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:

[1] Dioctyltin dilaurate, [2] Stannane, dioctyl-, bis(coco acyloxy) derivs.

EC Number: **222-883-3 [1], 293-901-5 [2]**

CAS Number: **3648-18-8 [1], 91648-39-4 [2]**

Index Number: **Not applicable**

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Version number: 2 **Date: July 7, 2017**

CONTENTS

1 PHYSICAL HAZARDS

1.1 Explosives

Not evaluated in this CLH Report.

1.2 Flammable gases (including chemically unstable gases)

Not evaluated in this CLH Report.

1.3 Oxidising gases

Not evaluated in this CLH Report.

1.4 Gases under pressure

Not evaluated in this CLH Report.

1.5 Flammable liquid

Not evaluated in this CLH Report.

1.6 Flammable solids

Not evaluated in this CLH Report.

1.7 Self-reactive substances

Not evaluated in this CLH Report.

1.8 Pyrophoric liquids

Not evaluated in this CLH Report.

1.9 Pyrophoric solid

Not evaluated in this CLH Report.

1.10 Self-heating substances

Not evaluated in this CLH Report.

1.11 Substances which in contact with water emit flammable gases

Not evaluated in this CLH Report.

1.12 Oxidising liquids

Not evaluated in this CLH Report.

1.13 Oxidising solids

Not evaluated in this CLH Report.

1.14 Organic peroxides

Not evaluated in this CLH Report.

1.15 Corrosive to metals

Not evaluated in this CLH Report.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1 Toxicokinetics in rats

Reference	Penninks, A. H.; Hilgers, L.; Seinen, W. (1987). The absorption, tissue distribution and excretion of di-n-octyltin dichloride in rats. Toxicology **44**, 107-120.
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	**Dioctyltin dichloride (DOTC)** CAS 3542-36-7 EC 222-583-2 Purity > 98%
Study design	The absorption, tissue distribution and excretion of DOTC were investigated in Wistar rats after oral and intravenous administration of [^{14}C]DOTC. Following a single i.v. administration with 1,2 mg [^{14}C]DOTC/kg bw or after oral administration with 6,3 mg [^{14}C]DOTC/kg bw, rats were terminated at time points of 1-7 days and blood, organs and tissues were analysed for radioactivity. Following a single i.v. or oral dose of 1,2 and 2 mg [^{14}C]DOTC/kg bw, respectively, the excretion of radioactivity in feces and urine was also determined.
Findings	The highest amount of radioactivity was found in liver and kidney and to a lesser degree in adrenal, pituitary and thyroid glands. The lowest activity was recovered from blood and brain. No selective accumulation was observed in thymus, although thymus atrophy is the most sensitive parameter of DOT toxicity in rats. The tissue radioactivity was 3-4 times higher after i.v. administration with 1,2 mg [^{14}C]DOTC/kg bw than after oral administration with 6,3 mg [^{14}C]DOTC/kg bw, but the relative accumulation of radioactivity between organs/tissues was indenpendant of administration route. The radioactivity declined time-dependantly in all organs and tissues except in kidney where the activity remained constant during the 7 days experimental period. Absorption was calculated to be approximately 20% of the oral dose. <u>Tissue (radioactivity, dpm/mg tissue, oral administration) day 1:</u> Liver (679), kidneys (144), adrenals (103), pituitary gland (95), thyroid gland (86), spleen (75), peripheral lymph nodes (64), lungs (50), pancreas (41), heart (38), submaxillary glands (31), epididymal adipose (31), parotid gland (30), perirenal adipose (27), inguinal adipose (25), thymus (23), skeletal muscle (20), testis (14), blood (13), brain (6). <u>Tissue (radioactivity, dpm/mg tissue, i.v. administration) day 1:</u>

Liver (2598), kidneys (831), adrenals (510), spleen (300), thyroid gland (243), pituitary gland (232), submaxillary glands (221), peripheral lymph nodes (217), heart (213), lungs (182), pancreas (156), parotid gland (106), perirenal adipose (94), epidydimal adipose (93), skeletal muscle (86), inguinal adipose (83), thymus (66), testis (48), blood (34), brain (26).

In the excretion studies, a single i.v. or a single oral (by gavage) dose of 1,2 mg and 2 mg [^{14}C]DOTC/kg bw respectively were given to rats, and urine and feces were separately collected for 25 days. Following i.v. administration, most of the radioactivity was excreted in feces and characterized by a biphasic excretion pattern. The first 4 days showed an increase in radioactivity excretion, while from day 5 the excretion gradually declined with a halflife of 8.3 days. The urinary excretion of radioactivity appeared to be independent of the body burden since the daily excretion was nearly constant during 25 days. From the orally administered dose, more than 80% was excreted in the feces the first 2 days which is in accordance to the higher tissue radioactivity after i.v. administration. From day 3, the excretion in feces followed first order kinetics with a halflife of 8,9 days. Also for oral administration, the urinary excretion of radioactivity appeared to be independent of the body burden.

Conclusion The results of this study show that DOTC distributes to various tissues after intravenous or oral administration, with an absorption of approximately 20% of the oral dose. The highest concentrations are found in liver and kidney, while DOTC concentrations in thymus are much lower. It was concluded that the selective thymus effects of DOTC can not be correlated with a specific distribution to this organ.

2.1.2 Toxicokinetics in rats

Reference Study report, Anonymous (1987). Summary included in the publically disseminated REACH Registration Dossier for dichlorodioctylstannane (ECHA, 2016a).

Guideline No guideline followed

Reliability Klimisch 2: reliable with restrictions according to Registrant(s).

Species / strain Rat (Wistar), female

Test material Dichlorodioctylstannane (dioctyltin dichloride, DOTC)

CAS 3542-36-7

EC 222-583-2

Purity not specified

Study design The distribution of DOTC were investigated in Wistar rats after oral administration of radiolabelled DOTC (^{113}Sn). Following a single administration with 25 mg DOTC/kg bw in peanut oil, the proportions in blood and organs were measured up to 72h after exposure.

Findings The proportions of concentrations (in ng DOTC-equivalents/g tissue) at 1 h post administration were as follows:

blood (1) < kidneys (1.7) < brain (2.6) < thymus (5.3) < liver (21.1)

and at 24 h post administration:

brain (1) < blood (2.9) < thymus (4.5) < kidneys (32.5) < liver (131.3).

Most of the ^{113}Sn_DOTC was found in the liver, where at 1 h post administration 0.2 % and after 24 h 1.2 % of the initial dose was measured. The activity in liver and all other organs except for brain did not decrease within 72 h. The blood levels were comparatively low but still increasing up to the last measurement in blood at 24 h. An evaluation of the time dependencies as e.g. DOTC showed a slight affinity to the corpuscular compartments of blood: the solid/liquid partition factor, calculated for three points of measure, was about 2 and seemed to decrease slowly.

Half-life of the substance was calculated to be 67 h.

Conclusion The results of this study show that DOTC distributes to various tissues after oral administration. The highest concentrations (at 24 h post administration) are found in liver and kidney, while DOTC

concentrations in thymus are lower.

2.1.3 Simulated gastric hydrolysis

Reference Naßhan H (2015).
Dioctyltin dilaurate [DOTL] CAS number: 3648-18-8. 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 Dioctyltin dilaurate
CAS 3648-18-8
EC 222-883-3
Purity >90 %
(Test material as cited in the study report. The study owner confirmed that the test material corresponds to the industrially manufactured UVCB substance.)

Study design Simulated gastric hydrolysis studies were performed using dioctyltin dilaurate.
The extent of hydrolysis was assessed under low pH (1.2) conditions at 37°C at a concentration of 13.4 mM in aqueous HCl. The degree of hydrolysis was measured after 0.5h and 4h respectively, after extraction in hexane and subsequent ^{119}Sn NMR analysis in toluene-d^8 which allowed positive identification of the hydrolysis product(s). Any remaining tin-residues in the aqueous phase (decomposition products and/or water soluble substances) was analysed by atomic absorption spectrometry (AAS).

Findings Simulated gastric hydrolysis studies demonstrate that dioctyltin dilaurate rapidly hydrolyses at low pH. Three major products were observed after 0.5h and assigned to $ClOct_2SnOSnOct_2Cl$ (14%, ^{119}Sn-NMR: δ (ppm) -92, -145), DOTLC - a mono-chloride mono-carboxylate species (43%, ^{119}Sn-NMR: δ (ppm) -35), and a non-assigned tin-species (43%, ^{119}Sn-NMR: δ (ppm) -150, broad peak). Only minor changes in product composition were observed from 0.5h to 4h.
The non-assigned tin-species can be associated to some polymeric structure distinct from DOTO (dioctyltinoxide). The recativity of the non-assigned tin-species was further analysed by addition of excess DOTC to the organic solvent, the non-assigned tin-species instantly formed DOTLC.
Only trace amounts of tin-residues were observed in the remaining water fractions.

