Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol; tetrabromobisphenol-A

EC Number: 201-236-9
CAS Number: 79-94-7

CLH-O-0000007043-83-01/F

Adopted

16 September 2021

16 September 2021

CLH-O-0000007043-83-01/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: **2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol; tetrabromobisphenol-A**

EC Number: **201-236-9**

CAS Number: **79-94-7**

The proposal was submitted by **Norway and Denmark** and received by RAC on **18 September 2020.**

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

PROCESS FOR ADOPTION OF THE OPINION

Norway and Denmark have 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: **Wendy Rodriguez**

Co-Rapporteur, appointed by RAC: **Ruth Moeller**

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 ATEs	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	604-074-00-0	tetrabromobisphenol-A; 2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol	201-236-9	79-94-7	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Wng	H410			
Dossier submitters proposal	604-074-00-0	2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol; tetrabromobisphenol-A	201-236-9	79-94-7	**Add** Carc 1B	**Add** H350	**Add** GHS08 **Modify** Dgr	**Add** H350			
Resulting entry in Annex VI if adopted by RAC and agreed by Commission	604-074-00-0	2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol; tetrabromobisphenol-A	201-236-9	79-94-7	Carc 1B Aquatic Acute 1 Aquatic Chronic 1	H350 H400 H410	GHS08 GHS09 Dgr	H350 H410			

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Tetrabromobisphenol-A (TBBPA) is a brominated flame retardant commonly used in epoxy coated circuit boards (Cannon *et al*., 2019), printed circuit boards, paper, and textiles (Dunnick *et al*., 2017). TBBPA has the largest worldwide production of any brominated flame retardant (Knudsen *et al*., 2017), with a global production volume over 100 000 tons per year (IARC, 2018).

TBBPA has a current entry in Annex VI to the CLP regulation with Aquatic Acute 1 and Aquatic Chronic 1 classifications and, in the C&L inventory, a self-classification as Carc. 2. TBBPA has also been classified as "probably carcinogenic to humans" (Group 2A) by the International Agency for Research on Cancer (IARC).

The CLH report has been created based on the REACH registration dossier, a technical Report on TBBPA from the U.S. National Toxicology Program (NTP, 2014) and a recent literature search (in early 2020). The proposal from the dossier submitter (DS) addressed the following endpoints: STOT RE, germ cell mutagenicity, carcinogenicity and reproductive toxicity.

HUMAN HEALTH HAZARD EVALUATION

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

Summary of the Dossier Submitter's proposal

Eight studies were presented by the DS:

- One 14week oral study on mice and rats (NTP, 2014)
- Five oral studies on rats, including three 90-day studies (Dunnick *et al*., 2017; Osimitz *et al*., 2016; Unnamed, 2002) and two 28-days studies (Borghoff *et al*., 2016; Van der Ven *et al*., 2008).
- One 14-day inhalation study on rats (Unnamed, 1975)
- One 3-week dermal study on rabbits (Unnamed, 1979)

While TBBPA exposure did not demonstrate a significant effect on mortality, body weight or food consumption compared to the control group in any of the repeated-exposure toxicity studies, a decrease in serum T4 was highlighted in all oral studies where T4 was measured (NTP, 2014; Osimitz, 2016; Unnamed, 2002; Van der Ven, 2008). After exposure to TBBPA, slight effects on liver were also detected in two 90-day studies (NTP, 2014; Dunnick, 2017), including enzyme activation and/or increases in liver weights.

In the rat inhalation study, only clinical effects (salivation, red or clear nasal discharge, excessive lacrimation) and a decrease in liver weight compared to control animals were detected, whereas the rat dermal study didn't induce any significant change except very slight erythema in test animals.

Overall, the DS concluded that there is some, but not significant nor severe toxic effects at moderate exposure concentrations, and therefore did not propose a classification as STOT RE.

Comments received during consultation

One MS supported the DS's view that the effects are not sufficiently severe for classification, considering that the effects on liver were not accompanied by lesions, that renal tubule cytoplasmic alterations occurred at doses outside the range of guidance values for STOT-RE classification and that thyroxine (T4) increases were not accompanied by decreases in triiodothyronine(T3) concentrations or increases in thyroid stimulating hormone (TSH) concentrations.

Two MS also supported this view, adding that the effects on T4 were only observed in rats.

Industry indicated that reductions in T4 alone are not considered adverse in the absence of any other relevant thyroid-related effects (EU, 2006; Health Canada, 2013), such as changes in T3, TSH, thyroid weights and histopathology. They noted that these effects didn't consistently accompany the decreases in levels of T4 after TBBPA exposure (Schroeder, 2002a; 2002b; 2003; van der Ven *et al*., 2008; NTP, 2013), and no neurobehavioral and neuropathology effects were detected during reproductive and developmental studies. Furthermore, Industry pointed to the conclusions of EFSA (2013) stating that, due to "the limitations and uncertainties in the database," it was inappropriate to use a BMDL10 for decreased T4 to establish a health-based guidance value. Also, Industry emphasised that a PROD increase is indicative of xenobiotic metabolism and detoxification (i.e., Cyp2b via CAR activation) rather than indicative of a liver disturbance or adversity. The same Industry asked all BMDL values to be reported with their corresponding benchmark response levels (e.g., BMR of 5%, 10%, 1SD, etc.), for context.

In their response, the DS confirmed the importance of thyroid hormones during neurodevelopment and was of the opinion that data on T4, T3 and TSH should be presented to ensure transparency. The DS added that the BMR was reported as Critical Effect Size (CES) by Van der Ven *et al*. (2008) and Lilienthal *et al*. (2008). A description of the CES was included in the CLH report Annex 3.10.1.3. The default CES reported in Van der Ven *et al*. was 10%. The exceptions were for testis weight and bone parameters, where the CES used was 5%. For liver weight and immune parameters a CES of 20% was used. Lilienthal *et al*. reported the CES used as 5%.

One industry source stated that TBBPA is not an endocrine disruptor. RAC notes that as ED properties are not currently a hazard class in the CLP regulation, the implications of an endocrine mode of action (MoA) will be considered under the relevant endpoints carcinogenicity and toxicity on reproduction.

Assessment and comparison with the classification criteria

The oral repeated dose toxicity studies with TBBPA are presented in the table below.

***Table 1**: Summary of the repeated dose oral toxicity studies (from Table 18 of the CLH report, slightly modified).*

Method, guideline, deviations if any, species, strain, sex, no/group Guidance value (GV) for STOT RE2 classification	**Exposure**	**Result**	**Reference**
14-week study in F344/NTAC rats and B6C3F1/N mice 10 male and 10 female rats and mice/group (core study) Additional special study group of 10 male and 10 female rats were administered the same doses for 23 days for hematology, clinical chemistry and thyroid hormone analysis. Similar to OECD TG 408. Reliability score 1 (DS) GV: 129 mg/kg bw/d (Haber's Rule)	TBBPA, purity > 99% 0, 10, 50, 100, 500, 1000 mg/kg bw/d (oral gavage in corn oil, 5×/week) for 14 weeks	All rats (core study) and mice survived to the end of the study. No changes in final body weights, body weight gains nor clinical sign were observed in rats and mice of dosed groups compared to controls. **<u>Rats:</u>** **500 and 1000 mg/kg bw/d:** Increase in T4 compared to control: Day 4: 4.78 ± 0.18 (500 mg/kg bw/d); 4.49 ± 0.30 (1000 mg/kg bw/d) for males and 4.05 ± 0.27 (500 mg/kg bw/d) and 3.87 ± 0.3 (1000 mg/kg bw/d) for females compared to 6.13 ± 0.18 (males) and 5.52 ± 0.16 (females) Day 23: 3.35 ± 0.19 (500 mg/kg bw/d); 3.78 ± 0.22 (1000 mg/kg bw/d) for males and 2.56 ± 0.25 (500 mg/kg bw/d) 2.64 ± 0.21 (1000 mg/kg bw/d) for females compared to 5.11 ± 0.31 (males) and 4.26 ± 0.25 (females) Week 14: 3.08 ± 0.12(500 mg/kg bw/d); 2.8 ± 0.13 (1000 mg/kg bw/d) for males and 1.83 ± 0.15 (500 mg/kg bw/d) 1.66 ± 0.1 (1000 mg/kg bw/d) for females compared to 4.66 ± 0.16 (males) and 3.33 ± 0.22 (females) Total bile acids in serum: transient increases (two-fold or greater) in males and females on day 4; essentially resolved by day 23. Biologically significant liver enzyme changes: increases in PROD activities (4 to 23 fold) in males and females at week 14. No treatment-related liver lesions were detected. Increases in the absolute and relative liver weights (males and females) **<u>Mice:</u>** **500 and 1000 mg/kg bw/d:** Decrease in acetanilide-4-hydroxylase, 7-ethoxyresorufin-O-deethylase, and PROD activities in the liver of males (30% to 40%) Significantly increased incidences of renal tubule cytoplasmic alteration (males): decrease or absence of the normal vacuoles present in the cortical proximal tubules. Increases in absolute and relative liver weights in 500 mg/kg bw/d males compared to control **1000 mg/kg bw/d:** PROD activity significant decreased (30%) at week 14 in females. Absolute and relative changes in some organs weight (including liver weights increase in males and females)	NTP, 2014

Method, guideline, deviations if any, species, strain, sex, no/group Guidance value (GV) for STOT RE2 classification	**Exposure**	**Result**	**Reference**
Female Wistar Han rats, (academic 13- week study) Reliability score 2 (by DS) GV: 138 mg/kg bw/d (Haber's Rule)	TBBPA, purity ≥ 99% 0, 25, 250, or 1000 mg/kg bw/d (oral gavage in corn oil, 5×/week) for 13 weeks	There were no treatment-related effects on body weights, liver or uterus lesions and the liver and uterine weights were within 10% of controls, so only the high dose animals were analyzed. The TBBPA hepatic transcripts included upregulation of Scd2 (steraroly-coenzyme A desaturase 2), Elovl-6 (fatty acid elongase 6), and FasN (fatty acid synthase). TBBPA exposure also increased levels of the Cyp2b6, and induced the interferon (IFN) pathway transcripts in the liver.	Dunnick *et al*. (2017)
CD/SD rats, male and female Key study 13 weeks OECD TG 408 Reliability score 1 (by DS) *Rapporteur addendum: two measurements of T4: at day 33 and day 90* GV at 90D: 100 mg/kg bw/d (Haber's Rule) GV at 33D: 273 mg/kg bw/d (Haber's Rule)	TBBPA, purity ± 99% 0, 100, 300 and 1000 mg/kg bw/d by oral gavage in corn oil Two recovery groups were included (control, 1000 mg/kg bw/d) 6 weeks post-treatment	TBBPA exerted no marked effect on mortality, clinical signs, body or organ weights, feed consumption, histopathology, urinalysis, ophthalmology, and neurological outcomes in a functional observation battery, motor activity, serum thyroid stimulating hormone, serum triiodothyronine, or other serum chemistries. **100, 300 and 1000 mg/kg bw/d** Significant decrease in **all dose groups** in mean serum T4 concentrations on day 33 and day 90 in males and on day 33 in females (* p<0.01). After recovery, T4 levels in males returned to control levels whereas levels stayed lower in females: <u>Males</u> D33 D90 0 mg/kg bw/d: 4.96±0.837* 5.09±0.797* 100 mg/kg bw/d: 3.66±0.878* 3.27±0.672* 300 mg/kg bw/d: 3.42±0.713* 2.61±0.874* 1000 mg/kg bw/d: 3.39±0.548* 3.09±0.910* Recovery: 5.32±0.944 (control) 5.9±1.538 (HD) <u>Females</u> D33 D90 0 mg/kg bw/d: 4.27±0.957* 5.41±1.036 100 mg/kg bw/d: 3.31±1.079* 5.22±1.234 300 mg/kg bw/d: 3.24±0.846* 4.95±1.316 1000 mg/kg bw/d: 3.33±0.844* 4.95±1.111 Recovery: 3.95±1.406 (control) 3.05±0.705 (HD) There was no effect on T3 and TSH in males and females. **300 and 1000 mg/kg bw/d** Statistically significant increase of total bilirubin values (females) after 13 wk (females: 0.13±0.05 mg/dL) in the females in the 300 mg/kg/day group (0.19±0.03 mg/dL) and 1000 mg/kg/day group (0.2±0.06 mg/dL). **1000 mg/kg bw/d** Statistically significant increase in total bilirubin values (males) after 13 wk and of mean serum ALP levels after 90 days (female). Both were comparable **after the recovery** period. No changes in liver weight nor in liver histopathology were observed. The effects were not considered to be biological or toxicological meaningful or adverse.	Osimitz *et al*., 2016 (appears to be the same study as Unnamed, 2002 reported by the DS)

Method, guideline, deviations if any, species, strain, sex, no/group Guidance value (GV) for STOT RE2 classification	Exposure	Result	Reference
Female Wistar Han rats, 6 per dose group 28 days Similar to OECD TG 407 study, but only females and 6 animals per dose Reliability score 2 (by the DS) GV: 300 mg/kg bw/d	TBBPA, purity: 98.83% 0, 50, 100, 250, 500 and 1000 mg/kg bw/d by oral gavage in corn oil daily for 28 days	There were no significant changes noted in body weight gain, final body weight, absolute liver or uterine weights at any dose level of TBBPA compared to vehicle control rats, nor were there any dose-related trends in liver or uterine weights at either 4- or 8-h post dose on day 28. There were **dose-related increases** in the concentration of TBBPA and its major conjugates, TBBPA-glucuronide and TBBPA-sulfate in liver, plasma and uterine tissue. The concentration of TBBPA-sulfate was higher in liver compared to TBBPA-glucuronide, while TBBPA-glucuronide concentration is higher compared to TBBPA-sulfate in the plasma and uterus at high dose levels. Overall, the ratio of the TBBPA-sulfate to TBBPA-glucuronide in all three tissues decreased with increasing dose level of TBBPA, suggesting the sulfation pathway becomes limited with increased dose of TBBPA.	Borghoff *et al*., 2016
Wistar rats, 10 animals/dose/ sex (also in control group). Repeated Dose 28-day Oral Toxicity Study in Rodents, enhanced for endocrine and immune parameters Conducted according to OECD TG 407. Reliability 2 (by the DS) GV: 300 mg/kg bw/d	TBBPA Purity 98% 0, 30, 100 and 300 mg/kg bw/d exposed by oral feed.	No effects on food intake, body weight, or organ weights in both sexes (notably not of the testis and male pituitary), immune, hematological or histological parameters Effects on thyroid hormone levels: Authors report T4 levels were decreased and T3 levels were increased (significant Dose-Response for males only, no pairwise statistics, for details on BMD modelling results see the text below).	Van der Ven *et al*., 2008

Summary of the table above: In the 14-week study (NTP, 2014), statistically significant, progressive, and dose-related decreases in total T4 concentrations (male and female) were observed in all groups of rats, males and females, exposed to TBBPA doses greater than or equal to 500 mg/kg bw/d for 5 days per week. This effect was also observed but less consistently (only statistically significant at week 14 in males -3.67±0.21 and at day 4 -4.52±0.18 in females) in the 100 mg/kg bw/d groups. On day 4, T4 was decreased by approximately 30% in the 1000 mg/kg bw/d animals; by week 14, it was decreased by approximately 45%. The decreases in T4 were not accompanied by decreases in T3 concentrations or increases in TSH concentrations. No histopathological changes were described and the weight of the thyroid was not provided.

Several indicators of hepatic function disturbance were also highlighted in rats, but despite these effects, no treatment-related liver lesions were observed in histopathological examinations.

Apart from a non-consistent decrease of T4 at 100 mg/kg bw/d and the extended estrus time, other effects observed after TBBPA exposure (haematology findings, changes in organ weights), were not seen at doses that warrant classification.

In mice, several effects on liver were detected (absolute and relative increase in liver weight in males and females and a decrease in enzyme activity), however these were less pronounced than in rats. A change in spleen and liver weight and renal tubule alteration was also highlighted. None of the effects detected in mice occurred at doses that warrant classification.

No significant general toxicity was seen in the oral rat 13-week study (**Dunnick *et al*., 2017**). There were few changes in the uterine transcriptome after TBBPA exposure, but none of the uterine transcripts were common among the three TBBPA exposure levels examined and were not considered to alter organ function. Several hepatic transcripts were upregulated in rats exposed to 1000 mg/kg bw/d for 5 days per week, especially the interferon (IFN) and other transcripts associated with IFN pathway regulation. The design of the study did not enable effects at doses that warrant a classification to be determined.

No general toxicity (except few mortalities due to dosing injury) was observed in an OECD TG 408 13 weeks study (**Osimitz *et al*., 2016**). T4 levels were significantly lower in all dose group (both sexes) compared to controls on day 33, and in all dose group for males on day 90. After 6 months of recovery (high dose group), T4 levels returned to control levels in males but stayed lower in females. RAC notes a relatively high standard deviation and also no clear dose-dependency, but levels were statistically significantly reduced ($p<0.01$) and it is noted that the effects are consistent with the other oral studies (see Tables 19 and 20 in CLH report). Mean TSH and T3 levels were comparable between control and treated animals at all time points. No gross or histopathological changes were reported for the thyroid. Some effects were also observed on alkaline phosphatase levels and bilirubin, however, these were not considered to be of toxicological relevance and were comparable to control after the end of the recovery period. Moreover, these effects were observed only at doses above those relevant for classification.

A 28-day gavage study was performed on female rats exposed to 50, 100, 250, 500 and 1000 mg/kg bw/d (**Borghoff *et al*., 2016**). No general toxicity was observed. It was highlighted that the ratio of the TBBPA-sulfate to TBBPA-glucuronide decreased with increasing dose level of TBBPA in the liver, plasma and uterus, suggesting that the sulfation pathway becomes saturated with increasing dose of TBBPA.

In another 28-day oral study performed on rats (**Van der Ven *et al*., 2008**), thyroid hormones were measured in plasma. Dose response analysis of effects was done from the best fitted curve obtained by using a nested family of purely descriptive (exponential) models using the PROAST software. Whether these findings are significant based on pairwise statistics remains unclear as such statistics were not reported by the authors. For males, the authors used a pre-defined critical effect size (CES) of 10% and modelled BMDL of 48 mg/kg bw/d (critical effect dose (CED) = 100.4 mg/kg bw/d, max. response -26.8%) for a significant decrease in T4, and a BMDL of 124 mg/kg bw/d for a significant increase in T3 (CED = 214.4 mg/kg bw/d, max. response reported +5.5%). The dose response relationships were reported to be significant in male rats for a decrease in T4 and an increase in T3, but not for females (indicated by +/- at the bottom of the table below). There were no TBBPA-induced histopathological changes in the thyroid gland. The thyroid weight was not reported. Specific immunohistochemistry of the pituitary did not highlight any changes in TSH expression in thyrotrophic cells. RAC considers the BMD modelling (and resulting BMDL) based on three dose levels as uncertain and looking at the published data, only the high dose of 300 mg/kg bw/d seemed to be relevant for T4 decrease. A T3 increase appears as inconsistent thyroid response to TBBPA treatment.

***Table**: Thyroid hormone levels measured in a 28-day TBBPA study (Van der Ven et al., 2008)*

	females			*males*		
TBBPA dose mg/kg bw	n	TT4 nmol/L	TT3 nmol/L	n	TT4 nmol/L	TT3 nmol/L
0	10	38.7 ± 7.3	1.05 ± 0.32	9	43.4 ± 10	1.07 ± 0.14
30	10	39.9 ± 10.9	1.07 ± 0.35	9	41 ± 7.7	1.09 ± 0.15
100	10	36.3 ± 5.9	1.2 ± 0.22	9	42 ± 7.6	1.1 ± 0.16
300	9	34.8 ± 7.3	1.22 ± 0.24	8	31.6 ± 7.6	1.22 ± 0.17
dose response		-	-		+	+

TBBPA has a low general hazard profile, but a statistically significant decrease of serum T4 was consistently observed in all oral studies in rats where this was measured. The concentration of T3 appeared unaffected in most studies, except in the Van der Ven *et al*. (2008) subacute and one-generation studies (more detail in the reproductive toxicity section) reporting an increase in T4, thus there was an inconsistent response. The method of oral administration of TBBPA (gavage versus dietary exposure) didn´t seem to affect the effect on T4.

No treatment-related lesions were observed in the uterus of Wistar Han rats, Fischer 344/NTac rats, or B6C3F1/N mice treated with TBBPA for 3-months (NTP, 2014). No treatment-related microscopic lesions were observed in the liver or uterus in a published experimental 3-month study in Wistar Han rats (Dunnick *et al*., 2017). Renal tubule cytoplasmic alteration was observed in mice (NTP, 2014), but at doses too high to warrant classification.

Meerts *et al*. 2000 demonstrated *in vitro* that TBBPA is a very potent competitor of T4 for the binding of human transthyretin (TTR). Nevertheless, recent results of Ren *et al*. (2020) seem to highlight that TBBPA did not bind to the human thyroxine-binding globulin (TBG) *in vitro*. No relevant *in vivo* data seem to currently be available. Therefore, that portion of T4 displaced from its TTR binding site would be available for metabolism and elimination, thereby leading to a decrease in serum levels. Humans may be less sensitive than rodents to TBBPA mediated decreases in T4 since, unlike rodents, they possess the high-affinity T4 and T3 carrier TBG.

The role of TBBPA mediated liver enzyme induction and UDP-glucuronosyltransferase (UGT)-metabolism mediated T4 decrease is unclear. TBBPA undergoes extensive first pass metabolism and TBBPA and TBBPA-glucuronide are metabolized by liver-GT while a decrease in UGT activity was seen in rats by NTP (NTP, 2014).
Because the decrease in T4 levels was not of sufficient magnitude to alter mean serum TSH or T3 levels, thyroid histopathology, thyroid weight, or other parameters indicative of thyroid pathology (e.g. body weight, etc.), RAC does not consider the decrease in serum T4 levels to be significant toxicity warranting a STOT RE classification.

The sub-acute inhalation study summarized in the following table was presented by the dossier submitter.

