muta. 2	H341	886
Repr. 1A/Repr. 1B	H360	112/866
STOT SE 1	H370	-
STOT RE 1/STOT RE 2	H372/H373	694/249
Aquatic Acute 1	H400	-
Aquatic Chronic 1/Aquatic Chronic 2	H410/H411	-

2.4.2 Current self-classification and labelling based on DSD criteria

N/A

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

DBTDL has CMR properties (reproductive toxicity and mutagenicity). There is no harmonized classification for DBTDL. Harmonized classification and labelling for CMR and respiratory sensitization is a community-wide action under Article 36 of the CLP. STOT RE is closely related to the classification with Repr and it is therefore relevant to consider both of these hazard classes. For the hazard classes Carc. and Resp. Sens. data is lacking and they are therefore not evaluated. The self-classifications notified by industry and published in the C&L Inventory shows a great degree of variety, also for the Repr/Muta/STOT RE properties of the substance. This justifies a harmonized classification for DBTDL.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

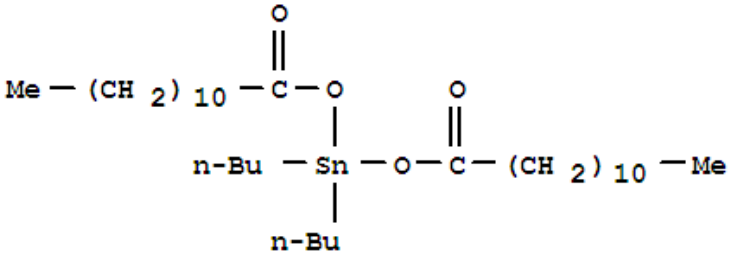
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	201-039-8
EC name:	dibutyltin dilaurate
CAS number (EC inventory):	77-58-7
CAS number:	77-58-7
CAS name:	stannane, dibutylbis[(1-oxododecyl)oxy]-
IUPAC name:	dibutyl[bis(dodecanoyloxy)]stannane
CLP Annex VI Index number:	None
Molecular formula:	$C_{32}H_{64}O_4Sn$
Molecular weight range:	631.56 g/mol

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Dibutyltin dilaurate (77-58-7)	>95%		Data from REACH registration

Current Annex VI entry: None

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Not relevant for the hazard classes evaluated in this classification proposal			Data from REACH registration

Current Annex VI entry: Not applicable

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No data available				

Current Annex VI entry: Not applicable

1.2.1 Composition of test material

The purity of the DBTDL tested in the studies is above 95% w/w where reported. Information on the actual composition used is provided in the relevant tables in this report, if available, and also in the associated IUCLID summaries (where provided).

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	clear colourless liquid	ECHA DBTDL dossier submitter	
Melting/freezing point	28.5°C	"	
Boiling point	205°C	"	
Relative density	1.043 g/mL at 28.5°C	"	
Vapour pressure	7.7 x 10^{-6} Pa at 25ºC	"	
Surface tension	No data	"	
Water solubility	≤ 1.43 mg/l at 20.0 ± 0.5ºC	"	
Partition coefficient n-octanol/water	2.77 x 10^{4} at 20.8 ± 0.5°C (log10 Pow 4.44)	"	
Flash point	191 ± 2°C	"	
Flammability	No data	"	
Explosive properties	No data	"	
Self-ignition temperature	≥400°C at atmospheric pressure	"	
Oxidising properties	No data	"	
Granulometry	No data	"	
Stability in organic solvents and identity of relevant degradation products	No data	"	
Dissociation constant	No data	"	
Viscosity	72 mPa s (dynamic) at 20°C	"	

2 MANUFACTURE AND USES

2.1 Manufacture

The total tonnage band is 100-1000 tonnes per annum (ECHA dissemination web site. Information as accessed May 20, 2014).

2.2 Identified uses

There is a wide range of organotin compounds that can be manufactured and placed on the market and these are used in a variety of industrial applications. Di-substituted organotins (usually in combination with mono-substituted organotins and, to a lesser extent, tri-substituted compounds) are used as stabilisers for PVC and as catalysts for various products (RPA, 2007).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not evaluated in this dossier.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

During simulated reaction studies at conditions (pH 1-2, 0.07 N HCl, 37°C) resembling the mammalian gastric system, DBTDL, which contains tin-ligand oxygen esters, hydrolysed into DBTC by 87.8% after 2 hours. The half-life was <0.5 hours (Schilt, 2004). No study appears to have looked at DBTDL hydrolysis *in vivo*, however, in a mice study having administrated ^{14}C-dibutyltin diacetate dissolved in methoxytriglycol by gavage, a large amount of unmetabolized compound was observed in the feces, indicating that stomach hydrolysis was far from complete (Kimmel et al. 1977). However, the gastric pH is higher in rodents than in humans (pH 1-2); fasted mice/rats have gastric pH ~4 whereas the pH drops to ~3 in fed animals (McConnell 2008). Presumably, the choice of solvent and the compound's chemical structure (ester bond strength, etc.) also influences the hydrolysis reaction. Importantly, similar toxicological effects from DBTDL and DBTC administration have been observed in animal studies.

The metabolism of DBTC in male Wistar rats following a single intraperitoneal administration of 4 mg DBTC/kg bw was investigated (Ishizaka et al. 1989). The rats were decapitated without anesthesia 6-168 h after administration. Blood and urine was collected and the liver, kidneys and brain were excised. The halftime of DBTC in liver, kidney and blood was 3-5 days. DBTC was present in liver, kidney and spleen already 6 h after treatment and had been metabolized to some extent. The accumulation of DBTC in brain was slower than in other organs. The highest concentration of DBTC in brain was observed three days after administration and corresponded to one fifth of the concentration found in the liver and kidneys. Butyl(3-hydroxybutyl)tin dichloride (M-2), butyl(4-hydroxybutyl)tin dichloride and butyltin trichloride were detected as acid-soluble metabolites by HPLC and mass spectrometry. M-2 may be formed in the liver and accumulated in the kidney. DBTC and M-2 are excreted into the bile and suggested to be involved in the induction of the biliary and hepatic lesions. The concentration of DBTC in the blood was about 1/20 of the concentration in the liver and kidneys.

Experimental data show that the oxygen esters in DBTDL can hydrolyze into dibutyltin (DBT; Bu_2Sn^{2+}) in the stomach (low pH, high HCl) producing the metabolite dibutyltin dichloride (DBTC) and free laurate chains, where the laurate chains are not expected to hydrolyze or react further. Likewise, it can be expected that oral administration of butyl(3-hydroxybutyl)tin dilaurate (3-OHDBTDL); investigated for developmental effects in an oral administration study by Noda et al. 1993) would hydrolyze into butyl(3-hydroxybutyl)tin dichloride, the major metabolite observed after DBTC administration (Ishizaka et al. 1989). For respiratory and dermal exposures, DBTDL is expected to be absorbed un-metabolized. However, hydrolysis of the oxygen esters in DBTDL can occur after aqueous dissolution (Schilt, 2005).

It is generally assumed that DBT, probably as the chloride, is the moiety (toxophore) responsible for the *in vivo* effects when animals are orally exposed to DBTDL. Thus, when considering classification of DBTDL for hazard classes that reside on exposure through the oral route, it is justified to take studies into account where DBTC and other rapidly acid-hydrolysable DBT substances have been administrated orally (the same metabolite, DBT).

4.1.2 Human information

None.

4.1.3 Summary and discussion on toxicokinetics

DBTDL is in simulated reaction studies shown to rapidly undergo acid-mediated hydrolysis into the metabolite dibutyltin dichloride (DBTC; the chloride salt of dibutyltin (DBT; Bu_2Sn^{2+})), a reaction expected to occur in the human stomach following oral exposure. Therefore DBTC and other stomach-hydrolysable DBT substances can be taken into consideration when classifying DBTDL for hazard classes that reside on exposure through the oral route. For DBTC, one rat study measured a halftime of 3-5 days in liver, kidney and blood (Ishizaka et al. 1989). The extent of hydrolysis may be influenced by chemical structure (bond strengths) and solvent used; for dibutyltin diacetate one gavage mice study found large amounts of unmetabolised compound in the feces, but rodents have higher gastric pH than humans.

4.2 Acute toxicity

Not evaluated in this dossier.

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

Not evaluated in this dossier.

4.4 Irritation

Not evaluated in this dossier.

4.5 Corrosivity

Not evaluated in this dossier.

4.6 Sensitisation

Not evaluated in this dossier.

4.7 Repeated dose toxicity

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

There are very few studies on DBTDL that are relevant for classification for repeated dose toxicity. The results from these studies are presented in table 10a.

Experimental data show that DBTDL can hydrolyse into DBT in the stomach producing DBTC. Studies on DBTC are therefore taken into consideration when classifying DBTDL for this hazard class. The results from these studies are presented in table 10b.

Table 10a: Summary table of relevant repeated dose toxicity studies for DBTDL

Method	Results	Remarks	Reference
Three rat trials with 4-5 dose groups of at least 5 female and 5 male weanlings or 8w olds were given chow containing up to 2000 ppm or 133 mg/kg bw/day DBTDL up to 13 weeks.	1000 ppm: weight gain and feed intake affected. Enlarged bile duct. No effects on thymus reported.	LOAEL = 1000 ppm or 60 mg/kg bw/day based on body weight loss. DBTDL given as Tinostat.	Study report 1961 Supporting study in ECHA database for self-classification of DBTDL
Five groups of 5-6 young male albino rats (90-100 g) were gavaged with 0, 2, 4, 8, or 16 mg/kg bw/day of DBTDL in peanut oil (2 ml/kg) for 5 days/week for 2 weeks.	A dose dependent reduction in thymus weight and cell counts, histological alterations and other functional disturbances were seen.	LOAEL = 8 mg/kg bw/day based on combined effects on thymus and lymph nodes. Findings similar to the thymic atrophy of DBTC. Purity of DBTDL was not given.	Subramoniam et al., 1994
Forty Wistar rats, 10 w old, male or female, 190±10 g, gavaged with DBTDL in corn oil at 0, 5, 10 and 20 mg/kg bw/day for 5 days/week for 7 weeks. (10 rats/dose group). Brains were analysed for enzyme activities, NO_2, proteins, DNA damage, apoptosis and ultrastructural changes.	DBTDL reduced: superoxide dismutase and glutathione peroxidase activities, DBTDL increased: malondialdehyde and nitric oxide content and nitric oxide synthase, DNA damage, apoptosis, number of brain cell remaining in the G0/G1 phase. All in a dose dependent manner.	LOAEL = 20 mg/kg bw/day DBTDL based on observed ultrastructural changes in the brain. 5 mg/kg bw/day resulted in enzyme activity and cellular reactions. Only clinical symptoms were euphoria and aggression in 2 animals in the high and 2 in the mid dose group.	Jin et al., 2012
Supplementing short study:			
Rats were given 17.5 mg/kg bw/day (1/10 of LD50) DBTDL in feed for 15 days. Liver enzyme activities were measured.	Weight loss, 20% mortality, most liver enzymes showed reduced activity in exposed animals, while some were unaffected and one enzyme had increased activity.	Supplementing short study, not well documented. LOAEL = 17.5 mg/kg bw/day based on mortality.	Mushtaq et al.,1981

Table 10b: Summary table of relevant repeated dose toxicity studies for DBTC

Method	Results	Remarks	Reference
Male and female Wistar rats, 9-10 w old, dosed 0, 5, 30, 200 ppm DBTC in diet for 41 days. Reproduction study with additional analysis of the effects on thymus.	Moderate thymus lymphoid depletion in mid dose, severe effects at high dose. Only females investigated.	LOAEL = 30 ppm (approx. 2 mg/kg bw/day) for thymus effects in dams. OECD 421 study.	Waalkens-Berendsen, D.H., 2003
160 CFE rats fed diets containing 0, 10, 20, 40 or 80 ppm DBTC for 90 days, 16 males and 16 females in each dose group.	No deaths, no changes in relative organ weights. No abnormalities seen at autopsy or histology. Thymus was examined. However, a slight reduction of body weight and food intake, mild anaemia was observed.	LOAEL = 80 ppm (approx. 4 mg/kg bw/day) a slight reduction of growth and food intake, mild anaemia.	Gaunt et al., 1968
Prenatal Developmental Toxicity Study. 20 pregnant Wistar rats were given 0, 1.0, 2.5, 5.0 or 10 mg/kg bw/day DBTC daily for 10 consecutive days from GD 6 to GD 15, killed on day 20.	At 10.0 mg/kg bw/day, a high number of animals showed thymus atrophy and reduced thymus weights. A non-significant number of animals with thymus atrophy was also seen at 5.0 and 2.5 mg/kg bw/day.	OECD 414, GLP Maternal LOAEL = 10 mg/kg bw /day based on a significant thymus atrophy and reduced food consumption	Study report 1993, report date 1994-11-11 Key study in ECHA database for self-classification of DBTDL Study extract is available at ECHA.
Wistar-derived (WU) rats, and inbred Swiss mice received DBTC mixed in a commercial chow at levels of 50 or 150 ppm for 4 weeks, 5-10 animals/group.	Dose dependent thymus effects in rats. High dose group had lowered antibody production to SRBC, but not to LPS. High dose effect on graft rejection. No effects on phagocytosis. Summarized as a toxic effect on T-cells. Mice tested showed no effects.	LOAEL = 2.5 mg/kg bw/day (nominal) based on thymus effects in rats. Liver effects seen in rats at 7.5 mg/kg bw/day	Seinen, W. et al., 1977a, 1977b
Sprague–Dawley rats; 96 males and females, and 45 pregnant (w/offspring). Dose groups were: 0, 0.9, and 1.9 mg DBTC/kg bw/ day, and 0, 1.0, and 2.5 mg/kg bw/day. Duration: 28 days. Dosed in drinking water. Additional groups where pups were given DBTC gavage in addition to maternal exposure.	No adverse effects on antibody production, delayed type hypersensitivity or NK cell cytotoxicity. There was a dose dependent difference between males and female pups.	Two similar studies, one sub-acute, one developmental study. Slightly different results were obtained. NOAEL = 2.5 mg/kg bw/day (highest dose).	DeWitt et al.,2005, 2006
Three feeding groups of 10 weanlings Wistar- derived rats (WU-CPB) received 0, 50 & 150 ppm DBTC in feed for 2 weeks. Parenteral single injections of 2.5, 5 and 10 mg/kg bw.	There was a dose dependent thymus and spleen weight reduction. Parenteral group and high dose group showed lymphoblast depletion in thymus and spleen. Liver effects in bile duct area at high dose.	LOAEL = 2.5 mg/kg bw/day based on thymus weights and lymphoblast depletion. 450 ppm in feed and 10 mg/kg bw parenteral was always lethal.	Penninks, A.H. & Seinen, W., 1982
Supplementing short studies:			
Male Wistar rats gavaged a single dose DBTC in two experiments. First, 21 rats were	There was a dose response relationship showing linear negate correlation between dose	Thymus toxicity is completely reversed after 5 days.	Snoeij et al., 1988