Hydrolysis of DOTL

Time	DOTLC (mol %)	$ClOct_2SnOSnOct_2Cl$ (mol %)	Non-assigned tin-species (mol %)
0.5 h	43 %	14 %	43 %
4 h	47 %	16 %	38 %

Conclusion Dioctyltin dilaurate is shown to be rapidly hydrolysed under conditions representative of the mammalian stomach to form three major products. The complex chemistry observed may be expected due the coordinating carboxylate ligands which can bind to the tin moiety in various ways (e.g. monodentate, bidentate, bridging). The generation of a common intermediate, identical to the major product observed upon hydrolysis of dioctyltin dichloride (see 2.1.4) supports the analogue

approach for read-across from dioctyltin dichloride to dioctyltin dilaurate.

2.1.4 Simulated gastric hydrolysis

Reference Naßhan H (2016).
Dioctyltin dichloride [DOTC] CAS number: 3542-36-7. 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 Dioctyltin dichloride
CAS 3542-36-7
EC 222-583-2
Purity >95 %

Study design Simulated gastric hydrolysis studies were performed using dioctyltin dichloride.
The extent of hydrolysis was assessed under low pH (1.2) conditions at 37°C at a concentration of 24.0 mM in aqueous HCl. The degree of hydrolysis was measured after 30 s, 1 h, and 4 h respectively, after extraction in hexane and subsequent ^{119}Sn NMR analysis in toluene-d^8 which allowed positive identification of the hydrolysis product.

Findings Simulated gastric hydrolysis studies demonstrate that dioctyltin dichloride rapidly forms the dimeric stannoxane $ClOct_2SnOSnOct_2Cl$ (^{119}Sn-NMR: δ (ppm) -92, -145) as the only observed hydrolysis product when exposed to conditions representative of the mammalian stomach. Small amounts (~10 mol%) of non-hydrolyzed DOTC remains after 4 hours. The recovery rate of organotins, defined by the isolated mass after extraction vs. the mass of the test sample was determined to 91-101%.

Conversion of DOTC to $ClOct_2SnOSnOct_2Cl$

Time	DOTC (mol %)	$ClOct_2SnOSnOct_2Cl$ (mol %)
30 s	46	54
1 h	23	77
4 h	10	90

Conclusion Dioctyltin dichloride is shown to be rapidly converted to $ClOct_2SnOSnOct_2Cl$ under conditions representative of the mammalian stomach. The generation of a common intermediate, identical to one of the hydrolysis products of dioctyltin dilaurate (see 2.1.3), supports the analogue approach for read-across from DOTC to dioctyltin dilaurate.

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

Not evaluated in this CLH Report.

3.9 Carcinogenicity

Not evaluated in this CLH Report.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Reproduction/developmental toxicity screening study in the rat

Reference Appel MJ and Waalkens-Berendsen DH. (2004). Dichlorodioctylstannane [CASRN # 3542-36-7]: Sub-chronic (13 week) oral toxicity study in rats, including a reproduction/developmental screening study. Testing laboratory: TNO Nutrition and Food Research. Report no.: V3964. Owner company: ORTEP. Report date: 2004-04-01.

Guideline OECD 421 (Reproduction/Developmental Toxicity Screeining Test)

Reliability Klimisch 1: reliable without restriction (guideline-compliant study with no or minor deviations not affecting the quality of the results, GLP-compliant study with certificate), according to Registrant(s).

Species / strain Rat (Wistar)

Test material Dichlorodioctylstannane (dioctyltin dichloride, DOTC)

CAS 3542-36-7

EC 222-583-2

Purity 92.1 %

Study design The repeated dose toxicity of the test material was studied using continuous administration via the diet for 13 consecutive weeks according to OECD 408 (study summarised in section 3.12.1.1). In satellite groups of female rats a reproduction/developmental screening test was performed according to OECD 421. The main 13-week study used four groups of 10 rats/sex and the satellite reproduction/developmental screening study used four groups of 10 female rats. For both studies the control group was kept on untreated diet and the three test groups received diets containing 10, 100 and 300 mg/kg of the test material.

In the satellite study administration of female rats started two weeks prior to the mating period and continued through mating, gestation, and up to PN 4 or shortly thereafter. After a premating period of 10 weeks, male rats from the main study were mated with female rats of the satellite groups, which were administered the same dose of test diet.

The study summary continued below refers to the satellite study, i.e. OECD 421 (Reproduction/Developmental Toxicity Screeining Test).

Animals were observed daily for mortality and, if necessary, clinical signs. Body weights were measured pre-test (day -5), on gestation day 0, 7, 14 and 21 and on postnatal day 1 and 4. All animals were weighed on the day of necropsy in order to determine their correct organ to body weight ratios. This was in addition to a weekly measurement being taken throughout the study.

The numbers of females placed with males, males mated with females, successful copulations, males that became sire and pregnant females were noted, as was pre-coital time. At the end of the gestation period, females were examined twice daily for signs of parturition. Any difficulties occurring during parturition were recorded.

At necropsy, ovaries, uterus (after counting of the implantation sites), thymus and gross lesions from all females were weighed, and samples of the organs were preserved. Microscopic examination of the thymus was performed. The ovaries and uterus of the females of the control and 300 mg/kg were microscopically examined. Furthermore, the reproductive organs of the males of the 10 and 100 mg/kg groups that failed to sire (did not mate or female was not pregnant) and the reproductive organs of females of the 10 and 100 mg/kg groups that were non-mated or non-pregnant were microscopically examined.

The total litter size and numbers of each sex as well as the number of stillbirths, live and dead pups and grossly malformed pups were evaluated on days 1 and 4 of lactation. The pups were weighed individually and litter weight was calculated for days 1 and 4 of lactation. Mean pup weight was calculated as litter weight/number pups. The number of runts (defined as pup weight less than 2 standard deviations from the litter mean) were noted and reported. A necropsy was performed on stillborn pups and pups dying during the study and macroscopic abnormalities were recorded. Pups were examined externally for gross abnormalities.

Findings **Parental animals *(Note: only maternal data is presented here. Please refer to section 3.12.1.1 for data on males)***

CLINICAL SIGNS AND MORTALITY (PARENTAL ANIMALS)

No clinical signs were observed during the premating period. During the gestation period piloerection

was observed in animal A115 (GD 21 -24) of the control group and animal D161 (GD 23-24) of the 300 mg/kg group. In addition, blepharospasm was observed in animal D161 (GD 23-24). During lactation piloerection was observed in animal C157 (PND 2-4) of the 100 mg/kg group and D163 (PND 2-4) and D167 (PN 1) of the 300 mg/kg group. Blepharospasm was observed in animal D167 of the 300 mg/kg group. Animals C149 (PND 4-5) and C157 (PND 4) of the 100 mg/kg group and animal D163 (PND 4) of the 300 mg/kg group were considered to be thin. In addition animals C149 and C159 showed a pale appearance. Some animals were sparsely haired during gestation and/or lactation; this finding is normal for this strain.

BODY WEIGHT AND FOOD CONSUMPTION (PARENTAL ANIMALS)

During the premating period no significant differences in mean body weight were observed. Mean body weight change was statistically significantly reduced in the 100 and 300 mg/kg groups during the first week of the premating period. During the gestation period, mean body weight was statistically significantly reduced from GD 7-21 in the 300 mg/kg group. Body weight change was statistically significantly reduced during the entire gestation period. During the lactation period, the mean body weight was statistically significantly reduced in the 300 mg/kg group.

During the premating period, mean food consumption (expressed as g/animal/day and as g/kg body weight/day) of the female animals of the 100 and 300 mg/kg groups was statistically significantly decreased. During the gestation period, food consumption (g/animal/day) of the females of the 100 mg/kg group was statistically significantly decreased from GD 7-14. Mean food consumption of the 300 mg/kg group (expressed as g/animal/day) was statistically significantly decreased during the entire gestation period and as g/kg body weight/day from GD 0-14. During the lactation period food consumption of the female animals of the 300 mg/kg group was statistically significantly decreased.

TEST SUBSTANCE INTAKE (PARENTAL ANIMALS)

The test substance intake of the female animals of the 10, 100, and 300 mg DOTC/kg diet was respectively:

Pre-mating period days 0-7: 0.6, 5.8 and 13.5 mg/kg bw/day

Pre-mating period days 7-14: 0.7, 5.9 and 16.4 mg/kg bw/day

Gestation period days 0-7: 0.7, 6.2 and 17.0 mg/kg bw/day

Gestation period days 7-14: 0.7, 6.2 and 17.0 mg/kg bw/day

Gestation period days 14-21: 0.5, 4.2 and 11.0 mg/kg bw/day

Lactation period days 1-4: 0.7, 5.0 and 8.4 mg/kg bw/day

The overall intake of the test substance for the 10, 100, and 300 mg DOTC/kg diet, respectively was approximately 0.7, 6.5 and 19.3 mg/kg bw/day in males.

FERTILITY, PARTURITION AND SEXUAL FUNCTION (PARENTAL ANIMALS)

Effects on sperm parameters and oestrous cycling were not investigated.

The pre-coital time was not statistically significantly different between groups (2.33, 2.40, 1.50 and 2.80 days in control, 10, 100 and 300 mg DOTC/kg diet respectively).

No effects on gestational length.

Female fecundity index, female fertility index and male fertility index were comparable for the control group and treated groups and ranged between 70-80%. The female mating index was 90% in control and 100% in the treated groups.

