Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Dibutyltin oxide

EC Number: 212-449-1
CAS Number: 818-08-6

CLH-O-0000007033-84-01/F

Adopted

16 September 2021

16 September 2012

CLH-O-0000007033-84-01/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 oxide**

EC Number: **212-449-1**

CAS Number: **818-08-6**

The proposal was submitted by **Austria** and received by RAC on **2 September 2020.**

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

PROCESS FOR ADOPTION OF THE OPINION

Austria 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 November 2020**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **29 January 2021**.

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 **16 September 2021** 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 oxide	212-449-1	818-08-6	Muta. 2 Repr. 1B Acute Tox. 3 STOT RE 1 Skin Corr. 1 Eye Dam. 1	H341 H360FD H301 H372 (immune system) H314 H318	GHS08 GHS06 GHS05 Dgr	H341 H360FD H301 H372 (immune system) H314		oral: ATE = 172 mg/kg bw	
RAC opinion	TBD	dibutyltin oxide	212-449-1	818-08-6	Muta. 2 Repr. 1B Acute Tox. 3 STOT RE 1 Skin Irrit. 2 Eye Dam. 1	H341 H360FD H301 H372 (immune system) H315 H318	GHS08 GHS06 GHS05 Dgr	H341 H360FD H301 H372 (immune system) H315 H318		oral: ATE = 170 mg/kg bw	
Resulting Annex VI entry if agreed by COM	TBD	dibutyltin oxide	212-449-1	818-08-6	Muta. 2 Repr. 1B Acute Tox. 3 STOT RE 1 Skin Irrit. 2 Eye Dam. 1	H341 H360FD H301 H372 (immune system) H315 H318	GHS08 GHS06 GHS05 Dgr	H341 H360FD H301 H372 (immune system) H315 H318		oral: ATE = 170 mg/kg bw	

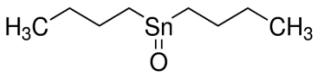
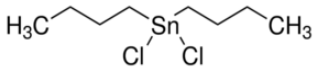
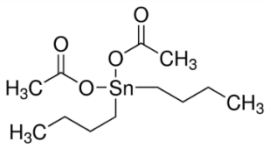
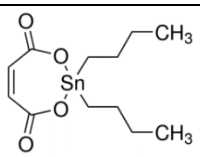
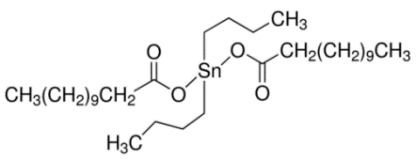
GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The dossier submitter (DS) proposed to classify dibutyltin oxide (abbreviated throughout this document as DBTO) for acute oral toxicity, skin corrosion, serious eye damage, mutagenicity, STOT RE and reproductive toxicity. In addition to studies performed with DBTO itself, reference was made to studies performed with the following substances as part of a read across, category approach: DBTC, DBTM, DBTA, DBTL and DBTP (see 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 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 (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% No further details (monoconstituent substance)
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 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 dialkyltin compounds form DBTC after hydrolysis. However, recent *in vitro* hydrolysis studies which used 119Sn-NMR spectroscopy showed that both DBTC and the related compounds DBTP and DBTM form the distannoxane $ClBu_2SnOSnBu_2Cl$.

The CLH dossier of DBTO includes one *in vitro* gastric simulation study, performed with GC-FPD, which showed a conversion under simulated gastric conditions to DBTC. It is noted that this detection method cannot distinguish whether the distannoxane $ClBu_2SnOSnBu_2Cl$ is formed, however considering the behaviour of the other category members, this is highly likely.

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

Considering the metabolism studies and similar toxicological profiles the RAC agrees with the read across approach proposed by the DS. In accordance with this approach, the classification proposal for DBTO for mutagenicity, STOT RE, and reproductive toxicity is mainly based on studies performed with DBTC, and supported by studies with related dibutyltin compounds. This is also consistent with the RAC opinions of dibutyltin dibutyltin di(acetate) (DBTA), dibutyltin maleate (DBTM), dilaurate (DBTL), dibutylbis(pentane-2,4-dionato)-OO')tin (DBTP) and dibutyltin bis(2-ethylhexanoate) (DBTE).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity – oral

Summary of the Dossier Submitter's proposal

There are 10 acute oral toxicity studies included in the CLH dossier, all performed with DBTO itself. One study was a modern OECD test guideline (TG) 423 study (GLP), four were OECD TG 401 studies performed in the period 1978-1983 and five were older non-guideline studies.

The OECD TG 423 study resulted in an LD_{50} of 500 mg/kg bw (Anonymous, 2019). Two of the older, non-guideline studies gave LD_{50} values in the same order of magnitude, namely 487 mg/kg bw (Anonymous, 1971) and 520 mg/kg bw (Klimmer, 1969). Two OECD TG 401 studies reported lower LD_{50} values of 172 (121-240) mg/kg bw and 260 (209-311) mg/kg bw, respectively (Anonymous, 1983 and Anonymous, 1978). Higher values were reported in Anonymous (1980a, b) with a value of > 794 mg/kg bw and Anonymous (1971) with a value of > 10000 mg/kg bw.

No explanation for the difference in outcomes was provided in the dossier. There was no relationship with sex of the animals, or the vehicle used. It was also noted that for none of the presented studies detailed information on the test substance, or on impurities that could explain the differences in outcome, was available.

Due to these considerations, the DS proposed to use the lowest LD_{50} value of 172 (121-240) mg/kg bw (males (m)/females (f)) justifying classification as Acute Tox. 3; H301, with an ATE value of 172 mg/kg bw.