Method	Results	Remarks	Reference
given15 mg /kg bw and killed after 1, 2, 3, 4, 5, 7 and 9 days, In a second study 4-5 rats were dosed between 5-35 mg/kg bw and killed 4 days after exposure.	and thymus weight. Time effect study showed decreasing thymus weights until day 4, returning to normal at day 9.	Supplementing short study. Only relevant for mechanism, no LOAEL.	
Male Long-Evans rats 250-350 g (Charles River) was administered DBTC by gavage. Study A: daily administration for 4 days, Study B: every 2 days for 12 days, at doses of 0, 10 and 20 mg/kg bw/day in corn oil by. Rats were decapitated 24 h after the last administration. Liver tissue was prepared for light microscopy and determination of prolyl hydroxylase activity.	Study A: Inflammation in portal tracts and biliary damage at both dose groups. Prolyl hydroxylase activity and in vitro collagen synthesis was increased in the higher dose group. Study B: extensive inflammation in portal tracts, biliary damage, fibrosis, necrosis, infarcted areas, and granulomatous lesions. In the higher dose group increases in hydroxyprolinecontent,prolyl hydroxylase activity, and relative collagen synthesis in vitro were observed. DBTC did not increase prolyl hydroxylase activity of L929 cells.	Supplementing short study. LOAEL = 10 mg/kg bw/day based on liver pathology.	Yermakoff, et al., 1979

DBTDL studies:

Type of study: **Repeated oral**

Reference: Study report 1961 as described in summary from applicant on ECHAs web pages.

Animal species & strain: Rat; Holtzman and white Simonsen.

Test substance: Tinostat (DBTDL 25%) was administered in ground Purina laboratory Chow. Rats were individually weighed, caged, and given feed and water ad libitum.

Doses, vehicle, duration: 1st range-finding test: 25-200 ppm for 4 weeks.

2nd additional study (named 23-61): 400 ppm.

3rd study (RST-26-62): 500, 1000, 1500, 2000 ppm using white Simonsen rats.

Group sizes: Four groups of 5 males and 5 females.

Tests performed: Rats were weighed, sacrificed and autopsied, and histopathology was performed on grossly abnormal appearing tissue.

Results: NOAEL is above 0.040% in feed (400 ppm or 26.6 mg/kg bw/day) after 13 weeks. Few tissue changes which were observed appeared physiological or infectious and no evidence of toxic changes was observed upon histopathological examination. At 1000 ppm, weight gain

and feed intake were significantly affected. An enlarged bile duct was the most common gross necropsy observation attributable to dose.

NOAEL = 26.6 mg/kg bw/day.

Type of study:	**Immunotoxicity study**
Reference:	Subramoniam A, Khandelwal S, Dwivedi PD, Khanna S and Shanker R (1994). Dibutyltin dilaurate induced thymic atrophy and modulation of phosphoinositide pathway of cell signalling in thymocytes of rats. *Immunopharmacol. Immunotoxicol.*, 16, 645–677.
Animal species & strain:	Rats, male albino (90-100 g) from the Industrial Toxicology Research Centre, Lucknow- 226 001, India. Unknown genetic and microbiological quality. Unknown cage and bedding quality, fed pelleted diet.
Test substance:	Dibutyltin dilaurate obtained from Fluka AG, Switzerland (DBTDL). Purity not given.
Doses, vehicle, duration:	Animals were gavaged with DBTDL in ground nut oil at dose levels of 0, 2, 4, 8 or 16 mg/kg bw/day for 5 days per week for 2 weeks.
Group sizes	Males, 5-6 in each dose group.
Tests performed	Weight of lymphoid organs, body weight, weight of other organs (kidney, liver, adrenal), histology of lymphoid organs; in vivo and in vitro effects of DBTDL on phosphoinositide metabolism in thymocytes were assessed. In vivo and in vitro effects on Protein kinase C (PKC) activity and intracellular Ca^{2+} in thymocytes were assessed.
Results:	A marked dose dependent reduction in thymus weight and its nucleated cell counts with histological alterations were observed in rats exposed to oral dibutyltin dilaurate for 2 weeks at 2, 4, 8, or 16 mg/kg bw/day:

Dose group	**Thymus**		**Spleen**		**Peripheral lymph nodes**	**Mesenteric lymph nodes**
mg/ kg bw/ day	Cell x10^6	Viab (%)	Cell x10^6	Viab (%)	Cell x10^6	Cell x10^6
0	106 ± 16.9	98.8	218 ± 17.3	98.6	107 ± 11.9	125 ± 17.3
2	92 ± 10.9	98.3	229 ± 13.3	98.5	98 ± 14.1	116 ± 11.7
4	80 ± 11.8*	98.6	194 ± 20.2	97.8	94 ± 10.5	110 ± 11.5
8	63 ± 15.4**	97.2	216 ± 18.1	96.8	66 ± 10.3**	89 ± 9.4*
16	19 ± 4.8***	93.4	130 ± 8.5***	96.0	56 ± 3.2***	78 ± 3.1***

Values are means ± SD (N=5)
Cell no. represents total cell counts/organ
$^{*}p<0.05$; $^{**}p<0.001$; $^{***}p<0.001$; significant difference from control

The incorporation of [^{3}H]-inositol into all the three major phosphoinositides was drastically reduced in thymocytes in a dose dependent manner. Furthermore, the basal and ConA stimulated [^{3}H]-inositol phosphates generation was diminished significantly in the 8

	mg/kg bw/day DBTDL group. However, in vitro incubation of DBTDL with thymocytes failed to evoke any change in phosphoinositide hydrolysis. A 130% and 600% enhancement of PKC activity in thymocytes was seen in the 4 mg/kg bw/day and 8 mg/kg bw/day DBTDL group, respectively. Addition of DBTDL to the cell free assay system of thymocytes resulted in a concentration dependent activation of the enzyme activity. A dose dependent increase in intracellular calcium was evident when DBTDL was added to thymocytes under in vitro conditions. LOAEL = 8 mg/kg bw/day based on significant organ weight reduction and reduced cell counts in thymus and lymph nodes combined.
Type of study:	**Neurotoxicity study**
Reference:	Jin M, Song P, Li N, Li X and Chen J (2012). A plastic stabilizer dibutyltin dilaurate induces subchronic neurotoxicity in rats. *Neural Regen. Res.*, 7, 2213-2220.
Animal species & strain:	Forty Wistar rats, aged 10 weeks, male or female, weighing 190±10g, were provided by the Animal Experimental Center of Jilin University, China.
Test substance:	DBTDL solution was prepared with corn oil (Sigma, St. Louis, MO, USA). Purity not reported.
Doses, vehicle, duration:	Animals were gavaged with DBTDL in corn oil at dose levels of 0, 5, 10 and 20 mg/kg bw/day for 5 days/week for 7 weeks. (10 rats/dose group)
Tests performed	The activities of superoxide dismutase, glutathione peroxidase and nitric oxide synthase was measured. In addition, the content of malondialdehyde, nitric oxide and the protein levels in brain homogenates was examined. Brain tissues were quantified for apoptosis, cell cycle and DNA damage in single cortical cells. EM was used for detecting ultrastructural changes.
Results:	DBTDL reduced superoxide dismutase and glutathione peroxidase activities, and increased the malondialdehyde content in rat brain tissue. DBTDL increased nitric oxide content and nitric oxide synthase activity in rat brain tissue. DNA damage and apoptosis was seen with increasing frequency and intensity with increased DBTDL dose. This coincided with an increased number of brain cell remaining in the G0/G1 phase. After exposure, two rats in each of the high dose and medium dose groups had mild euphoria and fought each other to the death. Other clinical symptoms were not reported. All above effects appeared in a dose dependent manner. 20 mg/kg bw/day resulted in apparent neuropil cavitation in the brains, as well as other ultrastructural changes, with glial filaments dissolving within the axon.

	LOAEL = 20 mg/kg bw/day is based on ultrastructural changes in brain and glial filaments dissolving within the axon.
Type of study:	**Subacute toxicity study 15 days**
Reference:	Mushtaq, M., Mukhtar, H., Datta, K. K., Tandon, S. G., & Seth, P. K. (1981).Toxicological studies of a leachable stabilizer di-n-butyltin dilaurate: Effects on hepatic drug metabolizing enzyme activities. Drug and Chemical Toxicology, 4, 75–88.
Animal species & strain:	Male albino rats (200 ± 10 g); source: Industrial Toxicology Research Centre, Lucknow, India. Strain, stock and microbiological status not specified.
Test substance:	DBTDL was a gift from National Chemical Laboratory, Poona, India, purity not reported.
Doses, vehicle, duration:	1/10 LD50 = 17.5 mg/kg bw/day by daily oral gavage in ground nut oil, for 15 days, rats decapitated 16h after last dosing on day 16.
Group sizes:	6 and 8 animals reported in two of the tables. Otherwise not described.
Tests performed:	Body weight, histopathology of liver, homogenates of brain and liver prepared for enzyme activity analysis. Barbiturate sleeping times were tested on days 1, 4, 8 and 15.
Results:	20% mortality in the experimental group. Unknown number of dead animals, probably 2 out of 10. The DBTDL group was lethargic and had a lower weight gain than controls. DBTDL had no significant effect on: organ weights (total or relative to body weight), activities of succinic dehydrogenase, Mg2+-adenosine triphosphatase, acid phosphatase, g-ALA synthetase, the activities of brain enzymes, succinic dehydrogenase, adenosine triphosphatase, acetycholine esterase and monoamine oxidase. DBTDL resulted in a decrease in: weight gain compared to controls, the specific activities of microsomal enzymes, glucose-6-phosphatase (35%), aniline hydroxylase (22%), benzo(a)pyrene hydroxylase (57%), aminopyrine-N-demethylase (32%), benzphetamine-N-demethylase (33%) and cytochrome P-450 content (32%). DBTDL resulted in an increased activity of heme oxygenase (75%) and pentabarbital induced sleeping time. Sections of rat livers from the DBTL-group showed only uniformly distributed cytoplasmolysis.

LOAEL lower than 17.5 mg/kg BW/day

DBTC studies:

Type of study	**OECD Guideline for Testing of Chemicals no. 421: Reproduction/Developmental Toxicity Screening Test**
Reference:	Waalkens-Berendsen, D.H., 2003. DBTC OECD 421 study, V4906 report, TNO, The Netherlands.
Animal species & strain:	Male and female Wistar outbred Crl:WI rats, 9-10 w old.
Test substance:	98.57% DBTC, ORTEP, Association Stabilizer Task Force, Lot no XFRDC296K.
Doses, vehicle, duration:	0, 5, 30, 200 mg/kg in diet mixed in house. Males fed 28 days, Females 41 days (end at PN4-PN6). Calculated doses in mg/kg bw/day: Male animals: 0.3- 0.4, 1.9-2.3 and 10.4-13.0 mg/kg bw/day Females, during the premating, gestation and lactation period: 0.3-0.4, 1.7-2.4 and 6.2-15.4 mg/kg bw/day (low-, mid- and high-dose group).
Group sizes	12 males and 12 females / dose group, N=96.
Tests performed	Reproduction parameters, food intake, body weight, organ weights, complete organ necropsy, pup examined before killed at PND 4, stillbirths necropsied, thymus histopathology was examined (females, N=48).
Results:	No mortalities or clinical signs were observed. A 1080 ppm group was terminated after 1 week because of low palatability and severe effects. Effects on pups: Males: Body weight of the high-dose group decreased from day 14 to day 28. Body weight change decreased during the entire study, in the mid-dose group day 14-21. Body weight change increased in the low- and mid-dose groups from days 0-7. Food consumption decreased in the high-dose group from day 7 to day 14. Females: The body weight of the high-dose group decreased compared to controls. Statistical significance for the difference in body weight was observed on day14 of the premating period, the entire gestation period and during the lactation period on PND 4. Statistical significance for the difference in body weight change was observed from days 0-7 of the premating period and during the gestation days (GD) 7-14 and 14-21. Food consumption of the high-dose group decreased GD 0-7, during the entire gestation period and during the lactation period from PND 1-4. Food consumption of the animals of the low- and mid-dose groups was comparable to the control group. Effects on the dams:

