Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Dibutylbis(pentane-2,4-dionato-O,O')tin

EC Number: 245-152-0
CAS Number: 22673-19-4

CLH-O-0000001412-86-184/F

Adopted

5 December 2017

5 December 2017

CLH-O-0000001412-86-184/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: **Dibutylbis(pentane-2,4-dionato-O,O')tin**

EC Number: **245-152-0**

CAS Number: **22673-19-4**

The proposal was submitted by **Sweden** and received by RAC on **1 December 2016.**

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

PROCESS FOR ADOPTION OF THE OPINION

Sweden 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 **16 December 2016**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **9 February 2017**.

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 **5 December 2017** by **consensus**.

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

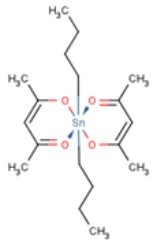
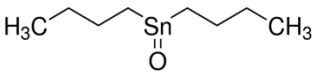
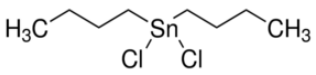
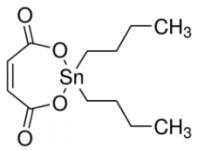
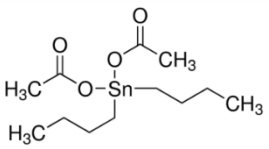
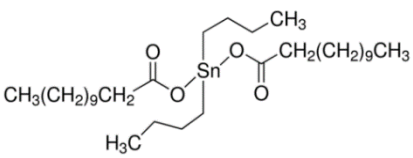
	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	Dibutylbis(pentane-2,4-dionato-O,O')tin	245-152-0	22673-19-4	Repr. 1B STOT RE 1	H360FD H372	GHS08 Dgr	H360FD H372			
RAC opinion	TBD	Dibutylbis(pentane-2,4-dionato-O,O')tin	245-152-0	22673-19-4	Repr. 1B STOT RE 1	H360FD H372 (immune system)	GHS08 Dgr	H360FD H372 (immune system)			
Resulting Annex VI entry if agreed by COM	TBD	Dibutylbis(pentane-2,4-dionato-O,O')tin	245-152-0	22673-19-4	Repr. 1B STOT RE 1	H360FD H372 (immune system)	GHS08 Dgr	H360FD H372 (immune system)			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The DS proposed to classify Dibutylbis(pentane-2,4-dionato)-OO')tin (abbreviated throughout this document as DBTP) for STOT-RE and reproductive toxicity. Although no studies with DBTP are available for these endpoints, 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 Table below for the full substance names and structures).

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

Substance	EC # / CAS #	Structure	Purity (studies)	Purity / Impurity details (REACH Dossier)
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 (di)acetate (DBTA)	213-928-8 / 1067-33-0		Not reported	No further details (monoconstituent substance)
Dibutyltin dilaurate (DBTL)	201-039-8 / 77-58-7		Not reported	95-100% Monoconstituent substance; potential presence of tributyl(lauryloxy) stannane

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

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 the 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 DBTP forms DBTC after hydrolysis.
However, in a recent *in vitro* hydrolysis study (Naßhan, 2015) a direct method of detection was used that allowed specific structural identification and demonstrated that both DBTP and DBTC form the same species, namely, the distannoxane $ClBu_2SnOSnBu_2Cl$ (Figure below).

