Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Dibutyltin di(acetate)

EC Number: 213-928-8
CAS Number: 1067-33-0

CLH-O-0000006851-71-01/F

Adopted

17 September 2020

17 September 2020

CLH-O-0000006851-71-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **Dibutyltin di(acetate)**

EC Number: **213-928-8**

CAS Number: **1067-33-0**

The proposal was submitted by **Norway** and received by RAC on **30 August 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Norway has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **23 September 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **22 November 2019**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Betty Hakkert**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **17 September 2020** by **consensus.**

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	Dibutyltin di(acetate)	213-928-8	1067-33-0	Muta. 2 Repr. 1B STOT RE 1	H341 H360FD H372 (immune system)	GHS08 Dgr	H341 H360FD H372 (immune system)			
RAC opinion	TBD	Dibutyltin di(acetate)	213-928-8	1067-33-0	Muta. 2 Repr. 1B STOT RE 1	H341 H360FD H372 (immune system)	GHS08 Dgr	H341 H360FD H372 (immune system)			
Resulting Annex VI entry if agreed by COM	TBD	Dibutyltin di(acetate)	213-928-8	1067-33-0	Muta. 2 Repr. 1B STOT RE 1	H341 H360FD H372 (immune system)	GHS08 Dgr	H341 H360FD H372 (immune system)			

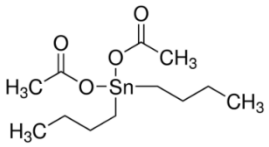
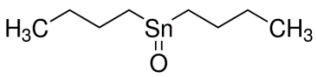
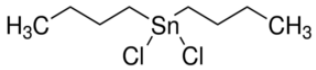
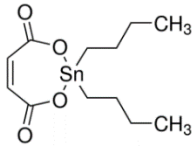
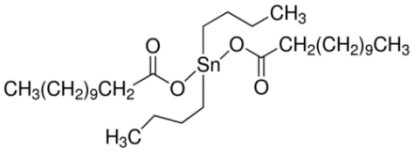
GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The dossier submitter (DS) proposed to classify dibutyltin di(acetate) (abbreviated throughout this document as DBTA) for mutagenicity, STOT RE and reproductive toxicity. In addition to studies performed with DBTA itself, reference was made to studies performed with the following substances as part of a read-across, category approach: DBTC, DBTM, DBTA, DBTL and DBTO (see the table below for the full substance names and structures).

Table: *Substance characteristics*, adapted from Table 10 in the CLH report*

* The structures are arbitrary as tin(IV) compounds may adopt various geometries and coordination numbers depending on the ligands.

Substance	EC # / CAS #	Structure	Purity (studies)	Purity / Impurity details (REACH Dossier)
Dibutyltin (di)acetate (DBTA)	213-928-8 / 1067-33-0		Not reported	No further details (monoconstituent substance)
Dibutylbis(pentane-2,4-dionato-O,O')tin (DBTP)	245-152-0 / 22673-19-4		>92%	>92% (TIB KAT 226) No further details (monoconstituent substance)
Dibutyltin oxide (DBTO)	212-449-1 / 818-08-6		Not reported	>97.5% No further details (monoconstituent substance)
Dibutyltin dichloride (DBTC)	211-670-0 / 683-18-1		96-99.7% where reported for studies	93-100% Monoconstituent substance; tributyltin chloride (0.25-1%) in some sources
Dibutyltin maleate (DBTM)	201-077-5 / 78-04-6		Not reported	No further details (monoconstituent substance)
Dibutyltin dilaurate (DBTL)	201-039-8 / 77-58-7		Not reported	95-100% Monoconstituent substance; potenital presence of tributyl(lauryloxy) stannane

The DS proposed to form this category for read-across purposes based on the common hydrolytic behaviour of its members. According to the DS proposal, the result of hydrolysis is a common tin compound that is responsible for the toxic effects observed. In addition, since all category members hydrolyse at neutral or low pH, it demonstrates that systemic exposure to the intact substances, following oral administration, was unlikely.

In the initial hydrolytic studies, an indirect detection method was used that could not determine the exact tin species that was formed; therefore, it was thought that dialkyltin compounds form DBTC after hydrolysis. However, recent *in vitro* hydrolysis studies which used 119Sn-NMR spectroscopy showed that both DBTC and the related compound DBTP form the distannoxane $ClBu_2SnOSnBu_2Cl$.

