Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Dibutyltin maleate

EC Number: 201-077-5
CAS Number: 78-04-6

CLH-O-0000007032-86-01/F

Adopted

16 September 2021

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CLH -O-0000007032-86-01/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 maleate**

EC Number: **201-077-5**

CAS Number: **78-04-6**

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)

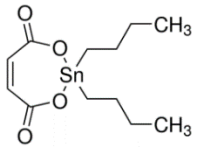
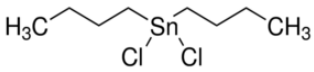
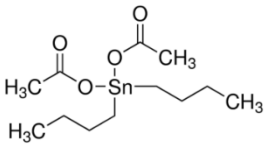
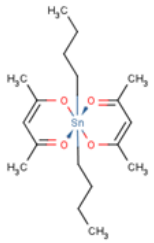
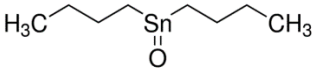
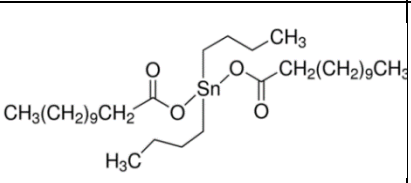
	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	dibutyltin maleate	201-077-5	78-04-6	Muta. 2 Repr. 1B Acute Tox. 2 Acute Tox. 4 STOT RE 1 Skin Corr. 1 Eye Dam. 1	H341 H360FD H330 H302 H372 (immune system) H314 H318	GHS08 GHS06 GHS05 Dgr	H341 H360FD H330 H302 H372 (immune system) H314		inhalation: ATE = 0,317 mg/L (dusts or mists) oral: ATE = 510 mg/kg bw	
RAC opinion	TBD	dibutyltin maleate	201-077-5	78-04-6	Muta. 2 Repr. 1B Acute Tox. 2 Acute Tox. 4 STOT RE 1 Skin Corr. 1 Eye Dam. 1	H341 H360FD H330 H302 H372 (immune system) H314 H318	GHS08 GHS06 GHS05 Dgr	H341 H360FD H330 H302 H372 (immune system) H314		inhalation: ATE = 0,317 mg/L (dusts or mists) oral: ATE = 510 mg/kg bw	
Resulting Annex VI entry if agreed by COM	TBD	dibutyltin maleate	201-077-5	78-04-6	Muta. 2 Repr. 1B Acute Tox. 2 Acute Tox. 4 STOT RE 1 Skin Corr. 1 Eye Dam. 1	H341 H360FD H330 H302 H372 (immune system) H314 H318	GHS08 GHS06 GHS05 Dgr	H341 H360FD H330 H302 H372 (immune system) H314		inhalation: ATE = 0,317 mg/L (dusts or mists) oral: ATE = 510 mg/kg bw	

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

The dossier submitter (DS) proposed to classify dibutyltin maleate (abbreviated throughout this document as DBTM) for acute oral toxicity, acute inhalation toxicity, skin corrosion, serious eye damage, mutagenicity, STOT RE and reproductive toxicity. In addition to studies performed with DBTM itself, reference was made to studies performed with the following substances as part of a read-across, category approach: DBTC, DBTO, 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*

Substance	EC # / CAS #	Structure	Purity (studies)	Purity / Impurity details (REACH Dossier)
Dibutyltin maleate (DBTM)	201-077-5 / 78-04-6		Not reported	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 oxide (DBTO)	212-449-1 / 818-08-6		Not reported	>97.5% 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 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 were 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 DBTM form the distannoxane $ClBu_2SnOSnBu_2Cl$.

The *in vitro* gastric simulation study with DBTM used an exposure time of 72 h at a pH of 1.2, after which DBTM was completely hydrolysed to the distannoxane $ClBu_2SnOSnBu_2Cl$ (Umweltbundesamt, 2019).

