Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Dibutyltin maleate

(DBTM)

EC Number: **201-077-5**

CAS Number: **78-04-6**

Index Number: **Not applicable**

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CONTENTS

In the following a detailed study description is provided for studies cited and refered in the CLH report for dibutyltin maleate (DBTM).

The studies described in the section Toxicokinetic (Chapter 1) and Reproductive Toxictity (Chapter 2.2) and Specific Target Organ Toxicity (Chapter 2.3) (except unpublished report, 2017) below have been described in the CLH-dossier for DBTP (EC 245-152-0/ CAS 22673-19-4) and assessed by RAC in 2017.

The studies decribed in the section germ cell mutagenicity (Chapter 2.1) have been described in the CLH dossier for Dibutyltin di(acetate) (DBTA) (EC 213-928-8, CAS 1067-33-0) which is currently under evaluation.

Where the description of the studies below are in quotes the text is a verbatim rendering of the text in the mentioned CLH-dossier for DBTP and DBTA.

Only the simulated gastric hydrolysis study carried out by Umweltbundesamt in 2019 (Chapter 1.1.2) and the prenatal developmental toxicity study with DBTO (unpublished report, 2017) are described for the first time in detail.

1 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

The studies described below have been described in the CLH-dossier for DBTP (EC 245-152-0) and assessed by RAC in 2017. Where the description of the studies below are in quotes the text is a verbatim rendering of the text in the mentioned CLH-dossier.

1.1 Simulated gastric hydrolysis

Reference	”Schilt R & Zondervan-van den Beuken EK (2004).
	Dibutyltin dilaurate (DBTL, CAS #77-58-7), Dibutyltin maleate (DBTM, CAS #78-04-6), Dibutyltin oxide (DBTO, CAS #818-08-6) and Dioctyltin oxide (DOTO, CAS #870-08-6): simulated gastric hydrolysis.
	TNO Nutrition and Food Research, Zeist, The Netherlands. TNO Report V5047.
Guideline	None followed
Reliability	Klimisch 2: reliable with restrictions (non-guideline study)
Species/strain	Not relevant: *in vitro* study
Test material	**DBTL**
	CAS 77-58-7
	EC 201-039-8
	Purity 98.2%
	DBTM
	CAS 78-04-6
	EC 201-077-5
	Purity 99.65%
	DBTO
	CAS 818-08-6
	EC 212-449-1
	Purity 99.2%
Study design	Gastric hydrolysis studies were performed under the auspices of the Organotin Environmental Programme (ORTEP) Association Stabilizer Task Force. Simulated gastric reaction studies were

performed using dibutyltin dilaurate (DBTL), dibutyltin maleate (DBTM) and dibutyltin oxide (DBTO) at approximate concentrations of 0.015-0.040 mM. The extent of hydrolysis was assessed under low pH (1-2) conditions (0.07 N HCl) at 37°C, simulating mammalian gastric contents. The degree of hydrolysis was measured by determination of the amount of DBTC formed after 0.5, 1, 2, and 4 hours, using GC-FPD.

Findings Simulated gastric hydrolysis studies indicate that dibutyltin substances undergo rapid conversion to dibutyltin chloride species when exposed to conditions representative of the mammalian stomach.

Conversion of dibutyltin compounds to DBTC

Time	DBTL	DBTM	DBTO
0.5 h	82%	100%	43%
1 h	78%	97%	65%
2 h	88%	98%	80%
3 h	-	-	-
4 h	87%	95%	87%

Conclusion DBTL, DBTM and DBTO are shown to be rapidly converted to dibutyltin chloride species under conditions representative of the mammalian stomach. The generation of a common intermediate supports the read-across approach and the formation of a category for these substances and for dibutylbis(pentane-2,4-dionato-O,O')tin."

1.2 Simulated gastric hydrolysis

Reference Umweltbundesamt (2019) NMR based investigation of the hydrolysis of DOTE and DBTM

Report No 0709, Vienna 2019

Guideline None followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study)

Species/strain Not relevant: *in vitro* study

Test material **Dibutyltin maleate (DBTM)**

CAS 78-04-6

EC 201-077-5

Purity >95 %

Study design Hydrolysis study was performed using dibutyltin maleate

The extent of hydrolysis was assessed under low pH conditions (0.1 mol/L of aqeous HCL, 72h, 40°C). The degree of hydrolysis was measured after workup with dichloromethane-D_2 by ^{119}Sn NMR.

Findings Hydrolysis studies demonstrate that DBTM is hydrolysed to the dimeric stannoxane $ClBu_2SnOSnBu_2Cl$.

Conclusion Dibutyltin maleate is shown to be converted to $ClBu_2SnOSnBu_2Cl$ under acidic conditions. The generation of a common intermediate, identical to the hydrolysis product of DBTC (see 1.4) and also to DBTP (see 1.3), supports the read-across approach for the involved substances in the category including DBTO and DBTM.

1.3 Simulated gastric hydrolysis

Reference	”Naßhan H (2015). Dibutylbis(pentane-2,4-dionato-O,O´)tin [DBTAcAc) CAS number: 22673-19-4. In-vitro Metabolism Study Galata Chemicals GmbH, Chemiestrasse 22, 68623 Lampertheim, Germany
Guideline	None followed
Reliability	Klimisch 2: reliable with restrictions (non-guideline study)
Species/strain	Not relevant: *in vitro* study
Test material	**Dibutylbis(pentane-2,4-dionato-O,O´)tin** CAS 22673-19-4 EC 245-152-0 Purity >90 %
Study design	Simulated gastric hydrolysis studies were performed using dibutylbis(pentane-2,4-dionato-O,O´)tin. The extent of hydrolysis was assessed under low pH (1.2) conditions at 37°C at a concentration of 23.2 mM. The degree of hydrolysis was measured after workup in hexane by ^{119}Sn NMR in toluene-d^8 which allowed positive identification of the hydrolysis product. Any remaining tin-residues (decomposition products and/or water soluble substances) was analysed by atomic absorption spectrometry (AAS).
Findings	Simulated gastric hydrolysis studies demonstrate that dibutylbis(pentane-2,4-dionato-O,O´)tin rapidly form the dimeric stannoxane $ClBu_2SnOSnBu_2Cl$ (^{119}Sn-NMR: δ (ppm) -91, -144) in almost quantitative yield when exposed to conditions representative of the mammalian stomach. Minor amounts (~2 mol%) of non-hydrolyzed DBTC was also detected.
Conclusion	Dibutylbis(pentane-2,4-dionato-O,O´)tin is shown to be rapidly converted to $ClBu_2SnOSnBu_2Cl$ under conditions representative of the mammalian stomach. The generation of a common intermediate, identical to the hydrolysis product of DBTC (see 1.4) and DBTM (see 1.2).”

1.4 Simulated gastric hydrolysis

Reference	”Naßhan H (2016). Dibutyltin dichloride [DBTC] CAS number: 683-18-1. In-vitro Metabolism Study. Galata Chemicals GmbH, Chemiestrasse 22, 68623 Lampertheim, Germany
Guideline	None followed
Reliability	Klimisch 2: reliable with restrictions (non-guideline study)
Species/strain	Not relevant: *in vitro* study
Test material	**Dibutyltin dichloride** CAS 683-18-1 EC 211-670-0 Purity >90 % (Tributyltin chloride (TBTC) was identified as impurity in small amounts)
Study design	Simulated gastric hydrolysis studies were performed using dibutyltin dichloride. The extent of hydrolysis was assessed under low pH (1.2) conditions at 37°C at a concentration of 33 mM. The degree of hydrolysis was measured after 30 s, 1 h, and 4 h respectively, after workup in hexane by ^{119}Sn NMR in toluene-d^8 which allowed positive identification of the hydrolysis product.
Findings	Simulated gastric hydrolysis studies demonstrate that dibutyltin dichloride rapidly form the dimeric stannoxane $ClBu_2SnOSnBu_2Cl$ (^{119}Sn-NMR: δ (ppm) -91, -144) as the only observed hydrolysis product when exposed to conditions representative of the mammalian stomach. Minor amounts (~6 mol%) of DBTC remains after 4 hours. The impurity tributyltin chloride remains unchanged during the hydrolysis. The recovery of total tin (as calculated from the isolated product mass) ranged from

80-97%.

Conversion of DBTC to $ClBu_2SnOSnBu_2Cl$

Time	DBTC	$ClBu_2SnOSnBu_2Cl$	TBTC
30 s	25 mol%	70 mol%	5 mol%
1 h	11 mol%	85 mol%	4 mol%
4 h	6 mol%	90 mol%	4 mol%

Conclusion Dibutyltin dichloride is shown to be rapidly converted to $ClBu_2SnOSnBu_2Cl$ under conditions representative of the mammalian stomach. The generation of a common intermediate, identical to the hydrolysis product of dibutyltin maleate (see 1.2) and dibutylbis(pentane-2,4-dionato-O,O´)tin (see 1.3), supports the read-across approach for the involved substances in the category."

1.5 Toxicokinetics in the mouse

Reference "Kimmel EC, Fish RH & Casida JE (1977)

Bioorganotin Chemistry. Metabolism of Organotin Compounds in Microsomal Monooxygenase Systems and in Mammals

Journal of Agriculture & Food Chemistry 25 (1):1-9.

Guideline None

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species/strain Mouse (Swiss Webster)

Test material **Dibutyltin (di)acetate**

CAS 1067-33-0

EC 213-928-8

Radiochemical purity >99%

Study design In an *in vivo* phase, groups of mice (group size not specified) were gavaged with a single oral dose of 1.1 mg/kg bw ^{14}C-butyl labelled dibutyltin (di)acetate (in methoxytriglycol). Urine and faeces were investigated for metabolites. Tissue levels of radioactivity were investigated at 138 hours following dosing.

In an i*n vitro* phase, the metabolites of ^{14}C butyl labelled dibutyltin (di)acetate were investigated in rat liver microsomal systems. The metabolism of unlabelled dibutyltin dichloride was also investigated.

Findings *In vitro*, rat microsomal systems were shown to generate ^{14}C butyl labelled dibutyltin (di)acetate to dibutyl and monobutyl species by both nonenzymatic destannylation and by a- and β-carbon hydroxylation and decomposition of the hydroxy derivatives.

The results of the *in vivo* phase indicate partial absorption of dibutyltin (di)acetate in the mouse following oral gavage; the faeces contained a proportion of non-metabolised test material and some non-labelled dibutyltin derivatives. Extensive cleavage of the tin-carbon bond was also indicated, with further metabolism of the liberated butyl group to (exhaled) carbon dioxide and small quantities of butene.

Conclusion The results of this study show that oral administration of dibutyltin (di)acetate to the mouse results in hydrolysis of the test material to form an unidentified dibutyltin compound and liberation of the acetate moieties, which are further transformed and incorporated into normal cellular metabolism."

1.5.1 Toxicokinetics in the rat

Reference "Ishizaka T, Suzuki T & Saito Y (1989)

Metabolism of Dibutyltin Dichloride in Male Rats

	Journal of Agricultural and Food Chemistry 37(4): 1096-1101.
Guideline	No guideline followed
Reliability	Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)
Species/strain	Rat (Wistar)
Test material	**Dibutyltin dichloride (DBTC)** CAS 683-18-1 EC 211-670-0
Study design	The metabolism of DBTC was investigated in male Wistar rats following a single intraperitoneal administration at a dose level of 4 mg/kg bw. Rats were terminated at time points of 6-168 hours after administration. Blood and urine samples were collected and the liver, kidneys and brain were removed and analysed for the presence of DBTC and its metabolites.
Findings	DBTC and its metabolites were detected in the liver, kidney and spleen at 6 hours after administration. The half-live of DBTC in the liver, kidney and blood was calculated to be between 3-5 days. The accumulation of DBTC in the brain was found to be relatively slow compared to the other tissues investigated in this study. 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, butyl(4-hydroxybutyl)tin dichloride and butyltin trichloride were detected by HPLC and MS. The authors suggest that butyl(3-hydroxybutyl)tin dichloride may be formed in the liver and accumulate in the kidney. DBTC and butyl(3-hydroxybutyl)tin dichloride were shown to be excreted into the bile. The concentration of DBTC in the blood was about 1/20 of the concentration in the liver and kidneys."

2 HEALTH HAZARDS

2.1 Germ cell mutagenicity

Detailed summaries of studies relevant to classification for mutagenicity are presented in this section.

The studies described below have been described in the CLH dossier for Dibutyltin di(acetate) (DBTA) (EC 213-928-8) which is currently under evaluation. Where the description of the studies below are in quotes the text is a verbatim rendering of the text in the CLH-dossier for DBTA.

2.1.1 Micronucleus assay (chromosome aberration). Key study.

Reference	**"**Anonymous, 1991 (*in vivo* mammalian somatic cell study: cytogenicity / erythrocyte micronucleus, registration dossier for DBTA on ECHAs dissemination site)
Guideline	Performed according to OECD guideline No. 474 (Mammalian Erythrocyte Micronucleus Test). Conducted according to GLP.
Reliability	Klimisch 2: reliable with restrictions. Key study.
Species/strain	Bone marrow erythrocytes from male and female mice (ICR).
Test material	**Dibutyltin dichloride (DBTC)** CAS 683-18-1 EC 211-670-0 Purity 97.7%
Study design	Male and female mice were given a single oral dose of DBTC (in corn oil) at 2, 10 or 50 mg/kg. 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. Dose selection was based on 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 (severe rales, piloerection, immobility, hunched posture and uneven respiration) and all were killed in extremis 4 hours (500 mg/kg) or 23 hours (125 and 250 mg/kg) following

dosing. All animals dosed at 62.5 mg/kg bw showed piloerection on the day following dosing, males were hunched and lethargic from day 3 until termination and all 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.

Dose groups consisted of 5 male/5 female in the 2 and 10 mg/kg bw groups and 15 males/15 females in 50 mg/kg bw and control group. Control animals were given corn oil at 10 ml/kg bw. The positive control group (5 male/5 female) were given Chlorambucil orally (30 mg/kg in aqueous 10% ethanol). The mice were housed in single sex groups of two or five.

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.

Findings Positive - a biologically and statistically significant increase in the incidence of micro-nucleated 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."

2.1.2 Micronucleus assay (chromosome aberration)

Reference "Anonymous, 1990 (in vivo mammalian somatic cell study: cytogenicity / erythrocyte micronucleus, registration dossier for DBTA on ECHAs dissemination site)

Guideline Internal Method No. 185.3, Experimental Toxicology+.
References:
- Schmid, W., The micronucleus test for cytogenetic analysis In: Hollaender, A. (ed.) Chemical Mutagens, vol. 4, Plenum Press, New York, 1976, p.31-53.
- Schmid, W., The micronucleus test In: Handbook of mutagenicity test procedures B.J. Kilbey et al. (eds.), Elsevier, Amsterdam, New York, Oxford, 1977, p.235-242.
Study did not identify if it was conducted in accordance with Good Laboratory Practices (GLP), however, quality assurance was equivalent.

Reliability Klimisch 2: reliable with restrictions.

Species/strain Male and female mice (NMRI)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design 0, 50, 100, and 200 mg/kg bw by gavage, single exposure. DBTC 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. Doses were based on findings in a preceeding acute toxicity study where toxic effects were seen at 200 mg/kg bw.

Negative control and test groups consisted of 15 males and 15 females (30 in total) with an additional 3 reserve animals of each sex in the high-dose group. Control animals were given the vehicle (arachis oil) at 10 ml/kg bw. The positive control was triaziquone (0.15 mg/kg bw; single i.p. treatment) given to 5 males and 5 females.

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: proportion of micronucleated PCE, proportion of micronucleated NCE and ratio of PCE/NCE.

Findings 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).”

2.1.3 DNA damage in rat cerebral cortical cells (single cell gel electrophoresis)

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.

Guideline Non-guideline. Non-GLP.

Reliability Klimisch 3: reliable with restrictions (non-guideline study published in a peer-reviewed journal, but of low quality and with major deviations particularly regarind methods and results).

Species/strain Wistar male/female rats

Test material **Dibutyltin dilaurate (DBTDL)**

EC number: 201-039-8

CAS Number: 77-58-7

Purity not reported

Study design Animals (40 in total, 10 rats/dose group) 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. 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.

Findings 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."

2.2 Reproductive toxicity

Detailed summaries of studies relevant to classification for reproductive toxicity (adverse effects on sexual function and fertility, adverse effects on development of the offspring, effects on or via lactation) are presented in this section.

The studies described below have been described in the CLH-dossier for DBTP (EC 245-152-0) and assessed by RAC in 2017. Where the description of the studies below are in quotes the text is a verbatim rendering of the text in the mentioned CLH-dossier.

2.2.1 Animal data

2.2.1.1 Reproductive/developmental toxicity screening study in the rat

Reference "Unpublished report (2003) TNO, The Netherlands. TNO Report V4906.

[Study summary included in the publically disseminated REACH Registration Dossier for dibutyltin dichloride]

Guideline OECD 421

Reliability Klimisch 2: reliable with restrictions (Guideline and GLP-compliant study, full report not available)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

98.57% purity

Study design Groups of 12 Wistar rats/sex were administered the test material in the diet at concentrations of 0, 5, 30 or 200 ppm for four weeks (males) or for two weeks prior to mating and to Day 4 or 6 *post partum* (females).

Animals were observed daily for mortality and clinical signs. Bodyweights were measured pre-test and on Days 0, 7 and 14 of the pre-mating period. Bodyweights of males were measured on Days 21 and 28; bodyweights of females were measured during mating (Days 0, 7 and 14); on Days 0, 7, 14 and 21 of gestation; on Days 1 and 4 of lactation and at termination. Food consumption was measured for both sexes during the pre-mating period (Days 0-7, 7-13); for males during the post-mating period (Day 21-28); for females during the gestation period (Days 0-7, 7-14, 14-21) and during the lactation period (Days 1-4).

At termination, weights of the kidney liver, spleen, testes, thymus and brain were recorded for parental animals. Gross necropsy was performed on all parental animals; the prostate, seminal vesicles and testes of male animals were investigated. The ovaries, uterus (including counting of implantation sites) and thymus were investigated in females. Pups were assessed for gross abnormalities.

