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 bis(2-ethylhexanoate)

EC Number: **220-481-2**

CAS Numbers: **2781-10-4**

Index Number: **Not applicable**

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Note on confidential information

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1 PHYSICAL HAZARDS

Not evaluated in this CLH Report.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Many of the studies described below are the same studies that were described in the CLH-dossier for DBTP (dibutylbis(pentane-2,4-dionato-O,O')tin) (EC no.:245-152-0/ CAS no.:22673-19-4) 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.

2.1.1 Simulated gastric hydrolysis

"Reference	Schilt R & Zondervan-van den Beuken EK (2004). Dibutyltin dilaurate (DBTDL, 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	**DBTDL** 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 (DBTDL), 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	DBTDL	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 DBTDL, 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. This study is also included in the publically disseminated REACH Registration Dossier for the substance and is used to justify read-across to toxicological studies with DBTC using oral administration."

2.1.2 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."

2.1.3 Microsomal metabolism *in vitro* and *in vivo*

"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, DBTA**

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 14C-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 in vitro phase, the metabolites of 14C 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 14C 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."

2.1.4 Simulated gastric hydrolysis

Reference Unnamed, 2000 (study 005, basic toxicokinetics, registration dossier for DBTE on ECHAs dissemination site)

Guideline No guideline

Reliability Klimisch 2: reliable with restrictions. Key study with supporting substance. Well documented study, included in an ORTEP summary report (Organotin Environmental Programme (ORTEP) Association, Stabilizer Task Force)

Species/strain Not relevant

Test material **dibutyltin bis EHMA** (2-ethylhexyl 4,4-dibutyl-10-ethyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate, CAS nr 10584-98-2)

Study design not described

Findings Under acidic conditions, mono- or di- alkyltin mercaptides undergo a tin-EHMA bond break releasing EHMA. The free EHMA undergoes additional hydrolysis with ethyl hexanol and thioglycolic acid as products. EHMA and ethyl hexanol are easily quantified at low ppm level by GC-AED. The water soluble thioglycolic acid could be determined indirectly by total sulfur analysis-ICP emission spectroscopy.

2.1.5 Simulated gastric hydrolysis

Reference Unnamed, 2000 (study 006, basic toxicokinetics, registration dossier for DBTE on ECHAs dissemination site)

Guideline No guideline

Reliability Klimisch 2: reliable with restrictions. Weight of evidence from supporting substance. Well documented study, included in an ORTEP summary report (Organotin Environmental Programme (ORTEP) Association, Stabilizer Task Force)

Species/strain Not relevant

Test material **dibutyltin bis EHMA** (2-ethylhexyl 4,4-dibutyl-10-ethyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate, CAS nr 10584-98-2).

Study design A direct injection gas chromatographic (GC) method has been developed to quantify monobutyltin trichloride (MBTC) and dibutyltin dichloride (DBTC) produced during the hydrolysis of Bu2Sn(EHMA)2 under simulated gastric conditions (37°C, pH = 1.2 or 4). DBTC can be quantified in the range of 0.1 to 5 µg/mL (as tin). MBTC can be quantified in the range of 0.2 to 5 µg/ml (as tin) at pH = 1.2, but can not be quantified at pH = 4. The repeatability of results is about ± 10%, relative.

Findings Results indicate that the hydrolysis of Bu2Sn(EHMA)2 is very rapid at pH = 1.2., in the order of minutes. Additionally, pepsin seems to have no affect on the hydrolysis rate.

2.1.6 Simulated gastric hydrolysis

Reference Unnamed, 2000 (study 007, basic toxicokinetics, registration dossier for DBTE on ECHAs dissemination site)

Guideline No guideline

Reliability Klimisch 2: reliable with restrictions. Weight of evidence from supporting substance. Well documented study, included in an ORTEP summary report (Organotin Environmental Programme (ORTEP) Association, Stabilizer Task Force)

Species/strain Not relevant

Test material **dibutyltin bis EHMA** (2-ethylhexyl 4,4-dibutyl-10-ethyl-7-oxo-8-oxa-3,5-dithia-4-stannatetradecanoate, CAS nr 10584-98-2). Lot/batch No.: NB#10777-64.

Study design Direct infusion electrospray MS is used to study the hydrolysis of Bu2Sn(EHMA)2 to its corresponding chloride derivative under simulated gastric conditions (pH = 1 and pH = 4).

Findings Experiment 1: In positive mode, organotins (M) appear as a sodium adduct ion labeled [M+Na]+.

Experiment 2: When water is replaced with pH 4 HCl in solution 2, the organotins were still detectable in the positive mode but the sensitivity of the sodium adduct peaks was found to be lower compared to experiment 1. The intermediate chlorides were detected as chloride adducts. When the flow rate of solution 2 is increased from 10 to 50 µl/min, detection of the initial organotins become more challenging in the positive mode. The decrease in sensitivity can be explained by a greater conversion of the organotins into their corresponding chloride species. In addition, using these conditions, Bu2SnCl2 was able to be detected as a chloride adduct in the negative mode.

Experiment 3: The starting materials were not dectected in the positive mode. The hydrolysis product Bu2SnCl2 was identified in the negative mode . The absolute intensity was similar to the one observed in Experiment 2 with flow rate of Solution 2 = 50 µl/min.

The results indicate that the hydrolysis occurs more completely at pH = 1 than at pH = 4. In addition, the same behavior is observed for the four organotins investigated in this study.