In addition, an older simulated hydrolysis study which used GC-FPD detection and showed that the half-time of DBTM under gastric conditions was <0.5 hours (Schilt & Zondervan-van den Beuken, 2004).

Moreover, the available developmental toxicity studies with DBTM itself show 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 DBTM 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 oxide (DBTO), dilaurate (DBTL) and dibutylbis(pentane-2,4-dionato)-OO')tin (DBTP).

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of oral acute toxicity

Summary of the Dossier Submitter's proposal

Three acute oral toxicity studies with DBTM are available, of which one is an OECD test guideline (TG) 401 study. This study was performed in rats by oral gavage and gave LD_{50} values of 422 mg/g bw for males and 647 mg/kg bw for females. Animals from all groups exhibited dyspnoea, curved position, and ruffled fur within 1 h after dosing, but no gross organ changes were reported (Anonymous, 1982a).

The other two studies were non-guideline studies with limitations in reporting and large deviations from the guidelines, reducing their reliability. One used wild trapped mice exposed by gavage and through feed. The gavage LD_{50} was 470 mg/kg bw (Schafer, 1985). For the other study, no more information was available other than that 10 rats were exposed to 50 mg/kg bw after which 4 died (Anonymous, 1975).

The DS based the classification proposal on the average LD_{50} of 510 mg/kg bw from the OECD TG 401 study. This results in a classification as Acute Tox. 4 with and ATE value of 510 mg/kg bw.

Comments received during consultation

Three comments were received from Member State Competent Authorities (MSCAs), all in support of the proposed classification in Acute Tox. 4. Two MSCAs commented on the ATE, one to indicate a preference to use the lower male LD_{50} of 422 mg/kg bw while the other supported the use of the mean value. The reasoning of the latter was the same as that of the DS, namely that there is no apparent difference in sensitivity between males and females for oral acute toxicity, and that there is a rather large range of confidence intervals for the LD_{50} values.

Assessment and comparison with the classification criteria

Of the three studies available, only one was of sufficient quality as regards both performance and reporting to be used as a key study for classification. This study was performed in rats and showed LD_{50} values of 422 and 647 mg/kg bw for males and females, respectively, with confidence limits of 263-777 mg/kg bw. Both LD_{50} values fall within the classification range of Acute tox 4 of > 300 and ≤ 2000 mg/kg bw.

Hence, **classification of DBTM in Acute Tox. 4; H302 (Harmful if swallowed) is warranted.**

As also brought up during the consultation, the broad confidence interval makes it difficult to judge whether the difference in outcome between males and females is due to actual differences in sensitivity or a chance finding. For this reason, RAC supports the proposal of the DS to use the mean LD_{50} value of **510 mg/kg bw** in the setting of the **ATE value**.

RAC evaluation of dermal acute toxicity

Summary of the Dossier Submitter's proposal

There are two acute dermal toxicity studies with DBTM, an OECD TG 402 study in rats and a non-guideline study in rabbits.

In the rat study semi-occlusive exposure to 2000 mg/kg bw DBTM in arachis oil for 24 h did not result in mortalities or systemic toxicity. There were signs of dermal irritation observed (Anonymous, 2010a).

The rabbit study was very old and only limited information is available. Shaved rabbits were exposed for 24 h to 200 mg/kg bw DBTM in propylene glycol. After 48 h 4/10 rabbits died, which increased to 5/10 after 72 h (Anonymous, 1950).

Due to the limitations in the rabbit study, the DS based the evaluation on the rat study. As the LD_{50} was above 2000 mg/kg bw, no classification was proposed.

Comments received during consultation

Two comments from MSCAs specifically addressed acute dermal toxicity. One agreed with the proposal for no classification. The second suggested that the higher sensitivity of rabbits may have to be considered as well.