Findings Mean male bodyweights were significantly lower from Day 14-28 of the study at the highest dose level. Weight gains by males at the highest dose level were significantly lower over the study period; weight gain by males at 30 ppm was also reduced from Day 14-21. There was no effect of treatment on weight gain in males administered 5 ppm DBTC. Weight gain by females at the highest dose level was reduced over the premating, gestation and lactation periods; mean bodyweights of females in this group were significantly lower at Day 14 of the premating period, over the entire gestation period and during the lactation period. Food consumption by males at the highest dose level was reduced; significantly at Day 7-14. Food consumption by females in this group was significantly reduced during the pre-mating, gestation and lactation periods. Food consumption by females in the other treatment groups was comparable to controls.

The number of pregnant females was comparable in all groups. A marked increase in post-implantation loss was seen at 200 ppm; only three females in this group had live offspring. Pup weight at birth and Day 4 at the highest dose level was also significantly lower than controls. Pup mortality in this group was markedly increased (50%) compared to controls (5%). One pup at the highest dose level had a missing tail tip.

Reproductive parameters

Dietary concentration (ppm)	**0**	**5**	**30**	**200**
Mated (#)	12	11	12	12
Pregnant (#)	9	8	7	7
Females with liveborn (#)	9	8	7	3
Gestation index	100%	100%	100%	43%
Live birth index	99%	99%	94%	56%
Litters with stillborn pups	1	1	3	3
Post-implantation loss	13.4%	7.5%	20.4%	87.6%

Gross necropsy and histopathology of males did not reveal any effects of treatment on the reproductive tract. Thymuses of males were not investigated histopathologically. Severe or very severe thymic lymphoid depletion was observed in females at 200 ppm; moderate to severe lymphoid depletion was seen in the majority of the pregnant (but not in the non-pregnant) females at 30 ppm. Lymphoid depletion was characterised by decreased size of the thymic lobules due to extensive loss of cortical and medullary lymphocytes. The border between the thymus cortical and medullary areas was therefore indistinct. In the most 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.

Conclusion Administration of DBTC in the diet at a concentration of 200 ppm caused an increase in post-implantation loss. The NOAEL for effects on reproduction for this study is therefore 30 ppm (equivalent to 1.7-2.4 mg/kg bw/d in females)."

2.2.1.2 Developmental toxicity study in the rat

Reference "Ema M & Harazono A (2000). Adverse effects of dibutyltin dichloride on initiation and maintenance of rat pregnancy. Reproductive Toxicology 14: 451-456.

Guideline No guideline followed. The study was designed to assess the effects of exposure to the test material on post-implantation loss following exposure of female rats during the early gestation period.

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

97% purity

Study design Mated female Jcl:Wistar rats (16-19/group) were gavaged with the test material (in olive oil) at dose levels of 0 (vehicle control), 3.8, 7.6 or 15.2 mg/kg bw/d on Gestation Day 0-3 or Gestation Day 4-7. Groups of food-restricted rats were provided with the same amount of diet as consumed by rats administered the test material at 15.2 mg/kg bw/d on GD 0-3 or on GD 4-7.

Rats were observed for mortality and signs of toxicity. Bodyweights and food consumption were measured daily. Female rats were terminated on Gestation Day 20 and the uterus assessed. Corpora lutea and implantation numbers were reported. Foetuses were assessed for viability, sexed, weighed and investigated for gross external malformations and malformations of the oral cavity.

Findings No deaths were seen in females of any group. After administration of the test material on GD 0-3,

female rats in the treated groups showed signs of toxicity (chromodacryorrhoea, piloerection and diarrhoea); the incidence of clinical signs increased in a treatment-related manner. Bodyweight gains on Days 0-4 were significantly reduced in all treated groups; weight loss was seen. Bodyweight gains on Days 4-20 and adjusted weight gains were significantly lower in females administered 7.6 and 15.2 mg/kg bw/d. Food consumption on Days 0-4 and Days 4-20 were significantly reduced at ≥3.8 mg/kg bw/d and at ≥7.6 mg/kg bw/d respectively. The proportion of non-pregnant females and the incidence of pre-implantation loss were both significantly higher at 7.6 mg/kg bw/d (compared to controls) and at 15.2 mg/kg bw/d (compared to the control and pair-fed groups). Only two dams at the highest dose level had litters with viable foetuses. In females with implantations, the numbers of implantations and live foetuses and the incidence of post-implantation loss in treated groups were comparable to controls. Mean foetal weights in treated groups were comparable to controls. Pair-fed controls showed a comparable weight loss to the highest dose level dams on GD 0-4; weight gain on GD 0-20 was less than controls but was notably higher than at the highest dose level. A slight increase in pre-implantation loss was seen in pair-fed controls, but not to the extent seen at the highest dose level; post-implantation loss was significantly higher than controls. Mean foetal weight was significantly reduced in the pair-fed controls.

Summary of findings: rats exposed GD 0-3

Group	Control	3.8	7.6	15.2	Pair-fed control
Mated (#)	19	16	16	16	17
Pregnant (#)	19	16	11*	2*	16
Non-pregnant (#)	-	-	5*	14*	1
Weight gain (g) D0-4	6	-2*	-14*	-20*	-20*
Weight gain (g) D4-20	100	104	74*	27*	75*
Adjusted weight gain (g)	35	29	16*	-5*	12
Food consumption (g) D0-4	51	35*	16*	13*	12*
Food consumption (g) D4-20	288	280	237*	197*	200*
Implantations (#)	15.0	15.0	10.1*	1.8*	13.4
Pre-implantation loss (%)	2.7	4.1	35.6*	87.9*	16.4
Litters (#)	19	16	11	2	16
Total resorption (#)	-	-	1	-	3
Corpora lutea (#)	15.0	15.6	15.6	14.5	16.2
Early resorptions (#)	1.0	1.0	3.0	1.0	4.3*
Late resorptions (#)	-	-	-	-	-
Post-implantation loss (%)	6.7	6.8	21.3	7.1	32.1*
Litter size (#)	14.1	14.0	11.6	13.0	10.0*
Foetal weight M (g)	3.42	3.50	3.48	3.25	3.09*
Foetal weight F (g)	3.25	3.26	3.28	3.02	2.95*

**significantly different to controls ($p<0.05$)*

After administration of the test material on Gestation Day 4-7, female rats in the treated groups showed signs of toxicity (chromodacryorrhoea, piloerection and diarrhoea); the incidence of clinical signs increased in a treatment-related manner. Weight gain over GD 4-8 was reduced in all treated groups, significantly at ≥7.6 mg/kg bw/d; food consumption over the same period was significantly reduced in all treated groups. Adjusted weight gain was significantly reduced in dams at 15.2 mg/kg bw/d. Pre-implantation loss was increased at 15.2 mg/kg bw/d; the number of total resorptions was significantly increased in this group and was slight increased at 7.8 mg/kg bw/d. Post-implantation loss was significantly increased in all treated groups, with a clear dose-response relationship. Litter size and mean foetal weights were significantly reduced at ≥7.6 mg/kg bw/d. Pair-fed controls also showed a significantly reduced weight gain over GD 4-8 and significantly reduced adjusted weight gain. A slight increase in post-implantation loss and significantly reduced mean foetal weights were

also seen in this group.

Summary of findings: rats exposed GD 4-7

Group	Control	3.8	7.6	15.2	Pair-fed
Mated (#)	16	16	16	17	17
Pregnant (#)	16	16	16	16	17
Non-pregnant (#)	-	-	-	1	-
Implantations (#)	15.0	14.0	15.0	14.1	14.6
Weight gain (g) D0-4	12	11	9	10	9
Weight gain (g) D4-8	8	4	-2*	-14*	-15*
Weight gain (g) D8-20	227	228	226	228	224
Adjusted weight gain (g)	35	32	30	5*	0*
Food consumption (g) D0-4	68	68	64	65	66
Food consumption (g) D4-8	57	46*	34*	25*	25*
Food consumption (g) D8-20	219	213	210	158*	145*
Pre-implantation loss (%)	2.4	4.5	4.4	32.7	5.9
Litters (#)	16	16	16	16	17
Total resorption (#)	-	-	3	14*	2
Corpora lutea (#)	15.4	15.4	16.2	16.3	15.7
Early resorptions (#)	1.1	2.1	6.3*	13.6*	2.5
Late resorptions (#)	-	-	-	-	-
Post-implantation loss (%)	7.0	13.9*	39.9*	91.5*	18.3
Litter size (#)	13.9	12.6	9.3*	1.3*	12.1
Foetal weight M (g)	3.45	3.38	2.99*	2.62*	2.98*
Foetal weight F (g)	3.22	3.16	2.85*	2.74*	2.74*

**significantly different to controls (p<0.05)*

In females with implantations, the numbers of *corpora lutea*, implantations, resorptions, dead and live foetuses, the incidence of totally resorption, the proportions of pre- and post-implantation loss were unaffected by treatment. Foetal bodyweight and sex ratio were comparable in all groups. No external foetal malformations were noted in any group.

Conclusion

The results of this study show that the administration of DBTC at dose levels of ≥7.6 mg/kg bw during very early gestation (GD 0-3) causes an increase in pre-implantation loss, including a high incidence of total litter loss. Maternal toxicity (clinical signs, reduced weight gain and food consumption) was seen in all treated groups.

Administration of DBTC at dose levels of ≥3.8 mg/kg bw during early gestation (GD 4-7) causes an increase in post-implantation loss. Maternal toxicity (clinical signs, reduced weight gain and food consumption) was seen in all treated groups. Reductions in litter size and foetal weight were seen at ≥7.6 mg/kg bw/d. Pair-fed control groups included in the design of this study show that maternal toxicity (reduced food consumption and weight gain) caused by exposure to the highest dose level of DBTC resulted in some effects (increased post-implantation loss, reduced foetal weight), but not to the same extent as seen in the DBTC-treated groups. Exposure to DBTC on GD 0-3 or GD 4-7 did not result in teratogenicity (external malformations or malformations of the oral cavity). A NOAEL of ≤3.8 mg/kg bw/d can be determined for this study, based on the significantly increased incidence of post-implantation loss in dams administered DBTC on GD 4-7."

2.2.1.3 Developmental toxicity study in the mouse

Reference "Ema M, Fujii S, Ikka T, Matsumoto M, Hirose A & Kamata E (2007a). Early pregnancy failure induced by dibutyltin dichloride in mice. Environmental Toxicology 22(1):44-52.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Mouse (CRlj:CD1(ICR)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

99.5% purity

Study design The effects of oral administration of DBTC during early gestation were investigated in the mouse. Groups of mated female ICR mice were gavaged with DBTC (in olive oil) at dose levels of 0 (vehicle control), 7.6, 15.2 or 30.4 mg/kg bw on GD 0-3 or GD 4-7. Mice were observed at least daily for signs of toxicity. Maternal bodyweights were recorded daily; food consumption was measured at regular intervals. Mice were terminated on GD 18 and the uterine contents examined. The uterus was weighed and the number of corpora lutea recorded. The numbers of implantations, live and dead foetuses and resorptions were counted. The uteri were placed in 10% ammonium sulphide for confirmation of pregnancy status. Foetuses were sexed, weighed and inspected for external malformations and malformations of the oral cavity. Placental weight was also measured. Terminal blood samples were taken from dams of control and highest dose groups for the measurement of serum progesterone and 17β-oestradiol.

Findings In mice administered DBTC on GD 0-3, mortality occurred in each treated group but without a dose-response relationship. It is unclear, therefore, if deaths are related to treatment. Signs of toxicity (vaginal discharge, hypoactivity, hypothermia) were observed in all treated groups; jaundice was additionally observed at 15.2 and 30.4 mg/kg bw. Bodyweight gain and food consumption were reduced in the treated groups; significantly at the highest dose level.

Maternal findings: dosing on GD 0-3

Dose level (mg/kg bw)	**0**	**7.6**	**15.2**	**30.4**
Mated (#)	12	12	12	12
Mortality (#)	-	2	1	1
Weight gain (g) GD 0-4	1.7	0.6	1.2	0.3*
Weight gain (g) GD 4-8	2.9	2.5	2.1	1.6
Weight gain (g) GD 8-18	20.1	9.9	7.9	5.3
Adjusted weight gain (g)	8.9	9.9	7.9	5.3
Food consumption (g) GD 0-4	1.82	15.0*	16.7	14.8*
Food consumption (g) GD 4-8	22.9	22.0	21.7	20.9
Food consumption (g) GD 8-18	71.7	71.0	64.6	57.8*

**significantly different to controls ($p<0.05$)*

The number of pregnant females was lower in al treated groups; significantly at 30.4 mg/kg bw and with a clear dose-response relationship; findings are associated with increased pre-implantation loss. Post-implantation loss was also increased in the treated groups, significantly at 15.2 mg/kg bw. Mean foetal weights were significantly reduced in all treated groups. There was no clear effect of treatment on the incidence of malformations. One foetus at 15.2 mg/kg bw showed findings characteristic of DBTC (cleft palate, kinked tail); however no findings were seen at the highest dose level (although the number of foetuses available for examination in this group was lower than other groups) and cleft palate was also seen in one control foetus.

Litter findings: dosing on GD 0-3

Dose level (mg/kg bw)	0	7.6	15.2	30.4
Mated (#)	12	12	12	12
Pregnant (#)	11	9	8	5*
Corpora lutea (#)	10.5	13.1	12.4	13.3
Implantations (#)	9.5	9.8	8.3	5.4
Pre-implantation loss (%)	9.7	29.7	34.0	58.3*
Total resorption (#)	-	-	1	1
Post-implantation loss (%)	10.1	14.1	41.3*	32.2
Live foetuses (#)	9.4	11.5	8.1	9.3
Foetal weight (M)	1.54	1.30*	1.14*	1.12*
Foetal weight (F)	1.42	1.28	1.08*	1.01*
Foetuses examined (#)	103	92	57	37
Malformations (#)	1 (1)	-	2 (1)	-
Cleft palate (#)	1 (1)	-	1 (1)	-
Kinked tail (#)	-	-	1 (1)	-

**significantly different to controls (p<0.05)*

In mice administered DBTC on GD 4-7, one death occurred at 15.2 mg/kg bw. Signs of toxicity (vaginal discharge, hypoactivity, hypothermia) were observed in all treated groups; jaundice was additionally observed at 30.4 mg/kg bw. Bodyweight gain and food consumption were reduced in the treated groups.

Maternal findings: dosing on GD 4-7

Dose level (mg/kg bw)	0	7.6	15.2	30.4
Mated (#)	12	12	12	12
Mortality (#)	-	-	1	-
Weight gain (g) GD 0-4	1.6	1.9	1.2	1.6
Weight gain (g) GD 4-8	3.1	1.9	0.5*	-0.3*
Weight gain (g) GD 8-18	24.9	14.9*	2.9*	2.4*
Adjusted weight gain (g)	8.3	8.1	3.2*	3.8*
Food consumption (g) GD 0-4	18.5	18.9	18.4	18.8
Food consumption (g) GD 4-8	21.8	19.2	16.4*	15.6*
Food consumption (g) GD 8-18	74.5	67.7	55.2*	57.2*

**significantly different to controls (p<0.05)*

The number of pregnant females was comparable in all groups. Pre-implantation loss was increased at 15.2 and 30.4 mg/kg bw. Total resorption was increased in all treated groups (significantly at 15.2 and 30.4 mg/kg bw) and with a clear dose-response relationship. Post-implantation loss was markedly increased in all treated groups and reached 100% at the highest dose level. Litter size was consequently reduced in all treated groups. Mean foetal weights were significantly reduced in all treated groups. There was no clear effect of treatment on the incidence of malformations. Two foetuses at 7.6 mg/kg bw showed malformations (omphalocoele, exencephaly); no malformations were seen at higher dose levels, however no foetuses were examined at 30.2 mg/kg bw/d and the numbers of foetuses examined at 15.2 was very low. A teratogenic effect of DBTC cannot therefore be excluded.

Litter findings: dosing on GD 4-7

Dose level (mg/kg bw)	0	7.6	15.2	30.4
Mated (#)	12	12	12	12

Pregnant (#)	11	11	10	11
Corpora lutea (#)	13.8	14.5	10.6	13.9
Implantations (#)	13.7	14.4	9.4	12.7
Pre-implantation loss (%)	8.9	8.9	24.7	18.3
Total resorption (#)	-	2	8*	10*
Post-implantation loss (%)	4.3	48.3*	94.4*	100*
Live foetuses (#)	13.1	7.2*	0.8*	-
Foetal weight (M)	1.45	1.23*	1.27	
Foetal weight (F)	1.39	1.18*	1.18	
Foetuses examined (#)	144	79	7	
Malformations (#)	-	2 (2)	-	
Omphalocoele (#)	-	1 (1)	-	
Exencephaly (#)	-	1 (1)	-	

**significantly different to controls (p<0.05)*

Serum progesterone levels were significantly lower in mice administered DBTC at 30.4 mg/kg bw/d on GD 0-3 and GD 4-7 of pregnancy (values represented graphically in the published paper).

Conclusion Administration of DBTC to pregnant mice during early gestation results in pregnancy failure, which is associated with reduced progesterone levels at high dose levels. Increased post-implantation loss was seen at all dose levels in this study, the NOAEL is therefore <7.6 mg/kg bw/d. There is no clear indication of teratogenicity in this study."

2.2.1.4 Mechanistic study in the rat

Reference "Ema M, Harazono A, Hirose A & Kamata E (2003). Protective effects of progesterone on implantation failure induced by dibutyltin dichloride in rats. Toxicology Letters 143(2):233-8.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain Rat (Jcl:Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

98% purity

Study design Groups of 14-15 mated female Jcl:Wistar rats were gavaged with DBTC (in olive oil) at dose levels of 0 (vehicle control), 7.6, or 15.2 mg/kg bw on GD 0-3, with or without progesterone supplementation (subcutaneous injection of 2 mg progesterone GD 0-8. Maternal bodyweight and food consumption were measured. Pregnancy outcome was determined on GD 9 and reproductive outcome was investigated. Numbers of corpora lutea and implantations were measured.

Findings Marked weight loss and reduced food consumption were observed at both dose levels of DBTC. Effects at 7.6 mg/kg bw/d were reduced slightly by the administration of progesterone; however progesterone administration had little effect at 15.3 mg/kg bw/d.