	The absolute and relative thymus weight of the high-dose groups was decreased. The relative thymus weight of the female mid-dose group was decreased whereas the absolute thymus weight was not decreased. No effect on thymus weight in the female low-dose group. Histopathology of the thymus revealed severe to very severe lymphoid depletion in 12/12 high-dose females, and moderate to severe lymphoid depletion in 6/12 (pregnant) mid-dose females (no lymphoid depletion of the thymus was observed in all 5 non-pregnant and one pregnant female). Lymphoid depletion was characterized by a decrease in the size of the thymic lobules because of an extensive loss of cortical and medullary small lymphocytes. The distinction between the cortical and medullary areas was blurred. In the severe cases the cortex was very small, or partially absent. The remaining cells visible in the cortical areas were mainly lymphoblasts and reticular epithelial cells. The small lymphocytes were absent in the collapsed thymic stroma. LOAEL for the dams = 1.7-2.4 mg/kg bw/day in females based on thymus effects. LOAEL for the pups = 6.2-15.4 mg/kg bw/day based on reduced bw.
Type of study:	**Subchronic similar to OECD Guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents)**
Reference:	Gaunt, I.F. et al., 1968. Acute and Short-term Toxicity Studies on Di-n-butyltin Dichloride in Rats. Fd Cosmet. Toxicol Vol., 6, pp.599–608.
Animal species & strain:	CFE rats, males: 181-189 g and females: 147-153 g.
Test substance:	DBTC (Albright & Wilson Ltd., London) stated to contain 99.7 % di-n-butyltin dichloride and 0.25 % tri-n-butyltin dichloride.
Doses, vehicle, duration:	Diets containing 0, 10, 20, 40 or 80 ppm for 90 days (recalculated to mg/kg bw/day according to Guidance to Regulation (EC) No 1272/2008)
Group sizes	16 male and 16 female weanling rats were housed four per cage (N=160).
Tests performed	Body weight and food consumption were measured weekly. Haematological investigations: week 6 and at the end of the study period(haemoglobin, haematocrit, erythrocytes, reticulocytes and total and differential leucocytes). Serum chemistry was examined by measuring glutamic-oxaloacetic, glutamic-pyruvio transaminases, amylase and serum-urea levels. Kidney function was investigated by urine analysis by colour, pH, microscopic constituents, and the content of protein, glucose, bile salts, blood and tin and activity of glutamic-oxaloacetic transaminase. The concentrating ability of the kidney was assessed by measuring the volume and specific gravity of urine produced under conditions of normal hydration, during a 6 h period of water deprivation, during a 2h

	period after a water load of 25 ml/kg and during a 4 h period commencing 16 h after the water load. All rats were necropsied; a careful search was made for gross changes to the bile duct and pancreas. The brain, pituitary, heart, thyroid, liver, spleen, kidneys, adrenals and gonads were weighed. The duodenal loop with the pancreas and bile duct in situ were fixed flat to retain their anatomical relationship. Paraffin-wax sections of these organs, together with salivary gland, trachea, lungs, diaphragm, various lymph nodes, thymus, stomach, ileum, colon, caecum, rectum, urinary bladder, sternum and uterus were HE stained for histopathological examination.
Results:	There were no deaths or any effects on the behavior or clinical conditions. There was a slight reduction of growth in both sexes fed 80 ppm (4 mg/kg bw/day) but this was statistically significant only in females. Some reduction in food intake was noted which may have been due to low palatability. In the preference test, even at 10 ppm, there was a marked preference for the basal diet. Haemoglobin concentrations were lowered at 4 mg/kg bw/day, and in females at week 6 and males at week 13. The decreases, although statistically significant were slight and not associated with reductions of other erythrocyte parameters or with a reticulocytosis. There were no deviations from normal in the serum-urea or -enzyme levels or in the urine parameters measured. Tin was not found in any urine sample. There were no changes in relative organ weights. No abnormalities were seen at autopsy. There were no differences between test and control animals in the findings in any of the tissues examined, including thymus. LOAEL = 4 mg/kg bw/day. There was a slight reduction of growth and food intake at this dietary level and a mild anemia.
Type of study:	**OECD Guideline 414 (Prenatal Developmental Toxicity Study)**
Reference:	Study report 1993 as described in summary from applicant on ECHA's web pages.
Animal species & strain:	Wistar Crl:CD (Wi) BR rats, specific pathogen free, Charles River Wiga GmbH, 97633 Sulzfeld, Germany.
Test substance:	>98% DBTC, in olive oil.
Doses, vehicle, duration:	0, 1, 2.5, 5, 10 mg/kg BW/day by daily oral gavage from GD6 to GD15, dams and fetuses killed on GD20
Group sizes:	25 females mated/ dose group, of these were 20 females/group analysed,
Tests performed:	

	N=100
Results:	Clinical observations, food intake, body weight, complete necropsy including evaluation of ovaries (number of corpora lutea) and uterus (resorption, implantation sites, stillbirths, placenta), fetuses weighed and examined before killed, histopathology examination of maternal thymus (weighed before fixation), liver, bile duct and mesenteric lymph node.
	No mortalities or clinical signs related to dosage were observed. Dosage of 10 mg/kg bw /day elicited maternal toxicity (reduced body weight gain and food consumption, and thymus atrophy). Although the incidence of fetuses with malformations was slightly increased at 10.0 mg/kg, this was due to three fetuses in two litters. This low incidence and the lack of a consistent malformation render this finding of equivocal toxicological significance.
	Administration of dibutyltin dichloride at a dose level of 5.0 mg/kg elicited slight maternal toxicity (slightly reduced body weight gain and non-significant thymus atrophy), but did not elicit embyrotoxicity or teratogenicity.
	Administration of dibutyltin dichloride at a dose level of 2.5 mg/kg revealed a non-significantly increased incidence of animals showing thymus atrophy at histopathology, but no embyrotoxicity or teratogenicity.
	Administration of dibutyltin dichloride at a dose level of 1.0 mg/kg did not elicit maternal toxicity, embyrotoxicity or teratogenicity.
	LOAEL for maternal toxicity = 10 mg/kg bw/day in females based on significant thymus effects.

Type of study:

Reference:	**Subacute, Developmental (immunotoxicity)**
Animal species & strain:	Seinen, W. et al., 1977b. Toxicity of Organotin Compounds . III . Suppression of Immunity in Rats by Di-n-Butyltindichloride and Di-n-Octyltindichloride Toxicol Applied Pharmacol, 42(1), pp.213–224.
Test substance:	Specific pathogen-free Wistar-derived (WU), inbred Wistar (WAG) and (WAG x B)F1 hybrid rats, inbred (sic) Swiss mice (TNO, Zeist, NL).
Doses, vehicle, duration:	Dibutyltin dichloride (purity > 98%) from Dr. E. J. Bulten, Institute for Organic Chemistry TNO, Utrecht. The Netherlands.
Group sizes	Adult rat and mouse studies: DBTC was mixed in a commercial chow at levels of 50 or 150 ppm for 4 weeks. Virgin rats were mated and received the test diets from GD 2. Postnatally the pups, standardized to six per litter, were exposed via milk and gastric intubation. 3 groups of pups were gavaged with 0, 1 and 3 mg/kg bw 3d/week until week 7.

Tests performed	5 to 10 animals per group.
Results	Delayed-Type Hypersensitivity to Tuberculin (skin test), (not reported) Allograft Rejection (WAG x B grafts will normally lead to graft rejection in WU rats after 10d). Antibody Formation after Sheep Red Blood cells (SRBC) or E. coli LPS immunization was evaluated in a hemolysin assay, a hemagglutinin assay and a plaque forming cell assay. *In Vivo* phagocytosis (Carbon clearance from blood after iv injection).
Results:	Allograft rejection (cellular immune response, rats) was delayed by DBTC in adult and offspring rats (dose dependent, borderline significant, not the most appropriate statistical test). Body weights were slightly lower in the high dose groups. The rat primary antibody response to SRBC, (needs the co-operation of T helper cells and B cells) was depressed at 150 ppm. Hemagglutination and hemolysin titers as well as the number of direct plaque-forming cells against SRBC per spleen, were decreased in a dose-related manner by DBTC. The rat antibody response to LPS (a T cell-independent antigen) was not affected by DBTC. The secondary antibody responses were not affected by DBTC. It was concluded that DBTC induce immune suppression in rats by a selective inhibition of T-lymphocyte activity. Immune suppression was most pronounced in animals exposed to the chemicals during the developmental phase of the lymphoid system. Carbon clearance was not affected in rats. Rats: LOAEL = 2.5 mg/kg bw/day based on effects on the immune response (nominal) Altered immune function was never observed in mice exposed to DBTC.
Type of study:	**Subacute**
Reference:	Seinen, W. & Vos, J.G., 1977a. Toxicity of Organotin. II. Comparative in Vivo and in Vitro Studies with Various Organotin and Organolead Compounds in Different Animal Species with special Emphasis on Lymphocyte Cytotoxicity. Toxicol Applied Pharmacol, 42, pp.197–212.
Animal species & strain:	Rats (Wistar-derived specific pathogen-free, weighing 45-50 g) and mice (Swiss, specific pathogen-free, weighing 10-12 g), in vitro test on thymus glands/tissue. Japanese quail (weighing 70-80 g).
Test substance:	Dibutyltin dichloride (purity > 98%) from Dr. E. J. Bulten, Institute for Organic Chemistry TNO, Utrecht. The Netherlands.
Doses, vehicle, duration:	Feeding study: 2 week for rats; 4 week feeding study for mice. Doses; 0, 50 and 150 ppm. Intravenous: repeated, but no specific timeframe given. In vitro: up to 40 h with 50 µg/l.

Group sizes	Oral Treatment Studies: Randomized groups of 10 weanling male and/or female rats , 10 weanling male mice
Tests performed	Body weights were recorded weekly. Necropsy: body weights and the weight of the thymus, spleen, popliteal lymph node, liver, kidneys and adrenals were recorded. Histopathological examination was performed with HE stains.
Results:	Rats: Mortality occurred in the 150-ppm group (4 females and 2 males) in the second week. The relative weights of thymus was reduced by 53% in the 50 ppm group and by 68-72% in the 150ppm group. as well as the spleen (16% and 33% for 50 and 150 ppm) and popliteal lymph nodes.(16 and 28% for 50 and 150 ppm). Necropsy: a pronounced reduction in size of the thymus was found in all animals fed DBTC. Thickened and dilated bile ducts accompanied by irregularly yellowish discolored livers were found in the animals that died and in two male and two female survivors fed 150 ppm of DBTC. In two males and two females that died in this group, abdominal edema was observed. Microscopically severe proliferation of bile duct epithelial cells and bile ductules were associated with pericholangiolitis and periportal fibrosis, in livers of 4 male and 6 female rats fed 150 ppm of DBTC. The most prominent effect found was lymphocyte depletion in lymphoid organs, and it was most pronounced in the thymic cortex of animals fed DBTC. At the 150-ppm level, the cortex was almost completely depleted. Signs of cell destruction were never observed. Also, lymphocyte depletion was present in the thymus-dependent areas of the spleen and popliteal lymph node (respectively, periarteriolar lymphocyte sheets and paracortical areas). Other treatment-related histopathological changes were not noted. Intravenous injections in rats supported the findings from the oral study. In vitro study demonstrated a cytotoxic effect of DBTC in the suspension cultures of rat thymocytes. Mice: No measurements were affected by DBTC. NOAEL for rats = 50 ppm in feed (approx. 3 mg/kg bw/day) for 2 weeks shows no effects on thymus cell populations, thymus weights were, however lowered. LOAEL for rats = 150 ppm in feed (approx. 8 mg/kg bw/day) for 2 weeks showed strong effects on thymus cell populations and thymus weights.

Type of study:	**Subacute**
Reference:	DeWitt, J.C., Copeland, C.B. & Luebke, R.W., 2005. Immune responses in Sprague-Dawley rats exposed to dibutyltin dichloride in drinking water as adults. J Immunotoxicol, 2(3), pp.151–60.
Animal species & strain:	60 days old individually housed male and female Sprague–Dawley (CD, Charles River) rats.
Test substance:	DBTC (96% purity, Batch 02211AO) purchased from Aldrich Chemical Company Inc.
Doses, vehicle, duration:	DBTC was emulsified in Alkamuls; ethoxylated castor oil, and diluted in drinking water at concentrations of 0, 10, or 25 mg /L (final concentration) in 0.5% Alkamuls. Dose groups were: 0, 0.9, and 1.9 mg/kg bw/day, and 0, 1.0, and 2.5 mg/kg bw/day for the replicate experiment. Duration: 28 days
Group sizes	Both experiments: 48 animals (8 males 8 females per dose) in each test.
Tests performed	Antibody Response: Primary (IgM) and secondary (IgG) T-cell-dependent antibody responses against sheep red blood cells (SRBC). Animals were immunized (day 24 of dosing) intravenously (2.0×10^8 SRBCs in 0.5 mL of sterile saline) were bled 5 days later (day 29). Booster immunization (2.0×10^8 SRBCs) was administered intravenously to the same animals at day 39. Blood collected 5 days later (day 44) was analysed for SRBC-specific IgG. The relative serum titer of SRBC-specific IgM and IgG antibodies were measured by ELISA (details provided for the analyses). Delayed-type hypersensitivity (DTH) and natural killer (NK) cell activity were evaluated in separate groups of treated and control animals on day 29 of exposure. Delayed-Type Hypersensitivity Response (DTH): Sensitized with purified bovine serum albumin (BSA; Sigma) in Freund's complete adjuvant subcutaneously into the caudal tail fold. Seven days later, animals were challenged by 0.1 ml BSA into the right rear footpad. The left rear footpad was the injection control. After 24 h, footpad thickness (triplicate measurements) was determined. Swelling was calculated by subtracting the mean saline-injected, left footpad thickness from the mean BSA-injected right footpad thickness. Natural Killer (NK) Cell Activity: Splenocyte single cell suspensions were prepared and cultured with 51Cr-labeled murine YAC-1 lymphoma target cells in 3 ratios. 51Cr release was determined in by liquid scintillation counting.
Results	Body Weights: no statistical effect was observed. Spleen and thymus absolute weights and relative weights did not vary statistically by dose. Water Consumption: was reduced in the 25 mg/L group, 17% less for males, 21% less for females ($p < 0.0001$).