Comments received during consultation

Two comments were received from Member State Competent Authorities (MSCAs) who agreed with the proposed classification and ATE. Six comments submitted by industry representatives disagreed with the classification in Category 3 and instead considered that classification in Category 4 based on the most recent OECD TG 423 study was more appropriate. The reasoning was that the impurities in the test material of the Anonymous (1983) study were unknown and the newer study is performed and reported according to modern standards. For these reasons, all studies except Anonymous (2019) and Anonymous (1980b) were given Klimisch scores of 4 by the registrants.

The DS replied that the OECD TG 401 study used as key study in the classification proposal was a well-documented guideline study. The study was also judged as valid and used as key study in the OECD SIDS Dossier for DBTO (2008). Moreover, the purity of the test substance was missing for all studies, including the recent OECD TG 423 study. For these reasons the DS disagreed with the registrants and considered that the outcome of the OECD TG 401 study cannot be dismissed.

Assessment and comparison with the classification criteria

A large amount of data is available on the acute oral toxicity of DBTO. Of the ten studies, five were performed according to OECD TGs. The LD_{50} values reported span a wide range of 172 to >794 mg/kg bw, excluding the oldest non-guideline studies, or 60-10 000 mg/kg bw if all studies are included. Gross necropsy revealed severe damage to the digestive system in deceased animals.

There was no clear link between the outcome of the studies and the rat strain or vehicle used. Both male and female rats were included and there was no notable difference in sensitivity. As no information was given on the impurities in the test material for any of the studies including the more recent study, it cannot be evaluated whether this was a factor of influence or not.

The guideline study with the lowest LD_{50} of 172 mg/kg bw (m/f) was an OECD TG 401 study in Tif:RA1f (SPF) rats, with six doses ranging from 50 to 525 mg/kg, with 5 animals/sex/dose.

RAC agrees with the DS that the limitations in reporting on the test material are not sufficient reasons to discard the outcome of this study. For all other parameters, the study was well reported and showed a very consistent pattern over the entire dose range.

In conclusion, considering the LD_{50} of 172 mg/kg bw (m/f), **RAC considers that classification of DBTO as Acute Tox. 3; H301 (Toxic if swallowed) is warranted** rounding down the **ATE value to 170 mg/kg bw**.

RAC evaluation of acute toxicity – dermal

Summary of the Dossier Submitter's proposal

Two acute dermal toxicity studies were performed with DBTO, an OECD TG 402 study in rats (Anonymous, 2010a) and an older non-guideline study in rabbits (Anonymous, 1975). In both studies, local irritation was observed, but there were no signs of systemic toxicity or deaths up to the top dose of 2000 mg/kg bw. The DS proposed no classification for acute dermal toxicity.

Comments received during consultation

One MSCA expressed support for no classification.

Assessment and comparison with the classification criteria

There is one guideline study in rats and a non-guideline study in rabbits available investigating acute dermal toxicity of DBTO. Both studies were performed with a top dose of 2000 mg/kg bw and no mortality was observed in either one.

As the criteria for classification are not fulfilled, **RAC considers that no classification for Acute toxicity by the dermal route is warranted**.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

There are two non-guideline studies with DBTC that investigated the effects of a single dose on the thymus of rats (Snoeij *et al*., 1989) and SCID-hu mice (SCID mice engrafted with human foetal thymus and liver tissue fragments; de Heer *et al*., 1995), respectively.

In the rat study, a single dose of 15 mg/kg bw was given by gastric intubation. DBTC induced rapid but reversible atrophy of the thymus in the 9-d observation period.

Lower doses of 0.03 and 1.0 mg/kg bw were given intraperitoneally to SCID-hu mice engrafted with human thymus and liver tissue fragments. DBTC induced a reduction in thymus cortex size.

Although effects occurred after single application below the guidance value for STOT SE Category 1 of 300 mg/kg bw, both studies are non-standard mechanistic studies with few animals and limitations in the description of the results. In addition, thymus effects were according to the DS already covered by the proposed STOT RE 1 classification. Hence, the DS proposed no classification for STOT SE.

Comments received during consultation

Four comments were received for STOT SE; three of these came from industry representatives and were general objections to the category approach.

One MSCA disagreed with the proposal for no classification and argued that classification as STOT SE Category 1 was warranted. The reasoning presented was that although both studies had limitations, their results were very much in line with those from repeated dose studies, including both target organ and effective dose range. It was also stated that reversibility and a proposed classification for STOT RE were no valid justifications to forfeit classification for STOT SE.

The DS replied that these studies had been previously considered for other category members, such as DBTP (Dibutylbis(pentane-2,4-dionato-O,O')tin), which then did not result in classification. It was also considered that the evidence for STOT RE was more comprehensive; hence, this classification was preferred for thymus toxicity.

The Industry representatives presented arguments against the use of the category approach and using read across between category members, arguing that new hydrolysis studies show that a dimer (DBDTC distannoxane dimer) is formed rather than DBTC itself (Ghobrial *et al*., 2019; Munschi *et al*., 2010; Patel *et al*., 2009; Alwyn, 2009). According to the comments, the dimeric structure is the only metabolite of the gastric hydrolysis and has a molecular weight of 1089.4 Dalton. With this high molecular weight, the dimeric distannoxane is by far too heavy to be biologically active and have a low reaction potential because the molecules are too large to pass through biological membranes, limiting their bioavailability. They also noted that further testing

related to the read across is planned. The DS replied that the available information indicates that common metabolites/intermediates are formed in vivo, and affect common biological targets, which supports the applicability of a read across from category members (e.g., DBTC) to DBTO for oral toxicity studies (germ cell mutagenicity, reproductive toxicity, specific target organ toxicity repeated exposure and specific target organ toxicity single exposure). The DS concluded, that based on available information, the read across is robust and it is valid to consider data of category members to read across for these endpoints. The DS also took note of the new studies.