Administration of progesterone alone had no effect on pregnancy rate or on the number of implantations. Both the pregnancy rate and the number of implantations were significantly lower in the groups administered DBTC; some reduction in pregnancy rate and the number of implantations were also seen in the groups administered progesterone and DBTC; although parameters were not affected to the same extent as in the groups administered DBTC alone.

Summary of findings [24]

Dose level (mg/kg bw/d)	0		7.6		15.2	
Progesterone +/-	**-P**	**+P**	**-P**	**+P**	**-P**	**+P**
Weight gain (g) D0-4	8	7	-24*	-24*	-31*	-28*
Weight gain (g) D4-9	12	14	-11*	-22*	-35*	-31*
Food consumption (g) D0-4	48	46	10*	9*	4*	3*
Food consumption (g) D4-9	80	78	25*	15*	2*	4*
Mated (#)	14	14	15	14	15	14
Pregnant (#)	14	14	7*	13	5*	9*
Implantations (#)	14.9	15.1	5.6*	11.6	2.9*	6.1*
Pre-implantation loss (%)	8.6	10.5	62.8*	25.9*	81.3*	60.0*

**significantly different to controls (p<0.05)*

Conclusion The study confirms other data by the same authors which demonstrates an adverse effect of DBTC on pregnancy rate and implantation numbers when administered to pregnant rats during very early gestation. There is some indication for a protective effect of progesterone on implantation failure; the authors therefore propose that implantation failure due to DBTC is due to a decline in progesterone levels.

NOAELs of <7.6 mg/kg bw/d for maternal toxicity and developmental toxicity can be determined for this study."

2.2.1.5 Mechanistic study in the rat

Reference "Harazono A & Ema M (2003). Suppression of decidual cell response induced by dibutyltin dichloride in pseudopregnant rats: as a cause of early embryonic loss. Reproductive Toxicology 17(4):393-9.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

No purity details

Study design Groups of pseudopregnant female Wistar rats were administered DBTC by gavage at dose levels of 0 (vehicle control), 3.8, 7.6 or 15.2 mg/kg bw on pseudopregnant day (PPD) 0-3 or PPD 4-7. Decidual cell response was induced by bilateral uterine scratch on PPD 4. Uterine weight (PPD 9) was used as an index of uterine decidualisation.

Findings Uterine weight and serum progesterone levels on PPD 9 were significantly decreased after administration of DBTC at 7.6 and 15.2 mg/kg bw (PPD 0-3 and 4-7). Treatment with DBTC had no effect on the serum oestradiol levels or the number of corpora lutea. Administration of progesterone reversed the suppression of uterine decidualisation seen in rats administered DBTC on PPD 0-3.

Conclusion The authors conclude that DBTC administration to the pregnant rat suppresses the uterine decidual cell response and decreases progesterone levels. It is proposed that these effects may be factors involved in the induction of early embryonic loss resulting from exposure to DBTC."

2.2.1.6 Developmental toxicity study in the rat

Reference "Noda T, Morita S & Baba A (1993). Teratogenic effects of various di-n-butyltins with different anions and butyl(3-hydroxybutyl)tin dilaurate in rats. Toxicology 85: 149-60.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Dibutyltin di(acetate) (DBTA)

CAS 1067-33-0

EC 211-670-0

Purity not reported

Dibutyltin maleate (DBTM)

CAS 78-04-6

EC 201-077-5

Purity not reported

Dibutyltin dilaurate (DBTL)

CAS 77-58-7

EC 201-039-8

Purity not reported

Dibutyltin oxide (DBTO)

CAS 818-08-6

EC 212-449-1

Purity not reported

Study design Groups of 10 mated female Wistar rats were gavaged with a single dose (equivalent to 80 µmol/kg bw) of five dibutyltin substances (in olive oil) on Gestation Day 8. A concurrent control group received the dosing vehicle only. Dams were observed daily for clinical signs; bodyweights and food consumption were measured daily. Dams were sacrificed on Gestation Day 20 and the uterine contents investigated. Foetuses were weighed, sexed and were assessed for external malformations and for skeletal malformations following staining with Alizarin Red S.

Findings There was no maternal mortality or signs of toxicity. Maternal bodyweights and food consumption were unaffected by treatment. No significant effects of treatment were seen on implantation numbers, implantation losses, litter size or foetal weight.

A significantly higher incidence of external foetal malformations was observed in all the treated groups; the nature of malformations was similar in all groups. Findings consisted predominantly of exencephaly and mandible findings (cleft mandible, cleft lower lip, ankyloglossia, schistoglossia).

External malformations

Group	Control	DBTA	DBTC	DBTM	DBTO	DBTL
Foetuses examined (#)	126	133	107	124	129	130
Malformations (%)	-	28.3**	17.3**	12.5	20.7*	30.6*
Malformations (#)	-	37 (7)**	18 (6)**	16 (5)**	28 (6)**	37 (6)**

Cleft mandible, cleft lower lip, ankyloglossia or schistoglossia	-	37 (7)**	8 (4)**	13 (5)**	23 (6)**	33 (6)**
Micrognathia	-	2 (1)	1 (1)	-	-	2 (1)
Peaked mandible	-	-	1 (1)	-	1 (1)	-
Exencephaly	-	18 (6)**	9 (4)**	-	7 (6)*	16 (5)**
Cleft upper lip	-	3 (1)	1 (1)	5(2)*	2(2)	4 (3)
Cleft palate	-	1 (1)	-	-	1(1)	2 (2)
Facial cleft	-	-	2 (2)	-	-	-
Asymmetric face	-	1(1)	1 (1)	-	-	-
Omphalocoele	-	-	-	-	-	-
Kinked tail	-	-	1 (1)	-	-	-
Vestigial tail	-	-	-	-	-	-
Pes varus	-	-	1 (1)	-	-	-
Pes valgus	-	-	-	-	-	-
Scoliosis	-	-	3 (1)	-	-	-

**significantly different to controls (p<0.05); **p<0.01*

Skeletal malformations were also observed with significantly increased incidences in all treated groups. Anomalies of mandibular fixation, fused mandibula, cranial hypoplasia, fused ribs, fused vertebral arches and cleft maxilla were commonly observed.

Skeletal malformations

Group	**Control**	**DBTA**	**DBTC**	**DBTM**	**DBTO**	**DBTL**
Foetuses examined (#)	126	133	107	124	129	130
Malformations (%)	-	21.9**	29.2*	9.3	26.2 *	28.1*
Malformations (#)	-	29 (7)**	29 (5)**	12 (4)	30 (6)**	34 (6)**
Anomaly of mandibular fixation	-	17 (6)**	29 (5)**	11 (4)	18 (6)**	25 (6)**
Fused mandibles	-	1 (1)	2 (2)	-	1 (1)	1 (1)
Fused mandibles / micromandible	-	2 (1)	2 (1)	-	-	2 (1)
Cranial hypoplasia	-	12 (5)**	3 (3)	3 (2)	4 (4)	15 (5)**
Fused ribs	-	9 (2)**	10 (4)**	-	12 (3)**	7 (3)*
Absent ribs	-	2 (1)	25 (4)**	-	6 (2)*	-
Fused cervical arches	-	1(1)	16 (4)**	-	3 (1)	-
Fused thoracic arches	-	5 (1)	6 (2)**	-	8 (3)**	3 (2)
Fused lumbar arches	-	-	16 (4)**	-	-	-
Cleft maxilla	-	3 (1)	2 (1)	-	2 (2)	3 (3)
Vertebral agenesis	-	-	2 (2)	-	-	-
Leg bone agenesis	-	-	2 (2)	-	-	-

**significantly different to controls (p<0.05); **p<0.01*

The incidences of skeletal variations were also significantly increased in all treated groups; the most

common findings were asymmetric/cleft sternebra and cervical rib

Skeletal variations

Group	Control	DBTA	DBTC	DBTM	DBTO	DBTL
Foetuses examined (#)	126	133	107	124	129	130
Variations (%)	1.4	70.2**	95.9**	33.2**	66.7**	65.3**
Variations (#)	2 (2)	93 (8)**	103 (8)**	39 (9)**	83 (9)**	82 (8)**
Asymmetric/cleft sternebra	-	19 (6)**	23 (7)**	1 (1)	11 (4)**	11 (5)**
Cervical rib	2 (2)	90 (8)**	100 (8)**	37 (8)**	80 (9)**	76 (8)**
Lumbar rib	-	-	1 (1)	-	1 (1)	1 (1)
Rudimentary lumbar rib	-	4 (2)	4 (2)*	2 (1)	2 (2)	7 (5)*
Bifurcated cervical arch	-	8 (5)**	15 (6)**	1 (1)	14 (5)**	13 (5)**
Bifurcated thoracic vertebra	-	11 (2)**	32 (5)**	-	20 (3)**	13 (4)**
Variations in numbers of vertebrae	-	3 (1)	13 (4)**	-	6 (2)*	-
Occipital dysplasia	-	1 (1)	3 (1)	-	-	-
Short 13th rib	-	-	5 (2)*	-	3 (1)	-

**significantly different to controls (p<0.05); **p<0.01*

Conclusion The results of the study demonstrate that the di-*n*-butyltin compounds cause a similar spectrum of foetal malformations when administered during a sensitive period of gestation. The di-*n*-butyltin moiety rather than the associated anionic group is therefore concluded to be responsible for teratogenicity. A NOAEL cannot be determined for this study."

2.2.1.7 Developmental toxicity study in the rat

Reference "Study report (1994). Summary included in the publically disseminated REACH Registration Dossier for dibutyltin dichloride; the full study report is not available. Anonymous.

Guideline OECD 414; no deviations reported

Reliability Klimisch 2: reliable with restrictions (Guideline and GLP-compliant study, full report not available)

Species / strain Rat (Wistar) Crl:CD(Wi)BR

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

>98% purity

Study design Groups of 25 mated Wistar female rats were gavaged with the test material (in corn oil) at dose levels of 0, 1, 2.5, 5 or 10 mg/kg bw/d on Days 6-15 of gestation. Dams were investigated for mortality and clinical signs. Bodyweights and food consumption were recorded at regular intervals.

Rats were sacrificed on Day 20 of gestation and the uterine contents investigated. All foetuses were assessed for external findings. Foetuses were sexed and weighed. Approximately half of the foetuses from each litter were assessed for visceral findings; the remainder of the foetuses were assessed for skeletal findings following staining with Alizarin Red.

Dams were subject to gross necropsy; the thymus, liver, bile duct and mesenteric lymph nodes were investigated histopathologically.

Findings No deaths occurred and no signs of maternal toxicity were observed during the study period. Reduced weight gain and food consumption were seen at 10 mg/kg bw/d; weight gain was also slightly reduced at 5 mg/kg bw/d. Gross necropsy of dams did not reveal any treatment-related

findings. Mean thymus weight was significantly lower in dams at 10 mg/kg bw/d. Histopathology of the thymus showed atrophy at 10 mg/kg bw/d and to a lesser extent at 5 and 2.5 mg/kg bw/d.

Mean litter size and foetal weights were comparable in all groups.

The incidence of foetuses with malformations was increased at 10 mg/kg bw/d; four foetuses from three litters had malformations. Oedema (anasarca) was observed in one foetus; the remaining three foetuses showed more severe malformations. One showed ankyloglossia, hydrocephaly, anophthalmia and diaphragmatic hernia. A second foetus exhibited agnathia, absent mandibles and malformed zygomatic arches. A third foetus had a filamentous and curly tail, scoliosis and an absence of sacral and caudal vertebrae and sacral vertebral arches.

Conclusion A NOAEL for maternal toxicity of 1 mg/kg bw/d can be determined for this study based on thymic atrophy at dose levels of ≥2.5 mg/kg bw/d; reduced weight gain at ≥5 mg/kg bw/d. A NOAEL for developmental toxicity of 5.0 mg/kg bw/d can be determined for this study based on an increased incidence of skeletal malformations at 10 mg/kg bw/d."

2.2.1.8 Developmental toxicity study in the rat

Reference "Farr CH, Reinisch K, Holson JF & Neubert D (2001). Potential teratogenicity of di-n-butyltin dichloride and other dibutyltin compounds. Teratogenesis, Carcinogenesis & Mutagenesis 21(6):405-15.

Guideline OECD 414

Reliability Klimisch 2: reliable with restrictions (guideline study summary, published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design A developmental toxicity study was conducted in the rat according to OECD guidelines and GLP. Groups of 25 mated female Wistar rats were gavaged with DBTC (in olive oil) at dose levels of 0, 1, 2.5, 5 or 10 mg/kg bw on GD 6-15. Evaluation of pregnancy outcome was performed on day 20 of pregnancy.

Findings Maternal toxicity (reduced food consumption, bodyweight gain and reduced thymus weight) were seen at 10 mg/kg bw. No evidence of embryotoxicity as assessed by numbers of total resorptions, viable foetuses or foetal weight was noted in any treated group. **A slightly increased frequency of total malformations was seen at 10 mg/kg bw (4/262 foetuses) compared to the control group (1/269 foetuses).** The authors consider that the nature and pattern of malformations does not suggest any effect of treatment; however the nature of findings (including single incidences of ankyloglossia, agnathia, mandibular defect) are consistent with the results of other studies and therefore indicate a relationship to treatment with DBTC

Maternal findings

Dose level (mg/kg bw)	0	1.0	2.5	5.0	10.0
Inseminated females (#)	25	25	25	25	25
Pregnant females (#)	20	25	23	19	20
100% intrauterine deaths (#)	0	1	0	1	0
Females with viable foetuses (#)	20	24	23	18	20
Malformed foetuses (#)	1/269	0-343	0-292	1/224	4/262
Maternal weight gain (g) GD 6-16	67.2	67.3	64.9	67.4	55.7*
Maternal food consumption (g) GD 6-16	25.5	25.5	25.2	25.9	23.7*

Maternal thymus weight (mg)	371	366	409	339	287**
Malformed foetuses (#)	1 (1)	-	-	1 (1)	4 (3)
Anasarca	-	-	-	1	1
Hydrocephaly	-	-	-	-	1
Ankyloglossia	-	-	-	-	1
Agnathia	-	-	-	-	1
Pulmonary valve atresia	1	-	-	-	-
Scoliosis	-	-	-	-	1
Anophthalmia	-	-	-	-	1
Mandible absent	-	-	-	-	1
Vertebrae / arches absent	-	-	-	-	1

* *significantly different to controls* p*<0.05; **p<0.01*

Conclusion A NOAEL of 5 mg/kg bw can be determined for teratogenicity and developmental toxicity, based on the slightly elevated incidence of characteristic foetal malformations at 10 mg/kg bw/d. A NOAEL of 5 mg/kg bw/d can be determined for maternal toxicity, based on reduced bodyweight gain, food consumption and reduced thymus weight at the highest dose level."

2.2.1.9 Developmental toxicity study in the rat

Reference "Ema M, Itami T & Kawasaki H (1991). Teratogenicity of di-n-butyltin dichloride in rats.Toxicology Letters 58(3): 347-356.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design Groups of mated female Wistar rats were gavaged with DBTC (in olive oil) at dose levels of 0 (vehicle control), 2.5, 5.0, 7.5 or 10 mg/kg bw/d on Days 7-15 of gestation. Dose levels were based on the individual bodyweights at Day 0 of gestation and were not subsequently adjusted. Animals were observed daily for mortality and clinical signs. Bodyweights and food consumption were also measured daily. Dams were terminated on Gestation Day 20; the numbers of live and dead foetuses and resorptions were recorded. Foetuses were sexed, weighed and investigated for external malformations and for malformations of the oral cavity. Placental weight was measured. Approximately two thirds of the foetuses from each litter were assessed for skeletal findings following staining with Alizarin Red S. The remaining foetuses were fixed in Bouin's solution and examined for internal malformations following freehand serial sectioning.

Findings The majority of rats administered 7.5 and 10.0 mg/kg bw/d DBTC showed signs of toxicity including chromodacryorrhoea and piloerection. A high level of mortality was seen in rats administered 7.5 mg/kg bw/d (5/12) and at 10 mg/kg bw/d (9/12) groups; deaths occurred on average at 8 and 6 days after dosing with 7.5 and 10 mg/kg bw/d, respectively. Necropsy of the decedent females revealed haemorrhagic stomachs. Maternal bodyweight gain on Gestation Days 7-15, 15-20 and 0-20 were markedly (and generally significantly) lower at 7.5 and 10 mg/kg bw/d compared to controls; adjusted weight gain was also significantly lower in these groups. Food consumption over Gestation Days 7-15, 15-20 and 0-20 was significantly lower at 7.5 and 10 mg/kg bw/d compared to controls. No significant effects on maternal bodyweight or food consumption were seen at 2.5 or

5 mg/kg bw/d.

Maternal findings

Dose level (mg/kg bw/d)	0	2.5	5.0	7.5	10
Pregnant rats (#)	11	10	11	12	12
Deaths (#)	0	0	0	5*	9*
Weight gain (g) GD 0-7	25	21	26	25	21
Weight gain (g) GD 7-15	38	34	27	-9*	6*
Weight gain (g) GD 15-20	65	61	59	-17*	30
Weight gain (g) GD 0-20	128	116	112	-2*	58*
Adjusted weight gain (g)	56	46	50	-20*	14*
Food consumption (g) GD 0-7	129	105	127	114	131
Food consumption (g) GD 7-15	140	126	118	80*	85*
Food consumption (g) GD 15-20	107	100	108	39*	69
Food consumption (g) GD 0-20	376	331	353	232*	285*

**significantly different to controls (p<0.05)*

Complete resorption was seen in at 7.5 mg/kg bw/d (5/7 surviving rats) and at 10 mg/kg bw/d (1/3 surviving rats); there were consequently only two dams with live foetuses at 7.5 and 10 mg/kg bw/d. Significantly higher numbers of resorptions and dead foetuses per litter, a significantly higher proportion of post-implantation loss and a significantly lower litter size were observed at 7.5 and 10 mg/kg bw/d. Mean foetal and placental weights were significantly lower at 5.0, 7.5 and 10 mg/kg bw/d.

