Committee for Risk Assessment

RAC

Annex 1
Background document
to the 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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

16 September 2021

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol; tetrabromobisphenol-A (TBBPA)

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

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

Index Number: **604-074-00-0**

Contact details for dossier submitter:

Norwegian Environment Agency

P.O. Box 5672 Torgarden,

7485 Trondheim, Norway

postmottak@miljodir.no

+47 73 58 05 00

Co-submitter: The Danish Environmental Protection Agency

Version number: 2.0 **Date: 18.09.2020**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 2,2',6,6'-TETRABROMO-4,4'-ISOPROPYLIDENEDIPHENOL; TETRABROMOBISPHENOL-A

CONTENTS

1 IDENTITY OF THE SUBSTANCE 1

1.1 NAME AND OTHER IDENTIFIERS OF THE SUBSTANCE 1
1.2 COMPOSITION OF THE SUBSTANCE 2

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING 3

2.1 PROPOSED HARMONISED CLASSIFICATION AND LABELLING ACCORDING TO THE CLP CRITERIA 3

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING 5

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL 5

5 IDENTIFIED USES 5

6 DATA SOURCES 6

7 PHYSICOCHEMICAL PROPERTIES 6

8 EVALUATION OF PHYSICAL HAZARDS 8

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION) 8

9.1 SHORT SUMMARY AND OVERALL RELEVANCE OF THE PROVIDED TOXICOKINETIC INFORMATION ON THE PROPOSED CLASSIFICATION(S) 10

10 EVALUATION OF HEALTH HAZARDS 13

10.1 ACUTE TOXICITY - ORAL ROUTE 13
10.2 ACUTE TOXICITY - DERMAL ROUTE 13
10.3 ACUTE TOXICITY - INHALATION ROUTE 13
10.4 SKIN CORROSION/IRRITATION 13
10.5 SERIOUS EYE DAMAGE/EYE IRRITATION 13
10.6 RESPIRATORY SENSITISATION 14
10.7 SKIN SENSITISATION 14
10.8 GERM CELL MUTAGENICITY 14
10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity 17
10.8.2 Comparison with the CLP criteria 18
10.8.3 Conclusion on classification and labelling for germ cell mutagenicity 18
10.9 CARCINOGENICITY 23
10.9.1 Short summary and overall relevance of the provided information on carcinogenicity 28
10.9.1.1 Mode of action (MoA) for uterine carcinogenesis in female rats and relevance to humans: 32
10.9.2 Comparison with the CLP criteria 36
10.9.3 Conclusion on classification and labelling for carcinogenicity 38
10.10 REPRODUCTIVE TOXICITY 61
10.10.1 Adverse effects on sexual function and fertility 61
10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility 64
10.10.3 Comparison with the CLP criteria 68
10.10.4 Adverse effects on development 69
10.10.5 Short summary and overall relevance of the provided information on adverse effects on development 70
10.10.6 Comparison with the CLP criteria 71
10.10.7 Conclusion on classification and labelling for reproductive toxicity 72
10.11 SPECIFIC TARGET ORGAN TOXICITY-SINGLE EXPOSURE 94
10.12 SPECIFIC TARGET ORGAN TOXICITY-REPEATED EXPOSURE 95
10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure 102
10.12.2 Comparison with the CLP criteria 104
10.12.3 Conclusion on classification and labelling for STOT RE 104

10.13 ASPIRATION HAZARD 112

11 EVALUATION OF ENVIRONMENTAL HAZARDS 112

12 EVALUATION OF ADDITIONAL HAZARDS 112

13 ADDITIONAL LABELLING 113

14 REFERENCES 113

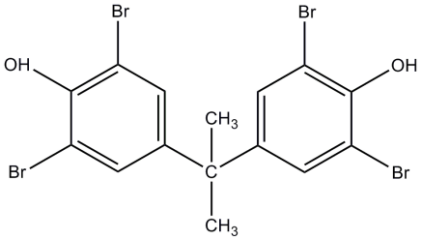
15 ANNEXES 116

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON 2,2',6,6'-TETRABROMO-4,4'-ISOPROPYLIDENEDIPHENOL; TETRABROMOBISPHENOL-A

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol; tetrabromobisphenol-A
Other names (usual name, trade name, abbreviation)	2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol 2,2-Bis(3,5-dibromo-4-hydroxyphenyl)propane; 2,2-bis(4-hydroxy-3,5-dibromophenyl)propane; 4,4′-isopropylidenebis(2,6-dibromophenol); 4,4′-(1-methylethylidene)bis(2,6-dibromophenol); 2,2′,6,6′-tetrabromobisphenol A; 3,3′,5,5′-tetrabromobisphenol A; 2,2′,6,6′-tetrabromo-4,4′-isopropylidenediphenol; Tetrabromodiphenylpropane; TBBPA
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	201-236-9
EC name (if available and appropriate)	2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol
CAS number (if available)	79-94-7
Other identity code (if available)	-
Molecular formula	C15H12Br4O2
Structural formula	
SMILES notation (if available)	CC(C)(C1=CC(Br)=C(O)C(Br)=C1)C1=CC(Br)=C(O)C(Br)=C1
Molecular weight or molecular weight range	543.88
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	-
Description of the manufacturing process and identity of the source (for UVCB substances only)	-
Degree of purity (%) (if relevant for the entry in Annex VI)	-

1.2 Composition of the substance

See confidential annex for information.

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	Concentration range (% w/w minimum and maximum in multi-constituent substances)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)
2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol; tetrabromobisphenol-A **EC no: 201-236-9** **CAS no: 79-94-7**	See confidential annex for information	Aquatic Acute 1, H 400 Aquatic Chronic 1, H410	Carc. 2, H351 (Number of notifiers: 706 (19.05.2020)

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The impurity contributes to the classification and labelling
See confidential annex for information				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	Function	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	Current self-classification and labelling (CLP)	The additive contributes to the classification and labelling
Not relevant					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	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	2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol; tetrabromobisphenol-A	201-236-9	79-94-7	Aquatic Acute 1 Aquatic Chronic 1	H400 H410	GHS09 Warning	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 Annex VI entry if agreed by RAC and COM	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			

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route	hazard class not assessed in this dossier	No
Acute toxicity via dermal route	hazard class not assessed in this dossier	No
Acute toxicity via inhalation route	hazard class not assessed in this dossier	No
Skin corrosion/irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity	**data conclusive but not sufficient for classification**	**Yes**
Carcinogenicity	**harmonised classification proposed**	**Yes**
Reproductive toxicity	**data conclusive but not sufficient for classification**	**Yes**
Specific target organ toxicity-single exposure	hazard class not assessed in this dossier	No
Specific target organ toxicity-repeated exposure	**data conclusive but not sufficient for classification**	**Yes**
Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

2,2',6,6'-tetrabromo-4,4'-isopropylidenediphenol; tetrabromobisphenol-A (TBBPA) has harmonised classifications for environmental hazards. The substance has a self-classification as Carcinogen cat. 2 in the registration dossier, as well as in the CLH inventory (see table 2 above for more details).

TBBPA has been assigned as ***probably carcinogenic to humans*** **(Group 2a)** by the Interagency for Research on Cancer (IARC) based on sufficient evidence for carcinogenicity found in the 2-year rodent studies and mechanistic information reported in the literature (Grosse et al., 2016; IARC 2018). The majority of the working group members supported a group 2A classification based on *sufficient evidence of carcinogenicity in experimental animals*, and strong mechanistic evidence on three key characteristics (TBBPA modulates receptor-mediated effects, is immunosuppressive and induces oxidative stress) that were shown to also operate in humans. A minority of the IARC working group considered that the data did not support a mechanistic upgrade to Group 2A. For a description of the IARC system, please see the 2019 revised IARC preamble (poster).

The substance is currently under evaluation for PBT and ED and is included in the CoRAP list.

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.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The substance has CMR properties (carcinogenicity). Harmonised classification and labelling for CMR is a community-wide action under article 36 of the CLP regulation.

STOT RE is closely related to the CMR properties and it is therefore relevant to consider this hazard class. This justifies a harmonised classification for TBBPA.

5 IDENTIFIED USES

Tetrabromobisphenol-A (TBBPA) is a brominated flame retardant (BFR) commonly used in electronics to meet fire safety standards and has the largest worldwide production of any BFR (Knudsen et al., 2017). It is estimated that the global production volume of TBBPA and its derivates is over 100,000 tons per year

(IARC, 2018). It is used in 90% of epoxy coated circuit boards (Cannon et al., 2019). It is also used in printed circuit boards, paper, and textiles (Dunnick et al., 2017). TBBPA is the most widely used brominated flame retardant worldwide and may be released from products into the environment (Birnbaum and Staskal, 2004).

6 DATA SOURCES

REACH registration dossier, including Chemical Safety Report and clarifications from registrant.

Technical Report on TBBPA from National Toxicology Program (NTP) 2014 is a central source.

Litterature search in PubMed, latest search early 2020.

References in peer reviewed scientific papers.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	Solid	ECHA Dissemination site, 2020	White solid crystalline powder.
Melting/freezing point	180 °C at 101 325 Pa	ECHA Dissemination site, 2020	
Boiling point	-	ECHA Dissemination site, 2020	In accordance with column 2 of REACH Annex VII, the boiling point study (required in section 7.3) does not need to be conducted as the substance decomposes at a temperature of 316 °C prior to boiling.
Relative density	2.17 at 20°C	ECHA Dissemination site, 2020	
Vapour pressure	0 Pa at 20 °C	ECHA Dissemination site, 2020	
Surface tension	-	ECHA Dissemination site, 2020	In accordance with column 2 of REACH Annex VII, the surface tension study (required in section 7.6) does not need to be conducted as the water solubility of the substance is below 1 mg/l, surface activity is not a desired property, and based on chemical structure surface activity is not predicted.
Water solubility	1.26 mg/L at 25 °C	ECHA Dissemination site, 2020	
Partition coefficient n-octanol/water	Log Kow 5.903 at 25 °C	ECHA Dissemination site, 2020	

Property	Value	Reference	Comment (e.g. measured or estimated)
Flash point	-	ECHA Dissemination site, 2020	In accordance with section 2 of REACH Annex XI, the study does not need to be conducted as it is technically not feasible due to the substance being a high-melting point solid.
Flammability	Non-flammable	ECHA Dissemination site, 2020	In accordance with section 1 of REACH Annex XI, this study is scientifically unnecessary as the main use of this substance is as a flame retardant, and it is well known that the substance exhibits flame retardant properties. It is therefore unrealistic and unnecessary to conduct an experimental study for this endpoint.
Explosive properties	Non explosive	ECHA Dissemination site, 2020	In accordance with column 2 of REACH Annex VII, the explosive properties study (required in section 7.11) does not need to be conducted as there are no chemical groups associated with explosive properties present in the molecule. The substance is not known to exhibit explosive properties with other materials.
Self-ignition temperature	-	ECHA Dissemination site, 2020	In accordance with section 1 of REACH Annex XI, this study is scientifically unnecessary as the main use of this substance is as a flame retardant, and it is well known that the substance exhibits flame retardant properties. It is therefore unrealistic and unnecessary to conduct an experimental study for this endpoint.
Oxidising properties	No	ECHA Dissemination site, 2020	In accordance with column 2 of REACH Annex VII, the oxidising properties study (required in section 7.13) does not need to be conducted as the substance is predicted to be incapable of reacting exothermically with combustible materials on the basis of the chemical structure. The substance is not known to exhibit oxidising effects
Granulometry	Mass Median Aerodynamic Diameter (MMAD) = ca. 42 µm.	ECHA Dissemination site, 2020	TBBPA is potentially inhalable, but not respirable.

Property	Value	Reference	Comment (e.g. measured or estimated)
Stability in organic solvents and identity of relevant degradation products	-	ECHA Dissemination site, 2020	In accordance with column 1 of REACH Annex IX, the stability in organic solvents study (required in section 7.15) does not need to be conducted as the stability of the substance in organic solvents is not considered to be critical.
Dissociation constant	pKa at 20°C: 9.4	ECHA Dissemination site, 2020	
Viscosity	-	ECHA Dissemination site, 2020	In accordance with section 2 of REACH Annex XI, the study does not need to be conducted as it is technically not feasible due to the substance being a high-melting point solid.

8 EVALUATION OF PHYSICAL HAZARDS

Not performed for this substance.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
Basic toxicokinetics in vivo Rat (Sprague-Dawley), 6 male Human volunteers, 3 male/2 female Rats: single oral gavage dose of 300 mg/kg bw, dose volume of 3.2 ml Humans: single gel capsule orally, 0.1 mg/kg bw Urine and blood concentrations of TBBPA and its metabolites were determined by LC/MS-MS. TBBPA-glucuronide and TBBPAsulfate were identified as metabolites of TBBPA in blood and urine of the human subjects and rats.	In blood, TBBPAglucuronide was detected in all human subjects, whereas TBBPA-sulfate was only present in blood from two individuals. Maximum plasma concentrations of TBBPA-glucuronide (16 nmol/l) were obtained within 4 h after administration. In two individuals where TBBPA-sulfate was present in blood, maximum concentrations were obtained at the 4-h sampling point; the concentrations rapidly declined to reach the limit of detection (LOD) after 8 h. Parent TBBPA was not present in detectable concentrations in any of the human plasma samples. TBBPA-glucuronide was slowly eliminated in urine to reach the LOD 124 h after administration. In rats, TBBPA-glucuronide and TBBPA-sulfate were also the major metabolites of TBBPA present in blood; in addition, a diglucuronide of TBBPA, a mixed glucuronide-sulfate conjugate of TBBPA, tribromobisphenol A, and the glucuronide of tribromobisphenol A were also present in low concentrations.	Registrant reliability index: 1 (reliable without restriction) Not GLP or specific testing guidelines. Registrant, key study	Schauer et al., 2006

Method	Results	Remarks	Reference
	TBBPA plasma concentrations peaked at 103 mmol/l 3 h after administration and thereafter declined with a half-life of 13 h; maximal concentrations of TBBPAglucuronide (25 mmol/l) were also observed 3 h after administration. Peak plasma concentrations of TBBPA-sulfate (694 mmol/l) were reached within 6 h after administration. The obtained results suggest absorption of TBBPA from the gastrointestinal tract and rapid metabolism of the absorbed TBBPA by conjugation resulting in a low systemic bioavailability of TBBPA. The results show that oral exposure of both humans and rodents to TBBPA results in low blood levels of TBBPA and its metabolites, and only a minor part of the given dose of TBBPA is excreted in urine due to the high molecular weight of TBBPA metabolites.		
Basic toxicokinetics in vivo Rat (Fischer-344), 4 male for each oral dose, 9 for iv dose Rats: oral (2, 20, 200 mg/kg) /iv (20 mg/kg) The effect of multiple doses and route of administraiton was investigated in rats using 14C-TBBPA.	The percent of dose eliminated as total radioactivity in feces at 72 h following three different single oral doses (2, 20, or 200 mg/kg) of 14C-TBBPA was 90% or greater for all doses. Most of the dose was eliminated in the first 24 h. At 72 h after administration of the highest dose, the amounts of 14C found in the tissues were minimal (0.2–0.9%). With repeated daily oral doses (20 mg/kg) for 5 or 10 days, the cumulative percent dose eliminated in the feces was 85.1 ± 2.8 and 97.9 ± 1.1, respectively. In all studies radioactivity recovered in urine was minimal, <2%. Repeated dosing did not lead to retention in tissues. Following iv administration, feces was also the major route of elimination. It is extensively extracted and metabolized by the liver and the metabolites (glucuronides) exported into the bile. About 50% of an oral dose (20 mg/kg) was found in the bile within 2 h. This extensive extraction and metabolism by the liver greatly limits exposure of internal tissues to TBBPA following oral exposures.	Registrant reliability index: 2 (reliable with restriction) Not GLP or specific testing guidelines. Registrant, key study	Kuester et al., 2007
Basic toxicokinetics in vivo Rat (albino Sprague-Dawley) 10 females (only); divided into three groups of 2	Approximately 95% of the administered 14C-activity was recovered in the feces within 72 hr. Less than 1.1% of the radioactivity was recovered in the urine within the same timeframe.	Registrant reliability index: 2 (reliable with restriction)	Unnamed, Study report 1979

Method	Results	Remarks	Reference
(sacrificed at 8hr, 24hr, and 72hr) and one group of four (used for blood monitoring) Single dose, equivalent to 5 mg/kg Absorption, distribution, and excretion of TBBPA were studied in rats following administration as a single oral dose (via corn oil).	14C-TBBPA was rapidly eliminated in the feces after oral dosing to the rat. Low bioaccumulation potential based on study results.	Not GLP or specific testing guidelines. Registrant, supporting study	
Skin absorption in vitro method Human skin (female), 10 samples Single dose applied to skin surface, with vehicle evaporation and observations for 24hours post-application. Nominal doses: 1.9 mg/cm2 Actual doses: 2.0 mg/cm2 Dose volume: 6.4 uL	in vitro skin permeability study in human (split thickness) skin of 14C-TBBPA: < 1% of dose absorbed. The stratum corneum was an efficient barrier to [14C]-TBBPA penetration.	Registrant reliability index: 1 (reliable without restriction) OECD Guideline 428 GLP compliant Registrant, key study	Unnamed, Study report 2005

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

There are extensive data available for TBBPA, which have been reviewed, inter alia, in the EU risk assessment: United Kingdom (TBBPA) (2008) (EU RAR TBBPA, 2008). Also NTP and IARC have published reports on TBBPA lately (2014, 2018).

In vivo studies of TBBPA's absorption, distribution, metabolism and elimination have been conducted in humans and rats. An in vitro study of TBBPA's potential for dermal absorption (using human skin) has also been performed. The in vivo studies indicate rapid absorption from the gastrointestinal tract with rapid metabolism to conjugates. The primary route of elimination is in the feces. The in vitro dermal absorption study indicated <1% of a dermal dose would be absorbed. Estimated half-lives are ~2 days and ~0.5 day in humans and rats, respectively.

Absorption:

TBBPA is rapidly absorbed by oral route in rats and humans. TBBPA was readily absorbed after oral administration of [14C]-labelled doses in male F-344 rats (Kuester et al., 2007). Studies with other strains and females indicated minimal sex and strain differences (Hakk et al., 2000; Knudsen et al., 2014). TBBPA was absorbed and metabolized rapidly in healthy human volunteers receiving a single oral dose of 0.1 mg/kg (Schauer et al., 2006).

In an *in vitro* skin permeability study in humans < 1% of dose was absorbed. The stratum corneum was an efficient barrier to [14C]-TBBPA penetration (Unnamed, 2005/EU RAR TBBPA, 2008).

TBBPA is a crystalline particle/powder with a moderately high molecular weight, low water solubility, and moderately high lipophilicity (Log P). Only approximately 4% of the TBBPA-particles

are <15 µm in diameter. Thus, only a minimal quantity of the particles present in TBBPA dust (<4%) are respirable (<10µm in diameter) and can be absorbed from the lung into the systemic circulation following inhalation exposure (EU RAR TBBPA, 2008).