The gestation index was 86, 100, 71 and 50% in the control, 10, 100 and 300 mg/kg groups, respectively. The number of females with liveborn pups was 6, 8, 5 and 4 for the control, 10, 100 and 300 mg/kg groups, respectively. The number of females with stillborn pups amounted to 1, 1, 4 and 3 for the control, 10, 100 and 300 mg/kg groups, respectively. The number of females with all stillborn pups was 0, 0, 2 and 1 in the control, 10, 100 and 300 mg/kg groups, respectively.

ORGAN WEIGHTS (PARENTAL ANIMALS)

Absolute and relative uterus and ovary weight were similar in all groups.

The absolute and relative thymus weights were decreased in all treated groups (but only stat. sign. at the

100 and 300 mg DOTC/kg diet) in a dose-dependent manner (-23/-24% (not stat. sign.), -38/-33%, $p<0.05$ and -69/-62%, $p<0.001$ in the low intermediate and high dose groups, respectively).

GROSS PATHOLOGY (PARENTAL ANIMALS)

No effects

HISTOPATHOLOGY (PARENTAL ANIMALS)

Microscopic examination revealed moderate to very severe lymphoid depletion in the thymus, which was considered related to treatment. Lymphoid depletion was characterised by a decrease in the size of the thymic lobules which can be ascribed to extensive loss of cortical en medullary small lymphocytes. Consequently, the distinction between the cortical and medullary areas was blurred. Lymphoid depletion was observed in 5/10 animals of the 10 mg/kg group and in all animals of the 100 and 300 mg/kg groups. Severity score was severe to very severe in all groups. One control animal (A115) also had very severe lymphoid depletion in the thymus. However, this was most probably associated with the fact that this animal was physiologically disturbed, as was demonstrated by 12 resorptions in the uterus and an abnormal kidney (gross changes: flabby and yellow patches). Some 10 mg/kg animals showed thymic involution as a result of pregnancy/lactation. This picture was similar to the thymic pregnancy/lactation involution in control animals and was characterised by a decreased size of thymic lobules exhibiting normal architecture. This phenomenon is a common observation in pregnant or lactating animals. However, the lymphoid depletion in the 10 mg/kg animals was similar to the thymic change in the 100 and 300 mg/kg animals. Therefore, lymphoid depletion in the 10, 100 and 300 mg/kg animals was considered related to treatment with the test substance. The other histopathological changes observed are common findings in rats of this strain and age or occurred in a single animal only.

Table 1: Maternal effects

Dose level	**0 mg/kg diet (Control)**	**10 mg/kg diet**	**100 mg/kg diet**	**300 mg /kg diet**
Test substance intake	0 mg/kg bw/day	0.5-0.7 mg/kg bw/day	4.2-5.9 mg/kg bw/day	8.4-17 mg/kg bw/day
Number of pregnant animals	7	8	7	8
Mortalitites	0	0	0	0
Clinical observation during pre-mating	0	0	0	0
Clinical observation during gestation	1/10 piloerection (GD 21-24)	0	0	1/10 piloerection and blepharospasm (GD 23-24)
Clinical observation during lactation	0	0	1/10 piloerection (PND 2-4), thin (PND 4) 1/10 thin and pale appearance (PND 4-5) 1/10 pale appearance (PND 4-5)	1/10 piloerection (PND 2-4) and thin (PND 4) 1/10 piloerection and blepharospasm (PND 1)
Food consumption [g/rat/day] during pre-mating (week 0 - 1)[d]	13.05 ± 0.230	12.79 ± 0.317	11.05 ± 0.134** (-15%)	8.81 ± 0.173** (-33%)
Food consumption [g/rat/day] during pre-mating (week	13.05 ± 0.255	13.20 ± 0.146	11.78 ± 0.177** (-10%)	10.73 ± 0.019** (-18%)

1 - 2) [d]				
Food consumption [g/rat/day] during gestation (d 0-7)[t]	14.37 ± 0.265 (N=6)	15.37 ± 0.431 (N=8)	13.04 ± 0.215 (N=7)	11.09 ± 0.482[#] (-23%) (N=7)
Food consumption [g/rat/day] during gestation (d 7-14)[t]	16.00 ± 0.356 (N=7)	15.82 ± 0.383 (N=8)	14.17 ± 0.418* (-11%) (N=7)	12.05 ± 0.453** (-25%) (N=8)
Food consumption [g/rat/day] during gestation (d 14-21)[t]	10.72 ± 0.802 (N=7)	12.02 ± 0.649 (N=8)	10.27 ± 0.439 (N=7)	8.19 ± 0.535* (-24%) (N=8)
Food consumption [g/rat/day] during lactation (d 1-4) [t]	15.25 ± 1.555 (N=6)	14.63 ± 1.791 (N=8)	9.71 ± 3.059 (-34%) (N=5)	4.92 ± 2.686* (-68%) (N=4)
Body weight [g] pre-mating day 0	197.88 ± 2.036	195.65 ± 2.240	197.91 ± 1.989	198.07 ± 1.953
Body weight [g] pre-mating day 7	202.68 ± 2.414	199.05 ± 2.660	198.19 ± 2.612	194.04 ± 1.794 (-4.4%)
Body weight [g] pre-mating day 14	206.29 ± 2.222	202.99 ± 2.670	201.30 ± 2.455	198.28 ± 2.772 (-3.9%)
Body weight gain [g] pre-mating day 0-7[d]	4.80 ± 1.077	3.40 ± 0.953	0.28 ± 1.399*	-4.03 ± 0.699**
Body weight gain [g] pre-mating day 7-14[d]	3.61 ± 1.019	3.94 ± 1.088	3.11 ± 0.787	4.24 ± 1.417
Body weight [g] GD 0	207.49 ± 3.083	205.95 ± 4.331	201.53 ± 2.823	195.01 ± 2.955
Body weight [g] GD 7	223.31 ±3.352	224.94 ± 4.136	217.77 ± 2.963	207.96 ± 4.490* (-6.9%)
Body weight [g] GD 14	248.07 ± 4.309	245.84 ± 4.234	236.74 ± 3.308	219.35 ± 4.555[#] (-11.6%)
Body weight [g] GD 21[d]	273.30 ± 8.546	275.58 ± 5.872	254.90 ± 4.975	229.39 ± 4.463[#] (-16%)
Body weight gain [g] GD 0-7[d]	15.83 ± 1.113	18.99 ± 1.065	16.24 ± 0.655	10.41 ± 2.149* (-34%)
Body weight gain [g] GD 7-14[d]	24.76 ± 1.532	20.90 ± 0.910	18.97 ± 1.464	11.39 ± 2.418[#] (-54%)
Body weight gain [g] GD 14-21[d]	25.23 ± 6.137	29.74 ± 2.453	18.16 ± 3.196	10.04 ± 3.239[#] (-60%)
Body weight [g] at lactation day 1[d]	200.13 ± 4.043	198.19 ± 5.017	189.18 ± 4.166	164.98 ± 6.147[#] (-18%)
Body weight [g] at lactation day 4	211.65 ± 2.814	212.93 ± 5.479	194.26 ± 6.470	168.85 ±12.768** (-20%)
Body weight gain [g] lactation day 1-4[d]	11.52 ± 3.198	14.74 ± 2.278	5.08 ± 6.072	3.88 ± 11.062
Relative thymus weight [%][d]	0.612 ± 0.0612 (N=6)	0.466 ± 0.0441 (-24%) (N=8)	0.408 ± 0.0511* (-33%) (N=5)	0.231 ± 0.0298[#] (-62%) (N=4)
Lymphoid depletion in thymus (revealed at histopathlogical examination)[f]	1/10 (1 very severe)	5/10 (4 severe, 1 very severe)	10/10*** (5 severe, 5 very severe)	10/10*** (3 severe, 7 very severe)

# p<0.001
* p<0.05
** p<0.01

*** p<0.001
(d) Anova & Dunnett test
(f) Fishers exact test
(t) t-test with Bonferroni correction

Tabel 2: Summary of reproductive data

Dose level	0 mg/kg diet (Control)	10 mg/kg diet	100 mg/kg diet	300 mg/kg diet
Test substance intake	0 mg/kg bw/day	0.5-0.7 mg/kg bw/day	4.2-5.9 mg/kg bw/day	8.4-17 mg/kg bw/day
Number of animals in the study (females + males)	10 + 10	10 + 10	10 + 10	10 +10
Number of mated females	9	10	10	10
Number of pregnant females -Thereof no. with only implantation sites at necropsy -Thereof no. with only stillborn pups	7 1 0	8 0 0	7 0 2	8 3 1
Number of females with liveborn pups	6	8	5	4
Female mating index[1]	90	100	100	100
Female fecundity index[2]	78	80	70	80
Female fertility index[3]	70	80	70	80
Male fertility index[4]	70	80	70	80
Gestation index[5]	86	100	71	50

1) female mating index = number of mated females/number of females placed w males
2) female fecundity index = number of pregnant females/number of females with confirmed mating
3) female fertility index = number of pregnant females/number of females placed with males
4) male fertility index = number of males that become sire/number of males placed with females
5) gestation index = number of females with live born/number of females with evidence of pregnancy

UTERINE OBSERVATIONS

The mean number of implantation sites were reduced in the 100 and 300 mg DOTC/kg diet dose groups (11.3 and 10.3 respectively, not stat. sign. compared to 12.6 in control).