Assessment and comparison with the classification criteria

RAC agrees with the replies by the DS regarding the use of the category approach and considers that based on the available data, read across between category members can used for STOT SE. Also see 'RAC general comment'.

Two studies are presented that specifically addressed the potential of DBTC to induce thymus toxicity after single exposure. Both studies have been included previously for category members as mechanistic evidence for the assessment of STOT RE. No additional evidence is available from the acute toxicity studies. Repeated dose studies consistently showed thymus toxicity but did not include examinations after one day.

The effect observed after a single exposure consists of reversible thymus atrophy. Although also reversible effects should be considered for classification, RAC did not consider that there was 'clear evidence of marked organ dysfunction' in these single exposure studies as required for the STOT SE classification. In addition, one of the studies was conducted intraperitoneally which is a less relevant route for STOT SE.

RAC also considered the classification for Acute Tox. 3 (oral). As the ATE value is with the guidance value range for STOT SE 1, classifying for both endpoints would result in double classification.

For these reasons, **RAC considers that no classification is warranted for STOT SE**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The CLH report includes three skin irritation studies, an OECD TG 404 study, a non-guideline study and a report from the OECD SIDS (OECD Screening Data Set). In addition, the findings from the two acute dermal toxicity studies were used as supporting evidence.

The OECD TG 404 study (Anonymous, 1994) in rabbits was negative for corrosivity under occlusive conditions for 3 minutes and 1 hour. After exposure for 4 hours under semi-occlusive conditions, DBTO induced erythema and oedema with mean (24, 48, 72h) scores of 1.78 and 0.72 for erythema and oedema, respectively. Maximum scores for both effects were 2. Erythema was not fully reversible within the observation period of 14 days as very slight to slight erythema were present on all sites.

In the non-guideline study (Anonymous, 1975) with three rabbits exposed for 24 hours, DBTO induced third-degree burns in two animals and second-degree burns in one animal. Mean scores of 4 were assigned for both erythema and oedema.

In the study cited by OECD SIDS (2008), four rabbits were exposed for 3-4 hours under semi-occlusive conditions, resulting in reddening and swelling with induration of the skin in all animals on days 5-9 and induration, demarcation and skin necrosis on days 6-10.

Based on the severe irritation, including skin burns, observed in the skin irritation study by Anonymous (1975) and in the acute dermal toxicity studies, the DS proposed classification of DBTO as Skin Corr. 1.

Comments received during consultation

Comments were received from six industry representatives and two MSCAs. All industry representatives objected to the proposed classification in Category 1 as no corrosion was observed in the OECD TG 404 study and the older skin irritation study, and also since the acute dermal toxicity studies used 24-h rather than 4-h exposure.

One MSCA preferred a classification in Category 2 rather than Category 1 as corrosion was only observed after 24-h occlusive exposure, which is not in line with the CLP criteria.

The other MSCA supported the proposed classification and suggested that as the effects in Anonymous (1994) occurred following 4-h exposure sub-category 1C could be considered.

The DS replied that although the OECD TG 404 study supports classification in Category 2, the other studies provide sufficient evidence for Category 1. In particular it was noted that as rats are less sensitive than rabbits, skin corrosivity in a rat dermal toxicity test indicates that a Category 1 classification is justified. It was noted that as the only study with 4-h exposure did not show corrosivity, it cannot be used to derive a sub-categorisation.

Assessment and comparison with the classification criteria

Three skin irritation and/or corrosion studies in rabbits are available, all studies performed with DBTO. The skin irritation studies included an OECD TG 404 study (Anonymous, 1994) and two older studies, for one of which there was only a secondary source. Of the two acute toxicity studies, one was performed in rats and one in rabbits. The studies are summarised in the table below.

***Table**: Overview of the results from the skin irritation and dermal toxicity studies. All studies were performed with DBTO.*

Method, test guideline, deviations if any	**Species, strain, sex, no/group**	**Dose levels, duration of exposure**	**Results** **-Observations and time point of onset** **-Mean scores/animal** **-Reversibility**	**Reference**
OECD TG 404 GLP	Rabbit, New Zealand White N=6 (3m+3f)	0.5 g/side Duration: 3 min / 60 min: for corrosion testing, occlusive 4 h: for irritation testing, semi-occlusive 4 h-exposure: DBTO was moistened with deionised water Purity: 98.5% Removal: wiped with tap water	Corrosion (3min / 60 min exposure): no effects Irritation (4h-exposure): Erythema score: 24h (mean 1.83, max 2) 48h (mean 1.67, max 2) 72h (mean 1.83, max 2) 14d (mean 1.83, max 2), desquamation Not fully reversible within 14 days. Oedema score: 24h (mean 1, max 2) 48h (mean 0.17, max 1) 72h (mean 0, max 0) Fully reversible	Anonymous, 1994

Method, test guideline, deviations if any	Species, strain, sex, no/group	Dose levels, duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
Primary skin irritation study	Albino rabbits	500 mg un-diluted Exposure: 24 h Occluded, abraded and intact skin Observation: 24 h, 72 h	Extremely irritating. Third-degree chemicals burns (24/48h): 2/3 Second-degree chemical burns (24/48h): 1/3	Anonymous, 1975 (OTS0570737)
- GLP	Rabbit N=4 (2m+2f)	500 mg / 0.19 mL peanut oil Semi-occlusive Exposure 3-4 h	Irritating. Reddening and swelling with induration of the skin in all animals on days 5-9. Induration, demarcation, and skin necrosis at days 6-10. No local effects at day 30.	OECD SIDS, 2008 Secondary source
OECD TG 402 GLP	Rat, Wistar N=5m/5f per dose	2000 mg/kg bw Semi occlusive, 24 h Removal of residual test material with arachis oil (vehicle)	Dermal reactions (erythema, very slight oedema, haemorrhage of dermal capillaries, small superficial scabs, glossy skin).	Anonymous, 2010a
Acute dermal toxicity study	Albino rabbit N=1/dose	200, 500, 2000 mg/kg bw slurry in 3% (w/v) aqu. methylcellulose Abraded skin	24 h: red, well defined erythema, moderate to severe oedema and second-degree burns (all animals) Day 7: well defined erythema, mild oedema, escharosis, and wrinkling Day 14: escharosis, and severe desquamation	Anonymous, 1975 (OTS0570737)

According to the CLP criteria, all existing human and animal data should be considered, including acute dermal toxicity studies provided that the dilutions used, and species tested, are equivalent. In the evaluation, the method of application, exposure time, and species should be considered due to the differences between studies performed under different guidelines.