The CLH dossier of DBTA did not include an *in vitro* gastric simulation study, however, such a study was provided by the registrants in the consultation. Similar to DBTC and DBTP, also DBTA formed the distannoxane $ClBu_2SnOSnBu_2Cl$.

In addition, the CLH dossier includes an *in vivo* toxicokinetic study of DBTA in mice, as well as an *in vivo* study performed with DBTC in rats. The study in mice noted cleavage of both the acetate and butyl group(s). The exact metabolites of DBTA were not determined, but this finding supports the hypothesis that the distannoxane $ClBu_2SnOSnBu_2Cl$ is formed.

Moreover, the available developmental toxicity studies with DBTA itself show effects very similar to those induced by other category members, and in particular DBTC.

Considering the metabolism studies and similar toxicological profiles, RAC agrees with the read-across approach proposed by the DS. In accordance with this approach, the classification proposal of DBTA is mainly based on studies performed with DBTC, and supported by studies with related dibutyltin compounds. This is also consistent with the RAC opinion of dibutyltin dilaurate (DBTL) and dibutylbis(pentane-2,4-dionato)-OO')tin (DBTP).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Although there are no studies with DBTA for STOT RE, a category approach, supported on the basis of the toxicokinetic and hydrolytic behaviour of the substances in the category, was used by the DS to justify that studies on DBTC and DBTDL can be taken into consideration when classifying DBTA for this hazard class.

Only one, rather old, 90-day study is available. This feeding study in rats (0, 10, 20, 40, 80 ppm DBTC in diet, corresponding to 0, 0.5, 1, 2, 4 mg/kg bw/d) indicated some slight effects such as reduced food consumption and body weight and mild anaemia at the highest dose (Gaunt *et al*., 1968). No abnormalities were seen at autopsy or histology (including the thymus).

A 28-day rat/mouse immunotoxicity study with doses of DBTC of 0, 50 and 150 ppm in the diet (corresponding to 0, 2.5, 7.5 mg/kg bw/d for rats and 0, 7.1, 21.4 mg/kg bw/d for mice) was included in the CLH report (Seinen & Vos, 1977). No treatment-related effects were observed in mice. In rats, mortality was observed in the 7.5 mg/kg bw/d group (4/10 females and 2/10 males). Further, clear dose-dependent effects on the thymus were observed. Reductions in relative organ weights were noticed for the thymus (2.5 mg/kg bw/d: 53%, 7.5 mg/kg bw/d: 68-72%), but also the spleen (2.5 mg/kg bw/d: 16%, 7.5 mg/kg bw/d: 33%) and popliteal lymph nodes (2.5 mg/kg bw/d: 16%, 7.5 mg/kg bw/d: 28%). A pronounced reduction in size of the thymus was found in all DBTC-treated animals. The most important effect observed was lymphocyte depletion in lymphoid organs, which was most pronounced in the thymic cortex of DBTC-treated animals. At the 7.5 mg/kg bw/d level, the thymic cortex was almost completely

depleted, although no signs of cell destruction were observed. Lymphocyte depletion was also present in the thymus-dependent areas of the spleen and popliteal lymph nodes.

An additional 2-week rat feeding study (0, 50, 150 ppm DBTC in diet, corresponding to 0, 2.5, 7.5 mg/kg bw/d) confirmed previous findings of clear effects on thymus (Penninks & Seinen, 1982). Relative thymus weight was reduced (<30% of control group at 7.5 mg/kg bw/d), and lymphocyte depletion was observed in thymus (mainly in the thymic cortex and in thymus-dependent lymphoid areas of the spleen).

An OECD TG 421 reproductive/developmental toxicity screening test (diet) in rats (Waalkens-Berendsen, 2003) showed a reduced relative thymus weight and moderate to severe lymphoid depletion in dams exposed to 1.7-2.4 mg/kg bw/d after ~41 days of exposure. A dose of 6.2- to 15.4 mg/kg bw/d induced reduced absolute and relative thymus weight and severe to very severe lymphoid depletion in dams.

An OECD TG 414 prenatal developmental toxicity study in rats (oral gavage; 0, 1, 2.5, 5, 10 mg/kg bw/d on GD 6-15) showed clear maternal toxicity (Study Report, 1994; Farr *et al*., 2001). Effects included reduced bw gain (≥5 mg/kg bw/d), reduced food consumption (≥10 mg/kg bw/d) and significantly increased number of animals with thymus atrophy (≥10 mg/kg bw/d). Maternal toxicity was not observed at 1 mg/kg bw/d.