Animal 115 of the control group and animals D161, D171 and D173 of the 300 mg/kg group showed only implantation sites at necropsy.

Mean percentage of incidences of post-implantation loss was 22.3, 21.0, 49.2 and 70.0% for the control, 10, 100 and 300 mg/kg groups, respectively.

VIABILITY (OFFSPRING)

The mean number of pups (live + dead) delivered per litter amounted to 11.7, 11.0, 10.3 and 8.6 or the control, 10, 100 and 300 mg/kg groups, respectively. There was an increased number of stillborn pups in the 100 and 300 mg DOTC/kg diet dose groups (34 and 17, respectively, $p<0.001$, compared to 1 in control) and the live birth index was reduced in the 100 and 300 mg DOTC/kg diet dose groups (99, 95, 53 and 60% in the control, 10, 100 and 300 mg/kg groups, respectively). Pup mortality on PND 1 was 1.4, 4.5, 47, and 40% in the control, 10, 100 and 300 mg/kg groups, respectively. All pups of the following animals died between PND 1-4: B137 of the 10 mg/kg group, C145 and C159 of the 100 mg/kg group and D165, D175 and D177 of the 300 mg/kg group. Pup mortality on PND 4 was 5.8, 8.3, 26 and 88 %. Viability index (PND 1 -4) was 94, 92, 74 and 12% in the control, 10, 100 and 300 mg/kg groups, respectively. The number of live pups per litter on PND 1 amounted to 11.5, 10.5, 7.6, 6.5 for the control, 10, 100 and 300 mg/kg groups, respectively and on PND 4 the number of live pups per litter amounted to 10.8, 11.0, 9.3 and 3.0 for the control, 10, 100 and 300 mg/kg groups, respectively.

No difference was observed in the sex ratio between the groups.

CLINICAL SIGNS (OFFSPRING)

On PND 1 and 4, the number of runts was statistically significantly increased in the 100 and 300 mg/kg groups (1, 7, 10 and 6 respectively in control, 10, 100 and 300 mg DOTC/kg diet). In addition the number of cold pups was increased in the 300 mg/kg group on PND 1.

BODY WEIGHT (OFFSPRING)

Mean pup weight and pup weight change were similar in the 10 and 100 mg/kg groups when compared to the control group. Pup weight of the 300 mg/kg group at PND 1 (3 litters, 3.9 g not stat. sign. compared to 4.76 g in control) and PND 4 (1 litter) was reduced.

SEXUAL MATURATION (OFFSPRING)

Not examined

ORGAN WEIGHTS (OFFSPRING)

Not examined

GROSS PATHOLOGY (OFFSPRING)

Macroscopic observations in stillborn pups and pups that died between PND 1 and 4 revealed no treatment related abnormalities in the pups.

HISTOPATHOLOGY (OFFSPRING)

Not examined

Table 3: Summary of pup data

Dose level	**Control**	**10 mg/kg diet**	**100 mg/kg diet**	**300 mg/kg diet**
Number of pregnant females	7	8	7	8
Mean number of implantations	12.6	13.4	11.3	10.3
Post implantation loss (%) Mean value Median value [N = number of females]	 22.33 ± 13.159 7 N=7	 20.98 ± 7.114 11 N=8	 49.23 ± 17.453 50 N=7	 69.99 ± 14.713 95[£] N=8
Pups delivered (total) (N)	70	88	72	43

Pups delivered (live + dead; mean) [N= number of litters]	11.67 ± 0.803 N=6	11.00 ± 0.707 N=8	10.29 ± 0522 N=7	8.60 ± 1.208 N=5
Mean viable litter size PND 1 [N= number of litters]	11.50 ± 0.719 N=6	10.50 ± 0.945 N=8	7.60 ± 1.631 N=5	6.50 ± 2.217 N=4
Total no. of liveborn pups[f] (Live birth index)	69 99	84 95	38[#] 53	26[#] 60
Total no. of stillborn pups[f] (% stillborn) [N = number of litters]	1 1.4 N=1	4 4.5 N=1	34[#] 47 N=4	17[#] 40 N=3
Total number of dead pups PND 0 to PND 4[f]	4	7	10[**]	23[#]
Total number of pups dying perinatally	5	11	44	40
Mean viability index PND 1-4	94	92	74	12
Mean viable litter size PND 4 [N= number of litters]	10.83 ± 0.601 N=6	11.00 ± 0.787 N=7	9.33 ± 0.667 N=3	3.00 ± 0.000 N=1
Pup weight (g) PND 1 (all viable pups) [N= number of litters]	4.76 ± 0.229 (N=6)	4.74 ± 0.229 (N=8)	4.19 ± 0.346 (-12%) (N=5)	3.90 ± 0.088 (-18%) (N=4)
Pup weight gain (g) PND 1 to PND 4	2.17 ± 0.257	1.86 ± 0.382	1.41 ± 0.584	-0.57 ± 0.000
Pup weight (g) PND 4 (all viable pups) [N= number of litters]	6.93 ± 0.447 N=6	6.69 ± 0.743 N=7	6.10 ± 0.719 N=3	3.10 ± 0.000 N=1
Total number of runts [ǂ] [N= number of litters]	1 N=1	7 N=3	10 N=3	6 N=1

(ǂ) runts = pups with weight below 2 standard deviations as compared to mean pup weight of control group at PND 0

(f) Fishers exact test
* $p<0.05$
** $p<0.01$
# $p<0.001$
(£) Statistical significant trend, $p<0.01$

Conclusion LOAEL for fertility and developmental effects was 100 mg DOTC/kg diet (equivalent to 6.5 mg/ kg body weight/day in males and 4.2-5.9 mg/kg body weight for females) according to the Registrant(s).

LOAEL for maternal toxicity was 10 mg DOTC/ kg diet (equivalent to 0.5-0.7 mg/kg body weight/day) based on the observed histological changes in the thymus (lymphoid depletion) according to the Registrant(s).

3.10.1.2 Prenatal developmental toxicity study in rats

Reference Study report (2014) Prenatal developmental toxicity study of DOTC administered orally in diet to Sprague Dawley rats.

Guideline	OECD 414 (Prenatal Developmental Toxicity Study)
Reliability	Klimisch 1: reliable without restriction (guideline-compliant study, GLP-compliant study with certificate), according to the Registrant(s).
Species / strain	Rat (Sprague Dawley)
Test material	Dichlorodioctylstannane (dioctyltin dichloride, DOTC) CAS 3542-36-7 EC 222-583-2 Purity 97.7 %
Study design	Groups of 25 mated females were administered the test material in the diet at concentrations of 0, 10, 100 or 300 mg/kg from gestation day (GD) 5 to 19. Animals were observed once daily for clinical signs of toxicity and twice daily for mortality/morbidity. Individual animal body weight was taken on GD 0, 3, 5, 8, 11, 14, 17, 19 and 20 (day of caesarean section). All surviving animals were subjected to detailed necropsy on the day of caesarean section (GD 20). The ovaries, uterus, thymus, spleen and liver were collected and preserved. and gross lesions from all females were weighed, and samples of the organs were preserved. The weight of the gravid uterus including cervix was recorded for each pregnant female. The foetuses were taken out and femals were sublected to macroscopic examination including numbers of corpora lutea, implantations, live and dead foetuses, and early and late resorptions. All foetuses were examined for sex, weight, external appearance (including oral cavity), and external anomalies. Approximately one half of live foetuses from each litter were examined for skeletal alterations, and the other half for visceral alterations.
Findings	PREGNANCY DATA A total number of 22, 21, 20 and 20 mated females were confirmed with pregnancy at a pregnancy rate of 88%, 84%, 80% and 80% at the time of caesarean section at 0, 10, 100 and 300 ppm respectively. MATERNAL DATA <u>General Tolerability</u> No deaths and abortions were noted during the experimental period. <u>Body weight, Body Weight Change and Corrected body weight</u> There was a statistically significant decrease in maternal body weight for the period of gestation days (GD) 17-20 and maternal body weight change for the periods GD 5-8, GD 11-14, GD 14-17 and GD 17-19 for dams in the 300 ppm group was noted. There were no statistically significant decreases in maternal body weight and maternal body weight change for dams in the 10 or 100 ppm dose groups on any gestation day when compared to control dams. No statistically significant decrease in body weight change GD 5-20 for dams in the 10 ppm and 100 ppm groups compared to controls was noted. A statistically significant decrease in body weight change GD 5-20 for dams in the 100 ppm (11.9%) and in 300 ppm (30.8%) groups as compared with controls was noted. The corrected body weight change and the percent change were similar to controls in the 10 and 100 ppm groups. In the 300 ppm group the corrected body weight change and the percent change (75.6%) were both statistically significantly decreased compared to controls. Gravid uterine weight was similar to controls at all doses. <u>Feed Consumption and Test Article Consumption</u> There were no treatment related differences in average feed consumption at any of the tested dose. Test Article consumption for each dose was calculated as 0.8, 7.2 and 22.4 mg/kg/day for the low, mid and high dose groups, respectively. <u>Gross Pathology</u> Macroscopic observations of reduced size of thymus in 7 of 25 females at 100 ppm and in all females (25 of 25) at 300 ppm were observed. These observations were judged to be treatment-related. No gross pathological observations were noted in 10 ppm animals.