Conclusion In conclusion, these data show that the hydrolysis of organotins such as Bu2Sn(EHMA)2 depends on the amount of acid HCl added to the solution, and hydrolysis is very fast at pH = 1. In addition, all the tested (Me2Sn(EHMA)2, BuSn(EHMA)3,Bu2Sn(EHMA)2and Oct2Sn(EHMA)2) organotins follow the same pattern of conversion into their chloride derivatives. Information on the rate of hydrolysis can not be determined due to the major experimental differences between these studies (high concentration of organic solvent, etc.) and actual gastric hydrolysis measurements.

2.1.7 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 119Sn NMR in toluene-d8 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 ClBu2SnOSnBu2Cl (119Sn-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 ClBu2SnOSnBu2Cl under conditions representative of the mammalian stomach. The generation of a common intermediate, identical to the hydrolysis product of DBTC (see 2.1.5), supports the read-across approach for the involved substances in the category including dibutylbis(pentane-2,4-dionato-O,O´)tin."

2.1.8 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 119Sn NMR in toluene-d8 which allowed positive identification of the hydrolysis product.

Findings Simulated gastric hydrolysis studies demonstrate that dibutyltin dichloride rapidly form the dimeric stannoxane ClBu2SnOSnBu2Cl (119Sn-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 ClBu2SnOSnBu2Cl

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

Conclusion Dibutyltin dichloride is shown to be rapidly converted to ClBu2SnOSnBu2Cl under conditions representative of the mammalian stomach. The generation of a common intermediate, identical to the hydrolysis product of dibutylbis(pentane-2,4-dionato-O,O´)tin (see 2.1.4), supports the read-across approach for the involved substances in the category including dibutylbis(pentane-2,4-dionato-O,O´)tin."

2.1.9 Dermal absorpsjon, *in vitro*

Reference Unnamed, 2003 (study dermal absorption, registration dossier for DBTE on ECHAs dissemination site)

Guideline Performed according to the draft version of the current OECD guideline (OECD 428, dermal absorption *in vitro*). Conducted according to GLP.

Reliability Klimisch 2: reliable with restrictions. Key study, using supporting substance.

Species/strain Human and Wistar rat epidermis.

Test material **Dibutyltin bis(2-ethylhexyl mercaptoacetate)**

CAS no. 10584-98-2.

Lot/batch No.: (Batch reference) R&D/S-vH 2002/38/9.

Study design The absorption of dibutyltin bis(2-ethylhexylmercaptoacetate), containing 18.5 % w/w tin, was measured *in vitro* through human and rat epidermis. The first phase of the study was to identify the highest dose that could practically be applied, which was also regarded as likely to be non-damaging to human epiderms, using rat epidermis as a model. During the second phase of the study, the absorption of tin was determined through human and rat epidermis from both occluded and unoccluded applications of this non-damaging dose (100 µL/cm^2 eq. 21120 µg tin/cm^2).

Findings 100 µL/cm^2 (= 21120 µg tin/cm^2) was found to alter the barrier function of the rat epidermis. At 100 µL/cm^2, approximately up to 18-45 % of the tin dose was unaccounted for, possibly due to adherence of the test material to the glass apparatus. The absorption of tin through human epidermis was very slow, when compared with the absorption rates of other penetrants. The proportions of dibutyltin bis(2-ethylhexylmercaptoacetate) absorbed through human epidermis were 0.0004% and 0.0010% (occluded and unoccluded respectively) after 24 hours exposure, compared to 0.261% and 0.189% through rat epidermis. The majority of the applied tin dose was washed from the surface of the epidermis during decontamination, only a relatively small proportion of the dose (human up to 1%; rat up to 10%) remained associated with the epidermis and therefore was not regarded as systemically available.

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

Not evaluated in this CLH Report.

3.2 Acute toxicity - dermal route

Not evaluated in this CLH Report.

3.3 Acute toxicity - inhalation route

Not evaluated in this CLH Report.

3.4 Skin corrosion/irritation

Not evaluated in this CLH Report.

3.5 Serious eye damage/eye irritation

Not evaluated in this CLH Report.

3.6 Respiratory sensitisation

Not evaluated in this CLH Report.

3.7 Skin sensitisation

Not evaluated in this CLH Report.

3.8 Germ cell mutagenicity

Many of the studies described below are the same studies that were described in the CLH-dossier for DBTP (dibutylbis(pentane-2,4-dionato-O,O')tin) (EC no.:245-152-0/ CAS no.:22673-19-4) 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.

3.8.1 In vitro data

3.8.1.1 *In vitro* gene mutation study in bacteria, Ames test. Key study.

Reference Unnamed, 2010 (in vitro gene mutation study in bacteria, registration dossier for DBTE on ECHAs dissemination site)

Guideline Performed according to OECD guideline (No. 471 "Bacterial Reverse Mutation Test"). Conducted according to GLP.

Reliability Klimisch 1: reliable without restrictions. Key study.

Species/strain Salmonella typhimurium strains TA1535, TA1537, TA98, TA100 and Escherichia coli strain WP2uvrA$^-$

Test material **Dibutyltin dilaurate (DBTDL)**

CAS 77-58-7

EC 201-039-8

Purity >95%

Batch number LA6K05N001

Study design Salmonella typhimurium strains TA1535, TA1537, TA98, TA100 and Escherichia coli strain WP2uvrA- were treated with the test material using both the Ames plate incorporation and pre-incubation methods at seven dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolising system (10% liver S9 in standard co-factors). The dose range was determined in a preliminary toxicity assay and was 0.05 to 5000 µg/plate in the first experiment. The experiment was repeated on a separate day (pre-incubation method) using fresh cultures of the bacterial strains and fresh test material formulations. The test material dose range was amended slightly (ranging between 0.005 and 5000 µg/plate) to allow for results from the first experiment and the change in test methodology. Additional

dose levels and an expanded dose range were selected in each experiment in order to achieve both four non-toxic dose levels and the toxic limit of the test material.