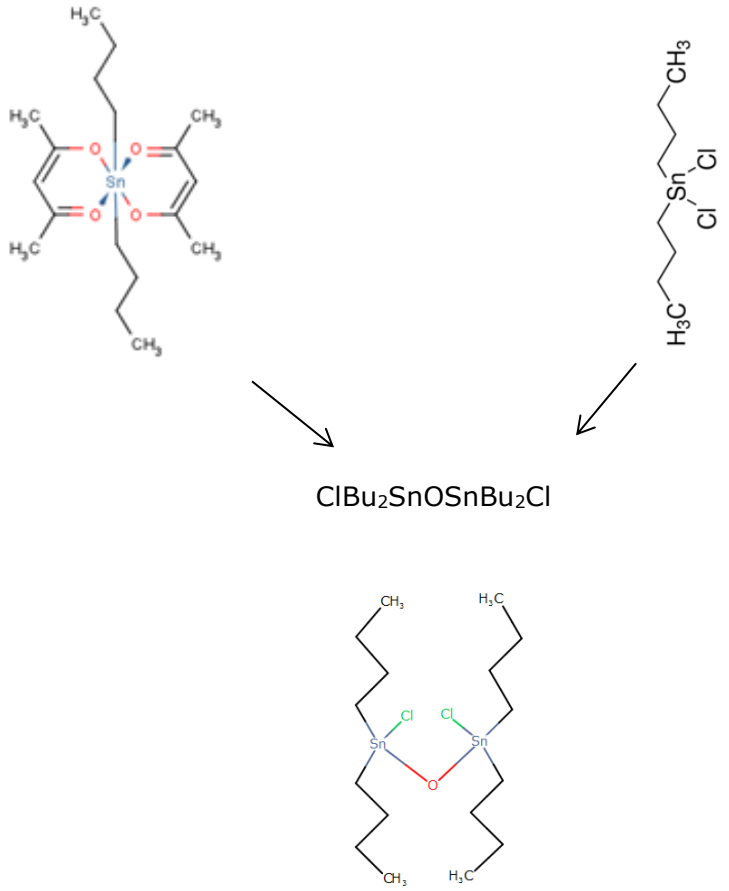


Figure: Overview of the hydrolysis of DBTP and DBTC as determined by Naßhan (2015)

In this study, DBTP was tested at a final concentration of 23.2 mM under low pH (~1-2) conditions (0.07 N HCl) at 37°C in order to simulate the hydrolytic action of mammalian gastric contents. DBTP rapidly formed the dimeric stannoxane $ClBu_2SnOSnBu_2Cl$ (119Sn-NMR: δ (ppm) -91, -144) in almost quantitative yield when exposed to conditions representative of the mammalian stomach. Minor amounts (~2 mol%) of non-hydrolysed DBTC were also detected.
Also DBTC formed the same bridged stannoxane (85% conversion after 1h and 90% conversion after 4 hours).

Complementary studies using indirect detection methods for other category members support the formation of common intermediate(s). This read-across approach is further supported by the

available toxicological data that show similar toxicological profiles for the substances in this category.

Considering the available hydrolysis 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 DBTP is mainly supported by studies performed with DBTC and other related dibutyltin compounds. This is also consistent with the RAC opinion issued for dibutyltin dilaurate (DBTL) in 2015.

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 DBTP for STOT RE, a category approach, based on the hydrolytic and toxicokinetic behaviour, and on the toxicological data, was used by the DS to justify that studies on DBTC can be considered when classifying DBTP for this hazard class. The CLH report does not contain any relevant studies with DBTP but it includes several animal studies with repeated oral exposure to DBTC. No studies are available for the dermal and inhalation route.

DBTC

The following studies were included in the CLH report:

- OECD TG 407 (comparable study) repeated dose 28-day oral toxicity in rat (Wister) (Seinen & Vos, 1977)
- OECD TG 407 (comparable study) repeated dose 28-day oral toxicity with Swiss mouse (Penninks & Seinen, 1982)
- OECD TG 408 (comparable study) in rat (CFE - Carworth Farms Elia) 90-d oral feeding (Gaunt *et al.*, 1968)
- OECD TG 421 reproductive/developmental toxicity screening test (diet) in rat (Wistar) (Waalkens-Berendsen, 2003)
- OECD TG 414 prenatal developmental toxicity study (gavage) in rat (Wistar) (Study Report, 1994)
- OECD TG 414 prenatal development toxicity studies (gavage) in rat (Wistar) (Farr *et al.*, 2001).
- 28 day drinking water sub-acute toxicity study of immune function in the rat (DeWitt *et al.*, 2005b)
- sub-chronic oral feeding study (dietary concentrations of max. 100 ppm) for up to 6 months (Barnes & Stoner, 1958).

A number of non-standard and mechanistic studies are also available for DBTC.