Several mechanistic immunotoxicity studies were included in the dossier. In general, these studies suffered from limitations including too low doses, the use of a single dose level or single exposure. However, the results confirmed that the thymus is a target organ of DBTA.

There was one neurotoxicity study with DBTDL included in rats (0, 5, 10 and 20 mg/kg bw/day for 5 days/week for 7 weeks), which was considered too poor to allow the addition of neurotoxicity as a target organ.

The DS considered the 28-day study (Seinen & Vos, 1977), 14-day study (Penninks & Seinen, 1982) and reproductive/developmental toxicity screening study (Waalkens-Berendsen, 2003) as key studies. All three studies showed thymus toxicity at low dose levels. After adjusting for the difference in molecular weight between DBTC and DBTA, the approximately equivalent doses of 2-3 mg/kg bw/d are clearly below the guidance value for STOT RE 1. The DS concluded that the data supported classification for specific target organ toxicity following repeated exposure as STOT RE 1 with the immune system as target organ.

Comments received during consultation

Four comments from member state competent authorities (MSCAs) were received, all indicated support for the category approach, and all were in favour of the proposed classification as STOT RE 1.

One industry representative commented on the proposal. They disagreed with the category approach, because new hydrolysis studies showed that a dimer was formed rather than DBTC itself. However, they also indicated that they self-classified DBTA as STOT RE 1 based on thymus effects.

Assessment and comparison with the classification criteria

Given that both DBTA and DBTC are hydrolysed to the same distannoxane, RAC is of the opinion that data on DBTC can be used for classification of DBTA for STOT RE (see also the RAC general comment).

The results of the studies with DBTC consistently showed that the immune system, in particular the thymus, was the target organ after repeated oral exposure. Effects included reduced thymus weight, thymus atrophy, and severe lymphoid depletion. At higher doses, also effects on liver, bile duct and pancreas have been reported; however, inconsistently in the studies, and therefore not sufficient to trigger classification. The studies with DBTDL provided supporting evidence.

When considering differences in molecular weight between DBTC and DBTA (DBTA: 351.03 g/mol, DBTC: 303.84 g/mol), the effective dose levels expressed as DBTA are:

- ≥2.9 mg/kg bw/d (combined rat subacute/developmental), respectively.
- ≥1.96-2.8 mg/kg bw/d (in a reproductive/developmental toxicity screening test exposure period of 41 days for adult animals in this study),
- ≥2.9 mg/kg bw/d (in a rat prenatal developmental toxicity study (dams were exposed during 10 days, GD 6-15),

Overall, RAC considers the effects on the immune system as sufficiently severe to fulfil the classification criteria for STOT RE. The effects on the immune system include morphological changes that provide clear evidence of marked organ dysfunction and are considered as significant organ damage noted at necropsy and/or subsequently seen or confirmed at microscopic examination.

Effective dose-levels for DBTA are below the extrapolated guidance values for classification as STOT RE 1 (i.e. 10, 30 and 60 mg/kg bw/d for a 90-day, 28-day and 14-day study, respectively). Setting of a specific concentration limit (SCL) is not considered necessary, given the small margin between the effective dose levels and the guidance values for STOT RE.

RAC therefore supports the conclusion of the DS that a classification is warranted for DBTA as **STOT RE 1, H372 (Causes damage to the immune system through prolonged or repeated exposure).**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

Only one *in vitro* gene mutation study in bacteria has been performed with DBTA itself, which was negative. The evaluation of mutagenicity was thus based on studies with DBTC and one study with DBTDL.

The study with DBTDL investigated *in vivo* DNA damage in rat cerebral cortical cells and found a significant, dose-dependent increase (Jin *et al*., 2012).