Distribution:

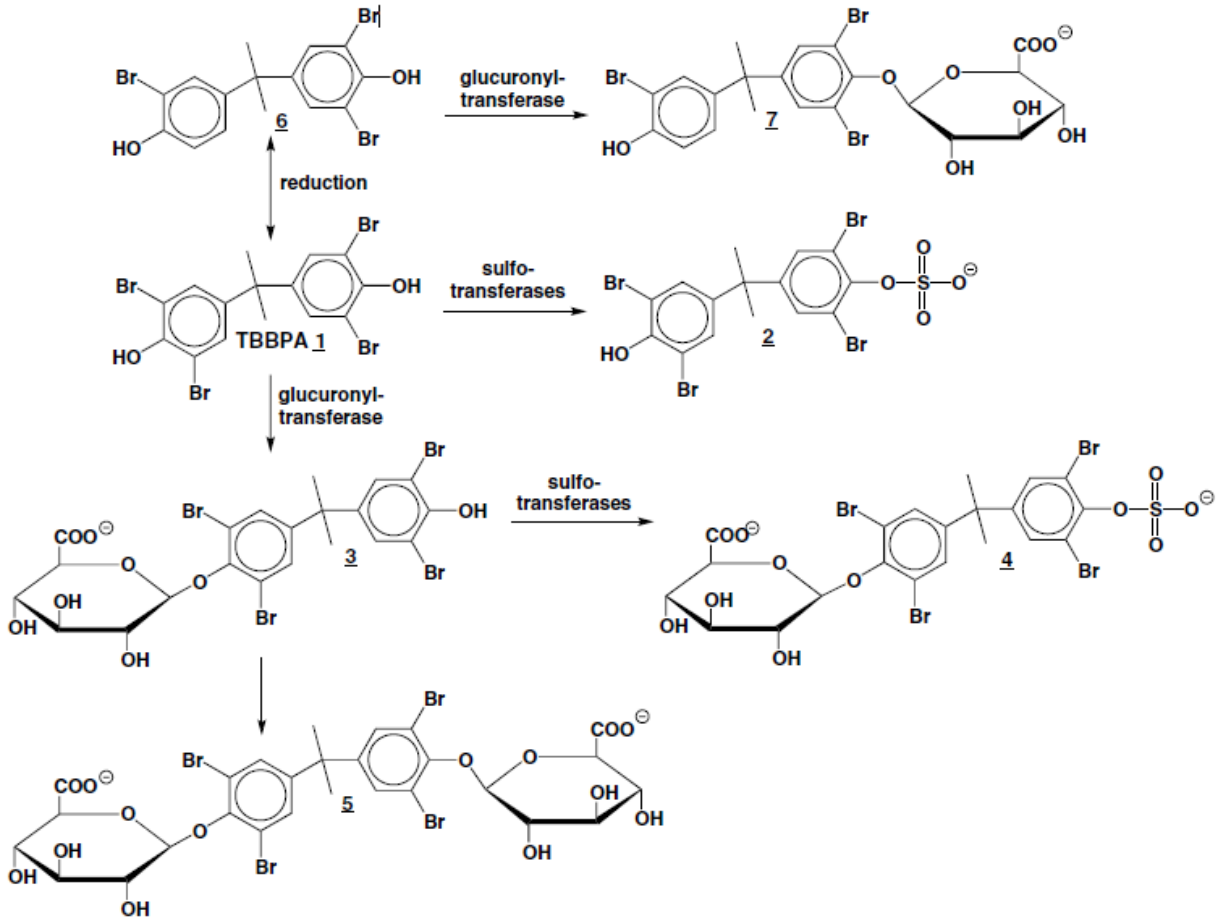
Maximum plasma concentrations of TBBPA-conjugates in humans were obtained within 4 h of dosing and rapidly declined to reach the limit of detection (LOD) after 8 h. Parent TBBPA molecule was not present in detectable concentrations in any of the human plasma samples. TBBPA plasma concentrations in rats peaked at 103 µmol/l 3 h after administration and thereafter declined with a half-life of 13 h; maximal concentrations of TBBPA-glucuronide (25 µmol/l) were also observed 3 h after administration. Peak plasma concentrations of TBBPA-sulfate (694 µmol/l) were reached within 6 h after administration. The results indicate rapid metabolism of the absorbed TBBPA by conjugation resulting in a low systemic bioavailability of TBBPA (Schauer et al., 2006).

The observed half-life of approximately 2 days of TBBPA in humans (Schauer et al., 2006, Hagmar et al., 2000, Sjodin et al., 2003). Elimination half-lives of TBBPA in experimental animals (~ 0.5 day) and humans do not differ considerably (EFSA 2011).

Metabolism:

TBBPA undergoes extensive first-pass metabolism in the gastrointestinal tract and the liver in rat in vivo to form conjugates (Kuester et al. 2007; Schauer et al. 2006). The major metabolic pathways for TBBPA are conjugation with either glucuronic acid or sulfate (see figure 1). Conjugates are also major metabolites of TBBPA formed in rat hepatocytes in vitro (Nakagawa et al. 2007) and Xenopus laevis, tadpoles, in vivo (Fini et al. 2012).

Figure 1: Biotransformation of TBBPA 1 in mammals. 2, TBBPA-sulfate; 3, TBBPA-glucuronide; 4, TBBPA-glucuronide/sulfate; 5, TBBPAdiglucuronide; 6, tribromobisphenol A; 7, tribromobisphenol A-glucuronide:



After a single oral dose, sulfate and glucuronide conjugates were identified as metabolites in blood and urine of human volunteers and rats. In blood, TBBPA-glucuronide was detected in all human subjects, whereas TBBPA-sulfate was only present in blood from two individuals. In rats, TBBPA-glucuronide and TBBPA-sulfate were also the major metabolites of TBBPA present in blood; in addition, a diglucuronide of TBBPA, a mixed glucuronide-sulfate conjugate of TBBPA, tribromobisphenol A, and the glucuronide of tribromobisphenol A were also present in low concentrations (Schauer et al., 2006).

The comparative in vitro metabolism of TBBPA was studied in rat and human. TBBPA is metabolised into the corresponding glucuronide (liver S9 fractions) and several other metabolites produced by cytochrome P450 dependent pathways (liver microsomes and liver S9 fractions). No major qualitative differences were observed between rat and human. TBBPA undergoes an oxidative cleavage near the central carbon of the molecule, that leads to the production of hydroxylated dibromo-phenol, hydroxylated dibromo-isopropyl-phenol and glutathione conjugated dibromo-isopropyl-phenol. The main metabolites of tetrabromo-bisphenol A are two molecules of lower polarity than the parent compound, characterised as a hexa-brominated compound with three aromatic rings and a hepta-brominated dimer-like compound, respectively. Both structures, as well as the lower molecular weight metabolites resulting from the breakdown of the molecule, suggest the occurrence of chemically reactive intermediates formed following a first step oxidation of TBBPA (Zalko et al., 2006).

Elimination:

The percent of dose eliminated as total radioactivity in feces at 72 h following three different single oral doses (2, 20, or 200 mg/kg) of 14C-TBBPA was 90% or greater for all doses. Most of the dose was eliminated in the first 24 h. At 72 h after administration of the highest dose, the amounts of 14C found in the tissues were minimal (0.2–0.9%). About 50% of an oral dose (20 mg/kg) was found in the bile within 2 h (Knudsen et al., 2014).

With repeated daily oral doses (20 mg/kg) for 5 or 10 days, the cumulative percent dose eliminated in the feces was 85.1 ± 2.8 and 97.9 ± 1.1, respectively. In all studies radioactivity recovered in urine was minimal, <2%. Repeated dosing did not lead to retention in tissues. Following iv administration, feces was also the major route of elimination (Kuester et al., 2007).

Systemic bioavailability of TBBPA is low (F < 0.05) due to extensive hepatic first pass biotransformation to glucuronides and sulfates, which are predominantly excreted with bile from the liver due to their high molecular weight. A delayed elimination was only observed after oral administration of a single dose of 1000 mg/kg bw, apparently due to saturation of conjugation reactions (Knudsen et al., 2014). At lower doses, over 95% of orally administered TBBPA is excreted, partially as parent compound and in the form of metabolites in feces within 72 hr after a single dose with associated little tissue retention or bioaccumulation (Colnot et al., 2014; Knudsen et al., 2014; Kuester et al., 2007).

Detection in human serum and milk:

TBBPA has been detected in the serum and milk, as a result of environmental or occupational exposure. It has been detected in milk (0.06 - 37.34 ng/g lipid weight) in surveys of the general population conducted in France and Norway and in the serum (0.15-1.8 ng/g lipids) of the general population in France and Norway, as well as from exposed workers (0.34-3.8 ng/g lipid weight) in Norway and Sweden (Cariou et al., 2008; Thomsen et al., 2001; Thomsen et al., 2002a; Thomsen et al., 2002b; Hagmar et al., 2000).

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Not performed for this substance.

10.2 Acute toxicity - dermal route

Not performed for this substance.

10.3 Acute toxicity - inhalation route

Not performed for this substance.

10.4 Skin corrosion/irritation

Not performed for this substance.

10.5 Serious eye damage/eye irritation

Not performed for this substance.

10.6 Respiratory sensitisation

Not performed for this substance.

10.7 Skin sensitisation

Not performed for this substance.

10.8 Germ cell mutagenicity

Table 9: Summary table of mutagenicity/genotoxicity tests in vitro

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
Bacterial Reverse Mutation Assay OECD TG 471	TBBPA the same test substance as used in the 2-year NTP studies, i.e. purity > 99% Test concentrations: 0, 100, 333, 1000, 3333 and 10000 µg/plate	Bacterial reverse mutation test in Salmonella typhimurium strains TA98 and TA100 and in Escherichia coli strain WP2 uvrA/pKM101 With and without meatobolic activation with 10% hamster S9	No mutagenicity detected in Salmonella strains or E. Colis strains, with or without metabolic activation from rat liver S9. Slight toxicity and precipitate on plate was only observed at 1000 and 3000 µg/plate in two parallels in TA100 without S9.	NTP (2014)
Bacterial Reverse Mutation Assay Similar to OECD TG 471 Cytotoxicity: N/A	TBBPA purity: N/A Test concentrations: 0, 50, 100, 250, 500, 1000, 6000 The highest dose was limited by the experimental design to 6000 µg/plate	Bacterial mutagenicity test in Salmonella typhimurium TA100, TA1535, TA1537 and TA98 With and without Aroclor 1254-induced rat and hamster metabolic activation systems (Also tested in yeast cells, but not reported here as considered not relevant since the deletion of TG 480 by OECD in 2014)	Negative. No evidence of mutagenicity with or without metabolic activation.	Mortelmans et al. (1986)
Bacterial Reverse Mutation Assay Similar to OECD TG 471	TBBPA purity: N/A Test concentrations: 0, 5, 10, 50, 100, 500 and 1000 µg/plate. Toxicity observed at	Bacterial reverse mutation test in Salmonella typhimurium TA92, TA98, TA100, TA1535, TA1537 and TA1538 With and without metabolic activation	No increase in the number of revertant colonies. Cytotoxicity at levels higher than the tested concentrations.	DOW Chemical Company (1985), reported in RAR TBBPA (2008)

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
	higher concentrartions			
Bacterial Reverse Mutation Assay Similar to OECD TG 471 Reliability score 2 (by registrant)	TBBPA purity: N/A 0.1, 1, 19, 100 and 500 µg/plate	Bacterial reverse mutation test in Salmonella typhimurium TA 92, TA98, TA100, TA1535, TA1537 and TA1538 With and without metabolic activation	No mutagenic response with or without metabolic activation. Evidence of some chemically-induced effects at highest dose tested.	Velsicol Chemical Company (1977), reported in EU RAR TBBPA (2008) Supporting study 5 in the REACH registration, Reliability indicated in the REACH registration: 2 (reliable with restrictions)
Bacterial Reverse Mutation Assay Similar to OECD TG 471 Reliability score 2 (by registrant)	TBBPA purity: N/A Test concentrations: 1, 10, 100 µg/plate	Bacterial reverse mutation test in Salmonella typhimurium TA98, TA100, TA1535 and TA1537 Study was carried out with and without metabolic activation, but no details about this is given	Negative with and without metabolic activation. No cytotoxicity observed.	Israel Institute for Biological Research (1978), reported in EU RAR TBBPA (2008) Key study 1 in the REACH registration, Reliability indicated in the REACH registration: 2 (reliable with restrictions).
Bacterial Reverse Mutation Assay Similar to OECD TG 471 Reliability score 2 (by registrant)	TBBPA purity: Test concentrations: 0.25, 0.5, 5 and 50 µg/plate	Bacterial reverse mutation test in Salmonella typhimurium TA92, TA98, TA100, TA1535, TA1537 and TA1538 With and without metabolic activation	Negative with and without metabolic activation. Evidence of chemically-induced physiological effects at highest dose.	Litton Bionetics Inc. (1976), reported in EU RAR TBBPA (2008) Supporting study 4 in the REACH registration, Reliability indicated in

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
				the REACH registration: 2 (reliable with restrictions).
Bacterial Reverse Mutation Assay Similar to OECD TG 471 Reliability score 2 (by registrant)	TBBPA purity: N/A Test concentrations: first study: 0.005, 0.015, 0.05, 0.15 and 0.5 mg/plate second study: 0.001, 0.003, 0.01, 0.3, 0.1 mg/plate	Bacterial reverse mutation test in Salmonella typhimurium TA98, TA100, TA1535, TA1537 and TA1538 with and without metabolic activation	No significant increase in the number of revertant colonies. Cytotoxicity was apparent at the higher concentrations	Ethyl Corporation (1981), reported in EU RAR TBBPA (2008) Key study 2 in the REACH registration, Reliability indicated in the REACH registration: 2 (reliable with restrictions
In vitro mammalian chromosome aberration test Study perfomed equivalent or similar to OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test) Reliability score 1 (by registrant)	TBBPA purity: 98.91% Test concentrations: Doses in main study: 0, 6.25, 25, 100 µg/ml without metabolic activation, and 0, 3.125, 12.5, and 50 µg/ml with metabolic activation.	In vitro mammalian chromosome aberration test in human peripheral blood lymphocytes with and without Aroclor-induced S9-activation system	At no concentration of TBBPA was the percentage of metaphases with structural and numerical aberrations statistically significantly greater than that of the solvent control. Cytotoxic at doses greater than or equal to 150 ug/ml.	BioReliance (2001), reported in EU RAR TBBPA (2008) Key study 3 in the REACH registration, Reliability indicated in the REACH registration: 1 (reliable without restriction).
In Vitro mammalian cell gene mutation tests using the hprt and xprt genes Study assumingly perfomed equivalent or similar to	TBBPA purity: N/A Test concentrations: Dose levels were 0, 5, 10, 20, 30, and 40 µg/ml in DMSO (final concentration 0.2%) in the	Intragenic Sp5/V79 and SPD8 recombination assays in mammalian cells (Chinese hamster cells)	TBBPA did not elicit an increase in the number of revertant colonies in either the SPD8 or the Sp5 assay at doses producing some toxicity (30-50% growth inhibition). Cloning efficiency and growth inhibition were assessed as a measure of cytotoxicity.	Helleday et al.(1999), reported in EU RAR TBBPA (2008)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
OECD Guideline 476	SPD8 assay and 0, 10, 20, 40, 70 µg/ml in DMSO (final concentration 0.2%) in the Sp5 assay. At 70 µg/ml, precipitation of the test substance was observed.			

Table10: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo

Method, guideline, deviations if any	Test substance	Relevant information about the study (as applicable)	Observations	Reference
Mouse peripheral blood micronucleus test Study assumingly perfomed equivalent or similar to OECD TG 474	TBBPA purity > 99% Test doses 0, 10, 50, 100, 500, 1000 mg/kg, 5 days per week for 14 weeks	Mouse peripheral blood micronucleus test. Male or female B6C3F1/N mice: At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice.	No increases in micronucleatedNCEs were observed in male or female B6C3F1/N mice following 3 months of administration og TBBPA by gavage. No effect on micronucleated NCEs was oberserved. No significant changes in the percentage of circulating polychromatic (immature) erythrocytes (PCEs) were observed in dosed mice, suggesting that tetrabromobisphenol A did not induce genotoxicity or other bone marrow toxicity over the dose range tested.	NTP (2014)

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

TBBPA was not mutagenic in bacteria reverse mutation tests in Salmonella typhimurium strains TA92, TA98, TA100, TA1535, or TA1537, TA1538 or in E. coli strain WP2 uvrA/pKM101, with or without exogenous metabolic activation. TBBPA was not mutagenic *in vivo* in the mammalian erythrocyte micronucleus test. No increases in micronucleated normochromatic erythro-cytes were observed in male or female B6C3F1/N mice following 3 months of administration of TBBPA by oral gavage; no significant changes in the percentage of circulating polychromatic erythrocyteswere observed in dosed mice, suggesting that TBBPA did not induce bone marrow toxicity over the dose range tested (NTP, 2014; EU RAR TBBPA, 2008). TBBPA did not exhibit the potential to induce structural or numerical chromosomal aberrations in a *in vitro* mammalian chromosome aberration test in human peripheral blood lymphocytes (BioReliance, 2001 reported in EU RAR TBBPA (2008)).

According to the RAR (EU RAR TBBPA (2008)), the Ames tests reported in the EU RAR were largely compatible with current regulatory guidelines. Also the chromosomal aberration study on human peripheral lymphocytes and the unconventional *in vitro* recombination assays were well-conducted, according to the EU RAR. All tests were negative.

The mammalian erythrocyte micronucleus test (OECD TG 474) is restricted to effects in bone marrow detected in either bone marrow *per se* or peripheral blood due to lack of validation of tests applied to other tissues. This restrict the usefulness of the micronucleus test for detection of effects in other target organs (OECD, 2017).

The dossier submitter notes NTP´s reasoning that negative results in the assays are not good predictors of noncarcinogenicity, even if positiv are good predictors of carcinogenicity (NTP, 2014). None of the available studies indicate that TBBPA is mutagenic or genotoxic in any way.

10.8.2 Comparison with the CLP criteria

All test results were negative, so no CLP criterias for classification is fulfilled.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

As all test results were negative, no classification is proposed for germ cell mutagenicity. The DS notes that negative results in bacterial mutagenicity assays and rodent micronucleus tests are not good predictors of noncarcinogenicity (Tennant et al., 1987; Zeiger, 1998; Witt et al., 2000).

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 (± S9) second study: 0.001, 0.003, 0.01, 0.3, 0.1 mg/plate (± S9)	Negative in both studies. Cytotoxicity was apparent at the higher concentrations	Ethyl Corporation (1981)*
***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.

Supplemental information - In depth analyses by RAC

RAC takes note of the recent Priority Existing Chemical Assessment Report of the Australian Government (NICNAS, 2020) presenting two further Ames test studies, one (Shibuya, 2001) with Salmonella strains TA98, TA100, TA1535, TA1537 and Escherichia coliWP2 uvrA with and without S9 (-S9: up to 156 μg/plate with TA1537, up to 625 μg/plate with TA1535, and over 2500 μg/plate with other strains; +S9: up to 313 μg/plate with TA1537 and up to 5000 μg/plate with other strains), where TBBPA did not cause an increase in mutation frequency in any strain with and without metabolic activation. TBBPA was negative in another briefly

reported study (Brusick, 1976, cited in EHC, 1995) which tested strains TA98, TA100, TA1535, TA1537, TA1538 and *S. cerevisiae* strain D4 with and without S9 up to 50 µg/plate .

10.9 Carcinogenicity

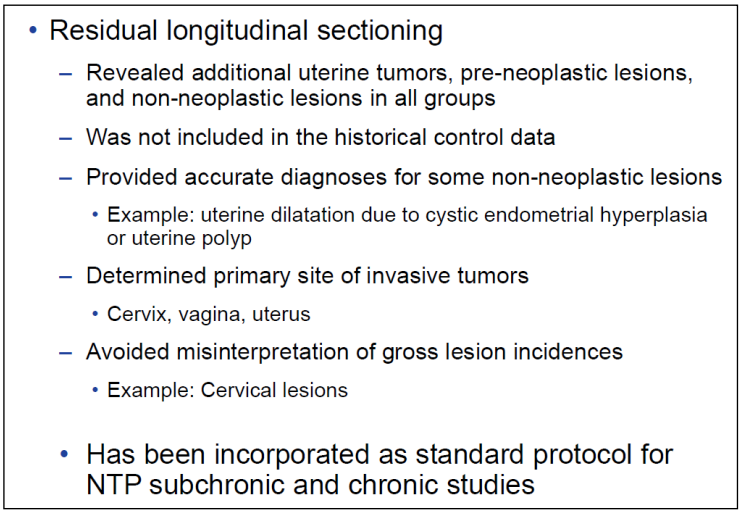
NTP has conducted 2-year studies in rats and mice. The studies complied with GLP and included multiple doses, large number of animals and both male and female animals (NTP, 2014). It was given Reliability score 1 by the REACH registrants. The DS agrees to this score. Historical control data are presented, according to the template, in table 13 ("Compilation of factors to be taken into consideration in the hazard assessment").