	Antibody Production: Effects of gender and experiment number vas seen in a non-systematic order. No effect of dose group was observed. DTH Response: No dose-dependent or sex-related differences. NK Cell Activity: There were no dose-dependent or sex-related differences in 51Cr release. NOAEL = 2.5 mg/kg bw/day (highest dose).
Type of study:	**Subacute, developmental**
Reference:	DeWitt, J.C., Copeland, C.B. & Luebke, R.W., 2006. Developmental Exposure to 1.0 or 2.5 mg/kg of Dibutyltin Dichloride Does Not Impair Immune Function in Sprague-Dawley Rats. Journal of immunotoxicology, 3(4), pp.245–52.
Animal species & strain:	Sprague–Dawley (CD) 9- to 10-week old, nulliparous rats were purchased from Charles River Laboratories (Raleigh, NC). The dams arrived at the EPA on gestational Day (GD) 2.
Test substance:	DBTC (96% purity, Batch 02211AO) purchased from Aldrich Chemical Company Inc.
Doses, vehicle, duration:	Dams: 0, 10, or 25 mg/L of DBTC (final concentration) in 0.5% Alkamul, exposure for 37 days. These concentrations corresponded to dosages of approximately 1.0 and 2.5 mg/kg bw/day during gestation and 2.0 and 4.4 mg/kg bw/day post-parturition, based on DBTC concentration in the drinking water, dam water consumption, and body weight. Pups: 0, 1.0, or 2.5 mg /kg bw/day administrated DBTC gavage 3 times a week, 10 doses in a total during 3-4 weeks. It should be noted that pups were exposed to both milk and gavage DBTC content. However, the milk DBTC concentration appears not to have been measured.
Group sizes	45 timed-pregnant dams were randomly subdivided into six groups. In three of the groups (1–3; maternal) only the dams were dosed and in three of the groups (4–6; maternal+direct) both the dams and the offspring were dosed. Groups were as follows: (1) 0 mg DBTC/L to dams only; (2) 10 mg DBTC/L to dams only; (3) 25 mg DBTC/L to dams only; (4) 0 mg DBTC/L to dams and 0 mg DBTC/kg bw/day to pups; (5) 10 mgDBTC/L to dams and 1.0 mgDBTC/kg bw/day to pups; (6) 25 mg DBTC/L to dams and 2.5 mg DBTC/kg bw/day to pups.
Tests performed	Antibody Responses: antibody responses to SRBC, primary and secondary immunizations. Delayed-Type Hypersensitivity Response (DTH): Sensitized with purified bovine serum albumin (BSA; Sigma) in Freund's complete adjuvant subcutaneously into the caudal tail fold. Seven days later,

animals were challenged by 0.1 ml BSA into the right rear footpad. The left rear footpad was the injection control. After 24 hr, footpad thickness (triplicate measurements) was determined. Swelling was calculated by subtracting the mean saline-injected, left footpad thickness from the mean BSA-injected right footpad thickness.

Natural Killer (NK) Cell Activity: Splenocyte single cell suspensions were prepared and cultured with 51Cr-labeled murine YAC-1 lymphoma target cells in 3 ratios. 51Cr release was determined by liquid scintillation counting.

Results

Pup body weights and mortality: At birth, there was no statistical effect of dose on the mean litter weights or litter sizes of offspring. However, there was pup mortality throughout the study (<10%). The mortality did not vary by dose, exposure regime, or sex.

Dose-dependent differences in mean body weight of male and female pups were observed. This effect began at PND 14 (males) or PND 17 (females). Pups from the 2.5 mg/kg bw/day gavage + maternal exposure group weighed 10–20% less than average weight for the remaining pups at every weighing after PND21.

Immune Function: There were no doses, exposure regime, or sex-related differences in the DTH responses. In the initial experiment, antibody responses differed by dose within each exposure regime for both male and female offspring. Relative to controls ($p < 0.05$), responses differed in offspring from the 25 mg/L maternal-only group; IgM titers were reduced 17.5% in females and IgG titers were elevated 26.8% in males. In both of the maternal + gavaged dose groups, IgM titers in female offspring were reduced approximately 20% relative to controls ($p = 0.05$).

IgG titers of male offspring from the 10 mg DBTC/L maternal-only group were also 21.8% lower relative to IgG titers of male offspring from the 1.0 mg DBTC/kg BW maternal + direct group ($p = 0.05$).

Antibody titers from the initial experiment and the replicate did not differ statistically. Therefore IgM titers from the initial experiment were combined with IgM titers from the replicate and IgG titers from the initial experiment were combined with IgG titers from the replicate. Antibody titers did not differ by dose, sex, or exposure regime.

NK cell activity did not differ statistically by exposure regime, but did differ in two instances by dose and by sex.

NOAEL = 2.5 mg/kg bw/day based on male offspring immunotoxicity. However, pups exposed through milk and gavaged 2.5 mg/kg bw/day showed a smaller body weight increase than the rest of the pups. LOAEL is not easily determined, but must be more than 2.5 mg/kg bw/day.

Type of study:	**Subacute feeding study, parenteral exposure, in vitro assessment of lymphocytotoxicity**
Reference:	Penninks, A.H. & Seinen, W., 1982. Comparative toxicity of alkyltin estertin stabilizers. Fd Chm Toxic, 20(6), pp.909–916.
Animal species & strain:	Specified pathogen-free Wistar- derived rats (WU-CPBJ from the Central Institute for the Breeding of Laboratory Animals, TNO, Zeist), In vitro testing on rat lymphocytes.
Test substance:	DBTC from Dr. E. J. Bulten, Institute for Organic Chemistry TNO, Utrecht. The Netherlands. Purity of DBTC is not reported, but in earlier studies by the same group the reported purity of DBTC from Dr. E. J. Bulten has been > 98%.
Doses, vehicle, duration:	Feeding study: 2 weeks, doses: 0, 50 and 150 ppm Parenteral: Single injection ip and iv 2.5 mg/kg bw In vitro: 0.5, 50 µg/ml dissolved in absolute ethanol just before addition to the cell suspensions. For all in vitro experiments the final ethanol concentration was always 0.5%, a concentration that did not affect the test system.
Group sizes	Ten weanling male rats in each group.
Tests performed	At necropsy: Body weights, thymus, spleen, liver, kidneys and adrenals were weighed, fixed and processed for histopathology (HE stains).
Results	The terminal body weight was 6% and 15% lower than in the control group for the 50 and 150 ppm group, respectively. The relative thymus weight was less than 30% compared with the controls, and there was lymphocyte depletion in the thymus, especially the thymic cortex and in thymus-dependent lymphoid areas of the spleen. Parenteral exposure caused a slight reduction in body weight gain and severe thymic atrophy. In vitro: In the presence of graded amounts of DBTC thymocyte survival decreased in a dose related and time-dependent fashion compared to the thymocyte loss in the control cultures. DBTC caused a progressive decrease in thymocyte survival until after a 24 h incubation with 50µg DBTC/ml, survival was only some 25-30% of the control values. With concentrations of 0.5 µg/ml (1.6 µM DBTC) thymocyte survival still showed a considerable decrease. LOAEL= 3 mg/kg bw/day based on thymus lymphocyte depletion.

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

No data available.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.2 Human information

No data available.

4.7.3 Other relevant information

None

4.7.4 Summary and discussion of repeated dose toxicity

For both DBTDL and DBTC the most sensitive organ system seems to be the immune system, with main findings in the thymus. These effects may be readily reversible at low concentrations. Liver effects and brain pathology was also observed, but at somewhat higher dosage levels. All findings are typically dose-dependent, and it is not clear at which dose adverse effects appear.

The studies of DBTDL are few and of such quality that classification cannot be based on these studies alone. Atrophy and cell depletion has been described for DBTDL in thymus and lymph nodes, with reduced organ size and cell counts. The LOAEL based on these combined effects are 8 mg/kg bw/day (Subramoniam et al 1994). This study is however of limited quality.

For DBTC, studies are numerous and better documented. However, different laboratories have slightly different conclusions regarding LOAELs. Notably, all studies having used animals and substance from TNO, the Netherlands, conclude with LOAELs at 1.7-2.5 mg/kg bw/day (Waalkens-Berendsen 2003, Seinen et al, 1977a) while studies from other laboratories conclude with a NOAEL at the same dosage (DeWitt et al, 2004, 2006). Thymus pathology of DBTC was observed, with organ atrophy, a shift in cell populations and loss of structure in the organ: LOAEL = 1,7-2.4 mg/kg bw/day (Waalkens-Berendsen 2003), in a study where TNO was the supplier of animals and substance. No functional defects on the immune responses were observed at the same level (DeWitt 2005, 2006) in a US EPA sponsored 28-day study, a study duration which is too short to be in conflict with the LOAEL of 1,7-2.4 mg/kg bw/day where the rats were given the DBTC in the diet for 41 days. Seinen et al (1977b) reported slight effects on immune function at 3 mg/kg bw/day, their source is TNO. For liver toxicity, LOAELs of 10 mg/kg bw/day (Yermakoff, et al, 1979) and 8 mg/kg bw/day were reported (Penniks et al, 1982, source: TNO). The LOAEL for DBTC on the thymus effects should be the basis for STOT RE. The liver effects appear at slightly higher doses.

It should be noted that the molecular weight of DTBDL (631.56 g/mol) is more than twice the molecular weight of DBTC (303.8 g/mol). This implies that dosages must be multiplied by 2.08 when concluding on dosages with respect to DBTDL from DBTC-data.

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

Not relevant for this dossier.

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

Not relevant for this dossier.

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

Not relevant for this dossier.

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

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Even though the data for DBTDL is not sufficient on its own for classification the available information indicate that the target organ for DBTDL is similar to the identified target organ for DBTC. The main effects of DBTDL and DBTC are on the immune system, either in the bone marrow or in the thymus. The histological changes (depletion of cell populations) in the rat thymus together with the dose dependent lowered thymus weights, is observed in most of the studies. The thymus effect is related to functional disturbances in the T-cells of the immune system. Necrosis and apoptosis is not described. However, the dose-dependent response with severe atrophy and loss of organ structure at high dose levels must invariably lead to a loss of thymus function and impaired immunity.

The classification for repeated dose toxicity of DBTDL thus relies on a weight of evidence using data for both DBTDL and DBTC. The meeting of TC C&L in 2006 recommended that classification of DBTDL should be based on studies with DBTC and be classified in the same way as DBTC (T; R48/25 (DSD)).

According to CLP Annex 1: 3.9.2.7.3, DBTDL should be classified according to the following criteria:
(d) significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination.
(f) morphological changes that are potentially reversible but provide clear evidence of marked organ dysfunction.

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

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

Experiments support that DBTDL hydrolyses in the human stomach to the active metabolite DBTC which has a harmonised classification with STOT RE1; H372. The critical effects on the immune system identified in the studies of DBTC occur at 2.5 mg/kg bw/day (Waalkens-Berendsen 2003).

When considering obtained threshold levels for DBTC, it should be taken into account that DBTDL has about twice the molecular weight of DBTC. Considering the double molecular weight of DBTDL compared to DBTC, LOAEL = 2.5 x 2.08 = 5.2 mg/kg bw/day for DBTDL.

Based on these criteria and a LOAEL of 5.2 mg/kg bw/day for DBTDL, DBTDL should have the same classification as DBTC.

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

Classification with STOT RE 1; H372 is proposed.

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

A Specific Concentration Limit (SCL) is allocated if the effective dose level or concentration is 10 times below the guidance values according to the CLP that corresponds to an effective dose below 1 mg/kg bw/day in 90-day studies (CLP 3.9.2.6). Thus, a SCL is not relevant for DBTDL.

4.9 Germ cell mutagenicity (Mutagenicity)

4.9.1 Non-human information

There are very few studies on DBTDL that are relevant for classification for mutagenicity. The results from these studies are presented in table 11.

For oral exposure, experimental data show that DBTDL can hydrolyse into DBT in the stomach producing DBTC. Studies on DBTC are therefore taken into consideration when classifying DBTDL for this hazard class. The results from these studies are presented in table 11.

Table 11: Summary table of genotoxicity studies for DBTDL and DBTC

Method	Quality assured	Metabolic activation	Results	Reference
DBTDL *in vitro* studies				
OECD guideline 471 (Bacterial Reverse Mutation Assay)	GLP	±S9 mix	negative	Bowles A & Thompson PW, 2010
Salmonella mutagenicity test	non-GLP	±S9 mix	negative	Zeiger E et al., 1987
Salmonella mutagenicity test	presently unclear	±S9 mix	negative	Dow Corning Corp, 1981
Salmonella mutagenicity test	presently unclear	±S9 mix	negative	E I du Pont de Nemours, 1977
DBTDL *in vivo* studies				
DNA damage in rat cerebral cortical cells (single cell gel electrophoresis)	non-GLP	no	positive	Jin M et al., 2012
DBTC *in vitro* studies				
Similar to OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	GLP	±S9 mix	positive*	Study report 1990, report date 1990-09-16
HGPRT/V79 mammalian cell gene mutation test	GLP	±S9 mix	negative	Lang R & Schmitt R, 1989
Similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay)	no (prior GLP)	±S9 mix	negative	Schering, LBI project 20998, 1979
Breakage of naked λ-DNA ±H_2O_2	no	no	negative	Hamasaki T et al. 1995
Salmonella typhimurium TA98 & TA100	no	no	positive	Hamasaki T et al., 1993

mutation testing				
Bacterial SOS chromotest (indication of genotoxicity)	no	no	positive	Hamasaki T et al. 1992
Bacterial rec-assay (indication of genotoxicity)	no	no	positive	Hamasaki T et al. 1992
Condensate formation with DNA	no	no	positive	Piro V. et al. 1992
Spindle disturbance in V79 Chinese hamster cells	no	no	positive	Jensen KG et al., 1991a
Aneuploidy in human peripheral lymphocytes	no	no	negative	Jensen KG et al., 1991b
Spindle-inhibition as chromosomal contractions in human lymphocytes	no	no	negative	Jensen KG et al., 1989
CHO cell/HGPRT gene mutation	no	no	positive	Li AP et al., 1982
DBTC *in vivo* studies				
OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	GLP	N/A	positive*	Dance CA, 1991
Similar to OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	GLP	N/A	negative	Lang R & Wedel J v, 1991

*Key study in the classification proposal for mutagenicity of DBTC previously concluded by the TC C&L.