- Single oral gavage rat study (Snoeij *et al.*, 1988)
- Mechanistic investigation of thymic atrophy in the rat, the mouse and the Guinea pig (Seinen *et al.*, 1977)
- 28 day drinking water sub-acute toxicity study of immune function in the rat (DeWitt *et al.*, 2005a)
- Sub-acute toxicity study of immune function in rats exposed during development (drinking water) (DeWitt *et al.*, 2006a)

- Developmental immunotoxicity study in the rat (dams: drinking water; pups: gavage or untreated) (DeWitt *et al.*, 2006b) (also reported by Luebke *et al.*, 2003; in conference abstract)
- Effect of single dose intraperitoneal injection on SCID mice engrafted with human foetal thymus and liver tissue fragments (de Heer *et al.*, 1995)

The immune system was clearly the target organ, as observed after oral exposure to DBTC. Effects included reduced thymus weight, thymus atrophy, immune system depression (e.g. inhibitory effect on T-lymphocyte activity), reduced weight gain in pups and reduced water and food consumption in some cases. The DS considered the two OECD TG 407 comparable 28-d repeated dose studies as key studies (Seinen & Vos, 1977 and Penninks & Seinen, 1982). In these studies a LOAEL of 2.5 mg/kg bw/d was derived based on reduced thymus, spleen and lymph node weight (M/F) and lymphocyte depletion in the thymic cortex and PALS. The DS concluded that the available 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 public consultation

Three MSCAs commented and agreed with the proposed classification for STOT RE during the public consultation. One of the MSCAs expressed their doubts regarding the study of Gaunt *et al.* (1968) being considered a key study. They proposed that it should be considered as supportive due a lack of identified effects on thymus. The DS agreed to this proposal.

Assessment and comparison with the classification criteria

Summary of the most relevant studies

An OECD TG 421 reproductive/developmental toxicity screening test (diet) in rats (Waalkens-Berendsen, 2003) showed, in addition to reduced body weight gain and food consumption in male and female animals, a reduced relative thymus weight and moderate to severe lymphoid depletion in dams exposed to 1.7-2.4 mg/kg bw/d (exposure 41 days for females and 28 days for males). A dose of 6.2- to 15.4 mg/kg bw/d induced a reduced absolute and relative thymus weight and a severe to very severe lymphoid depletion in dams. Lymphoid depletion was characterized by a decrease in size of thymic lobules due to an extensive loss of cortical and medullary small lymphocytes. The distinction between cortical and medullary areas was blurred. In the severe cases, the cortex was very small or partially absent. The effects on fertility and development, as observed in this study, are described and evaluated in the section RAC evaluation of Reproductive Toxicity.

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). Effects included reduced bw gain ($\geqslant$ 5 mg/kg bw/d), reduced food consumption ($\geqslant$ 10 mg/kg bw/d) and significantly increased number of animals with thymus atrophy ($\geqslant$ 10 mg/kg bw/d). Maternal toxicity was not observed at a dose of 1 mg/kg bw/d. The effects on development, as observed in this study, are described and evaluated in the section "RAC evaluation of Reproductive Toxicity".

A 90-d 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, reduced 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-d rat drinking water study (0, 0.9, 1.9 mg DBTC/kg bw/d in an initial experiment; 0, 1.0, 2.8 mg DBTC/kg bw/d in the replicate experiment), which focussed on immunotoxic effects, did not reveal any treatment-related effect on organ weight (including the thymus and spleen), antibody production, delayed type hypersensitivity response or natural killer cell activity. A slight reduction in water consumption was observed in the high dose group (DeWitt *et al.*, 2005b). Another study (DeWitt *et al.*, 2006b) also did not provide evidence that DBTC affected the rat immune system at low concentrations (1.0 to 4.4 mg/kg bw/d).

A 28d rat/mouse immunotoxicity study (rat: 2 weeks, mouse: 4 weeks) 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. In addition, effects on liver were observed and included thickened and dilated bile ducts accompanied by irregularly yellowish discoloured livers. These effects were found in the animals that died and in 2 male and 2 female survivors of the high dose group. Microscopic analysis revealed severe proliferation of bile duct epithelial cells and bile ducts which was associated with pericholangiolitis and periportal fibrosis in livers of 4 male and 6 female rats of the high dose group. Other treatment-related histopathological changes were not observed.

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), and lymphocyte depletion was observed in thymus (mainly in the thymic cortex and in thymus-dependent lymphoid areas of the spleen).