MATERNAL DATA (UTERINE OBSERVATIONS)

No treatment related differences in mean gravid uterus weight, number of corpora lutea per dam, number of implantation sites per dam, incidence of early and late resorptions, number of dead and live fetuses, pre and post-implantation losses and male/female sex ratio were noted at all the doses.

The occurrence of early resorptions at 100 ppm, late resorptions at 10 and 300 ppm, pre-implantation loss at 300 ppm and post-implantation loss at 10, 100 and 300 ppm were judged as incidental and not treatment related.

Number of abortions

No effects observed

Pre-Implantation Loss

No treatment-related pre-implantation loss was noted across all doses when compared to the vehicle control. The statistically significant difference in this parameter for the 300 ppm group was attributed to two dams [Ra6262 and Ra6270] which had pre-implantation losses of 44.4% and 60.4%, respectively. In the 100 ppm group two dams [Ra6248 and Ra6249] were noted with a pre-implantation loss of 45.5% and 62.5% respectively. These occurrences were judged as incidental and not treatment related.

Post-Implantation Loss

No statistically significant difference in the percentage of post-implantation loss was observed for any dose group when compared to vehicle controls.

The observed post-implantation losses were 6.9%, 4.9% and 6.9% in the 10, 100, and 300 ppm groups, respectively versus 0.8% in the vehicle control.

Total litter losses by resorption

No effects observed

Effects on pregnancy duration

No effects observed

Early Resorptions

The incidence of early resorptions was statistically significantly increased at the mid dose compared to the vehicle control. This was judged to be an incidental occurrence. One dam (Animal No. Ra6232) in this group had 4 early resorptions compared to a maximum of 2 early resorptions in any dam. In addition the incidence of early resorptions did not demonstrate a dose-response.

Late Resorptions

No statistically significant differences in the number of late resorptions per dam were observed across groups.

Dead fetuses

No dead fetuses were observed in the 100 or 300 ppm groups or in the controls. In the 10 ppm group, 2 dead fetuses were observed in a single litter.

Changes in pregnancy duration

No effects observed

Changes in number of pregnant

No effects observed

Other effects

No effects observed

FETAL DATA

Fetal Sex Ratio, Average Fetal Weight and Average Crown Rump Length

No treatment related effects on the fetal sex ratio, average fetal weight and average crown-rump length were noted at any of the dose.

External Examination

No gross external abnormalities were noted within any group after external examination of fetuses.

Visceral Examination

Malformations: Lateral ventricular dilation of the 3rd ventricle of the brain. No dose-response.

Variations: Abnormal liver lobation and renal pelvis dilation. No dose-response.

The noted observations, abnormal liver lobation and dilation of the renal pelvis, are common findings for rat fetuses and were judged as incidental occurrences.

Skeletal Examination

Malformations:

Statistically significant and treatment related increases were observed in the percentage of malformations of missing metacarpal No. 5 (11.4 and 34.6 % at 100 and 300 mg/kg, respectively, as compared to 0.8 % in the control), proximal phalanx No. 3 bilateral (14.3 and 28.0 % at 100 and 300 mg/kg, respectively, as compared to 0.8 % in the control) and proximal phalanx No. 4 (13.3 and 27.1 % at 100 and 300 mg/kg, respectively, as compared to 0.8 % in the control).

Observations of skeletal malformations were:

- 1 incidence in 132 foetuses (1 of 22 litters affected) at 0 mg DOTC/kg diet
- 11 incidences in 115 foetuses (8 of 21 litters affected) at 10 mg DOTC/kg diet
- 22 incidences in 105 foetuses (11 of 20 litters affected) at 100 mg DOTC/kg diet
- 47 incidences in 107 foetuses (19 of 20 litters affected) at 300 mg DOTC/kg diet

Split thoracic vertebra centrum no. 12 was noted as a single occurrence in a single litter at 10 ppm.

Missing caudal vertebral arch no. 2 on both sides was noted in 2 litters (2 fetuses) at 10 ppm and 2 litters (3 fetuses) at 300 ppm. Both these observations were judged to be incidental occurrence.

Variations:

Statistically significant and treatment related increases were observed in the percentage variations of poor ossification of sternum No. 5 (6.5 % at 300 mg/kg as compared to 0 % in the control) and sternum No. 6 (14.0 % at 300 mg/kg as compared to 0 % in the control). A dose dependent and treatment related increase in poor ossification of metacarpal No. 5 was observed (1.0 and 3.7 % at 100 and 300 mg/kg, respectively, as compared to 0 % in the control).

Observations of skeletal variations were:

- 6 incidences in 132 foetuses (5 of 22 litters affected) at 0 DOTC/kg diet
- 11 incidences in 115 foetuses (7 of 21 litters affected) at 10 DOTC/kg diet
- 10 incidences in 105 foetuses (4 of 20 litters affected) at 100 DOTC/kg diet
- 26 incidences in 107 foetuses (12 of 20 litters affected) at 300 DOTC/kg diet

Table 4: Main maternal and developmental effects

Nominal dose in the diet (Actual test substance intake)	**0 ppm** **(0 mg/kg/d)**	**10 ppm** **(0.8 mg/kg/d)**	**100 ppm** **(7.2 mg/kg/d)**	**300 ppm** **(22.4 mg/kg/d)**
Pregnancy data				
Initial animals per group	25	25	25	25
Mortalities	0	0	0	0
Confirmed pregnancy at necropsy	22	21	20	20
Pregnancy rate (%)	88	84	80	80

Maternal data				
Initial body weight (g) at GD 0	195.62 ± 12.45	197.88 ± 11.99	197.79 ± 9.62	198.01 ± 9.52
Body weight (g) at GD 5	211.44 ± 11.70	212.10 ± 11.95	213.88 ± 12.32	213.59 ± 9.70
Final body weight (g) at GD 20	305.34 ±18.98	300.90 ±18.42	296.62 ±18.08	278.54 ± 25.85*** (-8.8 %)
Body weight gain (g) from GD 5-20	93.9 ± 11.96	88.80 ± 12.92	82.74 ± 12.43*	64.95 ± 20.95 *** (-31.2 %)
Corrected body weight (g)	235.38	238.67	233.36	219.44
Corrected body weight change (g) GD 5-20	23.94 ± 15.48	26.57 ± 10.57	19.47 ± 11.98	5.85 ± 18.22***
Uterine observation				
Gravid uterus weight (g)	69.96 ± 15.06	62.23 ± 14.46	63.26 ± 16.20	59.10 ± 19.67
Corpora lutea (no.)	11.7 ± 2.1	11.2 ± 1.9	11.4 ± 1.8	11.6 ± 2.5
Total implantation per female (no.)	11.5 ± 2.1	11.1 ± 1.9	10.7 ± 2.6	10.7 ± 3.3
Live foetuses (no.)	11.4 ± 2.2	10.1 ± 2.8	10.1 ± 2.7	10.1 ± 3.8
Dead foetuses (no.)	0.0 ± 0.0	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0
Early resorptions (no.)	0.0 ± 0.2	0.4 ± 0.6	0.6 ± 1.1*	0.4 ± 0.6
Late resorptions (no.)	0.0 ± 0.2	0.1 ± 0.4	0.0 ± 0.0	0.2 ± 0.5
Pre-implantation loss (%) 4/22	1.5 ± 3.3 2/21	0.8 ± 2.4	7.0 ± 16.8 5/20 animals with pre-implantation loss (8.3, 8.3, 45.5, 62.5, 15.4%)	10.4 ± 17.1* 8/20 animals with pre-implantation loss (6.3, 44.4, 60.0, 20.0, 21.4, 27.3, 20.0, 8.3%)
Post-implantation loss (%) 1/22	0.8 ± 3.6 10/21	6.9 ± 10.2	4.9 ± 10.0 6/20 animals with post-implantation loss (40, 15.4, 9.1, 7.1, 9.1, 18.2)	6.9 ± 13.8 7/20 animals with post-implantation loss (40, 8.3, 50.0, 8.3, 9.1, 12.5, 9.19
Litter data	**0 ppm** **(0 mg/kg/d)**	**10 ppm** **(0.8 mg/kg/d)**	**100 ppm** **(7.2 mg/kg/d)**	**300 ppm** **(22.4 mg/kg/d)**

