Annex I to the 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 (TBBPA)

EC Number: 79-94-7

CAS Number: 201-236-9

Index Number: 604-074-00-0

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CONTENTS

1 PHYSICAL HAZARDS

Evaluation not performed for this substance.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1 Schauer et al., 2006

Study reference:

Registrant key study:

Schauer et al. (2006), Toxicokinetics of tetrabromobisphenol A in humans and rats after oral administration, Tox Sci 91(1)49-58

2,2',6,6'-tetrabromo-4,4'-isopropylidene diphenol (TBBPA), EU risk assessment, Environment Agency, Chemicals Assessment Section, United Kingdom, 2008

Test type

Determine systemic bioavailability after oral adminstration in rats; disposition in humans. Registrant indicate that the study is well designed and reported in the peer-reviewed literature. Not GLP or specific testing guidelines. Reliability: 1 (reliable without restriction).

Key study.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Constituent
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity*
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number*
- *Physicochemical properties that may be important when assessing toxicokinetics*

Detailed study summary and results:

Interpretation of results (migrated information): no bioaccumulation potential based on study results. TBBPA is rapidly metabolized and eliminated as glucuronide and/or sulfate conjugates by humans and rats, such that its systemic bioavailability is low. The conjugate(s) is/are the circulating form of TBBPA in blood.

Executive summary:

This study reports the characterization of the toxicokinetics of TBBPA in human subjects and in rats. A single oral dose of 0.1 mg/kg TBBPA was administered to five human subjects. Rats were administered a single oral dose of 300 mg TBBPA/kg body weight. Urine and blood concentrations of TBBPA and its metabolites were determined by LC/MS-MS. TBBPA-glucuronide and TBBPA-sulfate were identified as metabolites of TBBPA in blood and urine of the human subjects and rats. In blood, TBBPA-glucuronide 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 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. In conclusion, TBBPA is rapidly metabolized after absorption by conjugation resulting in a low systemic bioavailability of TBBPA.

Material and methods

- Species: other: rat, human
- Strain: Sprague-Dawley
- Sex: male/female
- Details on test animals and environmental conditions: 6 male rats; 3 male and 2 female human volunteers
- Route of administration: other: oral gavage (rat); gel capsule (human)
- Vehicle: corn oil
- Duration and frequency of treatment / exposure:
    - 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
- Doses / concentrations:
    - 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
- No. of animals per sex per dose: 6 male rats, 3 human males; 2 human females

- Control animals: no

Results

Metabolism:

Metabolized mainly to sulfate conjugates in the rat, and to glucuronide conjugates in humans.

Excretion:

Rats: fecal excretion of unchanged TBBPA (>80% dose), trace amounts of sulfate-congugate in urine; Human: <0.1% dose in urine; feces not collected

Distribution:

Rats: TBBPA-sulfate main form in plasma followed by TBBPA and trace amounts of TBBPA-glucuronide

Humans: TBBPA not detected in plasma, TBBPA-glucuronide was circulating form; trace amounts of TBBPA-sulfate in blood

Details on excretion: Results in rats indicate sulfate and glucuronide conjugates are cleaved in the feces to the parent molecule.

Metabolites identified: yes

Details on metabolites:

Rats: primarily TBBPA-sulfate

Humans: primarily TBBPA-glucuronide

Trace amounts of the disulfate and diglucuronide and/or tribombisphenol A conjugates

2.1.2 **Kuester et al., 2007**

Study reference:

Registrant key study:

The effects of dose, route and repeated dosing on the disposition and kientics of tetrabromobisphenol A in male F-344 rats Kuester et al., 2007, The effects of dose, route and repeated dosing on the disposition and kientics of tetrabromobisphenol A in male F-344 rats, Tox Sci 96(2):237-245

Test type

Basic toxicokinetics in vivo.

Registrant indicate that the study appears well-designed and reported. Published in the literature. Not GLP, no specific testing guidelines. Reliability: 2 (reliable with restrictions).

Key study.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Constituent

- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity:* 97% (chemical purity), 98.9% (radiochemical purity)
- *Impurities (or a note that the impurities do not affect the classification*
- *Batch number*
- *Physicochemical properties that may be important when assessing toxicokinetics*

Detailed study summary and results:

Conclusions:

No bioaccumulation potential based on study results.

Systemic bioavailabilty < 2%. Rapid and extensive extraction and metbolism by the liver. Rapid elimination in feces.

Material and methods

- Species: rat
- Strain: Fischer-344, without surgical alteration, with indwelling jugular vein cannula (JVC) or with cannulated bile ducts (BDC)
- Sex: male
- Age at study initiation: 8–9 weeks of age
- Weight at study initiation: 161–190 g
- Fasting period before study: Yes, 12 hours
- Housing: in Nalgene metabolism cages
- Diet (e.g. ad libitum): Teklad 4% Rat Diet 7001, ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: All animals were maintained in an Association for Assessment and Accreditation for Laboratory Animal Care–approved animal care facility for at least 1 week before experimentation. Except for the JVC and BDC animals, acclimation time was 5–7 days. The cannulated animals were acclimated only for 1 day to ensure that the cannula remained unobstructed
- Rate of preparation of diet (frequency):
- Mixing appropriate amounts with (Type of food):
- Storage temperature of food: N/A, used immediately
- Vehicle:
- Concentration in vehicle:
- Amount of vehicle (if gavage):