Assessment and comparison with the classification criteria

No mortalities were observed in an acute dermal toxicity study in rats with a top dose of 2000 mg/kg bw. On the other hand, mortality up to 50% occurred in a study in rabbits at 200 mg/kg bw. There are several possible reasons for this difference, including differences in sensitivity

between species, higher absorption due to the solvent used (arachis oil vs. propylene glycol), but also potential flaws in animal husbandry in the rabbit study. As the rabbit study is very old and limited information is available, it is not possible to draw a conclusion on this, but RAC agrees with the DS that the level of confidence for this study is rather low. It should also be noted that all of the other category members for which acute dermal toxicity studies are available have LD_{50} values > 2000 mg/kg bw (DBTO, DBTL, and DBTP). Considering the close similarities in toxicity between these substances, this increases the confidence for the use of the test guideline study in rats as the only key study.

In conclusion, RAC considers that **no classification is warranted for DBTM for Acute toxicity via the dermal route.**

RAC evaluation of inhalation acute toxicity

Summary of the Dossier Submitter's proposal

An acute inhalation study was performed in rats with doses of 0, 104, 212, 478 and 1004 mg/m^3 DBTM in ethanol administered by nose-only (Anonymous, 1982b). The exposure duration was 4 h with a 14-d observation period.

Control animals showed slight exophthalmus and ruffled fur. For animals exposed to 104 mg/m^3 slight dyspnoea, exophthalmus, ruffled fur and curved body position were documented. Higher exposure concentrations resulted in slight sedation, ventral body position and moderate dyspnoea, exophthalmus, ruffled fur and curved body position. At the 478 and 1004 mg/m^3 a significant decrease in bodyweight was seen. Gross pathology showed mottled effects in the lungs in some animals at all exposure concentrations. At 207 mg/m^3 and above oedema of the lungs are documented. Other effects such as mottled liver, stomach expansion and small/large intestine expansion were seen in some animals at exposure concentrations of 474 mg/m^3 and above. Based on these results an LC_{50} (m/f) of 317 (240-416) mg/m^3 was derived using the probit method. For males an LC_{50} value of 313 (214-459) mg/m^3 and females a value of 319 (198-504) mg/m^3 was derived.

Based on the LC_{50} value of 317 mg/m^3 the DS proposed classification of DBTM as Acute Tox. 2 (inhalation). An ATE of 0.317 mg/L was proposed.

Comments received during consultation

Three MSCAs indicated their support for the proposed classification.

Assessment and comparison with the classification criteria

One acute inhalation study in the rat is available with DBTM. The outcome showed both local and systemic toxicity including mortality after 4 h exposure. Although the study did not follow an OECD test guideline, it did use a generally recognised method and both methodology and results are sufficiently well described.

The LD_{50} values for males and females were very similar; hence, no difference in sensitivity between sexes is apparent and RAC agrees with the use of the mean LC_{50} value of 0.317 mg/L. This value falls within the boundaries for Acute Tox. 2 of 0.05 and 0.5 mg/L (4-h exposure). Hence, RAC agrees that **classification of DBTM as Acute Tox. 2; H330 (Fatal if inhaled) is warranted**. An **ATE of 0.317 mg/L (dusts or mists)** is considered warranted for both sexes.

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 and SCID-hu mice (SCID mice engrafted with human foetal thymus and liver tissue fragments)

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 (Snoeij *et al*., 1989).

Lower doses of 0.03 and 1.0 mg/kg bw were given intraperitoneally to SCID-humice engrafted with human thymus and liver tissue fragments. DBTC induced a reduction in thymus cortex size (de Heer *et al*., 1995).

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

One MSCA commented indicating their disagreement with the proposal for no classification, stating that reversibility and a classification proposal for STOT RE 1 are not valid arguments to not classify for STOT SE. Instead, they preferred classification as STOT SE 1 for immune system effects.

The DS responded that the main reason to not propose a classification was related to the limitations of the two studies available. The rat study used only one, relatively high, dose and only three animals per group. The mouse study had an unusual design by using engrafted mice with human thymus and liver tissue, as well as intraperitoneal exposure. It was also noted that both studies have been included in previous discussions for category members, which were not classified for STOT SE.