Findings Negative, the test material was considered to be non-mutagenic under the conditions of this test. In the first experiment (plate incorporation method) the test material caused a visible reduction in the growth of the bacterial background lawns of all of the Salmonella strains at 5000 µg/plate and also a substantial reduction in the frequency of revertant colonies from 500 µg/plate in both the presence and absence of S9-mix. No toxicity was noted to Escherichia coli strain WP2uvrA- at any dose level. In the second experiment (pre-incubation method) the test material induced a much stronger toxic response to the Salmonella strains with weakened bacterial background lawns initially noted from 50 µg/plate. A toxic response was also noted to Escherichia coli strain WP2uvrA- at 500 µg/plate in the absence of S9-mix. The sensitivity of the tester strains to the toxicity of the test material varied between strain type, exposures with or without S9-mix and experiment number. The test material was, therefore, tested up to the maximum recommended dose level of 5000 µg/plate or the toxic limit, depending on bacterial strain type, presence or absence of S9-mix and experiment number. A precipitate (oily in appearance) was noted at 5000 µg/plate, this observation did not prevent the scoring of revertant colonies. No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation or exposure method.

3.8.1.2 *In vitro* gene mutation study in bacteria, Ames test. Key study.

Reference Unnamed, 2002 (in vitro gene mutation study in bacteria, registration dossier for DBTE on ECHAs dissemination site)

Guideline Performed according to OECD guideline with minor deviations (No. 471 "Bacterial Reverse Mutation Test"). Conducted according to GLP.

Reliability Klimisch 1: reliable without restrictions. Key study.

Species/strain Salmonella typhimurium strains TA1535, TA1537, TA98, TA100 and Escherichia coli strain WP2uvrA$^-$

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

CAS 818-08-6

EC 212-449-1

Purity 98,09%

Batch number 1704

Study design Salmonella typhimurium strains TA1535, TA1537, TA98, TA100 and Escherichia coli strain WP2uvrA- were treated with the test material using both the Ames plate incorporation and pre-incubation methods at seven dose levels, in triplicate, both with and without the addition

of a rat liver homogenate metabolising system (10% liver S9 in standard co-factors). The dose range was determined in a preliminary toxicity assay and was 7 to 5000 µg/plate in the first experiment. The experiment was repeated on a separate day (pre-incubation method) using fresh cultures of the bacterial strains and fresh test material formulations. The test material dose range was amended slightly (ranging between 1.25 and 20 µg/plate) to allow for results from the first experiment and the change in test methodology.

Findings Negative, the test material was considered to be non-mutagenic under the conditions of this test. In both the absence and the presence of S9 -mix and in all strains, DBTO did not cause a more than two-fold or a dose-related increase in the mean number of revertant colonies appearing in the test plates compared to the background spontaneous reversion rate observed with the negative control. The mean number of his+ and trp+ revertant colonies of the negative controls were within the acceptable range, and the positive controls gave the expected increase in the mean number of revertant colonies. It is concluded that DBTO was not mutagenic under the conditions employed in this study.

3.8.1.3 *In vitro* gene mutation study in bacteria, Ames test.

Reference Unnamed, 2010 (in vitro gene mutation study in bacteria, registration dossier for DBTA on ECHAs dissemination site)

Guideline Performed according to OECD guideline (No. 471 "Bacterial Reverse Mutation Test"). Conducted according to GLP.

Reliability Klimisch 1: reliable without restrictions. Key study.

Species/strain *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* strain WP2*uvr*A$^-$

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

CAS 1067-33-0

EC 211-670-0

Purity >98%

Batch number 156 004 10/1

Study design Salmonella typhimurium strains TA1535, TA1537, TA98, TA100 and Escherichia coli strain WP2uvrA- were treated with the test material using both the Ames plate incorporation and pre-incubation methods at seven dose levels, in triplicate, both with and without the addition of a rat liver homogenate metabolising system (10% liver S9 in standard co-factors). The dose range was determined in a preliminary toxicity assay and was 0.5 to 500 µg/plate in the first experiment. The experiment was repeated on a separate day (pre-incubation method) using fresh cultures of the bacterial strains and fresh test material formulations. The test material dose range was amended slightly (ranging between 0.15 and 500 µg/plate) to allow for results from the first experiment and the change in test methodology.

Findings Negative, the test material was considered to be non-mutagenic under the conditions of this test. In the first experiment the test material caused a toxic response at and above 150 and 500 µg/plate to all of the tester strains, in both the presence and absence of S9-mix, for the Salmonella strains and Escherichia coli strain WP2uvrA-, respectively. A similar toxic response was noted in the second experiment to all of the tester strains, initially from 50 µg/plate (TA100 and TA1535), 150 µg/plate (TA98 and TA1537) and 500 µg/plate for Escherichia coli strain WP2uvrA-. The test material was, therefore, tested up to the toxic limit. No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation or exposure method.

3.8.1.4 *In vitro* mammalian cell gene mutation assay – lung fibroblast. Key study

Reference Unnamed, 1989 (*in vitro* mammalian cell gene mutation assay, registration dossier for DBTE on ECHAs dissemination site)

Guideline Internal Method No. 515.0 Main Dept. Exp. Toxicology. Equivalent or similar to OECD guideline No. 476 (*In vitro* Mammalian Cell Gene Mutation Test). Conducted according to GLP.

Reliability Klimisch 2: reliable with restrictions. Key study.

Species/strain Chinese hamster lung fibroblasts (V79)

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design Chinese hamster lung fibroblasts (V79) ± Aroclor 1254-induced rat liver S9 metabolic activation. Test concentrations, -S9: 0.000001, 0.000003, 0.000010, 0.000030, 0.000060 µl/ml; +S9: 0.00020, 0.00030, 0.00040, 0.00045, 0.00050 µl/ml.