4.9.1.1 DBTDL in vitro studies

Type of study:	**OECD guideline 471 (Bacterial Reverse Mutation Assay), stated GLP**
Reference:	Bowles and Thompson, 2010. A study extract is available at ECHA from the DBTDL dossier submitter.
Experimental model:	DBTDL (batch LA6K05N001, purity >95%) was tested at multiple doses in triplicates from 0.005/5 (WP2uvrA$^-$) up to 5000 µg/plate or the toxic limit using *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* strain WP2*uvr*A$^-$, ± a rat liver homogenate metabolizing system (10% liver S9 in standard co-factors). Positive and negative controls were used.
Results:	No significant increases in the frequency of revertant colonies over negative controls were observed for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation or exposure method. All of the positive controls induced marked increases in the frequency of revertant colonies. The test material was considered to be non-mutagenic.
Type of study:	**Salmonella mutagenicity test, non-guideline, non-GLP**
Reference:	Zeiger E. et al., 1987
Experimental model:	DBTDL (from Pfaltz & Bauer; purity not stated) was evaluated for mutagenicity in the *Salmonella*/microsome test as part of the National Toxicology Program (NTP) using a standard protocol. After dose range determination in a toxicity assay, DBTDL was tested in triplicates at doses of 0, 1, 3, 10, 33, 100, and 166 µg/plate

	in four *Salmonella typhimurium* strains (TA98, TA100, TA1535, and TA1537) ±S9 fraction from Aroclor-induced male rat or hamster liver. Concurrent solvent and positive controls were run with each trial.
Results:	DBTDL was negative in these tests.
Type of study:	**Salmonella mutagenicity test, use of guideline and quality assurance is presently unclear**
Reference:	Dow Corning Corp., 1981. [Abstract available from yosemite.epa.gov]
Experimental model:	The mutagenicity of DBTDL was evaluated in *Salmonella* tester strains TA1535, TA1537, TA1538, TA98, and TA100 ±metabolic activation by Aroclor-induced rat liver S9 fraction. An overlay plate test for mutagenicity was conducted on DBTDL diluted with DMSO at concentrations of 0.5, 5, 100, and 500 µg/plate and a spot plate test was conducted on undiluted test material. The source and purity of DBTDL is presently unknown.
Results:	DBTDL was negative in these tests.
Type of study:	**Salmonella mutagenicity test, use of guideline and quality assurance is presently unclear**
Reference:	E I du Pont de Nemours, 1977. [Abstract available from yosemite.epa.gov]
Experimental model:	The mutagenicity of DBTDL was evaluated in *Salmonella* tester strains TA98, TA100, TA1535, TA1537, and TA1538 (Ames test), ±metabolic activation by rat liver S9 fraction (inducer not reported). Based on preliminary bacterial toxicity determination, DBTDL was tested for mutagenicity at concentrations up to 250 µg/plate using the direct plate incorporation technique. The source and purity of DBTDL is presently unknown.
Results:	DBTDL was negative in these tests. The investigators reported that the test compound showed increased toxicity to tester strains with each trial in the activated assay with time, suggesting degradation of the compound with time.

4.9.1.2 DBTDL in vivo studies

Type of study:	**DNA damage in rat cerebral cortical cells (single cell gel electrophoresis). Non-GLP.**
Reference:	Jin M et al 2012.
Animal species & strain:	Wistar male/female rats. 40 in total.

Test substance:	DBTDL. Unclear source and purity.
Doses, vehicle, duration:	Animals were gavaged with DBTDL in corn oil at dose levels of 0, 5, 10 and 20 mg/kg bw/day for 5 days/week for 7 weeks.
Group sizes	10 rats/dose group. How many of each sex per dose appears not to be specified.
Analyses performed	The single cell gel electrophoresis assay (Comet assay) was performed by the modified Singh method (Singh NP et al (1988). A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res., 175, 184-191). 50 ethidium bromide stained cells were scored per slide and the DNA damage was divided into 5 levels (0 – 4). The method of isolating cerebral cortical cells from brain tissue appears not to have been specified.
Results:	Positive – a significant dose-dependent increase in DNA damage was seen in rat cerebral cortical cells. Also other toxic effects such as right parietal cortex cell cycle disturbance and increased apoptosis was observed.

4.9.1.3 DBTC in vitro studies

Type of study:	**Internal Method No. 449.1 similar to OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test). Stated GLP.**
Reference:	Study report 1990 as described in summary from applicant on ECHAs web pages.
Experimental model:	Human peripheral blood lymphocytes isolated from a healthy adult male. Tests using ± liver S9 from Aroclor 1254 treated rats. Name of test material (as cited in study report): ZK 22.663 (DBTC). Assay - S9 mix; 1st run: 0.001, 0.003, 0.006, 0.01, 0.03, 0.06, 0.1, 0.3, 0.6, 1.0 and 3.0 µg/ml; 2nd : 0.006, 0.008, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2 and 0.4 µg/ml. Assay +S9 mix: 1st run: 0.050, 0.075, 0.10, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0 and 7.5 µg/ml. 2nd : 0.05, 0.10, 0.25, 0.50, 0.75, 1.0, 2.0 and 3.0 µg/ml. Negative and positive controls used.
Results:	Positive. An evaluation of the clastogenic potential in the human lymphocyte test indicates a clastogenic potential of the test material in the human lymphocyte test *in vitro* at clearly cytotoxic concentrations. From the four assays conducted without and with an extrinsic metabolizing system in two independent studies, one assay without and one with S9 mix gave statistically significant ($P < 0.05$) increases in the frequency of chromosomal aberrations at the highest concentrations evaluated, whereby in the remaining assays the results were borderline negative. In each assay of this investigation, the test material was tested up to cytotoxic concentrations as

	indicated by an obvious reduction of the mitotic index.
Type of study:	**Guideline (internal method no. 515.0) equivalent or similar to OECD Guideline 476 (In vitro Mammalian Cell Gene Mutation Test). Stated GLP.**
Reference:	Lang R and Schmitt R, 1989. Schering report IC 16/89. A study extract is available at ECHA from the DBTC and DBTDL dossier submitters.
Experimental model:	Chinese hamster lung fibroblasts (V79) ± Aroclor 1254-induced rat liver S9 metabolic activation. Test material: ZK 22.663 (DBTC; purity not reported) from Schering. Test concentrations, -S9: 0.000001, 0.000003, 0.000010, 0.000030, 0.000060 µl/ml; +S9: 0.00020, 0.00030, 0.00040, 0.00045, 0.00050 µl/ml.
Results:	Negative - the test material did not show a mutagenic potential in the HGPRT/V79 mammalian cell gene mutation test neither - nor + S9 mix in two independently performed experiments. The test material was found to have cytotoxic effects –S9 at 0.00006 µl/ml and using +S9 a clear toxic effect could be observed at 0.0003 µl/ml in the first experiment and at 0.0005 µl/ml a second experiment. The positive control (ethylmethane sulfonate) was clearly mutagenic.
Type of study:	**Equivalent or similar to OECD Guideline 471 (Bacterial Reverse Mutation Assay. Pre-dates GLP.**
Reference:	Schering. LBI project number 20998, 1979. A study extract is available at ECHA from the DBTC dossier submitter.
Experimental model:	*S typhimurium* TA 1535, TA 1537, TA 1538, TA 98 and TA 100 ± Aroclor 1254-induced rat liver S9 metabolic activation. Test material: Di-n-butylzinndichlorid (dibutyltin dichloride, DBTC; purity not reported). Test concentrations: 0.5, 1.0, 10.0, 100.0, 500.0 and 1000.0 µg per plate (run 1). Use of negative and positive controls. After observing toxicity in all strains at 100, 500, and 1000 µg, the test was repeated at 1, 5, 25 and 100 µg/plate (run 2).
Results:	Negative - the test material did not demonstrate genetic activity in any of the assays conducted in this evaluation and was considered not mutagenic.
Type of study:	**Breakage of naked λ-DNA (±H_2O_2), non-guideline, non-GLP**
Reference:	Hamasaki T et al. 1995
Experimental model:	Purchased λ-DNA (0.5 µg, double stranded, from Gibco BRL) was incubated with DBTC (from Merck; purity not specified) ± 10 mM H_2O_2 in 10 mM sodium phosphate buffer (pH 7.4) at 37°C for 2 h. After purification by phenol/chloroform extraction and ethanol precipitation, the DNA was dissolved and checked for breaks using agarose gel electrophoresis.
Results:	Negative – DBTC did not induce dsDNA breaks in presence or absence of H_2O_2.

Type of study:	**Bacterial reverse mutation (Ames), non-guideline, non-GLP**
Reference:	Hamasaki T et al 1993
Experimental model:	Salmonella typhimurium TA98 & TA100 strains. Metabolic activation (S9) was not used. DBTC from Merck Co.; analytical grade, purity not reported.
Results:	DBTC (tested at 0.1-10 µg/tube) was found to be mutagenic in both strains. Positive without metabolic activation.
Type of study:	**Bacterial SOS chromotest and rec-assay, non-guideline, non-GLP**
Reference:	Hamasaki T et al. 1992
Experimental model:	Indication of genotoxicity in two bacterial assays: SOS chromotest (*sfi* A induction; a SOS system related gene) with *E. coli* PQ37, and, rec-assay with *Bacillus subtilis* (H17 Rec^+ and M45 Rec^-). Metabolic activation (S9) was not used. DBTC (analytical grade; purity not reported) from Merck.
Results:	DBTC indicated genotoxicity in both assays and showed SOS activity at an extremely low dose (0.01 µg/tube). Positive without metabolic activation, however, these tests do not measure genetic damage directly.
Type of study:	**Condensate formation with DNA, non-guideline, non-GLP**
Reference:	Piro V. et al. 1992
Experimental model:	DBTC (source and purity not stated) in ethanol solution was added to calf thymus DNA dissolved in aqueous buffer (1 mM Tris, 0.1 mM EDTA, pH 8) to give molar ratios *r* (*r* = [Sn]/DNA phosphate) of 0.48-1.00 (experiment 1) and 2.40 (experiment 2), respectively, followed by analysis of pellet formation.
Results:	Positive – DBTC formed pellets (condensates/solid phases) with DNA in both experiments.
Type of study:	**Effect on spindle structure in V79 Chinese hamster cells, non-guideline, non-GLP**
Reference:	Jensen KG et al 1991a
Experimental model:	V79 Chinese hamster cells were treated with 10^{-8} - 10^{-4} M DBTC for 30 min at 37°C. Several end-points were investigated: c-mitosis, spindle structure, survival, as well as bovine brain microtubule protein assembly. DBTC was from Aldrich (purity not specified).
Results:	Positive - in general, loss of stainable spindle could be demonstrated at slightly higher concentrations than c-mitosis (DBTC also induced c-mitosis).

Type of study:	**Aneuploidy in human peripheral lymphocytes**
Reference:	Jensen KG et al 1991b
Experimental model:	Human peripheral blood was obtained from a healthy female donor and lymphocytes were cultured in medium for 72 h (37°C) during PHA-M stimulation. Then, lymphocytes were treatment with 10^{-8} - 10^{-6} M DBTC (source not specified) for 48 h. After hypotonic treatment and fixation, approximately 100 metaphases selected at random were photographed and chromosomes counted.
Results:	Negative – no significant induction of hyperdiploid cells (aneuploidy) was observed.
Type of study:	**Effect on spindle-inhibition as chromosomal contractions in human lymphocytes, non-guideline, non-GLP**
Reference:	Jensen KG et al. 1989
Experimental model:	Lymphocyte cultures were prepared from human peripheral blood from donors selected at random. Incubation in media for 72 h (37.5 °C) followed by exposure to 10^{-9} – 10^{-3} mol dm^{-3} DBTC (source and purity not specified) for 24 h. After hypotonic treatment and fixation, the length of chromosome No. 1 was determined in 100 metaphases selected at random.
Results:	Negative - no effect on average chromosome length was seen in the range 10^{-9} – 3 x 10^{-7} mol dm-3 DBTC versus control. No results were obtained at higher concentrations (≥1 x 10^{-6}) due to toxicity of treatment.
Type of study:	**CHO cell/HGPRT gene mutation, non-guideline, non-GLP**
Reference:	Li AP et al. 1982
Experimental model:	Chinese hamster ovary (CHO) cell mutation. DBTC (from Organometallics Inc,, East Hamstead, NH; purity not specified) was tested at 0.05 – 0.3 µg/ml. Metabolic activation (S9) was not used.
Results:	DBTC induced mutations at the HGPRT gene locus in CHO cells. The mutant frequency increased with dose up to 0.2 µg/ml (0.66 µM). A decrease in mutant frequency was observed at higher concentrations. The LC_{50} value of DBTC for CHO cells, as determined by cloning efficiency, was approximately 0.35 µg/ml (1.12 µM). Positive without metabolic activation.