Assessment and comparison with the classification criteria

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. In the rat study, significant reductions of cell numbers in the thymus were observed after exposure to 15 mg/kg bw, with the lowest number at day 4 (-70%). At day 9 cell numbers had reversed back to control levels.

In the mouse study a reduction in cortex size of the human thymus graft was observed. This i.p. study is considered of limited relevance for STOT SE classification due to, amongst other reasons, the route of administration.

Although effects were seen in a single dose study in rats, RAC notes that the effects were reversible, the study does not allow to assess whether there is: "clear evidence of marked

disfunction" and the endpoint, immune system, is already taken into consideration in the STOT RE classification, for which there is a much stronger burden of proof.

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

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The dossier contains one dermal irritation study in rabbits, an acute dermal toxicity study in rats, and two *in vitro* skin corrosion assays. All studies were performed with DBTM.

The dermal irritation study exposed six rabbits to doses of 500 mg for four hours (Anonymous, 1988b). Mean scores for erythema were between 1 and 2 and for oedema between 0 and 1.67. The effects were irreversible in all animals in 14 days and in fact progressed to scores for erythema between 2 and 4 and for oedema between 0 and 2. Necrosis was documented for one animal and superficial necrosis for another animal.

Dermal irritation was also recorded in the acute dermal toxicity study in rats (Anonymous, 2010a). The dose was 2000 mg/kg bw, which is equivalent to about 400 mg/animal and animals were exposed semi-occlusive for 24 h. Erythema with scores up to 2 were seen in all treated rats. Small superficial scattered scabs were seen in three females on day 2 and haemorrhage of dermal capillaries were documented for 7 animals on day 3. Scabs and/or crust formation were seen in 9/10 animals. One female rat showed scar tissue at the end of the observation period. Effects in other animals were reversible.

The *in vitro* assays were both the human skin model EPISKIN (OECD TG 431), using respectively 10 and 20 mg test material (Anonymous, 2010b and c). Exposure periods were 15 min in one study and 3, 60, and 240 min in the other. Although both studies found a reduction in cell viability (at most 71.6% and 79.1%, respectively), both were above the threshold value of 50% for this test.

Based on the outcome of the studies in rats and rabbits, which both showed severe, irreversible effects after 4 h exposure, the DS proposed to classify DBTM as Skin Corr. 1.

Comments received during consultation

Two MSCAs indicated their support for classification as Skin Corr. 1. However, one added that sub-category 1C could also be considered as the exposure inducing the effects was 4 h. This was agreed on by the DS based on irreversibility.

Assessment and comparison with the classification criteria

There is one dermal irritation study performed with DBTM in six rabbits. Animals were exposed for 4 h to 500 mg and test sites were wiped at the end of this period. The individual scores are given in the CLH report, table 18. Notable is that, while all animals have comparable scores 30 minutes after exposure (1/2 for erythema, 0/1 for oedema), in three animals there is a pronounced increase in severity in the 14-d observation period (3/4 for erythema, 1/2 for

oedema), while the scores stayed practically the same in the other three. At the end of the observation period, desquamation (4), superficial necrosis (1), or necrosis (1) were observed.

Dermal irritation was also observed in the acute dermal toxicity study in rats. This study used a slightly lower dose of 400 mg, but an exposure period of 24 h. Erythema scores ranged from 0 to 2 and no oedema was observed. Also scabbing, crust formation and in one animal scar tissue were observed. Most effects were reversible in 14 days.

Two *in vitro* Human Skin Model Tests were negative with all exposure periods tested.

According to the CLP Regulation *"A substance is corrosive to skin when it produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis in at least one tested animal after exposure for up to 4 hours".* Visible necrosis was observed in two rabbits after 4 h exposure. Effects were also irreversible in all animals in this study. No information is available on the effects after shorter durations of exposure, which would allow division into a subcategory. Hence, RAC considers that no sub-categorisation can be done.