Strains of rats

It should be noted that in the 2-years study in rats, NTP (2014) used the strain Wistar Han, in contrast to previous studies where F344 rats were commonly used. This makes the historical control database limited to 150 animals. The acute, subchronic, developmental, reproductive and neurobehavioural studies were conducted with Sprague-Dawley and F344 rat strains. According to Lai et al. (2015)[1] the Wistar Han strain resemble the SD strain, which are known to contain elevated levels of estrogens and a higher estrogen/progesterone ratio.

Transverse and longitudinal sectioning of the uterus in the rat 2y study:

In the NTP rat 2-year study (NTP, 2014), there was findings of uterine tumours in female rats. Originally, transverse sections/evaluation through the cervix of the uterus were made to determine the primary location for adenocarcinomas in the cervix and vagina, and to review all the cervices for hyperplasia/fibrosis. In the following, these are called "original transverse uterine reviews", and consisted of transverse cuts through the uterine horns 0.5 cm from the cervix/body of the uterus. Following this, also logitudinal sections/evaluation were made to examine all remaining parts of the uterus, cervix, and vagina more completely. These are called "residual longitudinal uterine reviews". Residual longitudinal sectioning made it possible to determine the site of origin for grossly identified tumours, and find more neoplastic and non-neoplastic lesions.



Slide from NTP, see https://ntp.niehs.nih.gov/ntp/about_ntp/trpanel/2014/may/presentations/03uteruspathelmore_508.pdf

Studies and results

[1] The co-authors have declared a conflict of interest

Combined results from the two methods were also reported by NTP and named "combined original transverse and residual longitudinal reviews".

Table 11: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
2 years carcinogenicity study in Wistar Han rats OECD TG 451(/453) compliant[2] Reliability score 1 (by DS)	TBBPA purity > 99% Doses were based on the results from the 3-month study. 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 50 male and 50 female in each dose group, and 10 extra male and female animals in the control group and the highest dose group. These 10 males and 10 females were killed and used for interim evaluation after 3 months. This was done to compare with the 3-month endpoints in the F344/NTac rats, see section 10.12. 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.	Statistical significant results are indicated in **bold** text/numbers as significant in trend test (**trend**) or by pairwise comparison: Survival of dosed groups was similar to that of the vehicle control groups. There were no clinical findings related to TBBPA administration in male or female rats. The mean body weight of male rats in the two highest dose groups (500 and 1000 mg/kg bw) were generally at least 10 % lower after 25 weeks than in the control group. This did not occur in females where the body weights were similar to the controls throughout the study. At the 3-month interim evaluation, the absolute and relative thymus weights of 1000 mg/kg bw rats were significantly less than those of the vehicle control groups and the relative liver weights of these dosed groups were significantly greater than those of the vehicle controls. No treatment-related histopathological lesions were observed in 1000 mg/kg bw males or females at 3-months. *Non-neoplastic lesions:* In the original transverse review of the uterus, the incidences of cystic endometrial hyperplasia were increased (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, however the results were not significant (24/50, 31/50, 30/50, 32/50 at 0, 250, 500, and 1000 mg/kg bw). Atypical endometrial hyperplasia was identified in all dose groups (2/50, **13/50, 11/50, 13/50** at 0, 250, 500, and 1000 mg/kg bw) during the residual longitudinal review of the uterus. In the ovary, the incidences of rete ovarii cyst were 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 was 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	NTP (2014) Dunnick et al. (2015)

[2] The NTP conducts its studies in compliance with its laboratory health and safety guidelines and US FDA Good Laboratory Practice Regulations. The OECD TG is not mentioned in the NTP report. However, the studies are assumed to fulfill the OECD TG 451(/453) and considered robust and of high quality by the DS.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		lesion increased with increasing dose. *Neoplastic lesions:* *Female rats:* Increased incidence of cell proliferation at low dose and tumour formation in the uterus at medium and high dose: Clear dose-related carcinogenic effects were observed, as the incidence of uterine tumours in female rats - predominantly adenocarcinoma - was increased in the two highest dose groups (500 mg/kg bw and 1000 mg/kg bw). A continuum was seen from 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**)**, to uterine adenoma and carcinoma: *Adenoma* (original transverse review-0/50, 0/50, 3/50, 4/50, at 0, 250, 500, and 1000 mg/kg bw/d **trend test sign,** not parwise statistics; transverse and longitudinal combined 3/50, 2/50, 4/50, 6/50 at 0, 250, 500, and 1000 mg/kg bw/d); *adenocarcinoma* (original transverse review-3/50, 3/50, 8/50, 9/50, at 0, 250, 500, and 1000 mg/kg bw/d **trend**, not pairwise; 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**); malignant mixed Müllerian tumour (original transverse review- 0/50, 4/50, 0/50, 2/50 at 0, 250, 500, and 1000 mg/kg bw/d); adenoma, adenocarcinoma, or malignant mixed Müllerian tumour[3] (original transverse review-3/50, 7/50, **11/50**, **13/50** at 0, 250, 500, and 1000 mg/kg bw/d**, trend;** residual longitudinal 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**). Uterine tumour metastases were found as carcinomas throughout the body - in the intestine, liver, mesentery, pancreas, glandular stomach, adrenal cortex, lymph nodes, spleen, thymus, skeletal muscle, lung, kidney, and urinary bladder. The metastatic rate for malignant mixed Müllerian tumour was 76% (4/6) and for adenocarcinomas 24% (11/45). Latency in tumour induction: Reduced latency based on days of onset. Historical controls: The historical control incidence adenocarcinoma for 2-year studies was 7/150 (includes one endometrium carcinoma). The NTP historical control	

[3] uncommon tomour type

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		database 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. The historical control data for malignant mixed Müllerian tumours were 0/150 (all routes); and 7/150 (all routes) for all the uterine tumours (combined). For more details on historical control data, see section 10.9.1 below. The tumour types and cancer site (uterus) are relevant for humans. As discussed in Dunnick et al., 2017, endometrial tumours (especially carcinomas) are a common malignancy in women. Uterine cancer is predicted to be one of the three most common cancer type in women by 2030. Survival rate in rats was not affected by the TBBPA administration. Survival rates were 33/50, 28/50, 38/50, 39/50 in male rats and 35/50, 34/50, 29/50, 33/50 in female rats. *Male rats:* In male rats, there was a significant trend in the incidence of testicular interstitial cell adenoma (includes bilateral) (0/50, 0/50, 1/50, 3/50, **trend**). Historical control incidence was 4/150 (all routes), the incidence in the highest dose group exceeded this. At the three months-interim evaluation, the absolute and relative thymus weights of rats in the top dose were significantly less than those of the vehicle control groups. The relative liver weight in the dosed groups were significantly greater than in the control groups. Food consumption: N/A	
2 years carcinogenicity study in B6C3F1/N mice OECD TG 451(/453) compliant Reliability score 1 (by DS)	TBBPA purity > 99% Doses were based on the results from the 3-month study Doses: 0, 250, 500, 1000 mg/kg bw/d by oral gavage in corn oil, 5 days per week for up to 105 weeks 50 male and 50 female in each dose group	There was no treatment-related tumourigenic effects in female mice. Due to early mortality, tumour incidence data in the 1000 mg/kg bw group is not presented. Statistical significant results are indicated in **bold** text/numbers as significant in trend test (**trend**) or by pairwise comparison: Survival in the top dose group 1000 mg/kg bw was significantly less than that of the vehicle control groups. Survival rate was 33/50, 26/50, 39/50, **12/50**, at 0, 250, 500, 1000 mg/kg bw/d in male mice (in control, low dose, medium and high dose, respectively), and 40/50, 31/50, 36/50, **4/50**, at 0, 250, 500, 1000 mg/kg bw/d in female mice. Increased mortality was seen in male and female mice 6 months into the study and was possibly due to gastrointenstinal toxicity. Forestomach toxicity was evident and dose-related in male and female mice as ulcers, inflammation and/or hyperplasia.	NTP (2014) Dunnick et al. (2015)

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		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. Food consumption: N/A *Non-neoplastic lesions:* In the liver, the incidences of clear cell focus in medium dose 500 mg/kg bw males and of eosinophilic focus in the low dose 250 and medium dose 500 mg/kg bw males were statistically significantly increased; the incidence of mixed cell focus in the liver was increased in 500 mg/kg bw males, though not statistically significantly. In the kidney, incidences of renal tubule cytoplasmic alteration were significantly increased in all dosed groups of males and the severities increased with increasing dose; incidences of nephropathy in the 250 and 500 mg/kg bw groups were significantly decreased. In the forestomach, the incidences of ulcer, mononuclear cell cellular infiltration, inflammation, and epithelium hyperplasia were significantly increased in 500 and 1000 mg/kg bw males and all dosed groups of females. *Neoplastic lesions:* TBBPA showed evidence of liver and colon tumours in B6C3F1 male mice: The incidence of hepatocellular adenoma, multiple, was increased in the medium dose group (12/50, 20/50, **28/50**, at 0, 250, and 500 mg/kg bw/d) in male mice. The incidence of hepatocellular adenoma (includes multiple) was not increased (32/50, 33/50, 38/50, at 0, 250, and 500 mg/kg bw/d). There was a **treatment related increase** in the incidence of hepatoblastoma (2/50, **11/50**, 8/50, at 0, 250, and 500 mg/kg bw/d), hepatocellular carcinoma or hepatoblastoma combined (12/50, **24/50**, 20/50, at 0, 250, and 500 mg/kg bw/d, historical control range 24-48%), hemangiosarcoma (in all organs) (1/50, 5/50, **8/50**, at 0, 250, and 500 mg/kg bw/d, **trend**), and large intestine tumours in male mice (0/50, 0/50, **3/50**, at 0, 250, and 500 mg/kg bw/d, **trend**). The incidence of hepatolcellular carcinoma was not significantly increased in male mice (11/50, 15/50, 17/50, at 0, 250, and 500 mg/kg bw/d), and within historical control range of 22-44%. The incidence of hepatoblastoma in male mice exceeded the *historical control* ranges (0-12%) for corn oil gavage studies (and all routes of administration) in male B6C3F1/N mice. For more details on historical control data, see see section 10.9.1 below.	

For an easier overview of the uterine tumours in rats, see the following table:

Table 12: Neoplasms of the uterus in female Wistar Han rats in the 2 year gavage study:

Tumor	Control	250 mg/kg	500 mg/kg	1000 mg/kg
Original transverse review				
Adenoma, Adenocarcinoma, or Malignant Mixed Müllerian Tumor	3**	7	11*	13**
Residual longitudinal review				
Adenoma, Adenocarcinoma, or Malignant Mixed Müllerian Tumor	6**	10	16**	16**
Atypical hyperplasia	2	13**	11**	13**
Combined original transverse and residual longitudinal reviews				
Adenoma, Adenocarcinoma, or Malignant Mixed Müllerian Tumor	6**	11	16**	19**
Atypical endometrial hyperplasia	2	13**	11**	13**

* Positive trend test or significantly different ($p \leq .05$) from the control group by Poly 3 test

** Positive trend test or significantly different ($p \leq .01$) from the control group by Poly 3 test

In the coloumn with control data, asterisks indicate statistical significance in the incidences associated with the trend test. In the coloumns with the dosed groups, asterisks indicate statistical significant incidences by pairwise comparisons between the vehicle controls and that dosed group (Poly-3 test)
A more detailed table is given in annex I, 3.9.1.1.

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Dosing with TBBPA resulted in a dose-response increased incidence (positive trend) of uterine tumours in female rats, stat.sign. in the medium and high dose groups at 500 and 1000 mg/kg bw/d and of liver tumours of male mice in all dose groups (lowest dose 250 mg/kg bw/d) (NTP, 2014). The predominant tumour type in rats was uterine adenocarcinoma, which is also the predominant uterine tumour type in humans. The occurence of testis tumours in male rats and large intestine tumours and hemangiosarcoma in male mice was possibly related to dosing with TBBPA. NTP considered the findings in uterine tumours in female rats in the 2-year study with TBBPA as clear evidence for carcinogenic activity. Findings of testicular interstital cell adenoma in rats were considered as equivocal evidence for carcinogenic activity.

Regarding the neoplastic findings in liver in male mice: Hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma are considered to represent a biological and morphological continuum (NTP (2014) with reference to Takahashi et al. (2002). Takahashi et al. (2002) reported that altered hepatocellular foci developed first, followed subsequently by hepatocellular adenomas, and then carcinomas). Hepatoblastoma is a very rare and malignant tumour type.

Table 13: Compilation of factors to be taken into consideration in the hazard assessment, see also discussion in section 10.9.2 below

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
Wistar Han female rats	**Control animals:** Uterine adenocarcinoma 3/50[4], and 4/50[5]; malignant mixed Müllerian tumours 0/50[6]; adenoma, adenocarcinoma or malignant mixed Müllerian tumours 3/50[7] and 6/50[8] **Historical control data** (see details in table below): Uterine adenocarcinoma 7/150[9]; malignant mixed Müllerian tumours 0/150[10]	No	Yes, uterine adenocarcinomas and malignant mixed Müllerian tumours	Yes First incidence on days 668 (control), 548 (low dose), 321 (medium dose), 442 (high dose) for Original Transverse and Residual Longitudinal Reviews (Combined)	Not applicable (NA)	No	Oral by gavage	MoA: See section below table Findings are relevant to humans, see text below
Wistar Han male rats	**Control animals:** Intestitial cell adenoma in testis 0/50 **Historical control data:** Interstitial cell adenoma in testis 4/150	No	No	NA	NA	No	Oral by gavage	
B6C3F1/N male mice	**Liver:** **Control animals:** Hepatoblastoma 2/50	Liver, large intestine (only sign trend in large	Yes, hepatoblastoma is a rare malignant liver cancer	No First incidence on days 521 (control),	No, only treatment-related tumourigenic effects in male	Top dose animals not included in the carcinogenicity results	Oral, by gavage	

[4] original transverse examination

[5] original transverse and residual longitudinal reviews, combined

[6] original transverse examination

[7] original transverse examination

[8] original transverse and residual longitudinal reviews, combined

[9] original transverse examination

[10] original transverse examination

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
	Historical control data: Hepatoblastoma 9/250 (gavage), 40/949 (all routes) (see details in table below) **Large intestine:** **Control animals:** **A**denoma or carcinoma: 0/50 **Historical control data:** caecum or colon adenoma or carcinoma: 0/250 (gavage), 4/950 (all routes), (mean ± standard deviation 0.4% ±0.8%), 0-2% **Haemangiosarcoma (all organs):** **Control animals:** 1/50 **Historical control data:** 28/250 (mean ± standard deviation 11.2% ±6,4%), 2-18% (gavage), 92/950 (9.7%±4,5%), 2-18% (all routes)	intestine), hemangiosarcoma (all organs)		535 (low dose), 513 (medium dose)	mice	due to high toxicity and early mortality		
B6C3F1 /N female mice	No treatment-related tumourigenic effects in female mice	-	-	-	-	-	-	-

US National Toxicology Program (NTP)
The NTP used routinely to carry out two trend tests. One assumed that all tumours in dead or moribund animals were „fatal"; the other assumed all the tumours were non-fatal („incidental"). The current approach is that life-table tests or prevalence tests are no longer used. Instead, the poly-3 test with Bieler-Williams variance with a trend test and pair-wise tests with controls is used. Sometimes this test is used with k=1.5 and/or k=6 (source OECD website).

Historical Incidence of uterus neoplasms in control female Wistar Han rats (Copy of Table B3 from NTP, 2014):

TABLE B3
Historical Incidence of Uterus Neoplasms in Control Female Wistar Han Rats[a]

	Adenoma	Adenocarcinoma[b]	Malignant Mixed Müllerian Tumor	Adenoma, Adenocarcinoma or Malignant Mixed Müllerian Tumor[b]
Overall Historical Incidence: All Routes				
Total (%)	0/150	7/150 (4.7%)	0/150	7/150 (4.7%)
Mean ± standard deviation		4.7% ± 2.3%		4.7% ± 2.3%
Range		2%-6%		2%-6%

[a] Data as of June 2013
[b] Includes one endometrium carcinoma

Historical Incidence of liver neoplasms in control male B6C3F1/N Mice (Copy of Table C3a from NTP, 2014):

TABLE C3a
Historical Incidence of Liver Neoplasms in Control Male B6C3F1/N Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatoblastoma	Hepatocellular Carcinoma or Hepatoblastoma
Historical Incidence: Corn Oil Gavage Studies				
Ginkgo biloba extract (March 2005)	31/50	22/50	3/50	24/50
Indole-3-carbinol (April 2007)	26/50	12/50	3/50	15/50
Kava kava extract (August 2004)	27/50	20/50	0/50	20/50
N,*N*-Dimethyl-*p*-toluidine (October 2004)	29/50	22/50	1/50	22/50
Tetrabromobisphenol A (August 2007)	32/50	11/50	2/50	12/50
Total (%)	145/250 (58.0%)	87/250 (34.8%)	9/250 (3.6%)	93/250 (37.2%)
Mean ± standard deviation	58.0% ± 5.1%	34.8% ± 10.9%	3.6% ± 2.6%	37.2% ± 10.0%
Range	52%-64%	22%-44%	0%-6%	24%-48%
Overall Historical Incidence: All Routes				
Total (%)	594/949 (62.6%)	348/949 (36.7%)	40/949 (4.2%)	371/949 (39.1%)
Mean ± standard deviation	62.6% ± 9.1%	36.7% ± 11.4%	4.2% ± 3.5%	39.1% ± 11.6%
Range	48%-78%	22%-56%	0%-12%	22%-54%

[a] Data as of June 2013

10.9.1.1 Mode of action (MoA) for uterine carcinogenesis in female rats and relevance to humans:

According to IARC (2018), based on key characteristics of human carcinogens, there is strong evidence that TBBPA modulates receptor-mediated effects, induces oxidative stress and is immunosuppressive; there is moderate evidence that TBBPA induces chronic inflammation; and there is weak evidence that TBBPA is electrophilic, genotoxic or alters cell proliferation, cell death or nutrient supply.

TBBPA is an endocrine disruptor: In repeated-dose toxicity and reproductive toxicity studies, decreased thyroxine levels and other endocrine effects are seen (see section 10.10 and 10.12). It does not bind to the progesterone or the estrogen receptors in an agonistic or antagonistic significant way (IARC, 2018), but still affects the estrogen homestasis, especially in rats.

TBBPA-induced uterine tumours were seen in rats and not in mice, possibly because of differences between rats and mice as estrogen homeostasis is less affected in mice than in rats due to differences in capacity and/or capability of conjugating enzymes (Dunnick et al., 2015). TBBPA is affecting estrogen homeostasis by competing with estrogen for estrogen glucuronosyltransferases and/or estrogen sulfotransferases.

Uterine carcinogensis is driven at least partly by alterations in the *Tp53* tumour supression gene signalling pathway.TBBPA was not mutagenic in standard assays, but mutations in the *TP53 gene* (exon 5 to 8) was found in the tumours. This could be a direct or more probably an indirect effect of TBBPA, as TBBPA could lead to increased levels of circulating estrogens by competitive inhibition of estrogen conjucation resulting in promotion of pre-existing *TP53*-mutations in the uterus.