4.9.1.4 DBTC in vivo studies

Type of study:	**OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test). Stated GLP.**
Reference:	Dance CA 1991. Life Science Research Limited project no. 91/0357. A study extract is available at ECHA from the DBTC and DBTDL dossier submitters.
Animal species & strain:	ICR male/female mice.
Test substance:	DBTC (batch 5842-34, purity 97.7%; trace levels of mono-n-butyltin trichloride, no tributyltin chloride detected) from Atochem NA, Inc.
Doses, vehicle, duration:	Male and female mice were given a single dose of DBTC at 2, 10 or 50 mg/kg. In all cases DBTC was dosed orally, mixed with corn oil. Five males and five females from each group were scheduled for termination 24 hours after treatment; further lots of five males and five females, given DBTC at 50 mg/kg bw or the vehicle control, were scheduled for termination 48 and 72 hours after treatment. [Doses were selected based upon a preliminary toxicity test using DBTC dosages of 62.5, 125.0, 250.0 and 500.0 mg/kg. All animals dosed with DBTC at 125, 250 and 500 mg/kg bw showed adverse reactions to treatment including severe rales, piloerection, immobility, hunched posture and uneven respiration: all were killed in extremis 4 hours (500 mg/kg) or 23 hours (125 and 250 mg/kg) following dosing, and all were seen to have lost weight since dosing. All animals dosed at 62.5 mg/kg bw showed piloerection on the day following dosing, and both males were hunched and lethargic from day 3 until termination: all these animals lost weight over the 72 hour period. Slides were prepared and stained for all animals. Examination of slide preparations showed evidence of bone marrow toxicity (depression in bone marrow proliferation) in individual animals dosed at 62.5, 125.0 or 250.0 mg/kg. After consideration of these data, the highest DBTC dosage selected for the main micronucleus test was 50 mg/kg.]
Group sizes	Dose groups consisted of 5 male/5 female in 2 and 10 mg/kg bw; 15 male/15 female in 50 mg/kg bw. Control animals (15 male/15 female) were given corn oil at 10 ml/kg bw. Chlorambucil (30 mg/kg in aqueous 10% ethanol; administered orally) served as the positive control (5 male/5 female). The mice were housed in single sex groups of two or five.
Analyses performed	After sacrifice, bone marrow erythrocytes were isolated from the marrow canal in femurs. Smears of cells were fixed and stained on slides. At least one slide from each animal was randomly coded. A total of at least 2000 erythrocytes per animal were examined. Each erythrocyte scored was classed as polychromatic or mature: polychromatic cells stain blue/pink and the older cells stain red/pink. At least 1000 cells of each type were scored from each animal where possible, but where there was an appreciable deviation from unity in the ratio of polychromatic to mature erythrocytes, scoring continued

	until a minimum of 2000 of the predominant cell type were counted. In addition each erythrocyte scored was examined for the presence or absence of micronuclei. The frequencies of micronucleated cells per 1000 erythrocytes were then calculated. The ratio of polychromatic to mature cells was also determined; a decrease in this may indicate inhibition of cell division following treatment, and the incidence of micronuclei in the mature cell population 24 hours after treatment reflects the pretreatment situation, since most of these cells were produced before treatment. The frequency of micronuclei in polychromatic cells provides an index of induced genetic damage.
Results:	Positive - a biologically and statistically significant increase in the incidence of micronucleated polychromatic cells was observed in the bone marrow of mice treated with DBTC at 50 mg/kg bw and killed 48 and 72 hours later ($0.01<p<0.05$): this effect was seen more clearly in females than in males. No such effect was apparent for any group treated with DBTC and killed 24 hours later. Statistically significant increases over controls were also seen in positive control group animals given chlorambucil at 30 mg/kg bw ($p<0.01$). Other toxicities: at a dosage of 2 mg/kg, no animal showed reactions to treatment. At 10 mg/kg, 3 males showed hunched posture and piloerection on the day of dosing only: no signs were observed in females. No marked incidences of weight loss were apparent in animals of either group. At 50 mg/kg, one male was killed in extremis approximately 2 hours after dosing (as a result of inactivity, unstable gait, slow respiration and piloerection). All but one of the remaining animals showed reactions to treatment including hunched posture, piloerection, inactivity, rales, closing of one or both eyes, and yellow staining of the coat. In addition, one female was found dead at termination, although it was seen to be alive 2 hours previously. At the 24 hour termination time, 5 animals had lost weight and one had failed to gain weight. At the 48 hour termination time all animals were seen to have lost weight, and all but two animals had lost weight at the 72 hour termination time. All weight losses recorded at 48 and 72 hours were marked. Of the ten mice given chlorambucil, the positive control agent, seven lost weight during the 24 hour period before termination.
Type of study:	**Internal method no. 185.3 based upon Schmid, W. 1976; 1977 similar to OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test). Stated GLP.**
Reference:	Lang E and Wedel J. v. 1991. Schering report IC 19/90. Performed year 1990. A study extract is available at ECHA from the DBTC and DBTDL dossier submitters.
Animal species & strain:	NMRI male/female mice.
Test substance:	ZK22.663 (DBTC, batch no. 1299; purity ≥97%) from Schering.
Doses, vehicle,	0, 50, 100, and 200 mg/kg bw by gavage, single exposure. DBTC

duration:	was dissolved in arachis oil. 5 males and 5 females from each of the negative control and the test material groups were killed by cervical dislocation 24, 48 or 72 hours after treatment (the positive control animals were killed 24 hours after treatment). [A range-finding study was not performed. It was assumed from a preceeding acute toxicity study that 200 mg/kg bw would be a dose level at which toxic effects might be notated.]
Group sizes	Dose groups consisted of 15 males and 15 females (30 in total). 3 additional reserve animals of each sex for the high-dose group were also used. Control animals (15 male/15 female) were given the vehicle (arachis oil) at 10 ml/kg bw. Triaziquone (0.15 mg/kg bw; single i.p. treatment) served as the positive control (5 male/5 female).
Analyses performed	After sacrifice, bone marrow erythrocytes were isolated from both femurs. Smears of cells were fixed and stained on slides. The slides were coded and analyzed "blind" in random order. The slides were examined for the incidence of micronucleated cells per 2000 polychromatic (PCE) and 1000 normochromatic (NCE) erythrocytes per animal. The ratio of polychromatic to normochromatic erythrocytes was calculated on the basis of 1000 NCE scored. Any toxic effect of the test material on the immature nucleated cells may lead either to a reduction in cell division or to cell death. These effects in turn lead to a reduction in cell numbers and to compensate for this, peripheral blood is shunted into the bone marrow. Therefore, a decrease in the frequency of polychromatic erythrocytes is taken as being indicative of toxicity. A statistical analysis was conducted for each of the following variables: p1: proportion of micronucleated PCE p2: proportion of micronucleated NCE p3: ratio of PCE/NCE
Results:	Negative - the test material failed to show any evidence of mutagenic potential when administered by gavage up to the toxic dose level of 200 mg/kg. Triaziquone, the positive reference, gave the expected mutagenic response. Other toxicities: three days after application of 100 mg/kg one male died; after application of the high dose (200 mg/kg) three males died two days after application, one male and one female after three days. More than half of the animals of the two highest dose groups showed signs of toxicity (predominantly apathy, eyelid closure, ruffled fur).

4.9.2 Human information

None.

4.9.3 Other relevant information

None.

4.9.4 Summary and discussion of mutagenicity

Few mutagenicity studies are available for DBTDL and the existing bacterial *in vitro* studies are negative also in presence of metabolic activation (+S9). However, repeated DBTDL gavage administration in rats increased *in vivo* DNA damage in isolated cerebral cortical cells (Jin, 2012). Since DBTDL is known to be hydrolysed into DBTC in the stomach, it is suggested that mutagenicity studies with DBTC could be used to classify DBTDL. The same approach was used in the meeting of TC C&L in 2006 where DBTDL was recommended classified as Muta. Cat. 3; R68 according to the Dangerous Substances Directive 67/548/EEC (DSD). This corresponds to Muta. 2; H341 according to CLP. For DBTC there is a mixed outcome both for *in vitro* and *in vivo* studies (Table 11), but overall most studies are positive. No *in vivo* or *in vitro* study has compared DBTDL and DBTC directly. For DBTC, positive *in vitro* mutagenicity (Li, 1982; Study report 1990; Hamasaki, 1993) and genotoxicity tests exist where the latter indicate clastogenicity (Study report 1990; Dance, 1991) and effects on spindle formation during mitosis (Jensen, 1991a). The genotoxic mechanism is presently not known, but has been suggested to involve penta-coordinate organotin-DNA structure formation leading to DNA condensation (Li AP et al., 1982; Pagliarani A et al., 2013), which was shown to occur at high DBTC to DNA ratios (Piro V et al., 1992). For DBTC one *in vivo* GLP study (OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)) assessing mutagenicity in mice is positive, but a possibly issue is that effects were only observed at the highest dose (50 mg/kg bw) (Dance, 1991). A similar GLP *in vivo* study (also Mammalian Erythrocyte Micronucleus Test and also in mice) observed no mutagenicity up to 200 mg/kg bw (Lang and Wedel, 1991).

4.9.5 Comparison with criteria

For both DBTDL and DBTC there are no germ cell mutagenicity studies available, and Cat 1B is therefore not relevant. For DBTC, metabolism studies show that it reaches several organs from the systemic circulation and distribution into testis/ovaries can be expected. For DBTC there is one positive well conducted (GLP) *in vivo* somatic cell mutagenicity test (Dance, 1991) as well as support from positive results from *in vitro* mutagenicity/genotoxicity tests. Thus, DBTC is a suspected germ cell mutagen. Presently DBTC has a harmonised classification with Muta. 2; H341. Based upon stomach hydrolysis of DBTDL into DBTC, it is justified that DBTDL should have the same classification as DBTC. In support, one oral DBTDL rat study found evidence of genotoxicity in isolated brain cells (Jin, 2012). There is generally no threshold for mutagenicity.

4.9.6 Conclusions on classification and labelling

Classification with Muta.2; H341 is proposed.

4.10 Carcinogenicity

Not evaluated in this dossier.

4.11 Toxicity for reproduction

Relevant studies for classification of DBTL are presented in Table 12.

Table 12: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
FERTILITY STUDY WITH DBTC			
Fertility study with DBTC in 16-19 Wistar rats/group gastric intubated with DBTC (0, 3.8, 7.6, 15.2 mg/kg bw/day) on gestation day (GD) 0-3 or GD 4-7.	Fertility NOAEL = 3.8 mg/kg bw/day. Based on higher No. of non-pregnant females, lower No. of implantations per females and higher incidence of preimplantation loss compared to the controls. Systemic toxicity was observed as reduced bw only at highest dose.	Non-guideline, non-GLP study, but the quality of the study is satisfactory. Purity: 97%	Ema et al., 2000
DEVELOPMENTAL STUDIES WITH DBTC AND DBTDL			
Developmental study with Wistar rats (n =10). Oral administration of 80 µmol/kg (50.5 mg/kg bw) DBTDL, 80 µmol/kg (24.3 mg/kg bw) DBTC, and 80 (51.8 mg/kg bw) or 160 µmol/kg (103.6 mg/kg bw) 3-OHDBTDL on day 8 of gestation and sacrifice on day 20 of gestation.	Maternal NOAEL = 50.5 (DBTDL), 24.3 (DBTC), and 103.6 (3-OHDBTDL) mg/kg bw, based on no maternal effects observed. Foetal NOAEL < 50.5 (DBTDL) and < 24.3 (DBTC) mg/kg bw, respectively, based on significantly higher incidence of external and skeletal malformations for both DBTDL and DBTC. 3-OHDBTDL was less potent, but malformations were observed at the highest dose (LOAEL = 103.6 mg/kg bw).	Non-guideline, non-GLP study. Number of animals and dose groups were less than recommended in OECD Guideline 414 (10 animals/group); however, but the quality of the study is satisfactory. Purity of test material is not reported.	Noda et al., 1993
STUDY WITH DBTC TO SUPPORT DEVELOPMENTAL EFFECTS			
Developmental study in 10-12 Wistar rats/group given DBTC (di-n-butyltin dichloride) gavage (2.5, 5 and 10 mg/kg bw/day) GD7-15.	Maternal NOAEL = 5 mg/kg bw/day. Based on increased mortality. Foetal NOAEL = 2.5 mg/kg bw/day. Based on increased malformations.	Non-guideline, non-GLP study. Number of animals in each group is less than recommended in OECD Guideline 414. Purity of test material is not reported.	Ema et al., 1991
DEVELOPMENTAL STUDIES WITH DBTC: FOCUS ON TIME DEPENDENT SUSCEPTIBILITY TO DBTC			
Developmental study with pregnant Wistar rats given DBTC gavage. Experiment 1 (3 days exposure): 20 mg/kg bw/day (36-133 litters) given on GD 7-9, 10-12, or 13-15. Experiment 2 (1 day exposure): 20 or 40 mg/kg bw/day, (11 litters) given on GD 6, 7, 8 or 9. Pregnant rats were killed on GD 20.	Experiment 1: Those dosed on days 7-9 showed significantly and highly teratogenic effects (malformation), but there was no evidence of teratogenicity for those dosed on GD 10-12, or 13-15. Also resorption of litters and number of dead foetuses were significantly increased in GD 7-9 group. Experiment 2: Pregnant females dosed on GD 7 or 8 with either 20 mg/kg or 40 mg/kg DBTC resulted in an increased incidence of foetuses with malformations. The	Non-guideline, non-GLP study. Purity of test material is not reported.	Ema et al., 1992

	highest incidence of malformed fetuses occurred after treatment on GD 8. There were no effects for females dosed on days 6 or 9. Also resorption of litters was significantly increased, while number of foetuses decreased in GD 7 and 8 groups. NOAEL< 20 mg/kg DBTC based on malformation in fetuses and reduced fertility. Susceptibility for DBTC exposure starts at GD 7 and is highest at GD 8. The incidence of malformed fetuses was proportional to the dose of DBT.		
Developmental study with pregnant rats given DBTC gavage. Wistar-ST rats were given DBTC gavage (10 or 15 mg/kg bw/day) GD7-8.	Exposure at GD 7 and 8 with DBTC at doses of 10 or 15 mg/kg caused increases post-implantation embryolethality and fetal malformations with maternal toxicity, as evidenced by decreased weight gain.	Non-guideline, non-GLP study. Purity of test material is not reported.	Ema et al., 1995
Developmental study with pregnant rats given DBTC gavage. Wistar-ST rats were given DBTC gavage (50 or 100 mg/kg bw/day) GD13-15.	The findings of the study suggest that DBTC has no teratogenic effect when administered during late organogenesis at doses that induced maternal toxicity as evidenced by decreased weight gain. A higher incidence of postimplantation loss was observed per litter.	Non-guideline,,non-GLP study. Purity of test material is not reported.	Ema et al., 1996