- Duration and frequency of treatment / exposure: three different single, oral doses (gavage)(2, 20 and 200 mg/kg), repeated daily oral doses (gavage)(20 mg/kg) for 5 or 10 days or iv administration (20 mg/kg)
- Doses / Concentrations: 2, 20 and 200 mg/kg. The doses used in these studies were based on the results of published toxicity studies (Fukuda et al., 2004; IPCS/WHO, 1995). The doses of 2 and 20 mg/kg represent nontoxic doses, while 200 mg/kg may represent a minimally toxic dose. This lack of acute toxicity was confirmed following a single iv administration of unlabeled TBBPA (20 mg/kg). TBBPA was administered to male F-344 rats (N ¼ 4) by the indwelling JVC. Control JVC animals (N ¼ 2) were dosed with vehicle only.
- Control animals:
- Rationale for animal assignment (if not random):
- Tissues and body fluids sampled:

  Following iv (20 mg/kg) or oral (2, 20, or 200 mg/kg) dosing urine was collected at 6, 12, 24, 36, 48, and 72 h; feces were collected at 12, 24, 36, 48, and 72 h. At the end of the study, animals were subjected to euthanasia by CO2 inhalation and necropsy. All collected samples were analyzed immediately or stored at -80C until analyzed. Blood, bile, feces, and selected tissues (brain, heart, lung, thyroid, kidneys, liver, spleen, stomach, stomach contents, intestine, intestinal contents, cecum, cecum contents, testes, muscle, fat, and skin) were solubilized with Soluene-350 as described by Thomson and Burns (1996).

  For repeated dosing studies feces samples were collected just prior to the next dose administration. During the 1-day study, urine and cage rinse samples were collected at 6, 12, and 24 h; feces were collected at 12 and 24 h after dosing.
- Time and frequency of sampling: See above
- Statistics

Results

The effect of multiple doses and route of administration was investigated in rats using 14C-TBBPA. Rats eliminated > 90% of total radioactivity in feces by 72 hr following single oral doses of 2 to 200 mg/kg. Most of each dose was eliminated in the first 24 hr. At 72 hr after administration of the highest dose, the amounts of 14C found in the tissues were minimal (0.2 - 0.9% of the dose). With repeated daily oral doses (20 mg/kg) for 5 or 10 days, the cummulative 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, and the radioactivity in the blood decreased rapidly and was described by a biexponentil equation, consistent with a two-compartment model. The key calculated kinetic parameters are terminal elimination half-life (t1/2 Beta) = 82 min; area under the blood concentration-time curve from time 0 to infinity (AUC) = 1440 ug x min/ml;

and apparent clearance (CL) = 2.44 ml/min. Althougth readily absorbed from the gut, systemic bioavailability of TBBPA was low (< 2% of the dose). TBBPA underwent substantial first pass effect in the liver; TBBPA was rapidly metabolized and eliminated in the bile. Approximately 50% of an oral dose of 20 mg/kg was detected in the bile within 2 h. The extensive extraction and metabolism by the liver limits systemic exposure to TBBPA. For more details see figures and tables below.

Figures and table from Kuester et al., 2007:

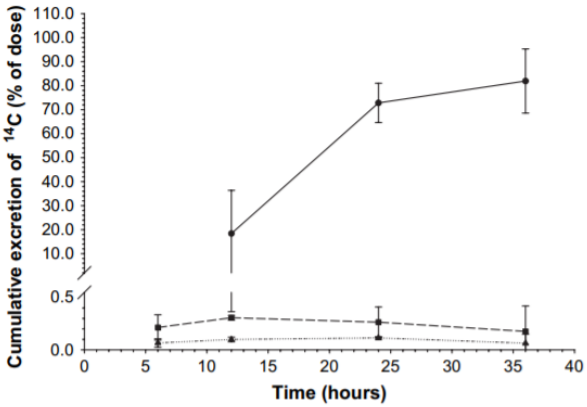

FIG. 3. Cumulative excretion of total radioactivity in urine (■), feces (●), and cage rinse (▲) expressed as percent of dose following iv administration of ^{14}C-TBBPA (20 mg/kg, 50 μCi/kg) to male Fischer-344 rats. Data are expressed as mean ± SD ($N = 4$ ± SD at 24 h and $N = 2$ at 36 h).

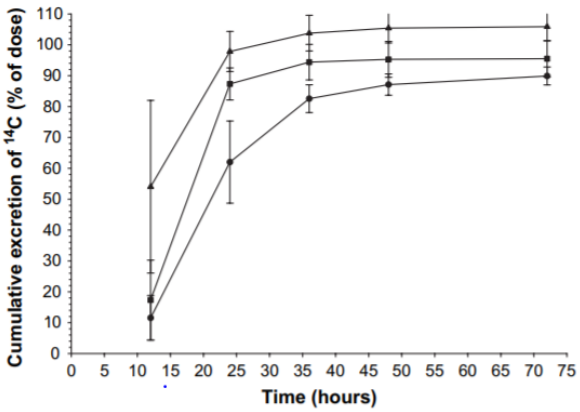

FIG. 4. Comparison of the cumulative excretion of total radioactivity in feces following administration of different oral doses of ^{14}C-TBBPA (2 mg/kg [■], 20 mg/kg [▲], and 200 mg/kg [●]) to male Fischer-344 rats. Data are expressed as the mean percent of dose $N = 4$ ± SD