In another study, dose- and time-effects of DBTC administration were studied (Snoeij *et al.*, 1988). Rats were given DBTC via oral gavage in a single dose of 15 mg/kg bw and killed after 1, 2, 3, 4, 5, 7 and 9 days. A second group of rats received doses varying between 5 and 35 mg/kg bw and were killed 4 days post-exposure. A dose-dependent reduction in thymus weight was observed, and further, thymus weights returned to normal at day 9 post-exposure.

Assessment and comparison with the criteria

The available data indicates that the immune system is clearly affected after oral exposure to DBTC.

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

Studies on DBTC revealed effects on the immune system. The effects included thymus atrophy with lymphoid depletion, loss of organ-structure and reduced immune response. The effective observed dose levels for DBTC are:

- ⩾ 1.7-2.4 mg/kg bw/d in a reproductive/developmental toxicity screening test (exposure period of 41 days for adult animals in this study) (Waalkens-Berendsen, 2003),
- ⩾ 5 mg/kg bw/d in a rat prenatal developmental toxicity study (dams were exposed during 10 days, GD 6-15) (Study Report, 1994),
- ⩾ 2.5 mg/kg bw/d in combined rat subacute/developmental toxicity studies (Seinen & Vos, 1977)

When considering differences in molecular weight between DBTC and DBTP (DBTP: 431.14 g/mol, DBTC: 303.84 g/mol), these effective dose levels would correspond to effective dose levels expressed as DBTP as:

- ⩾ 2.4-3.4 mg/kg bw/d (in a reproductive/developmental toxicity screening test exposure period of 41 days for adult animals in this study),
- ⩾ 7.1 mg/kg bw/d (in a rat prenatal developmental toxicity study, dams were exposed during 10 days, GD 6-15),
- ⩾ 3.6 mg/kg bw/d (combined rat subacute/developmental), respectively.

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

Effective dose-levels for DBTP 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).

RAC therefore supports the conclusion of the dossier submitter that DBTP should be classified as **STOT RE 1 (H372: Causes damage to the immune system through prolonged or repeated exposure).**

Setting of Specific Concentration Limits is not considered necessary, given the small margin between the effective dose levels and the guidance values for STOT RE.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

A category approach, supported on the basis of hydrolytic and toxicokinetic behaviour, and toxicological data, was used by the DS to justify that studies on DBTC can be taken into consideration when classifying DBTP for the hazard class reproductive toxicity.

Fertility

No data are available for DBTP, thus the evaluation was based on studies with DBTC and related substances.

The fertility effects of DBTC observed in the OECD TG 421 reproduction/developmental toxicity screening study (Waalkens-Berendsen, 2003) did not include noticeable effects on males. However, in females rats reduced weight gain was observed over the pre-mating, gestation, and lactation periods at the higher dose level (200 ppm). The corpora lutea numbers were not measured in this study and the full study report was also not available.

A fertility study with DBTC (Ema & Harazono, 2000a; but also reported in Ema *et al.*, 2000 and Ema & Harazono, 2000b), which was used as a key study for the classification of DBTC, is included

in the CLH report. Observed effects included an increase in the number of non-pregnant females, a reduced number of implantations and an increased incidence of pre-implantation loss upon exposure to DBTC on GD 0-3, and an increased incidence of early total resorption upon exposure to DBTC on GD 4-7.

In a developmental toxicity study in the mouse with DBTC (Ema *et al*., 2007a) a lower level of pregnancies was associated with pre-implantation losses and it followed a clear dose-response relationship. Post-implantation loss increase was also associated with an increase in dosing. In addition, there was a small number of deaths of dams observed across all dosing groups, however they were not considered to be treatment related due to the absence of a dose-response relationship.

Two supportive mechanistic studies explored the effect of progesterone on implantation failure induced by dibutyltin dichloride in rats (Ema *et al.*, 2003a,b). 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 pseudo-pregnancy.

Based on 1) the increased number of non-pregnant individuals among successfully mated females, the reduced number of implantations, the increased pre-implantation losses and increased early total resorptions in the key fertility study with DBTC; 2) the harmonised classification of DBTC as Repr. 1B for effects on fertility and sexual function; and 3) given the hydrolytic behaviour of the substances in the category at neutral and low pH, the DS considered that DBTP should have the same classification as DBTC. The DS therefore proposed Repr. 1B for effects on fertility and sexual function for DBTP.