The DS agrees with Lai et al. (2015) that TBBPA is expected to exhibit a threshold for adverse effects as the observed thyroxin hormone changes is compensated by the mammalian organism when the changes in thyroid hormones levels are small (thyroid hormones: total triiodothyronine T3, thyroid stimulating hormone TSH, and total thyroxine T4). Thyroid hormones levels are not reported in the 2-year studies in rats and mice, however no thyroid follicular hyperplasia was observed after dosing with TBBPA in rats or mice. In the NTP 90-day study in rats a significant fall in serum total T_4 levels at 500 and 1000 mg/kg bw/d in male and female rats were observed, without any thyroid histologic lesions. A similar fall in T_4 was not seen in the NTP 90-day study in mice (NTP, 2014). A significant decrease in serum T4 was also seen after 5 days dosing with 250 mg/kg bw oral to Wistar Han rats (Sanders et a., 2016). Serum T_4 is a prohormone, and T_3 is the ultimate active hormone. T_4 is metabolized to triiodothyronine (T3) peripherally by deiodination. A fall in T_4 without a fall in T_3 would assumingly not lead to manifestations of overt hypothyroidism. According to Wikoff et al. (2016)[11], the fall in T4 levels in the 90-day studies may be associated with the inhibition of sulfotransferase, as these enzymes are also involved in hormone metabolism.

The Mode of action of TBBPA-induced cancer of the uterus does probably also include other contributing factors, such as the formation of free radicals, and downregulation of gene products implicated in several immunologic pathways in uterine tissue, see table below.

Table 14: Scientific studies and reviews on the possible mode of action for TBBPA induced uterine carcinogenesis in rats

Possible modes of action	**Details/explanations**	**Reviewed or reported[12] by**
Disruption of estrogen homeostasis	TBBPA affecting estrogen homeostasis by competing with estrogen for estrogen glucuronosyltransferases and/or estrogen sulfotransferases.	NTP, 2014 (major hypothesis)

[11] Study funded by the North American Flame Retardant Alliance (NAFRA) of the American Chemistry Council (ACC)

[12] Preferably, reviews are mentioned, single references cited only if not included in review. For single references, please see review.

Possible modes of action	**Details/explanations**	**Reviewed or reported[12] by**
	Binding affinities for TBBPA and estradiol to sulfotransferases are similar. Competition could lead to decreased estrogen excretion, elevated levels of the estrogen in the uterus and increased formation of estrogen-derived reactive species (mutagenic estrogen metabolites) This could lead to increased risk of cancer at the site	Dunnick et al., 2015 Sanders et al., 2016
	TBBPA (via competitive inhibition of estrogen conjugation) could lead to elevated levels of the estrogen in the uterus which could produce uterine tumours by promoting pre-existing mutations in the *Tp53* tumour suppression gene. TBBPA has very low affinity to the estrogen receptors. TBBPA does not induce cytochrome P450 1A/B. Saturation of sulfation conjugation. (see figure below)	Lai et al., 2015 Borghoff et al., 2016
	The plausible molecular initiating event (MIE) in rats is TBBPAs ability to bind to and inhibit sulfotransferases (SULT1E1[13]) leading to increased bioavailability of unconjugated estrogens in uterine tissue.	Wikoff et al., 2016
	TBBPA may disrupt endocrine signalling through direct interaction with endocrine receptors/signalling (or through binding to estradiolsulfotransferase)	NTP, 2014
	TBBPA enhancing estrogen activity by inhibiting hydroxysteroid-dehydrogenase-17β (HSD 17β) *(*only studied in *in vitro assay)* HSD 17β converts active estradiol to less active estrone. Inhibition of HSD 17β results in increased estrogen activity	Dunnick et al., 2015
Disruption of thyroid hormone pathway - (Thyroxin T4, triiodothyronine T3, thyroid stimulating hormone TSH)	Decreased serum thyroxine concentration (T_4, prohormone to T_3) in 3-month study in male and female rats, but not decreased T_3 (hormone) or TSH level. No decrease in mice. T_3 not reduced, and TBBPA did not produce manifestations of overt hypothyroidism (no alterations in thyroid gland histopathology). T_3 pool not depleted. Humans less susceptible than mice to plasma T_4 depletion.	NTP, 2014 Lai et al., 2015
	Significant fall in serum T4 in rats (3-month study in rats). No change in T3 and TSH and no changes in thyriod histopathology.	Osimitz et al., 2016[14]
	No anatomical thyroid abnormalities in any of the rodent investigations reported in this review.	Colnot et al., 2014
Oxidative stress	Glucuronidase may free TBBPA from its conjugated form, thus increasing the potential for free radical formation at target sites.	NTP, 2014 Dunnick et

[13] the major estrogen sulfotransferase

[14] Conflict of interest, the American Chemistry Council's North American Flame Retardant Alliance (NAFRA) funded the study and preparation of the manuscript. Both TGO and AWH serve on NAFRA's Science Advisory Council and receive compensation for doing so. TGO does occasional paid scientific analysis and legislative testimony on behalf of ACC. WD is paid by Science Strategies, LLC, for her time on the project. AWH serves as editor for the Americas for Human & Experimental Toxicology.

Possible modes of action	**Details/explanations**	**Reviewed or reported[12] by**
	Oxidative cleavage of the TBBPA molecule.	al., 2015 (minor hypothesis)
	Induction of oxidative stress by TBBPA is well established by studies in human cells and other experimental systems *in vitro* and *in vivo.* Production of reactive oxygen species (ROS) with involvement if the superoxide anion.	IARC, 2018
Inflammation and immunosuppression	TBBPA is immunosuppressive. It affects human NK cells and activates inflammatory pathways in human placental cells Also clear effects on the immune system in experimental animals *in vivo* and in *in vitro* systems, with production of proinflammatory cytokines and activation of the macrophage COX-2 gene etc.	IARC, 2018
	Activation of the hepatic interferon pathway and metabolic networks in Wistar Han rats (not seen in uterus). These liver changes could affect hormone levels and play a role in liver cancer in mice. Immunomodulatory changes could contribute to carcinogenic processes in the uterus.	Dunnick et al., 2017
Genetic and related effects	Uterine carcinogensis driven at least partly by alterations in the *Tp53* signalling pathway, direct or indirect via a secondary nongenotoxic event. TBBPA is not genotoxic in *in vitro* and *in vivo* assays	NTP, 2014
	Increase in the frequency of Tp53 mutations and increased human growth factor receptor 2 gene expression in the uterine carcinomas from the NTP study from 2014 reexamined by Harvey et al. (2015)	Harvey et al., 2015
	Promotion of pre-existing mutations in the *Tp53* tumour suppression gene	Lai et al., 2015

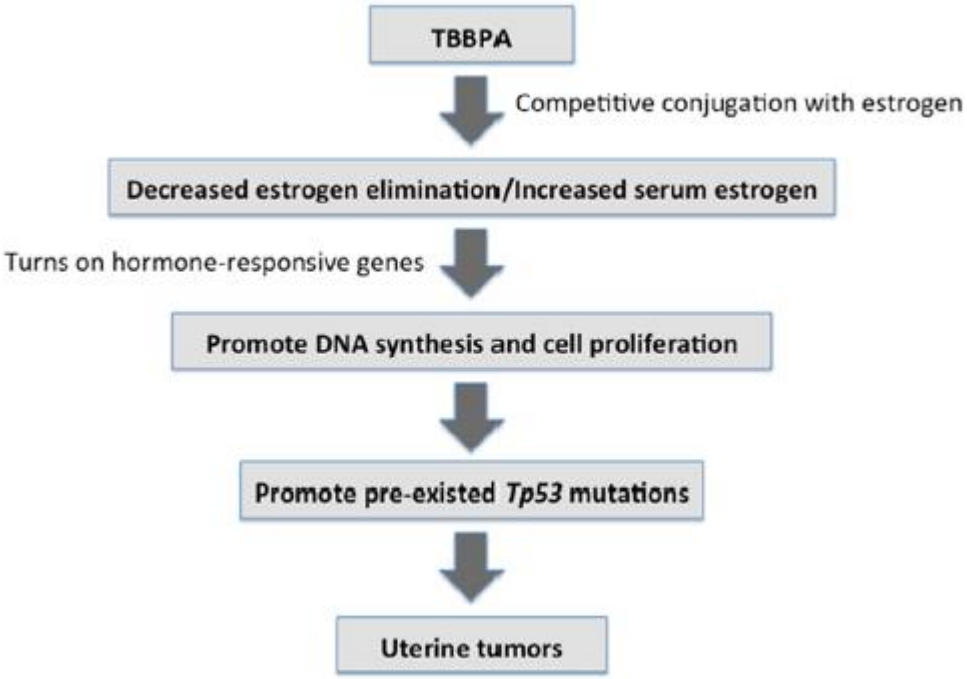


Figure 2: Proposed mechanism/MoA of TBBPA on uterus carcinogenesis in rats (copied from Lai et al., 2015).

In an evaluation of key events in a possible adverse outcome pathway for TBBPA-induced uterine carcinomas in Wistar Han rats, Wikoff et al. (2016) concludes that the plausible molecular initiating event (MIE) in rats is TBBPAs ability to bind to and inhibit sulfotransferases (SULT1E1) leading to increased bioavailability of unconjugated estrogens in uterine tissue and a disruption in estrogen homestasis. A proposed MoA for TBBPA-induced uterine tumours in the context of an AOP is presented in the paper.

The DS agrees with IARC which states that there is strong evidence that TBBPA both modulates receptor-mediated effects, induces oxidative stress and is immunosuppressive (IARC, 2018).

The estrogen/progesterone levels were not measured in the 2-year studies nor in the reproductive, developmental, neurobehavioural study by Cope et al., 2015[15]. In a study designed to test the hypothesis that disruption of estrogen homeostasis was a major MoA for the uterine carcinogenicity, the changes in expression of genes associated with specific pathways of estrogen biosynthesis and metabolism supported the proposed MoA (Sanders et al., 2016). In this study biological changes were assessed in serum, liver, and the proximal and distal 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 bw. In a follow-up study to Sanders et al. (2016) using the same animals to detect additional pathways perturbed by TBBPA, Hall et al. (2017) reported that the mechanism may be related to estrogen mediated immunosuppression, as down-regulating of several genes involved in immune system was observed in uterine tissue sections. In a 28-day oral gavage study in female Wistar Han rats, it was found that dosing of TBBPA up to 1000 mg/kg bw/d (0, 50, 250, 500 and 1000, i.e. identical to doses in the 2-year NTP study in rats) resulted in increased systemic circulation of conjugates and a disruption in the balance of conjugate reflected by a decrease in the TBBPA-S/TBBPA-GA ratio (Borghoff et al., 2016[16]). Concentration of TBBPA and its major conjugates TBBPA-GA and TBBPA-S was measured in liver, plasma and uterus tissue and increased with dose in all three. The metabolism of TBBPA at high doses appreared non-linear. The results suggested a saturation of sulfation conjugation of TBBPA at around 250 mg/kg/d in liver, plasma and uterus. The study demonstrated that sulfation is limited in the liver and in the uterus after dosing with TBBPA.

[15] Study funded by the Brominated Flame Retardant Industry Panel of the American Chemistry Council

[16] Conflict of interest; study funded by the North American Flame Retardant Alliance (NAFRA) Panel of the American Chemistry Council (ACC)

The data suggests that the concentration of TBBPA and conjugates in the uterus is due to the translocation from plasma, as the balance of conjugates was the same in the plasma and the uterus.

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.

TBBPA was tested by the IARC working group (IARC, 2018 pp. 63-64) across the full assay suite of ToxCast and Tox21[17] with data available for 836 assay end-points:

"Overall, tetrabromobisphenol A demonstrated strong cytotoxic effects that may have confounded the results from other end-points. It activated several stress pathways, in particular the oxidative stress pathway. It was also a promiscuous nuclear receptor modulator with higher potency towards PPARγ than other receptors, but also active for steroid hormone receptors and the xenobiotic receptor PXR. In assay end-points not currently mapped to the key characteristics of carcinogens, tetrabromobisphenol A disrupted steroidogenesis in H295R human adrenal corticocarcinoma cells through the upregulation of progesterone and hydroxyprogesterone."

The data from ToxCast/Tox21 contributes to the evidence that TBBPA is not an ER agonist (Wikoff et al., 2016). IARC describes *in vitro* studies with TBBPA in human cells and non-human mammalian cells, for this please see IARC (2018).

The significant increased incidence of mutations in Tp53 gene (exons 5 to 8) in uterine adenocarcinomas from TBBPA dosed animals (10/16, 63%) compared to spontaneous uterine adenocarcinomas (1/9, 11%) may be a result of a direct genotoxic event from TBBPA or the result of a secondary nongenotoxic event. The increased mutagenicity in dosed animals suggest that uterine carcinogenesis in TBBPA dosed animals is at least partly driven by alterations in the Tp53 pathway (NTP, 2014). This pathway is described as relevant in humans (Harvey et al., 2015), even if the type classification of the uterine carcinomas (only type II has Tp53 mutations) in rats is challenged by others (Wikoff et al., 2016).

In addition to significant tumour findings in a dose-response relationship, outside the range of the historical control data, especially for tumours in uterus in female rats and liver in male mice, it should be noted that these tumours types are relevant for humans. Uterine cancer in humans is of the same type as uterine tumours seen in rats. As discussed in Dunnick et al., 2017, endometrial tumours (especially carcinomas) are a common malignancy in women, and uterine cancer is predicted to be one of the three most common cancer type in women by 2030. According to Lai et al. (2015) uterine tumours induced by TBBPA in rats are *qualitatively* applicable to humans by the described MoA, but that it is unlikely that thos MoA is *quantitatively* plausible for humans, especially taking into account the ADME and kinetic factors. In the DS´s view, this argument is relevant for risk assessment, and not for classification. Wikoff et al. (2016) also questions the human relevance of the uterine cancer in rats based on the possible molecular initiating event (TBBPA binding to and inhibit estrogen sulfotransferase) operative at high repeated doses.

10.9.2 Comparison with the CLP criteria

No epidemiological data is available, so Category 1A is not warranted.

Category 1B, i.e. that the substance is presumed to have carcinogenic potential for humans, a classification largely based on animal evidence, is relevant as animal data in rats and mice are available. 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.

Strength of evidence:

In the NTP report, tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. For details on the statistical methods, please see the NTP report (NTP, 2014).

[17] High-throughput screening data generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast™) research programmes of the government of the USA

In female rats, the incidence of adenoma, adenocarcinoma or malignant Müllerian tumour (combined) was statistically significantly (stat.sign.) increased in the two highest dose groups (500 and 1000 mg/kg bw 5 d/w for 105 weeks) in the original transverse review and in the residual longitudinal review, as well as in the original transverse and residual longitudinal review combined.

The incidence of uterine adenocarcinoma was stat.sign. increased in the two highest dose groups in the residual longitudinal review, and in the original transverse and residual longitudinal review combined.

There was a significant trend in the incidence of uterine adenoma, adenocarcinoma, and in the incidence of adenoma, adenocarcinoma or malignant Müllerian tumour (combined) in the original transverse review. In the residual longitudinal review as well as in the original transverse and residual longitudinal review combined, the results were similar except for the adenomas where the trend was not significant.

In male rats there were few carcinogenicity stat.sign. findings, and only a significant trend for increased incidence in testis interstitial cell adenoma was observed.

In male mice, the incidence of liver hepatoblastoma and hepatocellular carcinoma or hepatoblastoma (combined) was stat.sign. increased in the low dose group (250 mg/kg bw 5d/w for 105 w) but not in the 500 mg/kg bw group. There was a finding of stat.sign. increased incidence of haemangiosarcoma in the 500 mg/kg bw group.

There was a significant trend in the incidence of liver adenoma or carcinoma (combined) and in the incidence of haemoangiosarcoma.

All in all there was a causal relationship between the substance and an increased incidence of tumours in female rats and male mice.

Additional considerations:

a) *tumour type and background incidence:* The majority of human uterine tumours are endometrial carcinomas, i.e. the same type of uterine tumours observed in rats after dosing with TBBPA. The incidence in the 2-year rat study exceeds the incidence of these tumours in the historical control database which is of limited magnitude due to a change of rat strain by NTP.

b) *multi-site response:* Not so evident. Only clear evidence of carcinogenicity in uterus: The DS agrees with NTP who concluded that there was 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 hepatoblastoma, and equivocal evidence of carcinogenicity in male rats based on the occurence of testicular adenoma. The increased incidence of large intestine neoplasms and hemangiosarcoma (all organs) may have been related to TBBPA.

c) *progression of lesions to malignancy:* Yes, in addition to the adenocarcinomas of the uterus, uterine tumour metastases were found as carcinomas throughout the body in female rats - in the intestine, liver, mesentery, pancreas, glandular stomach, adrenal cortex, lymph nodes, spleen, thymus, skeletal muscle, lung, kidney, and urinary bladder. The metastatic rate for malignant mixed Müllerian tumour was 76% (4/6) and for adenocarcinomas 24% (11/45). In male mice the evidence of carcinogenicity was not so clear as in female rats, but malignancy was observed as hepatocellular carcinomas and hepatoblastomas. Also hemangiosarcoma seen in some male mice is a rare malignant cancer type.

d) *reduced tumour latency:* Yes, indicated for the uterine tumours, based on days of onset

e) *single or both sexes response:* Not applicable for uterine tumours. Not observed for the tumours in mice, as only male mice was affected by the treatment.

f) *single or several species:* Clear evidence only in female rats.

g) *structural similarity:* TBBPA has little activity as an estrogen receptor agonist or antagonist compared to other bisphenols, e.g. bisphenol A.

h) *comparison of ADME between test animals and humans:* Comparative studies in experimental animals and humans shows that TBBPA was absorbed and metabolised rapidly in healthy volunteers as well as in experimental animals. No accumulation of TBBPA or metabolites found in uterus in female rats. TBBPA was metabolised by i.a. sulfate conjugation in humans and experimental animals, and excreted predominantly via bile. Elimination half life of TBBPA in experimental animals and humans do not differ considerably

i) *confounding effect of excessive toxicity at test doses:* No signs of toxicity in female rats. Due to early mortality, tumour incidence data in the 1000 mg/kg bw male mice group is not presented.

j) *relevance for humans of mode of action:* Yes, TBBPA is affecting estrogen homeostasis by competing with estrogen for estrogen sulfotransferases.

10.9.3 Conclusion on classification and labelling for carcinogenicity

We propose TBBPA to be classified as a Category 1B carcinogen based on conclusive data (carcinogenic in animal studies and relevant mode of action for humans).

TBBPA administered orally by gavage for two years was clearly carcinogenic in female rats resulting in uterine tumours. TBBPA also resulted in liver tumours in male mice. A nongenotoxic mode of action is assumed (threshold carcinogen) relevant to humans. The tumour type is also relevant to humans as the predominant tumour type in rats was uterine adenocarcinoma, which is also the predominant uterine tumour type in humans.

With reference to the strong link between CLP and the IARC classification criteria (CLP guidance 3.6.2.3.1) the DS notes that IARC has classified TBBPA in Group 2A – "probably carcinogenic to humans".

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.