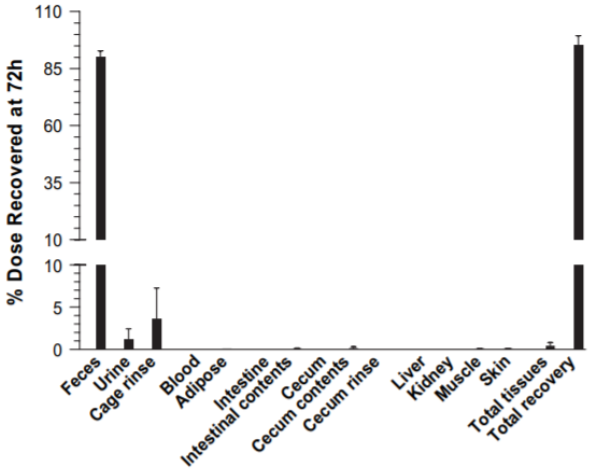

FIG. 5. Excretion and tissue distribution of TBBPA (200 mg/kg, 50 μCi/kg) following single po administration. Feces, urine, cage rinse, and tissues were collected, solubilized, and analyzed by LSC. Data expressed as mean percent of dose ± SD ($N = 4$)

TABLE 2
Percent of Dose Recovered One Day following Administration of ^{14}C-TBBPA (20 mg/kg, 50 μCi/kg, po daily dose) for 1, 5, or 10 Days to Male F-344 Rats ($N = 4$ ± SD)

	1 day	5 days	10 days
Feces	81.91 ± 3.59	85.05 ± 0.5	97.92 ± 1.12
Urine	0.4 ± 0.19	0.2 ± 0.01	0.35 ± 0.11
Cage rinse	0.1 ± 0.07	0.06 ± 0.01	0.12 ± 0.03
Brain	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cecum	0.17 ± 0.06	0.06 ± 0.04	0.01 ± 0.00
Cecum contents	8.43 ± 3.65	1.12 ± 0.23	0.69 ± 0.21
Cecum rinse	0.10 ± 0.07	0.06 ± 0.04	0.02 ± 0.01
Adipose tissues			
Kidney fat	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mesentary fat	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.02
Testes fat	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Thoracic fat	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total fat	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Heart	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Intestine	0.09 ± 0.03	0.04 ± 0.04	0.01 ± 0.01
Intestine contents	1.92 ± 0.52	0.38 ± 0.17	0.26 ± 0.17
Kidneys	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Liver	0.07 ± 0.03	0.01 ± 0.00	0.01 ± 0.00
Lung	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Muscle	0.05 ± 0.05	0.01 ± 0.01	0.01 ± 0.00
Skin	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
Spleen	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Stomach	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Stomach contents	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Testes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Thyroid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Bladder urine	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Total recovery	93.35 ± 5.89	86.99 ± 2.80	99.36 ± 0.93

2.1.3 **Unnamed, Study report 1979**

Study reference:

Registrant supporting study:

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

Test type

Absorption, distribution, and excretion of BP-4A were studied in albino Sprague-Dawley rats following administration as a single oral dose (via corn oil).

No GLP data; methodology predates or was not conducted according to standardized guidelines; no analytical verification of test compound concentrations. Reliability: 2 (reliable with restrictions).

Supporting study.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Constituent
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity*
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number:* MRI-77-161-19
- *Physicochemical properties that may be important when assessing toxicokinetics*
- *Substance type:* Monoconstituent
- *Physical state:* Solid
- *Specific activity (if radiolabelling):* 9.32 mCi/mM

Detailed study summary and results:

Conclusions: Interpretation of results (migrated information): low bioaccumulation potential based on study results 14C-TBBPA was rapidly eliminated in the feces after oral dosing to the rat.

Executive summary:

A single oral dose of 14C-TBBPA was administered to female rats. 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. The authors interpreted the high fecal recovery as poor absorption, when in

reality later studies have demonstrated TBBPA is well absorbed from the GI tract and undergoes substantial first pass elimination by the liver into the bile and feces such that systemic availability is low. The authors considered the absorbed 14C-TBBPA to follow a one compartment open model with a half life of 19.9 hours. They concluded that the absorbed fraction of the dose would be non-persistent and nonaccumulative because the maximum half-life in any tissue was <3 d.

Material and methods

- Species: rat
- Strain: Sprague-Dawley
- Sex: female
- Age at study initiation: Adult (inferred from weights)
- Weight at study initiation: 151 to 176g
- Fasting period before study: Yes, 4 hours
- Housing: Polycarbonate metabolism cages (Maryland Plastics)
- Diet (e.g. ad libitum): Purina laboratory animal food, ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: Three days
- Rate of preparation of diet (frequency): Once
- Mixing appropriate amounts with (Type of food): a stock solution of 14C BP-4A in corn oil was produced and diluted with additional corn oil to give final concentration
- Storage temperature of food: N/A, used immediately
- Vehicle: corn oil
- Concentration in vehicle: Final dosing concentration= 1mg (2.7uCi)/0.3 mL corn oil; administered to give 5 mg/kg
- Amount of vehicle (if gavage): 0.5 mL
- Duration and frequency of treatment / exposure: Once
- Doses / Concentrations:
- Single dose, equivalent to 5 mg/kgNo. of animals per sex per dose:10 females (only); divided into three groups of 2 (sacrificed at 8hr, 24hr, and 72hr) and one group of four (used for blood monitoring)
- Control animals: no
- Rationale for animal assignment (if not random): Random
- Tissues and body fluids sampled: Urine, faeces, blood, plasma, serum; Tissue samples: fat, kidney, liver, spleen, muscle, gonads
- Time and frequency of sampling: Urine & faeces: daily; Blood, plasma, serum: at 4, 8, 24, 48, 72 hours from 2 rats, at 16 hours from another two rats; Tissue samples from rats sacrificed at 8, 24, and 72 hours

- Statistics: Linear regression

Results

Absorption: Described as poorly absorbed. BP-4A was poorly absorbed from the gut, but appears to follow a one compartment open model system. See table below.