Litter size (no.)	11.4 ± 2.2	10.2 ± 2.8	10.1 ± 2.7	10.1 ± 3.8
Total (male + female) live foetuses (no.)	11.4 ± 2.2	10.1 ± 2.8	10.1 ± 2.7	10.1 ± 3.8
Live male foetuses (no.)	5.1 ± 1.6	4.7 ± 1.7	4.7 ± 1.8	5.4 ± 2.6
Live female foetuses (no.)	6.3 ± 2.0	5.8 ± 1.8	5.5 ± 2.3	4.8 ± 2.1*
Average fetal weight (g)	4.0 ± 0.3	4.0 ± 0.5	4.2 ± 0.2	4.0 ± 0.2
Fetal data				
Total no. of live fetuses	251	220	202	202
Total no. of foetuses examined for visceral examination	119	105	97	95
Total no. of foetuses examined for skeletal examination	132	115	105	107
Total foetuses available for gross external evaluation (no.)	11.4 ± 2.2	10.5 ± 2.2	10.1 ± 2.7	10.1 ± 3.8
Foetuses available for visceral examination (no.)	5.4 ± 1.2	5.0 ± 1.2	4.8 ± 1.4	4.8 ± 1.8
Foetuses available for skeletal examination (no.)	6.0 ± 1.0	5.5 ± 1.1	5.3 ± 1.3	5.4 ± 2.0
External Examination				
Malformations, no. of foetuses %	 0 0.0	 0 0.0	 0 0.0	 0 0.0
Variations no. of foetuses %	 0 0.0	 0 0.0	 0 0.0	 0 0.0
Visceral Examination				

Malformations no. of foetuses %	 0 0.0	 3 2.9	 1 1.0	 1 1.1
Variations no. of foetuses %	 0 0.0	 3 2.9	 1 1.0	 1 1.1
Skeletal examinations				
No. foetuses examined No. litters examined	132 22	115 21	105 20	107 20
Malformations (total) Foetal basis, no. (%) Litter basis, no. (%)	1 (0.8) 1 (4.5)	11 (9.6) 8 (38.0)	22** (21.0) 11 (55.0)	47*** (43.9) 19 (95.0)
Metacarpal no. 5 bilateral Foetal basis, no. (%) Litter basis, no. (%)	1 (0.8) 1 (4.5)	3 (2.6) 3 (14.3)	12 (11.4*) 6 (30.0)	37 (34.6*) 18 (90.0)
Proximal phalanx no. 3 bilateral Foetal basis, no. (%) Litter basis, no. (%)	1 (0.8) 1 (4.5)	9 (7.8) 7 (35.0)	15 (14.3 *) 10 (50.0)	29 (28.0*) 16 (80.0)
Proximal phalanx no.4 bilateral Foetal basis, no. (%) Litter basis, no. (%)	1 (0.8) 1 (4.5)	8 (7.0) 6 (28.6)	15 (13.3*) 9 (45.0)	29 (27.1*) 16 (80.0)
Variations (total) Foetal basis, no. (%) Litter basis, no. (%)	6 (4.5) 5 (22.7)	11 (9.6) 7 (33.3)	10 (9.5) 4 (20.0)	26* (24.3) 12 (60.0)
Split Thoracic vetrtebra centrum no. 12	0	1(1)	0	0
Mising caudal vertebral arch no 2	0	2(2)	0	3(2)
Poor or incomplete ossification of				

sternum no. 5 Foetal basis, no (%) Litter basis, no. (%)	0 0	1 (0.9) 1 (4.8)	0 0	7 (6.5*) 4 (20.0)
Poor or incomplete ossification of sternum no. 6 Foetal basis, no (%) Litter basis, no. (%)	0 0	0 0	2 (1.9) 1 (5.0)	16 (14.0*) 8 (40.0)
Poor or incomplete ossification of metacarpal no. 5 Foetal basis, no (%) Litter basis, no. (%)	0 0	0 0	1 (1.0) 1 (5.0)	4 (3.7) 3 (15.0)

* p<0.05

** p<0.01

*** p<0.001

Conclusion The main developmental effect was a dose dependent increase, starting at low dose ($p < 0.5$ at intermediate, and $p < 0.01$ at high dose compared to control) in the incidence of total skeletal malformations, where missing bones (metacarpal no 5 and proximal phalang no. 3, bilateral) of the forepaws was the predominant malformation.

LOAEL for both maternal toxicity and developmental toxicity was set to 100 mg DOTC/kg diet (7.2 mg/kg bw/day) by the the Registrant(s).

3.10.1.3 Extended one-generation reproductive toxicity study in rats

Reference Tonk *et al*. (2011) Developmental immunotoxicity of di-*n*-octyltin dichloride (DOTC) in an extended one-generation reproductive toxicity study. Toxicol. Lett. 204: 156-163.

Guideline Similar to OECD TG 443 – Extended one-generation reproductive toxicity study (EOGRTS) without the Cohorts 2 and 3* and without the extension of Cohort 1B to mate the F1 animals to produce the F2 generation.

(*) note that 8 F1 males per group were used for immune assessment, however, the design to assess the potential impact of chemical exposure on the developing immune system deviates substantially from that described for Cohort 3 in OECD TG 443.

Reliability Klimisch 1: reliable without restriction, according to Registrant(s), however, it is noted that GLP-compliance and purity of test substance are unknown.

Species / strain Rat (Wistar)

Test material di-*n*-octyltin dichloride (DOTC)

CAS 3542-36-7

EC 222-583-2

Purity not reported.

Study design Rats were randomly assigned to the treatment groups and received the test diets with 0, 3, 10 or 30 mg/kg DOTC during the premating period, mating, gestation and lactation and subsequently F1 were

exposed from weaning onwards. The dose levels were selected based on in house dose range finding studies (data not shown). At the end of the two-week pre-mating period, rats were mated at a ratio of 2 females:1 male. The day of sperm detection in the vaginal smear was considered day 0 of gestation and the mated F0 females were housed individually.

The morning after birth was considered postnatal day (PND) 1. Litters were not standardised and pups were weaned on PND 21. Evaluation of sexual maturation was performed using 1 pup/sex/litter.

Throughout the study, all animals were checked daily for clinical signs and abnormal behavior. The body weights of all males and females were recorded weekly during the premating period, and the body weights of the males weekly thereafter. Mated females were weighed on gestational days (GD) 0, 6, 14, and 21 and during lactation on days 1, 4, 8, 10, 13, 17, and 21. Pup body weights were recorded at PNDs 1, 4, 8, 10, 13, 17, and 21 and weekly from weaning.

During the premating period, food consumption was measured weekly for each cage by weighing the feeders. Individual food consumption of all mated females was recorded from GD 0–6, 6–14, and 14–21 and for all females with live pups from postpartum days 1–4, 4–8, 8–10, and 10–13. F1 food consumption was recorded weekly from weaning.

Subsets of F_1 male rats (n = 8/dose) originating from different litters, were evaluated at PNDs 21, 42, and 70 for changes in immune function. Terminal body weights were recorded, and the following organs were weighed: liver, thymus, spleen, kidneys, adrenals, heart, and testes.

The following immunotoxicological assessment was peformed:

- Subpopulations of spleenocytes and thymocytes were analysed using flow cytometric analysis of cell surface markers
- NK cell activity using in vitro ^{51}Cr-release assay
- NO/TNF-α production by adherent splenocytes stimulated with LPS
- Lymphoprolifertive responses assessed in splenocytes using ConA and LPS and in thymocytes using ConA.
- Cytokine production of splenocytes after stimulation with ConA
- T-cell antibody response to Keyhole Limpet hemocyanin (KLH) was assessed following subcutaneous immunizations with KLH on PNDs 21 and 35.
- Delayed type hypersensitivity response (DTH) against KLH was evaluated at PND 49.
- KLH-specific lymphoproliferative response and cytokine production of splenocyte cultures of KLH-immunized rats.

Findings

Parental generation

Parental animals showed no adverse behavior or clinical signs. No statistically significant effects of DOTC on the body weights of the F_0 rats were observed, except for the F_0 females during lactation. On lactation days 4, 8, 10, 17, and 21 the F_0 females in the mid and high dose groups (on day 8 only in the high dose) showed a slight (approximately 5%), but statistically significantly increased body weight when compared to controls. There were no effects of DOTC on the food consumption of the F_0 females during gestation or lactation. The substance intake for the treated F_0 females was 0.17–0.21, 0.56–0.71, 1.7–2.1 mg/kg bw/day during gestation and 0.27–0.55, 1.0–1.9, 2.9–5.2 mg/kg bw/day during lactation for the 3, 10, and 30 mg DOTC/kg diet dose groups, respectively.

Organ weights and Histopathology

No information available on F0 animals.

Fertility, parturition and sexual function

Mating and fertility indices were similar among all groups.

Precoital time similar among all groups (1.8 days in all groups except in the 10 mg DOTC/ kg diet group where the precoital time was 2.5 days, not statistically significantly different from control).

The gestation index was 100% at all dose levels

Mean duration of pregnancy was comparable between all test substance-treated groups and the controls (21.3-21.5 days).

Development

↑ post-implantation loss (12.2, 13.7 and 17.9 % in 3, 10 and 30 mg/kg dose groups, repectively, not stat. sign. compared to 8.8% in control)

Mean number of pups (live + dead) delivered per dam was not different among groups and there was no difference in live birth index or number of stillborn pups among groups.

↓ mean number of live pups per litter at PND 4 in high dose group (8.78, $p<0.05$ compared to 10.48).