Development

No data are available for DBTP, thus the evaluation was based on studies with DBTC and related substances.

Two guideline compliant studies are presented in the CLH report: a screening study (OECD TG 421) and a prenatal developmental toxicity study (PNDT, OECD TG 414). At the high dose of the screening study (12.0-15.4 mg/kg bw/d), the number of foetuses was strongly reduced (10 compared to 101 in the controls). The PNDT reported severe malformations in 4 foetuses from 3 high dose litters (10 mg/kg bw/d). Both studies reported maternal toxicity at these doses, in particular reduced body weight gain, however, the values were not reported.

In addition, several non-compliant PNDT studies were presented as supportive evidence.

Five studies by Ema *et al.* (2003a,b; 2007a,f,g) identified that the sensitive period for DBTC teratogenicity is GD 7 or 8. Exposure in this period resulted in a characteristic pattern of external and skeletal malformations, predominantly affecting the skull and jaw. Other reported effects included increased post-implantation loss, reduced litter size and reduced foetal weight. Although also maternal toxicity was observed at doses of 7.5 mg/kg bw/d and higher, foetal malformations occurred at 5 mg/kg bw/d in absence of significant maternal toxicity (Ema *et al.*, 1991).

Studies by Noda *et al.* (1992 a,c, 1993) showed that similar foetal malformations are induced by other di-n-butyltin moieties after administration on GD 8, including DBTA, DBTO, DBTL, and DBTM.

Foetotoxicity and increased post-implantation loss was also observed in mice and cynomolgus monkeys, but these did not show the malformations observed in rats, although the effects may have been masked by the high level of post-implantation loss.

Based on the clear and consistent evidence of effects on the developing foetus (post-implantation loss, skeletal and external malformations) in rat studies and in the absence of severe maternal

toxicity, the DS proposed to classify DBTP for reproductive toxicity (adverse effects on development) in Category 1B.

Comments received during public consultation

Two MSCAs agreed with the proposed classification as Repr. 1B (development). One asked for additional clarification regarding the read-across approach and purity of the compound.

The DS noted there are no indications that DBTC is an impurity of DBTP, and even if it would be, this would not change the classification as the read-across is based on DBTC.

It is also clarified that the hydrolysis studies of Schilt (2004) and Naβhan (2015, 2016) used different concentrations and analytical techniques. As a result, it is not certain that DBTL, DBTO, and DBTM also form the distannoxane, although they seem to form identical tin moieties.

One Company/Manufacturer submitted comments expressing their different interpretation of the hydrolysis data. Their main argument is that, since the hydrolysis of DBTP is faster than DBTC it will result in a different bioavailability of the tin compound. The company also considers that the other tin compounds that are part of the category (DBTA, DBTM) have different hydrolysis rates and initiate different products therefore making them not suitable for read across.

In their response, the DS considered that DBTC and DBTP have hydrolysis rates of equivalent magnitude and result in the same bridged stannoxane, thus supporting the category read-across approach based on hydrolysis and toxicokinetics. The DS also replied that they were not in the possession of gastric hydrolysis studies for the other dibutyltin compounds referred in the comment from industry (DBTL, DBTA and DBTM).

Furthermore, to further justify the inclusion of the category members, the DS indicated a comparative rat developmental study that included all category members. The findings of the study by Noda *et al*. (1992c and 1993) demonstrate that all category members cause similar characteristic external and skeletal foetal malformations (largely affecting the jaw/skull).

Assessment and comparison with the classification criteria

Fertility

The reprotoxic effects of DBTC observed in the OECD TG 421 reproduction/developmental toxicity screening (Waalkens-Berendsen, 2003) were a significant increase in the incidence of ovarian cysts in nine of the twelve high-dosed females (200 mg/kg diet; corresponding to 6.2-15.4 mg/kg bw/d), 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. In line with the conclusion of the dossier submitter, RAC considers these effects as relevant for classification for fertility of DBTC, although it is recognized that some of these effects (e.g. reduced number of live pups) could also be due to developmental toxicity.