Details on distribution in tissues: See table below.

Details on excretion: Excretion data indicated that BP-4A is poorly absorbed through the gut, and that 95% of administered 14C was accounted for in the faeces with 72 hours after exposure.

Excretion: 95% of radiolabelled dose excreted via the feces within 72h. 1.1% of radiolabelled dose excreted via the urine within 72h

Metabolites identified: no

Table 1: Elimination of radiocarbon in urine and faeces from rats administered in a single oral dose

	% of administered radiocarbon					
	Urine			Faeces		
Sampling time (hr)	Group I	Group II	Group III	Group I	Group II	Group III
4	0.03	0.04	0.05	0.0015	0.002	0.0015
8	0.02	0.04	0.09	0.0015	0.003	0.1450
16	--	0.12	0.12	--	25.771	7.4910
24	--	0.15	0.15	--	7.435	49.6420
48	--	--	0.30	--	--	36.2615
72	--	--	0.32	--	--	1.4545
Total	0.05	0.04	1.03	0.003	33.211	94.996

Table 2: Radiocarbon levels in blood of rats at the indicated times following a single oral exposure to 14C BP-4A

Time After Dosing (hrs)	Percent of administered dose
4	0.03
8	0.03
16	0.02
24	0.01
48	0.01

2.1.4 **Unnamed, Study report 2005**

Study reference:

Registrant key study:

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

2,2',6,6'-tetrabromo-4,4'-isopropylidene diphenol (TBBPA), EU risk assessment, Environment Agency, Chemicals Assessment Section, United Kingdom, 2008

Test type

OECD Guideline 428 (Skin Absorption: In Vitro Method).

GLP compliance: yes

Reliability: 1 (reliable without restriction)

Key study.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Constituent
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity*
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number:* Radiolabelled: No. CFQ14174; Non-radiolabelled: #TS6404
- *Physicochemical properties that may be important when assessing toxicokinetics*
- *Substance type:* Monoconstituent
- *Physical state:* Radiolabelled test item- dissolved in ethanol; Non-radiolabelled- white powder
  Analytical purity: Radiolabelled= 99.6%; Non-radiolabelled=99.20%
- *Composition of test material, percentage of components:*
  Non-radiolabelled: 99.20% TBBPA, 0.02% Tribromophenol, 0.75% TriBPA, 0.03% o,p-TBBPA
  Radiochemical purity (if radiolabelling): 99.6% (determined by HPLC)
- *Specific activity (if radiolabelling):* 1.0mCi/mL in ethanol; 56mCi/mmol when undissolved
- *Storage condition of test material:* Radiolabelled test item: at -20C in the dark; Non-radiolabelled test item: at ambient temperature in the dark

Detailed study summary and results:

Conclusions:

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.

Executive summary:

In an in vitro percutaneous absorption study of radiolabelled tetrabromobisphenol A (TBBPA) through human skin conducted to GLP in accordance with OECD Guideline 428, radiolabelled [14C]-TBBPA was applied in vitro in an acetone vehicle at 10 µL/cm2 to human split-thickness skin membranes mounted using flow-through

diffusion cells. The acetone evaporated rapidly from the skin surface leaving behind the [14C]-TBBPA (1.9 mg/cm2). Following this topical application of [14C]-TBBPA to human split-thickness skin in vitro, the absorbed dose and dermal delivery were 0.73% (14.60 µg.equiv./cm2) and 1.60% (32.05 µg.equiv./cm2) of the applied dose, respectively. At 8 h post dose, the dislodgeable dose was 61.52% of the applied dose. At 24 h post dose, a further 28.01% was dislodged from the skin. Therefore, the total dislodgeable dose was 89.53% of the applied dose. The stratum corneum contained a further 12.48% of the applied dose. The bulk of this (9.47%) was recovered in the first 5 tape strips. Since the bulk of the stratum corneum associated material was found in the first 5 tapes strips, this indicated that the [14C]-TBBPA was on the surface of the skin and that the stratum corneum was an efficient barrier to [14C]-TBBPA penetration.

Material and methods

- Species: human
- Sex: female
- Details on test animals and environmental conditions:
- Skin washing experiment: obtained from female surgery patients aged 19 to 49 years old
- Absorbtion experiment: obtained from female surgery patients aged 25 to 57 years old
- Temperature (°C): Stored at -20C, and thawed prior to use
- Air flow rate: about 1.5 mL/h
- Type of coverage: not specified
- Vehicle: acetone
- Duration of exposure: Single dose applied to skin surface, with vehicle evaporation and observations for 24 hours post-application.
- Nominal doses: 1.9 mg/cm2
- Actual doses: 2.0 mg/cm2
- Actual doses calculated as follows: Absorbed dose was 0.73% of the applied dose; Dermal delivery was 1.60% of the applied dose
- Dose volume: 6.4 uL
- No. of animals per group:10 samples of human skin
- Control animals: no
- Justification for use and choice of vehicle (if other than water): Solubility of TBBPA in acetone is greater than water, and acetone evaporates faster
- Amount(s) applied (volume or weight with unit): 6.4 uL
- Concentration (if solution): 1246 ug/6.4 uL
- Preparation of test site: Skin placed in a flow-through diffusion cell
- Area of exposure: 0.64 cm2
- Type of cover / wrap if used: N/A