Findings Negative - the test material did not show a mutagenic potential in the HGPRT/V79 mammalian cell gene mutation test neither - nor + S9 mix in two independently performed experiments. The test material was found to have cytotoxic effects –S9 at 0.00006 µl/ml and using +S9 a clear toxic effect could be observed at 0.0003 µl/ml in the first experiment and at 0.0005 µl/ml a second experiment. The positive control (ethylmethane sulfonate) was clearly mutagenic.

3.8.1.5 *In vitro* lymphocyte toxicity (gene mutation)

Reference Li AP, Dahl AR & Hill JO, 1982. In Vitro Cytotoxicity and Genotoxicity of Dibutyltin Dichloride and Dibutylgermanium Dichloride. Toxicology and applied pharmacology 64, 482-485 (supporting study in registration dossier for DBTE on ECHAs dissemination site)

Guideline No guideline followed. Said to be performed according to good basic scientific principles.

Reliability Klimisch 2: reliable with restrictions. Supporting study

Species/strain Lymphocytes from pooled thoracic lymph nodes from Fischer 344 rats.

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design Lymphocytes isolated from Fischer 344 rats were tested without metabolic activation. Negative and positive controls were not specified. Test concentrations: 9 to 75 µg/mL dissolved in DMSO.

Method of application: in medium.

Duration: 24 hours at 37 °C.

Stain (for cytogenetic assays): Trypan blue exclusion tests for viability.

Number of replications: Performed in triplicate.

Number of cells evaluated: 2 x 10^6 cells per tube.

Determination of cytotoxicity: mitotic index; cloning efficiency; relative total growth; *in vitro* Cunningham plaque assay for direct (IgM) antibody-forming cells (AFC) was performed.

Findings Positive without metabolic activation. The LC50 for lymphocytes as determined by dye-exclusion was approximately 50 µg/ml (0.16 mM). At the same concentration of DBTC, the number of antibody-forming cells (AFC) was reduced to approximately 10 % of the control.

3.8.1.6 *In vitro* mammalian cell gene mutation assay (gene mutation) Chinese hamster Ovary (CHO)

Reference Li AP, Dahl AR & Hill JO, 1982. *In Vitro* Cytotoxicity and Genotoxicity of Dibutyltin Dichloride and Dibutylgermanium Dichloride. Toxicology and applied pharmacology 64, 482-485 (supporting study in registration dossier for DBTE on ECHAs dissemination site)

Guideline No guideline followed. Said to be comparable to guideline study. Target gene HGPRT gene locus.

Reliability Klimisch 2: reliable with restrictions. Supporting study

Species/strain Chinese hamster ovary (CHO) cells clone K1-BH4.

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design Cytotoxicity and mutagenicity assay with CHO cells (conducted without metabolic activation): CHO cells were plated in growth medium one day before treatment. On the day of treatment, medium was changed to growth medium without serum. Graded concentrations of DBTC dissolved in DMSO were added. The final concentration of DBTC was 0.05 to 0.3 µg/ml. The final concentration of DMSO was kept at 1%. The cells were then incubated at 37°C for 3 hr after which they were trypsinized. Two hundred cells were plated in triplicate from each sample in 35-mm diameter tissue culture plates for the determination of cytotoxicity. These plates were incubated for 7 days after which the colonies that developed were fixed with 70% methanol, stained with l0% giemsa, and counted. Cytotoxicity data were expressed as relative survival which was the ratio of the cloning efficiency (C.E.) of the treated cells to that of the control: C.E. = No. colonies/No. cells plated; Relative survival = C.E. (treated) / C.E. (control).

To determine mutagenicity, the cells were subcultured for an 8-day expression period. The cells were then plated in selective medium consisting of 10 µM 6-thioguanine (Sigma) in hypoxanthine-free Ham's Fl2 medium (KC Biological Incorporation) supplemented with 5% dialyzed newborn calf serum. A number of 10ˆ6 cells per sample were selected for mutant frequency by plating 2 x 10ˆ5 cells in each of the five selection plates. A total of 200 cells were plated in triplicate in selective medium without 6-thioguanine in 35mm diameter tissue culture plates for the determination of cloning efficiency (C.E.). The plates were incubated for 9 days, after which the colonies that developed were fixed, stained, and counted. The mutant frequency (MF) was calculated as: MF = (No. of mutant colonies / No. of cells plated) x (1 / C.E.); MF was expressed as mutants per 10ˆ6 survivors.

Findings Positive without metabolic activation. The LC50 value of DBTC for CHO cells, as determined by cloning efficiency, was approximately 0.35 µg/ml (1.12 µM). DBTC induced mutations at the HGPRT gene locus in CHO cells.

3.8.1.7 *In vitro* mammalian chromosome aberration test. Key study.

Reference Unnamed, 1990 (*in vitro* mammalian chromosome aberration test, registration dossier for DBTA on ECHAs dissemination site)

Guideline Internal Method No. 449.1 Main Dept. Exp. Toxicology. Equivalent or similar to OECD guideline No. 473 (*In vitro* Mammalian Chromosome Aberration Test). Conducted according to GLP.

Reliability Klimisch 2: reliable with restrictions. Key study.

Species/strain Lymphocytes: whole blood culture from the peripheral blood

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Purity not reported

Study design Human peripheral blood lymphocytes isolated from a healthy adult male. Tests using ± liver S9 from Aroclor 1254 treated rats. Negative and positive controls used.

Assay -S9 mix; 1st assay: 0.001, 0.003, 0.006, 0.01, 0.03, 0.06, 0.1, 0.3, 0.6, 1.0, 3.0 µg/ml; 2nd assay: 0.006, 0.008, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2 and 0.4 µg/ml.