Another rat fertility study (non-guideline, non-GLP) with DBTC is presented (Ema & Harazono, 2000a). Successfully mated 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 after being exposed 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%). In addition, 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 preimplantation loss (high dose: 87.9%, mid dose: 35.6%, low dose: 4.1%, control: 2.7%, pair-fed: 16.4%) were observed in these DBTC-exposed groups as evidence for effects on

fertility. The fertility effects for the high dose group were statistically significantly different from the control group as well as from the pair-fed group, whereas effects for the mid dose groups were statistically significantly different from the control group. Slight general toxicity was observed and included reduced body weight and feed consumption in the high dose group (for details on body weight (BW) changes please refer to Annex I of the background document (BD))). Upon DBTC-exposure during GD 4-7 increased early total resorptions were observed in the high dose group (87.5%; statistically significantly different from the control (0%) and from the pair-fed groups (11.8%)). Also in these animals, some slight general toxicity was observed and included reduced adjusted bw gain (i.e. excl. the uterus) and reduced feed consumption (for details on BW changes please refer to Annex I of the BD). RAC evaluated the general toxicity effects and concluded that the reproductive effects are not due to a secondary non-specific consequence of parental toxicity.

In a developmental toxicity study with the CD1 mice, groups of mated female were administered DBTC (in olive oil) via gavage at dose levels of 0 (vehicle control), 7.6, 15.2 or 30.4 mg/kg bw/d on GD 0-3 or GD 4-7. Dam mortality occurred in all treated groups but without a dose-response relationship. Other signs of toxicity (vaginal discharge, hypo activity, hypothermia) were also observed at all dose levels and jaundice in the medium and high doses. Body weight and food consumption were also affected negatively. Regarding the number of pregnant females, there was a dose related increase in the pre-implantation loss with the dose administered (29.7% - 7,6 mg/kg bw/d, 34.0% - 15.2 mg/kg bw/d, 58.3% - 30.4 mg/kg bw/d at low, mid and high dose respectively) that was statistically significant in the high dose. As for the post implantation losses, they also were increased in a dose-dependent manner and the effect was statistically significant from the mid dose (15.2 mg/kg bw/d).

Four additional studies on the mechanism of toxicity of DBTC seem to support that DBTC negatively influences the levels of progesterone, hence causing the observed implantation losses (Ema *et al.*, 2003a,b; Harazono & Ema, 2003; Harazono & Ema, 2001).

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 fertility upon exposure to DBTC.

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

Altogether, RAC supports the conclusion of the DS that DBTP should be classified as toxic to reproduction for effects on sexual function and fertility as Repr. 1B (H360F: May damage fertility).

Development

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 bw/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 haemorrhages 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 5 mg/kg bw/d (i.e. without the presence of maternal toxicity).

Three additional studies on developmental toxicity conducted to assess the most sensitive gestation window for exposure to DBTC indicated that 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).

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, shows a comparable spectrum of effects (incidence and type of foetal malformations) for all substances, in the absence of maternal toxicity.

In addition, the sensitivity of the rat foetus to DBTC was confirmed by several *in vitro* studies.

There is some uncertainty whether the typical pattern of malformations observed is rat specific; however, as there is insufficient information on other species, RAC agrees with the DS that these findings should be considered relevant.

Overall, several studies consistently showed foetal effects (malformations, post-implantation loss and weight reduction) at doses with limited or no maternal toxicity, and there is no basis to question the human relevance of these effects. Thus, RAC considers that there is clear evidence of an adverse effect on development upon exposure to DBTC.

Given that both DBTP and DBTC are hydrolysed to the same distannoxane, RAC is of the opinion that data on DBTC can be used for classification of DBTP for effects on development (see also under *RAC general comment*).

RAC supports the conclusion of the DS that DBTP should be classified as toxic to reproduction for developmental toxicity in Category 1B, leading to the overall classification of **Repr. 1B** (**H360D; May damage the unborn child**).

Specific Concentration Limits (SCL)

Setting of Specific Concentration Limits for reproduction toxicity (effects on sexual function and fertility and on development) is not considered necessary in this case given that ED_{10}-values for DBTDL fall within the ranges of a medium potency group (i.e. 4 mg/kg bw/d < ED_{10} < 400 mg/kg bw/d) and modifying factors which might changing the potency group are considered not needed, resulting in the GCL of 0.3% (see ECHA Guidance on the Application of the CLP Criteria v. 5.0, section 3.7.2.5).

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).