***Table**: Summary of the repeated dose inhalation toxicity study (from Table 18 of the CLH report, slightly modified).*

Method, guideline, deviations if any, species, strain, sex, no./group **Guidance value (GV) for STOT RE2 classification**	**Exposure**	**Result**	**Reference**
Crj: CD(SD) Rats, 5 animals/dose/group Repeated dose 14-day inhalation (dust) study in rodents Similar to OECD TG 412 Reliability 2 (by the registrant) GV: 2 mg/l/6h/d (Haber's Rule)	TBBPA, Purity not given 0, 2, 6 , 18 mg/L 4 h/day, 5 days/week for 2 weeks	No deaths, and no changes in body weight gain, food consumption, haematological or biochemical parameters, or urinalysis were noted. A decrease in relative liver weight of females might have been compound related. No gross or microscopic lesions were observed in any of the treated rats. Inhalation of micronized TBBPA at doses at up to 18 mg/L air (ca. 18000 mg/m^3) did not result in adverse effects in rats.	Unnamed, 1975

Local irritation was observed, but it was assumed to be caused by a mechanical effect due to the high concentration of TBBPA. No specific effects due to TBBPA exposure were detected in rats when they were exposed via inhalation. It has to be noted that the study has limitations, for

example purity was not provided by the registrant and the animals were not exposed to the test chemical for a minimum of 6 hours per day over a 28 days.

The sub-acute dermal study summarized in the following table was presented by the dossier submitter.

***Table**: Summary of the repeated dose dermal toxicity study (from Table 18 of the CLH report, slightly modified).*

Method, guideline, deviations if any, species, strain, sex, no/group **Guidance value for STOT RE2 classification**	**Exposure**	**Result**	**Reference**
New Zealand White rabbits, 4 animals/dose/group Short-term repeated dose toxicity: dermal Short-term repeated dose toxicity: dermal Reliability 2 (by the registrant) GV: 1200 mg/kg bw/d (Haber's Rule)	TBBPA, Purity not given 0, 100, 500 and 2500 mg/kg/day 6 h/day, 5 days/week for 3 weeks	There was no mortality and no sign of overt toxicity (body weights, urinalysis, haematologic and biochemical parameters) or unusual behavior for the rabbits in any group. There were no compound induced gross or microscopic lesions in any of the tissues examined. On the skin of rabbits a dosage of 100 mg/kg/day occasionally elicited very slight erythema. The dosage of 500 and 2500 mg/kg/day evoked very slight erythema for almost all rabbits for varying lengths of time.	Unnamed, 1979

In this 3-week study, no gross lesions nor microscopic alterations were detected during dermal exposure of rabbits to TBBPA, as only a slight local effect was highlighted at doses too high to warrant classification. Also, this study report had limitations, including that the purity was not provided by the registrant.

To conclude on the STOT-RE classification, only a disturbance in thyroid hormone level observed as a T4 reduction at doses below the STOT-RE 2 guidance values was observed in the studies, while T3 and TSH concentration were unchanged. No thyroid weight changes nor histopathological variations were described. Therefore, the requirement for classification based on functional disturbance or morphological changes doesn't apply. Moreover, as suggested by *in vitro* studies, the decrease in T4 may be mediated by competitive binding of TBBPA to rodent TTR while no effect was seen on human high-affinity binding TBG. Therefore, the resulting increased clearance of free T4 in rats probably would be of questionable relevance to humans. No dose-related systemic adverse effects from TBBPA-treatment were observed in rodents and rabbits. Thus, some effects were seen, but as they were observed either above the respective guidance values or do not appear as severe nor significant, the requirements for classification with STOT-RE are not fulfilled. However, it is noted that the effects of TBBPA described above may suggest endocrine disruption activity.

RAC concurs with the DS's proposal and supports **no classification for STOT RE.**

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

TBBPA has been tested in several *in vitro* and in one *in vivo* genotoxicity assays.

In vitro, the substance was negative for gene mutation in bacteria (Ames test), mammalian cell gene mutation (Sp5/V79 and SPD8 cells; Chinese hamster cells), as well as for chromosomal aberrations in human peripheral blood lymphocytes. *In vivo*, up to 1000 mg/kg bw for 5 days per week during 14 weeks, TBBPA was not clastogenic in a mouse bone marrow micronucleus assay. The dossier submitter did not propose classification for germ cell mutagenicity.

Comments received during consultation

The proposal of the DS not to classify for mutagenicity was supported by three MS.

One industry source supported no classification for germ cell mutagenicity and noted that several international regulatory authorities have concluded that TBBPA is not mutagenic or genotoxic (EU RAR, 2006; Health Canada, 2013; U.S. EPA, 2015).

Assessment and comparison with the classification criteria

In vitro studies

Several *in vitro* genotoxicity studies covering bacterial gene mutation, mammalian gene mutation and chromosomal aberrations are available for TBBPA. The available data are summarised in the table below:

***Table**: Overview of in vitro genotoxicity studies presented by the DS*

Test method		Results	Reference
Ames test TA100, TA1535, TA1537 and TA98	0, 100, 333, 1000, 3333 and 10000 µg/plate ± S9	Negative. Precipitation, but no cytotoxicity, occurred at concentrations from 1000 µg/plate	Mortelmans *et al*. (1986) reported in NTP (2014) Reported in EU RAR as well-conducted
Ames test TA98, TA100 and WP2 uvrA/pKM101	0, 50, 100, 250, 500, 1000, 6000 µg/plate ± S9 (same TBBPA lot as that used for the 2-year NTP carc study)	Negative. No information reported on cytotoxicity	NTP (2014)
Ames test TA92, TA98, TA100, TA1535, TA1537 and TA1538	0, 5, 10, 50, 100, 500 and 1000 µg/plate ± S9	Negative. Cytotoxicity at the higher concentrations (decrease in colonies**)	DOW Chemical Company (1985)*
Ames test TA 92, TA98, TA100, TA1535, TA1537 and TA1538	0.1, 1, 19, 100 and 500 µg/plate ± S9	Negative. Evidence of some chemically-induced effects at highest dose tested.	Velsicol Chemical Company (1977)*
Ames test TA98, TA100, TA1535 and TA1537	1, 10, 100 µg/plate ± S9	Negative No cytotoxicity observed.	Israel Institute for Biological Research (1978)*
Ames test TA92, TA98, TA100, TA1535, TA1537 and TA1538	0.25, 0.5, 5 and 50 µg/plate ± S9	Negative. Evidence of chemically-induced physiological effects at highest dose.	Litton Bionetics Inc. (1976)*
Ames test TA98, TA100, TA1535, TA1537 and TA1538	first study: 0.005, 0.015, 0.05, 0.15 and 0.5 mg/plate	Negative in both studies.	Ethyl Corporation (1981)*

	(± S9) second study: 0.001, 0.003, 0.01, 0.3, 0.1 mg/plate (± S9)	Cytotoxicity was apparent at the higher concentrations	
***In Vitro* mammalian cell gene mutation tests using the hprt and xprt genes** Sp5/V79 and SPD8 recombination assays (Chinese hamster cells)	0, 5, 10, 20, 30, and 40 µg/ml in DMSO (SPD8 assay) 0, 10, 20, 40, 70 µg/ml in DMSO (Sp5 assay) Both -S9	Negative (At doses producing 30-50% growth inhibition)	Helleday et al. (1999)*
***In vitro* mammalian chromosome aberration test** Human peripheral blood lymphocytes	4 h exposure: 0, 6.25, 25, 100 µg/ml (-S9) 0, 3.125, 12.5, and 50 µg/ml (+S9) 20 h exposure: 0, 6.25, 25, 75 µg/ml (-S9)	Negative. Highest dose selected for the evaluation of chromosome aberrations induced at least 50% toxicity (mitotic inhibition)	BioReliance (2001)*

* reported in EU RAR TBBPA (2006)

** The DS states (CLH report, Table 9) cytotoxicity at concentrations higher than tested, while the EU RAR states cytotoxicity at the higher concentrations

Seven negative studies for gene mutation (**Ames test**) were provided on TBBPA. These studies, described in the RAR as compatible with the current regulatory guidelines (EU RAR TBBPA 2008), were negative with and without S9 factor. Cytotoxicity or precipitation at doses below 5 mg/plate were detected in most of the Ames tests (Mortelmans *et al*., 1986; DOW Chemical Company, 1985; Velsicol Chemical Company, 1977; Litton Bionetics Inc., 1976 and Ethyl Corporation, 1981). When reported (including in NTP, 2014; Mortelmans, 1986), the validity of the protocol was confirmed with a concurrent positive control, and the negative controls were valid. According to OECD TG 471, some limitations were noted: none of the available studies employed all five strains advised by the OECD guideline up to the maximum recommended concentration (5000 µg/plate for soluble non-cytotoxic substances). Nevertheless, considering that all the strains were tested with and without S9, at doses beyond the recommended concentration in NTP (2014), and that all the studies are negative, the impact of this uncertainty should be limited.

Another issue is that the purity of TBBPA was not reported for most studies, except in NTP 2014 (stated to be greater than 99%). Nevertheless, RAC agrees with the DS that TBBA did not induce gene mutation in bacteria. Negative mutagenicity results in the presence or absence of metabolic activation are also available from studies in yeast and are considered as supportive evidence by RAC (as mentioned in the RAR, 2006: *S. cerevisiae* D4 in Brusick, 1976 and Litton Bionetics 1976; *S. cerevisiae* D3 in DOW Chemical Company, 1985, Velsicol Chemical Company, 1977 and Litton Bionetics, 1976).

One unconventional study for **gene mutation in mammalian cells**, performed similarly to OECD TG 476, is available (Helleday *et al*, 1999). Cloning efficiency and growth inhibition were assessed as a measure of cytotoxicity. The study was concluded as negative at doses inducing 30-50% growth inhibition. At 70 µg/ml, precipitation of the test substance was observed.

However, there was no information provided on whether S9 factors were used, nor were results presented for the positive control (camptothecin).

An *in vitro* **mammalian chromosome aberration test** in human peripheral blood lymphocytes was performed with TBBPA (purity 98.91%), with and without S9 (BioReliance, 2001). A report of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) of Australia (NICNAS, 2020) mentions GLP compliance). The cells were exposed for 4 or 20 hours, and a minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined. The highest dose (100 and 50 µg/ml without and with metabolic activation, respectively) in the main study induced at least 50% toxicity. The solvent and positive controls gave the expected responses. At no concentration of TBBPA was the percentage of metaphases with structural and numerical aberrations statistically significantly greater than that of the solvent.

RAC notes that for this endpoint NICNAS (2020) mentions another *in vitro* chromosomal aberration test in Chinese hamster lung cells (± S9) conducted according to GLP and OECD TG 473 (Yamakage, 2001, reported in Japanese with an English summary). TBBPA did not cause structural chromosome aberrations or polyploidy when exposed in absence of S9 for 6 h up to 6.5 µg/mL and for 24 h up to 60 µg/mL, nor in the presence of S9, with cells treated for 6 h with TBBPA 0-30 µg/ml in DMSO.

According to the RAR (EU RAR TBBPA (2006)), the chromosomal aberration study on human peripheral lymphocytes and the unconventional *in vitro* recombination assays were well-conducted.

In vivo studies

A **peripheral blood micronucleus test** with TBBPA (assumed equivalent or similar to OECD TG 474) was performed on groups of males and females B6C3F1/N mice by NTP (NTP, 2014). The mice were exposed 5 days per week during 14 weeks to 0, 10, 50, 100, 500 and 1000 mg/kg bw/d of TBBPA (>99% purity) by corn oil gavage. Slides of the smear were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of five animals per dose group. No increases in micronucleated NCEs were observed in male or female mice suggesting that TBBPA did not induce genotoxicity. In addition, the percentage of polychromatic (immature) erythrocytes (PCEs) in a population of 1,000 erythrocytes in the peripheral blood was scored for each dose group as a measure of bone marrow toxicity. No significant changes in the percentage of circulating polychromatic (immature) erythrocytes (PCEs) were observed in dosed mice suggesting that TBBPA did not induce bone marrow toxicity over the dose range tested. The study assessed the micronuclei formation in the blood of B6C3F1/N mice from the 3-months NTP repeated dose toxicity study where mice were treated up to 1000 mg/kg bw/d. No positive control and no scoring controls were reported by NTP study. The dose levels were well tolerated and the results suggest some mild systemic effects. Due to an unchanged PCE ratio, it was not demonstrated that the bone marrow was reached. The DS assumed that chemical-related effects on liver enzymes, organ weights, and kidney lesions indicate that the test substance reached the general circulation. RAC notes that genotoxicity guideline requirements for proof of target organ exposure have not been fulfilled for the dosing regime and species in question. The effects of TBBPA on the liver as suggested by the DS did not provide a conclusive indication for systemic (and bone marrow) exposure after oral administration and it is also uncertain if available toxicokinetic studies suggest bone marrow exposure. Unfortunately, no TK studies are available in mice. TK studies in rats suggest an extensive liver first pass effect resulting in low systemic bioavailability to the parent with excretion mainly via faeces.

The oral absorption and metabolism seem rapid, resulting in a low systemic bioavailability of TBBPA (Schauer *et al*., 2006):

- Maximum plasma concentrations of TBBPA metabolites (TBBPA-glucuronide and TBBPA-sulfate) were obtained after 4 h in humans and after 6 h in rats (TBBPA-sulfate) after a single oral dose (rats: gavage dose of 300 mg/kg bw);
- Parent TBBPA was not present in detectable concentrations in any of the human plasma samples, but peaked after 3 h in rats;
- Oral exposure of both humans and rodents to TBBPA results in low blood levels of TBBPA and its metabolites;
- At low dose exposure (20 mg/kg), tissues contained little or no detectable [^{14}C] after 24 hours following 1, 5, or 10 consecutive daily oral doses of ^{14}C]-labelled tetrabromobisphenol A in male F344 rats (Kuester and al, 2007).
- Tetrabromobisphenol A had terminal half-lives of less than 5 hours and systemic bioavailability was less than 5% in these animals (Kuester and al, 2007);
- No accumulation in tissues of male Sprague Dawley rats receiving 14 consecutive daily doses of 1,000 mg/kg tetrabromobisphenol A was observed in Kang *et al*. (2009) (reported in NTP, 2014)

The excretion is high (Kuester *et al*., 2007):

- More than 90% of a unique oral dose was found within faeces after 3 days, and most of the dose was eliminated in the first 24 h. Moreover, NTP (2014) mentions that studies highlighting the rapid absorption, metabolism and excretion following oral exposure to TBBPA indicated minimal sex and strain differences in rats;
- About 50% of an oral dose (20 mg/kg) was found in the bile within 2 h.

TBBPA has a low bioaccumulation potential (Unnamed, Study report, 1979).

There is some evidence in carcinogenicity study (NTP, 2014) that oral exposure of TBBPA via gavage reach the reproductive organs and the germ cells: in rats, atrophy of the testicular germinal epithelium was identified from 250 mg/kg bw/d during 2 years exposure 5 days/week and an increase in the lesion severity with the dose. Seminiferous tubules were lined by low flattened epithelium with lumens devoid of spermatozoa. Females did not seem to be affected in this study.

Overall, although relying on the Klimisch scores provided in the CLH report and RAR, RAC notes some limitations in the genotoxicity data package (among others, poor reporting) but the available *in vitro* studies cover bacterial and yeast mutations, mammalian gene mutation, and mammalian chromosomal aberrations, thus the *in vitro* testing battery is quite complete and no concern has been identified as all test were unequivocally negative. As several Ames test were conducted and turned out negative, the fact that not all strains were tested in the individual studies is not considered a concern. An uncertainty relates to the *in vivo* test on micronuclei formation. While the study was negative, convincing evidence for target organ exposure has not been demonstrated in this study or in other relevant studies (in terms of species and dosing regimen), neither do TK data provide this evidence and in fact suggest low systemic bioavailability following extensive liver first pass effect and rapid excretion. Nevertheless, as the *in vitro* studies where negative for chromosomal aberrations as well as for mutagenicity, RAC agrees with the DS that **classification of TBBPA for germ cell mutagenicity is not warranted,** based on the available data which taken together indicate that TBBPA is not genotoxic.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Two oral carcinogenicity studies, reported in NTP (2014), were performed on rats and mice with TBBPA. Both have been classified as reliable by the DS (Klimisch score 1). The purity of TBBPA was more than 99% and mice and rats were exposed 5 days per week during 2 years.
The animals (50 males and 50 females in each dose group) were tested at 0, 250, 500 and 1000 mg/kg bw/d by gavage in corn oil. However, due to excessive mortality in mice (seen in males and females, attributed to gastrointestinal toxicity), tumour incidence data in the 1000 mg/kg bw group was not analysed in the original report.

Oral administration of TBBPA to rats resulted in increased incidences of four types of neoplastic lesions: uterine adenoma, uterine adenocarcinoma, Müllerian tumour and testicular interstitial cell adenoma. A continuum was seen between endometrial (uterine) atypical hyperplasia and uterine adenomas and carcinomas. The combined incidence of uterine adenoma, adenocarcinoma, or malignant mixed Müllerian tumours was dose-related and the increases were statistically significant (by trend test or pairwise comparison). Furthermore, uterine tumour metastases were found throughout the body, and a reduced latency based on days of onset was observed, although not in a dose-dependent manner. The survival rate in rats was not affected by the TBBPA administration at any dose level.

In male mice, incidences of four types of neoplastic lesions were statistically significantly increased: hepatocellular adenoma, hepatoblastoma, hemangiosarcoma and large intestine tumours (NTP, 2014). A dose-dependence was observed in hemangiosarcoma, whereas large intestine tumours were observed in the highest dose (500 mg/kg bw/d) without severe mortality. The incidence of hepatoblastoma in male mice exceeded the historical control ranges (0-6%) for corn oil gavage studies.

The DS proposed several potential key-characteristics in order to identify plausible cancer mechanisms for TBBPA-induced carcinogenicity in rodents. These are described below.

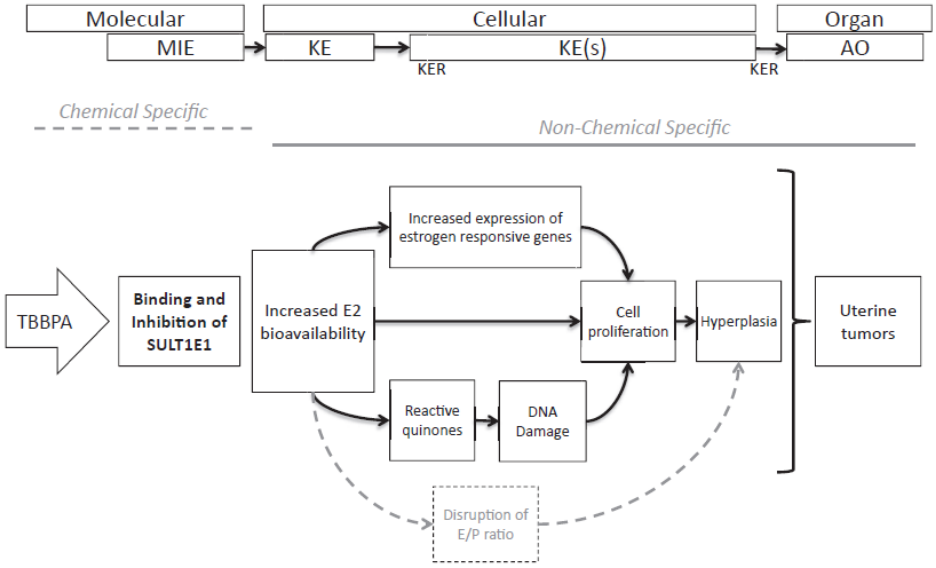
Receptor mediated effects

IARC stated that there is strong evidence that TBBPA presents receptor mediated effects (IARC, 2018).

<u>Disruption of oestrogen homeostasis</u>

TBBPA was suggested to affect oestrogen homeostasis by binding and inhibiting oestrogen glucuronosyltransferases and/or oestrogen sulfotransferases (major hypothesis of NTP, 2014).
This binding induces competition with oestrogen, which is assumed to lead to a decrease in oestrogen excretion, tissue-specific elevated levels of oestrogen (uterus and liver) and it was also proposed that it can increase formation of oestrogen-derived reactive metabolites (Sanders, 2016).

***Figure**: proposed MoA (Wikoff et al., 2016):*



The suggested molecular initiating event (MIE) is that TBBPA binds to and inhibits sulfotransferases SULT1E1 (Wikoff *et al*., 2016, figure above). In Dunnick *et al* (2015), it appears that binding affinities for TBBPA and oestradiol to sulfotransferases are similar.
The inhibition of sulfotransferases seems to lead to a non-linear metabolism of TBBPA at high doses. Borghoff *et al*. (2016) highlighted that saturation of conjugation of TBBPA occurs around 250 mg/kg/d in the liver, plasma and uterus, leading to a decrease in the TBBPA-S/TBBPA-GA ratio trend related to the dose, suggesting that at high TBBPA dose levels sulfation of TBBPA becomes limited. Nevertheless, as presented in Fig 12 of Borghoff *et al*. (2006)), due to the higher variability and lower concentration of these analytes in the uterus, statistical significance was only observed for the S/GA ratio at the 4-h time point (decreasing trend) and in the GA/TBBPA ratio (increasing trend) at the 8-h time point.

It was also proposed that higher serum levels of oestrogen may affect tumour-suppressor gene expression, including promotion of pre-existing *Tp53* mutations in the uterus through increased DNA synthesis and cell proliferation (Lai *et al.,* 2015) as, among others, the frequency of *Tp53* mutations was statistically significantly increased ($p<0.05$) in uterine carcinomas found in tetrabromobisphenol A-dosed rats compared to spontaneous tumours from control rats (NTP, 2014; Harvey *et al*., 2015)

<u>Disruption of thyroid hormone pathway</u>

This pathway was described in the CLH report as a possible carcinogenic key event (receptor-mediated effect) for uterine tumours (IARC, 2018). Nevertheless, where a decrease in serum T4 concentration in a 3-month study in rats was observed, the concentration of T3 and TSH remained unchanged. No decrease of T4 was observed in mice (NTP, 2014; Lai *et al*., 2015). In Colnot *et al*. (2014), no anatomical thyroid abnormalities in any of the rodent investigations reported was highlighted. Moreover, the link between the TSH pathway and uterine carcinoma is unclear.