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Type of study:	**Fertility study with dibutyltin dichloride (DBTC).**
Reference:	Ema M and Harazono A (2000).
Animal species & strain:	13 weeks Jcl:Wistar rats were mated and included in the study when vaginal smear was positive. The day sperm were detected in the vaginal smear was Day 0 of pregnancy
Test substance:	DBTC 97% pure (Tokyo Kasei Kogyo Co., Tokyo, Japan).
Doses, vehicle, duration:	Doses of 0, 3.8, 7.6 or 15.2 mg/kg bw was given on Day 0 through Day 3 of pregnancy or Day 4 through Day 7 of pregnancy. The animals were sacrificed on day 20 of gestation. The DBTC was diluted in olive oil. The control group was given olive oil at the same dose regime as the groups given DBTC. A food restricted group of pregnant rats were given the same amount of feed as the group given 15.2 mg/kg bw on Day 0 to 3 or Day 4 to 7 in addition to olive oil.

Group sizes:	16-19 pregnant rats/group.
Tests performed:	Non-guideline or - GLP study, but the quality of the study is sufficient for classification.
Results:	Dams given di-n-butyltin dichloride (DBTC) Day 0 to 3: The adjusted weight gain (excluding the uterus) was significantly higher in control group and at lowest dose group compared to the pair-fed group. However, the group given the highest dose had statistical significant lower bw than the pair-fed group. The reduced body weight gain was supported by reduced food consumption. Groups of dams given 7.5 and 15.2 mg/kg bw/day had statistical significant higher number of non-pregnant females (31.3-87%) compared with the control group, and for the 15.2 group the number were significantly higher than for the pair-fed group (11.8%). Also the number of implantations per females was lower and the incidence of preimplantation loss was higher compared with control and pair-fed groups. Successfully mated females treated with DBTC on Day 0 to 3 showed a clear reduced fertility compared to control groups and pair-fed group. Dams given DBTC Day 4 to 7: In the group given the highest dose or pair-fed had statistical significant lower adjusted body weight gain compared to the control group. The reduced body weight gain was supported by reduced food consumption. In the highest dose group it was observed 87.5% early total resorption in the dams. This finding was statistical significant higher compared with control and pair-fed group. *Fertility NOAEL = 3.8 mg/kg bw/day*

Table 13: Reproductive findings in rat given DBTC on Day 0 to 3 of pregnancy

Observations	0	3.8	7.6	15.2	Pair-fed
No. of females successfully mated	19	16	16	16	17
No. (%) of nonpregnant females	0(0)	0(0)	5(31.3)*	14(87)*†	1(5.9)
No. (%) of pregnant females	19(100)	16(100)	11(68.7)*	2(21.5)*†	16(94.1)
No. of implatations per female(a,b)	15±1.4	15±1.5	10.1±7.1*	1.8±4.8*†	13.4±4.3
Preimplantation loss per female (%) (b,d)	2.7	4.1	35.6*	87.9*†	16.4
No. of litters	19	16	11	2	16
No. (%) of litters totally resorbed	0(0)	0(0)	1(9.1)	0(0)	3(18.8)
No. of corpora lutea per litter (a,c)	15±1.2	15.6±0.9	15.6±1.1	14.5±0.7	16.2±1.1
No. of implantations per litter (a,c)	15±1.4	15.0±1.5	14.6±1.5	14.0±0	14.3±2.6
Preimplantation loss per litter (%) (c,d)	2.7	4.1	6.4	3.3	9.9
No. of resporption and dead foetuses per litter (a,c)	1.0±0.7†	1.0±0.8†	3.0±3.8	1.0±0	4.3±4.7*
Early stage	1.0±0.7†	1.0±0.8†	3.0±3.8	1.0±0	4.3±4.7*
Late stage	0	0	0	0	0
Postimplantation loss per litter (%)	6.7†	6.8†	21.3	7.1	32.1*
No. of live foetuses per litter	14.1±1.7†	14.0±1.8†	11.6±4.4	13.0±0	10±5.7*
Sex ratio of live foetuses (male/female)	138/130	110/114	65/63	18/8	76/84
Body weight of live foetuses (g) (a)					
Male	3.42±0.23†	3.50±0.13†	3.48±0.19†	3.25±0.10	3.09±0.22*
Female	3.25±0.20†	3.26±0.17†	3.28±0.13†	3.02±0.07	2.95±0.25*

a)Values are given as mean+/- SD. b)Values are obtained from females successfully mated. c)Values are obtained from pregnant females. d)(No of corpora lutea – no. of implantations)/no. of corpora lutea) x 100. e)(No. of resorptions and dead foetuses/no. of implantations) x 100. *Significantly different from the control, P<0.005. †Significantly different from the pair-fed group, P<0.005.

Table 14. Reproductive findings in rat given DBTC on Day 4 to 7 of pregnancy

Observations	0	3.8	7.6	15.2	Pair-fed
No. of females successfully mated	16	16	16	17	17
No. (%) of nonpregnant females	0(0)	0(0)	0(0)	1(5.9)	0(0)
No. (%) of pregnant females	16(100)	16(100)	16(100)	16(94.1)	17(100)
No. of implatations per female(a,b)	15±1.1	14±1.5	15±1.5*	14.1±3.8	14.6±1.9
Preimplantation loss per female (%) (b,d)	2.4	4.5	4.4	32.7f	5.9
No. of litters	16	16	16	16	17
No. (%) of litters totally resorbed	0(0)	0(0)	3(18.8)	14(87.5)*†	2(11.8)
No. of corpora lutea per litter (a,c)	15.4±0.9	15.4±1.3	16.2±1.1	16.3±0.6	15.7±1.3
No. of implantations per litter (a,c)	15.0±1.1	14.7±1.5	15.6±1.5	14.9±1.3	14.6±.7
Preimplantation loss per litter (%) (c,d)	2.4	4.5	4.4	10.3_g	5.9
No. of resporption and dead foetuses per litter (a,c)	1.0±0.9	2.1±2.1	6.3±5.7*†	13.6±3.6*†	2.5±4.1
Early stage	1.1±0.9	2.1±2.1	6.3±5.7*†	13.6±3.6*†	2.5±4.1
Late stage	0	0	0	0	0
Postimplantation loss per litter (%)	7.0	13.9*	39.9*†	91.5*†	18.3
No. of live foetuses per litter	13.9±1.2	12.6±2.3	9.3±5.6*	1.3±.6*†	12.1±4.8
Sex ratio of live foetuses (male/female)	122/112	99/103	77/71	12/9	95/110
Body weight of live foetuses (g) (a)					
Male	3.45±0.17†	3.38±0.21†	2.99±0.37*	2.62±0.13*	2.98±0.19*
Female	3.22±0.17†	3.16±0.21†	2.85±0.24*	2.74±0.22*	2.74±0.22*

a)Values are given as mean+/- SD. b)Values are obtained from females successfully mated. c)Values are obtained from pregnant females. d)(No of corpora lutea – no. of implantations)/no. of corpora lutea) x 100. e)(No. of resorptions and dead foetuses/no. of implantations) x 100. f)Value is obtained from four females (no. of corpora lutea was not determined in 13 females with early total resorptions). g)Value is obtained from three females (no. of corpora lutea was not determined in 13 females with early total resorptions). *Significantly different from the control, P<0.005. †Significantly different from the pair-fed group, P<0.005.

4.11.1.2 Human information

No data.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Type of study:	**Developmental study with DBTDL and DBTC.**
Reference:	Noda T, Morita S and Baba A (1993).
Animal species & strain:	Three-month old female and male Wistar rats.
Test substance:	Five di-n-butyltin compounds (DBTA, DBTC, DBTM, DBTO, DBT(2-EHMA), DBTDL) with different anions, and also butyl(3-hydroxybutyl)tin dilaurate (3-OHDBTDL) were examined for

teratogenic effects. Controls were given olive oil.

DBTDL was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), DBTC from Merck (Dermstadt, Germany), and 3-OHDBTDL from Sorl Lab. (Mie, Japan).

Doses, vehicle, duration: A single gavage dose of 80 µmol/kg (50.5 mg/kg bw) DBTDL, 80 µmol/kg (24.3 mg/kg bw) DBTC, and 80 (51.8 mg/kg bw) or 160 µmol/kg (103.6 mg/kg bw) 3-OHDBTDL was given on day 8 and the animals were sacrificed on day 20 of gestation. The organotin compounds were diluted in olive oil.

Group sizes: 10 pregnant rats

Tests performed: Non-guideline or - GLP study. Number of animals and dose groups were less than recommended in OECD Guideline 414; however, the study is generally acceptable. Purity of test material is not reported.

Results: Maternal: No effect on maternal mortality, body weight gain or food consumption or incidence of dead or resorbed foetuses in dams compared to control group. Thymus effects were not reported.

Foetuses: No treatment related effect on body weight, but statistical significant higher incidence of external and skeletal malformations compared to the control group was observed in foetuses exposed in utero to DBTDL, 30.6% and 28.1%, respectively (Table 13 and Table 14). A significant increase in the number of mandible malformations (cleft mandible, cleft lower lip, ankyloglossia or schistoglossia, and exencephaly). Anomalies of mandibular fixation, fused mandibula, cranial hypoplasia, fused ribs, fused vertebral arches, and cleft maxilla were observed. Skeletal variations were predominately cervical rib. The findings for the other di-n-butyltin compounds (di-n-butyl diacetate, -dichloride, -maleate and -oxide) were similar. In table 15 and 16 the findings for DBTC is presented together with those for DBTDL. Higher incidence of external malformation observed in DBTDL compared with DBTC group while similar prevalence of skeletal malformation was observed (Table 15 and Table 16). The effects of DBTDL and DBTC were similar, but more pronounced for the DBTDL group.

Table 15. External observations of foetuses from dams orally treated with control (olive oil), DBTDL (80 µmol/kg) and DBTC (80 µmol/kg).

Observations	Olive oil (2 ml/kg)	DBTDL	DBTC
No of fetuses examined	126	130	107
Incidence of fetuses with malformation (%)	0	30.6(6)a	17.3(6)b
No. of fetuses with malformation	0	37(6)b	18(6)b
-Cleft mandible, cleft lower lip, ankyloglossia or schistoglossia	0	33(6)b	8(4)b
-Micrognathia	0	2(1)	1(1)
-Peaked mandible	0	0	1(1)

-Exencephaly	0	16(5)b	9(4)b
-Clefy upper lip	0	4(3)	1(1)
-Cleft palate	0	2(2)	0
-Facial cleft	0	0	2(2)
-Asymmetric face	0	0	1(1)
-Omphalocele	0	0	0
-Kinky tail	0	0	1(1)
-Vestigial tail with internal haemorrhage	0	0	0
-Pes varus	0	0	1(1)
-Pes valgus	0	0	0
-Scoliosis	0	0	3(1)

The litter was used as the statistical unit for calculation of fetal values, thus these values represent mean of litter means within each group. [a]Significantly different from control. P<0.05. [b]Significantly different from control. P<0.01. () is No of conceived mother with case.

Table 16. Skeletal observations of foetuses

Observations	Olive oil (2 ml/kg)	DBTDL	DBTC
No. of foetuses examined	126	130	107
Incidence of foetuses with malformations (%)	0	28.1(6)b	29.2(5)b
No. of foetuses with malformation	0	34(6)c	29.2(5)b
-Anomaly of mandibular fixation	0	25(6)c	29(5)c
-Fused mandibular	0	1(1)	2(2)
-Micromandibula and fused mandibular	0	2(1)	2(1)
-Cranial hypoplasia	0	15(5)c	3(3)
-Fused ribs	0	7(3)b	10(4)c
-Absence of ribs	0	0	25(4)c
-Fused vertebral arches	0	3(2)	12(2)c
Fused cervical vertebral arches	0	0	16(4)c
Fused thoracic vertebral arches	0	3(2)	6(2)c
Fused lumbar vertebral arches	0	0	16(4)c
-Cleft maxilla	0	3(3)	2(1)
-Agenesis of sacro or coccygeal vertebra	0	0	2(2)
-Agenesis of fibula, tibia, femur or ilium	0	0	0 2(2)
Incidence of foetuses with variations (%)	1.4(2)	65.3(8)c	95.9(8)c
No. of foetuses with variations	2(2)	82(8)c	103(8)c
-Asymmetry or cleft sternebrae	0	11(5)c	23(7)c
-Cervical rib	2(2)	76(8)c	100(8)c
-Lumbar rib	0	1(1)	1(1)
-Rudimentary lumbar rib	0	7(5)b	4(2)b
-Splitting or difurcation of 1st cerrvical vertebral arch	0	13(5)c	15(6)c
-Splitting of ossification centres of thoracic vertebral bodies	0	13(4)c	32(5)c
-Variations in number of vertebrae	0	0	13(4)c
Cervical vertebrae	0	0	6(2)c
Thoracic vertebrae	0	0	7(2)c
Lumbar verterbrae	0	0	3(3)
Occipital dysplasia	0	0	3(1)
Shortened 13 rib	0	0	5(2)b

The litter was used as the statistical unit for calculation of fetal values, thus these values represent mean of litter means within each group. b Significantly different from control. P<0.05, c Significantly different from control. P<0.01. () is No of conceived mother with case.