Reproductive findings

Dose level (mg/kg bw/d)	0	2.5	5.0	7.5	10
Litters (#)	11	10	11	7	7
Implantations (#)	13.1	14.4	13.8	13.6	14.3
Resorptions (#)	1.3	2.3	2.5	10.0*	5.3
Post-implantation loss (%)	10.2	16.3	18.9	77.0*	37.9
Total resorption (#)	0	0	0	5*	1
Live foetuses (#)	11.8	12.1	11.4	3.6*	9.0
Foetal weight (g) M/F	4.05/3.92	3.84/3.63	3.36*/3.38*	2.50*/2.47*	2.80*/2.84*
Placental weight (g)	0.50	0.50	0.38*	0.29*	0.32*

**significantly different to controls (p<0.05)*

A dose-related increase in the incidence of foetuses with external malformations was observed at 5.0, 7.5 and 10 mg/kg bw/d. Craniofacial malformations predominated; most frequently cleft jaw and ankyloglossia. Cleft jaw varied in severity from mandibular hypoplasia and a small cleft on the midline of the lower jaw, to a large v-shaped cleft in the lower jaw. Mild findings were associated with fusion of the tongue at the midline of the lower lip; more sever cleft jaw was associated with ankyloglossia and/or cleft tongue. Micrognathia, cleft palate, omphalocoele, exencephaly, anal atresia, club foot and anomalies of the tail (vestigial, kinked and short tail) were also frequently observed in foetuses from the 5.0, 7.5 and l0 mg/kg bw/d dose groups. No external malformations were observed in the control or 2.5 mg/kg bw/d dose groups. In the 5.0 mg/kg bw/d group, 12% of the malformed foetuses had a single finding such as omphalocoele and exencephaly; 59% of the malformed foetuses had cleft jaw and ankyloglossia. The majority of affected foetuses in this group

had a relatively slight cleft jaw. At 7.5 mg/kg bw/d, 12% of the malformed foetuses had micrognathia only. 61% of the malformed foetuses had cleft jaw, ankyloglossia and/or cleft tongue. At 10 mg/kg bw/d, all malformed foetuses showed multiple findings; 88% of the malformed foetuses had cleft jaw, ankyloglossia and/or cleft tongue, and also had other types of malformation. The cleft jaw seen at 7.5 and 10 mg/kg bw/d was more severe than that seen at 5.0 mg/kg bw/d.

Incidence of external malformations

Dose level (mg/kg bw/d)	**0**	**2.5**	**5.0**	**7.5**	**10**
Examined	130 (11)	121 (10)	125 (11)	25 (2)	27 (2)
Total malformations (#)	-	-	18 (5)*	18 (2)*	16 (2)*
Cleft jaw (#)	-	-	10 (4)*	11 (2*)	14 (2)*
Micrognathia (#)	-	-	1 (1)	7 (1)	3 (1)
Cleft lip (#)	-	-	2 (2)	-	3 (1)
Cleft palate (#)	-	-	1 (1)	3 (2)*	8 (1)
Ankyloglossia (#)	-	-	10 (4)*	12 (2)*	14 (2)*
Cleft tongue (#)	-	-	-	2 (1)	7 (1)
Omphalocoele (#)	-	-	2 (2)	5 (1)	6 (2)*
Exencephaly (#)	-	-	1 (1)	3 (1)	1 (1)
Ecephalocoele (#)	-	-	-	5 (1)	2 (1)
Open eye (#)	-	-	-	1 (1)	-
Anal atresia (#)	-	-	4 (2)	1 (1)	1 (1)
Anasarca (#)	-	-	-	1 (1)	-
***Ectopia cordis* (#)**	-	-	-	3 (1)	-
Oligodactyly (#)	-	-	1 (1)	6 (1)	-
Club foot (#)	-	-	4 (2)	2 (1)	1 (1)
Tail anomaly (#)	-	-	3 (2)	2 (2)*	1 (1)

**significantly different to controls ($p<0.05$)*

A significant increase in the incidence of skeletal malformations was also observed at dose levels of 5.0 mg/kg bw/d 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. Defects of the mandible were found in foetuses with cleft jaw. The severity of the mandibular defect reflected the severity of cleft jaw and varied from separation of the right and left mandibles to small/short mandible and a wide separation between right and left mandibles. The incidence of fused ribs and deformed vertebral column was significantly increased at dose levels of 5.0 mg/kg bw/d and above. A dose-related increase in the incidence of foetuses with skeletal variations was also observed in these groups.

Incidence of skeletal malformations

Dose level (mg/kg bw/d)	**0**	**2.5**	**5.0**	**7.5**	**10**
Examined (#)	84 (11)	80 (10)	83 (11)	16 (2)	18 (2)
Total malformations (#)	-	-	18 (5)*	13 (2)*	10 (2)*
Mandibular defect (#)	-	-	5 (2)	13 (2)*	10 (2*)
Cervical arches fused/absent (#)	-	-	4 (2)	7 (2)*	4 (1)
Thoracic arches/bodies fused/absent (#)	-	-	7 (2)	8 (2)*	9 (2)*
Lumbar arches/bodies fused/absent (#)	-	-	1 (1)	-	-
Fused ribs (#)	-	-	12 (4)*	10 (2)*	8 (1)
Absent ribs (#)	-	-	3 (2)	1 (1)	-

Cleft sternum (#)	-	-	-	3 (1)	-
Fused sternebrae (#)	-	-	3 (3)	-	-

**significantly different to controls (p<0.05)*

Foetuses with internal malformations (undescended testis, hydrocephaly and microphthalmia) were observed at dose levels of 5.0 mg/kg bw/d and higher; findings were apparent in foetuses with external malformations.

Incidence of internal malformations

Dose level (mg/kg bw/d)	**0**	**2.5**	**5.0**	**7.5**	**10**
Examined	46 (11)	41 (10)	42 (11)	9 (2)	9 (2)
Total malformations	-	-	1 (1)	1 (1)	3 (1)
Undescended testes	-	-	1 (1)	-	-
Hydrocephaly	-	-	-	1 (1)	1 (1)
Microphthalmia	-	-	-	-	2 (1)

Conclusion Exposure to DBTC at dose levels of 5 mg/kg bw/d and above on Days 7-15 of gestation in the rat resulted in teratogenicity (predominantly craniofacial malformations). Dose levels of 7.5 and 10 mg/kg bw/d resulted in marked maternal toxicity (including mortality); however no maternal toxicity was apparent at 5.0 mg/kg bw/d. Administration of DBTC was also embryotoxic, resulting in complete resorption (at 7.5 and 10 mg/kg bw/d). Foetal weight was reduced at dose levels of 5.0 mg/kg bw/d and above; litter size was reduced at dose levels of 7.5 and 10 mg/kg bw/d.

Based on the results of this study, a NOAEL for developmental toxicity of 2.5 mg/kg bw/d can be determined. The NOAEL for teratogenicity is 2.5 mg/kg bw/d, based on increased incidences of craniofacial malformations at dose levels of 5.0 mg/kg bw/d and above. The NOAEL for maternal toxicity is 5.0 mg/kg bw/d, based on mortality and bodyweight effects at dose levels of 7.5 and 10 mg/kg bw/d."

2.2.1.10 Developmental toxicity study in the rat

Reference "Ema M, Itami T & Kawasaki H (1992). Susceptible period for the teratogenicity of di-n-butyltin dichloride in rats. Toxicology 73: 81-92.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design Groups of mated female Wistar rats were gavaged with DBTC (in olive oil) at a dose levels of 0 (vehicle control) or 20 mg/kg bw on Gestation Days 7-9, 10-12 or 13-15. Additional groups of mated female rats were gavaged with DBTC at dose levels of 0, 20 or 40 mg/kg bw on Gestation Days 6, 7, 8 or 9. Dose levels were based on bodyweights at Gestation Day 0 and were not subsequently adjusted. Dams were terminated on Gestation Day 20; the numbers of live and dead foetuses and resorptions were recorded. Foetuses were sexed, weighed and inspected for external malformations and malformations of the oral cavity. Approximately two thirds of the foetuses in each litter were examined for skeletal malformations following staining with Alizarin Red S. The remaining foetuses were fixed in Bouin's solution and assessed for visceral malformations following freehand serial sectioning.

Findings Dosing on Gestation Days 7-9, 10-12 or 13-15

Complete resorption was observed for five rats administered DBTC at 20 mg/kg bw/d on GD 7-9; six

litters contained live foetuses. A significantly higher number of resorptions and dead foetuses, a lower number of live foetuses and an increased incidence of post-implantation loss were observed in this group. Mean foetal weights in all treated groups were significantly lower than controls. The numbers of live foetuses, dead foetuses and resorptions and the proportion of post-implantation loss in rats administered DBTC on GD 10-12 or GD 13-15 were comparable to control.

No foetuses with external malformations were found in the control groups or in the groups treated with DBTC on GD 10-12 or GD 13-15. Treatment with DBTC on GD 7-9 resulted in a significant increase in the incidence of foetuses with external malformations; 26 of the 36 live foetuses in this group had external malformations. A significantly higher incidence of cleft jaw, ankyloglossia, omphalocoele, open eye, tail anomalies and club foot was seen, compared to controls. Of the 26 affected foetuses, one had a single malformation (omphalocoele), while the remainder had multiple findings. 54% of the malformed foetuses had omphalocoele and club foot. All foetuses with cleft jaw also showed ankyloglossia and/or cleft tongue. No skeletal malformations were observed in the control groups of the groups administered DBTC on GD 10-12 or GD 13-15. A significant increase in the incidence of foetuses with skeletal malformations was observed in the group treated with DBTC on GD 7-9; 14 of the 23 assessed foetuses had skeletal malformations. Deformity of the vertebral column including fusion and/or absence of the vertebral bodies and/or arches in the cervical and thoracic regions, fusion and/or absence of the ribs and cleft of the sternum were significantly increased in incidence. A significantly higher incidence of foetuses with visceral malformations was seen foetuses treated with DBTC on GD 7-9, but not in foetuses treated on GD 10-12 or GD 13-15. Eight of the 13 investigated foetuses showed internal malformations; the incidence of anophthalmia or microphthalmia was significantly increased. All internal malformations were found in foetuses also showing external malformations.

Reproductive and foetal findings in rats dosed on GD 7-9, 10-12 or 13-15

		Days of treatment		
	Controls	**GD 7-9**	**GD 10-12**	**GD 13-15**
Litters (#)	11	11	11	11
Implantations (#)	13.1	13.2	14.3	13.3
Resorptions (#)	1.3	9.9*	2.2	1.6
Post-implantation loss (%)	10.2	75.1*	15.4	14.0
Total resorption (#)	0	5*	0	0
Live foetuses (#)	11.8	3.3*	12.1	11.6
Foetal weight (g) M/F	4.05 / 3.92	2.43* / 2.38*	3.51*/ 3.29*	3.30* / 3.03*
External malformations				
Examined (#)	130 (11)	36 (6)	133 (11)	128 (11)
Malformations (#)	-	26 (6)	-	-
Skeletal malformations				
Examined (#)	84 (11)	23 (6)	87 (11)	85 (11)
Malformations (#)	-	14 (6)	-	-
Internal malformations				
Examined (#)	46 (11)	13 (5)	46 (11)	43 (11)
Malformations (#)	-	8 (4)*	-	-

** significantly different from control (p <0.05)*

Reproductive and foetal findings in rats dosed on GD 6, 7, 8 or 9

The incidence of total resorption was significantly increased in the groups treated with 40 mg/kg bw DBTC on Days 7 or 8; a significantly lower number of live foetuses per litter was also seen in these groups. An increased incidence of post-implantation loss was seen in the groups treated with DBTC

on GD 6, 7, 8 or 9. Administration of 40 mg/kg bw DBTC on GD 6, 7 or 8 caused a significant increase in post-implantation loss; a similar effect was seen with 20 mg/kg bw only when administered on GD 8. A dose-related decrease in mean foetal weight was observed in the treated groups.

Treatment on GD 7 or 8 with DBTC at 20 or 40 mg/kg bw resulted in a significant and dose-related increase in the incidence of external foetal malformations. The highest incidence of malformations (14/95 foetuses at 20 mg/kg bw 23/34 foetuses at 40 mg/kg bw) was seen after treatment on GD 8. 21% (at 20 mg/kg bw) and 20% (at 40 mg/kg bw) of the malformed foetuses had a single malformation such as exencephaly, omphalocoele and encephalocoele following treatment with DBTC on GD 7. 50% (at 20 mg/kg bw) and 13% (at 40 mg/kg bw) of the malformed foetuses had a single malformation such as omphalocoele, club foot and exencephaly following treatment with DBTC on GD 8.

Treatment with 20 mg/kg bw DBTC on GD 7 or with 20 or 40 mg/kg bw DBTC on GD 8 resulted in a significantly increased incidence of foetuses with skeletal anomalies. The highest increase in the incidence of skeletal malformations resulted treatment with DBTC on GD8; 21 of the 63 foetuses at 20 mg/kg bw and 22 of 23 foetuses at 40 mg/kg bw showed malformations. Cleft sternum was the predominant finding in foetuses treated with 20 mg/kg bw on GD 7. Following treatment on GD 8, a dose-related increase in malformations of the cervical, thoracic and lumbar vertebrae; fusion and absence of the ribs and fusion of the sternebrae were observed.

A significantly higher incidence of visceral malformations was observed for groups treated with 20 or 40 mg/kg bw DBTC on GD 7 or GD 8. The predominant malformations were anophthalmia or microphthalmia and dilatation of the cerebral ventricles (treatment on GD 7), absence or hypoplasia of the kidney (treatment on GD 8).

Reproductive and foetal findings in rats dosed on GD 6 or GD 7

	Day of treatment			
	GD 6		**GD 7**	
Dose level (mg/kg bw)	**20**	**40**	**20**	**40**
Litters (#)	11	11	11	11
Implantations (#)	14.0	14.2	14.1	14.4
Resorptions (#)	2.5	6.1	3.5	10.6*
Post-implantation loss (%)	18.9	43.5*	24.6	76.2*
Total resorption (#)	1	3	1	7*
Live foetuses (#)	11.5	8.1	10.5	3.7
Foetal weight (g) M/F	3.78 / 3.59	3.57 / 3.38*	3.30* / 3.23*	3.41/ 3.22*
External malformations				
No. examined (#)	127 (10)	89 (8)	116 (10)	41 (4)
Total malformations (#)	0	2 (2)	14 (6)*	5 (4)*
Skeletal malformations				
No. examined (#)	85 (10)	59 (8)	78 (10)	27 (3)
Total malformations (#)	0	1 (1)	13 (6)*	1 (1)
Internal malformations				
No. examined (#)	42 (10)	30 (8)	38 (10)	14 (4)
Total malformations (#)	0	2 (2)	16 (7)*	6 (4)*

**significantly different from controls (p <0.05)*

Reproductive and foetal findings in rats dosed on GD 8 or GD 9

	Day of treatment			
	GD 8		GD 9	
Dose level (mg/kg bw)	**20 mg/kg bw**	**40 mg/kg bw**	**20 mg/kg bw**	**40 mg/kg bw**
Litters (#)	11	11	11	11
Implantations (#)	14.6	13.3	14.1	14.2
Resorptions (#)	6.0	10.2*	1.3	4.0
Post-implantation loss (%)	42.8*	79.7*	8.6	31.7
Total resorption (#)	3	7*	0	3
Live foetuses (#)	8.6	3.1	12.8	10.2
Foetal weight (g) M/F	3.39*/ 3.26*	2.84*/ 2.49*	3.78 / 3.61	3.49* / 3.21*
External malformations				
No. examined (#)	95 (8)	34 (4)	141 (11)	112 (8)
Total malformations (#)	14 (6)*	23 (4)*	3 (2)	0
Skeletal malformations				
No. examined (#)	63 (8)	23 (4)	93 (11)	75 (8)
Total malformations (#)	21 (6)*	22 (4)*	3 (2)	5 (3)
Internal malformations				
No. examined (#)	32 (8)	11 (4)	48 (11)	37 (8)
Total malformations (#)	7 (4)*	7 (4)*	0	0

** significantly different from controls (p <0.05)*

Conclusion The results of this study identity Gestation Day 7-8 as the critical period for DBTC-mediated teratogenicity in the rat; the most sensitive period was shown to be GD 8. Malformations were not induced following exposure on GD 6 or on GD 9 or later. Exposure at later time points resulted in post-implantation loss, reduced litter size and reduced foetal weight.

A NOAEL of <20 mg/kg bw can be determined for this study."

2.2.1.11 Developmental toxicity study in the rat

Reference "Ema M, Kurosaka R, Amano H & Ogawa Y (1995b). Comparative Developmental Toxicity of Butyltin Trichloride, Dibutyltin Dichloride and Tributyltin Chloride in Rats. Journal of Applied Toxicology 15(4): 297-302.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design Groups of 10 mated female STD:Wistar-ST rats were gavaged with DBTC (in olive oil) at dose levels of 0 (vehicle control), 10 or 15 mg/kg bw (based on GD 0 bodyweight) on Days 7-8 of gestation. Maternal bodyweights were recorded. Dams were sacrificed on Day 20 of gestation. The

numbers of live and dead foetuses and resorptions were counted. Foetuses were sexed, weighed and inspected for external malformations and malformations of the oral cavity. Approximately two thirds of the foetuses in each litter stained with Alizarin Red S and examined for skeletal malformations. The remaining foetuses in each litter were fixed in Bouin's solution and examined for internal malformations by freehand serial sectioning.

Findings

Significantly decreased maternal weight gains on GD 7-9 and GD 0-20 was observed in both treated groups, compared to controls. Total resorptions were observed in both treated groups; the incidence of total resorption was significantly higher at 15 mg/kg bw. A significantly higher incidence of post-implantation loss, lower numbers of live foetuses and lower foetal weight were observed in both treated groups.

Maternal and litter findings

Dose level (mg/kg bw/d)	0	10	15
Pregnant (#)	10	10	10
Weight gain (g) GD 0-7	23	25	19
Weight gain (g) GD 7-9	8	-5**	-8**
Weight gain (g) GD 9-20	82	58	44
Weight gain (g) GD 0-20	113	78*	55**
Adjusted weight gain (g)	40	43	30
Total resorption (#)	-	2	4*
Post-implantation loss (%)	11.8	53.9**	71.2**
Litter size (#)	13.5	6.3*	4.4**
Foetal weight (g) M/F	3.88 / 3.74	3.20* / 2.87*	2.76* / 2.61*

**significantly different to controls (p<0.05); **p<0.01*

Administration of DBTC resulted in a marked and statistically significant increase in the incidence of external foetal malformations; malformation incidences were 37/63 foetuses (59%) at 10 mg/kg bw and 27/44 at 15 mg/kg bw (62%). Significantly increased incidences of exencephaly, cleft jaw, cleft lip, ankyloglossia and club foot were apparent at 10 mg/kg bw; the incidences of exencephaly, cleft tongue and omphalocoele were additionally significantly increased at 15 mg/kg bw.