<u>Other receptor mediated effects</u>

It was highlighted (mainly *in vitro*) that TBBPA is a promiscuous nuclear receptor modulator towards, among others, PPARγ, steroid hormone receptors and the PXR and disrupt

steroidogenesis in H295R human adrenal corticocarcinoma cells through the upregulation of progesterone and hydroxyprogesterone. These data were supported by ToxCast (IARC, 2018).

Oxidative stress

IARC stated that there is strong evidence that TBBPA induce oxidative stress (IARC, 2018). Several studies showed that an exposure to TBBPA can be linked to oxidative damage, via production of ROS (human neutrophil granulocytes) or via reaction of the 2,6-dibromobenzosemiquinone radical, a TBBPA metabolite (in Sprague–Dawley rat liver). It was also suggested that uterine glucuronidases might induce the release of free TBBPA from its conjugated form, increasing the potential for free radical formation at target-sites (NTP, 2014; Dunnick *et al*., 2015).

Inflammation and immunosuppression

TBBPA decreased the lytic and binding functions of isolated human natural killer cells and reduced the expression of cell-surface proteins needed for the attachment of human natural killer cells to target cells. Other data suggest that TBBPA may activate inflammatory pathways in human placental cells and mouse macrophages (IARC, 2018). Activation of the hepatic interferon pathway was seen in Wistar Han rats exposed to TBBPA (Dunnick *et al*., 2017).
Due to the absence of data on chronic effects *in vivo*, IARC stated that there is moderate evidence that TBBPA induce chronic inflammation, but strong evidence that TBBPA is immunosuppressive.

The DS concluded that there is clear evidence of carcinogenic activity in female Wistar Han rats based on the uterine tumours (predominantly uterine adenocarcinomas), some evidence in male mice based on the hepatoblastoma, equivocal evidence of carcinogenicity in male rats based on the occurrence of testicular adenoma and that the increased incidence in large intestine neoplasms and hemangiosarcoma (all organs) may have been related to TBBPA. Moreover, uterine tumour metastases were found as carcinomas throughout the body in female rats and malignancy was observed in some male mice as hepatocellular carcinomas, hepatoblastomas and hemangiosarcoma.
Regarding the plausible mechanism behind TBBPA-induced carcinogenicity, the DS concurred with IARC (2018) that there is strong evidence that TBBPA modulates receptor-mediated effects, induces oxidative stress and is immunosuppressive.

Overall, TBBPA was considered by the DS to be clearly carcinogenic in female rats, inducing uterine tumours, as well as liver tumours in male mice . The predominant tumour type in rats was uterine adenocarcinoma, which is also the predominant uterine tumour type in women. The DS was of the view that this finding is relevant to humans and therefore proposed a classification as Carc. 1B for carcinogenicity.

Comments received during consultation

Four MS supported classification in category 1B for carcinogenicity based on the different types of tumours found in two different species, metastasis, and the mode of action relevant to humans. One supporting MS correctly emphasised that if a consistent mode of action supports the observed effects, the absence of a full understanding of the MoA does not alleviate the level of evidence seen from experimental data. Also, no assessment was performed to clarify whether the competition with conjugation enzymes is specific to TBBPA or if it is relevant for other substances undergoing active conjugation. The MS highlighted that a potential threshold might be difficult to determine because non-linear dose-responses are observed.

One NGO also supported classification in category 1B for carcinogenicity.

Industry supported Cat 2 classification for carcinogenicity and provided several arguments for this proposal. These are described in detail below.

Differences between MoA and Key Characteristics

Regarding a proposed MoA, Industry argued that only disruption of oestrogen homeostasis was supported by studies. Evidence of other receptor-mediated effects, such as induction of oxidative stress and immunosuppression are related to Key Characteristics and are not representative nor equivalent to a mode of action and cannot be associated with non-carcinogenic effects as the biological significance of these mechanistic endpoints in the context of specific carcinogenic responses in animals or humans were not assessed according to the IPCS framework referenced in the ECHA guidance.

The DS agreed that an MoA is not the same as the Key Characteristics of carcinogens, but noted the strong link between CLP and the IARC classification criteria (ECHA, 2017a). In Monograph 115 of IARC, 2018, it was stated that: *"a majority of the Working Group considered that the strong mechanistic evidence that tetrabromobisphenol A can operate through three key characteristics of carcinogens and that these can be operative in humans warranted an upgrade to Group 2A."*

Species differences between rats and humans

Industry emphasised species differences in major metabolism pathways between rats (sulfate conjugation) and humans (glucuronide conjugation) which may influence the dose-dependence leading to sulfate saturation. Other structural similarity and kinetic aspects were also highlighted, such as that the endometrium is an oestrogen-responsive tissue in both rats and humans and is known to express oestrogen sulfotransferase in human tissue and that SULT1E1 is the isoform primarily responsible for oestrogen metabolism in humans (Coughtrie *et al*., 2002; Falany *et al*., 1998; Xu *et al*., 2012), but a tissue-specific evaluation of rat sulfotransferase messenger RNAs reported that they were not detected in the rat uterus (Dunn and Klassen, 1998). Other aspects, such as strain, gender, and dose differences may also influence kinetics related to sulfation in rats (Kuester *et. al*., 2007; Knudsen *et al*., 2014; Schauer *et al*., 2006).

The DS argued that even if TBBPA-sulfate was the major metabolite in rat plasma and urine and was only detected in a few individuals at some time points in humans, the evidence is not substantial enough to dismiss the effects of TBBPA in rats as non-relevant to humans, in accordance with ECHA endpoint specific guidance R.7.12. Regarding the kinetics, EFSA (2011) states that elimination half-lives do not differ considerably between experimental animals and humans: estimated half-lives are ~2 days and ~0.5 day in humans and rats (Sprague-Dawley).

Existence of a secondary mechanism of action

In addition, Industry emphasised that all of the modes of action for uterine carcinogenesis in female rats discussed in the dossier are associated with thresholds and that according to the CLP guidance, the existence of a secondary mechanism of action with the implication of a practical threshold may lead to a downgrading of a Category 1 to Category 2 classification. Indeed, the hormonal effects in the uterus are secondary to metabolic saturation and are specific to high dose exposure.

The DS reminded that a practical threshold *may* lead to a downgrading of the classifciation. Taking all available data into account and weighing the clear evidence of uterine cancer in female rats combined with some evidence in liver tumours in male mice as the most important evidence, DS reiterated their support for a Carc. 1B classification.

Structural similarity to other bisphenols

For Industry, the overall lack of oestrogen receptor activity identified for TBBPA and the fact that not all ER agonists can cause uterine tumours should be considered to decrease the strength of the evidence.

Concerning structural similarity, the DS noted that TBBPA is a bisphenol and is a brominated compound. Unlike bisphenol A, TBBPA does not bind significantly to the oestrogen receptor (ER) and this MoA is not targeted for carcinogenicity. Therefore, the structural similarity to other bisphenols, e.g. bisphenol A, is probably not so relevant for the carcinogenicity. TBBPA probably affects oestrogen homeostasis by competitive inhibition of oestrogen conjugation, thereby disturbing the oestrogen homeostasis.

Rat strain-specific sensitivity

Industry pointed out that Wistar Han rats were used instead of the commonly used F344 strain. Wistar rats have been shown to have elevated oestrogen levels and a higher oestrogen/progesterone ratio, which would cause this strain to be more susceptible to these effects than other rat strains (Lai *et al*., 2015).

In their response, the DS noted the wording of Lai *et al*. (2015): "*It is conceivable that Wistar Han strain rats resemble the SD strain, which are known to contain elevated levels of oestrogens as well as a higher oestrogen/progesterone ratio (Kacew et al., 1995) and this may account for the uterine carcinomas*". Moreover, according to the DS, the statement of Lai *et al* (2015) is misleading for two reasons - 1: SDs were Wistars almost a hundred years ago, and both stocks differ considerably between vendors/labs. 2: SDs were selected for endocrine experiments, while the Wistar is a much more general stock. There is no reason to conclude that Wistar Han rats are likely to be similar to Sprague-Dawley rats with respect to endocrine susceptibility.

Other uncertainties in the NTP study design

Industry also highlighted that there are unresolved questions regarding the design, in particular the utilisation of a novel histopathology technique (i.e., longitudinal sectioning, in contrast to a standard transverse section). For both cases, there were also very limited historical control data (HCD).

The DS argued that the residual longitudinal sectioning has now been incorporated as a standard protocol for NTP subchronic and chronic studies. For the HCD of the strain, as described in Greim *et al*. (2003), HCD can only be used if several requirements can be fulfilled, including same species and strain of experimental animal, same laboratory as the experimental data and same study design, experimental methods and assessment criteria. So even if the number of HCD is small (n=150) in the NTP report, they were considered by the DS more relevant than additional studies of Wistar Han rat uterine tumour background rates provided by Industry during the Consultation of the CLH report.

Strength of evidence according to CLP criteria

Industry contested that the CLP criteria requiring that animal experiments provide "sufficient" evidence had been met. They were of the view that clear evidence of carcinogenicity was limited to females (uterine tumours) and the carcinogenic findings on male mice may not be considered as strong evidence because of the lack of a clear dose-response relationship, the very high spontaneous control rate in control mice, and the approach for assessing incidence of hepatoblastomas. According to industry, it is recognized in the literature that hepatoblastomas should not be considered as a separate tumour type or incidence, because they represent a morphologically altered area of hepatocellular adenomas or carcinomas, rather than an

independently derived tumour (e.g., Turusov *et al*., 2002; Thoolen *et al*., 2010; Cattley *et al*., 2013).

Moreover, Industry highlighted that the evidence for carcinogenicity were restricted to a single experiment (NTP, 2014) and that as each tumour type was observed in only one sex and species. Multi-site response was also considered "not so evident" as carcinogenicity seems to be restricted to a narrow range of tissues or organs and that metastases were not described by the NTP as occurring to an unusual degree with regard to incidence, site, type of tumour, or age at onset.

The DS replied that classification of a substance involves both evaluation of strength of evidence (enumeration of tumours and statistical significance) and consideration of all other relevant information. Concerning the strength of evidence, the DS considered the findings in female rats to constitute clear evidence of carcinogenicity of TBBPA (uterine tumours, with extensive metastases). The carcinogenic potential of TBBPA is further supported by a statistically significant occurrence of some tumours in male mice (large intestine tumours and hemangiosarcoma and hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma), although not found to occur systematically in a dose-related manner. Moreover, uterine tumour metastases were found as carcinomas throughout the body in female rats, demonstrating malignancy.

The DS also stressed that literature is inconsistent regarding the origin of hepatoblastomas (hepatocellular adenoma, pluripotential hepatic stem cell, blastoma cells, neoplastic hepatocytes, oval cells) and that this tumour-type was mainly considered to represent some evidence of carcinogenicity in a WoE approach, but not clear evidence since combined incidences of hepatocellular carcinomas and hepatoblastomas were significant only in the 250 mg/kg group and the trend test was not statistically significant.

Assessment and comparison with the classification criteria

NTP (2014) reports two high quality carcinogenicity studies performed on rats and mice (see table below) exposed at 0, 250, 500 and 1000 mg/kg bw/d of TBBPA by gavage in corn oil. In each study, groups were composed of 50 animals per sex per dose. Supplementary groups composed of 10 animals per sex at 0 and 1000 mg/kg bw/d were added in the rat study.

In order to determine the primary site of invasive tumours and to avoid misinterpretation of gross lesion incidences, the histopathological protocol has changed at the NTP. This change has been implemented in rat 2-year study (NTP, 2014) for uterine zone sectioning. Originally, transverse sections/evaluation through the uterus horns were made to determine the primary location for adenocarcinomas in the cervix and vagina, and to examine for hyperplasia/fibrosis (original transverse uterine reviews). Following this, also longitudinal sections/evaluation were made to examine all remaining parts of the uterus, cervix, and vagina more completely (residual longitudinal uterine reviews), especially as cervix and vagina were not investigated in most animals in the original transverse review of uterine pathology. Residual longitudinal sectioning made it possible to determine the site of origin for grossly identified tumours, find more neoplastic/non-neoplastic lesions and to provide accurate diagnoses of some non-neoplastic lesions (and therefore avoid misinterpretations). These cuts were not included in the HCD, therefore comparisons of the HCD to NTP study controls were performed with the original transverse review of control sections.

***Table**: Summary of the carcinogenicity studies (from Table 11 of the CLH report, slightly modified).*

Method, guideline, deviations if any, species, strain, sex, no/group	**Exposure**	**Result** Statistically significant results are indicated **in bold** text/numbers as significant in trend test (**trend**) or by pairwise comparison (**value** statistically significant in bold text)	**Reference**
2 years carcinogenicity study in Wistar Han Rats Considered as OECD TG 451(/453) compliant by the DS 50 animals/sex/dose 10 extra animals/sex in control and high dose group for interim evaluation at 3 months	TBBPA purity > 99% Doses: 0, 250, 500, 1000 mg/kg bw/d by oral gavage in corn oil, 5 days per week for up to 104 weeks (male rats) or 105 weeks Complete necropsies and microscopic examinations were performed on all rats. At the 3-month interim evaluation, the heart, right kidney, liver, lung, right testis, and thymus were weighed.	No mortalities nor clinical findings were found in exposed groups compare to controls. Survival rates: 33/50, 28/50, 38/50, 39/50 in male rats and 35/50, 34/50, 29/50, 33/50 in female rats (at 0, 250, 500, 1000 mg/kg bw/d). The mean body weight of male rats in the two highest dose groups were generally at least 10 % lower after 25 weeks than in the control group. No changes in females. **<u>Non-neoplastic lesions:</u>** Cystic endometrial hyperplasia: statistically significant increase only with original transverse review of the uterus (8/50, 13/50, 11/50, **18/50** at 0, 250, 500, and 1000 mg/kg bw, **trend**). Combined with the results from the residual longitudinal review, the results were not significant (24/50, 31/50, 30/50, 32/50 at 0, 250, 500, and 1000 mg/kg bw). Rete ovarii cyst statistically significantly increased in 500 and 1000 mg/kg females (1/50, 0/50, **6/50, 6/50** at 0, 250, 500, and 1000 mg/kg bw). Atrophy of the testicular germinal epithelium identified in seven treated males (0/50, 4/50, 1/50, 2/50 at 0, 250, 500, and 1000 mg/kg bw), and the **severity of the lesions increased** with increasing dose. **<u>Neoplastic lesions:</u>** *Female rats*: Increase of incidence of uterine tumors in female rats in the two highest dose groups (500 mg/kg bw and 1000 mg/kg bw), a continuum was seen: Endometrial (uterine) atypical hyperplasia (2/50, **13/50, 11/50, 13/50**, at 0, 250, 500, and 1000 mg/kg bw/d as original transverse and residual longitudinal reviews, combined); Uterine adenoma (original transverse review- 0/50, 0/50, 3/50, 4/50, at 0, 250, 500, and 1000 mg/kg bw/d **trend**; transverse and longitudinal combined 3/50, 2/50, 4/50, 6/50 at 0, 250, 500, and 1000 mg/kg bw/d) – HCD: 0/150; Adenocarcinoma (original transverse review-3/50, 3/50, 8/50, 9/50, at 0, 250, 500, and 1000 mg/kg bw/d **trend**; residual longitudinal review 4/50, 9/50, **15/50, 15/50**, at 0, 250, 500, and 1000 mg/kg bw/d, **trend**; original transverse and residual longitudinal reviews, combined- 4/50, 10/50, **15/50, 16/50**, at 0, 250, 500, and 1000 mg/kg bw/d, **trend**) – HCD: 4.7% ± 2.3%, range 2-6%; Malignant mixed Müllerian tumor (original transverse review- 0/50, 4/50, 0/50, 2/50 at 0, 250, 500, and 1000 mg/kg bw/d) – HCD: 0/150; Adenoma, adenocarcinoma, or malignant mixed Müllerian tumour (original transverse review- 3/50, 7/50, **11/50, 13/50** at 0, 250, 500, and 1000 mg/kg bw/d, **trend**; residual longitudinal	NTP, 2014 Dunnick *et al*, 2015

Method, guideline, deviations if any, species, strain, sex, no/group	**Exposure**	**Result** Statistically significant results are indicated **in bold** text/numbers as significant in trend test (**trend**) or by pairwise comparison (**value** statistically significant in bold text)	**Reference**
		6/50, 10/50, **16/50, 16/50** at 0, 250, 500, and 1000 mg/kg bw/d, **trend**; original transverse and residual longitudinal reviews, combined-6/50, 11/50, **16/50, 19/50** at 0, 250, 500, and 1000 mg/kg bw/d, **trend**) - HCD: 4.7% ± 2.3%, range 2-6%. Uterine tumor metastases were found as carcinomas throughout the body *Male rats:* Increase of testicular interstitial cell adenoma incidence: 0/50, 0/50, 1/50, 3/50 (**trend**) - HCD: 2.7% ± 2.3%, range: 0 – 4%.	
2 years carcinogenicity study in B6C3F1/N mice Considered as OECD TG 451(/453) compliant by the DS 50 animals/sex/dose	TBBPA purity > 99% Doses: 0, 250, 500, 1000 mg/kg bw/d by oral gavage in corn oil, 5 days per week for up to 105 weeks	Reduced body weight was seen in top dose females. The body weights were 10-25% of vehicle controls after week 25. Statistical significance was not reported. Due to early mortality, tumor incidence data in the 1000 mg/kg bw group is not presented. Survival rate: 33/50, 26/50, 39/50, 12/50, at 0, 250, 500, 1000 mg/kg bw/d in male and 40/50, 31/50, 36/50, 4/50 in female mice (at 0, 250, 500, 1000 mg/kg bw/d) **Non-neoplastic lesions:** *Male mice* Liver: Statistically significantly increase in clear cell focus incidence (at 500 mg/kg bw) males and eosinophilic focus (at 250 and 500 mg/kg bw); increase incidence of mixed cell focus in the liver (at 500 mg/kg bw). Kidney: Renal tubule cytoplasmic alteration significantly increased (all dosed groups, severities increase with increasing dose) incidences of nephropathy in the 250 and 500 mg/kg bw groups were significantly decreased. Forestomach: significant increase of ulcer, mononuclear cell cellular infiltration, inflammation, and epithelium hyperplasia incidence in 500 and 1000 mg/kg bw males and all dosed groups of females. **Neoplastic lesions:** *Male mice* Liver: Increase in multiple hepatocellular adenoma (12/50, 20/50, **28/50**, at 0, 250, and 500 mg/kg bw/d) but incidences were still within the hepatocellular adenoma incidence range of HCD from oral studies (No HCD provided for multiple hepatocellular adenoma) Hepatocellular carcinoma (11/50, 15/50, 17/50, at 0, 250, and 500 mg/kg bw/d, HCD: 34.8% ± 10.9, range 22-44%), Hepatocellular adenoma (not mentioned if multiple adenoma were included) and carcinoma combined (39/50, 39/50, 43/50, at 0, 250, and 500 mg/kg bw/d, HCD: 75.6% ± 3.3, range 70-78% NTP website reports historical control for gavage with corn oil),	NTP, 2014 Dunnick *et al*, 2015

Method, guideline, deviations if any, species, strain, sex, no/group	Exposure	Result Statistically significant results are indicated **in bold** text/numbers as significant in trend test (**trend**) or by pairwise comparison (**value** statistically significant in bold text)	Reference
		Hepatoblastoma (2/50, **11/50**, 8/50, at 0, 250, and 500 mg/kg bw/d), and exceed the incidence of historical control ranges in exposed groups (HCD: 3.6% ± 2.6%, range 0-6%) Hemangiosarcoma (in all organs) (1/50, 5/50, **8/50**, at 0, 250, and 500 mg/kg bw/d, **trend**), HCD: 11.2% ± 6.4%, range 2-18%), Intestine*:* increase of large intestine tumors (0/50, 0/50, **3/50**, at 0, 250, and 500 mg/kg bw/d, **trend**), HCD: 0/250 *Female mice* Hepatocellular carcinoma (2/50, 3/50, 5/49, at 0, 250, and 500 mg/kg bw/d, HCD: 10.4% ± 5.6, range 4-18%), Hepatocellular adenoma multiple (1/50, 4/50, 4/49, at 0, 250, and 500 mg/kg bw/d, HCD not available)	

In the rat study, at the 3-months interim evaluation, absolute and relative thymus weights of 1000 mg/kg male and female rats were significantly less than those of the vehicle control groups, and there was a significant increase in relative liver weights in the 1000 mg/kg groups. No treatment-related histopathological lesions were observed in males or females.

An increased incidence in uterine tumours in female rats in the two highest dose groups was observed, with the presence of a continuum of uterine atypical hyperplasia, adenoma, adenocarcinoma and malignant mixed Müllerian tumour (see Table below). The increase in endometrial (uterine) atypical hyperplasia was statistically significant in all dose groups during the residual longitudinal review of the uterus, with a steep dose-response curve. Uterus endometrium atypical hyperplasia was not present in the cross sections of originally examined tissues but was only diagnosed in the longitudinally examined tissues. Despite the atypical features, these proliferative lesions were not considered adenomas as they did not form a distinct mass or compress the surrounding uterine architecture (NTP, 2014)

Except for the Müllerian tumour, all the tumour increases were dose-dependent, and statistically significant for the trend, or by pairwise comparison. Adenocarcinomas invaded distant organs, including the intestines, liver, mesentery, pancreas, adrenal gland, ovary, lymph node, spleen, thymus, subcutaneous tissue, skeletal muscle, lung, and kidney. Müllerian tumours were similar to adenocarcinomas in morphology. Tumours in four animals in the 250 mg/kg group had extensive metastases to the liver, mesentery, pancreas, stomach, ovary, spleen, subcutaneous tissue, lung, and kidney.