Assay +S9 mix: 1st assay: 0.050, 0.075, 0.10, 0.25, 0.50, 0.75, 1.0, 2.5, 5.0 and 7.5 µg/ml.

2nd assay: 0.05, 0.10, 0.25, 0.50, 0.75, 1.0, 2.0 and 3.0 µg/ml.

Findings Positive. The study indicates a clastogenic potential of the test material in the human lymphocyte test *in vitro* at clearly cytotoxic concentrations. From the four assays conducted without and with an extrinsic metabolizing system in two independent studies, one assay without and one with S9 mix gave statistically significant ($P < 0.05$) increases in the frequency of chromosomal aberrations at the highest concentrations evaluated, whereby in the remaining assays the results were borderline negative. In each assay of this investigation, the test material was tested up to cytotoxic concentrations as indicated by an obvious reduction of the mitotic index.

3.8.1.8 Breakage of naked λ-DNA (±H_2O_2)

Reference Hamasaki T, Sato T, Nagase H, and Kito H. (1995) Breakage of λ-DNA by inorganic tin and organotin compounds as environmental pollutants. Appl. Organomet. Chem. 9, 693-697.

Guideline/Reliability Non-guideline, non-GLP

Species/strain -

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Study design Purchased λ-DNA (0.5 µg, double stranded, from Gibco BRL) was incubated with DBTC (from Merck; purity not specified) ± 10 mM H2O2 in 10 mM sodium phosphate buffer (pH 7.4) at 37°C for 2 h. After purification by phenol/chloroform extraction and ethanol

precipitation, the DNA was dissolved and checked for breaks using agarose gel electrophoresis.

Findings Negative – DBTC did not induce dsDNA breaks in presence or absence of H2O2.

3.8.1.9 Bacterial reverse mutation (Ames)

Reference Hamasaki T, Sato T, Nagase H, and Kito H (1993). The mutagenicity of organotin compounds as environmental pollutants. Mutat. Res., 300, 265-271.

Guideline/Reliability non-guideline, non-GLP

Species/strain Salmonella typhimurium TA98 & TA100 strains. Metabolic activation (S9) was not used. DBTC from Merck Co.; analytical grade, purity not reported.

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Findings Positive without metabolic activation. DBTC (tested at 0.1-10 µg/tube) was found to be mutagenic in both strains.

3.8.1.10 Bacterial SOS chromotest and rec-assay

Reference Hamasaki T, Sato T, Nagase H, and Kito H (1992). The genotoxicity of organotin compounds in SOS chromotest and rec-assay. Mutat. Res., 280, 195-203.

Guideline/Reliability non-guideline, non-GLP

Species/strain *E. coli* PQ37, with *Bacillus subtilis* (H17 Rec^+ and M45 Rec^-).

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Study design Indication of genotoxicity in two bacterial assays: SOS chromotest (*sfi* A induction; a SOS system related gene) with *E. coli* PQ37, and, rec-assay with *Bacillus subtilis* (H17 Rec^+ and M45 Rec^-). Metabolic activation (S9) was not used. DBTC (analytical grade; purity not reported) from Merck.

Findings Positive without metabolic activation, however, these tests do not measure genetic damage directly. DBTC indicated genotoxicity in both assays and showed SOS activity at an extremely low dose (0.01 µg/tube).

3.8.1.11 Condensate formation with DNA

Reference Piro V, Di Simone F, Madonia G, Silvestri A, Giuliani AM, Ruisi G, and Barbieri R (1992). The interaction of organotins with native DNA. Appl. Organomet. Chem. 6, 537-542.

Guideline/Reliability Non-guideline, non-GLP

Species/strain Calf thymus DNA dissolved in aqueous buffer

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Study design DBTC (source and purity not stated) in ethanol solution was added to calf thymus DNA dissolved in aqueous buffer (1 mM Tris, 0.1 mM EDTA, pH 8) to give molar ratios *r* (*r* = [Sn]/DNA phosphate) of 0.48-1.00 (experiment 1) and 2.40 (experiment 2), respectively, followed by analysis of pellet formation.

Findings Positive – DBTC formed pellets (condensates/solid phases) with DNA in both experiments.

3.8.1.12 Effect on spindle structure in V79 Chinese hamster cells

Reference Jensen KG, Önfelt A, Wallin M, Lidums V, and Andersen O (1991a). Effects of organotin compounds on mitosis, spindle structure, toxicity and in vitro microtubule assembly. *Mutagenesis*, 6, 409-416.

Guideline/Reliability Non-guideline, non-GLP

Species/strain V79 Chinese hamster cells

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Study design V79 Chinese hamster cells were treated with 10^{-8} - 10^{-4} M DBTC for 30 min at 37°C. Several end-points were investigated: c-mitosis, spindle structure, survival, as well as bovine brain microtubule protein assembly. DBTC was from Aldrich (purity not specified).

Findings Positive - in general, loss of stainable spindle could be demonstrated at slightly higher concentrations than c-mitosis (DBTC also induced c-mitosis).

3.8.1.13 Aneuploidy in human peripheral lymphocytes

Reference Jensen KG, Andersen O, and Rønne M (1991b). Organotin compounds induce aneuploidy in human peripheral lymphocytes in vitro. Mutat. Res., 246, 109-112.

Guideline/Reliability

Species/strain Human lymphocytes from healthy female donor

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Study design Human peripheral blood was obtained from a healthy female donor and lymphocytes were cultured in medium for 72 h (37°C) during PHA-M stimulation. Then, lymphocytes were treated with 10^{-8} - 10^{-6} M DBTC (source not specified) for 48 h. After hypotonic treatment and fixation, approximately 100 metaphases selected at random were photographed and chromosomes counted.