In conclusion, RAC considers that **DBTM warrants classification as Skin Corr. 1; H314 (Causes severe skin burns and eye damage).**

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

Two *in vivo* eye irritation studies were performed with DBTM in rabbits.

In the first study in three rabbits DBTM showed scattered or diffuse or translucent corneal opacity at 24 and 48 h (mean scores: 1, 1, 1.33), iridial inflammation in all treated eyes (mean scores: 1, 0.67, 1) as well as moderate to severe conjunctival irritation (mean score redness: 2, 1.67, 2; chemosis: 2.33, 1.67, 2.33). In addition, pale area on the conjunctival membrane and/or petechial haemorrhage of the nictitating membrane were seen. One treated eye showed blepharitis and blood-stained discharge; this animal was killed for humane reasons (Anonymous, 2010d).

The second study with six animals found mean scores of ≥3 for conjunctivae redness and chemosis. Opacity score was ≥3 in 2 animals, however, two animals could not be scored due to severity of response. Iritis score was 3 for one animal and four animals could not be fully evaluated due to severity of response. Necrosis and ulceration were described for all animals. All animals were killed for humane reasons after 72 h observation period. Rabbits with washed eyes showed similar severe reactions, and observation until day 21 showed that redness and opacity did not recover for 2/3 animals. Necrosis was seen at all timepoints (Anonymous, 1988).

An available *in vitro* test (SkinEthic Reconstituted Human Corneal model, pre-validated method) found no irritative properties, but used only 10 min exposure (Anonymous, 2010e).

Based on the severe, irreversible effects observed in the study by Anonymous (1988), including redness, opacity, ulceration, and necrosis in eyes of all animals, the DS proposed classification as Eye Dam. 1.

Comments received during consultation

Two comments were received by MSCAs, both agreed with the proposal.

Assessment and comparison with the classification criteria

There are two *in vivo* eye irritation studies available in rabbits. Both showed consistent eye damage induced by DBTM, which was particularly severe in the Anonymous (1988) study, but also in the Anonymous (2010d) study one animal had to be killed for humane reasons (one treated eye showed blepharitis and blood-stained discharge).

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 hours after installation of the test material.

In Anonymous (1988) two out of three animals with washed eyes still showed conjunctival redness and cornea opacity after 21 days. Moreover, all six animals with unwashed eyes had such severe reactions that they had to be killed after 72 h.

As the criteria are met, RAC concludes that **DBTM warrants classification as Eye Dam. 1; H318 (Causes serious eye damage)**. As also classification for skin corrosion is proposed, classification for eye damage is the default classification.

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

Summary of the Dossier Submitter's proposal

There are no repeated dose studies available with DBTM itself. The classification proposal is based on read-across with DBTC and a PNDT study with DBTO and supported by the toxicokinetic and hydrolytic behaviour of the substances in the category.

In the developmental toxicity study with DBTO 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. 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 a reduced absolute and relative thymus weight and a severe to very severe lymphoid depletion in dams.

A 28-d rat/mouse immunotoxicity study with doses of 0, 50 and 150 ppm of 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 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 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 reproductive/developmental toxicity screening study (Unpublished report, 2003) to be key studies. All three studies showed thymus toxicity at low dose levels. As DBTM 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 (see RAC note on the 56-d study further down) studies 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 consultation

Comments were received from three MSCAs, indicating support for the proposed classification based on the category approach.

Assessment and comparison with the classification criteria

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

Seinen & Vos (1977) noted reductions in thymus weight 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 of exposure (it is unclear how the 56 days in the CLH dossier was calculated).

In addition, an OECD TG 414 study with DBTO was included for the assessment of STOT RE (Unpublished report, 2017). Mean absolute thymus weights were 19%, 34%, and 44% lower than the mean control values in the 0.75, 3.0, and 6.0 mg/kg bw/d dose groups, respectively, and relative to the adjusted GD 20 body weights, they were 20%, 35%, and 37% lower, respectively.