- Time intervals for shavings or clippings: N/A
- Removal of protecting device: N/A
- Washing procedures and type of cleansing agent: 50 uL of Radox Supersoap
- Time after start of exposure: 8hr after exposure
- Method type(s) for identification: Liquid scintillation counting
- Source of skin: Plastic surgery patients
- Ethical approval if human skin: Yes
- Type of skin: Breast and upper arm
- Preparative technique: placed in flow-through cell and acetone vehicle evaporated
- Thickness of skin (in mm): Full thickness (690 to 1550 um)
- Membrane integrity check: yes
- Storage conditions: at -20C until use
- Diffusion cell: Flow-through
- Receptor fluid: Ethanol and water, 1:1 v/v
- Solubility of test substance in receptor fluid: water solubility = 1.26 ppm and ethanol solubility = 80% w/w
- Static system: No
- Flow-through system: Yes
- Test temperature: 32C
- Occlusion: Yes
- Reference substance(s): None

Results

Signs and symptoms of toxicity: not specified. Dermal irritation: not specified. Absorption in different matrices:- Non-occlusive cover + enclosure rinse:

- Skin wash: 24.97% of applied dose when washed 8h after exposure

- Skin test site: 24hr mean total unabsorbed dose= 102.26% of radioactivity

- Skin, untreated site: 0.25% of radioactivity

- Blood: N/A

- Carcass: N/A

- Urine: N/A

- Expired air (if applicable): N/A

- Serial non-detects in excreta at termination: N/A

- Receptor fluid, receptor chamber, donor chamber (in vitro test system): ranged from 0.30% to 1.74% of applied dose

- Skin preparation (in vitro test system): See table below

- Stratum corneum (in vitro test system): The bulk of the radioactivity (9.47%) was recovered in the outermost 5 tape strips Total recovery:Total recovery ranged from 99.27 to 108.8%

Table 3: Distribution of Radioactivity (% Applied Dose) at 24 h Post Dose Following Topical Application of TBBPA in Acetone Vehicle to Human Split-Thickness Skin

	Cell Number and Donor Number											
	Cell 40	Cell 41	Cell 42	Cell 43	Cell 44	Cell 54	*Cell 55*	Cell 57	*Cell 58*	Cell 59		
	0095	0100	0109	0112	0113	0100	*0109*	0113	*0112*	0098	Mean	SD
Skin Wash 8 h	27.95	28.61	17.45	39.54	28.69	27.08	*12.70*	19.57	*17.31*	10.89	24.97	8.76
Tissue Swab 8 h	0.84	2.89	5.09	0.11	3.20	3.18	*4.58*	0.29	*2.00*	3.44	2.38	1.77
Pipette Tips 8 h	0.08	0.05	0.01	0.31	0.05	0.09	*0.04*	0.14	*0.43*	0.05	0.10	0.09
Total Q-Tips	31.19	36.04	27.96	60.37	37.64	32.21	*37.77*	29.89	*46.73*	17.33	34.08	12.28
Dislodgeable Dose 8 h	60.05	67.59	50.51	100.32	69.57	62.55	*55.08*	49.89	*66.47*	31.71	61.52	19.84
Tissue Swab 24 h	1.49	1.34	1.52	0.15	0.87	1.02	*2.39*	1.70	*6.24*	1.28	1.17	0.49
Cell Wash	26.85	18.52	33.36	0.95	22.06	31.07	*32.97*	26.54	*17.09*	55.38	26.84	15.30
Total Dislodgeable Dose	88.39	87.45	85.39	101.42	92.49	94.64	*90.45*	78.13	*89.80*	88.37	89.53	6.87
Stratum Corneum 1-5	6.78	8.69	9.34	1.41	8.06	5.45	*7.37*	21.11	*10.54*	14.88	9.47	6.04
Stratum Corneum 6-10	0.85	1.73	1.46	0.15	0.97	0.73	*2.05*	4.96	*1.51*	1.32	1.52	1.47
Stratum Corneum 11-15	0.39	0.43	0.95	0.06	0.99	0.40	*1.44*	2.44	*0.48*	1.23	0.86	0.75
Stratum Corneum 16-20	0.42	0.37	0.61	0.05	0.15	0.24	*0.46*	0.54	*0.32*	0.91	0.41	0.27
Stratum Corneum 21-25	0.21	0.48	0.22	0.01	0.01	0.32	*0.54*	0.18	*0.25*	0.29	0.22	0.16
Total Stratum Corneum	8.65	11.71	12.59	1.69	10.19	7.14	*11.86*	29.22	*13.09*	18.62	12.48	8.31
Unexposed Skin	0.09	0.16	0.27	0.06	0.05	0.13	*0.09*	0.08	*0.11*	1.16	0.25	0.37
Total Unabsorbed	97.12	99.31	98.24	103.17	102.74	101.91	*102.40*	107.43	*103.00*	108.16	102.26	4.03
Exposed Skin	1.14	0.87	1.49	0.26	0.78	1.63	*4.22*	0.63	*2.02*	0.16	0.87	0.53
Receptor Fluid	1.00	0.84	1.74	0.49	0.30	0.39	*0.75*	0.44	*3.61*	0.54	0.72	0.48
Receptor Rinse	0.01	0.01	0.02	0.00	0.00	0.01	*0.02*	0.01	*0.18*	0.02	0.01	0.01
Total Absorbed	1.01	0.85	1.76	0.50	0.31	0.40	*0.76*	0.46	*3.79*	0.56	0.73	0.48
Dermal Delivery	2.15	1.71	3.25	0.76	1.09	2.03	*4.98*	1.09	*5.81*	0.72	1.60	0.86
Mass Balance	99.27	101.02	101.49	103.92	103.83	103.94	*107.38*	108.51	*108.81*	108.88	103.86	3.41