Male pup mean body weights were statistically significantly increased on PNDs 8, 10 and 13 in the high dose group compared to control.

After weaning, no effects on body weight, food consumption and sexual maturation were observed (according to study authors, no data available).

F_1 animals showed no differences in the animals' appearance, general condition or behavior among treatment and control groups.

At necropsy no treatment-related macroscopic changes were observed and no treatment-related organ weight changes in kidneys, adrenals, heart and testes of F1 animals were reported.

↓ absolute (-22%, $p<0.05$) and relative (-20%, $p<0.05$) thymus weight and thymus cellularity (-36%, $p <0.05$) in high dose group on PND 42 compared to control.

Relative liver weigth was statistically significantly increased in low and mid dose groups at PND 70.

Table 5: Delivery and offspring data

DOTC (mg/kg diet)	0	3	10	30
Test substance intake (F0 females; mg/kg bw/day)	0	0.17–0.21 (gestation) 0.27–0.55 (lactation)	0.56–0.71 (gestation) 1.0–1.9 (lactation)	1.7–2.1 (gestation) 2.9–5.2 (lactation)
Parameters of reproductive performance				
Females mated	24	24	24	20
Mating index (%)[a]	100	100	100	100
Fertility[b]/fecundity[c] index (%)	88	100	96	90
Gestation index (%)[d]	100	100	100	100
Precoital time (days)[e]	1.8 ± 1.1	1.8 ± 0.9	2.5 ± 1.2	1.8 ± 0.9
Females pregnant	21	24	23	18
Gestation time (days)[e]	21.3 ± 0.46	21.4 ± 0.52	21.5 ± 0.52	21.5 ± 0.53
Number of females with implanation loss	8(12)[f]	11(19)	13(18)	7(13)
Implantation loss per animal (%)[e,g]	8.8 ± 8.6	12.2 ± 17.3	13.7 ± 13.6	17.9 ± 25.1
Females with liveborn pups	21	24	23	18
Females with still born pups	3	0	0	0
Offspring data				
Pup delivered	10.76 ± 2.09	10.5 ± 3.09	10.39 ± 2.08	9.78 ± 2.24

(mean)[e]				
Live birth index (%)[h]	99	100	100	100
Sex ratio day 1 (%)i	50	54	53	47
Live pups/litter				
Day 1[e]	10.62 ± 2.01	10.50 ± 3.09	10.39 ± 2.08	9.72 ± 2.24
Day 4[e]	10.48 ± 2.02	10.13 ± 3.13	10.00 ± 1.95	8.78* ± 12.60
Day 21[e]	10.48 ± 2.02	9.88 ± 3.01	9.78 ± 2.13	8.78 ± 2.60
Viability index day 1–4 (%)[j]	98.7	96.6	96.6	91.0
Vialbility index day 4–21 (%)[k]	99.6	97.8	97.6	100

a (No. of females mated/no. of females placed with males) ×100.
b (No. of females pregnant/no. of females placed with males) × 100.
c (No. of females pregnant/no. of females mated) × 100.
d (No. of females with live pups/no. of females pregnant) × 100.
e Values are means ± SD.
f No. of females evaluated.
g (No. of implantation sites–number of live pups/no. of implantation sites) × 100).
h (No. pup born alive/no. of pups born) × 100.
i (No. live male pups/no. of live pups) × 100.
j (No. pups surviving 4 days/no. of liveborn pups at day 1) × 100.
k (No. pups surviving 21 days/no. of liveborn pups at day 4) ×100.
* $p < 0.05$.

Immunotoxicological assessment of F1

Lymphocyte subpopulations – spleen

On PND 42 the absolute and relative number of CD3+, CD3+CD4+ and CD3+CD8+ cells showed statistically significant decrease in the high dose group together with a decreased T:B cell ratio. The decrease in CD3+CD4+ splenocytes was no longer statistically significant at PND 70 (see table 6)

Lymphocyte subpopulations – thymus

On PND 42 the absolute number of CD4-CD8+, CD4+CD8+, immature (CD3low) and mature (CD3high) thymocytes were statistically significantly decreased in the high dose group compared to the control group. Same trend at PND 70, however, not statistically significant (see table 6).

NK cell activity

No effect.

NO/TNF-α production by adherent splenocytes

No effect.

Lymphoproliferative responses

No effect.

Cytokine production

See table 6.

T-dependent antibody response

No statistically significant effects.

Delayed-type hypersensitivity (DTH)

The DTH response to KeyHole Limpet Hemocyanin (KLH) was evaluated at PND 49. There was an increased DTH response in all dose groups compared to the control, reaching statistical significance in the low and high dose groups (37% and 52% increase in thickening of the ear compared to control).

KLH-specific lymphoproliferative response and cytokine production

No effect.

Table 6: Immunotoxicological assessment

	PND 21				PND 42				PND 70			
DOTC (mg/kg diet)	0	3	10	30	0	3	10	30	0	3	10	30
Hematology												
MPV								↓				
RDW								↑			↑	
Cytometric analysis of splenocytes in F1 males												
Spleen weight												
cellularity spleen												↓
T Cell (CD3+)								↓				↓
CD4+CD8-								↓				↓
CD4-CD8+								↓				↓
NK cell								↓				↓
B Cell								↓				↓
Cytometric analysis of thymocytes in F1 males												
Thymus weight								↓				↓
cellularity thymus								↓				↓
CD3 high								↓				↓
CD3 low								↓				↓
CD4-CD8+								↓			↓	↓
CD4+CD8+								↓			↓	↓
CD4-CD8-								↑			↓	↑
CD4+CD8-								↓			↓	↓
ConA stimulated cytokine production by splenocytes in F1 males												
IL-4						↑	↑	↑			↑	↑
IL-13		↓					↓	↑			↑	↑
IL-10								↑				
IFN-γ				↑								

Conclusion Main finding was a statistically significant decrease in the mean number of live pups per litter at PND 4 in high dose group, and decreased absolute and relative thymus weight and thymus cellularity

in F1 high dose animals on PND 42 compared to control. There was also an increased DTH response in all dose groups compared to the control, reaching statistical significance in the low and high dose groups.

3.11 Specific target organ toxicity – single exposure

Not evaluated in this CLH Report.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Sub-chronic oral toxicity study in the rat

Reference Appel MJ and Waalkens-Berendsen DH. (2004). Dichlorodioctylstannane [CASRN # 3542-36-7]: Sub-chronic (13 week) oral toxicity study in rats, including a reproduction/developmental screening study. Testing laboratory: TNO Nutrition and Food Research. Report no.: V3964. Owner company: ORTEP. Report date: 2004-04-01.

Guideline OECD 408 (Repeated Dose 90-day Oral Toxicity Study in Rodents)

Reliability Klimisch 1: reliable without restriction (guideline-compliant study with no or minor deviations not affecting the quality of the results, GLP-compliant study with certificate), according to Registrant(s).

Species / strain Rat (Wistar)

Test material Dichlorodioctylstannane (dioctyltin dichloride, DOTC)

CAS 3542-36-7

EC 222-583-2

Purity 92.1 %

Study design The repeated dose toxicity of the test material was studied using continuous administration via the diet for 13 consecutive weeks according to OECD 408. In satellite groups of female rats a reproduction/developmental screening test was performed according to OECD 421 (study summarised in section 3.10.1.1). The main 13-week study used four groups of 10 rats/sex and the satellite reproduction/developmental screening study used four groups of 10 female rats. For both studies the control group was kept on untreated diet and the three test groups received diets containing 10, 100 and 300 mg/kg of the test material.

In the satellite study administration of female rats started two weeks prior to the mating period and continued through mating, gestation, and up to PN 4 or shortly thereafter. After a premating period of 10 weeks, male rats from the main study were mated with female rats of the satellite groups, which were administered the same dose of test diet.

The study summary continued below refers to the main study, i.e. OECD 408 (Repeated Dose 90-day Oral Toxicity Study in Rodents).

Clinical observations (daily), growth (body weight recorded once weekly), food consumption (measured weekly), food conversion efficiency, neurobehavioural testing, ophthalmoscopy (prior to the start of treatment in all animals and towards the end of the treatment period in all surviving animals of the control group and the 300 mg/kg group), haematology (at necropsy at the end of treatment), clinical chemistry (at necropsy at the end of treatment), renal concentration test (shortly before the end of treatment), urinalysis (shortly before the end of treatment), organ weights and gross examination at necropsy (in the week after the end of study), and microscopic examination of various organs and tissues (samples preserved during gross examination) were used as criteria for detecting effects of the treatment.