Twelve *in vitro* studies and two *in vivo* studies with DBTC are presented in the CLH report. A GLP-compliant (similar to OECD TG 473) *in vitro* mammalian chromosome aberration test (± S9-mix) was reported with positive results (Anonymous, 1990a). Two bacterial reverse mutation tests were reported with one demonstrating positive results (no metabolic activation applied) (Hamasaki *et al*., 1993) and the other presenting negative results (± S9-mix) (Anonymous, 1979). A CHO/HGPRT gene mutation assay (non-GLP and non-guideline; Li *et al*., 1982) showed positive results (no metabolic activation applied), whereas an OECD TG 476-compliant *in vitro* mammalian cell gene mutation test using Chinese hamster lung fibroblasts (V79) showed negative results (± S9-mix) (Anonymous, 1989). Furthermore, a study with bacterial SOS-assay and a bacterial rec-assay (Hamasaki *et al*., 1992) showed positive results from both assays (no metabolic activation applied).

In addition, various non-guideline, non-GLP studies were included in the CLH report as well, reporting both positive and negative results: DBTC was shown to induce breakage of naked λ-DNA (Hamasaki *et al*., 1995), to form condensates with DNA (Piro *et al*., 1992), and to affect spindle structure during mitosis in V79 Chinese hamster cells (Jensen *et al*., 1991a), but did not affect chromosomal length in human peripheral lymphocytes (Jensen *et al*., 1989), nor did DBTC induce hyperdiploid cells (aneuploidy) in human peripheral lymphocytes (Jensen *et al*., 1991b).

In the OECD TG 474 and GLP-compliant *in vivo* micronucleus study, mice received DBTC via a single oral gavage. Dose levels of 2, 10 or 50 mg DBTC/kg bw were applied (vehicle: corn oil). A statistically significant increase in the incidence of micro-nucleated polychromatic cells was observed in bone marrow 48h and 72h after exposure of mice to DBTC at 50 mg/kg bw, with effects more clearly in female than male animals. No positive result was obtained upon DBTC-exposure at the post-treatment time-interval of 24h.

The positive mutagenic result for DBTC was not confirmed in a second *in vivo* mouse micronucleus study. Mice received a single oral gavage exposure of DBTC of 0, 50, 100 or 200 mg/kg bw (vehicle: arachis oil). The results of this second micronucleus test indicated that DBTC failed to show any evidence of mutagenic potential up to the (toxic) dose level of 200 mg/kg bw as measured at 24h, 48h and 72h post-treatment.

Overall, for DBTC there was a mixed outcome both for *in vitro* and *in vivo* studies, but in general most studies were positive.

The DS concluded that given the absence of germ cell mutagenicity studies for DBTA or other members of its category, there is insufficient evidence to warrant classification in category 1B.

There was a positive *in vivo* somatic cell mutagenicity test as well as supportive evidence from positive results from *in vitro* mutagenicity/genotoxicity tests with DBTC, which had been previously classified as Muta. 2.

The DS proposed to classify DBTA also as Muta. 2 based on the category approach.

Comments received during consultation

Four MSCAs expressed their support for the proposed classification for Muta. 2 based on the category approach.

One industry representative disagreed with the classification proposed because they rejected the category approach.

Assessment and comparison with the classification criteria

The classification proposal for mutagenicity is based solely on the category approach, as the only available study with DBTA itself is a negative *in vitro* gene mutation study in bacteria. As DBTA forms at least in part the same metabolite as DBTC, RAC considers the proposed read-across valid for germ cell mutagenicity (see also *RAC general comment*).

Overall, the results of the *in vitro* tests performed with DBTC were variable with both positive and negative results. Additionally, two *in vivo* mouse micronucleus studies with DBTC are presented in the CLH report. One study showed positive effects at the highest dose only (50 mg/kg bw) (Anonymous, 1991), whereas a similar study did not show positive effects at doses up to 200 mg/kg bw (Anonymous, 1991).

Both mouse micronucleus studies included a sufficient number of animals. Positive as well as negative controls were included with appropriate results in both studies, and toxicity was

observed in both studies. After full evaluation, no clear explanation could be found for the discrepancy in results. Without any reason to discard one of the two *in vivo* mouse micronucleus studies, the positive result of the first study is taken forward for the evaluation.

In vivo mammalian germ cell mutagenicity tests are not available for DBTA or DBTC. However, a positive result was obtained from a well-performed OECD- and GLP-compliant *in vivo* mouse micronucleus test with DBTC. The positive result is supported by indications from one *in vivo* test with DBTDL (*in vivo* Comet assay, non-GLP). Further, the formation of micronuclei in the bone marrow suggest systemic availability.