For 3-OHDBTDL, foetal malformations (peaked mandible: the tip of lower jaw with acute angle) were observed first at the highest dose 160 µmol/kg bw (103.6 mg/kg bw = LOAEL).

Type of study:	**Developmental study to support effects with DBTC**
Reference:	Ema M, Itami T and Kawasaki H (1991).
Animal species & strain:	Female Wistar rats (Jbc:Wistar) 14 weeks of age were mated overnight with males from the same supplier. The day when sperm was detected in the vaginal smear was considered to be Day 0 of gestation.
Test substance:	DBTC. Controls were given olive oil.
Doses, vehicle, duration:	Rats were given a gavage dose on GD7 to 15 by gastric intubation. The doses were 0, 2.5, 5, 7.5 and 10 mg/kg bw/day. The animals were sacrificed on day 20 of gestation. The DBTC was diluted in olive oil and the control group was given olive oil.
Group sizes:	10-12 pregnant rats per group.
Tests performed:	Non-guideline and -GLP study. Number of animals in each group is less than recommended in OECD Guideline 414; however, the study has been evaluated to be acceptable for classification of DBTDL. Purity of test material is not reported.
Results:	The majority of the rats given 7.5 and 10 mg/kg bw DBTDL showed reddish-brown staining of the facial fur and pilorection. A significantly high mortality of pregnant rats was also observed in these groups. Of the 12 pregnant rats, 5 and 9 deaths were noted in the 7.5 and 10 mg/kg bw groups, respectively in the time period GD 6-8. Almost all of the dead rats had haemorrhage in the stomach. The maternal bw from 7.5 was markedly lower from GD7 to 20 compared to control and lower dose groups. Food consumption was also reduced in the same period of time. No significant changes in maternal parameters were observed in the 2.5 and 5 mg/kg bw DBTDL groups. Hence, 5.0 mg/kg bw was suggested as maternal NOAEL. The reproductive findings observed in the study were complete resorption of all implanted embryos in 5 and 1 pregnant rats in 7.5 and 10 mg/kg bw groups, respectively. Only live foetuses were derived from 2 dams in these groups. A significantly higher number of resorptions and dead foetuses per litter, the incidence of post-implantation loss per litter and a lower number of live foetuses per litter were noted in the 7.5 mg/kg group. Body weight of the live foetuses was significantly lower in dams given 5.0 and higher doses of

DBTDL compared to the control.

Increased incidence of foetuses with external and skeletal malformations was noted from 5.0 mg/kg bw group. Various malformations increased in accordance with the increase in dosage. Cleft jaw and ankyloglossia were the most frequent malformations observed. A significantly high incidence of fetuses with these malformations was found from 5.0 mg/kg bw.

Micrognathia, cleft palate, omphalocele, exencephaly, anal atresia, club foot and anomalies of the tail such as vestigial, kinky and short tail were frequently observed from 5.0, mg/kg bw. No fetus with external malformations was found in the control and 2.5 mg/kg groups.

In the 5.0 mg/kg group, about 12% of the malformed fetuses had a single anomaly such as omphalocele and exencephaly. About 59% of the malformed fetuses had cleft jaw and ankyloglossia. In the 7.5 mg/kg group, about 12% of the malformed fetuses had micrognathia only. About 61% of the malformed fetuses had cleft jaw, ankyloglossia and/or cleft tongue. In the 10.0 mg/kg group, all malformed fetuses had multiple anomalies. About 88% of the malformed fetuses had cleft jaw, ankyloglossia and/or cleft tongue, and these fetuses also had other types of malformation. Most of the cleft jaw observed in the 7.5 and 10.0 mg/kg groups was more serious than that in the 5.0 mg/kg group.

A significant increase in the incidence of fetuses with skeletal malformations was observed at doses of 5.0 mg/kg and above. Defects of the mandible, fusion of the ribs and deformity of the vertebral column including fusion and/or absence of the vertebral bodies and/or arches in the cervical and/or thoracic regions were significantly increased in these groups. A few fetuses with internal malformations such as undestended testis, hydrocephaly and microphthalmia were found at doses of 5.0 mg/kg bw and above. These malformations were seen in fetuses with external malformations

The author report that cleft jaw and ankyloglossia, which were the most frequent malformations observed in this study, have never been seen in the background data, and that these types of malformation have been found following administration of dibutyltin diacetate throughout pregnancy in rats. The appearance of these unusual types of malformation in both studies suggests that both dibutyltin compounds possess selective teratogenicity and have a similar mechanism of action.

Table. 17 Morphologic defects in foetuses of dams given oral DBTDL

Dose of DBTDL (mg/kg bw)	**0**	**2,5**	**5**	**7,5**	**10**

External malformations					
No. external fetuses (litters) examined	130(11)	121(10)	125(11)	25(2)	27(2)
No. fetuses (litters) with malformations	0	0	18(5)*	18(2)*	16(2)*
No. fetuses (litters) with:	0	0	10(4)*	11(2)*	14(2)*
Cleft jaw	0	0	1(1)	7(1)	3(1)
Micrognathia	0	0	2(2)	0	3(1)
Cleft lip	0	0	1(1)	3(2)*	8(1)
Cleft palate	0	0	10(4)*	12(2)*	14(2)*
Ankyloglossia	0	0	0	2(1)	7(1)
Cleft tongue	0	0	2(2)	5(1)	6(2)*
Omphalocele	0	0	1(1)	3(1)	1(1)
Exencephaly	0	0	0	5(1)	2(1)
Encephalocele	0	0	0	1(1)	0
Open eyelids	0	0	4(2)	1(I)	1(1)
Anal atresia	0	0	0	1(1)	0
General edema	0	0	0	3(1)	0
Ectopia cordis	0	0	1(1)	6(1)	0
Oligodactyly	0	0	4(2)	2(1)	1(1)
Club foot	0	0	3(2)	2(2)*	1(1)
Anomaly of tail					
Skeletal malformations					
No. fetuses (litters) examined	84(11)	80(10)	83(11)	16(2)	18(2)
No. fetuses (litters) with malformations	0	0	18(5)*	13(2)*	10(2)*
No. fetuses (litters) with:	0	0	5(2)	13(2)*	10(2)*
Defect of mandible					
Fusion and/or absence of cervical vertebral arches	0	0	4(2)	7(2)*	4(1)
Fusion and/or absence of thoracic vertebral bodies and/or arches	0	0	7(2)	8(2)*	9(2)*
Fusion and/or absence of lumbar vertebral bodies and/or arches	0	0	1(I)	0	0
Fusion of ribs	0	0	12(4)*	10(2)*	8(1)
Absence of ribs	0	0	3(2)	1(1)	0
Cleft of sternum	0	0	0	3(1)	0

Fusion of sternebrae	0	0	3(3)	0	0
Internal malformations					
No. fetuses (litters) examined	46(11)	41(10)	42(11)	9(2)	9(2)
No. fetuses (litters) with malformations	0	0	1(1)	1(1)	3(1)
No. fetuses (litters) with:	0	0	1(1)	0	0
Undescended testis	0	0	0	1(1)	1(1)
Hydrocephaly	0	0	0	0	2(1)
Microphthalmia					

*Significantly different from the control value ($P < 0.05$).

Maternal NOAEL is 5.0 mg/kg bw.

Foetal NOAEL is 2.5 mg/kg bw.

Review of studies that have examined the most sensitive window for exposure to DBTC:

In the studies by Ema and coworkers (Ema et al. 1992, 1995 and 1996) the result show that exposure to DBTC on GD 7 and 8 caused increased incidence of malformation similar to the effects observed previously in other developmental studies with DBTC and DBTDL (Noda et al., 1993, Ema et al., 1991). The teratogenic effects were most pronounced at GD 8, while the effects did not appear when the dams were exposed at GD 6 or from GD 9 and later on in the gestation period. Embryolethality was observed at a broader time range; from GD 6 to 15. To conclude; the teratogen effects of DBTC is observed when exposure to DBTC is on GD 7-8. The most sensitive window for teratogenic effects of DBT-exposure is GD 8, while the embryolethality was observed at all tested time points for exposure in the gestation.

Review of studies published on DBTC after harmonised classification of DBTC: Several studies have been published from 2003 to 2009 by Ema and co-workers. The studies support previous results. In addition, these studies aim at investigating the mechanism for the early embryo loss and lethality caused by exposure to DBTC. In rodent studies DBTC reduced the level of serum progesterone and uterine weight in the dams. Administration of progesterone in addition to DBTC reversed the suppression of uterine decidualization in rats (Harazono and Ema, 2003) and was found to protect, in part, against DBTC-induced implantation failure (Ema et al., 2003). Low levels of serum progesterone were also observed in mice exposed to DBTC. Timing of exposure also seemed to influence the early pregnancy failure. DBTC adversely affected the initiation and maintenance of pregnancy when administrated during early pregnancy in mice and the decline in serum progesterone was suggested by the authors as the mechanism for the pregnancy failure (Ema et al., 2007). Two studies have also been conducted in cynomolgus monkeys where DBTC was found to be embryolethal but not teratogenic (Ema et al., 2007; Ema et al., 2009).

4.11.2.2 Human information

No data.

4.11.3 Other relevant information

None

4.11.4 Summary and discussion of reproductive toxicity

Based on the similar findings of malformations found in foetuses after DBTDL, DBTM, DBTO, DBT(2-EHMA), and DBTC exposure (Noda et al., 1993), one assumes that it is the di-n-butyltin (DBT) moiety, which these substances are degraded to in the stomach, that is the active component, rather than the anionic group. 3-OHDBTDL (or its expected stomach hydrolysis product 3-OHDBTC) is not the critical substance of teratogenicity (Noda et al., 1993). Since DBTDL is known to be hydrolysed into DBTC in the stomach, it is suggested that reproduction studies with DBTC could be used to classify DBTDL. The same approach was used in the meeting of TC C&L in 2006 where DBTDL was recommended classified as Repr. Cat 2; R60-61 according to DSD. This corresponds to Repr. 1B; H360FD according to CLP.

For DBTDL there are no fertility studies available. Hence, for fertility effects of DBTDL a study with DBTC by Ema and co-workers (Ema et al. 2000), which was used for classification for fertility of DBTC, is included. The study is not GLP and OECD, but is still evaluated as sufficient for classification of DBTC and DBTDL. In this study, among successfully mated females, 87% were non-pregnant. Also the number of implantations per female was lower and the incidence of pre-implantation loss was higher compared to the control groups. Statistical significantly higher incidence of early total resorption, higher number of non-pregnant females, lower number of implantations per females and higher incidence of preimplantation loss compared to the controls (from 7.6 mg/kg bw/day) in animals exposed to DBTC from GD 0-3 was observed. Based on these findings, NOAEL for fertility was set at 3.8 mg/kg bw/day (Ema et al. 2000).

For developmental effects, Noda et al., 1993 is the only relevant study that has examined effects of DBTDL. In this study the effects of several di-n-butyltin compounds were examined and developmental effects of DBTDL could be compared with the effects of DBTC. The Noda study is neither GLP nor OECD, but the quality of the study design is sufficient for classification of DBTDL. This was also the conclusion by TC C&L in 2006. Only one dose was tested (50.5 mg DBTDL/kg bw/day). No maternal effects were observed, but higher incidence of external and skeletal malformations compared with the control was found in the foetuses (30.6% and 28.1%, respectively). The effects observed for the other di-n-butyltin compounds (di-n-butyl diacetate, -dichloride, -maleate and -oxide) were similar to the developmental effects of DBTDL. In the Ema et al., 1991 study developmental effects of DBTC was examined and their results support the findings of Noda et al., 1993. Malformations in the fetuses were similar to the effects observed in the other developmental studies and were observed at 5 mg/kg bw/day without maternal toxicity at this dose level.

4.11.5 Comparison with criteria

Based on significantly increased external and skeletal malformations in foetuses exposed to DBTDL in utero, without maternal toxicity (Noda et al., 1993), classification of DBTDL for developmental effects is justified.

The key study (Ema et al., 2000) used for classification of effects on fertility of DBTC showed that among successfully mated females most of the dams were non-pregnant. Also the number of implantations per females was lower and the incidence of pre-implantation loss was higher compared to the control groups. High incidence of early total resorption in the dams was also

observed. Based on these findings DBTC was classified for fertility Repr. Cat. 2; R60 according to DSD.

DBTC has a harmonised classification with Repr. 1B; H360FD. DBTC is a metabolite formed rapidly in the stomach after oral exposure to DBTDL due to HCl-mediated (low pH) hydrolysis of DBTDL. In addition, similar developmental effects were observed for DBTDL and DBTC in the Noda study that support hydrolysis of DBTDL and that the active metabolite is the DBTC. Based on the above observations DBTDL should have the same classification as DBTC. Hence, DBTDL should be classified Repr. 1B; H360FD.

4.11.6 Conclusions on classification and labelling

Classification with Repr.1B; H360FD is proposed.

4.12 Other effects

No harmonised classification proposed.

5 ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Not evaluated in this dossier.

6 OTHER INFORMATION

None.

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