Table 4: Distribution of [^{14}C]-TBBPA (µg equiv./cm^2) at 24 h Post Dose Following Topical Application of TBBPA in Acetone Vehicle to Human Split-Thickness Skin

Cell Number and Donor Number

	Cell 40	Cell 41	Cell 42	Cell 43	Cell 44	Cell 54	*Cell 55*	Cell 57	*Cell 58*	Cell 59		
	0095	0100	0109	0112	0113	0100	*0109*	0113	*0112*	0098	Mean	SD
Dislodgeable Dose 8 h	1202.93	1353.96	1011.84	2009.68	1393.62	1253.09	*1103.44*	999.34	1331.44	635.30	1232.47	397.44
Total Dislodgeable Dose	1770.57	1751.82	1710.45	2031.63	1852.86	1895.77	*1811.87*	1565.05	*1798.82*	1770.29	1793.56	137.62
Stratum Corneum	173.19	234.49	252.13	33.80	204.14	143.11	*237.62*	585.39	*262.30*	373.06	249.91	166.49
Total Unabsorbed	1945.48	1989.41	1967.95	2066.63	2058.08	2041.57	*2051.27*	2151.97	*2063.38*	2166.61	2048.46	80.82
Total Absorbed	20.18	16.95	35.34	9.94	6.11	7.95	*15.29*	9.12	*75.85*	11.23	14.60	9.61
Dermal Delivery	43.06	34.30	65.17	15.21	21.84	40.61	*99.72*	21.76	*116.38*	14.48	32.05	17.32
Total Recovery	1988.53	2023.72	2033.12	2081.84	2079.91	2082.18	*2151.00*	2173.73	*2179.75*	2181.09	2080.52	68.39

Cell 55 was rejected from mean + SD as this was an outlier for dermal delivery

Cell 58 was rejected from mean + SD as this was damaged during the 8 h washing process

3 HEALTH HAZARDS

3.1 Acute toxicity - oral route

Evaluation not performed for this substance.

3.2 Acute toxicity - dermal route

Evaluation not performed for this substance.

3.3 Acute toxicity - inhalation route

Evaluation not performed for this substance.

3.4 Skin corrosion/irritation

Evaluation not performed for this substance.

3.5 Serious eye damage/eye irritation

Evaluation not performed for this substance.

3.6 Respiratory sensitisation

Evaluation not performed for this substance.

3.7 Skin sensitisation

Evaluation not performed for this substance.

3.8 Germ cell mutagenicity

3.8.1 In vitro data

3.8.1.1 [Study 1] NTP TR 587, 2014 p 140

National Toxicology Program. 2014; Toxicology studies of teterabromobisphenol A (Cas no. 79-94-7) in F344/NTac rats and B6C3F1/N mice and toxicology and carcinogeogenesis studies of tetrabromobisphenol A in Wistar Han [Crl:WI(Han)] rats and B6C3F1/N mice (gavage studies). NTP Technical Report 587

Detailed study summary and results:

Test type

Bacterial reverse mutation test in Salmonella typhimurium strains TA98 and TA100 and in Escherichia coli strain WP2 uvrA/pKM101 , OECD TG 471

- *number of replicates:* Triplicate
- *number of doses, justification of dose selection:* At least five doses. The highest dose was limited by the experimental design to 6,000 μg/plate. All trials were repeated.
- *positive and negative control groups and treatment:* Each trial consisted of triplicate plates of concurrent positive and negative controls. The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-o-phenylenediamine (TA98), and methyl methanesulfonate (E. coli). The positive control for metabolic activation with all strains was 2-aminoanthracene.
- *details on slide preparation*: N/A (not available in the NTP report, but assumingly available in full study report if required - this applies to all fields labelled N/A)
- *number of metaphases analysed*: N/A
- *justification for choice of vehicle*: buffer
- *solubility and stability of the test substance in vehicle if known*: N/A
- *description of follow up repeat study*: N/A
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations)* : N/A

The study was GLP compliant, as National Toxicology Program (NTP) require studies to be conducted in accordance with GLP and only contract GLP compliant laboratories.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Yes, the same test substance as used in the 2-year NTP studies

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable:* S. typhimurium strains TA98 and TA100 and in Escherichia coli strain WP2 uvrA/pKM101. (Histidine-independent (for the S. typhimurium strains) or tryptophan-independent (for the E. coli strain) mutant colonies arising on these plates were counted following incubation for 2 days at 37º C)
- *Type and composition of metabolic activation system:*
  - *species and cell type:* Incubation in either buffer or S9 mix (from induced Sprague Dawley rat liver)
  - *quantity*: N/A
  - *induced or not induced:* Induced
  - *chemicals used for induction*: N/A
  - *co-factors used*: N/A
- *Test concentrations, and reasoning for selection of doses if applicable:* Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of tetrabromobisphenol A. The high dose was limited to 6,000 µg/plate. All trials were repeated.
- *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)* Solvent buffer.
- *Statistical methods:* N/A

Results and discussion

- *Justification should be given for choice of tested dose levels (e.g. dose-finding studies): N/A?*
- *Cytotoxic concentrations with and without metabolic activation*
  Slight toxicity and precipitate on plate was only observed at 1000 and 3000 µg/plate in two parallels in TA100 without S9.
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:*
  No mutagenicity detected in Salmonella strains or E. Colis strains, with or without metabolic activation from rat liver S9.