Although distribution into testes/ovaries can be expected, no experimental evidence is available which demonstrates a direct interaction of the substance or its metabolite with the genetic material of germ cells. Therefore, RAC considers classification in category 1B not appropriate. RAC concludes that classification for DBTA as **Muta. 2**, **H341 (Suspected of causing genetic defects)** is warranted.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Sexual function and fertility

No data are available for DBTA; thus, the evaluation was based on studies with DBTC.

The OECD TG 421 reproduction/developmental toxicity screening study (Waalkens-Berendsen, 2003) showed body weight effects in both females and males at the high dose (200 ppm, 12.0-15.4 mg/kg bw/d). In female rats, reduced weight gain was observed over the pre-mating, gestation, and lactation periods at the higher dose level. The corpora lutea numbers were not measured in this study. No reproductive toxicity was observed in males. There was a significant increase in the incidence of ovarian cysts in the high-dose females. Furthermore, the number of pregnant females was reduced in mid (30 ppm, 1.7-2.4 mg/kg bw/d) and high dose groups (7/12 in both vs 9/12 in the control) and only 3/7 pregnant high dose females delivered offspring. This resulted in a reduction in the number of live pups (10 vs 101).

A fertility study with DBTC (Ema & Harazono, 2000) was reported in which female rats were exposed via gastric intubation to DBTC in olive oil (0-3.8-7.6-15.2 mg/kg bw/d) on GD 0-3 or GD 4-7. In addition to a control group (olive oil), also a pair-fed group (feed restricted to same amounts as high dose DBTC-group) was included. A significantly higher number of non-pregnant dams was observed upon exposure to the mid and high dose of DBTC on GD 0-3 (high dose: 87%, mid dose: 31.3%, low dose: 0%, control: 0%, pair-fed: 5.9%). Further, a reduced number of implantations (high dose: 1.8±4.8, mid dose: 10.1±7.1, low dose: 15±1.5, control: 15±1.4, pair-fed: 13.4±4.3) and increased incidences of pre-implantation loss (high dose: 87.9%, mid dose: 35.6%, low dose: 4.1%, control: 2.7%, pair-fed: 16.4%) was observed as well in these DBTC-exposed groups as evidence for effects on fertility.

In a developmental toxicity study in the CD1 mice, DBTC (in olive oil) was administered by gavage to pregnant females 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. Mortality occurred in all treated groups, but without a dose-response relationship. Other signs of toxicity (vaginal discharge, hypoactivity, hypothermia) were also observed at all dose levels and jaundice was seen in the mid and high doses. Body weight and food consumption were also affected negatively. Regarding the number of pregnant females, there was an increase in the pre-implantation loss with the dose administered (29.7% - 7,6 mg/kg bw, 34.0% - 15.2 mg/kg bw, 58.3% - 30.4 mg/kg bw) that was statistically significant in

the high dose. Post-implantation losses increased with the dosing and the effect at the mid dose (15.2 mg/kg bw) was also statistically significant.

A supportive mechanistic study explored the effect of progesterone on implantation failure induced by DBTC in rats (Ema *et al*., 2003). The other two supportive studies (Harazono & Ema, 2003; Harazono & Ema, 2001) also focused on the effects of DBTC in decidual cell response and progesterone levels during pseudopregnancy.

Based on the increased number of non-pregnant females among successfully mated females, the reduced number of implantations, the increased pre-implantation losses and increased early total resorptions, as well as the previous harmonised classification of DBTC as Repr. 1B for adverse effects on sexual function and fertility, the DS considered that DBTA should have the same classification as DBTC. The DS therefore proposed Repr. 1B for adverse effects on sexual function and fertility for DBTA.

Development

There are three developmental toxicity studies available with DBTA, as well as a large number of studies with DBTC and one study performed with DBTDL, DBTM and DBTO, in addition to DBTA and DBTC.

The three studies performed by Noda *et al*. with DBTA had as main purpose to characterise the critical parameters of DBTA-induced teratotoxicity. In particular the critical window of exposure was investigated, the results of which are effectively illustrated by the figure below.