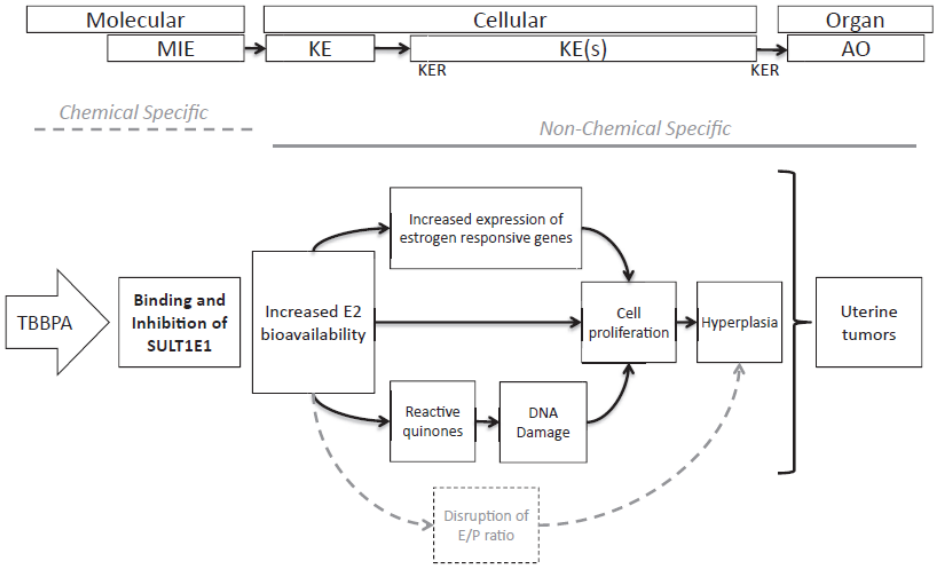
Receptor mediated effects

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)

Disruption of thyroid hormone pathway

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.

Other receptor mediated effects

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	TBBPA purity > 99% Doses: 0, 250, 500, 1000	No mortalities nor clinical findings were found in exposed groups compare to controls.	NTP, 2014 Dunnick *et al*, 2015

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	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.	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 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), 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%),	NTP, 2014 Dunnick *et al*, 2015

		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.

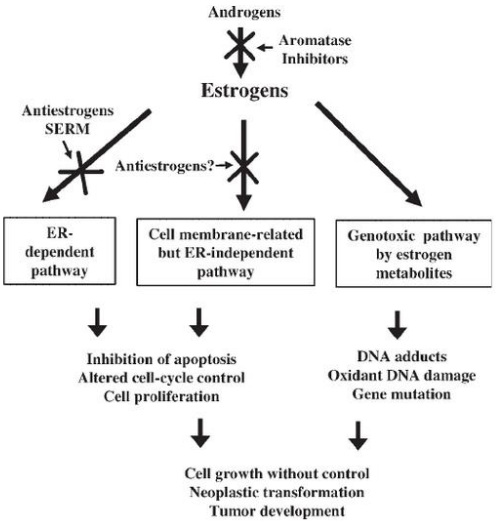
***Table**: Tp53 mutation pattern observed in uterine carcinoma in NTP (2014) rat study on TBBPA*

	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 ≤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	(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.

well-conducted study, ideally conducted under Good Laboratory Practice, can also provide sufficient evidence.		
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.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table15: Summary table of animal studies on adverse effects on sexual function and fertility

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Two Generation Reproduction Toxicity Study with a developmental neurotoxicity component in the F2 generation OECD TG 416, GLP-study Sprague-Dawley rat, male/female. 30 males and 30 females in each dose group For neurobehavioral studies 40 males and 40 females from each dose group in the F2 generation were randomly selected. Neurobeavioral tests were motor activity, learning and mobility (passive avoidance test and water M-maze) and auditory startle habituation. Additional 20 males and 20 females from each dose group from the F2 generation were retained for neuropathologic studies Reliability score 1 (by DS)	TBBPA Purity 98.91% Doses: 0, 10, 100 and 1000 mg/kg bw/day (actual ingested) by oral gavage in corn oil, daily for 36 weeks.	Statistical significant results are in marked in **bold (p<0.05)**. <u>Parental generation</u> 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. <u>F1-generation</u> In the F1 generation there were no general toxicity effects on clinical signs, mortality/viability, sexual maturation, gross pathological and histopathological findings. Lower body weight and body weight gain was observed in F1 males at 1000 mg/kg/day. Lower body weight was obsereved for several weekly intervals and lower weight gain (7%) were observed in the premating period week 1-11. No other effects on body weight and body weight gain was observed. <u>Thyroid hormones:</u> Treatment related thyroid effects were observed in both P and F1 generations. Serum thyroxine (T4) levels were reduced in both sexes in the P and F1-generations. Reduction of serum levels of tri-iodothyronine (T3) was observed in P-generation males. T4-levels: P-males T4-levels were 4.7, 5.08, **3.9** and **3.38** ng/dL for the 0, 10, 100 and 1000 mg/kg/day groups, respectively. P-females T4 levels were 4.23, 3.45, 3.5 and **2.39** ng/dL for the 0, 10, 100 and 1000 mg/kg/day groups, respectively. F1-males T4 levels were 6.29, 5.98, **3.91** and **3.33** ng/dL for the 0, 10, 100 and 1000 mg/kg/day groups, respectively. F1-females T4 levels were 6.00, 4.42, **3.40** and **3.41** ng/dL for the 0, 10, 100 and 1000 mg/kg/day groups, respectively. T3-levels: P-males T3 levels were 102.7, 92.8, 97.5 and **83.2** ng/dL for the 0, 10, 100 and 1000 mg/kg/day groups, respectively <u>Neurobehavioral toxicity (F2 animals)</u>: *Motor activity* test No differences in activity and emotionality at PND 13. At PND 17, females had decrease in horizontal activity in the 15-20 min segment of the test in the 10 mg/kg/day group and in the 20 min period in the 100 mg/kg/day group. At PND 21 there was a	Unnamed, 2002 EU RAR TBBPA, 2008 Cope et al., 2015

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
		significant reduced horizontal activity and distance travelled in the 5.10 min segment and over the 20 min test as a whole in females in the 100 mg/kg/day group compared to controls. At PND 60 males had reductions in horizontal activity in the 0-5 min segment of the test at 100 and 1000 mg/kg/day groups and during the 5-10 min segment in the 1000 mg/kg/day group. No other significant changes were reported Tests on *learning and memory*, *the passive avoidance test* Males exposed to 1000 mg/kg bw/day had a decrease in time spent in light. No differences were detected on day 1 and 3. Females: No differences were detected at any of the concentrations or timepoints. *Water M-maze test:* Males and females had no treatment related effects in the water M-maze test. <u>Neuropathology</u> (F2-animals) *Morphometric measurements:* Decrease of parietal cortex thickness of the 1000 mg/kg/day pups sacrified at PND 11. Thickness (parietal cortex) in males was 1.61, 1.56, 1.49 and **1.23** mm at 0, 10, 100 and 1000 mg/kg, respectively Thickness (parietal cortex) in females was 1.60, 1.46, 1.56, **1.33** mm at 0, 10, 100 and 1000 mg/kg, respectively. There were also reduction in parietal thickness observed at 10 and 100 mg/kg/day groups, but these changes were not significant. No histological changes observed in the parietal cortex. Parietal cortex thickness at PND 60 in the control and 1000 mg/kg/day pups were not different (thickness measured as 2.13 and 2.09 mm in males and 2.10 and 2.06 mm in females in controls and 1000 mg/kg/day groups, respectively).	
One generation reproduction toxicity study for endocrine and immunological endpoints and additional analysis for bone and neurophysiological parameters Similar to OECD TG 415 Rats (Wistar), 10 parental animals/sex/dose Reliability score 2 (by DS)	TBBPA Purity 98% Doses: 0, 3, 10, 30, 100, 300, 1000, 3000 mg TBBPA/kg bw/day Administration: oral, mixed with standard rat feed without soy. Duration: Exposure start P-males at 10 weeks and P-females at 2 weeks	<u>Reproduction effects:</u> There were no effects on reproduction endpoints such as mating success, number of implantation sites and litter size. No change in the duration of the estrus cycle and dustribution of stages during the cycle. No difference in sex ratio in the F1 litters. In F1 female pups it was a decrease in the anogenital distance at PND 7, but not at PND 4 and 21, and a delayed time for vaginal opening. The author uses benchmark doses and their lower 90% confidence interval enabling calculation of a lower 5% confidence interval (BMDL) which was reported to be around the highest concentration. Increased weight of reproductive organs at weaning were reported for male pups (BMDL of 0.5 mg/kg bw/day). During lactation it was a dose dependent decrease in mortality (BMDL of 4.8 mg/kg bw/day) and a decrease in rate of litters with mortality (BMDL of 33 mg/kg bw/day). The first 4-7 weeks it was a decrease in bodyweight around 10% for the F1 animals with a BMDL around the highest dose. <u>Thyroid hormones:</u>	Van der Ven et al. (2008) Lilienthal et al. (2008)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	premating and throughout mating, gestation and lactation. Offspring were fed the same diets as their respective mothers throughout life. Necroscopy carried out at week 14 (±1 week).	There were effects on thyroid levels in the F1 animals. Plasma T4 levels were decreased in males and females (BMDL of 30.8 and 16.1 mg/kg bw/day, repectively). T4 concentrations were 34.3, 33.5, 38.0, 41.2, 27.1, 23.2, 22.2 and 18.4 nmol/L in females and 53.4, 40.7, 45.7, 47.6, 43.0, 31.5, 26.5 and 27.9 nmol/L in male rats for 0, 3, 10, 30, 100, 300, 1000 and 3000 mg/kg bw/day exposure groups, respectively. T3 levels were 0.7, 0.8, 0.8, 0.9, 1.0, 0.9, 1.0 and 1.0 nmol/L in females for 0, 3, 10, 30, 100, 300, 1000 and 3000 mg/kg bw/day exposure groups, respectively. Female rats also had increased levels of plasma T3 (BMDL 2.3 mg/kg bw/day). Food intake: Reduced food intake in P-generation the first two weeks for the animals exposed to high concentrations. Females had reduced food intake the first two weeks after gestation, BMDL was close to the highest concentration. Weight loss was also reported in females until gestation week 3. Similarly, reduced weight gain was reported in females premating and during gestation with BMDLs at 94 and 298 mg/kg bw/day, respectively. Organ weight: Significant increase in liver weight (maximum increase 11.4%) and a dose dependent increase in adult testis weight (BMDL 0.5 mg/kg bw/day) in F1 males. Dose-dependent increase of pituitary weight, also correlated to weights of testis and to BAEP variables (but not to thyroid hormones). There were a correlation between female uterine weight, endometrium thickness and CYP19 activity in the ovary to the increased male gonad weight at PND21 or necropsy. Immunotoxic and hematological effect: Increase in total spleen cell counts. Neurobehavioral effects: Effects in both sexes on increase of hearing latencies at low frequencies and the increased hearing threshold reported in females were statistically supported by correlations between these parameters. The BMDL of hearing latencies and for decrease in T4 levels were also in the same range. There were effects on auditory responses measured by BAEPs. Exposure to TBBPA gave a dose-related elevation of BAEP threshold in female offspring. Increases were detected in the low frequency range up to 4kHz. The difference measured 13 dB at 0.5kHz in the 3000 mg/kg bw/day group compared to control. Significant fits to dose-response curves were obtained at 0.5 and 2 kHz with a BMDL value of 0.9 mg/kg bw/day. There were no effects in males. Slight exposure-related effects were seen on latency of wave II, but wave II latency was not significantly altered after click stimulation in both sexes. Prolonged wave IV latencies in the low frequency range was observed in exposed animals and was somewhat more pronounced in males. The prolongations measured 0.56 and 0.70	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		ms at 0.5 kHz in females and males, respectively when the highest exposure level was compared to controls (Benchmark analysis of wave IV latencies showed significant effects of TBBPA at 0.5 kHz in both sexes and also 2 kHz in males, BMDL around 8 mg/kg bw/day). There were significant latency increases of wave IV after stimulation with clicks of 60 dB in female rats, difference with controls measured 0.16 ms in the highest exposure group (BMDL 34 mg/kg bw/day). There were also effects on interpeak latencies II-IV. These differences measured 0.42 and 0.52 ms at 0.5 kHz in female and male rats, respectively, when the highest exposure level was compared to the control. Sweet preference: No effects in males. In females there were minor signs of supernormality. The results indicated an inverted U-shape relationship of percentage to dose and was highest at the medium exposure group (groups exposed to 30, 100 and 300 mg/kg bw/day) on the first 2 days of the measurement period. The differences were not significant There were no effects on conditional fear (cue or context).	

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Two available studies were assessed by the DS. A Two Generation Reproduction Toxicity Study including a developmental neurotoxicity component in the F2-generation (OECD TG 416) and a One Generation Reproduction Toxicity Study for Endocrine and Immunological endpoints and additional analysis for bone and neurophysiological parameters (conducted according to OECD TG 415).

In the Two Generation Reproduction Toxicity Study (Unnamed 2002, EU RAR TBBPA 2008 and Cope et al 2015) 30 male and 30 female rats were given TBBPA via gavage at concentrations 0, 100 and 1000 mg/kg bw/day daily during 36 weeks.

In the parental generation there were no general toxicity effects observed in clinical signs, food consumption and compound intake. There were no effects on organ/body weight and non-neoplastic histopathological finding. There were no effects on reproductive function, either estrus cycle or sperm evaluations and primordial follicle counts. There were no effects on reproductive performance.

In the F1-generation there were no general toxicity effects observed in clinical signs, mortality/viability, sexual maturation, gross pathological findings and histopathological finding, estrus cycle, reproductive performance, gestation/lactation, food consumption, gestation length, litter data, on macroscopic and microscopic evaluations, organ weights, sperm evaluations and primordial follicle counts. However, in the F1-males, lower body weights and body weight gain was observed in animals exposed to 1000 mg/kg bw/day. The lower weights was observed for several weekly intervals and the lower weight gain (7 %) was observed over week 1-11 premating period. For the F1-parental females there were no change in body weight and body weight gain.

In the F2 pups, there were no changes in body weight, sex ratio, survival to weaning or macroscopic findings or organ weigh.

Effects on serum levels of thyroid hormones were reported for parental and F1-generation. Both sexes in the P- and F1-generation had treatment related reduced levels on total thyroxine (T4) in TBBPA treated groups. In the P-generation, the effects were seen in the 100 mg/kg/day exposed males and in both sexes exposed to 1000 mg/kg/day. The conentrations of T4 was 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/day groups, respectively. Reductions in tri-iodothyronine (T3) values were also observed for P-generation male rats given 1000 mg/kg/day. The serum T3 concentrations were 102.7, 92.8, 97.5 and **83.2** ng/dL for the 0, 10, 100 and 1000 mg/kg/day exposed males, respectively. There were mild inconsistent alterations in T3 values for some female rats that were considered of equivocal relationship to TBBPA. In the F1-generation, the effects were seen in the 100 mg/kg/day and 1000 mg/kg/day groups for both sexes. The conentrations of T4 was 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/day groups, respectively. No significan changes in T3 leves the F1 generation for both sexes. Mean serum TSH-levels were comparable to the controls in both P and F1 generations. Summary of the Thyroid hormone values can be found in table 16 below. Significant changes are marked in bold.

Table16: Summary of Mean Thyroid Hormone Values in P and F1 generation

Endpoint	0 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day
P generation - male				
TSH ng/mL	15.10	11.47	14.4	14.93
T4 ng/dL	4.70	5.08	**3.9***	**3.38***
T3 ng/dL	102.7	92.8	97.5	**83.2***
TSH ng/mL	15.10	11.47	14.4	14.93
P generation – female				
TSH ng/mL	10.80	9.77	10.32	9.70
T4 ng/dL	4.23	3.45	3.5	**2.39***
T3 ng/dL	94.8	96.0	87.5	90.8
TSH ng/mL	10.80	9.77	10.32	9.70
F1 generation - male				
TSH ng/mL	11.92	10.67	13.34	9.12
T4 ng/dL	6.29	5.98	**3.91***	**3.33***
T3 ng/dL	116.8	112.6	105.9	108.2
TSH ng/mL	11.92	10.67	13.34	9.12
F1 generation – female				
TSH ng/mL	10.23	8.90	11.74	7.40
T4 ng/dL	6.00	4.42	**3.40***	**3.41***
T3 ng/dL	112.7	102.2	101.3	140.7
TSH ng/mL	10.23	8.90	11.74	7.40

* significantly different from controls, $p<0.05$

In the study the thyroid tissue was not examined, but there were no microscopic changes in the pituitary gland and liver. The reported mechanisms for decrease in T4 in the P and F1 generation and T3 in the male F1 generation is unclear. It is suggested by the author that it can be caused by induction of hepatic T4-uridine diphosphate glucuronyl transferase (UDP-GT), which is an enzyme involved in removal of circulating T4. However, there are no data that support this statement.

Neurobehavioral effects were investigated in the F2 generation. The tests performed were on motor activity, auditory startle habituation and learning and memory (passive avoidance test and water M-maze test). Reported data from the tests were found the EU RAR TBBPA (2008), except for the auditory startle habituation, were

no data were reported. For each test, 10 animals from each sex per exposure group were investigated for Animals in the F2 generation. The authors suggests that even though there are som effects in these tests (motor activity and passive avoidance test), there are a lack of consistency in the data ant that these results, although significant, can be considered to be chance findings and without toxicological relevance.

Motor activity was assessed at PND 13, 17, 21 and 60.There were some findings in terms of activity or emotionality at PND 17, PND 21 and PND 60. At PND 17, in females, it was reported a significant decrease in horizontal activity in the 15-20 min segment of the test in the 10 mg/kg bw/day group and in the 20 min period in the 100 mg/kg bw/day group. At PND 21 there was a significant reduced horizontal activity and distance travelled in the 5-10 min segment and over the 20 min test as a whole in females in the 100 mg/kg/day group compared to controls. At PND 60, in males, there were significant reductions in horizontal activity in the 0-5 min segment of the test at 100 and 1000 mg/kg/day groups and during the 5-10 min segment in the 1000 mg/kg/day group. No other significant changes were reported.

There were two tests conducted on learning and memory (passive avoidance test and water M-maze test), the same animals were used for both tests. The passive avoidance test was conducted on PND 22 and 60, once a day for 3 consecutive days. At PND 22, the test showed a significant decrease in time spent in light for males at day 2 in the 1000 mg/kg/day group. No differences were detected on day 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. No differences were detected for the other days and no differences were detected for females at any of the concetrations or timepoints. The water M-maze test were performed in the same animals at PND 110. There were no treatment related effects from the test.

Neuropathology studies were conducted in the F2 generation at PND 11 and 60. – At PND 11 animal brains were collected from 10 animals/sex/group. At PND 60 this was done for control and animals exposed to 1000 mg/kg bw/day. The main results from these evaluation was for the morphometric measurements, where there was observed significant decrease of parietal cortex thickness of the 1000 mg/kg/day pups sacrified at PND 11. The thickness was 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, respectively. There were also reduction in parietal thickenss observed at 10 and 100 mg/kg/day groups, but these changes were not significant. There were no histological changes observed in the parietal cortex. At PND 60 there were no significant differences observed (thickness measured as 2.13 and 2.09 mm in males and 2.10 and 2.06 mm in females in controls and 1000 mg/kg bw/day exposure groups, respectively).

The changes in the parietal thickness was seen on PND 11 at the highest dose. No such differences were observed at PND 60. No other changes were reported. The EU RAR TBBPA (2008) concludes that the lack of findings on the parietal cortex at PND 60 and no other microscopic findings at PND 11 and PND 60 indicate that the decreased thickness of the parietal cortex is regarded as transient or a by chance finding that is unlikly to be toxicological significant.

In summary, in the Two Generation Reproduction Toxicity Study there were no effects on reproduction and fertility. There were effects on Thyroide Hormones, T4 levels were decreased in both sexes for the P and F1 generations (at 100 and 1000 mg/kg/day for P males and F1 animals and 1000 mg/kg/day in P females). T3 levels were increased in P males exposed to 1000 mg/kg bw/day. 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 Toxicity Study was performed as a part of the FIRE project with financial support from the European Commision (Van der Ven et al., 2008 and Lilienthal et al., 2008). The authors of the study stated that it was conducted according to OECD TG 415. However, there are some differences. The study is designed to evaluate benchmark doses and contain 8 exposure groups with 10 animals/per sex in each group. The study was conducted on rats exposed to 0, 3, 10, 30, 100, 300, 1000 and 3000 mg/kg bw/day. The studies have received some criticism as reported by Strain et al., 2009 and Banasik et al., 2009, both these letters were responded to by Lilienthal et al., 2009 and Van der Ven et al., 2009. The critism was related to the way the BAEP was performed, and the use of Benchmark dose levels in the statistical analysis.