***Table**: Overview of uterine tumours observed in a rat carcinogenicity study with TBBPA (NTP, 2014)*

Females Rats	**Gavage dose of TBBPA (mg/kg bw/d) – 5 times/week**				**Historical incidence: all routes *****
Original transverse review	**0**	**250**	**500**	**1000**	
Adenoma	0/50**	0/50	3/50	4/50	0/150 (0%)
Adenocarcinoma	3/50*	3/50	8/50	9/50	7/150 (4.7%)
Malignant Mixed Müllerian Tumour	0/50	4/50	0/50	2/50	0/150 (0%)
Adenoma, Adenocarcinoma, or Malignant Mixed Müllerian Tumour	3/50**	7/50	11/50*	13/50**	7/150 (4.7%)
Residual longitudinal review	**0**	**250**	**500**	**1000**	**Not comparable**
Adenoma	3/50	2/50	1/50	3/50	/
Adenocarcinoma	4/50**	9/50	15/50**	15/50**	/
Malignant Mixed Müllerian Tumour	0/50	0/50	0/50	1/50	/
Adenoma, Adenocarcinoma, or Malignant Mixed Müllerian Tumour	6/50**	10/50	16/50**	16/50*	/
Atypical hyperplasia	2/50	13/50**	11/50**	13/50**	/
Combined original transverse and residual longitudinal reviews	**0**	**250**	**500**	**1000**	**Not comparable**
Adenoma	3/50	2/50	4/50	6/50	/
Adenocarcinoma	4/50**	10/50	15/50**	16/50**	/
Malignant Mixed Müllerian Tumour	0/50	4/50	0/50	2/50	/
Adenoma, Adenocarcinoma, or Malignant Mixed Müllerian Tumour	6/50**	11/50	16/50**	19/50**	/
Atypical endometrial hyperplasia	2/50	13/50**	11/50**	13/50**	/

* Positive trend test or significantly different (p ≤0.05) from the control group by Poly 3 test

** Positive trend test or significantly different (p ≤0.01) from the control group by Poly 3 test

*** Residual longitudinal sectioning was not included in the HCD

The NTP HCDbase contains all 2-year studies for each species, sex, and strain/stock with histopathology findings in control animals completed within the most recent 5-year period at the time (for uterus neoplasms in females Wistar Han Rats: 2013). The historical control incidence for uterine adenocarcinoma was 7/150 (includes one endometrium carcinoma), 0/150 for malignant mixed Müllerian tumors (all routes) and 7/150 (all routes) for all the uterine tumors (combined). Therefore, the findings were within the same order of magnitude as the incidence of neoplasms in the control group of the original transverse review for NTP studies (2014), i.e. 0/50 for adenoma, 3/50 for adenocarcinoma and 3/50 for adenoma, adenocarcinoma or malignant mixed Müllerian tumors combined. The historical control range for endometrial (uterine) atypical hyperplasia was not found. A comparison with HCD is useful to evaluate whether the concurrent control performs as expected within the normal range of variation. This is the case and the concurrent control is the most important control for the assessment of TBBPA-related uterine tumours. There is no reason to believe that the incidences from the residual longitudinal review should not be compared to concurrent control.

In males, the incidences of interstitial cell adenomas were slightly increased in 500 and 1000 mg/kg male groups (1/50 and 3/50 respectively). The incidence at the highest dose exceeded the historical control incidence for all administration routes (4/150). Atrophy of the testicular germinal epithelium was identified in 7/150 treated males, and the severity of the lesion was

dose-dependently increased. Approximately 50% to 90% of seminiferous tubules were affected in most cases and had lumens devoid of spermatozoa.

In the mouse study, increased mortality was seen in males and females at 6 months, which was attributed to gastrointestinal toxicity (NTP, 2014). Therefore, results at 1000 mg/kg were not statistically analysed, but the incidences were nevertheless presented in the NTP report. Adverse effects indicating forestomach toxicity included dose-related ulcer and related effects such as inflammation, hyperplasia and/or mononuclear cell infiltrate in both sexes and were observed in 500 and 1,000 mg/kg males and all dosed groups of females. Other non-neoplastic lesions consisted of renal tubule cytoplasmic alterations characterized by a decrease or absence of the vacuoles normally present in the cortical proximal tubules and liver effects. The mortality increase did not seem to correlate directly with the TBBPA dose, as the percent probability of survival at end of the study was higher at 500 mg/kg bw/d than at 200 mg/kg bw/d (66, 50, 78 and 25% for males and 80, 62, 72, and 8 % for females at 0, 250, 500 and 1000 mg/kg bw/d respectively). It has to be noted that the mortality occurs much earlier in female mice than in male mice, leading to less of 35% of survival after 75 weeks whereas the survival in male group is still two times higher in males at that time point (around 70% of survival, see fig 7 in NTP, 2014).

In the liver, a statistically significant increase of multiple hepatocellular adenoma was observed at 500 mg/kg bw/d (28/50), whereas the incidence of hepatocellular simple adenoma (20/50, 13/50, 10/50, at 0, 250, and 500 mg/kg bw/d) was not statistically significantly increased. The incidence of hepatocellular adenoma was within historical control range provided for hepatocellular adenoma in corn oil gavage studies (52-64%). The historical control range for multiple hepatocellular adenoma was not found. The concurrent control and HCD show that hepatocellular adenomas appear at high control incidences. The slightly increased incidence at the 500 mg/kg bw/d dose group is therefore probably of low concern. However, malignant tumours were also found, although the incidence did not always increase dose-dependently. An increased incidence of hepatoblastoma and hepatocellular carcinoma was observed in male mice at all doses (2/50, 11/50, 8/50 and 11/50, 15/50, 17/50, at 0, 250, and 500 mg/kg bw/d, respectively). Only the increase of hepatoblastoma was statistically significant (pairwise comparison) at 250 mg/kg bw/d. The significant increase in hepatoblastoma was also outside of the historical control range (0-6%) at both 250 and 500 mg/kg bw/d. The incidence of hepatocellular carcinoma was within the HCD at all the doses tested. While no clear dose-response relationship is evident, RAC notes that this phenomenon is observable also in other studies and for other effects, with a levelling-off at doses of 300-500 mg/kg bw/d and higher (see the reproductive toxicity section). Also, the exclusion of the high dose from analysis hampers a proper dose-response assessment being conducted because only two dose levels remained. The fact that adenoma, carcinoma and hepatoblastoma showed higher incidences in treatment groups raises a concern.

In males, the incidences of hemangiosarcoma (all organs) occurred with a significant positive trend and the incidence was significantly increased (pairwise) in the 500 mg/kg group (1/50, 5/50, 8/50, at 0, 250, and 500 mg/kg bw/d). These lesions occurred in a variety of organs such as the bone marrow, liver, lung serosa, lymph nodes, skin, spleen, and vertebra.

The incidences of adenoma or carcinoma (combined) of the large intestine (caecum or colon) occurred with a significant positive trend in males. However, this is based only on one dose, as the incidence in controls and at the low dose was zero. A firm conclusion on a whether there is a dose-response relationship is therefore not possible. The incidence in the 500 mg/kg group exceeded the HCD for corn oil gavage studies, which was 0/250. The concurrent control thus was in line with the HCD, indicating the tumour type was rare.

RAC assessment of uterine tumours

To summarise, NTP (2014) studies with rats highlighted a continuum from endometrial atypical hyperplasia to malignant tumours. Both adenocarcinomas and mixed Mullerian tumours appeared to be invasive and of high malignancy: the metastatic rate was 76% for the malignant mixed Müllerian tumour (4/6) and 24% (11/45) for adenocarcinomas. The findings on the first day of tumour onset was considered to indicate reduced latency. The first incidence of tumours was reduced in all exposed groups compared to controls for carcinomas, indicating reduced tumour latency (713d, 548d, 321d, 442d at 0, 250, 500, and 1000 mg/kg bw/d, respectively), in original transverse and residual longitudinal reviews (combined). For malignant mixed Müllerian tumours a decrease in latency was difficult to establish, as the incidence is zero for the controls. Moreover, it appears that early mortalities were slightly increased in female rats at the low and mid doses (moribund: 8, 14, 15, 10 and natural deaths: 4, 2, 6, 3 for 0, 250, 500, and 1000 mg/kg bw/d) and it could not be excluded that this could lead to bias when it comes to assessing reduced latency.

Strains of rats

NTP (2014) used the Wistar Han strain, in contrast to previous studies where F344 rats were commonly used. This limited the HCD database to 150 animals. The acute, sub-chronic, developmental, reproductive and neurobehavioral studies were conducted using Sprague-Dawley and F344 rat strains. According to Lai *et al*. (2015) "It is conceivable that Wistar Han strain rats resemble the SD strain, which are known to contain elevated levels of oestrogens as well as a higher oestrogen/progesterone ratio (Kacew *et al*., 1995) and this may account for the uterine carcinomas". RAC is of the opinion that the metabolic differences could potentially be relevant. However, as a result, RAC would not expect qualitative differences relevant for hazard classification, and discussion of differences in sensitivity linked to the strain relate to risk assessment. There is no justification to disregard the Wistar Han strain from consideration for a carcinogenicity classification. In addition, as in the NTP study the Wistar Han concurrent negative control was considered reliable, and a HCD database is available, there is no reason to not take into account the statistically significant and dose dependent increase in uterine tumours detected in the exposed groups. In addition, RAC takes note of a 3-month interim evaluation performed on Wistar Han rats in order to compare with the 3-month endpoints in the F344/NTac rats (NTP, 2014). According to the study report, the results of the 3-months interim evaluation in the 2-year Wistar Han rat study (vehicle control and 1,000 mg/kg groups) were similar to those in the 3-month F344/NTac rat study.

Plausible MoA and discussion on Key Characteristics

TBBPA is not mutagenic in standard assays and has a very low affinity to ER and other steroid hormone receptors and only induces ER-dependent cell proliferation at excessively high concentrations (Lai *et al*. 2015). According to Dunnick *et al*. (2015), evidence indicates that debromination by cleavage of a bromine-carbon bond and resulting formation of DNA-damaging free radicals and adducts is not a major metabolic pathway for TBBPA in rats.

In the NTP (2014) study, a statistically significant increase (Fisher's exact test, $p<0.05$) in the incidence of point mutations in the rat Tp53 gene was observed in uterine adenocarcinomas from TBBPA-exposed animals (10/16; 63%) compared to spontaneous uterine adenocarcinomas in control animals (1/9; 11%) (see the Table below). Tp53 is one of the most commonly altered tumour suppressor genes in multiple types of cancers including uterine carcinomas. Additionally, uterine adenocarcinomas from two rats exposed to TBBPA harboured multiple mutations.

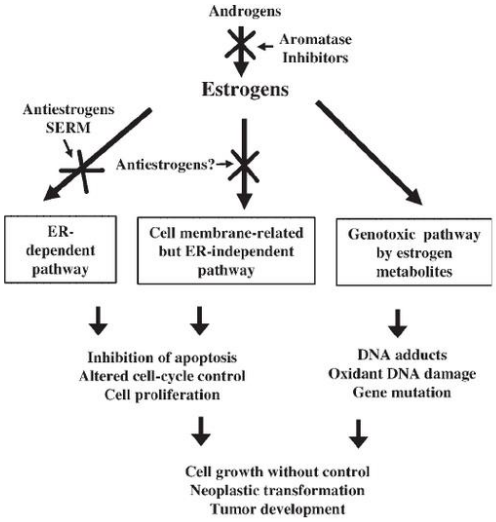
***Table**: Tp53 mutation pattern observed in uterine carcinoma in NTP (2014) rat study on TBBPA*

	Mutation Frequency (%)	Exon 5	Exon 6	Exon 7	Exon 8
Control					
Total incidence	1/9 (11%)	0	1	0	0
Tetrabromobisphenol A-dosed					
250	3/3 (100%)	1	2[b]	1[b]	0
500	3/7 (43%)	1	0	1	1
1,000	4/6 (67%)	1	2[b]	0	2[b]
Total incidence	10/16* (63%)	3	4[b]	2[b]	3[b]

*Significantly different ($P < 0.05$) from total control incidence.
[a]Female Wistar Han rats were administered 0, 250, 500, or 1,000 mg/kg tetrabromobisphenol A in corn oil by gavage for 2 years. Silent mutations are not included.
[b]Includes at least one animal with double mutations.

It was suggested by the DS that mutations in the *Tp53* gene (exon 5 to 8) found in the tumours could be caused by an indirect effect of TBBPA, and that TBBPA, by inhibiting the binding of oestrogen to glucuronosyl-transferases and/or sulfotransferases, leads to an increase in the circulating oestrogen level. This results in promotion of pre-existing *Tp53*-mutations in the uterus by promoting proliferation of cells, including cells with pre-existing mutations, thereby leading to tumour formation. Uterine tumours were observed after long-term administration of several hormonally active chemicals such as 17ß-estradiol or tamoxifen in rats.

***Figure**: The MoA suggested by the DS for uterine adenocarcinoma in rats treated with TBBPA*



In the view of RAC, Tp53 mutations are commonly found in different types of malignant tumours and the increased incidence in Tp53 mutations in TBBPA-related uterine tumours can be considered as a general marker for tumour progression and malignancy.

Regarding the MoA for metabolism-mediated increases in oestrogen levels, RAC notes that there are several significant uncertainties, therefore definitive conclusions are not possible. The reproductive studies with TBBPA have not demonstrated a significant oestrogen-mediated response (e.g., accelerated vaginal opening) consistent with either oestrogen receptor agonist activity or increased circulating oestrogen levels due to inhibition of oestradiol sulfation. In addition, no information on oestrogen homeostasis is available in the NTP (2014) or Borghoff *et al*. (2016) studies. High oestrogen levels in the blood would provide strong evidence for this MoA, but results from measurements of oestrous-cycle -dependent hormones are also needed. In other words, important key and associated events of this hypothesis seem either not to have been investigated or proven. RAC also questions the assertion that Tp53 expression is a specific key

event of the uterine carcinogenicity adverse outcome pathway (AOP). As a general remark, no validated AOP with its initiating and key events has been presented to RAC. In that sense, it is not considered crucial whether Tp53 mutation higher incidence was dependent on TBBPA dose or also harboured multiple mutations per tumour such that a strict dose-dependency of Tp53 mutations may not be expected.

In addition, RAC notes that no other relevant oestrogen-sensitive malignant lesions in other organs have been reported from the NTP studies, i.e. no lesions in the ovary or mammary gland in mice or rats. The predominant tumour type in rats was uterine adenocarcinoma, a tumour, which is also the predominant uterine tumour type in humans. The NTP and the DS consider the findings in uterine tumours in female rats in the 2-year study with TBBPA to be clear evidence for carcinogenic activity and RAC agrees with this conclusion. Sanders *et al*. (2016) assessed biological changes in serum, liver, and sections of the uterine horn of Wistar Han rats 24 h following administration of the last of five daily oral doses of 250 mg/kg TBBPA. Changes in the liver and uterus were observed for expression of genes associated with receptors, biosynthesis and metabolism of oestrogen (down-regulation of Hsd17b2 in uterus). Hsd17b2 gene encodes 17β-HSD type 2, an enzyme that converts E2 to the less active estrone (E1). CYP1B metabolizes E1 and E2 to catechol (hydroxyestrogens), and redox cycling. Some of these metabolites can lead to formation of DNA-reactive semiquinones. In Sanders *et al*. (2016), Cyp1b1 was significantly upregulated in different sections of the uterus following TBBPA exposure. These data may support the potential for increased formation of reactive oestrogen-derived metabolites in tissues of TBBPA-treated rats. Increased expression of Cyp2b1 and Cyp2b2 was observed in the liver of TBBPA-treated rats. An increase in liver CYP2B1and CYP2B2 has been observed in tamoxifen-treated Sprague Dawley rats. Tamoxifen is also known to provoke an estrogenic effect in the uterus in humans resulting in an increased risk of cancer in that tissue (Sanders *et al*., 2016). Overall, RAC considers that this MoA is plausible but that no firm conclusion is possible.

IARC (2018) also propose oxidative stress as a key characteristic that can be involved in the MoA of carcinogenicity of TBBPA. It was demonstrated that TBBPA activates several stress pathways, in particular the oxidative stress pathway, in *in vitro* studies on human neutrophil Granulocytes, where TBBPA induced significant dose-dependent increases in the production of reactive oxygen species (ROS) and increased intracellular calcium concentrations. The effect on oxidative stress was supported by several *in vitro* non-human mammalian systems. It was also suggested in NTP (2014) and Dunnick *et al*. (2015) that uterine glucuronidases might induce the release of free TBBPA from its conjugated form, increasing the potential for free radical formation at target-sites. While RAC acknowledges that oxidative stress is one of the probable key characteristics of carcinogens, no MoA data have been presented to clearly link an oxidative stress pathway to TBBPA-induced uterine tumours and thus, it remains a hypothesis.

The DS raised the hypothesis of linking uterine tumours to the thyroid hormone modulation. Sanders *et al* (2016) showed that expression of the gene (Thra) encoding the thyroid hormone receptor (TRα) is increased in the liver and sections of the uterus. Further, serum T4 decreased in TBBPA-treated rats. Nevertheless, serum T3 and TSH were not significantly affected, and no changes were observed in the histological morphology of the thyroid (NTP, 2014). In the view of RAC, such MoA hypothesis seem speculative und uncertain.

Several studies have addressed the possible agonistic or antagonistic properties of TBBPA on receptors in various human cell lines (IARC, 2018). As summarized by the DS, TBBPA is a promiscuous nuclear receptor modulator with higher potency towards PPARγ than other receptors, but is also active for steroid hormone receptors and the xenobiotic receptor PXR. While receptor-mediated effects are, in general, relevant for human carcinogenicity and the discussion on receptor-mediated effects may be valid, RAC considers that a concrete MoA hypothesis is missing

and no conclusions can be drawn that would be of relevance for the carcinogenicity classification proposal at hand.

IARC (2018) and Dunnick *et al*. (2017) also proposed immune suppressive effects as a potential key characteristic of TBBPA carcinogenicity, as decreases in immune function can facilitate cancer development. Some findings suggesting effects on the immune system after TBBPA exposure were detected, but RAC cannot extract any firm evidence in support of a specific MoA.

To summarize the above discussion, most of the proposals presented by the DS correspond to key characteristics of carcinogen – as published by IARC – and all such key characteristics resembling a multitude of diverse MoA, rather than offering one or several complete and specific MoA for TBBPA uterine carcinogenicity. Amongst those MoAs described specifically for uterine carcinoma (Yoshida *et al*. 2015), none has been proven or sufficiently investigated based on the crucial key events. A modulation and imbalance of oestrogen/progesterone ratio has not been investigated, while some indications exist for a modulation of oestrogen metabolism via induction of CYPs as well as a decreased E2 excretion and increased E2 levels in the blood due to modulation phase 2 drug metabolism enzymes (i.e. sulfoxylation).

The most complete proposal for RAC indeed is related to disruption of estrogen homeostasis and many uncertainties remain. During consultation of the CLH report it was raised that differences in metabolism between rats and human, especially for species differences between sulfate conjugation, can make the relevance to humans disputable. In the view of RAC, as long as the sulfate conjugation exists in humans, the saturation of this pathway cannot be excluded and this may lead to the toxic effects. There is no strong data showing that it is not possible to reach a dose level leading to saturation of sulfate conjugation in humans. The metabolic differences are of quantitative nature rather than qualitative nature and therefore do not enable disregarding the relevance for humans of the uterine tumors. Moreover, as many uncertainties remain regarding the MoA of TBBPA in the development of uterine carcinoma (or several MoA potentially operating), it is not possible to conclude that these are not-relevant to humans, based on a single proposed MoA hypothesis.

Therefore, RAC's view is that one very well documented study highlighted a continuum from atypical hyperplasia to high malignancy uterine tumors, with presence of metastases in female rats. The increase in incidence of high malignancy tumors is statistically significant, dose-dependent and outside of the comparable HCD (original transverse view). Signs of decreased latency were observed. Considering both sexes is not relevant for this type of tumor, but no uterine tumors were seen in female mice. Nevertheless, the mortality of female mice was extensive and occurred very early during the study, hampering carcinogenicity assessment in female mice (see "RAC assessment of liver tumors"). Some structural similarities appear with other chemicals that induce uterine carcinoma (bisphenol A), but there is a high level of probability that the MoA between these two substances are different, as TBBPA was not detected to bind ER directly. Nevertheless, the evidence does not appear to be sufficiently convincing to describe the suggested MoA as not relevant to humans, and the metabolism is not fundamentally different between rodents and humans. No excessive toxicity was found in female rats with adenocarcinomas. NTP considered the uterine tumors as clear evidence for carcinogenicity. Therefore, RAC is of the view that uterine tumors are relevant for classification.

RAC assessment of testis adenomas

An increase of testicular interstitial cell adenoma was observed in male rats. The increase was dose dependent (0/50, 0/50, 1/50 and 3/50 in control, 250, 500 and 1000 mg/kg bw/d, respectively) and exceeded the historical control incidence (2.7% ± 2.3% for all routes) in the highest dose group. Comparing the incidences to that of the concurrent control group (0/50), the data indicate a relationship to treatment, although specific HCD for corn oil gavage was not

provided. The increase is statically significant by a trend test but not by pairwise comparison. No testis adenoma nor carcinoma were described in mice and the latency of first occurrence onset did not decrease between mid and high dose in rats. In the same study, atrophy of the testicular germinal epithelium, with severity of the lesions increasing with the dose, was described in rats. No shift to malignancy of testicular adenomas was reported. NTP considered the testis interstitial cell adenoma as "equivocal evidence" of carcinogenicity, as the occurrence of the increase was statistically significant (trend test) but the control incidences were at the low end of the historical control range for this tumor and the incidence in the high dose group was, in their opinion, still low. RAC does not see any convincing reason not to rely on the concurrent control, as it is still within the HCD. Nevertheless, RAC agrees that testis adenomas may be considered as a plausible indication of carcinogenicity, but do not play a central role in the decision to classify for carcinogenicity.

RAC assessment of liver tumours

In male mice, liver tumours were detected, in addition to large intestine tumours and hemangiosarcomas,. More specifically, an increase in multiple hepatocellular adenoma, hepatocellular carcinoma and hepatoblastoma was highlighted in male mice exposed to TBBPA compared to the control group (NTP, 2014). Hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma are considered to represent a biological and morphological continuum in the NTP study. Hepatoblastoma is a malignant tumour type, very rare in adult humans. No increase in neoplastic incidences were found in rat liver after TBBPA exposure. The data are presented in the table below.