Findings Negative – no significant induction of hyperdiploid cells (aneuploidy) was observed.

3.8.1.14 Effect on spindle-inhibition as chromosomal contractions in human lymphocytes

Reference Jensen KG, Andersen O, and Rønne M (1989). Spindle-inhibiting effects of organotin compounds. II. Induction of chromosomal supercontraction by di- and tri-alkyl and -aryl compounds. Appl. Organomet. Chem., 3, 225-229.

Guideline/Reliability Non-guideline, non-GLP

Species/strain Human lymphocytes from random donors.

Test material **Dibutyltin dichloride (DBTC)**

CAS 683-18-1

EC 211-670-0

Study design Lymphocyte cultures were prepared from human peripheral blood from donors selected at random. Incubation in media for 72 h (37.5 °C) followed by exposure to 10^{-9} – 10^{-3} mol dm^{-3} DBTC (source and purity not specified) for 24 h. After hypotonic treatment and fixation, the length of chromosome No. 1 was determined in 100 metaphases selected at random.

Findings Negative - no effect on average chromosome length was seen in the range 10^{-9} – 3 x 10^{-7} mol dm-3 DBTC versus control. No results were obtained at higher concentrations (≥1 x 10^{-6}) due to toxicity of treatment.

3.8.2 Animal data

3.8.2.1 Micronucleus assay (chromosome aberration). Key study.

Reference Unnamed, 1991 (*in vivo* mammalian somatic cell study: cytogenicity / erythrocyte micronucleus, registration dossier for DBTE 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 micronucleated polychromatic cells was observed in the bone marrow of mice treated with DBTC at 50 mg/kg bw and killed 48 and 72 hours later ($0.01<p<0.05$): this effect was seen more clearly in females than in males. No such effect was apparent for any group treated with DBTC and killed 24 hours later. Statistically significant increases over controls were also seen in positive control group animals given chlorambucil at 30 mg/kg bw ($p<0.01$).

Other toxicities: at a dosage of 2 mg/kg, no animal showed reactions to treatment. At 10 mg/kg, 3 males showed hunched posture and piloerection on the day of dosing only: no signs were observed in females. No marked incidences of weight loss were apparent in animals of either group. At 50 mg/kg, one male was killed in extremis approximately 2 hours after dosing (as a result of inactivity, unstable gait, slow respiration and piloerection). All but one of the remaining animals showed reactions to treatment including hunched posture, piloerection, inactivity, rales, closing of one or both eyes, and yellow staining of the coat. In addition, one female was found dead at termination, although it was seen to be alive 2 hours previously. At the 24 hour termination time, 5 animals had lost weight and one had failed to gain weight. At the 48 hour termination time all animals were seen to have lost weight, and all but two animals had lost weight at the 72 hour termination time. All weight losses recorded at 48 and 72 hours were marked. Of the ten mice given chlorambucil, the positive control agent, seven lost weight during the 24 hour period before termination.

3.8.2.2 Micronucleus assay (chromosome aberration)

Reference Unnamed, 1990 (in vivo mammalian somatic cell study: cytogenicity / erythrocyte micronucleus, registration dossier for DBTE 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).

3.8.2.3 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 2: reliable with restrictions.

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.

3.8.3 Human data

Not available

3.8.4 Other data

Not available

3.9 Carcinogenicity

Not evaluated in this CLH Report.

3.10 Reproductive toxicity

3.10.1 Animal data

Many of the studies described below are the same studies that were described in the CLH-dossier for DBTP (dibutylbis(pentane-2,4-dionato-O,O')tin) (EC no.:245-152-0/ CAS no.:22673-19-4) 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.

3.10.1.1 Reproductive/developmental toxicity screening study in the rat – indicated as key study in this registration

"Reference Waalkens-Berendsen DH (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. There was a significant increase in the incidence of ovarian cysts in the high-dose females.

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.

Table 1. 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)."

3.10.1.2 Developmental toxicity study in the rat – indicated as a key study in this registration

"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 were. 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."

3.10.1.3 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 DBRC 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.

Table 2. Reproductive and foetal findings in rats dosed on GD 7-9, 10-12 or 13-15

	Controls	**Days of treatment**		
		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).

Table 3. 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)*

Table 4. 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."

3.10.1.4 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 (DBTDL)

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).

Table 5. External malformations

Group	Control	DBTA	DBTC	DBTM	DBTO	DBTDL
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.

Table 6. Skeletal malformations

Group	Control	DBTA	DBTC	DBTM	DBTO	DBTDL
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.

Table 7. Skeletal variations

Group	Control	DBTA	DBTC	DBTM	DBTO	DBTDL
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."

3.10.1.5 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.

Table 8. 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.

Table 9. 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."

3.10.1.6 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.

Table 10. 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.

Table 11. 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.

Table 12. 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.

Table 13. 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.

Table 14. 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."

3.10.1.7 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.

Table 15. 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.

Table 16. 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."

3.10.1.8 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.

Table 17. 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."

3.10.1.9 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.

Table 18. 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)*

Figure 1. Incidence of dead or resorbed fetuses (A) and of fetuses with external (▧) and skeletal (▨) malformations (B) from the dams treated orally with DBTA (15 mg/kg) at different gestational stages.

Facsimile from Noda et al. 1992a

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.

3.10.1.10 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, however dams with living fetuses at 15 mg/kg bw/day did not have reduced weight gain (corrected body weight gain is not specified in the study). 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.