In the P generation food intake was reduced temporarily during week 1 and 2 of exposure in the high dose groups for both sexes. Females had reduced food intake at the highest doses during the first two weeks after gestation. The BMDL was 207 mg/kg bw/day. Reduced food intake also affected the body weights in females before mating (BMDL close to highest dose at 3000 mg/kg bw/day). Significant weight loss was reported in dams until gestation week 3. Similarly, it was reported a significant reduced weight gain in females premating and during gestation (BMDL 94 and 298 mg/kg bw/day, respectively).

There were no effects on endpoints of reproduction such as mating success, number of uterine implantation sites and litter size. In the F1 litters there were no differences in sex ratio. Female pups showed decreased anogenital distance at PND 7, but not at PND 4 and PND 21, and a delayed time to vaginal opening (BMDL around the highest concentration). Male pups showed increased weight of the reproductive organs at weaning (BMDL of 0.5 mg/kg bw/day). There was a dose dependent decrease in mortality during lactation (BMDL of 4.8 mg/kg bw/day) and a decrease in rate of litters with mortality (BMDL 33 mg/kg bw/day). Overall, mortality rates were higher in male pups compared to female pups (17.1 and 8.8 %, respectively). During the first 4-7 weeks F1 body weights showed a decrease around 10% (BMDL around the highest concentration).

There were some effects on organ weights in F1 animals. In male rats there was a significant dose-dependent increase in liver weight (maximum increase 11.4%). There was a significant dose-dependent increase in adult testis weight (BMDL of 0.5 mg/kg bw/day). Pituitary weight was dose-dependently increased in males. Average pituitary weights were also correlated to weights of the testis and to BAEP variables, but not to effects in thyroid hormones. For female rats there were correlations of uterine weight, endometrium thickness and CYP19 activity in the ovary to the increased male gonad weight at PND21 or necropsy.

For endocrinology effects, there were no change in the duration of the estrus cycle or in the distribution of stages during the cycle. Necropsy was targeted at the time of diestrus, but there was only 41% concordance with histological staging of vagina and endometrium. There were no dose-dependent effects on testosterone and 17-betaestradiol in male plasma or CYP19 activity in ovaries. However, there was a correlation between testosterone and CYP19 with increased testis weight. For thyroid hormone levels there were changes in levels in exposed animals compared to controls. Plasma T4 levels was decreased in both sexes (BMDL of 30.8 mg/kg bw/day for males and 16.1 mg/kg bw/day for females). Plasma T3 levels were increased in females (BMDL of 2.3 mg/kg bw/day). TBBPA had no effect on immunotoxic and hematologic effects in F1 animals, except an increase in total spleen counts. But the spleocytes counts and the B-cell count were reported as statistical uncertain. TBBPA exposure directly to the splenocyte cells indicated splenocyte growth promoting effects, however, these data were not shown. There was an increase in monocytes, however the results were reported as statistical uncertain.

Neurobehavioral effects were studied by BAEP, sweet preference and conditional fear testing.
BAEPs were recorded from 93 rats (46 females and 47 males), 5-6 animals/sex/exposure group between postnatal days 50 and 110. Recordings were performed within 3 weeks to minimize the effect of age. Results from the BAEPs showed that TBBPA exposure caused dose-related elevation of BAEP thresholds in female offspring. Increases were detected in the low frequency range up to 4kHz. The difference measured 13 dB at 0.5kHz in the 3000 mg/kg bw/day-group compared to controls. Benchmark analysis was performed, significant fits to dose-response curves were obtained at 0.5 and 2 kHz with a BMDL value of 0.9 mg/kg bw/day. The lowest critical effect dose and benchmark dose level measuring 7 and 1 mg/kg body weight were found at 2kHz. There were no effects in male rats. Increase in click thresholds were not significant in both sexes. There were only slight exposure related effects on latency of wave II. The wave II latency data could be fitted to dose-response curves at 0.5kHz in female rats. With and absolute increase of 0.14 ms at the highest exposure level compared to controls (BMDL of 110 and 33 mg/kg bw, respectively). Wave II latency was not altered after click stimulation in both sexes. Exposed rats exhibited prolongations of wave IV latencies in the low frequency range which were somewhat more pronounced in males. Benchmark analysis of wave IV latencies revealed significant effects of TBBPA at 0.5 kHz in both sexes and at 2kHz in male rats. In addition there were significant latency increases of wave IV after stimulation with clicks of 60 dB in female rats, the difference to controls measuring 0.16 ms in the highest exposure group. There were also effects on interpeak latencies II-IV, reflecting increases in signal transmission time in the brainstem. These differences measured 0.42 and 0.52

ms at 0.5 kHz in female and male rats, respectively, when the highest exposure level was compared to controls. Trend analysis revealed significant trends for all parameters for which significant fits to dose-response models according to benchmark analysis were obtained. There were no significant influences of age on BAEP thersholds and latencies when age at testing was included as covariate. Overall, Lilienthal et al., 2008 suggests that TBBPA causes a predominant cochlear effect in female rats while in males neuronal effects are more apparent.

Sweet preference. Analysis of sweet preference as well as absolute consumption of saccharin solution did not indicate any effects in males. Minor signs of supernormality were found in females. Results indicated an inverted U-shape relationship of percentage to dose, being highest at the medium exposure levels on the first 2 days of measurement period. These differences missed statistical significance. Basal water intake was not affected by TBBPA.

In summary for the reproduction study by Van der Ven et al. 2008 and Lilienthal et al 2008 did not report any effects on fertility and reproduction. Van der Ven et al., 2008 reported a significant dose-dependent increase in liver weight (maximum increase 11.4%), adult testis weight (BMDL of 0.5 mg/kg bw/day) and pituitary weight in males. In F1 female pups it 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 concentration. Thyroid hormone levels were affected in both sexes og the F1 generation. Plasma T4 levels were decreased in both sexes (BMDL of 30.8 mg/kg bw/day for males and 16.1 mg/kg bw/day 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). Plasma T3 levels were increased in females (BMDL of 2.3 mg/kg bw/day), 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 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. Van der Ven et al., 2008 discusses that the effects in both sexes on increase of hearing latencies at low frequencies and the increased hearing threshold reported in females may relate to observed changes in thyroid hormone levels. The 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. Further correlation analysis showed that average pituitary weights were correlated to weights of the testis and to BAEP variables, but not to effects in thyroid hormones. For female rats there were correlations of uterine weight, endometrium thickness and CYP19 activity in the ovary to the increased male gonad weight at PND21 or necropsy. IARC 2018 has also noted that the results from Lilienthal et al., 2008 and Van der Ven et al., 2008 may reflect and effect of TBBPA on thyroid hormone regulated developmental events, including hearing and testis weight, however that there is a lack of studies addressing this directly.

10.10.3 Comparison with the CLP criteria

Category 1A

Known human reproductive toxicant The classification of a substance in Category 1A is largely based on evidence from humans.

No human data is available, classification not warranted.

Category 1B

Presumed human reproductive toxicant. The classification of a substance in Category 1B is largely based on data from animal studies.

Category 2

Suspected human reproductive toxicant

There were no effects on sexual function and fertility, no classification is warranted.

10.10.4 Adverse effects on development

Table17: Summary table of animal studies on adverse effects on development

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Prenatal Development Toxicity Study OECD TG 414 Sprague-Dawley rats 25 females per dose and in control Reliability score 1 (by DS)	TBBPA 0, 100, 300 and 1000 mg/kg/day (actual ingested) by oral (gavage) exposure. Daily exposure for 20 days from GD 0 to GD 19	No toxic effects in maternal animals. Slight significant lower liver weight in maternal animals treated with 100 mg/kg/day, but liver weight did not differ significantly in the 300 and 1000 mg/kg/day groups. No other effects of treatment were seen from clinical observations, gestational parameters and from the uterine implantation data in the maternal animals. In the fetuses, no embryotoxic/teratogenic effects were reported. No effects on fetal body weight, sex distribution or from external observation and visceral and skeletal examinations. Litter incidences did not differ from controls.	Unnamed, 2001 EU RAR TBBPA, 2008 Cope et al., 2015
OECD TG 426 (developmental neurotoxicity study), deviation: only 2 test doses, Wistar pregnant rats, 20/dose level Reliability score 2 (by DS)	TBBPA 0, 50, 250 mg/kg bw/d Dosing was from gestation day (GD) 7 to postnatal day (PND) 17	No exposure-related effects on either terminal body weight or any of the investigated organ weights were observed in any of the three age groups (PND 15, 22 and adult animals). At PND 15 and PND 22, no treatment-related effects were observed in the brain or any of the investigated reproductive organs during the histopathological examinations. No exposure-related effects on serum T3 and T4 levels were found in males at PND 22. The concentrations of NA, DA, and 5-HT in the brains of PND 22 and adult animals did not differ significantly between treated and control animals. Overall, this study provides limited evidence of changes in the habituation behaviour of female offspring and learning and memory in male offspring in the 250 mg/kg/day group. However, it is not possible to draw definitive conclusions from this study because the size 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 is weakened by absence of consistent changes in the two genders and the lack of histopathological investigations that could provide corroborative findings.	Hass et al., 2003 not published, but reported in EU RAR TBBPA, 2008
Non-guideline developmental study Cjr:CD®(SD)IGS rats 8 dams per exposure group	TBBPA 0, 100, 1000 and 10 000 ppm (≈ 0, 10, 90 and 800 mg/kg bw/ day	Dams showed increased body weight from day 9-20 after delivery in the 10 000 ppm exposure group. At day 20 the weight was unchanged compared to controls. No effects on food consumption during gestation and lactation. No effects on duration of pregnancy. No effects in relative thyroid weights compared to controls at day 20 after delivery. A dose-unrelated increasing tendency for relative thyroid weight of all treatment groups and an incidience of diffuse thyroid follicular cell hypertrophy showed a marginal increase from 1000 ppm.	Saegusa et al., 2009

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
and in controls Reliability 2	during gestation) Oral exposure through a soy free diet Exposure from GD 10 to GD 20 after delivery (day after weaning)	In offspring, there were no abnormalities in the clinical observations, number of implantation sites, number of live offspring, male ratio, body weight, organ weights or angiogenital distance at PND1. No effects on onset of puberty in either sex, however, higher body weight was reported in males exposed to 10 000 ppm compared to controls at the onset of puberty. No effect on estrus cycle in females. Male offspring showed a dose-unrelated decrease of serum T3 levels at 100 and 1000 ppm on PND 20, but not in the 10 000 ppm exposed group. No change in T4 and TSH. At post natal week 11, there were no effects on thyroid hormone levels, no change in body and organ weights. In females decreased relative kidney and uterus weights were reported for the 1000 and 10 000 ppm exposure groups. No findings in brain morphometric assessments.	

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

Three different studies assessed developmental effects of TBBPA. In addition developmental neurotoxicity and immunotoxicity was studied in the two reproduction studies (Unnamed 2002, EU RAR TBBPA 2008, Cope et al 2015, Van der Ven et al 2008 and Lilienthal et al 2008), a summary of these developmental findings can be found in section 10.10.2.

In the three studies investigated, one was a standard guideline study (Unnamed 2003, Cope et al 2015) another was a non-published guideline study with some modifications (Hass et al. 2003) and the last a non-guideline developmental study (Saegusa et al. 2009). None of the studies show conclusive evidence of developmental toxicity.

In the prenatal developmental toxicity study (OECD TG 414) by Unnamed 2003 and Cope et al 2015 (also reported in EU RAR TBBPA 2008), there were no toxic effects in maternal animals or in the fetuses. In the maternal animals it was a slight significant lower liver weight observed in animals treated with 100 mg/kg/day. However, this effect on liver weight did not differ significantly in the 300 and 1000 mg/kg/day groups. 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 observation and visceral and skeletal examinations. Litter incidences did not differ from controls.

In non-published developmental neurotoxicity study (OECD TG 426) by Hass et al. 2003, no exposure-related effects on either terminal body weight or any of the investigated organ weights were observed in any of the three age groups (PND 15, 22 and adult animals). At PND 15 and PND 22, no treatment-related effects were observed in the brain or any of the investigated reproductive organs during the histopathological examinations. No exposure-related effects on serum T3 and T4 levels were found in males at PND 22. The concentrations of NA, DA, and 5-HT in the brains of PND 22 and adult animals did not differ significantly between treated and control animals.

Overall, for the neurobehavioral effects the study by Hass et al. (2003) provides limited evidence of changes in the habituation behaviour of female offspring and learning and memory in male offspring in the 250 mg/kg/day group. However, it is not possible to draw definitive conclusions from this study because the size 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 is

weakened by absence of consistent changes in the two genders and the lack of histopathological investigations that could provide corroborative findings.

In a non-guideline developmental study by Saegusa et al. 2009, dams showed increased body weight from day 9-20 after delivery in the 10 000 ppm exposure group. At day 20 the weight was unchanged compared to controls. No effects on food consumption during gestation and lactation. No effects on duration of pregnancy. No effects in relative thyroid weights compared to controls at day 20 after delivery. A dose-unrelated increasing tendency for relative thyroid weight of all treatment groups and an incidience of diffuse thyroid follicular cell hypertrophy showed a marginal increase from 1000 ppm.

In offspring, 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, however, higher body weight was reported in males exposed to 10 000 ppm compared to controls at the onset of puberty. No effect on estrus cycle in females. Male offspring showed a dose-unrelated decrease of serum T3 levels at 100 and 1000 ppm on PND 20, but not in the 10 000 ppm exposed group. No change in T4 and TSH. At post natal week 11, there were no effects on thyroid hormone levels, no change in body and organ weights. In females decreased relative kidney and uterus weights were reported for the 1000 and 10 000 ppm exposure groups. No findings in brain morphometric assessments.

10.10.6 Comparison with the CLP criteria

Category 1A

Known human reproductive toxicant The classification of a substance in Category 1A is largely based on evidence from humans.

No human data is available, classification not warranted.

Category 1B

Presumed human reproductive toxicant The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Category 2

Suspected human reproductive toxicant 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.

Unnamed, 2002, Cope et al., 2015 and EU RAR TBBPA, 2008 all reported results for the same Two Generation Reproduction Toxicity Study. Among the findings were a thinning of the parietal cortex only seen at PND 11 of the F2 generation in the highest exposed group. These effects were not seen at the highest dose group at PND 60 compared to control.

Van der Ven et al., 2008 and Lilienthal et al., 2008 both reported on the same One Generation Reproduction Toxicity Study. There was a significant dose-dependent increase in liver weight (maximum increase 11.4%), adult testis weight (BMDL of 0.5 mg/kg bw/day) and pituitary weight in males. In F1 female pups it 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 concentration. 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 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.

Both reproduction studies had effects of thyroid hormone levels. In the two generation study T4 levels were decreased in both sexes for the P and F1 generations (at 100 and 1000 mg/kg/day for P males and F1 animals and 1000 mg/kg/day in P females). T3 levels were increased in P males exposed to 1000 mg/kg bw/day. However, there were no effects on TSH-levels. The study showed no relevant neurobehavioral effects (Unnamed, 2002, Cope et al., 2015 and EU RAR TBBPA, 2008). In the one generation reproduction study 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/day for males and 16.1 mg/kg bw/day for females). Plasma T3 levels were increased in females, BMDL of 2.3 mg/kg bw/day (Van der Ven et al., 2008), while they were decreased in the two generation study reported above.

Van der Ven et al., 2008 discusses that the effects in both sexes on increase of hearing latencies at low frequencies and the increased hearing threshold reported in females may relate to observed changes in thyroid hormone levels. The 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. Further correlation analysis showed that average pituitary weights were correlated to weights of the testis and to BAEP variables, but not to effects in thyroid hormones. For female rats there were correlations of uterine weight, endometrium thickness and CYP19 activity in the ovary to the increased male gonad weight at PND21 or necropsy. IARC 2018 has also 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 that there is a lack of studies addressing this directly.

10.10.7 Conclusion on classification and labelling for reproductive toxicity

No classification proposed for reproductive toxicity based on conclusive data, but not sufficient for classification. None of the studies show conclusive evidence of developmental toxicity. The animal studies do indicate some effects on development, but they were not characterized as sufficiently adverse. No classification is proposed.

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.

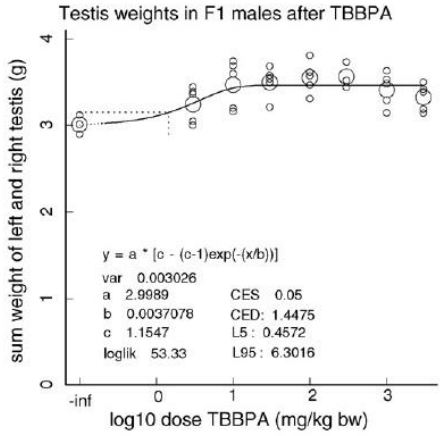
For F1 male juvenile rats a significant dose-response curve in reproductive organ weight at weaning PND21 (the publication refers to both terms testis weight and reproductive organ weight, CES 5%; max. response +15.5%, CED = 1.5 mg/kg bw/d and / BMDL = 0.5 mg/kg bw/d) and in adults testis weight (L+R) 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

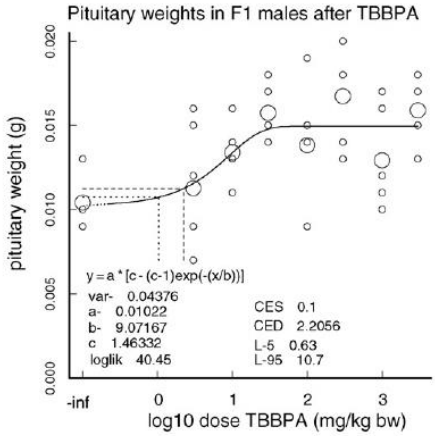
changes are evident from 30 mg/kg bw/d for the testis and pituitary (see figures below). For the testis, although it potentially being due to a low performing control group, no testis histopathology reported and no weights of the prostate, seminal vesicles or the epididymis, which are more sensitive 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

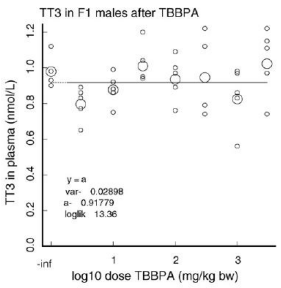
	anogenital distance (mm)			day vagina open
TBBPA dose mg/kg bw	d4	d7	d21	
0	3.3 ± 0.7	4.4 ± 0.8	11.3 ± 1.4	33.0 ± 1.9
3	3.6 ± 0.5	5.0 ± 0.8	12.7 ± 1.9	33.1 ± 2.0
10	3.0 ± 0.7	4.8 ± 1.6	12.4 ± 1.6	33.3 ± 2.4
30	3.4 ± 0.7	4.7 ± 1.0	12.0 ± 1.2	33.9 ± 3.2
100	3.2 ± 0.8	4.5 ± 0.8	11.2 ± 1.0	33.2 ± 2.4
300	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	+

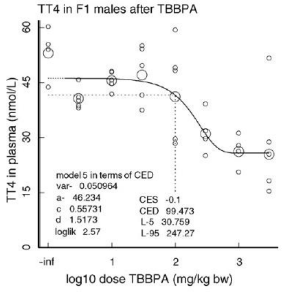
Endocrinology F1

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)*

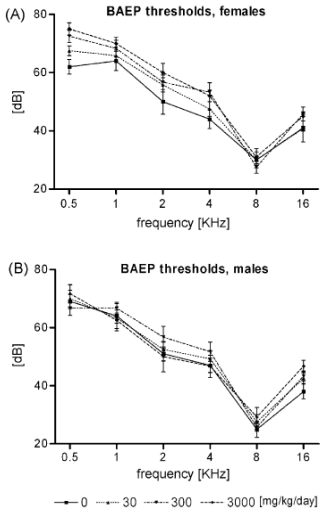


Fig. 2. BAEP thresholds in female (panel A) and male (panel B) rats (controls, groups exposed to TBBPA at 30, 300, or 3000 mg/kg body weight/day). Thresholds were elevated at frequencies of 0.5–4 kHz in exposed females, but not in males. Benchmark analyses revealed significant dose–response relationships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

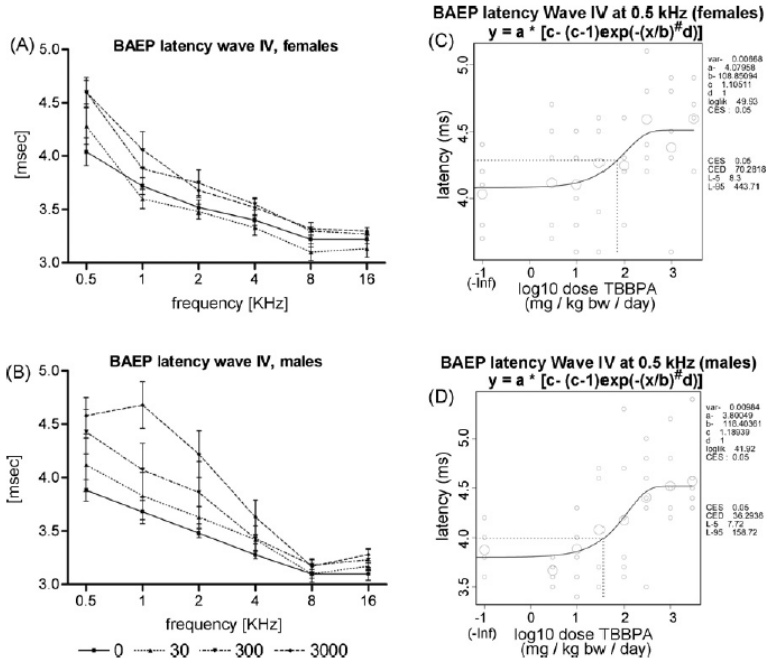


Fig. 4. Latencies of wave IV in female (panel A) and male (panel B) rats (controls, groups exposed to TBBPA at 30, 300, or 3000 mg/kg body weight/day). Exposed females and, in particular, males exhibited marked prolongations of wave IV latency in the low-frequency range. According to benchmark analysis, 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[18]). 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

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

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);
- 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.