***Table**: Overview of liver neoplastic lesions observed in mice carcinogenicity study (NTP 2014 study)*

	Gavage dose of TBBPA (mg/kg bw/d) – 5 times/week			
Male Mice	**0**	**250**	**500**	**1000***
Hepatoblastoma	2/50	11/50**	8/50	3/50
Hepatocellular adenoma simple	20/50	13/50	10/50	9/50
Hepatocellular adenoma multiple	12/50	20/50	28/50**	12/50
Hepatocellular carcinoma	9/50	11/50	12/50	7/50
Hepatocellular carcinoma multiple	2/50	4/50	5/50	2/50
Female mice	**0**	**250**	**500**	**1000***
Hepatocellular adenoma simple	12/50	9/50	11/49	1/49
Hepatocellular adenoma multiple	1/50	4/50	4/49	0/49
Hepatocellular carcinoma	2/50	3/50	5/49	1/49
Hepatocellular carcinoma multiple	0/50	1/50	0/49	0/49

* Values provided for indication but as the percent probability of survival at end of study was 25% in males and 8% for females at this exposure

** Positive trend test or significantly different ($p \leq 0.05$) from the control group by Poly 3 test

A dose dependent increase up to 500 mg/kg bw/d was observed in male mice for multiple hepatocellular adenoma, hepatocellular carcinoma and multiple hepatocellular carcinoma. No statistical significance was observed in the liver for simple adenoma and carcinoma (simple or multiple) and the incidences were within the HCD provided (58.0% ± 5.1% and 34.8% ± 10.9% respectively for corn oil gavage studies). The increases in multiple adenomas was statistically significant at 500 mg/kg bw/d ($P<0.05$). No HCD were provided for multiple hepatocellular adenomas or multiple carcinomas. Six hepatocellular carcinoma metastases were found at 500 mg/kg bw/d (in the mesentery, lymph node, lung). Nevertheless, it has to be noted that a high rate of hepatocellular carcinoma metastases were found in the lung, including in the control group (5 tumours were found in both the control and low dose groups). RAC takes note of a dose-dependent shift from adenoma to multiple adenoma and malignant carcinoma in male mice. A

dose dependent increase up to 500 mg/kg bw/d was observed in female mice for multiple hepatocellular adenoma and hepatocellular carcinoma (see table above). The increase in hepatocellular carcinomas and hepatocellular adenomas were not statistically significant (by trend or pairwise comparison). The HCD for females, available on the NTP website (24.8% ± 9.6%, range 14%-34% for hepatocellular adenoma and 10.4% ± 5.6%, range 4%-18% for hepatocellular carcinoma), highlighted that the occurrences in the exposed groups were still within the HCD. Nevertheless, as mentioned previously, in addition to an increase in mortality, it appears that the female mice died before the males in the highest dose group (see figure 7 of NTP study, 2014). The earliest incidence for hepatocellular carcinoma in females occurred at 552d (also corresponding to the general first incidence in males). By visual inspection of figure 7 of NTP study, RAC notes that, during this period of the study, the survival in females was already decreased to approximately 35%, whereas survival was two times higher in males (70%). Therefore, the early death of females may have interfered/masked liver tumour development.

The first day of incidence of hepatocellular carcinoma was slightly decreased in a dose dependant manner in females when compare to the control group (729d, 718d and 552d for control, 250 and 500 mg/kg bw/d, respectively), whereas the first day of incidence of hepatocellular carcinoma did not change with the dose in males (521d, 589d and 513d in control, 250 and 500 mg/kg bw/d, respectively). The first day of onset of adenomas did not consistently change in females and even increased in males (663d, 619d, 688d and 374d, 470d and 522d in control, 250 and 500 mg/kg bw/d in females and males, respectively).

Hepatoblastomas were detected only in male mice. The increase was not dose-dependent, but incidences at 250 and 500 mg/kg bw/d were both outside of the related HCD (3.6% ± 2.6% for corn oil gavage studies) and the incidence in the concurrent control was within the HCD (4%). Moreover, the pairwise comparison was statically significant at 250 mg/kg bw/d ($p=0.006$ and $p=0.052$ at 250 and 500 mg/kg bw/d, respectively). Metastases of the hepatoblastoma were found in the lung, but also in controls (1/50, 2/50 and 1/50 at 0, 200, 500 mg/kg bw/d, respectively). The pairwise comparison for hepatocellular carcinoma or hepatoblastoma was also statically significant at 250 mg/kg bw/d ($p=0.008$ at 250 mg/kg bw/d). Hepatoblastoma is a distinct form of hepatic neoplasm and is described as an age-related tumour in B6C3F1 mice at low incidences (Haschek and Rousseaux´s Handbook of toxicologic pathology), however increase in the incidence of hepatoblastoma can also be test-substance related and treatment groups usually show associated hepatocellular neoplasia and they often occur adjacent to, or arising from, hepatocellular adenomas or carcinomas. In that regard, with the increase of carcinomas and multiple adenomas, they may indicate general liver carcinogenicity despite the high spontaneous incidences of hepatocellular neoplasia (in particular simple adenoma). Hepatoblastoma were considered by NTP to be neoplastic effects providing some evidence for carcinogenic activity, but not clear evidence, because the combined incidences of hepatocellular carcinomas and hepatoblastomas were significant only in the 250 mg/kg group and the trend test was not significant.

RAC's view is that all types of liver tumours should be considered: hepatocellular adenoma, hepatocellular carcinoma and hepatoblastoma, and, since the information in the literature is not consistent, the origin of the hepatoblastoma should not be used to discard them (see DS response to industry). Adenoma, hepatocellular carcinoma and hepatoblastoma were considered to represent a biological and morphological continuum by the NTP study authors, with progression to malignancy. Nevertheless, hepatocellular adenomas and carcinomas (simple) cannot be considered as "strong evidence" of carcinogenicity, due to the lack of statistical significance and the high rate of spontaneous incidences in control groups (incidences were within the HCD). In contrast, hepatoblastoma are considered as rare and aggressive tumors that are known to occur in humans. The increase in incidence in exposed groups of mice was already statistically significant in the lowest dose group and the incidence in control mice was very low, including in

the HCD. No MoA was proposed for this tumour type. No indication that these tumours can be non-relevant to humans has been provided. Taking all this together, RAC considers the hepatoblastomas relevant for classification.

RAC assessment of large intestine tumours

Large intestine tumours were found in male mice (NTP, 2014). No specific MoA nor key characteristics were proposed for these tumours. No dose-dependent incidence increase in non-neoplastic lesions (including inflammation) were discovered in the intestines of male or female rats or mice (NTP, 2014). The large intestine neoplastic lesions appeared at incidences of 0/50, 0/50 and 3/50, at 0, 250, and 500 mg/kg bw/d, respectively. No large intestine tumours were found in male mice high dose group, probably due to the high mortality and the rare occurrence of this tumour. A dose-dependence is difficult to determine based exclusively on increased incidence at the highest dose, and the calculation of a reduction in latency is also not possible. One adenoma in the mid dose group and one leiomyoma in low dose group were found in large intestine of females. No dose dependency was observed, and these occurrences may be chance findings.

Nevertheless, NTP considered the large intestinal tumours in males to be equivocal evidence of carcinogenic activity because the occurrence is significant by the trend test (p=0.039), and because this type of tumour is very rare (absence of this type of tumour in controls and in the HCD for the same mode of administration). Moreover, although detected only at one dose, tumours are present in the highest dose group without excessive mortality. Therefore, large intestine tumours may have been related to chemical exposure. No indication is provided that these tumours can be non-relevant to humans, therefore RAC considers these tumours as a plausible indication of carcinogenicity, but places less weight to this tumour for classification than to some of the other tumours seen in this study.

RAC assessment of hemangiosarcoma

A dose-dependent increase in the incidence of hemangiosarcoma (all organs) was found for male mice (NTP, 2014) with incidence of 1/50, 5/50 and 8/50, at 0, 250, and 500 mg/kg bw/d, respectively (HCD: 11.2% ± 6.4%, range 2-18%). These are summarised in the table below.

Table: Overview of hemangiosarcoma observed in mice carcinogenicity study on TBBPA (NTP 2014)

	Gavage dose of TBBPA (mg/kg bw/d) – 5 times/week			
Male Mice	**0**	**250**	**500**	**1000***
Liver Hemangiosarcoma	0/50	4/50	3/50	2/50
Mesentery Hemangiosarcoma	0/3	0/3	0/4	1/2
Bone marrow Hemangiosarcoma	0/50	2/50	1/50	0/50
Spleen Hemangiosarcoma	1/50	3/48	4/50	3/49
Skin Hemangiosarcoma	0/50	0/50	2/50	0/50
Bone Vertebra, Hemangiosarcoma	0/50	0/50	1/50	0/50
Lung, Serosa, Hemangiosarcoma	0/50	0/50	1/50	0/50
Kidney, Hemangiosarcoma	0/50	0/50	0/50	1/48
All organs (incl liver) Hemangiosarcoma **	1/50***	5/50	8/50***	Not provided

* Values provided for indication but as the percent probability of survival at end of study was 25% in males and 8% for females at this exposure

** Sum as provided in the Statistical Analysis of NTP studies

*** Positive trend test or significantly different (p ≤0.05) from the control group by Poly 3 test

In one very well documented study (NTP, 2014), a dose-dependent increase of hemangiosarcoma (all organs combined) was detected. This tumour was found in one species (mice), and only in males. The occurrence increase of these tumors is significant by the trend statistic (p=0.014) and pairwise Poly-3 statistic in the 500 mg/kg bw/d group (p=0.019) (NTP, 2014). These lesions occurred in a variety of organs such as the bone marrow, liver, lung serosa, lymph nodes, skin, spleen, and vertebra. Nevertheless, it seems that the tumors can appear spontaneously relatively frequently, as the incidence of these tumors is still within the HCD range. It has to be noted that the HCD has a rather large standard deviation and the incidence of the hemangiosarcoma is close to the upper limit of the HCD range. The whole dose range was not statistically analysed by NTP due to high mortality in the high dose animals, but looking at the high dose animals nevertheless, incidences are increased as well (7 hemangiosarcoma detected in liver, mesentery, spleen and kidney in the highest dose group). Hemangiosarcoma may not be attributable to confounding effect of excessive toxicity, as the mortality in low and mid dose group does not differ statically significantly from controls and because body weights of all dosed groups of males and of 250 and 500 mg/kg females were generally similar to those of the vehicle control groups throughout the study. One metastasis was detected in the lymph node of a male from the 500 mg/kg bw/d dose group. No reduced tumour latency was detected (first day of onset: 645d, 602d and 730d for hemangioma or hemangiosarcoma in controls, 250 and 500 mg/kg bw/d, respectively). No indication that these tumours could be not relevant to humans has been provided.

For reasons described above, hemangiosarcoma were considered equivocal findings by NTP and thus "may have been" related to chemical administration. RAC is of the view that the hemangiosarcomas are a plausible indication of carcinogenicity, but puts less weight on this tumour for classification than on some other tumours seen in this study.

Summary and conclusion

The dossier submitter proposed classification as Carcinogen Category 1B.

According to the CLP guidance, Category 1B applies to "*presumed human carcinogens",* i.e. substances "*presumed to have carcinogenic potential for humans, classification is largely based on animal evidence".*

The classification in Category 1B is based on *"strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from:*

- *animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen)."*

According to the CLP guidance*,* Category 2 applies to *"suspected human carcinogens". "The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.*

In order to decide on the appropriate classification, RAC evaluates whether the evidence is sufficient to classify in Category 1B. According to the CLP guidance, to determine if experimental data represent sufficient or limited evidence of carcinogenicity, several aspects have to be taking into account:

Evidence of robustness: *Sufficient evidence of carcinogenicity*

Regarding the CLP guidance, to determine if experimental data represent sufficient or limited evidence of carcinogenicity, several aspects have to be taking into account:

Condition in the guidance	Sufficient evidence	Limited evidence
Appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) two or more independent studies in one species carried out at different times or in different laboratories or under different protocols. An increased incidence of tumors in both sexes of a single species in a well-conducted study, ideally conducted under Good Laboratory Practice, can also provide sufficient evidence.	(a) Statistically significant increase of malignant tumors in female rats (uterus) and male mice (hepatoblastoma) (b) Two studies in two species, (c) GLP-compliant, performed to a high standard by NTP	(a) Each tumor type was observed in only one sex and species, except the hepatocellular carcinoma, although the increase was not statistically significant in female mice.
The evidence of carcinogenicity is restricted to a single experiment	Two studies available	Conditions met for "Sufficient evidence"
There are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies	The study is well conducted and the design adequate	Conditions met for "Sufficient evidence"
The agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential;	Very high malignant tumors were observed, with metastasis, in both species and sex	Conditions met for "Sufficient evidence"
The evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs	Several organs are target and the studies are not limited to tumor promotion but complete carcinogenicity	Conditions met for "Sufficient evidence"

Additional consideration: *weight-of-evidence for carcinogenicity*

Additional consideration for classification	RAC evaluation for TBBPA
Rare and malignant tumors appear in two species, different sexes	Females rats: Uterine carcinoma tumors presenting the whole continuum including hyperplasia Male mice: Hepatoblastoma and large intestine tumors
More frequent tumors appear in both sexes of one species	Malignant hepatocellular carcinoma were found in both males and female mice, but without significance. Nevertheless, the premature death of females resulted in limitations for tumour detection
Multi-site response: several different organs were affected in one species, one sex	Some indications available for multi-site response in male mice: liver tumours, intestine large tumours, hemangiosarcoma

No negative carcinogenicity study	To some extent, all species and sexes seem to be affected (including male rats with testicular adenomas)
Genotoxicity	No
MoA relevant to human	Key elements of MoA (with uncertainties) were provided for uterine carcinoma only, and relevance to humans cannot be excluded
Threshold MoA	Likely but uncertain
Reduced tumor latency	Uncertain. A decrease in time of onset was observed in uterine tumors (but this was not dose dependent) and for liver carcinoma in females, but not systematically in other neoplastic lesions
Progression to malignancy	A high grade of tumor progression and malignancy is observed for uterine tumors based on metastasis and Tp53 expression, increasing the concern In male mice liver, a dose-dependent shift from predominantly benign adenoma to malignant carcinoma is observed and also show a continuum of findings
Structural similarity	Similarity with Bisphenol seem not relevant as TBBPA does not bind significantly to the estrogenic receptor
Route of exposure	Gavage is considered a relevant route of administration for classification and labelling
ADME between animals and humans	Glucuronide conjugates are major metabolites in humans, whereas sulfate conjugates are major metabolites in rats. Nevertheless, both metabolic activities are present in rats and humans and consideration of quantitative differences are relevant for risk assessment but cannot overrule hazard classification
Confounding effect	Results from the group with excessive toxicity (high dose group in mice) were removed from statistical analysis. No other appreciable signs of excess toxicity were detected. Therefore, specific scrutiny to avoid confounding effects seems to have been be applied by the study author
Dose-dependency	Yes, for uterine carcinoma and testis adenoma. In mice, from low to mid dose (high dose excluded from evaluation), dose dependency is observed in liver adenomas and carcinomas, hemangiosarcoma, and large intestine tumours.

In conclusion, in two reliable independent OECD guideline and GLP compliant carcinogenicity studies, dose-dependent induction of malignant tumours was observed in two species and two sexes, including uterine carcinoma in rats and hepatoblastoma in male mice. Evidence that points towards classification and indicating multi-site response were seen in male mice (with liver tumours, large intestine tumours, and hemangiosarcoma). Uterine carcinomas were found to be highly malignant with metastases at distant sites, furthermore, no evidence is available that would allow to conclude non-relevance to humans for any of these tumour types. RAC agrees with the dossier submitter that TBBPA fulfils the criteria for category 1B. In the view of RAC, elements that decrease a concern such as the lack of genotoxicity and presumption of a thresholded MoA are considered not sufficient to downgrade the classification.

RAC concludes that **classification of TBBPA in Category 1B; H350 (May cause cancer) is warranted**.

No route of exposure is stated as it is not conclusively proven that no other routes of exposure can cause the hazard.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS presented five studies on reproduction toxicity, two studies on sexual function and fertility, a Two-Generation Reproduction Toxicity Study (Unnamed, 2002; EU RAR TBBPA, 2008; Cope *et al*., 2015) which includes a developmental study in F1 and a developmental neurotoxicity component in the F2-generation (OECD TG 416), and a One-Generation Reproduction Toxicity Study (Van der Ven *et al*. 2008; Lilienthal *et al*. 2008) for Endocrine and Immunological endpoints and additional analysis for bone and neurophysiological parameters (conducted according to OECD TG 415). Three studies on development, an OECD TG 414 (developmental toxicity, (Unnamed, 2002; EU RAR TBBPA, 2008; Cope *et al*., 2015)), an OECD TG 426 (developmental neurotoxicity Hass *et al*., 2003) and a non-guideline developmental study (Saegusa *et al*., 2009) were assessed.

Sexual function and fertility

In the Two Generation Reproduction Toxicity Study (Unnamed, 2002; EU RAR TBBPA, 2008; Cope *et al*., 2015) there were no effects on reproduction and fertility.

There were effects on thyroid hormones, T4 levels were decreased in both sexes for the P and F1 generations (at 100 and 1000 mg/kg bw/d for P males and F1 animals and 1000 mg/kg bw/d in P females). T3 levels were decreased in P males exposed to 1000 mg/kg bw/d. However, there were no effects on TSH levels. The study showed no relevant neurobehavioral effects. However, at PND 11 of the F2 generation, the highest exposed group had a decreased parietal cortex thinning. These effects were not seen at the highest dose group at PND 60 compared to control.

The one-generation reproduction study did not report any effects on fertility and reproduction.

In this study Van der Ven *et al*., 2008 reported a significant dose-dependent increase in F1 liver weight (maximum increase 11.4%), adult F1 testis weight (BMDL of 0.5 mg/kg bw/d) and pituitary weight in F1 males. In F1 female pups there was a decrease in the anogenital distance at PND 7, but not at PND 4 and 21, and a delayed time for vaginal opening with a BMDL around the highest dose.

Thyroid hormone levels were affected in both sexes of the F1 generation. Plasma T4 levels were decreased in both sexes (BMDL of 30.8 mg/kg bw/d for males and 16.1 mg/kg bw/d for females). These results are in concordance with the changes in T4 levels observed in other studies (Unnamed, 2002, Cope *et al*., 2015 and studies assessed in the STOT RE section 10.12.1 of the CLH report). The DS reported plasma T3 levels were increased in females (BMDL of 2.3 mg/kg bw/d at necropsy which was at average age of 14 weeks), while they were decreased in the Two-Generation study reported above.

Effects on neurobehavioral parameters were reported by Lilienthal *et al*, (2008). Brainstem auditory evoked potentials (BAEPs) were used to study auditory responses in the offspring. The results showed an increase in the BAEP thresholds and wave IV latency in F1 exposed females in the low frequency range. The thresholds were unaffected in male rats, but absolute latency of wave IV and interpeak latencies II-IV showed exposure related increases at low frequencies. In their paper, Van der Ven *et al*. (2008) discussed the possibility that the effects in both sexes on increase of hearing latencies at low frequencies and the increased hearing threshold reported in females may have been related to the observed changes in thyroid hormone levels. The DS

reported on several statistical correlation analyses conducted by the authors, i.a. the link between BAEP hearing latency and threshold and thyroid hormones is reported to be statistically supported by correlations between these parameters, and also because the BMDL of hearing latencies and of the decrease in serum T4 were in the same range. IARC (2018) has noted that the results from Lilienthal *et al*. (2008) and Van der Ven *et al*. (2008) may reflect an effect of TBBPA on thyroid hormone regulated developmental events, including hearing and testis weight, however they also noted that there is a lack of studies addressing this directly.

The DS concluded that the studies do not warrant classification for sexual function and fertility as there were no effects on sexual function and fertility.

Developmental toxicity

Three different studies assessed developmental effects of TBBPA (one is published in the Cope paper but conducted separately from the Two-Generation study). In addition, the developmental neurotoxicity and immunotoxicity was studied in the two reproduction studies summarised above.

For the three developmental studies, according to the DS, none of these showed conclusive evidence of developmental toxicity.

In the prenatal developmental toxicity study (OECD TG 414, published by Cope *et al*, 2015), there were no toxic effects in maternal animals or in the foetuses. Despite a slightly lower liver weight in the maternal animals no other effects of treatment were seen from clinical observations, gestational parameters and from the uterine implantation data in the maternal animals. In the foetuses, no embryotoxic/teratogenic effects were reported. No effects on fetal body weight, sex distribution or from external observations or visceral and skeletal examinations. Litter incidences did not differ from controls.

The non-published developmental neurotoxicity study (OECD TG 426, Hass *et al*., 2003 reported in the EU RAR for TBBPA, 2008) provided limited evidence of neurobehavioral effects with changes in the habituation behaviour of female offspring and learning and memory in male offspring in the 250 mg/kg bw/d group, but considered that it was not possible to draw definitive conclusions from this study because the extent of the reported changes was very small and there was not a convincingly consistent pattern of changes in investigations conducted at different time points. Also, the evidence of developmental neurotoxicity was weakened by the absence of consistent changes in both sexes and the lack of histopathological investigations that could provide corroborative findings.

In the non-guideline developmental study for the offspring (Saegusa *et al*., 2009), there were no abnormalities in the clinical observations, number of implantation sites, number of live offspring, male ratio, body weight, organ weights or anogenital distance at PND1. No effects on onset of puberty in either sex, but higher body weight was reported in males exposed to 10000 ppm compared to controls at the onset of puberty. No effect was seen on the oestrus cycle in females. Male offspring showed a non dose-related decrease in serum T3 levels at 100 and 1000 ppm on PND 20, but not in the 10000 ppm exposed group. No Changes were reported in T4 and TSH. At post-natal week 11, there were no effects on thyroid hormone levels, or any change in body and organ weights. In females, decreased relative kidney and uterus weights were reported for the 1000 and 10000 ppm groups. No findings were noted in brain morphometric assessments.

The DS proposed no classification for developmental toxicity as none of the five studies show conclusive evidence of developmental toxicity. The animal studies did indicate some effects on development, but they were not characterized as sufficiently adverse.

Comments received during consultation

Three Member State Competent Authorities commented on reproduction toxicity.

Two MSCA supported the DS proposal for no classification.

One MSCA considered the effects warranting at least category 2 classification for developmental toxicity. This conclusion was based on the decrease in T4 that should be considered in the assessment of the neurodevelopmental potential of TBBPA and which supported the significant changes observed in the Two- and One-Generation studies, including the changes in motor activity observed in the F2 offspring in the Two-Generation study (considered not incidental by the MSCA) and the effects on hearing capacities reported in F1 in the One-Generation study. In addition, the transient effect on parietal cortex thickness at PND 11 observed in the Two-Generation study was not disregarded as irrelevant by this MSCA. The MSCA highlighted that T4 was consistently decreased in many of the repeated dose studies and that thyroid hormones play an important role in foetal and postnatal development and in particular, in the development of the central nervous system.