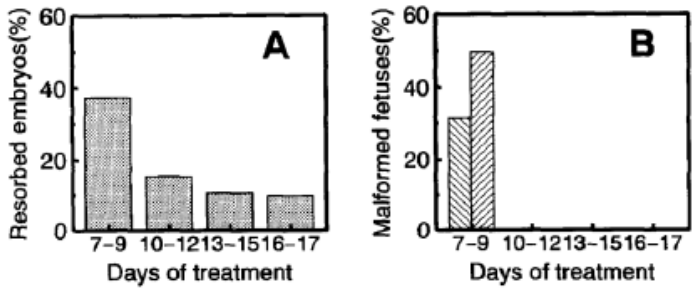


Figure 1. Incidence of dead or resorbed fetuses (A) and of fetuses with external (▨) and skeletal (▨) malformations (B) from the dams treated orally with DBTA (15 mg/kg) at different gestational stages.

Figure : Facsimile from Noda et al. 1992a, as included in Annex 1 of the CLH report

It can be observed that three days of exposure to 15 mg/kg bw on GD 7-9 results in a clear rise in resorbed embryos and in skeletal and external malformations. The malformations included cleft mandible, cleft lower lip, ankyloglossia or schistoglossia, exencephaly, anomaly of mandibular fixation, cranial hypoplasia, and fused ribs. Experiments with single doses showed that GD 8 was the critical time-point for these effects.

Noda *et al*. (1992b) also reported maternal effects after exposure to DBTA during GD 7-17, which consisted of reduced weight gain, albeit not in dams with living foetuses, and dose-related thymus atrophy with statistical significance at 5 mg/kg bw/d and above.

The developmental effects observed in this study are summarised in the table below.

Table: *Summary of developmental effects of DBTA in Noda et al. (1992b)*

Dose level (mg/kg bw/d)	**0**	**1.7**	**5**	**10**	**15**
Mated (#)	14	13	14	14	16
Pregnant (#)	14	12	14	14	16
Body wt (g)	314±12.1	312±18.6	309±17.6	298±14.5	254±40.7
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	3.2/2.9	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 ($p<0.01$)

The third study by Noda *et al* (2001) applied single doses of 0 (vehicle control), 7.5, 10, 15 or 22 mg/kg bw on GD 8 and investigated the effect of the age of the dams at the time of mating on the susceptibility to DBTA toxicity. In the group with dams of 7.5 months, maternal body weight gain, but not adjusted body weight gain, was statistically significantly decreased at the top dose. The effects on the pups were similar to the previous studies and included post-implantation loss, reduced pup weight, and external and skeletal malformations. The LOAEL for external malformations was the lowest dose of 7.5 mg/kg bw. There was no clear relationship between the age of the dams and DBTA effects, mainly because the implantation loss in older dams (12 months) was very high in all groups.

A comparative study with DBTC, DBTA, DBTM, DBTL and DBTO (Noda *et al*., 1993) using a single gavage administration of 80 µmol/kg bw on GD 8 (28 mg/kg bw DBTA), showed a comparable spectrum of effects for all substances, in the absence of maternal toxicity. Treatment lead to a comparable incidence and type of foetal malformations for all substances.

In addition to the studies with DBTA, also a large body of evidence exists with DBTC, which has been previously discussed in the RAC opinions on DBTC, DBTL and DBTP.

The reprotoxic effects of DBTC observed in the OECD TG 421 reproduction/developmental toxicity screening study (Waalkens-Berendsen, 2003) included an increase in the number of dams with post-implantation loss, a reduction in the number of live pups and a reduction in the gestation index.

An OECD TG 414 study with DBTC reported severe malformations in four pups at 10 mg/kg/d, including anasarca, ankyloglossia, hydrocephaly, agnathia and other skeletal defects. Maternal toxicity at this dose level consisted of reduced weight gain and food consumption.

In a supportive rat developmental toxicity study, rats were exposed during the gestation period (GD 7-15) via oral gavage to DBTC in olive oil (0, 2.5, 5, 7.5, 10 mg/kg bw/d) (Ema *et al.*, 1991).

Clear maternal toxicity was observed at the two highest dose levels and effects included significantly higher mortality in dams (5/10 and 9/10 dams died in the 7.5 and 10 mg/kg bw/d dose groups, respectively) with stomach hemorrhages observed in dead animals. In the 7.5 and 10 mg/kg bw/d dose-groups, total resorptions were observed in the remaining 5/10 and 1/10 pregnant rats, respectively. *In utero* exposure of foetuses resulted in developmental effects such as increased incidences of external and skeletal malformations, with cleft jaw and ankyglossia being the most frequently observed type of malformations. Although observed at the two highest dose levels in the presence of clear maternal toxicity, these developmental effects were also observed at the dose level of 5 mg/kg bw/d (i.e. without the presence of maternal toxicity).