<u>Non-guideline developmental study in SD rats</u>

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[19]) 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

[19] 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)

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 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.

Supplemental information - In depth analyses by RAC

RAC briefly summarizes four further studies on developmental toxicity that have not been considered by the DS.

Developmental toxicity study in rats (Noda et al., 1985)

In this developmental toxicity study, pregnant Wistar rats (22 to 24 per treatment group) were orally administered 0, 280, 830 and 2,500 mg/kg bw/d TBBPA throughout gestation. The

EU RAR concluded that based on these results, TBBPA did not produce adverse effects on development in the rat at dose levels up to 2,500 mg/kg.

Teratology range finding study in rats

Mated female rats were used in a range finding study to determine the dosage levels of TBBPA for a teratology study (Velsicol Chemical Corporation, 1978). This study is also reported in the REACH registration dossier. TBBPA was administered by gavage at dosage levels of 0, 30, 100, 300, 1,000, 3,000 and 10,000 mg/kg bw/d from GD 6 to GD 15 to five pregnant females. The EU RAR concluded that were no adverse effects on the developmental parameters assessed up to very high dose levels producing severe maternal toxicity (three death animals in high dose group).

Developmental neurotoxicity study in mice

In a developmental neurotoxicity in mice, TBBPA was administered to neonatal male NMRI-mice as a single oral dose on postnatal day 10 (Eriksson *et al*., 1998, Eriksson *et al*., 2001). The amount of TBBPA administered was 0.75 and 11.5 mg/kg to treatment groups of mice from 3-4 litters. No differences in behavioural tests conducted at the age of 2 and 4 months on eight mice randomly selected (locomotion, rearing, total activity and all types of vibration within the cage caused by mouse movements, shaking (tremors) and grooming) were reported and no observations in the swim maze test at 5 months of age on 16-18 mice randomly selected mice, nor any clinical signs.

Non-standard studies in newborn rats

In non-standard studies, Fukuda *et al*. (2004) investigated the effects of oral administration of TBBPA in newborn and young Sprague-Dawley rats. In a sighting study, 5 newborn rats /sex/group were administered a suspension of 0, 40, 200, 1000 mg/kg bw/d TBBPA in 0.5% (w/v) carboxymethylcellulose PND 4 to PND 21 by gavage. In the main study, 6 newborn rats/sex/group) were administered 0, 40, 200 or 600 mg/kg bw/d TBBPA suspension on PND 4 to PND 21. A recovery group with 6 animals/sex/group was included and sacrificed at 12 weeks of age (9 weeks without exposure to TBBPA). The EU-RAR summarised the findings as follows: *"These studies show an effect on the kidneys (polycystic lesions associated with the dilatation of the tubules) of newborn rats dosed from day 4 up to day 21 after birth by gavage with 200 and 600 but not 40 mg/kg TBBP-A. However, no similar effect was observed in 5-week old rats administered by gavage 2,000 and 6,000 mg/kg TBBP-A for 18 days and in a comprehensive GLP- and OECD-compliant rat 2-generation study with gavage doses of up to 1,000 mg/kg/day. It is considered that this effect is likely to be the consequence of the unconventional direct gavage administration of very high doses of TBBP-A to such young animals. Therefore, the relevance to human health of this isolated finding is considered questionable".*

10.11 Specific target organ toxicity-single exposure

Not performed for this substance.

10.12 Specific target organ toxicity-repeated exposure

Table18: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**

14-week study in F344/NTAC rats and B6C3F1/N mice 10 male and 10 femal 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)	TBBPA, purity > 99% 0, 10, 50, 100, 500, 1000 mg/kg bw (oral gavage in corn oil, 5×/week) for 14 weeks	All rats (core study) and mice survived to the end of the study. The final mean body weights and body weight gains of rats and mice of dosed groups were similar to controls. No clinical findings related to TBBPA administration were observed in rats or mice. Rats: Consistent, progressive, and dose-related decreases in total T_4 concentrations occurred in 500 and 1,000 mg/kg male and female rats; this effect was observed with less consistency in the 100 mg/kg groups. On day 4, T4 was decreased by approximately 30% in the 1,000 mg/kg 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. See copy of tables from NTP (2014) below (serum thyroid hormone levels). On day 23 and at week 14, the hematology findings suggested small (≤10%) decreases in the estimators of the circulating red cell mass in 500 and 1,000 mg/kg males and females. Serum concentrations of total bile acids, a marker of hepatic function/injury and cholestasis, demonstrated transient increases (twofold or greater) in 500 and 1,000 mg/kg males and females on day 4; the effect had essentially resolved by day 23. Decreases in cytochrome P450 enzyme and UDP glucuronosyl transferase activities were seen on day 23 and at week 14 in dosed groups of males and females; however no liver enzyme changes were considered to be biologically significant with the exception of 4- to 23-fold increases over the vehicle control value in 7-pentoxyresorufin-O-dealkylase (PROD) activities in 500 and 1,000 mg/kg males and females at week 14. The increased levels indicated some disturbance of liver function, but this was not accompanied by treatment-related liver lesions. There were significant increases in the absolute and relative liver weights of 500 and 1,000 mg/kg males and females. Significant decreases occurred in the absolute and relative spleen weights of 500 and 1,000 mg/kg males and the absolute thymus weight of 1,000 mg/kg males. There were no significant differences between the reproductive organ weights or sperm parameters of dosed and vehicle control groups of male rats. Dosed females exhibited a slight but significant increase in time in extended estrus compared to females in the vehicle control group. TBBPA dosing did not lead to endometrial alterations in F344 rats. Mice: Acetanilide-4-hydroxylase, 7-ethoxyresorufin-O-deethylase, and PROD activities in the liver of 500 and 1,000 mg/kg males were significantly less (30% to 40%) than those of the vehicle controls at the end of the study; in 1,000 mg/kg females, PROD activity was significantly decreased (30%) at week 14. These effects were less pronounced in mice than in rats in the 3-month study. Compared to those of the vehicle controls, absolute and relative liver weights were significantly increased in 500 mg/kg males and 1,000 mg/kg males and females; absolute and relative	NTP, 2014

		spleen weights in 1,000 mg/kg males were also significantly increased. Absolute and relative kidney weights were significantly decreased in 1,000 mg/kg male mice. Significantly increased incidences of renal tubule cytoplasmic alteration occurred in 500 and 1,000 mg/kg male mice. Renal tubule cytoplasmic alteration was characterized by a decrease or absence of the normal vacuoles present in the cortical proximal tubules.	
Female Wistar Han rats, (academic 13-week study) Reliability score 2 (by DS)	TBBPA 0, 25, 250, or 1000 mg/kg bw (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 analysed. 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, a transcript induced by phenobarbital and in xenobiotic metabolism. TBBPA exposure 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)	TBBPA 0, 100, 300 and 1000 mg/kg bw/d by oral gavage in corn oil Two recovery groups were included (control, 1000 mg/kg/day) 6 weeks post-treatment	TBBPA exerted no marked effect on the rate of 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. There was no effect on T3 and TSH in males and females. Significant decrease in all dose groups in mean serum T4 concentrations on day 33 and day 90 in males compared to control. In the high dose recovery group T4 levels returned to control levels after 6 weeks recovery. Significant decrease in all dose groups in mean serum T4 concentrations on day 33 in females compared to control. In the high dose recovery group T4 levels were lower than control levels after 6 weeks recovery.	Osimitz et al., 2016
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 DS)	TBBPA 0, 50, 100, 250, 500 and 1000 mg/kg by oral gavage in corn oil daily for 28 days	There were no significant changes noted in the 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 an inverse relationship was observed 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

Repeated Dose 28-day Oral Toxicity Study in Rodents, enhanced for endocrine and immune parameters Conducted according to OECD TG 407. Wistar rats Each dose group had 10 animals per sex (also control group). Reliability 2 (by DS)	TBBPA Purity 98% 0, 3, 100 and 300 mg/kg bw/day exposed by oral feed.	Effects on thyroid hormone levels - T4 levels were significantly decreased with a BMDL of 48 mg/kg bw/day. T3 levels were significantly increased with a BMDL of 123.8 mg/kg bw/day. These results were similar to what observed in the reproduction study by Van der Ven et al. (2008).	Van der Ven et al., 2008
Repeated Dose 90-day Oral Toxicity Study in Rodents, Conducted according to OECD TG 408 Sprague-Dawley Rats The control and 1000 mg/kg/day group had 15 male and 15 female animals in each group. The 100 and 300 mg/kg/day had 10 male and 10 female animals in each group. Controls and 1000 mg/kg/day group (5 animals /sex/group) were evaluated over a 6 weeks post treatment period (recovery animals) Reliability 1 (by the reg)	TBBPA Purity: 98.71 to 98.87 % 0, 100, 300 and 1000 mg/kg/day by oral gavage in corn oil	No effects were observed on mortality, body weight and weight changes, organ weight and organ/body weight ratios, food consumption and compound intake, neurobehavioral, histopathological and pathological, ophtamlmological, haematological and urinalysis findings Six females (2 controls and four in the 1000 mg/bw/day group) died or were euthanized in extremis. The mortality/moribundity was not considered to be caused by the treatment, but by dosing injury. Serum thyroid hormone levels (TSH, T3 and T4) were measured at Day 33, 90 and at Recovery Sacrifice. Effects were observed on T4 levels. T4 levels were significantly lower compared to controls on day 33 in the 100, 300 and 1000 mg/kg/day groups (both sex). The T4 levels were also significantly lower in males exposed to 100, 300 and 1000 mg/kg/day TBBPA on day 90 when compared to controls. Serum T4 concentrations in male and female rats at day 33 and 90 are shown in Table 21. At recovery euthanasia T4 levels were comparable in the control and 1000 mg/kg/day group (both sex). The change in T4 levels were reversible on recovery. No differences were observed for TSH and T3 levels at any of the time points tested (day 33, 90 and Recovery Sacrifice). After 90-days of dosing, total bilirubin values were statistically higher then the control (males: 0.14±0.05; females: 0.13±0.05 (unit not reported)) in the males in the 1000 mg/kg/day dose group (0.34±0.024) and in females in the 300 mg/kg/day group (0.19±0.03) and 1000 mg/kg/day group (0.2±0.06). Serum alkaline phosphatase levels (ALP) was significantly higher for the female 1000 mg/kg/day group (98.9±49.47) after 90 days of exposure compared to the control (58.4±28.46). Both serum bilirubin and ALP levels were comparable for control and treated group in the end of the recovery period and the effects were not considered to be biological or toxicological meaningful or adverse.	Unnamed, 2002

Repeated dose 14-day inhalation study in rodents Similar to OECD TG 412 Crj: CD(SD) Rats 5 male and 5 female in each dose group (also control) Reliability 2 (by reg)	TBBPA, Purity not given 0, 2,6,18 mg/L 4 h/day, 5 days/week for 2 weeks	Excessive salivation, red or clesar nasal discharge, and excessive lacrimation were noted during the course of the study in rats at the two highest dose levels. These effects are likely related to physical effects of the extremely high doses. No deaths, and no changes in body weight gain, food consumption, haematological and biochemical parameters, and 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 up to 18 mg/L air (ca. 18000 mg/m3) for 4 h daily, 5 d/wk for two weeks did not result in adverse effects in rats.	Unnamed, 1975
Short-term repeated dose toxicity: dermal No guideline available New Zealand White rabbits 4 male and 4 female in each dose group Reliability 2 (by reg)	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 or unusual behavior for the rabbits in any group. On the skin of rabbits at 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. There were no other signs of skin irritation or any signs of toxicity. No changes considered to be related to compound were seen in body weights, hematologic and biochemical parameters and urinalysis. There were no compound induced gross or microscopic lesions in any of the tissues examined. No compound-related organ weight variations occurred.	Unnamed, 1979

Table 19: Serum thyroid hormone levels in F344/NTac rats in 3-month study (NTP, 2014) (Thyroxin T4, triiodothyronine T3, thyroid stimulating hormone TSH)

TABLE F1
Hematology and Clinical Chemistry Data for F344/NTac Rats in the 3-Month Gavage Study of Tetrabromobisphenol A

	Vehicle Control	10 mg/kg	50 mg/kg	100 mg/kg	500 mg/kg	1,000 mg/kg
Male (continued)						
Clinical Chemistry						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Total thyroxine (µg/dL)						
Day 4	6.13 ± 0.18	5.94 ± 0.19	6.12 ± 0.14	5.56 ± 0.17	4.78 ± 0.18**	4.49 ± 0.30**
Day 23	5.11 ± 0.31	5.71 ± 0.34	5.52 ± 0.27	4.72 ± 0.22	3.35 ± 0.19**	3.78 ± 0.22**[d]
Week 14	4.66 ± 0.16	4.78 ± 0.25	4.61 ± 0.13	3.67 ± 0.21**	3.08 ± 0.12**	2.80 ± 0.13**
Total triiodothyronine (µg/dL)						
Day 23	151.2 ± 6.2	190.9 ± 14.7	167.6 ± 6.8	184.4 ± 7.5*	164.4 ± 9.7	199.6 ± 10.6**[d]
Week 14	105.9 ± 5.6[c]	109.4 ± 8.2[b]	106.7 ± 6.7[b]	96.7 ± 5.5	97.6 ± 4.5	102.4 ± 5.2
Thyroid stimulating hormone (ng/dL)						
Day 4	5.37 ± 0.39[b]	5.70 ± 0.29	5.06 ± 0.43	4.80 ± 0.35[b]	4.84 ± 0.35b	4.78 ± 0.22[b]
Day 23	7.41 ± 0.43	8.10 ± 0.54[b]	8.49 ± 0.49	6.95 ± 0.41	6.22 ± 0.30	6.50 ± 0.33[d]
Week 14	8.04 ± 0.42	7.94 ± 0.49	8.19 ± 0.37	7.83 ± 0.42	5.99 ± 0.29**	7.38 ± 0.34*
Female						
Total thyroxine (µg/dL)						
Day 4	5.52 ± 0.16	5.63 ± 0.12	5.18 ± 0.22	4.52 ± 0.18**	4.05 ± 0.27**	3.87 ± 0.30**
Day 23	4.26 ± 0.25[d]	4.51 ± 0.26	4.05 ± 0.25	3.75 ± 0.30[d]	2.56 ± 0.25**	2.64 ± 0.21**
Week 14	3.33 ± 0.22	3.58 ± 0.17[d]	3.07 ± 0.20	2.76 ± 0.19	1.83 ± 0.15**[d]	1.66 ± 0.10**
Total triiodothyronine (µg/dL)						
Day 23	180.4 ± 8.1[d]	177.5 ± 11.9	180.5 ± 12.1	167.1 ± 5.5[d]	143.8 ± 3.7**	168.1 ± 7.2
Week 14	116.2 ± 6.9[b]	115.8 ± 8.5	115.9 ± 10.7	128.3 ± 8.3	117.7 ± 7.2	113.1 ± 8.2[b]
Thyroid stimulating hormone (ng/dL)						
Day 4	4.95 ± 0.48	5.00 ± 0.36	4.65 ± 0.29	4.77 ± 0.27	4.26 ± 0.18	3.89 ± 0.09*
Day 23	5.26 ± 0.29[b]	6.46 ± 0.42[b]	5.85 ± 0.33	5.16 ± 0.17[d]	5.06 ± 0.23	4.89 ± 0.18
Week 14	7.36 ± 0.39	7.47 ± 0.69[d]	7.79 ± 0.47	8.87 ± 0.64	7.65 ± 0.39[d]	7.00 ± 0.46

* Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test
** P≤0.01
[a] Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=8
[d] n=10
[e] n=7
[f] n=4
[g] n=5

Table 20: Treatment-related thyroid responses in male and female rats (copied from Osimitz et al., 2016)

males:

Dose (mg/kg/day)	Study day	Mean T_4 (ng/dL)	SD	*N* (number of measures used to calculate mean)
0	33	4.96	0.837	15
	90	5.09	0.797	15
	Recovery	5.32	0.944	5
100	33	3.66[b]	0.878	10
	90	3.27[b]	0.672	10
300	33	3.42[b]	0.713	10
	90	2.61[b]	0.874	10
1000	33	3.39[b]	0.548	15
	90	3.09[b]	0.910	15
	Recovery	5.90[c]	1.538	5

NA: not applicable; ND: not determined per study protocol;
[a]No statistical significance observed.
[b]Significantly different from control ($p < 0.01$).
[c]Compared to recovery control.

females:

Dose (mg/kg/day)	Study day	Mean T_4 (ng/dL)	SD	*N* (number of measures used to calculate mean)
0	33	4.27	0.957	15
	90	5.41	1.036	12
	Recovery	3.95	1.406	4
100	33	3.31[b]	1.079	10
	90	5.22	1.234	10
300	33	3.24[b]	0.846	10
	90	4.95	1.316	10
1000	33	3.33[b]	0.844	15
	90	4.95	1.111	11
	Recovery	3.05[c]	0.705	4

NA: not applicable; ND: not determined per study protocol;
[a]No statistical significance observed.
[b]Significantly different from control ($p < 0.05$).
[c]Compared to recovery control.