In their response to comments, the DS agreed with the MSCA that there are some indications of effects on development, but the DS considered the effects as not sufficiently adverse to propose a classification.

Industry provided comments on reproduction toxicity supporting no classification.

The industry association concluded that the data indicate that decreased serum concentrations of T4 appear to have little adverse impact on parameters associated with a disruption in thyroid homeostasis in rat. The industry association further commented on a potential secondary UDP-GT mediated MoA for decreases in T4 informing that this (and other) proposed MoA have been reviewed by Lai *et al*. (2015), with the induction of UDP-GT considered the most plausible and supported MoA, based on decreases in T4 without concurrent compensatory increases in serum TSH or associated decreases in serum T3. The DS considered that it is very likely that increased TH clearance by the liver causes the observed serum T4 reductions, however referring to the ECHA/EFSA guidance that *in the absence of substance-specific data which provide proof of the contrary, humans and rodents are considered to be equally sensitive to thyroid-disruption (including cases where liver enzyme induction is responsible for increased TH clearance*).

The industry association further clarified that the assessment of Anogenital distance (AGD) and vaginal opening with a BMDL around the highest dose (the BMDL which is considered equivalent to a NOAEL) suggests that there is no effect. The DS clarified that the effect was small and that there was a significant dose-response relationship with the BMDL around the high dose. Deviations for timing of measurements for neuro-morphometric analysis in relation to OECD TG 416 were highlighted, and uncertainties in the results of the brainstem auditory evoked potentials (BAEPs) were raised. Industry pointed out that for analysis of parietal thickness with changes noted in high-dose groups on PND 11 but not on PND 60 do not comply with OECD 416. DS pointed out that in the study reported by Unnamed (2002); EU RAR TBBPA (2008); Cope *et al*. (2015), the parietal cortex was measured at PND 11 and PND 60 which is in line with the OECD TG 426.
Some further comments were made asking for clarifications, such as the correlations described in the CLH report were considered to be insufficiently described (in the statistical context). All BMDL values should be reported with their corresponding benchmark response levels for context and results total spleen cell counts to be clarified further. The DS provided responses aiming to clarify all these comments.

Assessment and comparison with the classification criteria

Sexual function and fertility

Two available studies were assessed by the DS. These two studies were also assessed in the EU RAR:

- GLP-compliant OECD TG 416 Two Generation Reproduction Toxicity Study including a developmental neurotoxicity component in the F2-generation – assessed as Klimisch score 1 by the DS (Unnamed, 2002, Cope *et al*., 2015);
- OECD TG 415 One Generation Reproduction Toxicity Study for Endocrine and Immunological endpoints and additional analysis for bone and neurophysiological parameters – assessed Klimisch score 2 by DS (Van der Ven *et al*. (2008), Lilienthal *et al*. (2008)).

Two Generation Reproduction Toxicity Study

In the Two Generation Reproduction Toxicity Study in SD rats (Unnamed 2002, Cope *et al*., 2015), 30 animals/sex/group were given TBBPA (purity 98,91%) via gavage at doses of 0, 10, 100 and 1000 mg/kg bw daily for 36 weeks. This study is described in detail in the EU RAR. In addition to sexual function and activity parameters, the study assessed thyroid hormone serum levels in the P and F1 generations, and the neurobehavioral effects and neuropathology in the F2 generation. For neurobehavioral studies, 40 animals/sex randomly selected from each F2 dose group were studied; tests included motor activity, learning and mobility (passive avoidance test and water maze) and auditory startle habituation. Additional 20 animals/sex from each F2 dose group were retained for neuropathologic studies.
The study was sponsored by the Brominated Flame Retardant Industry Panel of the American Chemistry Council and the publication was financially supported by the American Chemistry Council's North American Flame Retardant Alliance. RAC consulted the scientific publication to assess TBBPA effects.

Parental generation

There were no general toxicity effects on clinical signs, food consumption and compound intake, organ weight findings including organ/body ratios and non-neoplastic histopathological findings. No effects on reproductive function and reproductive performance are reported. Results from the P generation are summarised in the table below.

***Table**: Effect of TBBPA on reproductive parameters parental generation in the 2-Gen study (Cope et al. 2015)*

Parameter	TBBPA dose mg/kg BW			
	Control Parental generation	10	100	1000
Estrus cycle length (mean ± SD)	4.8 ± 0.69	4.6 ± 0.63	4.6 ± 0.69	4.4 ± 0.68
Female mating index	96.7	93.3	93.3	100.0
Female fertility index	80.0	86.7	83.3	96.7
Male mating index	96.7	93.3	93.3	100.0
Male fertility index	80.0	86.7	83.3	96.4
Sperm percent motility (mean ± SD)	97.4 ± 2.01	98.4 ± 1.25	97.2 ± 2.29	97.4 ± 2.21
Sperm percent progressive motility (mean ± SD)	77.4 ± 6.21	79.1 ± 6.70	73.9 ± 6.10	71.3 ± 8.68
Total sperm concentration/caudaepididymus × 10^8 (mean ± SD)	3.262 ± 0.5391	3.467 ± 0.4970	3.195 ± 0.5959	3.256 ± 0.5856
Percent abnormal sperm (mean ± SD)	0.70 ± 0.934	0.45 ± 0.562	1.07 ± 1.187	1.20 ± 1.595

F1-generation

In the F1 generation there were no general toxicity effects on clinical signs, mortality/viability, sexual maturation, gross pathological and histopathological findings, oestrus cycle, reproductive

performance, gestation/lactation, food consumption, gestation length, litter data, on macroscopic and microscopic evaluations, organ weights, or primordial follicle counts.
According to the publication data, sperm evaluation showed a significant ($p<0.05$) decrease in total sperm concentration/cauda epididymis in the F1 at the high dose (-13%, 3.365±0.538, 3.350±0.7549, 3.308±0.6111, 2.941±0.6338 for control, low, mid and high dose, respectively) not evident in the P-generation. Percent abnormal sperm was dose-dependently increased, but with a large standard deviation and not statistically significant. Details are provided in the table below.
Lower body weight and body weight gain was observed in F1 males at 1000 mg/kg/day for several weekly intervals and lower weight gain (7%) were observed in the premating period week 1-11.

***Table**: Effect of TBBPA on reproductive parameters F1 generation in the 2-Gen study (Cope et al. 2015)*

Parameter	TBBPA dose mg/kg BW			
	Control F1 Generation	10	100	1000
Female mating index	100	92.9	86.2	89.3
Female fertility index	86.7	92.9	69.0	75.0
Male mating index	100.0	92.9	86.2	89.3
Male fertility index	86.2	92.9	69.0	75.0
Sperm percent motility (mean ± SD)	96.5 ± 3.29	88.1 ± 20.87*	96.3 ± 2.85	95.7 ± 4.92
Sperm percent progressive motility (mean ± SD)	78.8 ± 5.63	73.5 ± 18.73	78.9 ± 6.30	75.9 ± 9.54
Total sperm concentration/cauda epididymus × 10^8 (mean ± SD)	3.365 ± 0.5380	3.350 ± 0.7549	3.308 ± 0.6111	2.941 ± 0.6338*
Percent abnormal sperm (mean ± SD)	0.19 ± 0.489	0.39 ± 0.550	1.31 ± 1.064	2.24 ± 1.935

* Significantly different from control $p < 0.05$.

F1/2 pups

The CLH report states there were no changes in bodyweight, clinical finding, sex ratio, survival to weaning, macroscopic findings or organ weight data for the F1 and F2 pups (see also section on developmental toxicity). According to the publication, F2 pups were selected for clinical examination, motor activity and neuropathology studies, and also for sexual maturation hallmarks. No data were, however, presented to enable RAC to conduct an independent evaluation regarding clinical findings, body weights, sexual maturation hallmarks.

Thyroid Hormones (P and F1)

The DS presented thyroid hormone data as these play an important role in neurodevelopment. Treatment related thyroid effects were observed in both P and F1 generations (see table 16 of the CLH report). **Serum T4 was statistically significantly reduced** in both sexes in the P generation (4.7, 5.08, 3.9 and 3.38 ng/dL in males and 4.23, 3.45, 3.5 and 2.39 ng/dL in females for the 0, 10, 100 and 1000 mg/kg/d groups, respectively) and F1-generation (seen in 100 mg/kg/d and 1000 mg/kg/d groups for both sexes with 6.29, 5.98, 3.91 and 3.33 ng/dL in males and 6.00, 4.42, 3.40 and 3.41 ng/dL in females for the 0, 10, 100 and 1000 mg/kg/d groups, respectively). Reduced serum T3 was observed in P-generation males (102.7, 92.8, 97.5 and 83.2 ng/dL for the 0, 10, 100 and 1000 mg/kg/d exposed males, respectively) with mild inconsistent responses in P females and no changes in the F1 generation. Mean serum TSH-levels were comparable to the controls in both P and F1 generations. The thyroid tissue was not examined and no microscopic changes in the pituitary gland or liver were noted. Thus, the mechanism of the T4 decrease is unclear. RAC takes note that no data are additionally presented that would suggest that a hepatic mediated UDP-GT MoA was involved in the removal of circulating T4, and that indeed, although a plausible hypothesis, the evidence for this hypothesis is weak. RAC notes that decreases in T4 were consistently also observed in repeated dose toxicity studies assessed under STOT-RE.

Neurobehavioral toxicity (F2 animals)

Motor activity was assessed at PND 13, 17, 21 and 60 for ten F2 pups/sex/group placed in an activity chamber with recording of horizontal and vertical activity counts, total distance travelled and emotionality assessment. No effects are reported for PND 13. Some findings in terms of activity or emotionality at PND 17, PND 21 and PND 60 were noted (and are described below).

At PND 17 in low dose females a significant decrease in horizontal activity in the 15-20 min segment of the test and in the 20 min period in the mid dose (but not for the individual segments). There were no statistically significant differences between controls and treated females for distance travelled, vertical activity or emotionality and no effects in males. Thus, there was no dose-response and no effects in males, also the decrease in horizontal activity was not accompanied by changes in distance travelled. At PND 21 there was a significantly reduced horizontal activity and distance travelled in the 5-10 min segment and over the 20-min test as a whole in mid dose females compared to controls. Otherwise there were no statistically significant differences between controls and treated animals. At PND 60, in males, there were significant reductions in horizontal activity in the 0-5 min test segment at mid and high dose and during the 5-10 min segment in the 1000 mg/kg bw/d high dose group. But there were no statistically significant effects on vertical activity or emotionality and no statistically significant differences between female controls and treated groups for horizontal and vertical activity and emotionality. Furthermore, no statistically significant effects were reported at other dose levels or time periods, including over the total 20 minutes duration of the test. These effects in males could be a treatment-related effect but could also be a chance finding.

Learning and memory assessed in the passive avoidance test (light and dark-side chamber) with ten F2 pups/sex/group conducted on PND 22 and PND 60 once a day for three consecutive days, showed on PND 22 a significant decrease in time spent in light for males on day 2 at the high dose. No differences were detected on days 1 and 3 in males. At PND 60, on day 1, there were significant reductions in time spent on the light side for all exposure groups when compared to controls, but not for the other days. According to the EU RAR, the difference on day 1 is attributed to only 3/10 control animals crossing from the light to the dark side against 8/10 or 10/10 from the treatment groups, respectively, suggesting unexpected control performance and questionable reliability of the test. No differences were detected for females at any time point nor in males on other days. RAC agrees with the conclusion that the findings in the passive avoidance test are of equivocal toxicological relevance. The water M-maze tests were performed in the same animals at PND 110 and there were no treatment related effects from the test, as reported by the DS, either on short-term or long-term memory.

Neuropathology (F2 animals)

For Neuropathology, ten F2 pups were investigated regarding brain weight and neuropathological evaluation of the brain, spinal cord and peripheral nerves on PND 60, the thickness of the parietal cortex was measured in ten control and high dose males and ten control and nine high dose females. At PND 11, ten F2 pups/sex/group were subjected to neuropathological evaluation and morphometric measurements including measurements of the thickness of the parietal cortex, hippocampus, the external granular, molecular and Purkinje/internal granular layers of the cerebellum, and thalamus. The main result was obtained from morphometric measurements showing a statistically significant decrease in parietal cortex thickness in the high dose pups sacrificed at PND 11 with a decreased thickness of 1.61, 1.56, 1.49 and 1.23* mm in males and 1.60, 1.46, 1.56, 1.33* mm for females at 0, 10, 100 and 1000 mg/kg bw/d, respectively. The change was seen also at 10 and 100 mg/kg bw/d, but these were not statistically significant and no dose-response relationship was apparent in females. There were no histological changes observed in the parietal cortex (including degeneration, necrosis, cell loss, demyelination,

proliferative changes, or changes in neuronal cell density). The decreased thickness was a transient observation as no significant change was observed at PND 60.

The DS summarised the findings of the Two Generation Reproduction Toxicity Study. There were no effects on reproduction performance and fertility. RAC notes that the sperm concentration in F1 males was dose-dependently reduced, this finding being statistically significant at the high dose of 1000 mg/kg bw/d ($p<0.05$), and the abnormal sperm count was non statistically significantly but dose-dependently increased for both P and F1 generations, but reproductive function was not negatively affected. There were effects on serum levels of thyroid hormone, with decreased T4 was in both sexes for the P and F1 generations at the mid and high dose for P males and F1 animals and at the high dose in P females. T3 was decreased in P high dose males only and there were no effects on TSH. The study showed no relevant neurobehavioral effects. In the neuropathology at PND 11 of the F2 generation the highest dose group had a transiently decreased parietal cortex thinning compared to the controls, the toxicological relevance of which is unclear. The study authors recommended to take this image analysis finsing with caution as it was not observed at later development stages and it was not associated with histological changes in the parietal cortex nor with significant test-article related changes in pre-weaning motor activity, step-through passive avoidance test performance, auditory startle test performance, forelimb and hind grip strength, emotionality, grooming behaviours, rearing, backing or water M-maze test performance. The authors derived a NOEL for brain parietal cortex thinning (day 11) at the mid dose of 100 mg/kg bw/d (and a modelled BMD of 700 and 170 mg/kg bw/d for males and females for one standard deviation of the mean assumed for the Benchmark response, and corresponding BMDL (95th lower limit) of 160 and 73 mg/kg bw/d, respectively), although the biological relevance is unclear and probably a chance finding because of lack of detectable functional and pathological deficits (the study authors assigned the NOEL for neuro-functional examinations at ≥ 1000 mg/kg bw/d). The EU-RAR interpreted this finding as toxicologically not relevant or a chance finding due lack of a dose-response relationship, transience and lack of other lesions and functional defects.

<u>One Generation Reproduction Toxicity Study</u>

The One-generation study for endocrine and immunological endpoints and additional analysis for bone and neurophysiological parameters (Van der Ven *et al*. (2008), Lilienthal *et al*. (2008)) was conducted similar to OECD TG 415 on Wistar rats. The study report was not available to the EU RAR rapporteur at that time. The DS assigned Klimisch 2 to account for deficiencies, while the registrant disregarded the study as Klimisch 3 arguing that there were major methodological deficiencies based on inappropriate use of BMDL modelling to derive risks, methodological confounding factors that invalidate findings and lack of consistent patterns. TBBPA (98%) was applied at doses of 0, 3, 10, 30, 100, 300, 1000, 3000 mg/kg bw/d orally via feeding to ten parental Wistar rats/sex/dose with dosing starting at ten weeks for males and at two weeks for females premating and through mating, gestation and lactation. Offspring received the same diets and necropsy was carried out on week 14 (+/- 1 week). The DS noted there were some differences to the OECD TG 415. Eight exposure groups were included, thereby aiming to evaluate benchmark doses. RAC notes as an important deviation from the guideline also the number of animals per dose differs from the TG, with 8-10 pregnant dams instead of achieving twenty pregnant females (and not less than 16). Neurobehavioral effects in F1 were studied by BAEP (brainstem audiometry evoked potential), sweet preference and conditional fear testing. BAEP was recorded from 93 rats (46 females and 47 males), using 5-6 animals/sex/exposure group between PND 50 and PND 110. Recordings were performed within three weeks to minimize the effect of age. The DS informed that criticism as reported by Strain *et al*. (2009) and Banasik *et al*. (2009) on these studies concerned the way the BAEP was performed, and the use of Benchmark dose levels in the statistical analysis.

With PROAST, BMD modelling performed with 8 dose groups was conducted from the best fitted curve and a critical effect dose (CED / BMD) was calculated for a default critical effect size (CES / BMR) of 10%. For testis weight and bone parameters CES of 5% were used, for liver weight a CES of 20% was pre-set. Calculation of a 5% lower confidence bound of the CED estimate was conducted and this value was considered as the BMDL (benchmark dose lower confidence bound 5%). The CED/BMDL was used as a measure for the statistical uncertainty in a data set; a 10-fold difference between CED and BMDL was used as a practical limit for an informative value. The controls were included as zero value input for the modelling calculations, although for graphical representation on a log-scale, an arbitrary value (but lower than the lowest dose) was used. According to the dossier, parameters from the study which showed sensitive effects, i.e. at BMDL in the low- to mid-dose range, were used for correlation testing against all other parameters. The correlation coefficients were based on group averages rather than comparison by individual, to allow comparisons across age cohorts and across sexes; this method ignores variability within groups, and these correlations should therefore be considered as indicative for clustering, according to the DS.

Parental generation

Changes in food intake and weight loss were recorded. For temporarily reduced food intakes, significant dose responses were obtained for the first two weeks of treatment with the test substance in the higher dose animals (both sexes, apparent at 1000 and 3000 mg/kg bw/d) and the first two weeks of gestation for females in the higher dose groups apparent at 1000 and 3000 mg/kg bw/d (BMDL 207 mg/kg bw/d). For body weights of females, significant dose-responses were obtained before mating, but RAC notes only slightly reduced body weights were apparent for the two high doses of 1000 and 3000 mg/kg bw/d (accordingly the BMDL was modelled to 3000 mg/kg bw/d), and in dams until gestation week 3 again affecting the two high doses (range -7 to -14%). The weight gain was also lower premating and during gestation for the females by up to -22% (BMDL 94 and 298 mg/kg bw/d).

Reproduction effects and F1 development

The CLH dossier reported no effects on reproduction endpoints including mating success, number of uterine implantation sites and litter size. No difference in sex ratio in the F1 litters was reported. Bodyweights at week 4-7 were decreased by about 10% for the F1 animals at the top dose. The CED (CES = 10%) was calculated by the authors with the BMD modelled to be around the top dose, the BMDL slightly lower.
The CLH report indicates a dose dependent decrease in pup mortality during lactation (BMDL of 4.8 mg/kg bw/d) and a decrease in rate of litters with mortality during lactation (BMDL of 33 mg/kg bw/d). Consulting the scientific publication, RAC noted the interpretation of the actual data based on a modelled BMDL. The actual data (see table below) showed that a decrease in mortality at the higher doses was preceded by a 3-fold increase in the rate of litters with mortality for the lower doses compared to the controls, which is only then followed by return to levels of the control or lower in the higher dose range (% rate of litters with mortality: 30, 40, 70, 89, 33, 33, 25, 11% for control, 3, 10, 30, 100, 300, 1000, 3000 mg/kg bw/d, respectively). A similar trend was evident for mortality rate during lactation on a foetal basis (see the table below):

***Table**: Reproduction parameters and F1 mortality during lactation in TBBPA one-generation study (Van der Ven et al., 2008)*

TBBPA dose mg/kg bw	litters [b] *count*	uterine implantation sites *count*	litter size *count*	sex ratio f/m	rate of litters with mortality % [c]	*n*	mortality rate during lactation (f + m)
0	10	11.9 ± 2.5	11.7 ± 1.6	1.0 ± 0.4	30	117	13.7
3	10	12.8 ± 1.9	10.0 ± 2.8	12.2 ± 30.9 [d]	40	100	13.6
10	10	11.2 ± 2.2	10.9 ± 1.9	1.3 ± 0.8	70	110	27.5
30	9	12 ± 1.2	10.0 ± 1.9	1.3 ± 1.2	89	94	28.7
100	9	11.9 ± 2.5	11.1 ± 1.5	1.0 ± 0.4	33	100	9.0
300	9	11.8 ± 1.6	10.9 ± 2.7	1.0 ± 0.5	33	98	8.2
1000	8	10.8 ± 1.2	8.8 ± 3.5	0.8 ± 0.4	25	70	4.3
3000	9	11.9 ± 2.5	9.7 ± 0.9	1.2 ± 0.9	11	89	1.1
dose response		a	-	-	+		+

The range of sex ratios (f/m) were 0.5-1.8 in control nests and 0.7-3.5 in top dose nests.

+, Significant dose response (for results see Table 1 in the text); *a*, absence of effect concluded from average values, dose-response analysis not performed.
[b] The number of litters represent the number of successful matings
[c] Dose-response analysis on average of dose groups, that is, n=1 per dose group
[d] The high variation in dose group 3 mg/kg bw is due to a single nest with only female pups.
Mortality was generally equally distributed among nests. Mortality was higher in male pups, throughout all groups.

RAC finds it difficult to draw definite conclusions, however the presentation of a BMDL for a decrease in litter mortality rate, i.e. the dose that induced almost 90% litter-based mortality with a 3-fold increase compared to control, was considered to be misleading. The BMD model seem not appropriate for the inverted u-shaped dose-response relationship. Such unclear dose-responses with a levelling-off or protective effect at higher doses appeared also for other parameters in this study and it could potentially be a relevant effect. It appears unlikely that it is just an issue of a low performing control group or direct test substance intake by the juveniles, which would result in a more variable response. But looking at other parameters that could potentially form a continuum of a toxic response in the juvenile animals, no growth retardation based on a decrease in body weight and marked effects on sexual maturation hallmarks are reported for the lactation period and no neonatal mortality was reported either. The study used less animals and produced less than 50% of the pregnant dams and litters per dose group as required as a minimum by the relevant guidelines. A lower number of animals does not necessarily invalidate the study, but it is a case-by-case evaluation whether effects observed are causally related to test-substance administration. It remains unclear whether low litter numbers or other confounding factors introduced bias and potentially compromised evaluation of the result. Overall, the published information was too limited to draw firm conclusions.