The incidences of foetal skeletal malformations were significantly increased after treatment with DBTC at 10 and 15 mg/kg bw; malformations were observed in 22/43 foetuses (51%) at 10 mg/kg bw and in 15/29 foetuses at 15 mg/kg bw (52%). Significantly increased incidences of the vertebral column deformity (cervical and thoracic regions) and ribs were observed in both treated groups; mandibular defects and fusion of the sternebrae were additionally observed at 15 mg/kg bw. A significantly increased incidence of foetal visceral malformations was also seen in the DBTC-treated groups; malformation incidences were 12/20 (60%) at 10 mg/kg bw and 10/15 (75%) at 15 mg/kg bw. The most frequent malformations were anophthalmia and microphthalmia.

Foetal malformations

Dose level (mg/kg bw/d)	0	10	15
Examined (#)	135 (10)	63 (8)	44 (6)
Total external malformations (#)	-	37 (8)**	27 (6)**
Exencephaly	-	25 (7)**	19 (6)**
Encephalocoele	-	8 (3)	4 (3)*
Spina bifida	-	1 (1)	-
Cleft jaw	-	14 (6)**	11 (4)**
Micrognathia	-	6 (3)	2 (1)

Cleft lip	-	11 (4)*	10 (5)**
Ankyloglossia	-	18 (5)**	7 (4)**
Cleft tongue	-	5 (3)	3 (3)*
Cleft palate	-	2 (2)	-
Omphalocoele	-	2 (1)	3 (3)*
Kinked tail	-	1 (1)	-
Club foot	-	10 (5)**	3 (3*)
Hind limb deformity	-	1 (1)	1(1)
Anasarca	-	-	3 (2)
Total skeletal malformations (#)	-	22 (7)**	15 (6)**
Mandibular defect	-	10 (3)	6 (5)**
Fused/absent cervical arch/body	-	13 (5)**	11 (6)**
Fused/absent thoracic arch/body	-	10 (4)*	9 (4)**
Fused/absent lumbar arch/body	-	2 (1)	-
Fused/absent ribs	-	14 (6)**	12 (5)**
Fused sternebrae	-	6 (3)	4 (3)*
Total visceral malformations (#)	-	12 (7)**	10 (4)**
Anophthalmia/microphthalmia	-	8 (5)**	9 (4)**

**significantly different to controls (p<0.05); **p<0.01*

Conclusion The results of this study demonstrate that the administration of DBTC to maternal rats at dose levels of 10 and 15 mg/kg bw on Days 7-8 of gestation results in embryolethality and teratogenicity. Findings were associated with maternal toxicity (reduced weight gain). Teratogenicity was characterised by increased incidences of external, skeletal and visceral malformations; malformations (predominantly exencephaly and mandibular defects) are characteristic of those induced by dibutyltin compounds in other studies. A NOAEL for teratogenicity of <10 mg/kg bw can be determined for this study."

2.2.1.12 Developmental toxicity study in the rat

Reference "Ema M, Kurosaka R, Amano H & Ogawa Y (1996b). Comparative Developmental Toxicity of Di-, Tri- and Tetrabutyltin Compounds after Administration during Late Organogenesis in Rats. Journal of Applied Toxicology 16(1): 71-76.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design Groups of mated female STD:Wistar-ST rats were gavaged with DBTC (in olive oil) at dose levels of 0 (vehicle control; 11 females), 165 (11 females) or 330 µmol/kg bw (13 females) on Days 13-15 of gestation (dose levels equivalent to 50 or 100 mg/kg bw/d). Maternal weight gain was measured on Days 13, 16 and 20. Dams were sacrificed on Day 20 of gestation. The numbers of live and dead foetuses and resorptions were counted. Foetuses were sexed, weighed and inspected for external

malformations and malformations of the oral cavity. Approximately two thirds of the foetuses in each litter stained with alizarin red S and examined for skeletal malformations. The remaining foetuses in each litter were fixed in Bouin's solution and examined for internal malformations by freehand serial sectioning.

Findings Maternal deaths occurred in the low dose group (1/11) and in the high dose group (3/13). Weight gain and adjusted weight gains were significantly lower in both of the treated groups compared to controls. Post-implantation loss was also slightly (but not significantly) higher in the treated groups. Mean foetal weights were significantly lower in the treated groups compared to controls. Three foetuses in the low dose group showed external (one foetus with cleft palate, one foetus with tail anomaly and anal atresia) or skeletal malformations (fuse sternebra). No malformations were observed in the control or high dose groups; the malformations observed in the low dose group are not considered to be related to treatment with DBTC.

Summary of findings

Dose level (µmol/kg bw)	**0**	**165**	**330**
Pregnant (#)	11	11	13
Deaths (#)	-	1	3
Weight gain (g) DG 0-13	47	46	50
Weight gain (g) DG 13-16	17	-13**	-13**
Weight gain (g) DG 16-20	40	0**	-22**
Weight gain (g) DG 0-20	104	31**	12**
Adjusted weight gain (g)	38	-13**	-26**
Implantations (#)	13.4	13.6	14.2
Total resorption (#)	-	-	2
Post-implantation loss (%)	9.8	22.0	34.4
Live foetuses (#)	12.1	10.5	9.1
Foetal weight (g) M/F	3.80 / 3.67	2.68**/2.43**	2.52**/2.19**

**significantly different to controls (p<0.05); **p<0.01*

Conclusion In the absence of any foetal malformations in the high dose group, it can be concluded that maternal exposure to DBTC on Days 13-15 of gestation does not result in teratogenicity in the rat. Developmental effects clearly related to treatment were limited to reduced foetal weight, associated with reduced maternal weight gain. A NOAEL cannot be determined for this study due to findings at both dose levels investigated. The relevance of the study for the purposes of classification is limited by the level of mortality seen."

2.2.1.13 Developmental toxicity study in the rat

Reference "Noda T, Nakamura T, Shimizu M, Yamano T & Morita S (1992a). Critical gestational day of teratogenesis by di-n-butyltin (di)acetate in rats. Bulletin of Environmental Contamination & Toxicology 49(5):715-722.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin (di)acetate (DBTA)**

CAS 1067-33-0

EC 211-670-0

Purity not reported

Study design Groups of pregnant Wistar-rats were gavaged with dibutyltin acetate (DBTA) at a dose level of 15 mg/kg bw DBTA on 2 or 3 consecutive days of gestation or were gavaged with single doses of 15 and 30 mg/kg bw on three different days of gestation; or were gavaged with DBTA at dose levels of 5.0, 7.2, 10.5, 15.2 or 22.0 mg/kg bw on GD 8. DBTA was dissolved in olive oil. Maternal animals were observed for signs of toxicity; bodyweight and food consumption were measured. Rats were sacrificed on GD 20 and were assessed for pregnancy status and foetal malformations.

Findings Rats treated with DBTA at 15 mg/kg bw for 2 or 3 consecutive days were most susceptible to teratogenesis on GD 7-9 (higher number of resorptions and malformed foetuses were observed). Rats administered single doses of DBTA on GD 8 had the highest proportion of foetal malformations; treatment on GD 7 resulted in a lower frequency of malformations. The incidence of foetal malformations was significantly increased at the highest dose of DBTA. External malformations observed in the DBTA treated rats included cleft mandible, cleft lower lip, ankyloglossia, schistoglossia and exencephaly. Maternal thymus weights on GD 20 were unaffected by single doses of DBTA on GD 8.

External and skeletal foetal observations of foetuses from dams treated with DBTA on GD 8

DBTA (mg/kg bw)	0	**5.0**	**7.2**	**10.5**	**15.2**	**22.0**
Foetuses/dams	115/9	140/10	138/10	120/10	117/10	103/9
External observations						
Foetuses with malformations (%)	0.9 (1)	-	0.6 (1)	-	1.9 (2)	26.3 (7)**
Foetuses with malformations (#)	1 (1)	-	1 (1)	-	2 (2)	18 (7)**
Cleft mandible, cleft lower lip, ankyloglossia or schistoglossia	-	-	-	-	2 (2)	14 (7)**
Exencephaly	-	-	-	-	-	8 (3)**
Cleft upper lip		-	-	-	-	4 (1)
Peaked mandible	9 (1)	-	-	-	-	0
Agnathia	-	-	-	-	-	1 (1)
Microcephaly	-	-	-	-	-	1 (1)
Vestigial tail	-	-	1 (1)	-	-	0
Club foot	-	-	-	-	-	1 (1)
Skeletal observations						
Foetuses with malformations (%)	0.8 (1)	0	1.2 (2)	0	0.7 (1)	22.4 (5)**
Foetuses with malformations (#)	1 (1)	0	2 (2)	0	1 (1)	13 (5)**
Anomaly of mandibular fixation	0	0	0	0	0	9 (5)**
Cranial hypoplasia	0	0	0	0	0	8 (3)**
Fused ribs	0	0	0	0	0	6 (1)*
Fused cervical or thoracic vertebral arches	0	0	0	0	0	5 (1)*
Fused mandibles	1 (1)	0	0	0	0	0
Agenesis of sacro-coccygeal or coccygeal vertebrae	0	0	2 (2)	0	1 (1)	0
No. of foetuses with cervical ribs	4 (4)	3 (2)	8 (6)	9 (4)	34 (8)**	62 (9)**

** significantly different from control ($p<0.05$); ** ($p<0.01$)*

Conclusion The study demonstrates that the administration of DBTA to the rat on GD 8 results in a characteristic spectrum of external and skeletal foetal malformations. The authors conclude that the GD8 is the critical period for the teratogenesis of DBTA in the rat. A NOAEL of 10.5 mg/kg bw can be determined for this study, based on increased incidences of foetal malformations at dose levels of ≥15.2 mg/kg bw."

2.2.1.14 Developmental toxicity study in the rat

Reference "Noda T, Yamano T, Shimizu M, Saitoh M, Nakamura T, Yamada A & Morita S (1992b). Comparative teratogenicity of di-n-butyltin (di)acetate with n-butyltin trichloride in rats. Archives of Environmental Contamination & Toxicology 23(2):216-22.

Guideline Comparable to OECD 414

Species / strain Rat (Wistar)

Test material **Dibutyltin acetate (DBTA)**

CAS 1067-33-0

EC 213-928-8

Purity not reported

Study design Groups of 13-16 mated female Wistar rats were gavaged with DBTA (in olive oil) at dose levels of 0 (vehicle controls), 1.7, 5.0, 10.0 or 15.0 mg/kg bw on GD 7-17. Rats were observed daily for signs of toxicity; bodyweights and food consumption were also measured daily. Rats were terminated on GD 20 and pregnancy status assessed. Maternal thymus weight was reported. Foetuses were weighed, sexed and investigated for external and skeletal malformations.

Findings Reduced maternal weight gain during late gestation was observed at the highest dose level of 15 mg/kg bw/d; no effects of treatment were seen on food consumption. A single rat at 15 mg/kg bw/d showed piloerection and vaginal bleeding. Thymic atrophy of the pregnant rats was observed in a dose-dependent manner by DBTA treatment.

The incidences of dead or resorbed foetuses and total foetal resorption were increased at the highest dose level. The proportion of foetuses with external malformations (mainly cleft mandible, cleft lower lip, ankyloglossia and schistoglossia) was increased in a dose-dependent manner by DBTA treatment at dose levels of ≥5.0 mg/kg bw/d. The proportion of foetuses with skeletal malformations (anomalies of mandibular fixation, fused ribs, fused cervical vertebral arches and fused thoracic vertebral arches) was also increased at 10.0 and 15.0 mg/kg bw. No visceral malformations were observed in any group. Similar effects were not seen with monobutyltin chloride, a major metabolite of DBTA.

Summary of effects

Dose level (mg/kg bw/d)	**0**	**1.7**	**5**	**10**	**15**
Mated (#)	14	13	14	14	16
Pregnant (#)	14	12	14	14	16
Dams with viable foetuses (#)	14	12	14	14	7**
Total resorption (#)	-	-	-	-	9**
Implants (#)	13.6	13.8	14.3	14.3	13.7
Early resorption (%)	5.9	4.6	2.9	10.7	69.5**
Late resorption (%)	-	-	0.4	2.1	4.9
Litter size (#)	12.9	13.3	14.0	12.8	4.3
Foetal weight (g) M/F	3.2/3.0	3.2/.9	3.0/2.8	2.6**/2.5**	2.3**/2.3**
External malformations (#)	-	-	2 (2)	43 (10)**	19 (7)**

External malformations (%)	-	-	1.0	25.1**	38.9**
Skeletal malformations (#)	-	-	-	20 (9)**	18 (7)**
Skeletal malformations (%)	-	-	-	22.7**	54.7**

***significantly different to controls (p<0.01)*

Conclusion The results of this study demonstrate that DBTA is teratogenic in the rat; the absence of similar effects with a metabolite indicate that teratogenicity is an effect of dibutyltin and not monobutyltin. A NOAEL of 1.7 mg/kg bw can be determined for this study, based on increased incidences of foetal malformations at ≥5 mg/kg bw. A NOAEL for maternal toxicity of 10 mg/kg bw can be determined."

2.2.1.15 Developmental toxicity study in the rat

Reference "Noda T, Yamano T & Shimizu M (2001). Effects of maternal age on teratogenicity of di-n-butyltin (di)acetate in rats. Toxicology 167(3):181-9.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin (di)acetate (DBTA)**

CAS 1067-33-0

EC 213-928-8

Purity details not reported

Study design Groups of 12-14 mated female Wistar rats (aged 3, 7.5 or 12 months at mating) were gavaged with a single dose DBTA at dose levels of 0 (vehicle control), 7.5, 10, 15 or 22 mg/kg bw on GD 8. Maternal bodyweight and food consumption were measured daily. Dams were terminated on GD 20; uterus weights were recorded and the uterine contents examined following Caesarean section. Foetuses were weighed and sexed and were stained with Alizarin Red S for the assessment of skeletal findings.

Findings Maternal weight gain and gravid uterus weight decreased with age and were also significantly reduced by treatment with 22 mg/kg bw in 7.5 month old dams. The number of dams with viable foetuses was markedly reduced in the 12-month old group; reduced conception rat and increased total resorption were apparent. In 7.5 month-old dams, numbers of viable foetuses were reduced, foetal weight was reduced, resorption and implantation loss were increased at 15 and 22 mg/kg bw. In 3 month-old dams, increased implantation loss and resorption rate were observed only at 22 mg/kg bw.

Reduction in litter size was seen in all treated groups, most notably in the older dams. Death of most of the foetuses of the 12-month dams precluded accurate evaluation of malformation incidences. In litters from the 3-month old dams, external foetal malformations typical of DBTA (cleft mandible, cleft lower lip, ankyloglossia and/or schistoglossia) were observed at ≥15 mg/kg bw. Similar malformations were seen in the litters of 7.5-month old dams at dose levels of ≥10 mg/kg bw. The incidences of these malformations at 15 and 22 mg/kg bw were similar to those seen in litters from 3-month old dams.

Summary of maternal and litter findings

Dose level (mg/kg bw)		**0**	**7.5**	**10**	**15**	**22**
Weight gain (g)	**3M**	111	115	112	107	105
	7.5M	91	86	78	79	61*
	12M	36	40	36	39	23

Gravid uterus weight (g)	**3M**	72	73	71	68	61
	7.5M	56	54	47	52	31*
	12M	13	10	12	13	16
Adjusted weight gain (g)	**3M**	39	42	42	39	44
	7.5M	35	32	31	27	30
	12M	36	33	36	36	30
Mated (#)	**3M**	12	12	12	12	12
	7.5M	12	13	14	13	13
	12M	12	14	13	12	13
Pregnant (#)	**3M**	12	11	12	11	11
	7.5M	11	13	12	13	11
	12M	8	11	8	9	9
Litters with viable foetuses (#)	**3M**	12	11	12	11	11
	7.5M	11	13	12	13	6*
	12M	4	9	4	3	1
Total resorption (#)	**3M**	-	-	-	-	-
	7.5M	-	-	-	-	5*
	12M	4	2	4	6	8
Implantation loss (%)	**3M**	3.4	6.6	11.4	7.1	19.2*
	7.5M	16.7	20.1	27.6	14.2	37.8*
	12M	79.2	52.5	79.0	86.7	95.2
Foetal weight (g) M/F	**3M**	3.4/3.2	3.3/3.2	3.3/3.1	3.2/3.1	2.7*/2.7*
	7.5M	3.2/3.0	2.9/2.8	3.0/2.8	2.8*/2.6*	2.2*/2.2*
	12M	2.6/2.5	2.4/2.3	2.3/2.2	2.5/2.0	2.1/1.6

*significantly different to controls ($p<0.01$)

External and skeletal malformations (typically cleft mandible, cleft lower lip, ankyloglossia, schistoglossia, fused ribs, fused cervical and vertebral arches) were observed in foetuses from 3 month-old and 7.5 month-old females. The incidence of exencephaly was also markedly increased at 22 mg/kg bw. Malformations were observed only in a single foetus from 12 month-old females due to the high level of foetal mortality in this group.

Summary of foetal findings [18]

Dose level (mg/kg bw)		**0**	**7.5**	**10**	**15**	**22**
Foetuses examined (#)	**3M**	166	155	166	148	139
	7.5M	122	140	110	143	43
	12M	8	14	8	8	3
External malformations (%)	**3M**	-	-	-	28.4*	61.8*
	7.5M	-	1.3*	7.9*	34.8*	64.0*
	12M	-	5.6	12.5	8.3	-
Skeletal	**3M**	-	-	-	30.2*	62.6*

malformations (%)	**7.5M**	-	-	7.0	32.0*	81.3*
	12M	-	-	-	8.3	-

*significantly different to controls ($p<0.01$)

Conclusion The study confirms that GD 8 is the susceptible period for teratogenesis caused by DBTA. The spectrum of foetal malformations is comparable to that induced by DBTC. The results of this study also indicate an influence of maternal age on the susceptibility of the rat to the developmental toxicity of DBTA. Effects on foetal survival were more marked in older dams; results also indicate that teratogenicity may be more marked in older dams, although findings in the oldest (12 month-old) dams may have been masked by the high level of foetal loss in this group.