Table 21: Treatment related serum T4 levels (ng/dL) in male and female rats (unnamed, 2002)

TBBPA	**0 mg/kg/day**	**100 mg/kg/day**	**300 mg/kg/day**	**1000 mg/kg/day**
Day 33 – male				
T4 ng/dL	4.96±0.84	**3.66±0.88***	**3.42±0.71***	**3.39±0.55***
Day 33 – female				
T4 ng/dL	4.27±0.96	**3.31±1.08***	**3.24±0.85***	**3.33±0.84***
Day 90 – male				
T4 ng/dL	5.09±0.80	**3.27±0.67***	**2.61±0.87***	**3.09±0.91***
Day 90 – female				
T4 ng/dL	5.41±1.04	5.22±1.23	4.95±1.32	4.95±1.11

Significant different levels compared to control (p<0.01) are marked in bold with asterisk (*)

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

NTP conducted 3-month studies in F344/NTac rats and B6C3F1/N mice (NTP, 2014) previous to the 2-year studies (and also conducted an interim 3-month evaluation in the 2-year Wistar Han rat study). In the 3-month-

studies in F344/NTac rats and B6C3F1/N mice, doses were 0, 10, 50, 100, 500 and 1000 mg/kg/bw/d. The TBBPA treatment did not cause mortality or changes in body weights. Increases in liver weights (9-14%) were seen in the two highest dose groups in male mice and female and male rats, and in the highest dose group in female mice. Increase in liver CYP2B activity were seen in the two highest dose groups in mice and rats. Treatment-related decreases in thyroxine (T4) concentration were seen in male and female rats. The results of the 3-month 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. A 90 day oral gavage study in male and female Sprague-Dawley rats no effects were found on mortality, body weight, food consumption, compound intake, food efficiency, ophtalmology, haematology, urinalysis, neurobehavioral and functional observation battery. There were treatment related effects on serum thyroid hormone T4 levels, but not T3 and TSH. Serum T4 levels were significantly reduced in male and females at day 33 for all treatment concentrations (100, 300 and 1000 mg/kg/day). At day 90, serum T4 levels were reduced at all treatment concentrations in males, but not in females. There were no difference in T4 levels at Recovery euthanasia between control animals and those treated with 1000 mg/kg/day. It is suggested by Meerts et al. 2000 that the reduction in T4 can be caused by competitive displacement by TBBPA from transthyretin (TTR), a major serum T4 -binding protein. However, this has not been demonstrated in *in vivo* studies. Some effects were also observed on alkaline phosphatase levels and bilirubin, however, these were not considered to be of toxicological relevance (Unnamed, 2002). In an old 14 day inhalation study in male and female Crj: CD(SD) rats, no systemic toxic effects were observed with concentrations up to 18 mg/L. The rats were exposed for 4 hours per day. Local irritation was observed as excessive salivation, red or clear nasal discharge but this was assumed to be caused by mechanical effects due to the high concentration of TBBPA (Unnamed, 1975). In an old 3 week dermal toxicity study in male and female New Zealand White rabbits no systemic toxicity or unusual behaviour was observed. Very slight erythema was observed in all exposures. No compound induced gross lesions were observed in any of the rabbits at the terminal sacrifice. There were no compound-related microscopic alterations observed in any of the tissues examined (Unnamed, 1979).

TBBPA exposure induced liver changes (upregulation) in Wistar Han rats exposed to 1000 mg/kg bw for 13 weeks (Dunnick et al., 2017). As the liver is involved in estradiol metabolism, these changes could affect hormone levels. TBBPA also induced the interferon (IFN) pathway transcripts in the liver. Some of these may be involved in hepatic cancer (Li et al., 2014).

TBBPA has little activity as an estrogen receptor agonist or antagonist, but a feedback loop between estrogen signalling and IFN signalling has been reported. Also, TBBPA can cause oxidative damage and disruption of thyroid hormone signalling (see references in Dunnick et al., 2017). The relationship between IFN-related mechanisms and uterine carcinogenesis is not yet clear.

TBBPA has a low hazard profile in the available studies, but in rodents high dosages lead to some changes in the levels of thyroid hormones (T_4 /T_3), primarily a decrease of serum T_4 i.e. the circulating thyroid hormone functional reserve pool, and not the circulating pool of ultimate active T3 hormone (Lai et al., 2015).

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 in the liver or uterus in an academic experimental 3-month study in Wistar Han rats (Dunnick et al., 2017).

There was significant decrease in all dose groups in mean serum T4 concentrations on day 33 in males and females and day 90 in males compared to control. In the high dose recovery group T4 levels returned to control levels after 6 weeks recovery for males. For females T4 levels were lower than control levels after 6 weeks recovery. There was no effect on T3 and TSH in males and females (Osimitz et al., 2016).

In rats exposed to 0, 3, 100 and 300 mg/kg bw/day in a 28 day oral repeated dose toxicity study, plasma levels of thyroid hormones were measured. The author uses benchmark doses and their lower 90% confidence interval enabling calculation of a lower 5% confidence interval (BMDL). The T4 levels were significantly decreased with a BMDL of 48 mg/kg bw/day in rats. T3 levels were significantly increased with a BMDL of 123.8 mg/kg bw/day (Van der Ven et al. 2008).

There were dose-related increases in the concentration of TBBPA and its major conjugates, TBBPA-glucuronide and TBBPA-sulfate for all dose groups in liver, plasma and uterine tissue in rats exposed up to 1000 mg/kg bw TBBPA for 28 days. There were, however, no significant changes noted in the 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 (Borghoff et al., 2016).

10.12.2 Comparison with the CLP criteria

STOT RE Category 1:

Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: — reliable and good quality evidence from human cases or epidemiological studies; or — observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.

We do not have human data. The animal studies do indicate some, but not severe toxic effects at low exposure concentrations.

STOT RE Category 2:

Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

The animal studies do indicate some, but not significant or severe toxic effects at moderate exposure concentrations. Some reduction in T4 was seen in several studies. No dose-related systemic adverse effects from the TBBPA-treatment were observed in rodents and rabbits.
Some effects were seen, but these do not fulfill the requirements for classification with STOT-RE.

10.12.3 Conclusion on classification and labelling for STOT RE

No classification is proposed for STOT RE.

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:**	NTP, 2014

		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)	
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)

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.**

Supplemental information - In depth analyses by RAC

Two further oral studies were described in the registration dossier. The first was a 28-day study in rats exposed to a diet containing 1, 10, 100, and 1000 ppm of TBBPA (Unnamed, 1972, reliability 2 by the registrant). No treatment-related effects were observed at any dosage level and bromine levels in liver and fat were slightly elevated, but similar in control and high dose group. The second one is a 90-day study on rats exposed via the diet to 0.3, 3, 30, 100 mg/kg/day of TBBPA (Unnamed, 1975, reliability 2 by the registrant). None of the effects (slight but statistically significant decrease in the haematocrit and decrease in the serum glutamic pyruvic transaminase activity in female rats in the 100 mg/kg/day dose group) were considered to be of toxicological significance. Neither of these studies provided GLP data as the methodology predated or was not conduced according to standardized guidelines. Also, no analytical verification of test compound concentrations was performed. The DS didn't include them as these studies were old, non-GLP and non-guideline. These studies were therefore considered to be of limited relevance for the specific target organ toxicity evaluation of TBBPA after repeated exposure.

10.13 Aspiration hazard

Not performed for this substance.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

Not performed for this substance.

12 EVALUATION OF ADDITIONAL HAZARDS

Not performed for this substance.

13 ADDITIONAL LABELLING

Not relevant.

14 REFERENCES

Banasik M., Hardy M., Harbison RD., Hsu CH., Stedeford T. Tetrabromobisphenol A and model-derived risks for reproductive toxicity. Toxicology 260 (2009) 150-152

Birnbaum LS, Staskal DF: Brominated flame retardants: Cause for concern? Environmental Health Perspectives 112 (1) 9-17, 2004

Borghoff SJ, Wikoff D, Harvey S, Haws L (2016). Dose- and time-dependent changes in tissue levels of tetrabromobisphenol A (TBBPA) and its sulfate and glucuronide conjugates following repeated administration to female Wistar Han Rats, Toxicology Reports, Vol. 3, 190-201.

Cannon RE, Trexler AW, Knudsen GA, Evans RA, Birnbaum LS: Tetrabromobisphenol A (TBBPA) alters ABC transport at the blood-brain barrier. Toxicol. Sci.: 169(2): 475-484 (2019)

Cope RB, Kacew S and Dourson MA: A reproductive, developmental and neurobehavioral study following oral exposure of tetrabromobisphenol A on Sprague-Dawley rats. Toxicology 329 (2015) 46-59

Dunnick JK, Morgan DL, Elmore SA, Gerrish K, Pandiri A, Ton TV, Shockley KR, Merrick BA: Tetrabromobisphenol A activates the hepatic interferon pathway in rats. Toxicol Lett. 2017 January 15; 266: 32-41

Dunnick JK, Sanders JM, Kissling GE, Johnson C, Boyle MH, Elmore: Environmental chemical exposure may contribute to uterine cancer development: studies with tetrabromobisphenol A: Toxicol Pathol. 2015 June; 43(4): 464-473

EFSA (2011) EFSA panel on contaminants in the food chain (CONTAM): scientific opinion on tetrabromobisphenol A (TBBPA) and its derivatives in food EFSA panel on contaminants in the food chain. EFSA J9:2477 (61)

EU RAR TBBPA (2008) EU risk assessment: United Kingdom (TBBPA) (2008). RISK ASSESSMENT of 2,2’,6,6’-TETRABROMO-4,4’-ISOPROPYLIDENE DIPHENOL. Environment Agency Chemicals Assessment Section United Kingdom. Report date: 2008-01-29.

Fini, J.-B., Riu, A., Debrauwer, L., Hillenweck, A., Le Mével, S., Chevolleau, S., Boulahtouf, A., Palmier, K., Balaguer, P., Cravedi, J.-P., Demeneix, B.A., and Zalko, D. (2012). Parallel biotransformation of tetrabromobisphenol A in Xenopus laevis and mammals: Xenopus as a model for endocrine perturbation studies. Toxicol. Sci. 125, 359-367.

Hagmar, L., Sjödin, A., Höglund, P., Thuresson, K., Rylander, L., and Bergman, Å. (2000). Biological half-lives of polybrominated diphenyl ethers and tetra-bromobisphenol A in exposed workers. Organohalogen Compounds 47, 198-201.

Hakk H, Larsen G, Bergman Å, Örn U, (2000), Metabolism, excretion and distribution of the flame retardant tetrabrombisphenol-A in conventional and bile-duct cannulated rats, Xenobiotica, Vol. 30, No. 9, 881-890.

Hall SM, Coulter SJ, Knudsen GA, Sanders JM, Birnbaum LS: Gene expression changes in immune response pathways following oral administration of tetrabromobisphenol A (TBBPA) in female Wistar Han rats. Toxicol Lett. 2017 April 15; 272: 68-74

Harvey JB, Osborne TS, Hong HHL, Bhusari S, Ton TV, Pandiri AR, Masinde T, Dunnick J, Peddada S, Elmore S, Hoenerhoff MJ (2015). Uterine Carcinomas in Tetrabromobisphenol A-Exposed Wistar Han Rats Harbor Increased Tp53 Mutations and Mimic High-Grade Type I Endometrial Carcinomas in Women. Toxicol Pathol. 2015 December ; 43(8): 1103–1113

Grosse Y et al. on behalf of the International Agency for Research on Cancer Monograph Working Group. The Lancet, News| Volume 17, Issue 4, 419-420, April 01, 2016

S1470-2045(16)00137-6

IARC (2018) Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 115 Some Industrial Chemicals, pp. 247-end

Knudsen GA, Sanders JM, Sadik AM, Birnbaum LS (2014). Disposition and kinetics of tetrabromobisphenol A in female Wistar Han rats. Toxicol Rev, 1:214–23.

Kuester R, Sólyom AM, Rodriguez VP, Sipes IG, (2007). The effects of dose, route and repeated dosing on the disposition and kientics of tetrabromobisphenol A in male F-344 rats, Tox Sci 96(2):237-245

Lai DY, Kacew S, Dekant W[20]. Tetrabromobisphenol A (TBBPA): Possible modes of action of toxicity and carcinogenicity in rodents. Food and Chemical Toxicology: 80 (2015) 206-214

Li C, Wang J, Zhang H, Zhu M, Chen F, Hu Y, Liu H, Zhu H. Interferon-stimulated gene 15 (ISG15) is a trigger for tumorigenesis and metastasis of hepatocellular carcinoma. Oncotarget. 2014; 5(18):8429–8441. [PubMed: 25238261]

Lilienthal, H., Verwer, CM, van der Ven, LTM., Piersma, A.H. and Vos, J.G. Exposure to tetrabromobisphenol A (TBBPA) in Wistar rats: Neurobehavioral effects in offspring from one-generation reproduction study. Toxicology 246 (2008) 45-54

Lilienthal H., Slob W., van der Ven LTM., Piersma AH. Measurment and evaluation of neurobehavioral effects induced by tetrabromobisphenol A (TBBPA)- Response to Strain et al. (2009)

Meerts IATM., van Zanden JJ., Luijks EAC., van Leeuwen-Bol I., Marsh G., Jakobsson E., Bergman Å. and Brouwer A. Potent Competitive Interactions of Some Brominated Flame Retardants and Related Compounds with Human Transthyretin in vitro. Toxicological Sciences, 56,95-104 (2000)

Nakagawa Y, Suzuki T, Ishii H, Ogata A (2007). Biotransformation and cytotoxicity of a brominated flame retardant, tetrabromobisphenol A, and its analogues in rat hepatocytes. Xenobiotica, 37(7):693–708.

National Toxicology Program. 2014; Toxicology studies of teterabromobisphenol A (Cas no. 79-94-7) in F344/NTac rats and B6C3F1/N mice and toxicology and carcinogeogenesis studies of tetrabromobisphenol A in Wistar Han [Crl:WI(Han)] rats and B6C3F1/N mice (gavage studies). NTP Technical Report 587 https://ntp.niehs.nih.gov/publications/reports/tr/500s/tr587/index.html

OECD: Overview of the set of OECD Genetic Toxicology Test Guidelines and updates performed in 2014-2015, Series on Testing & Assessment, No. 238 - 2nd edition (2017)

Osimitz TG, Droege W, Hayes AW (2016). Subchronic toxicology of tetrabromobisphenol A in rats. Human and Experimental Toxicology , Vol. 35(11) 1214–1226.

Hagmar L, Sjödin A, Höglund P, Thuresson K, Rylander L, Bergman Å (2000). Biological half-lives of polybrominated diphenyl ethers and tetrabromobisphenol A in exposed workers. Organohalogen Compd, 47:198–201.

Hass, U., & Wamberg, C. (2002). Developmental neurotoxicity study of the brominated flame retardant tetrabromobisphenol A in rats. Poster session presented at 30th Conference of European Teratology Society, Hannover, Germany. Also reported in EU RAR TBBPA, 2008 as Hass et al., 2003.

Hass et al., 2003 (not published), reported in EU RAR TBBPA, 2008 p. 94-100.

Sanders JM, Coulter SJ, Knudsen GA, Dunnick JK, Kissling GE, Birnbaum LS (2016). Disruption of estrogen homeostasis as a mechanism for uterine toxicity in Wistar Han rats treated with tetrabromobisphenol A

Schauer, U.M.D., Völkel, W, Dekant, W., (2006). Toxicokinetics of tetrabromobisphenol A in humans and rats after oral administration, Toxicological Sciences 91(1)49-58

Sjodin, A., Patterson, D.G., Jr., Bergman, A., 2003. A review on human exposure to brominated flame retardants – particularly polybrominated diphenyl ethers. Environ. Int. 29, 829–839.

[20] The co-authors have reported conflict of interest

Strain GM., Banasik M., Hardy M. and Stedeford T. Tetrabromobisphenol A (TBBPA) and model-derived risks for neurobehavioral effects in offspring from a one-generation reproduction study. Toxicology 260 (2009) 155-157

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor, R. (1987). Prediction of chemical carcinogen-icity in rodents from in vitro genetic toxicity assays. Science 236, 933-941.

Thomsen C, Lundanes E, Becher G (2001). Brominated flame retardants in plasma samples from three different occupational groups in Norway. J Environ Monit, 3(4):366–70.

Thomsen C, Lundanes E, Becher G (2002a). Brominated flame retardants in archived serum samples from Norway: a study on temporal trends and the role of age. Environ Sci Technol, 36(7):1414–8.

Thomsen, C., Leknes, H., Lundanes, E., and Becher, G. (2002b). A new method for determination of halo-genated flame retardants in human milk using solid-phase extraction. J. Anal. Toxicol. 26, 129-137.

Unnamed, Study report 2001, Repeated Dose 90-Day Oral Toxicity in Rodents.

Unnamed, Study report 1979, 3 weeks study, Short-term repeated dose toxicity: dermal

Unnamed, Study report 1975, 2 weeks study, Subacute Inhalation Toxicity

Unnamed, Study report 1979, Toxicokinetics of tetrabromobisphenol A in humans and rats after oral administration.

Unnamed, Study report 2005, Dermal absorption in vitro / ex vivo

Unnamed, Study report 2002, Two Generation Reproduction Toxicity Study with a developmental neurotoxicity component in the F2 generation. Conducted according to standardized guideline (OECD TG 416).

Van der Ven LT, Van de Kuil T, Verhoef A, Verwer CM, Lilienthal H, Leonards PE, Schauer UM, Cantón RF, Litens S, De Jong FH, Visser TJ, Dekant W, Stern N, Håkansson H, Slob W, Van den Berg M, Vos JG, Piersma AH. Endocrine effects of tetrabromobisphenol-A (TBBPA) in Wistar rats as tested in a one-generation reproduction study and a subacute toxicity study. Toxicology. 2008 Mar 12;245(1-2):76-89

Van der Ven LT., Slob W., Piersma AH., Opperhuizen A. The benchmark approach is the preferred method to describe the toxicology of tetrabromobisphenol A (TBBPA) – Response to Banasik et al. (2009). Toxicology 260 (2009) 153-154

Wikoff DS, Rager JE, Haws LC, Borghoff SJ (2016). A high dose mode of action for tetrabromobisphenol A-induced uterine adenocarcinomas in Wistar Han rats: A critical evaluation of key events in an adverse outcome pathway framework. Regul. Toxicol. Pharmacol. 77, 143-159

Witt, K.L., Knapton, A., Wehr, C.M., Hook, G.J., Mirsalis, J., Shelby, M.D., and MacGregor, J.T. (2000). Micronucleated erythrocyte frequency in peripheral blood of B6C3F1 mice from short-term, prechronic, and chronic studies of the NTP Carcinogenesis Bioassay Program. Environ. Mol. Mutagen. 36, 163-194.

Zalko, D., Prouillac, C., Riu, A., Perdu, E., Dolo, L., Jouanin, I., Canlet, C., Debrauwer, L., and Cravedi, J.-P. (2006). Biotransformation of the flame retardant tetrabromo-bisphenol A by human and rat sub-cellular liver fractions. Chemosphere 64, 318-327.

Zeiger, E. (1998). Identification of rodent carcinogens and noncarcinogens using genetic toxicity tests: Premises, promises, and performance. Regul. Toxicol. Pharmacol. 28, 85-95.

Additional references
Binding and Activity of Tetrabromobisphenol A Mono-Ether Structural Analogs to Thyroid Hormone Transport Proteins and Receptors; Ren *et al*.; Environmental Health Perspectives; 2020 NICNAS, Tetrabromobisphenol A, Priority Existing Chemical Assessment Report Assessment No. 42, May 2020 Curren RD, Kmetz J, Schechtman LM (1981). Activity of T1685 in the Salmonella/microsomal assay for bacterial mutagenicity final report. Prepared by Microbiological Associates for Ethyl Corporation. Submitted to the U.S. Environmental Protection Agency under TSCA Section 8D. Danish EPA (1999). Danish Environmental Protection Agency. Brominated flame retardants: substance flow analysis and assessment of alternatives. Haschek and Rousseaux´s Handbook of toxicologic pathology (2013). Haschek WM, Rousseaux CG, Wallig MA Editors, Academic Press.

15 ANNEXES

ANNEX I to the CLH report

Confidential annex