For the OECD TG 404 study with six rabbits (Anonymous, 1994), the following criteria apply with regard to severity:

a. Classification as skin corrosive – Category 1 if destruction of skin tissue (visible necrosis through the epidermis and into the dermis) occurs in at least one animal after exposure up to 4 hours.

b. Classification as skin irritant – Category 2 if at least 4 out of 6 rabbits show a mean score per animal of ≥ 2.3 ≤ 4.0 for erythema/eschar or for oedema.

In the short exposure periods of 3 min and 1 h, no corrosion was observed. No individual scores after 4 hr exposure were included in the CLH report, but the max scores were 2 for both erythema and oedema. This means that the severity criterium for irritation was not met.

Classification for irritation can also be warranted due to persistence of the effects:

Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling

Erythema and desquamation persisted over the duration of the study of 14 days, while oedema was reversible after 72 h. Although no individual data are given it was described by the DS as "*very slight to slight erythema were present on all sites at study termination on day 14.*" Considering the erythema persisted to the end of the observation period, the criterium for classification based on irreversibility is met.

The second study was an older non-guideline skin irritation study (Anonymous, 1975) in which three rabbits were exposed for 24 h, on occluded, abraded and intact skin. All three rabbits showed chemical burns and scores of 4 for erythema and oedema after 24 h and 72 h.

The interpretation of this study is more difficult, as the exposure is longer and it takes place under more severe conditions than in the guideline studies. The Guidance on the application of the CLP criteria (CLP guidance, ECHA, 2017) states the following on the interpretation of such studies: "*Calculation of mean scores should normally be restricted to the results obtained from intact skin. In case of pronounced responses at the 72 h-time point an expert judgement is needed as to whether the data is appropriate for classification.*" It is not entirely clear from the available information whether one or two rabbits with intact skin were included. On the other hand, all rabbits showed strong irritation reactions. Overall, this study supports classification for irritation, but is of insufficient quality to be used as the key study.

The third irritation study was cited from a secondary source (OECD SIDS, 2008). Four rabbits were exposed for 3-4 h under semi-occlusive conditions. No scores were given for erythema or oedema, but these effects were described after 5-9 days, increasing in severity at days 6-10 to induration, demarcation and skin necrosis. The effects were no longer observed after 30 days. Due to the limitations in the available information, it is difficult to judge the reliability of this study. Remarkable in this case is the apparent delay in the induction of irritation. Although this was not noted in the other irritation studies with DBTO, two dermal irritation studies in the CLH dossier for DBTM showed a very similar delay. Again, this study can only be used as supportive information as it is currently presented.

In addition to the skin irritation studies, the effects observed in the two acute dermal toxicity studies in rats and rabbits are relevant for the evaluation of skin irritation. Both studies reported clear effects indicative of irritation and/or corrosion after 24-h exposure. It should be noted that the rabbit skin was abraded, which resulted in effects indicative of corrosion. Unfortunately, no scoring was provided and there is no information on the effects after 4 h of exposure.

In conclusion, only one guideline study with 4 h exposure is available, which indicates some, but not severe skin irritation, that did not fully recover within 14 days. A secondary source noted more severe effects after 4 h, but the information was very limited and there was a delay in effects not observed in any other study. Several studies with longer exposure times showed strong irritation/corrosion but did not include shorter observation periods and some used abraded skin. Taking into account these factors, **RAC considers that classification as Skin Irrit. 2; H315 (Causes skin irritation) is warranted**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

Three *in vivo* eye irritation tests in rabbits were included as well as one *in vitro* SkinEthic Reconstituted Human Corneal model.

In an OECD TG 405 (Anonymous, 2010b), corneal opacity, blepharitis, and redness, chemosis and discharge of the conjunctiva were seen in one rabbit after administration of 93 mg DBTO to one eye. The effects increased in severity over time and were not reversible within 14 days.

A second non-guideline *in vivo* study in three rabbits (Anonymous, 1975) reported chemical burns and corrosion in all animals after exposure to 100 mg, without reversibility in 14 days. 29.7

The third study was a description from the SIDS dossier (2008), in which 72 mg was instilled into the eyes of six rabbits. The maximum average score was 19.5 (of 110 possible) at 72 h. Secondary effects like corneal oedema, hypopyon, corneal neovascularisation (including pannus) and the formation of scleral vesicles were described. At 21 days, corneal irritation in two animals was still present.

The *in vitro* study (Anonymous, 2010c) was based on transformed human keratinocytes that form a corneal epithelial tissue. The test is based on the hypothesis that irritant chemicals are able to penetrate the corneal epithelial tissue and are sufficiently cytotoxic to cause cell death (measured by a reduction of MTT (3[4,5dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide). Exposure was 10 min with 30 mg DBTO. The resulting tissue viability was 93.6% vs 100% in the negative control. As the threshold for irritation is 60%, DBTO was not irritating. It was noted that the test was not in line with the OECD TG 492, and amongst others the exposure time was too short.

