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**

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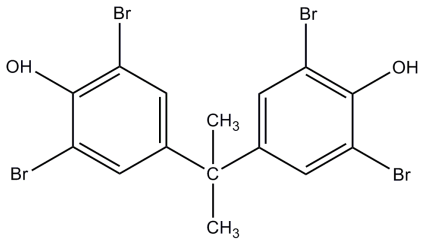
CONTENTS

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-AEC 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.

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	

Property	Value	Reference	Comment (e.g. measured or estimated)
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	
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

Property	Value	Reference	Comment (e.g. measured or estimated)
			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.
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. 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.	Registrant reliability index: 1 (reliable without restriction) Not GLP or specific testing guidelines. Registrant, key study	Schauer et al., 2006
Basic toxicokinetics in vivo Rat (Fischer-344), 4 male for each oral dose, 9 for iv dose Rats: oral (2, 20, 200 mg/kg)	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	Registrant reliability index: 2 (reliable with restriction)	Kuester et al., 2007

Method	Results	Remarks	Reference
/iv (20 mg/kg) The effect of multiple doses and route of administraiton was investigated in rats using 14C-TBBPA.	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.	Not GLP or specific testing guidelines. Registrant, key study	
Basic toxicokinetics in vivo Rat (albino Sprague-Dawley) 10 females (only); divided into three groups of 2 (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).	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. 14C-TBBPA was rapidly eliminated in the feces after oral dosing to the rat. Low bioaccumulation potential based on study results.	Registrant reliability index: 2 (reliable with restriction) Not GLP or specific testing guidelines. Registrant, supporting study	Unnamed, Study report 1979
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:

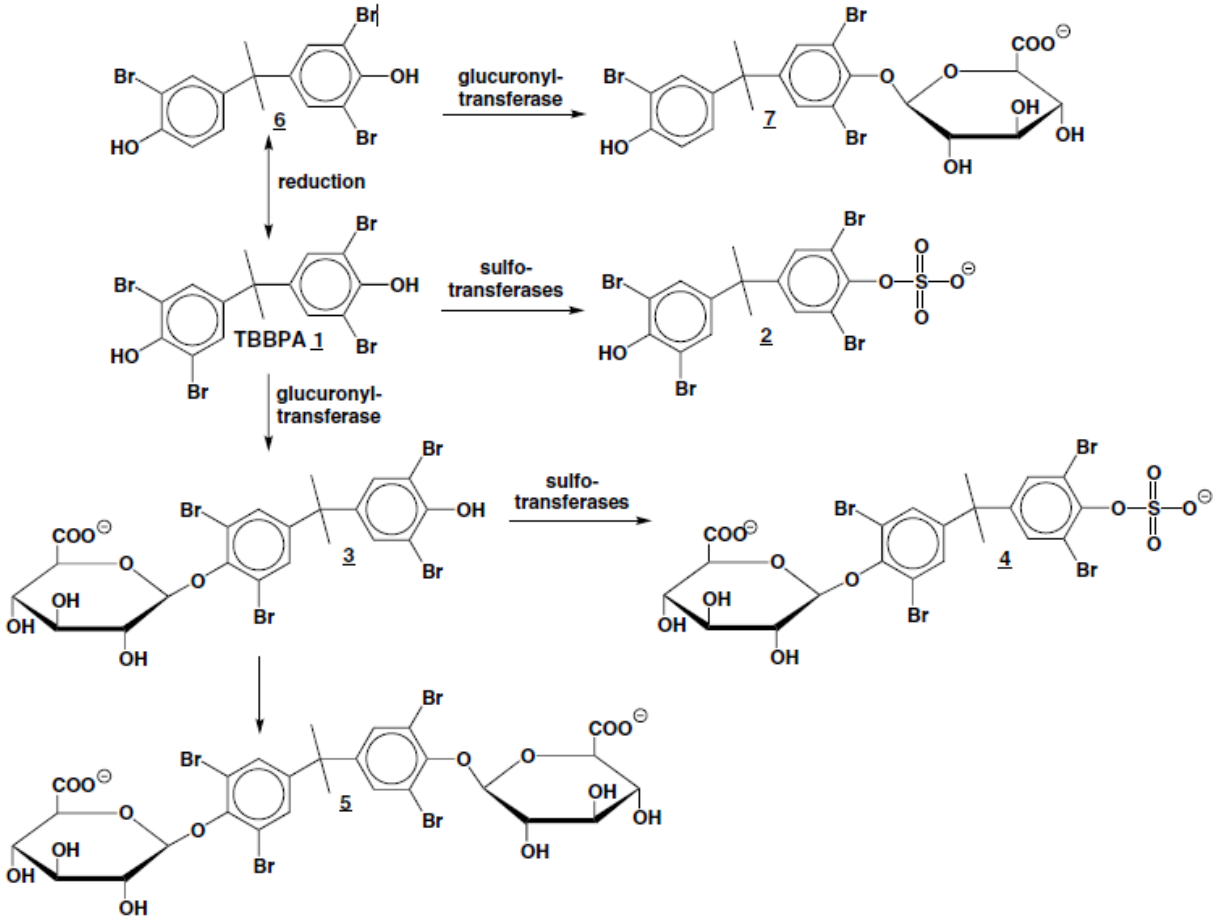
Maximum plasma concentrations of TBBPA-conjugates in humans were obtained within 4 h of dosing and rapidly declined to reach the limit of detection (LOD) after 8 h. Parent TBBPA molecule was not present in detectable concentrations in any of the human plasma samples. TBBPA plasma concentrations in rats 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	TBBPA purity: N/A Test concentrations: 0, 5, 10, 50,	Bacterial reverse mutation test in Salmonella typhimurium TA92, TA98, TA100, TA1535, TA1537 and TA1538	No increase in the number of revertant colonies. Cytotoxicity at levels higher than the tested concentrations.	DOW Chemical Company (1985), reported in RAR TBBPA