A NOAEL of <7.5 mg/kg bw can be determined for this study, based on reduced litter size in all treated groups. Teratogenicity (increased incidences of craniofacial malformations) was seen at dose levels of ≥10 mg/kg bw."

2.2.1.16 Developmental toxicity study in the monkey

Reference "Ema M, Fukunishi K, Matsumoto M, Hirose A, Kamata E & Ihara T (2007b). Developmental toxicity of dibutyltin dichloride in cynomolgus monkeys. Reproductive Toxicology 23(1):12-19.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Cynomolgus monkey

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

98% purity

Study design Groups of cynomolgus monkeys were administered DBTC (in olive oil) by nasogastric intubation at dose levels of 0 (vehicle control), 2.5 or 3.8 mg/kg bw/d on GD 20-50 (the period of organogenesis). Maternal animals were observed for signs of toxicity; bodyweight and food consumption were measured. Pregnancy outcome was determined on GD 100. Foetuses were weighed, sexed and measured (crown-rump length and tail length); anogenital distance was also recorded. Foetuses were assessed for external, visceral and skeletal malformations and variations.

Findings Maternal toxicity (soft stool, yellowish stool and/or diarrhoea) was observed in females of both treated groups; a significant increase in the incidence of females exhibiting these symptoms was observed. Soft stool and/or diarrhoea were also observed in one control female. In both treated groups, yellowish stool was noted in 8 females and vomiting was observed in 3 females. Maternal weight gain was reduced at 3.8 mg/kg bw/d; food consumption was decreased in 2.5 and 3.8 mg/kg bw/d during the treatment phase. Higher plasma progesterone levels were observed in treated dams compared to controls, however the difference was not statistically significant and no differences in 17β-estradiol were observed. Foetal survival was decreased in both treated groups, significantly at 2.5 mg/kg bw/d. There was no effect of treatment on foetal weight, crown-rump length, tail length, sex ratio, anogenital distance or placental weight. No external, visceral or skeletal malformations were observed in any group; similarly there was no effect of treatment on the incidence of visceral variations, skeletal variations or on the extent of foetal skeletal ossification. A significant decrease in absolute brain and lung weight, and an increase in the relative spleen weight of male foetuses at 3.8 mg/kg bw; no significant difference in relative brain or lung weight or absolute spleen weight were detected. There were no other significant differences in absolute and relative foetal organ weights.

Maternal and reproductive findings

	Control	**2.5 mg/kg bw**	**3.8 mg/kg bw**
Pregnant females (#)	12	12	10
Soft stool/diarrhoea (#)	1	12*	10*

Yellowish stool (#)	0	8*	8*
Vomiting (#)	0	3	3
Weight gain (g) GD 0-20	76 ± 114	42 ± 160	73 ± 142
Weight gain (g) GD 20-51	57 ± 237	-242 ± 423	-556 ± 526*
Weight gain (g) GD 51-100	710 ± 162	755 ± 174	848 ± 263
Females with embryonic/foetal loss (#)	1	8*	4
Females with live foetuses (#)	11	4*	6
Live foetuses (#)	11	4*	6

** significantly different from control (p <0.05)*

Maternal food consumption

Food consumption (g/day)	**Control**	**2.5 mg/kg bw**	**3.8 mg/kg bw**
GD 20-21	99 ± 18	93 ± 23	76 ± 33
GD 23-24	91 ± 27	71 ± 31	55 ± 31*
GD 27-28	77 ± 28	47 ± 19*	37 ± 34*
GD 30-31	63 ± 32	33 ± 15*	22 ± 10*
GD 34-35	88 ± 25	53 ± 42	23 ± 17*
GD 37-38	86 ± 28	53 ± 42*	25 ± 24*
GD 41-42	87 ± 27	59 ± 59	36 ± 29*
GD 44-45	95 ± 22	62 ± 40	41 ± 31*
GD 48-49	98 ± 18	70 ± 48	59 ± 44
GD 51-52	94 ± 20	97 ± 24	71 ± 39
GD 55-56	102 ± 12	107 ± 2	100 ± 20
GD 58-59	106 ± 7	108 ± 0	104 ± 10
GD 62-63	106 ± 7	108 ± 0	106 ± 5
GD 80-81	108 ± 0	108 ± 0	108 ± 0
GD 90-91	106 ± 7	108 ± 0	108 ± 0
GD 99-100	108 ± 0	108 ± 0	108 ± 0

** significantly different from control (p <0.05)*

Conclusion The results of this study show that the administration of DBTC causes embryofoetal lethality, but not teratogenicity, in the monkey. The NOAEL for this effect is <2.5 mg/kg bw/d. Findings were associated with maternal toxicity (clinical signs, weight loss)."

2.2.1.17 Developmental toxicity study in the monkey

Reference "Ema M, Arima A, Fukunishi K, Matsumoto M, Hirata-Koizumi M, Hirose A & Ihara T (2009). Developmental toxicity of dibutyltin dichloride given on three consecutive days during organogenesis in cynomolgus monkeys. Drug & Chemical Toxicology 32(2):150-7.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline study published in a peer-reviewed journal)

Species / strain Cynomolgus monkey

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1
EC 211-670-0
98% purity

Study design Groups of Cynomolgus monkeys were administered DBTC (in olive oil) by nasogastric intubation at a dose levels of 7.5 mg/kg bw on GD 19-21, 21-23, 24-26, 26-28, 29-31, 31-33 or 34-36. Control data (animals administered olive oil on GD 20-50) were available from a recent previous study (see Study 21 below). Maternal animals were observed for signs of toxicity; bodyweight and food consumption were measured. Pregnancy outcome was determined on GD 100. Foetuses were weighed, sexed and measured (crown-rump length and tail length). Foetuses were assessed for external, visceral and skeletal malformations and variations.

Findings Maternal toxicity (vomiting) was observed in all treated groups. Soft stool and/or diarrhoea were observed in all groups including the control. Significant increases in the incidence of females showing soft stool and/or diarrhoea after administration of DBTC on GD 19-21, 21-23, 24-26 or 26-28 were noted. Significant increases in the incidence of vomiting after administration of DBTC on GD 19-21 were noted. Maternal body weight gain was reduced over days 20-51 in dams given DBTC on GD 24-26, 26-28, 29-31 and 34-36, however differences were not statistically significant. A significant reduction in food consumption was observed on days 27-28 in the dams administered DBTC on GD 26-28; no other effects on food consumption were observed. Embryofoetal loss was observed in one female given DBTC on GD 19-21, in two females given DBTC on GD 24-26 and one female given DBTC on GD 34-36. There were no effects of treatment on developmental parameters in surviving foetuses, including foetal weight, crown-rump length, tail length or placental weight. No external, visceral or skeletal malformations were observed in any group. Treatment with DBTC similarly did not affect the incidence of skeletal variations or the level of skeletal ossification.

Reproductive findings

	Control	**7.5 mg/kg bw DBTC**						
GD dosing	**20-50**	**19-21**	**21-23**	**24-26**	**26-28**	**29-31**	**31-33**	**34-36**
Pregnant (#)	12	5	5	5	5	5	5	5
Embryofoetal loss (#)	1	1	0	2	0	0	0	1
Females with live foetuses (#)	11	4	5	3	5	5	5	4
Foetal weight (g)	126	122	124	100	110	117	111	124

Conclusion The results of this study show that the administration of DBTC causes embryofoetal lethality, but not teratogenicity, in the monkey. The NOAEL for this effect is 7.5 mg/kg bw/d."

2.2.1.18 Developmental toxicity study in the rat

Reference Unpublished report (2017). Dibutyloxide: an oral prenatal developmental toxicity study in rats

Guideline OECD 414

Reliability Klimisch 1: reliable without restrictions (Guideline and GLP-compliant study, full report available)

Species / strain Hsd: Sprague Dawley® SD®

Test material **Dibutyltin Oxide (DBTO)**
CAS 818-08-6
EC 212-449-1
Purity 97.3%

Vehicle Peanut oil

Dose levels Study 0, 0,75, 3 und 6 mg/kg bw/day

design

Groups of 25 mated female SD rats were gavaged with DBTO (in peanut oil) at dose levels of 0, 0.75, 3 and 6 mg/kg bw/d on days 0-19 of gestation. Dams were investigated for clinical signs and mortality. Body weights and food consumption were recorded at regular intervals. Rats were sacrified at day 20 and ovarian and uterine were examined. Foetuses were individually weighted sexed, tagged, and examined for external malformations and variations.

Approximately half of the foetuses from each litter were assessed for visceral findings; the remainder of the foetuses were assessed for skeletal findings following staining with Alizarin Red.

Dams were subject to gross necropsy. Emphasis was placed on structural abnormalities or pathologic changes that may have influenced the pregnancy. Thymus gland from each animal was collected, weighted, and preserved for possible further evaluation.

Findings

No death occurred in the control and in the 0,75 and 3 mg/kg bw group. Two animals in the highest dose group were euthanised *in extremis* on GD 12 and 9. Animals had clinical signs of toxicity that included decreased activity, low body carriage, red material around the nose/mouth, hunched posture, pale body collar, and thin unkempt appearance accompanied by body weight loss and low food consumption. These effects are considered test article related. In the 0.75 and 3 mg/kg bw/d group no adverse effects were observed. In these dose groups some animals showed also red material around the nose in the early phase of the study (week 1). In the 6 mg/kg bw/d group clinical signs that included low body carriage, red material around the nose, thin appearance, loss of skin elasticity and pale body color were considered adverse and substance related.

No effect of DBTO at dose levels of 0.75 and 3.0 mg/kg/day was observed on gestation bodyweights and body weight change. At 6.0 mg/kg/day, mean body weights were statistically lower than mean control values on GD 18 (-8%) and 20 (-9%).

Gestation body weight values (n = 22-24)

Dose group/ Study Interval (d)	**0 mg/kg bw (Mean ± SD)**	**0.75 mg/kg bw (Mean ± SD)**	**3.0 mg/kg bw (Mean ± SD)**	**6.0 mg/kg bw (Mean ± SD)**
0	187.7 ± 13.87	188.1 ± 14.45	187.3 ± 14.93	186.3 ± 13.16
3	205.0 ± 15.09	205.2 ± 13.75	206.1 ± 16.25	199.5 ±15.07
6	215.4 ± 14.72	216.3 ± 14.32	218.3 ± 17.18	210.0 ± 16.60
9	232.4 ± 15.62	232.5 ± 14.84	234.7 ± 18.18	221.1 ± 22.80
12	248.2 ± 16.57	247.2 ± 17.27	250.4 ± 20.35	233.6 ± 29.55
15	265.5 ± 16.66	265.7 ± 17.17	270.0 ± 21.28	248.5 ± 38.22
18	303.9 ± 17.65	310.0 ± 20.51	311.5 ± 26.81	280.3[a] ± 51.34
20	334.4 ± 19.82	344.6 ± 24.61	344.9 ± 30.34	305.4[a] ± 62.95

[a] *significantly different from control (p<0.05)*

The mean body weight change in this group was lower than mean control values over much of gestation and statistically lower over GD 0 to 3 (-24%), GD 6 to 9 (-34%) and over the entire GD 0 to 20 treatment period (-19%). Considerable variability in gestation body weight change was observed in the 6.0 mg/kg/day dose group.

This changes are attributed to low weight gain and/or weight loss in four animals (#4510, 4511, 4516, and 4524) that failed to retain pregnancies with fetuses and at GD 20 had uterine implantations comprised entirely of resorbing fetuses (100% post-implantation loss). These effects on gestation body weights and body weight change at 6.0 mg/kg/day were considered test article related correlating with adverse pregnancy outcomes in several animals.

The individual gestation body weight values (g) and the individual gestation body weight change values (g) of these four animals receiving 6 mg/kg bw/day on GD 0 and GD 20 are depicted in the table below.

Animal Number #	**GD 0**	**GD 20**	**GD 0-20**
	Individual gestation		**Individual gestation body**

	body weight values		weight change values
4510	176g	238g	62 g
4511	189g	255g	36 g
4516	165g	145g	-20 g
4524	181g	151g	-30 g

Mean gravid uterine weights, adjusted GD 20 body weights and adjusted weight change GD 0 to 20 in the DBTO-treated groups were comparable to mean control values.

The adjusted final body weight (GD 20 body weight minus gravid uterine weight) in the control group is 260.7 ± 15.5 and adjusted body weight change (GD 0 to 20) is 73.0 ± 7.98.

A comparison of data of the four animals with control data indicates that for animal #4510 and 4511 no significant difference is between gestation body weight values (below 10%), whereas for animals #4516 and 4524 no increase in gestation body weight was observed.

No clinical or minor clinical observations (material around mouth, red thin) were detected for #4510, 4511. Whereas, #4516 and 4524 were in bad conditions and clinical signs such as loss of skin elasticity, posture hunched, scabbed area, skin discolored and thin were detected observed mainly between GD 9-20.

Summary of gravid uterine weight and ajusted body weight/body weight change values is provided in the following table:

Endpoint	**0 mg/kg bw/d (Mean ± SD) n=24**	**0.75 mg/kg/bw/d (Mean ± SD) n=24**	**3.0mg/kg bw/d (Mean ± SD) n=23**	**6 mg/kg bw/d (Mean ± SD) n=18**
Gravid Uterine Weight, g	73.7 ± 10.76	81.9 ± 11.93	80.1 ± 12.17	68.8 ± 15.14
Final Body Weight, g	334.4 ± 19.82	344.6 ± 24.61	344.9± 30.34	331.1 ± 25.13
Adjusted Final Body Weight, g	260.7 ± 15.50	262.7 ± 16.49	264.8 ± 22.03	262.3 ± 15.88
Adjusted Weight Change from Day 0, g	73.0 ± 7.98	74.6 ± 10.45	77.4 ± 12.28	74.1 ± 11.11

Reduced thymus weights were observed at all DBTO treatment levels and an increased incidence of small thymus observed macroscopically in the 6.0 mg/kg bw/day animals. No histopathological examinations have been performed in this study.

In the following table reduced thymus weight and thymus weight/adjusted GD20 body weight is depicted

Endpoint	**0 mg/kg bw/day (Mean ± SD)**	**0.75 mg/kg bw/day (Mean ± SD)**	**3 mg/kg bw/day (Mean ± SD)**	**6 mg/kg bw/day (Mean ± SD)**
Thymus, g	0.239 ± 0.062	0.193^{x} ±0.042	0.158^{x} ± 0.043	0.134^{x} ±0.046
Thymus/adjusted GD 20 BWT, %	0.0891 ± 0.0192	0.0716^{x} ± 0.0123	0.0581^{x} ± 0.0143	0.0558^{x} ± 0.0108

^{x}statistically significant form control values (p<0.01)

Pregnancy index was 96%, 96%, 92% and 88% in the 0, 0.75, 3.0, and 6.0 mg/kg bw/day groups, respectively. There were one, one, two, and three nonpregnant females in the 0, 0.75, 3.0, and 6.0 mg/kg/day groups, respectively. Two of the nonpregnant females in the highest dose group were euthanized in extremis. Four females in the highest dose group (#4510, 4511, 4516, and 4524) had uterine implantations comprised entirely of resorbing fetuses (100% post-implantation loss) characterised by early resorptions. Overall, there were 24, 24, 23, and 18 litters with GD 20 fetuses for evaluation in the 0, 0.75, 3.0, and 6.0 mg/kg/day dose groups, respectively.

The increased incidence of females with all resorption sites in utero is considered to be related to DBTO

administration and adverse.

No substance related effects were observed on GD 20 uterine implantation parameters at 0.75 and 3.0 mg/kg/day.

In the 6 mg/kg bw/day group notable increases in mean postimplantation loss (25.70%) and in mean number of resorption sites (total and early)/dam (2.7) relative to controls (5.40% and 0.7, respectively) were observed in this group along with a decrease in number of viable fetuses/dam (9.7 vs. 12.5 in controls). Changes in these parameters in the 6.0 mg/kg/day dose group were also outside the range of recent historical control data for the laboratory (maximum study values were 5.35% for postimplantation loss, 0.7 resorption sites (total and early)/dam and 13.5 viable fetuses/dam) and these changes were largely attributable to the four females in the group with all resorption sites in utero (100% postimplantation loss). These effects are considered substance related and adverse.

Maternal and developmental observations at uterine examination

Endpoint	**0 mg/kg bw**	**0.75 mg/kg bw**	**3.0 mg/kg bw**	**6.0 mg/kg bw**
No of dams	25	25	25	25
No not pregnant	1	1	2	3
Pregnancy Index (Percent)	96.0	96.0	922.0	88.0
No Females with All Resorptions	0	0	0	4
No Females with viable foetuses day 20 gestation	24	24	23	18
Endpoint	**0 mg/kg bw (Mean ± SD)**	**0.75 mg/kg bw (Mean ± SD)**	**3.0 mg/kg bw (Mean ± SD)**	**6.0 mg/kg bw (Mean ± SD)**
Corpora Lutea No. per Animal	15.4 ± 2.30	16.1 ± 2.02	16.0 ± 3.01	15.4 ± 2.43
Implantation Sites No. per Animal	13.2 ± 1.89	14.3 ± 2.35	14.2 ± 1.67	12.5 ± 2.42
Preimplantation Loss % per Animal	12.91 ± 14.05	11.03 ± 9.76	9.80 ± 10.11	14.66 ± 15.27
Viable Fetuses No. per Animal	12.5 ± 1.96	14.1 ± 2.36	13.5 ± 1.78	9.7 ± 5.42
Postimplantation Loss % per Animal	5.40 ± 5.626	1.46 ± 2.952	4.89 ±5.687	25.70 ± 39.370 (18.3 ± 32.7[b])
Litter Size No. per Animal	12.5 ± 1.96	14.1[a] ± 2.36	13.5 ± 1.78	9.7 ± 5.42
Resorptions: Early + Late No. per Animal	0.7 ± 0.75	0.2[a] ± 0.41	0.7 ± 0.82	2.7 ± 4.26
Resorptions: Early No. per Animal	0.7 ± 0.75	0.2[a] ± 0.41	0.7 ± 0.83	2.7 ± 4.22

[b] *data without considering animal #4516 and 4524 (high toxicity observed in these dams)*

No effect of DBTO was observed on fetal sex ratio, fetal body weight, or fetal external, visceral, or skeletal examinations.