- *Indicate test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they affect the selection of test concentrations or interpretation of the results:* Precipitate occurred from 250 and 500 µg/plate in two parallels in TA100, from 500 in TA98 without S9, and from 3000 µg/plate with 10% S9 in both strains. Precipitate occurred from 250 and 3000 µg/plate in two parallels in E. coli without S9 and 500 and 3000 µg/plate.
- *Concurrent negative (solvent/vehicle) and positive control data*
  The positive controls were clearly positive, and the negative clearly negative

Copy of Table E2 from the NTP TR 587 (2014):

TABLE E2
Mutagenicity of Tetrabromobisphenol A in Bacterial Tester Strains[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% rat S9	With 10% rat S9
TA100					
	0	69 ± 3	87 ± 7	91 ± 9	87 ± 1
	50	62 ± 2	59 ± 4	74 ± 3	
	100	54 ± 2	48 ± 4	70 ± 2	85 ± 4
	250	37 ± 2[b]	42 ± 7	62 ± 7	68 ± 4
	500	37 ± 8[b]	33 ± 5[b]	43 ± 4	55 ± 3
	1,000	14 ± 0[c]	26 ± 4[b]	52 ± 5	49 ± 3
	3,000	20 ± 7[c]	49 ± 3[b]	53 ± 8[b]	33 ± 4[b]
	6,000				58 ± 6[b]
Trial summary		Negative	Negative	Negative	Negative
Positive control[d]		470 ± 10	524 ± 14	750 ± 21	491 ± 34
TA98					
	0	18 ± 4	20 ± 2	23 ± 4	16 ± 2
	50	15 ± 1	21 ± 2	24 ± 4	
	100	11 ± 1	16 ± 3	23 ± 4	24 ± 3
	250	13 ± 1	13 ± 2	21 ± 1	18 ± 4
	500	6 ± 1[b]	12 ± 1[b]	15 ± 1	15 ± 1
	1,000	9 ± 2[b]	9 ± 4[b]	21 ± 2	14 ± 2
	3,000	8 ± 1[b]	12 ± 5[b]	12 ± 1[b]	8 ± 1[b]
	6,000				8 ± 0[b]
Trial summary		Negative	Negative	Negative	Negative
Positive control		528 ± 31	629 ± 17	1,878 ± 79	1,078 ± 55
***Escherichia coli* WP2 *uvrA*/pKM101 (analogous to TA102)**					
	0	138 ± 8	128 ± 8	118 ± 6	187 ± 11
	50		166 ± 13	119 ± 5	
	100	112 ± 2	155 ± 13	113 ± 9	165 ± 4
	250	114 ± 3[b]	161 ± 9	103 ± 11	184 ± 13
	500	112 ± 15[b]	127 ± 27	121 ± 12[b]	159 ± 6
	1,000	95 ± 4[b]	102 ± 4	78 ± 6[b]	131 ± 3
	3,000	120 ± 9[b]	94 ± 8[b]	98 ± 8[b]	117 ± 8[b]
	6,000	143 ± 2[b]			122 ± 9[b]
Trial summary		Negative	Negative	Negative	Negative
Positive control		1,450 ± 73	1,123 ± 65	1,399 ± 20	1,286 ± 39

[a] Study was performed at ILS, Inc., using lot M032607KA. Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.
[b] Precipitate on plate
[c] Slight toxicity and precipitate on plate
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

3.8.1.2 [Study 2] Mortelmans et al., 1986

Study reference:

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., and Zeiger, E. (1986). Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environ. Mutagen. 8 (Suppl. 7), 1-119.

Detailed study summary and results: Standardised test, study commissioned from NTP

Test type

Bacterial mutagenicity test in Salmonella typhimurium TA100, TA1535, TA1537 and TA98

- *number of replicates:* When negative results were obtained in the initial assay, the chemicals were retested in all strains with and without metabolic activation.
- *number of doses, justification of dose selection*
- *positive and negative control groups and treatment:* The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-o-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.
- *details on slide preparation*: N/A
- *number of metaphases analysed*: N/A
- *justification for choice of vehicle:* N/A. The study concerns a high number of substances and the vehicle for TBBPA was not specified further: "The solvent of choice was distilled water; dimethyl sulfoxide (DMSO) was used for chemicals that were not soluble in water or soluble at low concentrations only. Ethanol (95%) or acetone was used for chemicals insoluble in water or DMSO."
- *solubility and stability of the test substance in vehicle if known*: N/A
- *description of follow up repeat study*: N/A
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations)*

Details on GLP not given, but the study was commissioned by NTP and assumed to be GLP compliant.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* TBBPA, but is a different lot of chemical than was used in the NTP animal studies, as it is a previously performed test, but it is equivalent to the substance identified in the CLH dossier.
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- Degree of purity*:* N/A
- *Impurities (or a note that the impurities do not affect the classification):* N/A
- *Batch number:* N/A

Administration/exposure

- Salmonella typhimurium tester strains TA98, TA100, TA1535, and TA1537 (*NB! TA1537 was replaced by TA97 by NTP in tests after 1983, so this test must have been done before 1983 (Mortelmans et al., 1986)*
- *Type and composition of metabolic activation system:* Incubation for 20 minutes / 2 days at 37º C
  - S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver)
  - *chemicals used for induction:* Aroclor 1254
  - *co-factors used:* metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver
- *Test concentrations, and reasoning for selection of doses if applicable:* 0, 100, 333, 1000, 3333 and 10000 µg/plate. The dose level used for each positive control chemical was selected by the laboratory (SRI) and was based on the dose-response curves of the chemicals in the laboratory.
- *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water):* see above
- *Statistical methods:* N/A