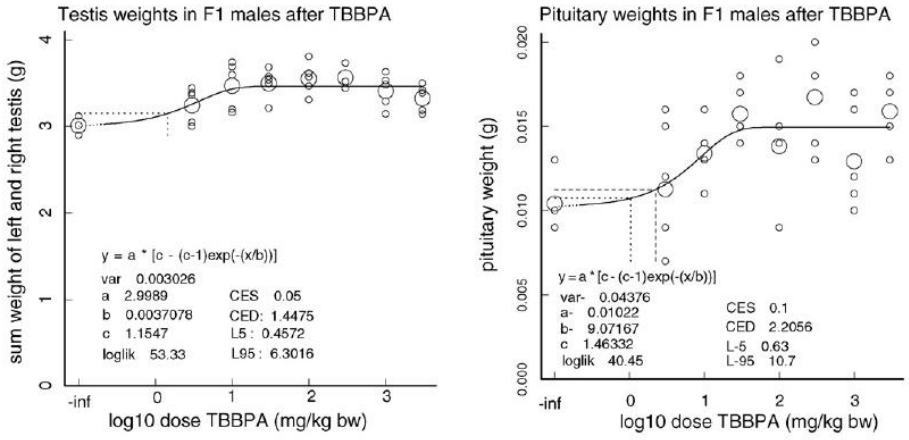
For F1 male juvenile rats a significant dose-response curve in reproductive organ weight at weaning PND21 (the publication refers to both terms testis weight and reproductive organ weight, CES 5%; max. response +15.5%, CED = 1.5 mg/kg bw/d and / BMDL = 0.5 mg/kg bw/d) and in adults testis weight (L+R) at necropsy (CES 5%; max. response +15.5%, CED = 1.4 mg/kg bw/d and / BMDL = 0.5 mg/kg bw/d) are reported. At necropsy a significant dose-dependent increase in liver weight below the CES (CES 20%; maximum response +11.4%) and increased pituitary weights (CES 10%; CED = 2.2 mg/kg bw/d / BMDL = 0.6 mg/kg bw/d, max. response +46.3%) are reported for F1 males. These data as published by Lilienthal seem to refer to absolute organ weights. It is evident from the Van der Ven (2008) publication that the dose-responses are unclear (an issue that was already raised by EFSA (2011)), since PND21 data show no clear dose-response for reproductive organs, with a low control group with varying increases between 8 and 25% which is not dose-dependent, while adult testis weights and pituitary weights have an onset at low dose and a levelling-off at mid dose. The dose-response curves (spanning over a large range) thus are very flat and no real changes are evident from 30 mg/kg bw/d for the testis and pituitary (see figures below). For the testis, although it potentially being due to a low performing control group, no testis histopathology reported and no weights of the prostate, seminal vesicles or the epididymis, which are more sensitive to hormonal and anti-hormonal effects, were reported. There were no histological or histochemical changes in the pituitary and

no histopathology changes in the liver. For liver weights, it appears to be a low performing control issue rather than a relevant effect.

RAC notes that the OECD guidelines require 20 animals/sex/group for evaluation of reproduction and general toxicity parameters. The publication indicates that two F1 animals per sex from each litter (study designed with 10 P-animals/litters) were euthanised for inspection of PND 21 reproductive organs, the actual number of animals analysed between 7-17 per dose group. The data on necropsy organ weights refer to an average ± SD (grams) of 5 replicates per dose group, occasionally 4, indicating clearly animal numbers below guideline requirements introducing uncertainty (on the other hand more dose groups are tested).

***Table and Figure**: Organ weights at necropsy and male reproductive organ weights at PND21 in TBBPA one-generation study (Van der Ven et al., 2008)*

TBBPA dose mg/kg bw	body weight	pituitary	liver	testis L+R	d21
0	414 ± 30	0.011 ± 0.002 [4]	13.8 ± 1.4	3.01 ± 0.11 [b]	339.4 ± 54.8
3	433 ± 32	0.012 ± 0.004	15.3 ± 2.7	3.25 ± 0.21	414.1 ± 112.6
10	453 ± 18	0.014 ± 0.002	16.2 ± 1.0	3.48 ± 0.28	369.2 ± 47.2
30	461 ± 51	0.016 ± 0.002	15.0 ± 2.4	3.50 ± 0.18	405.8 ± 81.6
100	478 ± 32	0.014 ± 0.004	15.9 ± 0.3	3.55 ± 0.18	426.4 ± 43.2
300	454 ± 46	0.017 ± 0.003	15.6 ± 1.8	3.56 ± 0.15 [b]	380.2 ± 73.1
1000	461 ± 41	0.013 ± 0.003	15.8 ± 1.3	3.41 ± 0.20	414.1 ± 60.4
3000	472 ± 56	0.016 ± 0.002	16.9 ± 1.7	3.32 ± 0.15	402.9 ± 45.2
dose response		+	+	+	+



According to the CLH report, female pups showed decreased anogenital distance at PND 7, but not at PND 4 and PND 21, and a delayed time to vaginal opening. The BMDL is reported to be around the highest dose. In Van der Ven *et al*. (2008), it appears that the CED for female AGD (CES = 10%) was calculated by the BMD model to be 4558 mg/kg bw/d, which is outside the tested dose range, and the BMDL was calculated to be 2736 mg/kg bw/d. The publication provides a maximum response of -6.3%. RAC cannot identify biologically significant effects for AGD based on this information, and BMD derivation far outside the testing range is questionable. For vaginal opening, the maximum response was +11.2%, the CED (CES 10%) was 2993 mg/kg bw/d and the BMDL was calculated by the authors to be 2745 mg/kg bw/d, i.e. also around the top dose. The dose-response curve (see below) in the publication shows a delay in vaginal opening of > 3 days for the top dose. Juvenile growth data do not indicate general growth retardation to be associated, but it remains a high dose observation and it is unclear if it is significant based on

pairwise statistics due to a standard deviation (36±4.6 days). The number of animals analysed is unclear, between 19-44 provided in the publication (starting point 8-10 produced litters per group).

***Table**: Date of anogenital distance and vaginal opening in TBBPA one-generation study (Van der Ven et al., 2008)*

female rats

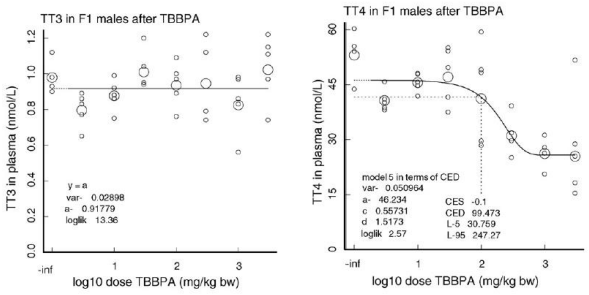
	anogenital distance (mm)			day vagina open
TBBPA dose mg/kg bw	d4	d7	d21	
0	3.3 ± 0.7	4.4 ± 0.8	11.3 ± 1.4	33.0 ± 1.9
3	3.6 ± 0.5	5.0 ± 0.8	12.7 ± 1.9	33.1 ± 2.0
10	3.0 ± 0.7	4.8 ± 1.6	12.4 ± 1.6	33.3 ± 2.4
30	3.4 ± 0.7	4.7 ± 1.0	12.0 ± 1.2	33.9 ± 3.2
100	3.2 ± 0.8	4.5 ± 0.8	11.2 ± 1.0	33.2 ± 2.4
300	3.4 ± 0.5	4.5 ± 0.6	11.7 ± 1.2	33.3 ± 2.0
1000	2.9 ± 0.7	4.5 ± 0.5	11.4 ± 0.6	32.6 ± 1.6
3000	3.2 ± 0.7	4.3 ± 0.4	11.2 ± 1.1	36.6 ± 4.6
dose response	a	+	a	+

Endocrinology F1

There was no change in the duration of the oestrus cycle and distribution of stages during the cycle, no dose-dependent effects on testosterone and 17-betaestradiol in male plasma or CYP19 activity in ovaries. Regarding thyroid, according to the CLH report and the published data presented there, Plasma T4 levels were decreased in both sexes. The CED (CES = 10%) was 99.5 (Maximum response: -44.3%) and 35.6 (Maximum response: -45.8%) mg/kg bw/d for males and females, respectively, the corresponding BMDL(10) were 30.8 and 16.1 mg/kg bw/d. T3 had a significant dose-response curve for an increase for females with a CED of 14 mg/kg bw/d (Maximum response:+26.5%, BMDL(10) = 2.3 mg/kg bw/d) (see table and figure below).

***Table and Figure**: T4 and T3 F1 serum levels in TBBPA one-generation study (Van der Ven et al., 2008)*

	females			*males*		
TBBPA dose mg/kg bw	n	TT4 nmol/L	TT3 nmol/L	n	TT4 nmol/L	TT3 nmol/L
0	4	34.3 ± 2.2	0.7 ± 0.1	4	53.4 ± 6.9	1.0 ± 0.1
3	5	33.5 ± 7.7	0.8 ± 0.1	5	40.7 ± 3.1	0.8 ± 0.1
10	5	38.0 ± 6.9	0.8 ± 0.1	5	45.7 ± 2.6	0.9 ± 0.1
30	5	41.2 ± 10.1	0.9 ± 0.1	5	47.6 ± 7.8	1.0 ± 0.1
100	5	27.1 ± 10.1	1.0 ± 0.1	5	43.0 ± 13.5	0.9 ± 0.1
300	5	23.2 ± 7.5	0.9 ± 0.1	4	31.5 ± 5.9	1.0 ± 0.2
1000	5	22.2 ± 4.7	1.0 ± 0.2	4/5	26.5 ± 4.4	0.8 ± 0.2
3000	5	18.4 ± 3.8	1.0 ± 0.2	5	27.9 ± 14.3	1.0 ± 0.2
dose response		+	+		+	-



RAC notes that while the T4 decrease is consistent with other TBBPA repeated dose studies (see STOT-RE section), a T3 increase for females appears rather inconsistent. In the Two-Generation study, T3 levels in P-males were decreased. Looking at the actual dose response, it could be a case of low control values. In repeated dose studies, T3 levels from males and females are comparable (e.g. subacute study, Van der Ven and al, 2008, supplementary data: (1.05 +/- 0.32 for females and 1.07 +/- 0.14 for males). The juvenile females control level in this study (0.7 nmol/L) is the only dose group outside the 0.8-1.0 nmol/L range for males and females across all groups. RAC notes that also for endocrine parameters a limited number of animals seem to have been investigated, i.e. the thyroid hormone data refer to 5 replicates per sex per dose group, for the controls and in two higher dose groups in males only 4 animals have been evaluated according to the publication, introducing uncertainties into the data.

TBBPA had no effect on immunotoxic and hematologic effects in F1 animals, except an increase in total spleen counts attributable to an increase in all major spleen cell populations (as clarified

in the DS response to comments received during the consultation of the CLH report). Splenocyte counts and B-cell counts as well as an increase monocytes were reported by the DS to be published as statistically uncertain.

Neurobehavioral effects

Brainstem auditory evoked potentials (BAEP) were used to study auditory responses in the offspring.

Auditory thresholds following tone pips and clicks:

According to the CLH report, BAEP showed dose-related elevation of BAEP thresholds in female offspring in the low frequency range up to 4 kHz. Significant fits to dose-response curves ($p<0.05$) were obtained for 0.5 and 2 kHz. The difference measured at 0.5 kHz in the top dose group was 13 dB compared to controls. The lowest CED and BMDL was obtained from the 2 kHz curve (CED = 6.6 mg/kg bw/d, BMDL = 0.9 mg/kg bw/d). RAC notes that the dossier does not show a dose-response relationship for individual frequencies and the description relies on the CLH report and publication showing the following threshold in females graphically, no effects on auditory thresholds for tone pips were noted for males. Increases in click thresholds were not significant in either sex.

***Figure and table**: BAEP effects (threshold and peak latencies) in TBBPA one-generation study (Lilienthal et al., 2008)*

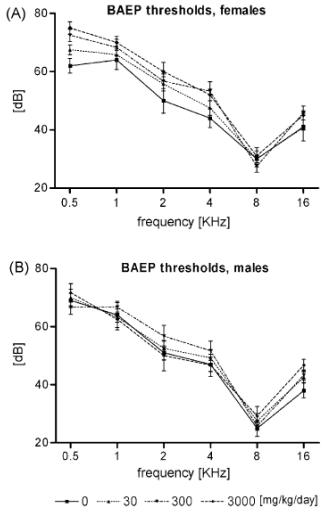


Fig. 2. BAEP thresholds in female (panel A) and male (panel B) rats (controls, groups exposed to TBBPA at 30, 300, or 3000 mg/kg body weight/day). Thresholds were elevated at frequencies of 0.5–4 kHz in exposed females, but not in males. Benchmark analyses revealed significant dose–response relationships at 0.5 and 2 kHz ($p<0.05$).

CED and BMDL values for effects on the BAEP (mg/kg body weight/day)

	CED	BMDL	Ratio	Maximum response (%)
Females				
Thresholds				
0.5 kHz	198	41.5	4.8	12
2 kHz	6.6	0.9	7.2	13
Latencies, wave II				
0.5 kHz	113	33.2	3.4	10
Latencies, wave IV				
0.5 kHz	70.3	8.3	8.5	10
Click, 60 dB	129	33.7	3.8	8
Males				
Latencies, wave IV				
0.5 kHz	36.3	7.7	4.7	19
2 kHz	383	55.9	6.8	21
Interpeak latencies II–IV				
0.5 kHz	597	353	1.7	25
1 kHz	343	238	1.4	49
2 kHz	61.0	22.9	2.7	42
4 kHz	409	99.6	4.1	14

Latencies of wave II and IV for tone pips and clicks:

Slightly prolonged latencies of wave II were noted for females, non-significant, with significant fit of the dose-response only for the lowest tone frequency 0.5 kHz. No significant effects were noted for males. Wave IV latency prolongations (see figure below) were reported for males and females, with significant fits of the dose-response curves obtained for both sexes for the 0.5 kHz frequency, and for males with the 2 kHz frequency. Corresponding 0.5 kHz frequency CED (CES = 5%) were 36 and 70 mg/kg bw/d for males and females, respectively (BMDL of approx. 8 mg/kg bw/d, see table 1 above). From visual inspection, a more pronounced peak is visible for 1 kHz in high dose males.

***Figure**: BAEP effects (peak latencies) in TBBPA one-generation study (Lilienthal et al., 2008)*

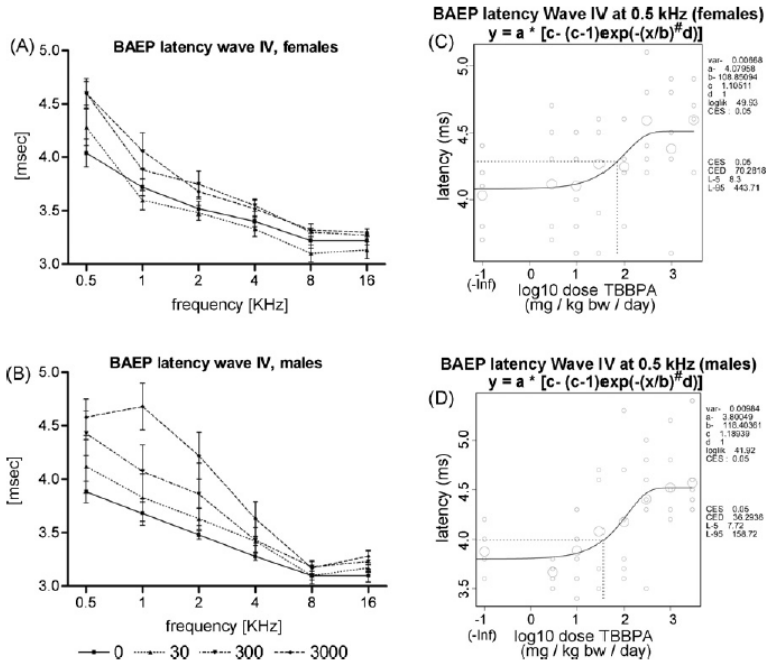


Fig. 4. Latencies of wave IV in female (panel A) and male (panel B) rats (controls, groups exposed to TBBPA at 30, 300, or 3000 mg/kg body weight/day). Exposed females and, in particular, males exhibited marked prolongations of wave IV latency in the low-frequency range. According to benchmark analysis, significant dose–response relationships were found at 0.5 kHz in exposed males and females and at 2 kHz in males. Panels C and D show the results for 0.5 kHz in females and males, respectively ($p < 0.05$). The methods for response analyses and the parameters describing the resultant curve are given in Slob (2002). The background value is referred to as a. Log likelihood is used to determine the extent by which the fitted curve deviates from no effect ($y = a$). The critical effect dose (CED) gives the dose at which a deviation of 5% from the background value (critical effect size, CES) is detected.

Due to a more pronounced shifts of wave IV latencies compared to wave II, the interpeak latencies for males showed shifts suggestive of increased signal transmission time in the brainstem. CED values between 61 and 597 mg/kg bw/d were obtained for the different frequencies (BMDL 23-353 mg/kg bw/d), see below:

***Table**: Interpeak latencies II-IV of tone evoked BAEPs in TBBPA one-generation study (Lilienthal et al., 2008)*

	0.5 kHz	1 kHz	2 kHz	4 kHz	8 kHz	16 kHz
Females						
Controls	1.78 ± 0.15	1.60 ± 0.05	1.60 ± 0.04	1.60 ± 0.06	1.68 ± 0.06	1.60 ± 0.03
3	2.00 ± 0.18	1.65 ± 0.04	1.60 ± 0.06	1.60 ± 0.05	1.58 ± 0.06	1.57 ± 0.04
10	1.83 ± 0.11	1.73 ± 0.15	1.63 ± 0.07	1.68 ± 0.06	1.62 ± 0.03	1.63 ± 0.06
30	2.07 ± 0.13	1.60 ± 0.08	1.58 ± 0.05	1.58 ± 0.04	1.58 ± 0.05	1.58 ± 0.05
100	1.98 ± 0.10	1.77 ± 0.14	1.92 ± 0.17	1.73 ± 0.18	1.62 ± 0.05	1.60 ± 0.04
300	2.13 ± 0.10	1.80 ± 0.20	1.77 ± 0.09	1.72 ± 0.03	1.72 ± 0.03	1.63 ± 0.03
1000	1.88 ± 0.14	1.55 ± 0.07	1.55 ± 0.06	1.77 ± 0.03	1.68 ± 0.03	1.68 ± 0.06
3000	2.20 ± 0.11	1.82 ± 0.13	1.62 ± 0.10	1.66 ± 0.04	1.66 ± 0.07	1.66 ± 0.02
Males						
controls	**1.66 ± 0.09**	**1.62 ± 0.05**	**1.52 ± 0.06**	**1.56 ± 0.05**	1.60 ± 0.06	1.56 ± 0.06
3	**1.50 ± 0.10**	**1.52 ± 0.12**	**1.50 ± 0.04**	**1.53 ± 0.02**	1.53 ± 0.05	1.52 ± 0.05
10	**1.75 ± 0.12**	**1.68 ± 0.09**	**1.53 ± 0.08**	**1.62 ± 0.03**	1.67 ± 0.02	1.62 ± 0.05
30	**1.90 ± 0.21**	**1.70 ± 0.09**	**1.65 ± 0.07**	**1.65 ± 0.03**	1.58 ± 0.03	1.60 ± 0.03
100	**1.87 ± 0.21**	**1.97 ± 0.19**	**1.62 ± 0.05**	**1.60 ± 0.04**	1.62 ± 0.04	1.62 ± 0.05
300	**2.13 ± 0.18**	**1.98 ± 0.20**	**1.87 ± 0.14**	**1.58 ± 0.04**	1.63 ± 0.03	1.65 ± 0.07
1000	**2.07 ± 0.24**	**1.72 ± 0.12**	**2.08 ± 0.27**	**1.77 ± 0.06**	1.65 ± 0.06	1.65 ± 0.06
3000	**2.18 ± 0.07**	**2.50 ± 0.14**	**2.17 ± 0.15**	**1.75 ± 0.15**	1.58 ± 0.03	1.63 ± 0.04

A significant fit to dose–response models was obtained in the frequency range of 0.5–4 kHz in male rats (bold figures, $p < 0.05$), see Table 1 for CED and BMDL values; means ± S.E.M., $n = 5$–6/group.

The publication also reported significant wave IV increase after click stimulation with 60 dB in female rats ($p<0.05$), but no significant changes in wave latencies were reported for 80 dB or wave II shifts or for males following any click stimulation. RAC notes a low effect size for this finding of max. 8% response, also the dose-response relationship appears not convincing (pre-set CES: 5%), 3.8±0.09, 3.8±0.09, 3.73±0.07, 3.75±0.08, 3.9±0.18, 4.13±0.16, 4.07±0.14, 3.96±0.05 for control, 3, 10, 30, 100, 300, 1000, 3000 mg/kg bw/d, respectively.

RAC considered whether the generic CES of 5% for wave latencies is appropriate and whether the effect size represents a meaningful response. No convention and laboratory reference value database has been mentioned by the author. Regarding further uncertainties, the EFSA CONTAM Panel noted that the ratios between the BMDL and their corresponding BMD value were rather large, indicating a high uncertainty in these outcomes. According to the publication, only 5-6 animals/sex/group were assessed between postnatal days 50 and 110. Wave latencies are only presented for three dosing groups. Thus, the significance obtained by the model maybe questionable. The EFSA panel also noted that increased thresholds in the BAEPs are difficult to interpret and have to be confirmed by other independent investigations.

Overall, the data indicate effects of TBBPA on BAEP auditory thresholds and wave latencies, however the information provided with the publication and experimental and statistical set-up raises questions and it is difficult to derive firm conclusions.

In the consultation of the CLH report, industry highlighted the criticism brought forward by Strain *et al*. (2009) questioning the results on BAEP from Lilienthal *et al*. (2008) by raising several questions on the statistical analysis and interpretation of the results.

Sweet preference study including absolute consumption of saccharin solution detected no effects in males, for females minor statistically non-significant inverted U-shaped results on the first 2 days of measurement period were noted.