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 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 DBTM (346.99 g/M), this applies to the equivalent values of DBTM 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 **DBTM 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 (Krul, 2002) has been performed with DBTM 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. 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 (Reimann & Gramlich, 1990). 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 it did not affect chromosomal length in human peripheral lymphocytes (Jensen *et al*., 1989), or 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 exposure. 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 females compared to males. 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 24h, 48h and 72h post-treatment.

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

The DS concluded that given the absence of germ cell mutagenicity studies for 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 DBTM also as Muta. 2 based on the category approach.

Comments received during consultation

Comments were received from three MSCAs, indicating support for the proposed classification based on the category approach.

Assessment and comparison with the classification criteria

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

Overall, the results of the *in vitro* tests performed with DBTC were variable with both positive and negative results. Additionally, two *in vivo* mouse micronucleus studies with DBTC are presented in the CLH 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, 1990).

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 DBTM 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.

RAC concludes that **DBTM 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 DBTM; thus, the evaluation was based on studies with DBTC. This evaluation has been made previously for several category members, including DBTA, DBTP and DBTDL.

The OECD TG 421 study (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 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 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 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 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 (Ema *et al*., 2003). The other two supportive studies (Harazono & Ema, 2003; Harazono & Ema, 2001) also focused on the effects of DBTC in decidual cell response and progesterone levels during pseudopregnancy.

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

Adverse effects on development

There is one comparative study performed with DBTM in addition to DBTDL, DBTA, DBTO, and DBTC (Noda *et al*., 1993). All studies except one performed with DBTO have been evaluated previously for category members. The CLH dossier divides the studies into four groups: the Noda *et al.* (1993) , the study with DBTO, studies with DBTC, and studies with DBTA.

The comparative study with <u>DBTM, DBTC, DBTA, DBTO, and DBTL</u> (Noda *et al*., 1993) using a single gavage administration of 80 µmoL/kg bw on GD 8 (28 mg/kg bw DBTM), showed a comparable spectrum of developmental effects for all substances, in the absence of maternal toxicity. For DBTM external malformations were observed in 12.5% of the pups (n=16), consisting of cleft mandible, cleft lower lip, ankyloglossia, schistoglossia or cleft upper lip. Skeletal malformations had an incidence of 9.3% (n=12), consisting of mandibular fixation, and cranial hypoplasia. Treatment showed a comparable incidence and type of foetal malformations for all organotin substances.

<u>Study with DBTO</u>

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 6 mg/kg bw/d. 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 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.

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 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) with category members and evidence that DBTM induces similar effects and in the absence of data indicating that effects are not relevant to humans, the DS proposed classification of DBTM as Repr. 1B; H360D.

Comments received during consultation

Comments were received from three MSCAs, indicating support for the proposed classification based on the category approach.

Assessment and comparison with the classification criteria

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 DBTM and DBTC are hydrolysed to the same distannoxane, RAC is of the opinion that data on DBTC can be used for classification of DBTM for adverse effects on sexual function and fertility (see also under 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 Guidance on the application of the CLP criteria; CLP guidance, 2017) the 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 **classification of DBTM for adverse effects on sexual function and fertility as Repr. 1B; H360F (May damage fertility) is warranted**.

Development

There is only one developmental toxicity study with DBTM itself, which was the study by Noda *et al*., 1993, in which single doses of several dibutyltins, including DBTM, were given on the critical day for organotin toxicity, namely GD 8. DBTM showed an induction in foetal malformations similar to those induced by other category members. There was no maternal mortality or signs of general toxicity.

In addition, there are numerous studies with DBTC, DBTO, 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 is 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 DBTM.

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

Specific concentration limit

Setting of an SCL is not considered necessary for effects on development, given that the ED10-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).

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).