At final necropsy in the 13-week study, the following organs were weighed (paired organs together) as soon as possible after dissection to avoid drying:

- adrenals
- ovaries
- brain
- spleen
- epididymides
- testes
- heart
- thymus
- kidneys
- thyroid (with parathyroids)
- liver

Histopathological examination was performed on all tissues and organs listed above - except those marked with an asterisk - of all animals of the control group (group A) and of the 300 mg/kg group (group D). In addition, lungs, liver, kidneys and gross lesions were examined microscopically in all rats of the intermediate dosegroups. Since treatment-related changes were found in the thymus of males and females of the 300 mg/kg group, histopathology on this organ was extended to males and females of the intermediate-dose groups.

adrenals
parathyroid
aorta
* parotid salivary glands
*axillary lymph nodes pituitary
brain (brain stem, cerebrum, cerebellum)
prostate
caecum
rectum
colon
* seminal vesicles with coagulating glands
epididymides
* skeletal muscle (thigh)
* exorbital lachrymal glands
skin (flank)
eyes
small intestine (duodenum, ileum, jejunum)
*femur with joint spinal cord (at three levels)
GALT (gut associated lymphoid tissue, including Peyer's patches)
spleen
sternum with bone marrow
* Harderian gland stomach (glandular and non-glandular)
heart
sublingual salivary glands
kidneys
submaxillary salivary glands
liver
testes
lungs
thymus
mammary gland (females)

thyroid
* mandibular (cervical) lymph nodes
* tongue
mesenteric lymph nodes
trachea/bronchi
* nasal cavity
urinary bladder
nerve-peripheral (sciatic)
uterus (with cervix)
oesophagus
* vagina
ovaries
* Zymbals gland
pancreas
all gross lesions.

(*) The tissues marked with an asterisk were preserved but not processed for histopathological examination, since histopathological examination was not considered necessary on the basis of the results of gross observations.

Findings

CLINICAL SIGNS AND MORTALITY

No treatment-related clinical signs or mortalities were observed.

BODY WEIGHT AND WEIGHT GAIN

Body weights were statistically significantly decreased by about 9% in males and females of the 300 mg/kg group and females throughout the study. The decrease in body weight accompanied by reduced food intake in males and females of the 300 mg/kg group was in the study report considered to be due to reduced palatability of the test diet.

FOOD CONSUMPTION AND COMPOUND INTAKE

Food consumption was slightly decreased in males and females of the 300 mg/kg group (by about 8 and 11%, respectively). On a number of days the difference reached the level of statistical significance. Food consumption was generally similar among the control, 10 and 100 mg/kg groups in males and females. An occasional statistically significant difference was seen among these groups.

The overall intake of the test substance for the 10, 100, and 300 mg DOTC/kg diet, respectively was approximately 0.7, 6.5 and 19.3 mg/kg bw/day in males and 0.7, 6.8 and 19.8 mg/kg bw/day in females.

FOOD EFFICIENCY

Food conversion efficiency was similar among the groups in males and females throughout the study. An occasional statistically significant difference was seen.

OPHTHALMOSCOPIC EXAMINATION

No treatment-related ocular changes were observed.

HAEMATOLOGY

In the 300 mg/kg group decreases in haemoglobin, packed cell volume, mean corpuscular haemoglobin, total white blood cells, absolute numbers of lymphocytes and an increase in

prothrombin time were observed.

The following statistically significant changes in haematology parameters were observed:

- Hb was decreased in females of the 300 mg/kg group;
- PCV was decreased in females of the 300 mg/kg group;
- MCV was decreased in males of the 100 mg/kg group;
- MCH was decreased in the 100 (males) and 300 mg/kg groups (males and females);
- Reticulocytes were decreased in males of the 100 mg/kg group;
- Prothrombin time was increased in females of the 300 mg/kg group;
- Total WBC was decreased in males of the 300 mg/kg group. Atlthough not statistically significant, a similar decrease was also seen in emales of the 100 and 300 mg/kg groups;
- The absolute number of lymphocytes was decreased in males of the 300 mg/kg group;
- The absolute numbers of monocytes were decreased in females of all treated groups.

CLINICAL CHEMISTRY

In the 100 and 300 mg/kg groups increases in alkaline phosphatase and bilirubin were observed.

The following statistically significant changes in clinical chemistry parameters were observed:

- ALP was increased in males and females of the 100 and 300 mg/kg groups;
- TP was decreased in females of the 300 mg/kg group;
- The A/G ratio was increased in the 10 (females) and 300 mg/kg groups (males and females);
- Total bilirubin was increased in females of the 100 and 300 mg/kg groups;
- Direct bilirubin was increased in females of the 300 mg/kg group;
- Cholesterol was decreased in females of the 300 mg/kg group;
- Bile acids were increased in males of the 300 mg/kg group. Although not statistically significant, a similar increase was also seen in females of the 300 mg/kg group;
- Phospholipids was increased in males of the 10 mg/kg group and decreased in females of the 300 mg/kg group;
- Calcium was decreased in females of the 300 mg/kg group;
- Sodium was decreased in males of the 100 and 300 mg/kg groups.

URINALYSIS

Urinary volume and density were similar among the groups. Urinary crystals were statistically significantly increased in females of the 300 mg/kg group. Further semi-quantitative and microscopic urinary observations were similar among the groups.

NEUROBEHAVIOUR

No neurotoxic effects of treatment were observed from neurobehavioural measures and motor activity assessment in any of the groups at any time point during the 13-week treatment period. Some abnormalities were observed in individual animals that were not considered to be related to treatment. On one occasion during arena testing, a tilted head was observed in one female. This single observation was not considered to be related to treatment.

Tiptoe walking was observed in some females of different groups in various weeks of the study. Tiptoe walking was not considered to be related to treatment, for it was observed in females only, but in all groups, including the control group. Further, it was not consistently observed in the concerned animals from first occurrence towards the end of the test period and the severity of this gait abnormality did not increase over time.

The results of the neurobehavioural observations and motor activity assessment did not indicate any neurotoxic potential of the test substance.

ORGAN WEIGHTS

Statistically significant changes in organ weights were observed in the 300 mg/kg group for relative kidney weight (8.4 % increase in females), relative liver weight (6.2 and 16.4 % increase in males

and females, respectively), relative testis weight (9.7 % increase), absolute spleen weight (11.6 % decrease in females) and absolute adrenal weight (16.4 % decrease in females). A statistically significant decrease of 12.2 % was observed for relative adrenal weight in females of the 100 mg/kg group.

A marked and dose-related decrease in absolute and relative thymus weight was observed. In males the absolute and relative thymus weights in all treated groups were decreased in a dose-response manner, statistically significant ($p<0.01$) at 100 mg DOTC/kg diet (-47/-48%) and 300 mg DOTC/kg diet (-75/-73%) compared to control.

In females, the absolute thymus weight in all treated groups was decreased in a dose-dependent manner (-14%, $p<0.05$, -68%, $p<0.01$, -73%, $p<0.01$ in 10, 100 and 300 mg DOTC/kg diet groups compared to control) as well as the relative thymus weight in all treated groups in a dose-dependent manner (-14%, $p<0.05$, -69%, $p<0.01$, -70%, $p<0.01$ in 10, 100 and 300 mg DOTC/kg diet groups compared to control).

GROSS PATHOLOGY

At necropsy, treatment-related gross changes were not observed.

HISTOPATHOLOGY: NON-NEOPLASTIC

At microscopical examination, treatment-related histopathological changes were observed in the thymus. The histopathological changes comprised lymphoid depletion, characterised by a decrease in the size of the thymic lobules which can be ascribed to extensive loss of cortical en medullary small lymphocytes. Consequently, the distinction between the cortical and medullary areas was blurred. The microscopic appearance of the affected thymus resembled thymus atrophy described in the literature for organotin compounds.

Lymphoid depletion was observed in the thymus in 5/10 (severity score slight to moderate) and 9/9 (severity score, moderate to severe) males of the 100 and 300 mg/kg group, respectively, and in 10/10 and 9/9 females (severity score was slight to very severe) of the 100 and 300 mg/kg group, respectively. Lymphoid depletion was not observed in any of the animals of the control group or the 10 mg/kg group.

All other histopathological changes were common findings in rats of this strain and age. They were about equally distributed amongst the different treatment groups or occurred in one animal only. Therefore, they were not considered to be related to treatment.

Table 7: Incidence of lesions in the thymus

	Males				Females			
	0 mg/kg diet	10 mg/kg diet	100 mg/kg diet	300 mg/kg diet	0 mg/kg diet	10 mg/kg diet	100 mg/kg diet	300 mg/kg diet
Lymphoid depletion								
Number of animals examined	10	10	10	9	10	10	10	9
Slight	0	0	3	0	0	0	1	0
Moderate	0	0	2	4	0	0	4	2
Severe	0	0	0	5	0	0	5	5
Very severe	0	0	0	0	0	0	0	2
Total score	0	0	5*	9***	0	0	10***	9***

* $p<0.05$

** p<0.01

*** p< 0.001

Conclusion Administration of dioctyltin chloride in the diet at the concentrations of 10, 100 and 300 mg/kg caused a decrease in thymic weight which was correlated with histopathological effects (lymphoid depletion) observed in the 100 and 300 mg DOTC/kg diet dose groups and were considered as adverse effects. The decreased absolute and relative thymus weights in females of the 10 mg/kg group, although not accompanied by histopathological changes, were also considered to reflect a toxicologically-relevant change in the thymus, which was in accordance with the shown toxicity profile of the test substance (i.e. thymotoxicity). Thus, no NOAEL could be set in the present study.

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.

4.5 Acute and/or chronic toxicity to other aquatic organisms

Not evaluated in this CLH Report.