There were no effects on conditional fear (cue or context).

To summarize, the CLH report suggests that the most sensitive effects of this reproduction study, based on BMDL, were increases in testis and pituitary weights and the modulation of thyroid hormones T3 and T4. RAC noted that for several parameters in this study, the BMD model results appear uncertain and dose-response curves, where available to RAC, were unclear and for some parameters relied on a rather small number of animals. Increased testis weights were not observed in the preceding 28-day study by the authors, nor were testis and pituitary weight changes observed in the Two-Generation reproduction study in rats with doses up to 1000 mg/kg bw/d (presented above, Cope *et al*. 2015). For neurobehavioral changes, the results of the study indicated an increase in the BAEP thresholds and wave IV latency in exposed females in the low frequency range and increases in absolute latency of wave IV and interpeak latencies II-IV at low frequencies in males (without an effect threshold). According to the study authors it is suggested that TBBPA causes a predominant cochlear effect in female rats while in males neuronal effects were more apparent. The authors further suggested it may relate to observed changes in thyroid hormone levels and that this link was statistically supported by correlations between these parameters, and the BMDL of hearing latencies and for decrease in serum T4 were in the same range. Surprisingly a potentially very adverse effect, an increase in juvenile pup mortality during lactation affecting up to 90% on a litter basis, was not discussed in the CLH report. The endpoint displayed an unclear (i.e. inverted u-shape) dose-response relationship with dose-dependently increased mortality over 3, 10, 30 mg/kg bw/d followed by decreasing rates for doses of 100 mg/kg bw/d up to 3000 mg/kg bw/d.

RAC has general remarks on the study and the data as presented in the CLH report. The study was conducted as a part of the tiered screening program of the FIRE project, which aimed at the

toxicological characterization of brominated flame retardants with a focus on endocrine disrupting and immunological effects. The study as presented in the CLH report doesn't seem useful for classification and labelling. Dose response curves were insufficiently presented and discussed, and study results presentation mainly relies on BMDL values. Modelled BMDL values were based on arbitrary – not internationally agreed - choices of meaningful magnitudes of effect, e.g. EFSA recommends for continuous data 5% as default BMR and 10% (extra risk) for quantal data, while especially for the neurodevelopmental investigations (e.g. BAEP) HCD are needed for interpretation of the results. The BMD uncertainty was assessed based on a practical CED/BMDL ratio threshold, however as already pointed out by EFSA, in order to take fully into account uncertainty, the BMDU/BMDL ratio should be considered (EFSA, 2016[1]). Some CED and BMDL were presented as having doses around the high dose and even outside the tested dose range (ie exceeding the high doses), which in the view of RAC is questionable. Atypical dose-response curves were obtained for some parameters, with levelling-off at higher doses or inverted U-shaped curves, which however is not obvious when solely looking at the reported BMDL values. It was clarified by the DS in response to a request from RAC, that BMD models are monotonic, unable to handle U-shaped or inverted U-shaped curves. In such cases, the model result essentially is misleading. The study authors however mainly relied on statistical analysis. This included also correlation analysis, however parallel responses for many estimates may be due to confounding factors rather than due to toxicological reasons. Such correlations are insufficient to establish causality, mode of action and adversity for classification purposes. As the relevance and robustness of these correlation exercises for classification is questionable, the results were not further considered by RAC. Statistical significance was not analysed based on pair-wise statistics, but on trend-test using the whole data set in the BMD approach. While RAC acknowledges the power of these statistics, based on visual inspection of response curves the biological significance of findings is not always obvious. The full dose-response curves are not published for all parameters (e.g. 3/7 dose groups for BAEP Wave IV latency prolongations graphically presented) and for some parameters the number of animals or groups included in the statistical analysis is not clear. This study reports results from ten or fewer animals. Overall, the study design, the result evaluation and reporting suffer from several important limitations and RAC has reservations regarding the reliability of the study for hazard classification.

Conclusion on sexual function and fertility

In TBBPA studies on reproductive function and fertility, neither the Two-Generation or the One-Generation study showed effects that warrant classification for sexual function and fertility. RAC therefore agrees with the dossier submitter proposal that **no classification is warranted.**

Findings on neurobehavioral toxicity and neuropathology arising from these studies will be considered for assessment of developmental toxicity (below).

Developmental toxicity

Three studies on developmental toxicity were assessed by the DS. Two of these studies were also assessed in the EU RAR (Cope *et al*., 2015; Hass *et al*., 2003).

- OECD TG 414 Developmental Toxicity Study in SD rats – assessed as Klimisch score 1 by the DS (Unnamed, 2002; Cope *et al* 2015);
- OECD TG 426 Developmental Neurotoxicity Study in Wistar rats – assessed as Klimisch score 2 by the DS (Hass *et al*., 2003);

[1] EFSA 2016: Update: use of the benchmark dose approach in risk assessment.

- Non-guideline developmental study in SD rats - assessed as Klimisch score 2 by the DS (Saegusa *et al*., 2009).

In addition, the findings on neurobehavioral toxicity and neuropathology from the reproduction studies described above were considered:

- Developmental neurotoxicity component in the F2-generation of the GLP-compliant OECD TG 416 Two Generation Reproduction Toxicity Study - assessed as Klimisch score 1 by the DS (Unnamed, 2002; Cope *et al*. 2015);
- Endocrine and Immunological endpoints and additional analysis for bone and neurophysiological parameters as part of the OECD TG 415 One Generation Reproduction Toxicity Study (Van der Ven, 2008; Lilienthal, 2008) – assessed as Klimisch score 2 by the DS.

OECD TG 414 Developmental Toxicity Study (Unnamed 2002, Cope *et al*. 2015)

Effects of TBBPA on development were assessed according to OECD TG 414 prenatal developmental toxicity study protocol in 25 female SD rats at doses of 0, 100, 300 and 1000 mg/kg bw/d by oral gavage administered daily for 20 days from GD 0 to GD 19.

No toxic effects were observed in maternal animals and **no embryotoxic/teratogenic effects** were reported in the foetuses. No effects on foetal body weight, sex distribution or external observation and results from visceral and skeletal examinations are reported from the study. A slight tendency to an increase in pre- and post-implantation loss and early resorption is noted, however also a high SD for these parameters and the unaffected pregnancy index is noted. Litter incidences did not differ from controls. Results are presented in the following table:

***Table**: Reproductive and pre-natal develomental parameters in TBBPA Developmental study (Cope et al., 2015)*

Endpoint evaluated	TBBPA dose			
	Control	100 mg/kg BW/day	300 mg/kg BW/day	1000 mg/kg BW/day
Number of females on study	25	25	25	25
Number not pregnant	0	1	0	1
Number pregnant	25	24	25	24
Pregnancy index	100.0	96.0	100.0	96.0
Number died during pregnancy	0	0	1	0
Number of abortions	0	0	0	0
Number of early deliveries	0	0	0	0
Number of females with viable fetuses on day 20 gestation	25	24	24	23
Number of coprora lutea per animal (mean ± SD)	16.8 ± 2.72	16.6 ± 2.21	16.8 ± 3.60	18.0 ± 2.57
Number of implantation sites per animal (mean ± SD)	15.4 ± 1.76	15.3 ± 2.48	15.4 ± 3.52	15.6 ± 8.059
Preimplantation loss %/animal (mean ± SD)	7.25 ± 7.540	7.77 ± 9.699	10.18 ± 15.696	10.65 ± 8.059
Viable fetuses number/animal (mean ± SD)	14.6 ± 1.68	14.5 ± 2.64	14.1 ± 3.71	14.3 ± 3.43
Viable fetuses/implant %/implant (mean ± SD)	95.05 ± 6.636	94.63 ± 7.523	92.34 ± 12.560	90.25 ± 20.023
Fetal sex ratio; % males/animal (mean ± SD)	52.9 ± 12.20	50.7 ± 15.95	47.5 ± 16.58	52.5 ± 13.64
Postimplantation loss; %implants/animal (mean ± SD)	4.95 ± 6.636	5.37 ± 7.523	7.66 ± 12.560	9.75 ± 20.023
Non-viable fetuses; number/animal (mean ± SD)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Early resporptions; number/animal (mean ± SD)	0.8 ± 1.12	0.8 ± 1.13	1.3 ± 2.01	1.3 ± 1.73
Early resoprtions/implant; %/impant (mean ± SD)	4.95 ± 6.636	5.37 ± 7.523	7.68 ± 12.560	9.75 ± 20.023
Late resorptions; number/animal	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
Gravid uterine weight (g; mean ± SD)	83.0 ± 8.21	81.3 ± 14.17	77.5 ± 19.54	83.7 ± 9.71
Male fetal weight (mean ± SD)	3.81 ± 0.258	3.81 ± 0.319	3.67 ± 0.240	3.75 ± 0.357
Female fetal weight (mean ± SD)	3.62 ± 0.262	3.63 ± 0.276	3.53 ± 0.208	3.56 ± 0.293
Male + female fetal weight (mean ± SD)	3.72 ± 0.254	3.72 ± 0.296	3.59 ± 0.221	3.66 ± 0.322
Forelimb external observations				
Digits, ectrodactyly malformations Number of litters (%)	0	0	0	0
Digits, ectrodactyly malformations Number of foetuses (%)	0	0	0	0
Abnormal forelimb flexure variations Number of litters (%)	0	0	1	0
Abnormal forelimb flexure variations Number of foetuses (%)	0	0	1	0

It is concluded that TBBPA had no adverse maternal and developmental effects in this study up to doses of 1000 mg/kg bw/d.

OECD TG 426 Developmental Neurotoxicity Study

TBBPA effects on developmental neurotoxicity were investigated in an OECD TG 426 study in 20 pregnant Wistar rats per dose level of 0, 50, 250 mg/kg bw/d, from gestation day 7 to PND 17. In deviation from the guideline only two dose levels were assessed. As a general remark, no tabulated result data was presented in the CLH report and the information was only assessed indirectly by the DS based on what is available in the EU RAR because the study report was not published (conference presentation). Delivered pups were inspected for sex and anomalies, decedent pups were examined macroscopically, if possible. Gross pathology and histopathology were conducted on reproductive organs, the thyroid and brain. Thyroid hormones and neurotransmitters were analysed PND 22. Postnatal development was assessed by measuring bodyweight on PND 6 and PND 13, anogenital distance (AGD) at birth, areola/nipples on PND 13 and PND 14, age and bodyweight of animals upon reaching sexual maturation, sexual maturation was evaluated by examining vaginal opening and balano-preputial separation. After weaning on PND 21, one male and one female from each litter were randomly selected for the behavioural testing. These tests included motor activity of dams and offspring (adult animals at 12 weeks and in offspring on PND 21 and 27), play behaviour of offspring (PND 31), learning and memory test based on Morris water maze and radial arm maze (ages 9, 13 and 17 weeks), sweet preference test months 5, and 8 arm radial maze at months 6-7. The non-published study results were presented based on information given in the EU RAR.

General toxicity, weights, histopathology, thyroid hormones, neurotransmitters

Maternal bodyweight gains during pregnancy, gestation lengths, litter sizes, frequency of neonatal death and birth weights were similar between control and treated animals. No adverse effects were observed on body and organ weights for any age group (PND 15, PND 22 and adult animals), including no effects on AGD, areolas/nipples, timing of sexual maturation, and histopathology of reproductive organs or brain (PND 15 and PND 22). No exposure-related changes on serum thyroid hormones in males (PND 22) or brain neurotransmitter levels investigated after sacrifice on PND 22, including 5-hydroxytryptamine (5-HT), noradrenaline (NA) and dopamine (DA), were observed.

Behavioural observation

No significant differences are reported on play behaviour or sweet preference.
Some changes in habituation behaviour of female offspring at PND 21 were detected based on higher motor activity in the second 15-min segment of a 30 minute observation period at the high dose compared to controls and the low dose. However, the 30 minute observation period overall showed no changes, males showed no effects, neither females nor males showed effects for this test on PND 28. For adults tested at 12 weeks of age, again no differences were reported between treated and control groups in males, and no differences over the entire 30 minute period between treated and control females. For females some inconsistent changes were reported for the individual observation segments, for segment 1 the activity in the 50 mg/kg bw/d group showed a non-significant reduction compared to controls while for the second segment, activity in the 250 mg/kg bw/d group females was higher than that in the control group. The DS noted that there was insufficient justification for the statistical test chosen. RAC agrees with the DS that these observations on habituation behaviour appear not to be consistent over observation days, segments, sex and doses. Also, the fact that only two doses were tested compromised interpretation of the results.
In the water Morris maze test, significant differences were observed only very occasionally, and there was no consistent pattern of changes across the 12 trials. The DS thus considered it unlikely that the results indicate a treatment-related effect on memory. No significant treatment-related differences were reported in the "reversal learning" part of the study. Occasional differences in "new learning" but without any consistent pattern were considered a chance finding. Some

marginal effect on the learning ability and memory of top dose male rats was suggested based on increased errors, statistically significant, observed for high dose males in week 1 in the radial arm maze test. The DS explained that overlapping SD of the means suggests that the finding might not be significant when analysed with routine statistical tests.
Overall, RAC agrees with the DS that firm conclusions from this study are not possible and that it suffers from small changes, inconsistent pattern of changes for different times and sex and the lack of histopathological corroborative findings.

Non-guideline developmental study in SD rats

In a non-guideline developmental study in rats, TBBPA was administered via feeding levels of 0, 100, 1000 and 10000 ppm, corresponding to 0, 10, 90, and 800 mg/kg bw/d, respectively, during gestation, to Cjr:CD®(SD)IGS dams from GD 10 until day 20 after delivery (day after weaning).

Dams: The only effect on body weight was a transient increase in high dose dams on days 9-20 after delivery, being normal at day 20 compared to the controls, while no effects on food consumption were reported. The treatment had no effect on pregnancy duration. A tendency for increased relative thyroid weights was noted but there was no dose-response relationship, and a marginal diffuse thyroid follicular hypertrophy was not significant.

Offspring: Offspring parameters did not show abnormalities in clinical observations, number of implantation sites, number of live offspring, male ratio, body weight, organ weights or anogenital distance at PND 1, onset of puberty in either sexes, and oestrus cycle in females. Higher body weight was reported for high dose males at the onset of puberty. According to the CLH report, male offspring showed a dose-unrelated decrease in serum T3 levels at the low and mid doses at PND 20, but not at the high dose, while T4 and TSH were unchanged and no changes in hormones were detected at PNW 11. Adult females showed, at PNW 11, decreased relative kidney and uterus weights at the mid and high doses, while body and organ weights for adult male offspring was unchanged. No treatment-related effects were observed in the histopathological assessment at PND 20 or PNW 11. There were no findings from the brain morphometric assessments in terms of neuronal migration and oligodendroglial development in male offspring at the adult stage.

Further studies on developmental toxicity

RAC notes that the EU RAR presents four further studies on developmental toxicity that had not been considered in the CLH report. The studies are briefly summarised in the Supplemental information section. Upon request, the DS clarified that these studies were evaluated in detail in the EU RAR. Overall, the data do not provide strong evidence of the potential for TBBPA to act as a developmental toxicant or neurotoxicant. The three first studies did not show any effects on development. However, there were some effects in the Fukuda *et al* (2004) study especially on nephrotoxicity. The EU RAR considered these effects as administration related (likely to be the consequence of the unconventional direct gavage administration of very high doses of TBBPA to such young animals. Therefore, the relevance to human health of this isolated finding is considered questionable). In the opinion of the DS, the EU RAR assessed the existing data accurately.

Conclusion on Developmental toxicity

In summary, none of the five developmental toxicity studies presented by the DS suggest adverse maternal effects, structural visceral or skeletal abnormalities in the offspring, or altered foetal growth or retardation. Neurodevelopmental investigations indicate a potential concern and findings need consideration in a weight-of-evidence assessment.

For the Two-Generation study, RAC acknowledges that effects in motor activity at PND 60 in males at the mid and high doses could potentially be a treatment related effect. However, the pattern in females with lack of dose-response and inconsistence in times/ages, differences in sex and no changes for males at any other age (PND 13, 17, 21) raises the possibility that it could be a chance finding, since the evidence appears rather weak. For the passive avoidance test assessed on PND 22 and PND 60, results also do not allow robust conclusions on substance-related effects on learning and memory. In general, test responses might be related to methodological issues due to a high error-variance and reduced sensitivity of the passive avoidance test. No effects were observed for females. The result pattern for males was inconsistent for PND 22 and PND 60 and, according to the RAR, an unexpected performance of the control animals at PND 60 raised questions on the reliability of the test system. Animals had no treatment related effects in the water M-maze test.

The reduced thickness of the parietal cortex was a transient finding at PND 11 not confirmed at PND 60, but the observation was apparent for both sexes at the high dose. No histological changes were evident in the parietal cortex, no effect on PND 60 was seen on brain weights, no microscopic alterations were observed in the brain, spinal cord, nerves or ganglia in PND 60 F2 animals, nor were neuro-functional deficits evident. Due to the transient nature of this change at the high dose only, RAC agrees that the toxicological relevance of this finding is equivocal.

The DS summarised the Hass *et al*. (2003) study on developmental neurotoxicity based on the EU-RAR (2008) (cf. Annex I to the CLH report). Some changes in motor activity were reported for females also in this study, indicating a decreased habituation activity in females exposed to 250 mg/kg/day at PND 21, but no robust evidence for PND 28 and no effects in males. It appears also in this study, that these observations on habituation behaviour appear not consistent along observation days, segments, sex and doses. Also, the fact that only two doses were tested compromises interpretation of the results. Similar to the Two-Generation study, the results can only be considered in a weight-of-evidence assessment. It is to be noted that the developmental neurotoxicity study used a lower top dose than the Two-Generation study. All other changes in this study were described as occasional by the EU-RAR and the DS.

In the One-Generation study, concerns are raised from the BAEP measurements indicating effects on the developing auditory system. The results suggest an increase in the BAEP thresholds and wave IV latency in exposed females in the low frequency range and increases in absolute latency of wave IV and interpeak latencies II-IV at low frequencies in males (without an effect threshold). The study authors suggested that TBBPA causes a predominant cochlear effect in female rats while in males neuronal effects were more apparent. The authors further suggested it may relate to observed changes in thyroid hormone levels as T4 levels were decreased in F1 males and females and the modulation of thyroid hormones T4 without concomitant TSH change or histopathology is a consistent finding for TBBPA observed in several repeated dose toxicity studies. For the context of assessing classification and labelling, RAC however has highlighted the uncertainties coming with the limitations in study design, data reporting and result evaluation, including the low number of animals analysed for BAEP, and uncertainty in BMD model results, including those on BAEP and thyroid hormones.

During the consultation of the CLH report, one Member State highlighted the role of the thyroid hormones in development considering the evidence from the above studies on neurodevelopment warranting at least a category 2 classification for adverse effects on development. IARC (referenced in the CLH report) concluded that no experimental studies exist addressing the effects of TBBPA on thyroid hormones regulated developmental events (including hearing and

testis weight). RAC notes an Adverse Outcome Pathway (AOP[1]) on Nuclear receptor induced TH Catabolism and Developmental Hearing Loss is under development based on evidence with PCB for which a correlation between the severity of functional auditory impairment and the degree of thyroid hormone depletion has been observed, with a critical post-natal exposure period. For TBBPA however no data are available in support of this AOP, except for Key Event T4 decrease and limited evidence for the Adverse Outcome, but independent confirmatory studies on TBBPA adverse effects on developmental hearing loss would be needed. RAC agrees that thyroid hormones play an important role in foetal and postnatal development and in particular in the development of the central nervous system, as highlighted by one MS. RAC also acknowledges the role of hypothyroxinemia (IMH), the presence of low maternal T4 in the absence of TSH elevation, in brain development and risk factor for impaired mental and motor neurodevelopment and neuropsychiatric diseases of the offspring. TBBPA consistently resulted in reduced T4. The mechanism of thyroid hormone regulation is still not resolved. Despite the uncertainties in the study results on neurodevelopment flagged above, RAC considers that based on the information provided, the causality of thyroid hormone modulation to any downstream developmental effects have not been adequately investigated and proven.

Category 2 classification criteria are as follows:

Suspected human reproductive toxicants
"Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification."

The DS assessed the data and considered that the effects on neurobehavioral parameters are insufficient for classification of TBBPA as a developmental toxicant. RAC agrees with the DS overall, that the effects observed are not sufficient for classification of TBBPA for developmental toxicity, although some evidence indicated a concern for developmental neurotoxicity. The uncertainties in the view of RAC are too high to draw firm conclusions for reasons which include the inconsistencies in results or lack of corroborative or correlative findings (e.g. functional deficit following neuropathology change parietal cortex thinning), and the limited reliability of the One-Generation study for hazard classification purpose.

One MSCA, supporting no classification, raised the lack of a developmental toxicity study in a second species in the REACH registration dossier. Indeed, all the data were obtained from rats and although five studies were assessed by the DS, the package thus still has uncertainties regarding interspecies variability. The additional four studies discussed under the Supplemental information section include one study in NMRI mice on developmental neurotoxicity but not an OECD 414 developmental toxicity / teratology study.

RAC concludes that **no classification for developmental toxicity** is warranted based on five studies (guideline and non-guideline) on developmental toxicity, developmental neurotoxicity and developmental immunotoxicity and endocrinology investigations performed in rats. In a

[1] https://aopwiki.org/wiki/index.php/Aop:8: Upregulation of Thyroid Hormone Catabolism via Activation of Hepatic Nuclear Receptors, and Subsequent Adverse Neurodevelopmental Outcomes in Mammals (Short name: Nuclear receptor induced TH Catabolism and Developmental Hearing Loss); MIE: PXR activation → KE: Upregulation of glucuronyltransferase activity → KE: Increase biliary excretion TH glucuronide → KE: decrease serum T4 → KE: decrease T4 in neuronal tissue → KE: altered hippocampal gene expression → KE: altered hippocampal anatomy → KE: altered hippocampal physiology → Adverse Outcome: loss of cochlear function)

weight of evidence assessment, the studies indicate some concern for developmental toxicity, but the data is considered not sufficient evidence for classification due to several limitations and uncertainties.

RAC notes that the DS did not assess effects of TBBPA on or via lactation. RAC therefore does not consider this endpoint.

Additional references

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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).