Three additional studies on potential developmental toxicity in relation to the most sensitive window for exposure to DBTC indicated that DBTC-induced teratogenic effects were observed following exposure on GD 7-8 and were most pronounced when dams were exposed on GD 8 (Ema *et al.*, 1992, 1995, 1996). Embryo-lethality was observed at all tested time-points for exposure during gestation (GD 6-15).

The sensitivity of the rat foetuses to DBTC was confirmed by several *in vitro* studies.

A single study performed in CD1 mice found a clear increase in post-implantation loss, up to 100% at 30.4 mg/kg bw/d. No significant increase in foetal malformations was found, however this is unsurprising considering the small number of foetuses investigated.

Two studies in cynomolgus monkeys gave unclear results.

Based on the clear and consistent evidence of effects on the developing foetus (post-implantation loss, skeletal and external malformations) in rat studies with only mild or no overt maternal toxicity and in the absence of data indicating that effects are not relevant to humans, classification of DBTA for adverse effects on development in Category 1B is proposed.

Comments received during consultation

Four MSCAs expressed their support for the proposed classification for both fertility and developmental toxicity based on the category approach and the effects observed in the studies with DBTA itself.

One industry representative disagreed with the classification proposed as they rejected the category approach and claimed that the developmental effects were caused by maternal toxicity.

Assessment and comparison with the classification criteria

Sexual function and fertility

The effects of DBTC on reproduction have been investigated in a reproduction/developmental toxicity screening study in rats and two studies with exposure in early pregnancy in respectively rats and mice. The studies showed consistent decreases in the number of pregnant dams and number of implantations. Maternal toxicity in the form of reduced body weight gain and food consumption was observed, but mainly at the high dose, while reproductive effects also appeared at the mid dose levels, in particular in the rat studies. Moreover, the pair-fed group (Ema & Harazono, 2000) confirmed that the reproductive effects could not be explained by reduced food consumption.

Considering that several studies consistently showed fertility effects (non-pregnant dams, reduced number of implantations), at doses with limited or no maternal toxicity, that supportive studies indicate that DBTC have an adverse effect on progesterone levels and that there is no basis to question the human relevance of these effects, RAC considers that there is clear evidence

of an adverse effect on sexual function and fertility upon exposure to DBTC, as also concluded in the RAC opinion on DBTC itself.

Given that both DBTA and DBTC are hydrolysed to the same distannoxane, RAC is of the opinion that data on DBTC can be used for classification of DBTA for effects on sexual function and fertility (see also the RAC general comment).

Altogether, RAC supports the conclusion of the DS that a classification is warranted for DBTA as **Repr. 1B**; **H360F (May damage fertility)**.

Setting of an SCL is not considered necessary for adverse effects on sexual function and fertility, given that the ED_{10}-values (cf. section 3.7.2.5 of the Guidance on the Application of the CLP Criteria, version 5.0) for DBTA fall within the ranges of a medium potency group (i.e. 4 mg/kg bw/d < ED_{10} < 400 mg/kg bw/d) and there are no modifying factors which might change the potency group, resulting in the GCL of 0.3%.

Development

There are numerous studies which consistently show dose-dependent increases in foetal effects (malformations, post-implantation loss and weight reduction) after exposure to either DBTA or DBTC. Maternal effects were minimal or absent at the lowest doses that induced foetal effects. It should be noted that it is highly likely that the reduced body weight gain at higher doses is caused by the sharp increase in post-implantation loss, as dams with live foetuses did not show this effect. Moreover, dose-related foetal toxicity was observed even after single exposure and has a clear critical window, which makes it very unlikely that there is a causative relationship with maternal effects. There is no basis to question the human relevance of these effects. RAC considers that there is clear evidence of an adverse effect on development upon exposure to DBTA.

Altogether, RAC supports the conclusion of the DS that classification is warranted for DBTA as **Repr. 1B; H360D (May damage the unborn child**).

Setting of an SCL is not considered necessary for adverse effects on development, given that the ED_{10}-values fall within the ranges of a medium potency group (i.e. 4 mg/kg bw/d < ED_{10} < 400 mg/kg bw/d) and there are no modifying factors which might change the potency group, resulting in the GCL of 0.3% (cf. section 3.7.2.5 of the Guidance on the Application of the CLP Criteria, version 5.0).

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).