As DBTO induced irreversible, worsening effects on cornea, iris and conjunctiva in a recent guideline study with mean scores (24, 48, 72 h) of 0.67, 1, 2, 2.33 for cornea opacity, iris, conjunctival redness and chemosis, respectively. Severe eye effects were also observed in an older eye irritation study and a secondary source.

Based on these effects, the DS proposed to classify DBTO as Eye Dam 1. It was noted by the DS that due to their proposed classification for Skin Corr. 1, classification for Eye Dam. 1 is already implicit.

Comments received during consultation

Two MSCAs and one industry representative commented specifically on the proposal for eye damage. All agreed that the available data support classification in Category 1. However, one MSCA and the industry representative disagreed with the classification for Skin Corr. 1 and noted that if DBTO is not classified for skin corrosion, the automatic classification for Eye Dam. 1 should be removed.

Assessment and comparison with the classification criteria

There are three *in vivo* studies in rabbits available, including one guideline study, which consistently show that DBTO induces irreversible eye damage.

According to the criteria, a substance should be classified in Category 1 if it produces:

(a) in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or
(b) in at least 2 of 3 tested animals, a positive response of:
    (i) corneal opacity ≥ 3; and/or
    (ii) iritis > 1,5
calculated as the mean scores following grading at 24, 48 and 72 h after installation of the test material.

Only the study cited in the OECD SIDS had an observation period of 21 days, in which no full reversion was observed in two animals. However, the other two studies (Anonymous, 1975 and

Anonymous, 2010b) showed such severe effects after 14 days that reversibility was deemed highly unlikely. The effects observed in Anonymous (1975) also met the (b) criterium for severity.

As both criteria are met, **RAC concludes that DBTO warrants classification as Eye Dam. 1; H318 (Causes serious eye damage)**.

As no classification for skin corrosion is proposed, a separate classification for eye damage is necessary.

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

Summary of the Dossier Submitter's proposal

There is one prenatal developmental toxicity study (PNDT, OECD TG 414) available with DBTO itself. In addition, 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 can be taken into consideration when classifying DBTO for this hazard class.

In the PNDT study in rats, a significant reduction in both absolute and relative thymus weights was observed at GD 20 after exposure to 0.75, 3 and 6 mg/kg bw/d DBTO. The weight reduction showed a clear dose-response relationship (Unpublished report, 2017).

Only one 90-d study is available, and it was performed with DBTC. 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 reproductive/developmental toxicity screening study according to OECD TG 421 (diet) with DBTC in rats (Unpublished report, 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 exposure. A dose of 6.2-15.4 mg/kg bw/d induced reduced absolute and relative thymus weight and severe to very severe lymphoid depletion in dams.

A 28-d rat/mouse immunotoxicity study with doses of 0, 50 and 150 ppm DBTC 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 dossier (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 old 6-month non-guideline study in rats showed reduced weight gain, food consumption and mortality, with a LOAEL of 2.5 mg/kg bw/d (Barnes and Stoner, 1958).

Two OECD TG 414 studies in rats (both oral gavage; 0, 1, 2.5, 5, 10 mg/kg bw/d) showed clear maternal toxicity (Study Report, 1994; Farr *et al*., 2001). Effects included reduced bw gain (10 mg/kg bw/d), reduced food consumption (10 mg/kg bw/d) and significantly increased number of animals with thymus atrophy (≥ 2.5 mg/kg bw/d). Maternal toxicity was not observed at a dose of 1 mg/kg bw/d.

Further investigation of the effects of DBTC on the immune system was reported by DeWitt *et al.* (2005, 2006), both in rats. Both dams and offspring were exposed to relatively low levels of DBTC (up to 5 mg/kg bw/d for direct exposure of offspring) via oral route but no effects on the immune system were reported.

Several mechanistic immunotoxicity studies were included in the CLH 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 DBTC.

The DS considered the 28-d study (Seinen & Vos, 1977), 14-d study (Penninks & Seinen, 1982) and reproduction/developmental screening study (Unpublished report, 2003) to be the key studies. All three studies showed thymus toxicity at low dose levels. As DBTO showed similar potency to DBTC in the study by Noda *et al*. (1993), no adjustment was proposed for molecular weight. The effective doses from the 28-d and 56-d studies (see RAC note on the 56-d study further down) were extrapolated to the 90-d equivalents of 0.8-1.25 mg/kg bw/d, which are clearly within the guidance value range 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 the target organ.

Comments received during public consultation

Three comments from MSCAs were received, all indicating support for the category approach, and all were in favour of the proposed classification as STOT RE 1.

Seven industry representatives commented, presenting arguments against the use of the category approach and using read across between category members, arguing that new hydrolysis studies show that a dimer (DBDTC distannoxane dimer) is formed rather than DBTC itself (Ghobrial *et al*., 2019; Munschi *et al*., 2010; Patel *et al*., 2009; Alwyn, 2009). According to the comments, the dimeric structure is the only metabolite of the gastric hydrolysis and has a molecular weight of 1089.4 Dalton. With this high molecular weight, the dimeric distannoxane is by far too heavy to be biologically active and have a low reaction potential because the molecules are too large to pass through biological membranes, limiting their bioavailability. They also noted that further testing related to the read across is planned. The DS replied that the available information indicates that common metabolites/intermediates are formed in vivo, and affect common biological targets, which supports the applicability of a read across from category members (e.g., DBTC) to DBTO for oral toxicity studies (germ cell mutagenicity, toxicity to reproduction, specific target organ toxicity repeated exposure, specific target organ toxicity single exposure). The DS concluded, that based on available information, the read across is robust and it is valid to consider data of category members to read across between these endpoints. The DS also took note of the new studies.