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
OECD TG 471	100, 500 and 1000 µg/plate. Toxicity observed at higher concentrartions	With and without metabolic activation		(2008)
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	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

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
score 2 (by registrant)				study 4 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: 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	TBBPA purity: N/A Test concentrations: Dose levels were 0, 5, 10,	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
Study assumingly perfomed equivalent or similar to OECD Guideline 476	20, 30, and 40 µg/ml in DMSO (final concentration 0.2%) in the 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).

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").

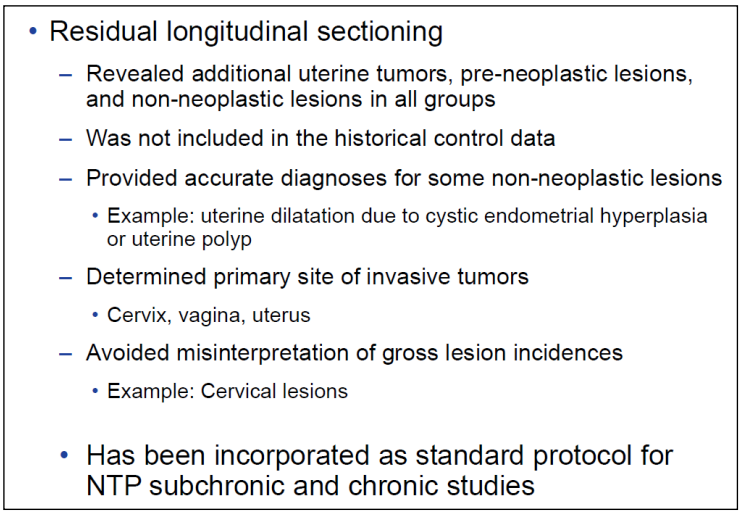
Strains of rats

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.

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

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

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)	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	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	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
compliant[2] Reliability score 1 (by DS)	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.	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 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,	

[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
		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 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,	