Although no dose-related effect of DBTO was observed from the fetal visceral examinations it is noteworthy, that the litter incidence of an irregular palatal rugae pattern, a visceral variation, in the control, 0.75, and 3.0 mg/kg/day treated groups was 4.2%, 12.5%, and 26.1%, respectively. The higher incidence of this variation in these treated groups did not differ statistically from controls and in the absence of a similar

finding among fetuses in the 6.0 mg/kg/day group, was not considered test article related.

In the following table individual visceral observation are summarized:

Endpoint	**0 mg/kg bw (Mean ± SD)**	**0.75 mg/kg bw (Mean ± SD)**	**3.0mg/kg bw (Mean ± SD)**	**6.0 mg/kg bw (Mean ± SD)**
No. of litters evaluated	24	24	23	18
No. of foetuses evaluated	150	169	154	106
Head - Retina folded No. of litters (%) No. of foetuses (%)	 0 (0) 0 (0)	 0 (0) 0 (0)	 1 (4.3) 1 (0.6)	 0 (0.0) 0 (0.0)
Mouth – Palate, rugae irregular No. of litters (%) No. of foetuses (%)	 1 (4.2) 3 (2.0)	 3 (12.5) 3 (1.8)	 6 (26.1) 6 (3.9)	 0 (0) 0 (0.0)
Thyroid, smaller than normal No. litters (%) No. foetuses (%)	 0 (0.0) 0 (0.0)	 0 (0.0) 0 (0.0)	 0 (0.0) 0 (0.0)	 1 (5.6) 1 (0.9)

Maternal thymus weight (absolute and relative) was reduced at GD 20. Mean absolute thymus weights were 19%, 34%, and 44% lower than the mean control value in the 0.75, 3.0, and 6.0 mg/kg/day dose groups, respectively and relative to the adjusted GD 20 body weights, were 20%, 35%, and 37% lower, respectively. The differences are statistically significant and are considered as test article related. An increased incidence of small thymus were observed macroscopically in the 6 mg/kg/d animals. No histopathological examination has been carried out, therefore the significance of this decrease is unclear.

Conclusion A NOAEL for maternal toxicity and developmental toxicity of 3 mg/kg bw/day DBTO can be determined for the study. At a dose level of 6 mg/kg bw dams had clinical signs and lower body weights, lower gestation body weights and lower food consumption. Lower thymus weights were observed in all substance treated groups and an increased incidence of small thymus were observed macroscopically in the 6 mg/kg/d animals. At a dose level of 6 mg/kg bw/day 4/23 femals at GD 20 had uterine implantation sited comprised entirely of resorbing foetuses (100% postimplantation loss). This observation is considered adverse and substance related. At dose levels of 0.75, 3.0, and 6.0 mg/kg/day DBTO was not teratogenic in the rat. An irregular palatal rugae pattern - a visceral variation- was dose dependent increased up to the dose level of 3 mg/kg bw/day. No findings of the variations was found at the 6 mg/ kg bw/group. Thus, study authors consider the variation as not treatment related. No effect of DBTO was observed on fetal sex ratio, fetal body weight, or fetal external and or skeletal examinations.

2.2.2 Human data

No human data are available.

2.2.3 Other data (e.g. studies on mechanism of action)

2.2.3.1 Cultured rat embryo study

Reference "Ema M, Iwase T, Iwase Y & Ogawa Y (1995a). Dysmorphogenic effects of di-n-butyltin dichloride in cultured rat embryos. Toxicology In Vitro 9(5):703-9.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material	**Dibutyltin dichloride (DBTC)** CAS 683-18-1 EC 211-670-0 No purity details
Study design	Wistar rat embryos were explanted on GD 8 and cultured for 68 hours in the presence of DBTC at concentrations of 0 (controls), 3, 10 or 30 ng/mL. At the end of the culture period the embryos were examined for the development of body and yolk sac vascularisation; yolk sac diameter, crown-rump length and the number of somite pairs were measured. Foetuses were given a morphological score and external anomalies were recorded.
Findings	Embryos cultured in the presence of 30 ng/mL DBTC showed a marked and statistically significant reduction in the incidence of well-developed vascularization in the body and yolk sac. Yolk sac diameter, crown-rump length and number of somite pairs were also reduced at this concentration. A concentration-dependent decrease in the overall morphological score and an increase in the incidence of embryos with anomalies were observed at all concentrations; differences compared to controls were statistically significant for embryos exposed to 10 and 30 ng/mL DBTC. The observed anomalies were mainly open anterior neuropore and craniofacial abnormalities.
Conclusion	The study indicates that exposure of explanted GD 8 rat embryos to DBTC *in vitro* at concentrations of ≥3 ng/mL causes dysmorphogenesis."

2.2.3.2 Cultured rat embryo study

Reference	"Ema M, Iwase T, Iwase Y, Ohyama N & Ogawa Y (1996a). Change of embryotoxic susceptibility to di-n-butyltin dichloride in cultured rat embryos. Archives of Toxicology 70(11):742-8.
Guideline	No guideline followed
Reliability	Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)
Species / strain	[*In vitro* study]
Test material	**Dibutyltin dichloride (DBTC)** CAS 683-18-1 EC 211-670-0 No purity details
Study design	Rat embryos explanted on GD 8.5, 9.5 or 11.5 were cultured for 68, 46 and 48 hours and were exposed to a range of DBTC concentrations for the first 24, 46 and the last 46 hours of culture, respectively.
Findings	In GD 8.5 embryos, exposure to DBTC resulted in significant decreases in placental diameter (at concentrations of ≥10 ng/mL) and in the number of somite pairs and the morphological score (at 30 ng/mL). In GD 9.5 embryos, significant decreases in yolk sac diameter and crown-rump length were seen at 100 ng/mL, a reduction in the number of somite pairs was seen at ≥50 ng/mL and a reduction in the morphological score was seen at ≥30 ng/mL. No adverse effects on these parameters were detected in embryos cultured from GD 11.5, even at the highest concentration tested of 300 ng/mL. Dysmorphogenesis was seen in embryos cultured from GD 8.5 (≥10 ng/mL), GD 9.5 (≥50 ng/mL) and GD 11.5 (300 ng/mL). Incomplete turning and craniofacial defects in embryos cultured from GD 8.5 and GD 9.5 and defects of the forelimb buds and tail in embryos cultured from GD 11.5 were most frequently observed.
Conclusion	The study shows that exposure to DBTC interferes with normal embryonic development during three different stages of organogenesis, and that susceptibility to the embryotoxicity and dysmorphogenic potential of DBTC varies with developmental stage."

2.2.3.3 Cultured rat embryo limb bud study

Reference	"Yonemoto J, Shiraishi H & Soma Y (1993). *In vitro* assessment of teratogenic potential of organotin compounds using rat embryo limb bud cell cultures. Toxicology Letters 66(2):183-91.
Guideline	No guideline followed
Reliability	Klimisch 4: not assignable (insufficient experimental detail)
Species / strain	[*In vitro* study]
Test material	**Dibutyltin dichloride (DBTC)** CAS 683-18-1 EC 211-670-0 No purity details
Study design	Rat embryo limb bud cell cultures were used to assess the relative teratogenic potential of tributyltin oxide and its metabolites including dibutyltin chloride and monobutyltin chloride. Fifty percent inhibition concentrations for cell proliferation (IP_{50}) and cell differentiation (ID_{50}) and P/D ratio were calculated.
Findings	With the exception of monobutyltin chloride, all of the organotin compounds investigated in this study showed very strong inhibition of cell differentiation (ID_{50} :0.13-1.71 μM) and cell proliferation (IP_{50}: 0.12-2.81 μM).
Conclusion	The authors suggest that dibutyltin is directly teratogenic."

2.3 Specific target organ toxicity – single exposure/repeated exposure

Detailed summaries of studies relevant to classification for specific target organ toxicity – single exposure/repeated - are presented in this section.

The studies described below have been described in the CLH-dossier for DBTP (EC 245-152-0) and assessed by RAC in 2017. Where the description of the studies below are in quotes the text is a verbatim rendering of the text in the mentioned CLH-dossier.

Studies of reproductive or developmental toxicity are also reported in this section where relevant endpoints were assessed.

2.3.1 Animal data

2.3.1.1 Mechanistic investigation of thymic atrophy in the rat

Reference	"Snoeij NJ, Penninks AH & Seinen W (1989). Thymus Atrophy and Immunosuppression Induced by Organotin Compounds. Archives of Toxicology S13: 171-174.
Guideline	No guideline followed
Reliability	Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)
Species / strain	Rat (Wistar)
Test material	**Dibutyltin dichloride (DBTC)** CAS 683-18-1 EC 211-670-0 >98% purity
Study design	Male Wistar rats were gavaged with DBTC (in ethanol/corn oil) at dose levels of 0 (vehicle control) or 15 mg/kg bw; bodyweights and thymus weights (3 rats per group) were measured at 1, 2, 3, 4, 7 and 9 days after dosing. Suspensions of the thymus were prepared for the analysis of total cell count, cell sizing and the incorporation of radiolabelled DNA, RNA and protein precursors.
Findings	A single oral dose of DBTC was associated with a decrease in absolute and relative thymus weights

from the second day after dosing. Thymus weight reduction was maximal at Day 4, but was shown to recover by Day 9. The number of cells isolated from the thymus was significantly reduced at Days 3, 4 and 7, with recovery by Day 9. The number of large cells (volume >225 μm^3) was decreased at Days 1 and 2, the numbers of small (volume <130 μm^3) and intermediate cells were not affected until Day 3. Cell populations were normal by Day 9. The incorporation of radioactivity was reduced on Days 1 and 2, but subsequently returned to control values

Conclusion Based on the reduction in thymus weight and loss of cellularity, the authors conclude that a single oral dose of 15 mg/kg bw DBTC induces a rapid but reversible atrophy of the thymus in the rat. Based on the thymus cell profile, the authors speculate that thymic atrophy is caused by the selective reduction of rapidly proliferating thymic lymphoblasts (which generate small cells populating the thymic cortex). Recovery is shown by a rise in the number of large cells and an increase in macromolecular synthesis."

2.3.1.2 Sub-chronic dietary toxicity study in the rat

Reference "Gaunt IF, Colley J, Grasso P, Creasey M & Gangolli SD (1968) Acute and Short-term Toxicity Studies on Di-n-butyltin Dichloride in Rats. Food & Cosmetic Toxicology 6: 599-608.

Guideline None

Species / strain Rat (CFE)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

99.7% purity

Study design Groups of SPF-derived rats (16/sex) were fed diets containing DBTC at concentrations of 0 (control), 10, 20, 40 or 80 ppm for 90 days. Animals were observed daily for signs of toxicity. Bodyweights and food consumption were measured weekly. Blood samples were taken during Week 6 (control, 40 and 80 ppm groups) for the assessment of haematological parameters; haematological parameters were also assessed in terminal blood samples taken from rats of all groups. Terminal blood samples were also assessed for AST and ALT activity; serum amylase activity was additionally measured in the control and 80 ppm dose groups as a marker of pancreatic damage. Urinalysis was also performed. Renal function tests were performed during Week 6 and prior to termination. Investigations comprised assessment of the concentrating ability of the kidney by measuring the volume and specific gravity of urine produced under conditions of normal hydration, during a 6-hour period of water deprivation, during a 2-hour period following a water load of 25 mL/kg bw and during a 4-hour period commencing 16 hours after the water load.

Gross necropsy was performed on all rats; weights of the brain, pituitary, heart, thyroid, liver, spleen, kidneys, adrenals and gonads were recorded. These organs and additionally the salivary gland, trachea, lungs, diaphragm, lymph nodes, thymus, pancreas, stomach, ileum, colon, caecum, rectum, urinary bladder, sternum and uterus were investigated histopathologically. The duodenal loop with the pancreas and bile duct *in situ* were fixed flat so as to retain their anatomical relationship.

Findings There were no deaths and no signs of toxicity in any group. A slight reduction in weight gain was seen in both sexes at 80 ppm and was statistically significant in females. Some reduction in food intake was noted and was attributed to an effect of the test material on dietary palatability. Haematology revealed statistically significantly reduced haemoglobin concentrations at 80 ppm in females at Week 6 and in males at Week 13. Decreases were slight and were not associated with changes in other erythrocyte parameters or an indication of reticulocytosis. Clinical chemistry and urinalysis did not reveal any effects of treatment. Gross necropsy did not show any treatment-related findings; organ weights were comparable in all dose groups. Histopathology did not show any effects of treatment on any organ or tissue investigated (including the thymus).

Mean body weight values

Dietary level (ppm)	Body weight (g)			
	Week 0	Week 4	Week 8	Week 13
Males				

0	187	367	457	543
10	183	368	464	544
20	189	393	474	561
40	189	374	472	556
80	181	345	438	512
Females				
0	153	240	283	316
10	148	231	274	301
20	151	237	283	318
40	147	239	287	330
80	147	227	267*	299*

*Significantly different to controls, *P <0.05 Students t-test*

Haematology parameters at Week 6 and Week 13

Dietary level (ppm)	**Hb (g/dL)**	**Hct (%)**	**RBC (10^6/mm^3)**	**Retics (% of RBC)**	**Leucocytes**				
					Total (10^3/mm^3)	**Differential (%)**			
						N	**E**	**L**	**M**
Males – Week 6									
0	14.5	47	7.48	1.24	23.6	13	1	85	1
40	14.6	46	7.72	1.32	19.0	14	1	85	0
80	14.3	47	7.01	1.17	19.5	13	1	86	0
Females – Week 6									
0	14.7	45	7.44	1.66	23.2	9	0	91	0
40	14.1	44	7.22	1.38	18.8	12	2	85	1
80	13.7*	44	7.45	1.80	16.9	12	1	87	0
Males – Week 13									
0	14.6	45	7.42	1.30	7.4	16	2	79	3
10	14.3	46	7.61	1.14	6.0	14	1	81	4
20	13.9	40	7.41	1.21	5.9	13	1	82	4
40	13.9	44	7.49	1.47	6.0	16	3	78	3
80	13.2**	46	7.33	1.13	5.0	19	3	74	4
Females – Week 13									
0	14.2	43	6.52	1.32	4.1	13	1	83	3
10	13.6	44	6.46	1.29	3.2	18	3	76	3
20	14.0	43	6.76	1.36	3.1	19	2	75	4
40	13.5	43	6.27	1.42	3.5	14	1	82	3
80	14.0	45	6.61	0.99	3.2	15	1	81	3

*Significantly different to controls, *P <0.05, **P <0.01*

Conclusion Sub-chronic administration of DBTC to the rat resulted in a slight reduction in weight gain and a marginal effect on haemoglobin concentration at the highest dose level of 80 ppm (equivalent to approximately 4 mg/kg bw/d). A NOAEL of 40 ppm (equivalent to approximately 2 mg/kg bw/d) can therefore be determined for this study. No effects on the thymus were apparent at the highest dose level (4 mg/kg bw/d) in either sex."

2.3.1.3 Reproductive/developmental toxicity screening study in the rat

Reference "Unpublished report (2003)

[Full report not available: study details taken from the publically disseminated REACH Registration Dossier and the 2014 CLH report on dibutyltin dilaurate]

Guideline OECD 421

Reliability Klimisch 2: reliable with restrictions (guideline study, full report not available)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

98.57% purity

Study design Groups of 12 Wistar rats/sex were administered the test material in the diet at concentrations of 0, 5, 30 or 200 ppm for four weeks (males) or for two weeks prior to mating and to Day 4 or 6 *post partum* (females).

Animals were observed daily for mortality and clinical signs. Bodyweights were measured pre-test and on Days 0, 7 and 14 of the pre-mating period. Bodyweights of males were measured on Days 21 and 28; bodyweights of females were measured during mating (Days 0, 7 and 14); on Days 0, 7, 14 and 21 of gestation; on Days 1 and 4 of lactation and at termination. Food consumption was measured for both sexes during the pre-mating period (Days 0-7, 7-13); for males during the post-mating period (Day 21-28); for females during the gestation period (Days 0-7, 7-14, 14-21) and during the lactation period (Days 1-4).

At termination, weights of the kidney liver, spleen, testes, thymus and brain were recorded for parental animals. Gross necropsy was performed on all parental animals; the prostate, seminal vesicles and testes of male animals were investigated. The ovaries, uterus (including counting of implantation sites) and thymus were investigated in females. Pups were assessed for gross abnormalities.

Findings Mean male bodyweights were significantly lower from Day 14-28 of the study at the highest dose level. Weight gains by males at the highest dose level were significantly lower over the study period; weight gain by males at 30 ppm was also reduced from Day 14-21. There was no effect of treatment on weight gain in males administered 5 ppm DBTC. Weight gain by females at the highest dose level was reduced over the premating, gestation and lactation periods; mean bodyweights of females in this group were significantly lower at Day 14 of the premating period, over the entire gestation period and during the lactation period. Food consumption by males at the highest dose level was reduced; significantly at Day 7-14. Food consumption by females in this group was significantly reduced during the pre-mating, gestation and lactation periods. Food consumption by females in the other treatment groups was comparable to controls.

Gross necropsy and histopathology of males did not reveal any effects of treatment on the reproductive tract. Thymuses of males were not investigated histopathologically. Severe or very severe thymic lymphoid depletion was observed in females at 200 ppm; moderate to severe lymphoid depletion was seen in the majority of the pregnant (but not in the non-pregnant) females at 30 ppm. Lymphoid depletion was characterised by decreased size of the thymic lobules due to extensive loss of cortical and medullary lymphocytes. The border between the thymus cortical and medullary areas was therefore indistinct. In the most 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.

Conclusion Administration of DBTC in the diet at concentrations of 30 and 200 ppm resulted in thymic lymphoid depletion in females. A NOAEL of 5 ppm can therefore be determined for this study."