Assessment and comparison with the classification criteria

Given that both DBTO and DBTC are hydrolysed to the same distannoxane, RAC is of the opinion that data on DBTC can be used for classification of DBTO for STOT RE. RAC agrees with the DS's arguments and concludes that the read across is robust and valid and that data from the category members can be used to assess STOT RE. Also see '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.

The only study with DBTO itself that used multiple dose levels as well as a repeated dosing regime is the OECD TG 414 study (Unpublished report, 2017). Mean absolute thymus weights were 19%, 34%, and 44% lower than the mean control value in the 0.75, 3.0, and 6.0 mg/kg bw/day dose groups, respectively, and relative to the adjusted GD 20 body weights, they were 20%, 35%, and 37% lower, respectively (see the table below).

***Table**: Thymus weight, and thymus weight adjusted for bw on GD20, after treatment with DBTO (Unpublished report, 2017).*

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

**statistically significantly different compared to control values (p<0.01)*

No histopathology was performed on the thymus in this study, which increases the uncertainty on the severity of this effect. On the other hand, similar reductions in thymus weight were observed in the studies with DBTC and were accompanied by lymphoid depletion. In particular Seinen & Vos (1977) noted reductions of 53% at 2.5 mg/kg bw/d and 68-72% at 7.5 mg/kg bw/d after 28 days of exposure, which were accompanied by marked lymphocyte depletion. The Unpublished report (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 exposure (in the CLH report the exposure length is indicated as 56 days but it is not clear to RAC how this was calculated).

The outcome of these studies confirms that DBTO has similar toxicity compared to DBTC as well as similar potency.

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 DBTC are within the extrapolated guidance value ranges for classification as STOT RE 1 (i.e., 10, 30 and 60 mg/kg bw/d for a 90-d, 28-d and 14-d study, respectively). As there is only a small difference in molecular weight between DBTC (303.84 g/M) and DBTO (248.92 g/M), this applies to the equivalent values of DBTO as well.
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 **DBTO warrants classification 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 DBTO itself, and it was negative. The evaluation of mutagenicity was thus based on studies with DBTC as well as on one study with DBTDL. These studies have been evaluated previously for other category members, most recently for DBTA (RAC opinion, 2020). Apart from the bacterial reverse mutation assay, no new information was added in this evaluation.

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 dossier. 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–guideline, GLP not specified; 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) (Lang and Schmitt , 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 dossier, 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 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 48 h and 72 h after exposure of mice to DBTC at 50 mg/kg bw, with effects more clearly seen in female compared to male animals. No positive result was obtained upon DBTC-exposure at the post-treatment time-interval of 24 h.

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). In this second micronucleus test DBTC did not show any evidence of

mutagenic potential up to the (toxic) dose level of 200 mg/kg bw as measured at 24 h, 48 h and 72 h 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 DBTO or other members of its category, there is insufficient evidence to warrant classification in Category 1B. There is 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 has been previously classified as Muta. 2.

The DS proposed to classify DBTO also as Muta. 2 based on a category approach.

Comments received during consultation

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

Six industry representatives commented. They presented arguments against the use of the category approach and using read across between category members, arguing that new hydrolysis studies show that a dimer (DBDTC distannoxane dimer) is formed rather than DBTC itself (Ghobrial *et al*., 2019; Munschi *et al*., 2010; Patel *et al*., 2009; Alwyn, 2009). According to the comments, the dimeric structure is the only metabolite of the gastric hydrolysis and has a molecular weight of 1089.4 Dalton. With this high molecular weight, the dimeric distannoxane is by far too heavy to be biologically active and have a low reaction potential because the molecules are too large to pass through biological membranes, limiting their bioavailability. They also noted that further testing related to the read across is planned. The DS replied that the available information indicates that common metabolites/intermediates are formed in vivo, and affect common biological targets, which supports the applicability of a read across from category members (e.g., DBTC) to DBTO for oral toxicity studies (mutagenicity, toxicity to reproduction, specific target organ toxicity repeated exposure, specific target organ toxicity single exposure). The DS concluded, that based on available information, the read across is robust and it is valid to consider data of category members to read across between these endpoints. The DS also took note of the new studies.

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 DBTO itself is a negative *in vitro* gene mutation study in bacteria. This result is in line with results of category members, which do not indicate mutagenic effects in bacterial mutagenicity assay either. RAC agrees with the DS replies on the use of the category approach. As DBTO forms at least in part the same metabolite as DBTC, RAC considers the proposed read across valid for germ cell mutagenicity. Also see '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 dossier. 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 DBTO or DBTC. However, a positive result was obtained from a well-performed OECD TG- 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 suggests 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.

Taking all available data into account, RAC concludes that **DBTO warrants classification for germ cell mutagenicity as Muta. 2; H341 (Suspected of causing genetic defects).**

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Adverse effects on sexual function and fertility

No data are available for DBTO; thus, the evaluation was based on studies with DBTC. This evaluation has been made previously for several category members, including DBTA (RAC opinion, 2020), DBTP (RAC opinion, 2017) and DBTDL (RAC opinion, 2015.

The OECD TG 421 study in rats with DBTC (Unpublished report, 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 mid and high dose group vs. 9/12 in the controls) and only 3/7 pregnant high dose females delivered offspring. This resulted in a reduction in the number of live pups (10 vs. 101 in controls).

A fertility study with DBTC (Ema & Harazono, 2000) was reported in which female rats were exposed via oral gavage to DBTC in olive oil (0, 3.8, 7.6 and15.2 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 in the mid and high dose exposed on GD 0-3 (number of pregnant dams in high dose: 2/16, mid dose: 11/16, low dose: 16/16, control: 19/19, pair-fed: 16/19). Further, a reduced number of implantations (number of implantations in 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.