[3] uncommon tomour type

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		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. 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;	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
		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	**Control animals:** Uterine adenocarcinoma	No	Yes, uterine adenocarcinomas and malignant	Yes First incidence	Not applicable (NA)	No	Oral by gavage	MoA: See section 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
rats	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]		mixed Müllerian tumours	on days 668 (control), 548 (low dose), 321 (medium dose), 442 (high dose) for Original Transverse and Residual Longitudinal Reviews (Combined)				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 **Historical control data:** Hepatoblastoma 9/250 (gavage), 40/949 (all routes) (see details in table	Liver, large intestine (only sign trend in large intestine), hemangiosarcoma (all organs)	Yes, hepatoblastoma is a rare malignant liver cancer	No First incidence on days 521 (control), 535 (low dose), 513 (medium dose)	No, only treatment-related tumourigenic effects in male mice	Top dose animals not included in the carcinogenicity results due to high toxicity and early mortality	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
	below) **Large intestine:** **Control animals:** Adenoma 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)							
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. 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	NTP, 2014 (major hypothesis) Dunnick et al., 2015 Sanders et al., 2016
	TBBPA (via competitive inhibition of estrogen conjugation) could	Lai et al.,

[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
	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)	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. Oxidative cleavage of the TBBPA molecule.	NTP, 2014 Dunnick et 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	IARC, 2018

[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
	superoxide anion.	
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

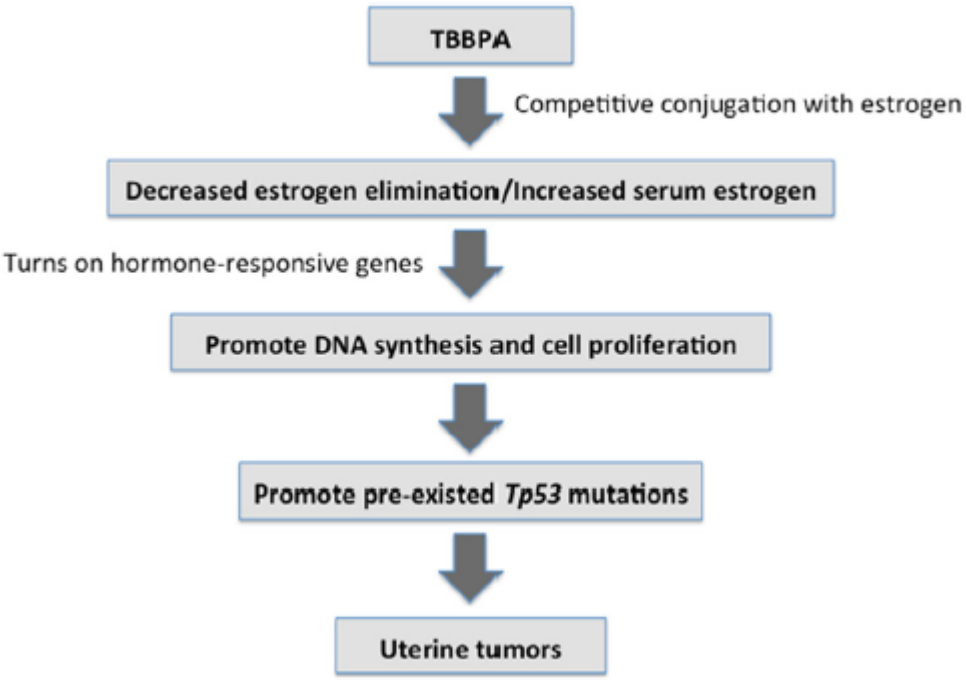


Figure 2: Proposed mechanism/MoA of TBBPA on uterus carcinogenesis in rats (copied from Lai et al., 2015).

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. 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).

[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)

[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 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).

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".

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	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	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
(by DS)		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 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. Neuropathology (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	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:	Reproduction effects: 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	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
animals/sex/dose Reliability score 2 (by DS)	Exposure start P-males at 10 weeks and P-females at 2 weeks 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).	(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> 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). <u>Food intake:</u> 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. <u>Organ weight:</u> 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. <u>Immunotoxic and hematological effect</u>: Increase in total spleen cell counts. <u>Neurobehavioral effects:</u> 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.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		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 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	TBBPA 0, 100, 1000 and	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	Saegusa et al., 2009

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
Cjr:CD®(SD)IGS rats 8 dams per exposure group and in controls Reliability 2	10 000 ppm (≈ 0, 10, 90 and 800 mg/kg bw/ day during gestation) Oral exposure through a soy free diet Exposure from GD 10 to GD 20 after delivery (day after weaning)	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 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.

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.

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.

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15 ANNEXES

ANNEX I to the CLH report

Confidential annex