Table 1. Effects of di-n-butyltin diacetate (DBTA) on maternal thymus weights on day 20 of gestation

	Olive oil 2 ml/kg	DBTA (mg/kg) 1.7	5.0	10.0	15.0
Body wt (g)	314 ± 12.1	312 ± 18.6	309 ± 17.6	298 ± 14.5	254 ± 40.7**
Thymus wt (mg)	174 ± 15.7	124 ± 18.3	81 ± 32.4**	57 ± 13.4**	63 ± 16.0**

Pregnant rats were treated orally with DBTA during days 7–17 of gestation.
Values are the means ± SD of 12–16 animals per group.
**Significantly different from control, p<0.01.

Facsimile from Noda *et al.*, 1992b

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.

Table 19 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.

3.10.1.11 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 rate 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.

Table 20. 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
13Mated (#)	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.

Table 21 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 malformations (%)	3M	-	-	-	30.2*	62.6*
	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."

3.10.1.12 Reproductive 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.

Table 22. 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	18.2	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 all 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.

Table 23. 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.

Table 24. 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 30.4 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.

Table 25. 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."

3.10.1.13 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.

Table 26. 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)*

Table 27. 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)."

3.10.1.14 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.

Table 28. 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."

3.10.1.15 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

Table 29. 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."

3.10.2 Human data

No human data is available.

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

3.10.3.1 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."

3.10.3.2 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."

3.10.3.3 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.

Table 30. Summary of findings [24]

Dose level (mg/kg bw/day)	**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."

3.10.3.4 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."

3.11 Specific target organ toxicity – single exposure

Not evaluated in this CLH Report.

3.12 Specific target organ toxicity – repeated exposure

Many of the studies described below are the same studies that were described in the CLH-dossier for DBTP (dibutylbis(pentane-2,4-dionato-O,O')tin) (EC no.:245-152-0/ CAS no.:22673-19-4) 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.

3.12.1 Animal data

3.12.1.1 Sub-chronic dietary toxicity study in the rat – indicated as a key study in the registration

"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).

Table 31. 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*

Table 32. Haematology parameters at Week 6 and Week 13

Dietary level (ppm)	Hb (g/dL)	Hct (%)	RBC (10^6/mm^3)	Retics (% of HBC)	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."

3.12.1.2 Reproductive/developmental toxicity screening study in the rat – indicated as a key study in the registration

"Reference Waalkens-Berendsen DH (2003). TNO, The Netherlands. TNO Report V4906. [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."

3.12.1.3 Short-term repeated dose oral toxicity study

Reference Unnamed, 1961 (study 005, repeated dose toxicity study, registration dossier for DBTE on ECHAs dissemination site)

Guideline/Reliability Klimisch 2: reliable with restrictions. Study conducted in accordance with generally accepted scientific principles, possibly with incomplete reporting or methodological deficiencies, which do not affect the quality of the relevant results. Predates GLP, lacks information on test substance purity, no LD50 determined.

Species / strain Rat (Holtzman and white Simonsen)

Test material **Dibutyltin dilaurate (DBTDL)**

CAS 77-58-7

EC 201-039-8

Study design Tinostat (DBTDL 25%) was administered in ground Purina laboratory Chow. Rats were individually weighed, caged, and given feed and water ad libitum. Four groups of 5 males and 5 females. Age at study initiation: Either weanlings or 8 weeks old.

1st range-finding test: 4 weeks; 25, 50, 100, 200 ppm in diet: 5 males and 5 females per dose group.

2nd additional study: 3 months; 25, 50, 100, 200 and 400 ppm in diet: 6 males and 6 females per dose group.

3rd study: 13 weeks; 500, 1000, 1500, 2000 ppm in diet: 10 males and 10 females per dose group using white Simonsen rats.

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

Findings NOAEL is above 0.040% in feed (400 ppm or 26.6 mg/kg bw/day) after 13 weeks. Clinical signs and mortality: 1 rat, 5 rats and 7 rats died in the 1000, 1500 and 2000 ppm dose groups, respectively - significance not reported. Few tissue changes which were observed appeared physiological or infectious and no evidence of toxic changes was observed upon histopathological examination. At 1000 ppm, weight gain and feed intake were significantly affected. An enlarged bile duct was the most common gross necropsy observation attributable to dose. No effects on thymus reported.

Conclusion NOAEL > 26.6 mg/kg bw/day. The maximum no effect dose lies between 500 and 1000 ppm, and the maximum tolerated dose lies between 1000 and 1500 ppm.

3.12.1.4 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.

Table 33. 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."

3.12.1.5 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.

Table 34. 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)."

3.12.1.6 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), 10 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 ×108 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 ×108 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 51Cr-labeled murine YAC-1 lymphoma target cells. 51Cr 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."

3.12.1.7 Developmental immunotoxicity study in the rat

"Reference DeWitt JC, Copeland CB & Luebke RW (2006). Developmental Exposure to 1.0 or 2.5 mg/kg of Dibutyltin Dichloride Does Not Impair Immune Function in Sprague-Dawley Rats. Journal of Immunotoxicology 3(4):245-252.

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 pregnant female Sprague-Dawley (CD) rats were given drinking water containing DBTC (in 0.5% Alkamuls) at concentrations of 0, 10, or 25 mg/L from GD 6 until the weaning of pups, a total of 37 days. Offspring were subsequently untreated (maternal administration only), or were administered DBTC by gavage at dose levels of 0, 1.0 and 2.5 mg/kg bw/d on three days a week to PND 24 (maternal + direct administration).

Maternal bodyweights were recorded twice weekly. Litters were sexed, weighed, and culled to 8 (4/sex) on PND 2. From PND 3, the litters from half of the dams of each group were gavaged with DBTC at 0, 1.0, or 2.5 mg/kg bw three times a week for a total of ten doses. Delayed-type hypersensitivity (DTH), antibody synthesis, and natural killer (NK) cell activity were evaluated in immunologically mature offspring (6/sex/group).