In a developmental toxicity study in the CD1 mice (Ema *et al*., 2007a), 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 dose groups. Body weight and food consumption were also affected negatively. Regarding the number of pregnant females, there was an increase in the pre-implantation loss in dams treated on GD 0-3 with the

dose administered (29.7% at 7,6 mg/kg bw, 34.0% at 15.2 mg/kg bw, 58.3% at 30.4 mg/kg bw) that was statistically significant in the high dose group. An increase in pre-implantation loss was seen in the high dose group also in dams treated on GD 4-7; however, not statistically significant. Post-implantation losses increased with the dosing and the effect at the mid dose (15.2 mg/kg bw) was also statistically significant (see 'Adverse effects on development').

A supportive mechanistic study explored the effect of progesterone on implantation failure induced by DBTC in rats. DBTC administration (7.6 and 15.2 mg/kg bw/d on GD 0-3 or GD 4-7) lead to reduced uterus weight and serum progesterone levels in dams treated GD 0-3 and GD 4-7. Oestradiol levels and corpora lutea numbers were unaffected by treatment. Administration of progesterone reversed the suppression of uterine decidualisation (Harazono & Ema, 2003).

Administration of progesterone caused slightly lower pre-implantation loss after exposure on GD 0-3, but offered no complete protection (8.6%, 62.8%, 81.3% at dose levels of 0, 7.6, 15.2 mg/kg bw, respectively, without progesterone; 10.5%, 25.9% and 60.6%, respectively, with application of progesterone) (Ema *et al*., 2003).

Based on the increased number of non-pregnant females among successfully mated females, the reduced number of implantations, and 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 DBTO should have the same classification as DBTC. The DS therefore proposed Repr. 1B; H360F for adverse effects on sexual function and fertility for DBTO.

Adverse effects on development

There is one developmental toxicity study available with DBTO (Unpublished report, 2017), as well as a large number of studies with DBTC or DBTA and one study performed with DBTDL, DBTM and DBTA in addition to DBTO and DBTC. All studies except the one performed with DBTO have been evaluated previously for category members. The CLH dossier divides the studies into three groups: studies with DBTO, studies with DBTC, and studies with DBTA.

Studies with DBTO

The study with DBTO (Unpublished report, 2017) was an OECD TG 414 study in which 25 SD rats/dose were exposed on GD 0-19 at dose levels of 0, 0.75, 3, and 6 mg/kg bw/d. The selection of the top dose was based on a range finding study in which 40% of the dams had to be euthanised at 9.5 mg/kg bw/d. Effects were only observed at the high dose. Maternal effects consisted of lower body weights (-8% at GD 18, -9% at GD 20), lower body weight gain, and lower food consumption and clinical findings (low body carriage, red material around the nose, thin appearance, loss of skin elasticity, and pale body colour). Two dams were euthanised in extremis due to general toxicity; these dams were not pregnant. An overview of the developmental effects observed in this study is given in the table below.

Table*: Maternal and developmental observations at uterine examination (Unpublished report, 2017)*

Endpoint	0 mg/kg bw	0.75 mg/kg bw	3.0 mg/kg bw	6.0 mg/kg bw
No. of dams	25	25	25	25
No. non-pregnant	1	1	2	3
Pregnancy index (%)	96.0	96.0	92.0	88.0
No. of females with total resorption	0	0	0	4
No. of females with viable foetuses GD 20	24	24	23	18

Endpoint	0 mg/kg bw (Mean ± SD)	0.75 mg/kg bw (Mean ± SD)	3.0 mg/kg bw (Mean ± SD)	6.0 mg/kg bw (Mean ± SD)
Corpora lutea No. per animal	15.4 ± 2.30	16.1 ± 2.02	16.0 ± 3.01	15.4 ± 2.43
Implantation sites No. per animal	13.2 ± 1.89	14.3 ± 2.35	14.2 ± 1.67	12.5 ± 2.42
Preimplantation loss % per animal	12.91 ± 14.05	11.03 ± 9.76	9.80 ± 10.11	14.66 ± 15.27
Viable foetuses No. per animal	12.5 ± 1.96	14.1 ± 2.36	13.5 ± 1.78	9.7 ± 5.42
Post-implantation loss % per animal	5.40 ± 5.626	1.46 ± 2.952	4.89 ±5.687	25.70 ± 39.37 (18.3 ± 32.7[b])
Litter size No. per animal	12.5 ± 1.96	14.1[a] ± 2.36	13.5 ± 1.78	9.7 ± 5.42
Resorptions: early + late No. per animal	0.7 ± 0.75	0.2[a] ± 0.41	0.7 ± 0.82	2.7 ± 4.26
Resorptions: early No. per animal	0.7 ± 0.75	0.2[a] ± 0.41	0.7 ± 0.83	2.7 ± 4.22

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

An increase in post-implantation loss was observed in the high dose, in particular in four females that resorbed all of their foetuses. Two of these displayed signs of maternal toxicity, while the other two did not. Another female with 75% post-implantation loss also showed no clinical signs or altered body weight. No other effects on the developing foetus were observed in this study.

A comparative study with DBTO, DBTC, DBTA, DBTM, and DBTL (Noda *et al*., 1993) using a single gavage administration of 80 µmoL/kg bw on GD 8 (20 mg/kg bw DBTO), showed a comparable spectrum of effects for all substances, in the absence of maternal toxicity. For DBTO, external malformations were observed in 20.7% of the pups (n=28), mainly consisting of cleft mandible, cleft lower lip, ankyloglossia or schistoglossia and exencephaly. Skeletal malformations had an incidence of 26.2% (n=30), of which anomaly of mandibular fixation, fused ribs, absent ribs, and fused thoracic arches were significantly increased. Treatment showed a comparable incidence and type of foetal malformations for all organotin substances.