2.3.1.4 Sub-acute toxicity study in the rat

Reference "Seinen W & Vos JG (1977). 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. Toxicology & Applied Pharmacology 42:197-212.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Mouse (Swiss)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity >98%

Study design Groups of rats (10/sex) or 10 male mice were administered DBTC in the diet at concentrations of 0 (controls), 50 or 150 ppm for 4 weeks. Bodyweights were recorded weekly. Gross necropsy was performed on all animals; weights of the thymus, spleen, popliteal lymph node, liver, kidneys and adrenals were recorded; these tissues were also investigated histopathologically.

Findings Mortality occurred in rats administered 150 ppm DBTC (2 males, 4 females) in the second week of the study. Relative thymus weight was reduced at 50 ppm (by 53%) and at 150 ppm (by 68-72%); spleen weights (16% and 33%) and popliteal lymph node weights (16% and 28%) were also reduced at 50 ppm and 150 ppm, respectively. Gross necropsy revealed a marked reduction in the size of the thymus was found in all treated animals. Yellow discoloration of the liver, thickened and dilated bile ducts were also observed in a small number of rats at 150 ppm. Histopathology revealed severe proliferation of bile duct epithelial cells and bile ductules, associated with pericholangiolitis and periportal fibrosis in rats at 150 ppm.

The most prominent effect found was lymphocyte depletion in lymphoid organs; this was most pronounced in the thymic cortex. At 150 ppm, the cortex was almost completely depleted; however signs of cell destruction were not observed. Lymphocyte depletion was also observed in the thymus-dependent areas of the spleen (periarteriolar lymphocyte sheets) and popliteal lymph node (paracortex).

No effects of treatment were observed in mice.

Body weight and relative organ weights (means ± SD)

Dietary level (ppm)	Body weight (g)	Liver (g/kg)	Thymus (g/kg)	Spleen (g/kg)	Popliteal lymph nodes (mg/kg)
Males					
0	115.3 ± 3.9	42.5 ± 0.9	3.77 ± 0.19	3.62 ± 0.20	73 ± 10
50	107.7 ± 2.4*	42.9 ± 0.7	1.70 ± 0.11*	3.01 ± 0.13*	57 ± 3*
150	92.1 ± 4.5*	49.3 ± 1.0*	1.04 ± 0.12*	2.41 ± 0.11*	52 ± 6*
Females					
0	106.4 ± 2.3	49.7 ± 0.9	3.76 ± 0.15	3.20 ± 0.12	62 ± 4
50	102.2 ± 0.9*	49.3 ± 1.3	1.79 ± 0.10*	2.39 ± 0.12*	50 ± 3*
150	86.0 ± 7.0*	50.8 ± 2.3	1.20 ± 0.18*	2.18 ± 0.08*	52 ± 6*

Significantly different to controls, *p <0.001 Students t-test

Conclusion A NOAEL of <50 ppm (equivalent to approximately 2.5 mg/kg bw/d) can be determined for this study, based on effects on the thymus, spleen and lymph nodes (lymphoid depletion) in both groups

of treated rats."

2.3.1.5 Sub-acute toxicity study in the rat

Reference "Penninks AH & Seinen W (1982). Comparative toxicity of alkyltin and estertin stabilisers Food & Chemical Toxicology 20:909-916.

Guideline None followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**
CAS 683-18-1
EC 211-670-0
Purity unknown

Study design Groups of ten male Wistar (WU-CPB) rats (bodyweight 40-45 g) were administered DBTC in the diet at concentrations of 0 (controls), 50 or 150 ppm for 14 days. Bodyweights were measured weekly. Gross necropsy was performed and the weights of the thymus, spleen, liver, kidneys and adrenals were recorded. These organs were investigated histopathologically.

Findings Two rats in the 150 ppm group died during Week 2 of the study and are reported to have showed signs of severe jaundice. A dose-related reduction in bodyweight gain was seen in the treated groups. Relative weights of the thymus and spleen were reduced in both treated groups; the decrease in thymus weight was pronounced and was equivalent to a reduction of greater than 70% at 150 ppm.

Gross necropsy showed yellow liver discolouration in some rats at 150 ppm; relative liver weight was increased in this group. Microscopically, rats administered 150 ppm showed hepatotoxicity (severe proliferation of the bile duct epithelium, associated with pericholangitis, periportal fibrosis and accumulation of bile pigment in hepatocytes). The most prominent histopathological feature in all treated animals was lymphocyte depletion; this findings was noted particularly in the thymic cortex, but was also apparent in the splenic periarteriolar lymphocyte sheets.

Summary of findings

Concentration (ppm)	0	50	150
Terminal bodyweight (g)	115.3	107.7**	92.1**
Liver weight (%)	4.25	4.29	4.93**
Thymus weight (%)	0.38	0.17**	0.10**
Spleen weight (%)	0.36	0.30**	0.24**
Kidney weight (%)	1.07	1.04	1.06
Adrenal weight (%)	0.025	0.021	0.022

***significantly different to controls (P<0.001)*

Conclusion A NOAEL of <50 ppm can be determined for this study based on reduced thymus and spleen weights and associated histopathology (lymphocyte depletion) in both treated groups."

2.3.1.6 Sub-chronic dietary toxicity study in the rat

Reference "Barnes JM & Stoner HB (1958). Toxic properties of some dialkyl and trialkyl tin salts British Journal of Industrial Medicine 15:15-22.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain Rat (unspecified)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity unknown

Study design Groups of 12 rats were administered DBTC in the diet at concentrations of 0 (controls), 20, 50, 75 or 100 ppm for periods of up to six months.

Findings In groups of rats administered DBTC for 54 or 55 days, a dose-related reduction in weight gain and food consumption was apparent in all groups; weight gain was significantly reduced at dietary concentrations of 50 ppm and above.

Bodyweight and food consumption effects

Dietary concentration	54 days		55 days	
	Weight gain (g)	Food consumption (g)	Weight gain (g)	Food consumption (g)
20 ppm	-11%	-2%		
50 ppm	-19%*	-21%	-22%	-23%
75 ppm			-35%*	-26%
100 ppm	-42%**	-29%	-30%*	-19%

Rats administered 20 ppm DBTC for 6 months grew normally and showed no lesions at gross necropsy. At 50 ppm, growth and food intake were reduced; gross necropsy showed thickening and dilatation of the bile duct and fibrosis of the pancreas. At 75 and 100 ppm, rats showed some mortality and a greater depression of growth. Gross necropsy of animals surviving to termination showed variable levels of bile duct damage.

Conclusion A NOAEL of 20 ppm (equivalent to approximately 1 mg/kg bw/d) can be determined for this study based on reduced weight gain at 50 ppm (equivalent to approximately 2.5 mg/kg bw/d)."

2.3.1.7 Developmental toxicity study in the rat

Reference "Study report (1994) included in the publically disseminated REACH Registration Dossier for dibutyltin dichloride.

Guideline OECD 414; no deviations

Reliability Klimisch 2: reliable with restrictions (guideline study, full report not available)

Species / strain Rat (Wistar) Crl:CD(Wi)BR

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

>98% purity

Study design Groups of 25 mated Wistar female rats were gavaged with the test material (in corn oil) at dose levels of 0, 1, 2.5, 5 or 10 mg/kg bw/d on Days 6-15 of gestation. Dams were investigated for mortality and clinical signs. Bodyweights and food consumption were recorded at regular intervals.

Dams were subject to gross necropsy; the thymus, liver, bile duct and mesenteric lymph nodes were investigated histopathologically.

Findings No deaths occurred and no signs of maternal toxicity were observed during the study period. Reduced weight gain and food consumption were seen at 10 mg/kg bw/d; weight gain was also slightly reduced at 5 mg/kg bw/d. Gross necropsy of dams did not reveal any treatment-related findings. Mean thymus weight was significantly lower in dams at 10 mg/kg bw/d. Histopathology of

the thymus showed atrophy at 10 mg/kg bw/d and to a lesser extent at 5 and 2.5 mg/kg bw/d.

Conclusion A NOAEL for maternal toxicity of 1 mg/kg bw/d can be determined for this study based on thymic atrophy at dose levels of ≥2.5 mg/kg bw/d; reduced weight gain was seen at ≥5 mg/kg bw/d."

2.3.1.8 Developmental toxicity study in the rat

Reference "Farr CH, Reinisch K, Holson JF & Neubert D (2001). Potential teratogenicity of di-n-butyltin dichloride and other dibutyltin compounds. Teratogenesis, Carcinogenesis & Mutagenesis 21(6):405-15.

Guideline OECD 414

Reliability Klimisch 2: reliable with restrictions (guideline study summary, published in a peer-reviewed journal)

Species / strain Rat (Wistar)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design A developmental toxicity study was conducted in the rat according to OECD guidelines and GLP. Groups of 25 mated female Wistar rats were gavaged with DBTC (in olive oil) at dose levels of 0, 1, 2.5, 5 or 10 mg/kg bw on GD 6-15. Evaluation of pregnancy outcome was performed on day 20 of pregnancy.

Findings Maternal toxicity (reduced food consumption, bodyweight gain and reduced thymus weight) were seen at 10 mg/kg bw. No evidence of embryotoxicity as assessed by numbers of total resorptions, viable foetuses or foetal weight was noted in any treated group. A slightly increased frequency of total malformations was seen at 10 mg/kg bw (4/262 foetuses) compared to the control group (1/269 foetuses). The authors consider that the nature and pattern of malformations does not suggest any effect of treatment; however the nature of findings (including single incidences of ankyloglossia, agnathia, mandibular defect) are consistent with the results of other studies and therefore indicate a relationship to treatment with DBTC

Maternal findings

Dose level (mg/kg bw)	**0**	**1.0**	**2.5**	**5.0**	**10.0**
Inseminated females (#)	25	25	25	25	25
Pregnant females (#)	20	25	23	19	20
100% intrauterine deaths (#)	0	1	0	1	0
Females with viable foetuses (#)	20	24	23	18	20
Malformed foetuses (#)	1/269	0-343	0-292	1/224	4/262
Maternal weight gain (g) GD 6-16	67.2	67.3	64.9	67.4	55.7*
Maternal food consumption (g) GD 6-16	25.5	25.5	25.2	25.9	23.7*
Maternal thymus weight (mg)	371	366	409	339	287**
Malformed foetuses (#)	1 (1)	-	-	1 (1)	4 (3)
Anasarca	-	-	-	1	1
Hydrocephaly	-	-	-	-	1
Ankyloglossia	-	-	-	-	1
Agnathia	-	-	-	-	1
Pulmonary valve atresia	1	-	-	-	-

Scoliosis	-	-	-	-	1
Anophthalmia	-	-	-	-	1
Mandible absent	-	-	-	-	1
Vertebrae / arches absent	-	-	-	-	1

* *significantly different to controls* p*<0.05; **p<0.01*

Conclusion A NOAEL of 5 mg/kg bw can be determined for teratogenicity and developmental toxicity, based on the slightly elevated incidence of characteristic foetal malformations at 10 mg/kg bw/d. A NOAEL of 5 mg/kg bw/d can be determined for maternal toxicity, based on reduced bodyweight gain, food consumption and reduced thymus weight at the highest dose level."

2.3.1.9 Sub-acute study of immunotoxicity in the rat

Reference "DeWitt JC, Copeland CB & Luebke RW (2005). Immune responses in Sprague-Dawley rats exposed to dibutyltin dichloride in drinking water as adults. Journal of Immunotoxicology 2(3):151–60.

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions (non-guideline mechanistic study published in a peer-reviewed journal)

Species / strain Rat (Sprague-Dawley CD)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

96% purity

Study design Groups of 60-day old Sprague-Dawley (CD) rats (8/sex) were administered DBTC in drinking water containing 0.5% Alkamuls at concentrations of 0 (controls), 0 or 25 mg/L for 28 days. Achieved dose levels were equivalent to 0, 0.9 and 1.9 mg/kg bw/d for the initial study; 0, 1.0 and 2.5 mg/kg bw/d for the confirmatory study. Water bottles were changed and water consumption monitored twice weekly; body weights were recorded weekly. Delayed-type hypersensitivity (DTH), primary and secondary antibody responses to sheep red blood cells (SRBCs), and natural killer (NK) cell activity were evaluated in groups of treated and control animals on Day 29 of the study.

Primary (IgM) and secondary (IgG) T-cell-dependent antibody responses against SRBCs were assessed in animals were immunized on Study Day 24 (intravenous injection of 2×10^8 SRBCs in 0.5 mL sterile saline); blood samples were taken on Study Day 29. The same animals were administered a booster immunization (intravenous injection of 2×10^8 SRBCs in 0.5 mL sterile saline) on study Day 39. Blood samples collected on study Day 44 were analysed for SRBC-specific IgG. The relative serum titre of SRBC-specific IgM and IgG antibodies were measured by ELISA.

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 was measured in splenocyte single cell suspensions prepared and cultured with ^{51}Cr-labeled murine YAC-1 lymphoma target cells. ^{51}Cr release was determined using liquid scintillation counting.

Findings No statistically significant effects were seen on bodyweight. Water consumption by males (-17%) and females (-21%) was significantly decreased at the highest concentration. Absolute and relative thymus and spleen weights were unaffected by treatment. No clear effects of treatment were seen on antibody production, DTH response or NK cell activity. Different results for antibody responses in male rats were obtained in two experimental replicates. In the first replicate, IgG was elevated at the highest dose level whereas in the second replicate, IgM was suppressed.

Conclusion A NOAEL of 2.5 mg/kg bw/d can be determined for this study, in the absence of any effects of treatment."

2.3.1.10 Sub-acute toxicity study of immune function in the rat

Reference "DeWitt J, Copeland C & Luebke R (2006). Immune Function In Rats Developmentally Exposed To Dibutyltin Dichloride. Toxicological Sciences 90(1-S):388.

The study is reported as a conference abstract only, and appears to report findings by the same authors reported elsewhere [DeWitt et al., 2005b]

Guideline No guideline followed

Reliability Klimisch 4: not assignable (insufficient experimental detail)

Species / strain Rat (Sprague-Dawley)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Study design Individually housed pregnant female SD rats were given administered DBTC (in 0.35% Alkamuls) in the drinking water at concentrations of 0, 10 or 25 mg/L from GD 6 to PND 21. Litters were sexed, weighed and culled to 8 pups (4/sex) on PND 2. From PND 3, the litters from half of the dams of each group were gavaged with DBTC (in 0.5% Alkamuls) at dose levels of 0, 1.0, or 2.5 mg bw, three times a week for a total of ten doses. Delayed-type hypersensitivity (DTH), primary and secondary antibody responses and natural killer (NK) cell activity were evaluated in offspring (6/sex/group) after PND 42.

Findings Weight gain by litters gavaged with 2.5 mg/kg bw DBTC was decreased, but recovery was seen and bodyweights reached control levels by PND 50. DTH response and NK cell activities were unaffected by treatment. In female offspring, IgM was lower in some treated groups relative to control groups. In male offspring, IgG was elevated in the 25 mg/L group relative to controls. Findings were, however, not replicated in a second study assessing antibody production.

Conclusion No clear effects of DBTC treatment were seen under the conditions of this study."

2.3.1.11 Mechanistic investigation of thymic atrophy in the rat, the mouse and the Guinea pig

Reference "Seinen W, Vos JG, van Krieken R, Penninks A, Brands R & Hooykaas H (1977). Toxicity of organotin compounds. III. Suppression of thymus-dependent immunity in rats by di-n-butyltindichloride and di-n-octyltindichloride. Toxicology and Applied Pharmacology 42(1):213-24.

Guideline No guideline followed

Species / strain Rat (Wistar WU, WAG inbred)

Mouse (Swiss)

Guinea pig (Hartley)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

>98% purity

Study design Groups of rats and mice were administered diet containing DBTC at concentrations of 0 (control), 50 or 150 ppm.

After three weeks of treatment, male WU rats were sensitised by subcutaneous injection of complete adjuvant; delayed hypersensitive response was tested by intradermal tuberculin injection after 5 or six weeks. At termination, weights of the thymus, spleen, adrenals and popliteal lymph node were

recorded.

Tail skin grafts from WAG x B F1 hybrid rats were performed on WAG rats; allograft rejection was assessed microscopically.

Immune response in rats was also assessed using plaque forming cell, haemagglutination, haemolysis and *in vitro* phagocytosis (carbon clearance) assays.

Findings Allograft rejection was significantly delayed by DBTC at 150 ppm (11.9 days) compared to controls (9.4 days), but not at 50 ppm (10.1 days). The antibody response against *E. coli* LPS, was unaffected by DBTC. The humoral immune response against sheep red blood cells (SRBC) was depressed by DBTC. Haemagglutination and haemolysin titres and the number of direct plaque-forming cells against SRBC were decreased in a dose-related manner by DBTC. Altered immune functions were not found in mice or guinea pigs exposed to DBTC.

Conclusion The authors conclude that DBTC causes immunotoxicity in rats by a selective inhibition of T-lymphocyte activity. Effects were most pronounced in animals exposed to the chemicals during the developmental phase of the lymphoid system."

2.3.2 Human data

No human data are available.

2.3.3 Other data

2.3.3.1 Mechanistic study

Reference "de Heer C, Schuurman HJ, Houben GF, Pieters RH, Penninks AH & van Loveren H (1995). The SCID-hu mouse as a tool in immunotoxicological risk assessment: effects of 2-acetyl-4(5)-tetrahydroxybutyl-imidazole (THI) and di-n-butyltin dichloride (DBTC) on the human thymus in SCID-hu mice. Toxicology 100(1-3):203-11.

Guideline No guideline followed

Species / strain Mouse (SCID-hu)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design 36 female SPF-derived homozygous C.B-17 *scid*/*scid* (SCID) mice aged 4-5 weeks were engrafted with human foetal thymus and liver tissue fragments. Mice were exposed to a single dose of DBTC by intraperitoneal injection at dose levels of 0 (vehicle), 0.3 or 1.0 mg/kg bw and sacrificed five days later. The human thymus transplants were removed and assessed morphometrically and histopathologically.

Findings Bodyweights were unaffected by treatment with DBTC. Relative spleen weight was increased in the treated groups, a finding attributed to increased extramedullary haematopoiesis. DBTC treatment resulted in reduced cortical size of the human thymus graft. Histopathological examination of the human thymus grafts of SCID-hu mice exposed to DBTC showed a reduction in the relative size of the thymus cortex.

Conclusion The results of this study indicate that the human thymus is a target for DBTC."