Findings Mean intakes were reported to be approximately 1.0 and 2.5 mg/kg bw during gestation, 2.0 and 4.4 mg/kg bw during lactation.

Litter size and mean foetal weight at birth were unaffected by treatment. Litters exposed to 2.5 mg/kg bw DBTC had a slightly (10-20%) but significantly lower mean bodyweight compared to the other groups from PND 14 (males) or PND 17 (females). DTH responses and antibody synthesis were unaffected by treatment. NK cell activity in the offspring of dams administered DBTC in the drinking water at 10 mg/L (but not treated by gavage) was greater in males. In female offspring exposed by gavage, cytotoxicity increased at the 25:1 effector: target cell ratio.

Conclusion The authors suggest that developmental immunotoxicity is unlikely at the concentrations of DBTC in drinking water (from PVC pipes) as effects in the rat were seen only at concentrations several orders of magnitude higher."

3.12.1.8 Sub-acute toxicity study in the rat and mouse

"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.

Table 35. 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."

3.12.1.9 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."

3.12.1.10 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 μm3) was decreased at Days 1 and 2, the numbers of small (volume <130 μm3) 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."

3.12.1.11 Developmental toxicity study in the rat

"Reference Study summary (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."

3.12.1.12 Immunotoxicity study

Reference Subramoniam A, Khandelwal S, Dwivedi PD, Khanna S and Shanker R (1994). Dibutyltin dilaurate induced thymic atrophy and modulation of phosphoinositide pathway of cell signalling in thymocytes of rats. *Immunopharmacol. Immunotoxicol.*, 16, 645–677.

Guideline non-guideline

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

Species / strain Rats, male albino.

Test material **Dibutyltin dilaurate (DBTDL).**
CAS 77-58-7
EC 201-039-8
Purity not given.

Study design Animals were gavaged with DBTDL in ground nut oil at dose levels of 0, 2, 4, 8 or 16 mg/kg bw/day for 5 days per week for 2 weeks. Males, 5-6 in each dose group. Weight of lymphoid organs, body weight, weight of other organs (kidney, liver, adrenal), histology of lymphoid organs; in vivo and in vitro effects of DBTDL on phosphoinositide metabolism in thymocytes were assessed. In vivo and in vitro effects on Protein kinase C (PKC) activity and intracellular Ca^{2+} in thymocytes were assessed.

Findings A marked dose dependent reduction in thymus weight and its nucleated cell counts with histological alterations were observed in rats exposed to oral dibutyltin dilaurate for 2 weeks at 4, 8, or 16 mg/kg bw/day:

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

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

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

bw/day DBTDL group. However, in vitro incubation of DBTDL with thymocytes failed to evoke any change in phosphoinositide hydrolysis. A 130% and 600% enhancement of PKC activity in thymocytes was seen in the 4 mg/kg bw/day and 8 mg/kg bw/day DBTDL group, respectively. Addition of DBTDL to the cell free assay system of thymocytes resulted in a concentration dependent activation of the enzyme activity. A dose dependent increase in intracellular calcium was evident when DBTDL was added to thymocytes under in vitro conditions.

Conclusion LOAEL = 8 mg/kg bw/day based on significant organ weight reduction and reduced cell counts in thymus and lymph nodes combined.

3.12.1.13 Neurotoxicity study

Reference Jin M, Song P, Li N, Li X and Chen J (2012). A plastic stabilizer dibutyltin dilaurate induces subchronic neurotoxicity in rats. *Neural Regen. Res.*, 7, 2213-2220.

Guideline Non-guideline.

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

Species / strain Rat (Wistar)

Test material **Dibutyltin dilaurate (DBTDL).**
CAS 77-58-7
EC 201-039-8
Purity not reported.

Study design Animals were gavaged with DBTDL in corn oil at dose levels of 0, 5, 10 and 20 mg/kg bw/day for 5 days/week for 7 weeks (10 rats/dose group). The activities of superoxide dismutase, glutathione peroxidase and nitric oxide synthase was measured. In addition, the content of malondialdehyde, nitric oxide and the protein levels in brain homogenates was examined. Brain tissues were quantified for apoptosis, cell cycle and DNA damage in single cortical cells. EM was used for detecting ultrastructural changes.

Findings The only clinical signs were cases of mild euphoria in two rats in each of the high dose and medium dose groups who fought each other to death. DBTDL reduced superoxide dismutase and glutathione peroxidase activities, and increased the malondialdehyde content in rat brain tissue in all dose groups. DBTDL increased nitric oxide content (all dose groups) and nitric oxide synthase activity (high dose group) in rat brain tissue. DNA damage and apoptosis was seen in all dose groups with increasing frequency and intensity with increased DBTDL dose. The percentage of cells at the G0/G1 phase in the right parietal cortex gradually increased (P

< 0.05), and was significantly higher in the high-dose and medium-dose dibutyltin dilaurate groups compared with the normal control group (P < 0.05). All above effects appeared in a dose dependent manner. 20 mg/kg bw/day resulted in apparent neuropil cavitation in the brains, as well as other ultrastructural changes, with glial filaments dissolving within the axon.

Conclusion LOAEL = 20 mg/kg bw/day is based on ultrastructural changes in brain and glial filaments dissolving within the axon.

3.12.1.14 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 (co-implanted under the renal capsule). 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." LOAEL < 0.3 mg/kg bw.

3.12.2 Human data

No human data available.

3.13 Aspiration hazard

Not evaluated in this CLH Report.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

Not evaluated in this CLH Report.

4.2 Bioaccumulation

Not evaluated in this CLH Report.

4.3 Acute toxicity

Not evaluated in this CLH Report.

4.4 Chronic toxicity

Not evaluated in this CLH Report.