Studies with DBTC

The developmental toxicity effects of DBTC observed in the OECD TG 421 (Unpublished report, 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 (Farr *et al*., 2001) 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 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.

The sensitivity of the rat foetus to DBTC was confirmed by several *in vitro* studies (Ema *et al*., 1995a, 1996a; Yonemoto *et al*., 1993).

A single study performed in CD1 mice (Ema et al., 2007b) 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 (Ema et al., 2007b, 2009).

Studies with DBTA

The three studies performed by Noda *et al*. (1992a, 1992b, 2001) with DBTA had as main purpose to characterise the critical parameters of DBTA-induced teratogenicity. In particular the critical window of exposure was investigated. It was observed that three days of exposure to 15 mg/kg bw on GD 7-9 resulted in a clear rise in resorbed embryo's and 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 window of exposure 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 were an increase in early resorptions, increases in external and skeletal malformations and a decrease in foetal weight.

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 7.5-month-old dams, 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 the dams and DBTA effects, mainly because the implantation loss in older dams (12 months) was very high in all groups.

Based on the clear and consistent evidence of effects on the developing foetus (post-implantation loss, skeletal and external malformations) in rat studies with DBTO and with category members and in the absence of data indicating that effects are not relevant to humans, the DS proposed classification of DBTO as Repr. 1B; H360D.

Comments received during consultation

Three MSCAs agreed with the proposed classification for reproductive toxicity, based on the data from category members and the OECD TG 414 study with DBTO.

Six industry representatives commented. They disagreed with the category approach and using read across between category members, arguing that new hydrolysis studies show that a dimer (DBDTC distannoxane dimer) is formed rather than DBTC itself (Ghobrial *et al*., 2019; Munschi *et al*., 2010; Patel *et al*., 2009; Alwyn, 2009). According to the comments, the dimeric structure is the only metabolite of the gastric hydrolysis and has a molecular weight of 1089.4 Dalton. With this high molecular weight, the dimeric distannoxane is by far too heavy to be biologically active and have a low reaction potential because the molecules are too large to pass through biological membranes, limiting their bioavailability. It was also commented that the rejection of the category approach is supported by absence of teratogenic effects in the OECD TG 414 study with DBTO. They also noted that further testing related to the read across is planned. The DS replied that the available information indicates that common metabolites/intermediates are formed in vivo, and affect common biological targets, which supports the applicability of a read across from category members (e.g., DBTC) to DBTO for oral toxicity studies (germ cell mutagenicity, toxicity to reproduction, specific target organ toxicity repeated exposure, specific target organ toxicity single exposure). The DS concluded, that based on available information, the read across is robust and it is valid to consider data of category members to read across between these endpoints. They also noted that the OECD TG 414 study referred to by Industry representatives demonstrates that DBTO has an adverse impact on thymus integrity, which is a target organ for category members, further supporting the validity of the category approach. In the study, reduced thymus weights were observed (relative and absolute, up to -38-44%) in a dose-dependent manner and also an increased incidence of small thymus was observed in dams at the highest dose applied. The DS also took note of the new studies.

Assessment and comparison with the classification criteria

RAC agrees with the DS's replies and concludes that the read across is robust and valid and that data from the category members can be used to assess reproductive toxicity. Also see 'RAC general comment'.

Adverse effects on sexual function and fertility

The effects of DBTC on sexual function and fertility 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 has 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. This was also concluded in the RAC opinion for DBTC itself.

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

Specific concentration limit

Setting of an SCL is not considered necessary for adverse effects on sexual function and fertility, given that (cf. section 3.7.2.5 of the CLP guidance, ECHA, 2017) ED_{10}-values for DBTO 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 change the potency group are considered not needed, resulting in the GCL of 0.3%.

Altogether, RAC supports the conclusion of the DS that **DBTO warrants classification for adverse effects on sexual function and fertility as Repr. 1B; H360F (May damage fertility)**.

Adverse effects on development

There are two developmental toxicity studies with DBTO available. The first was an OECD TG 414 study (Unpublished report, 2017) that showed an increase in post-implantation loss at the highest dose of 6 mg/kg bw/d. At this dose also maternal toxicity occurred, consisting of lower body weights (-8% at GD 18, -9% at GD 20), lower body weight gain, lower food consumption and clinical findings. Two dams in the high dose group were sacrificed in extremis on GD 9 and 12, respectively. However, it was noted that three dams with high resorption rates did not show clear signs of toxicity.

The second study with DBTO was the study by Noda *et al*. (1993), in which single doses of several dibutyltins were given on the critical day for organotin toxicity, namely GD 8. DBTO showed a statically significant higher incidence of in foetal malformations similar to those induced by other category members. There was no maternal mortality or signs of general toxicity.

In addition to these studies with DBTO, there are numerous studies with DBTC and DBTA that consistently show dose-dependent increases in foetal effects (malformations, post-implantation loss and weight reduction). 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 maternal body weight gain at higher doses was caused by the sharp increase in post-implantation loss, as dams with live foetuses at the same dose 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, and RAC considers that there is clear evidence of an adverse effect on development upon exposure to DBTO.

Specific concentration limit

Setting of an SCL is not considered necessary for adverse effects on development, given that the ED_{10}-values fall within the range of the medium potency group (i.e., 4 mg/kg bw/d < ED10 < 400 mg/kg bw/d) and modifying factors which might change the potency group are considered not needed, resulting in the GCL of 0.3% (cf. section 3.7.2.5 of the CLP guidance, ECHA, 2017).

Altogether, RAC supports the conclusion of the DS that **DBTO warrants classification for adverse effects on development as Repr. 1B; H360D (May damage the unborn child)**.

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).