Results and discussion

- *Justification should be given for choice of tested dose levels (e.g. dose-finding studies):* N/A
- *Cytotoxic concentrations with and without metabolic activation:* N/A
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* Negative. No evidence of mutagenicity with or without metabolic activation.
- *Concurrent negative (solvent/vehicle) and positive control data:* The positive controls were clearly positive, and the negative clearly negative.
- *Indicate test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they affect the selection of test concentrations or interpretation of the results:* Precipitate occurred on plate from 333 µg/plate without S9 in all strains and from 1000 µg/plate with 10% rat/hamster S9 also in all strains.
- *Statistical results:* N/A
- *Provide information that may be needed to adequately assess data for reliability*
  - *frequency of reversions/mutations/aberrations, polyploidy*
  - *mean number of revertant colonies per plate and standard deviation, number of cells with chromosome aberrations and type of chromosome aberrations given separately for each treated and control culture,*
  - *precipitation concentration if applicable*
  - *mitotic index*

Copy of Table E1 from the NTP TR 587 (2014):

TABLE E1
Mutagenicity of Tetrabromobisphenol A in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% hamster S9	With 10% hamster S9	With 10% rat S9	With 10% rat S9
TA100							
	0	105 ± 5	93 ± 4	104 ± 3	87 ± 3	112 ± 2	94 ± 9
	100	99 ± 13	99 ± 13	95 ± 5	107 ± 4	125 ± 7	115 ± 9
	333	110 ± 6[b]	74 ± 2[b]	91 ± 20	85 ± 6	114 ± 6	93 ± 10
	1,000	90 ± 9[b]	78 ± 3[b]	86 ± 8[b]	69 ± 4[b]	97 ± 4[b]	84 ± 3[b]
	3,333	88 ± 3[b]	79 ± 5[b]	79 ± 11[b]	79 ± 4[b]	92 ± 3[b]	96 ± 6[b]
	10,000	100 ± 2[b]	76 ± 3[b]	98 ± 9[b]	77 ± 9[b]	78 ± 8[b]	90 ± 5[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		372 ± 10	345 ± 18	1,521 ± 83	1,133 ± 40	645 ± 9	441 ± 9
TA1535							
	0	27 ± 3	28 ± 4	10 ± 2	6 ± 0	10 ± 1	10 ± 1
	100	23 ± 2	26 ± 1	12 ± 1	9 ± 2	8 ± 2	8 ± 2
	333	22 ± 1[b]	24 ± 3[b]	9 ± 2	8 ± 1	7 ± 1	7 ± 1
	1,000	14 ± 2[b]	20 ± 1[b]	8 ± 1[b]	8 ± 1[b]	5 ± 2[b]	4 ± 0[b]
	3,333	18 ± 1[b]	25 ± 3[b]	8 ± 3[b]	7 ± 1[b]	7 ± 1[b]	6 ± 1[b]
	10,000	17 ± 3[b]	24 ± 2[b]	7 ± 1[b]	6 ± 1[b]	6 ± 0[b]	9 ± 2[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		274 ± 13	324 ± 21	344 ± 9	452 ± 10	159 ± 20	187 ± 10
TA1537							
	0	4 ± 1	7 ± 3	5 ± 1	6 ± 2	6 ± 1	3 ± 1
	100	4 ± 1	5 ± 0	6 ± 2	10 ± 3	8 ± 1	10 ± 3
	333	4 ± 1[b]	4 ± 0[b]	4 ± 1	5 ± 1	8 ± 1	8 ± 1
	1,000	3 ± 0[b]	4 ± 1[b]	4 ± 1[b]	5 ± 2[b]	3 ± 0[b]	7 ± 1[b]
	3,333	4 ± 1[b]	4 ± 1[b]	3 ± 0[b]	4 ± 1[b]	3 ± 0[b]	4 ± 0[b]
	10,000	4 ± 1[b]	3 ± 1[b]	4 ± 0[b]	7 ± 1[b]	5 ± 1[b]	6 ± 1[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		192 ± 6	154 ± 24	342 ± 12	339 ± 18	108 ± 4	105 ± 6
TA98							
	0	19 ± 1	15 ± 2	17 ± 1	30 ± 1	25 ± 2	25 ± 4
	100	18 ± 1	14 ± 1	28 ± 3	35 ± 3	26 ± 7	38 ± 3
	333	16 ± 1[b]	12 ± 3[b]	24 ± 2	18 ± 1	24 ± 3	20 ± 5
	1,000	12 ± 2[b]	12 ± 1[b]	13 ± 0[b]	20 ± 3[b]	17 ± 3[b]	20 ± 3[b]
	3,333	15 ± 3[b]	12 ± 2[b]	11 ± 1[b]	15 ± 2[b]	15 ± 2[b]	15 ± 3[b]
	10,000	16 ± 2[b]	11 ± 0[b]	14 ± 3[b]	23 ± 3[b]	16 ± 1[b]	13 ± 1[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		844 ± 37	354 ± 31	1,665 ± 37	1,444 ± 62	541 ± 11	404 ± 30

[a] Study performed at SRI International. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol and these data are presented by Mortelmans *et al*. (1986). 0 µg/plate was the solvent control.

[b] Precipitate on plate

[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

3.8.1.3 [Study 3] Dow Chemical Company, 1985, reported in EU RAR TBBPA, 2008

Study reference:

The Dow Chemical Company, 1985, summarized in EU risk assessment: United Kingdom, EU RAR TBBPA, 2008.

Detailed study summary and results (only a summary available in RAR):

Test type

Bacterial reverse mutation test in Salmonella typhimurium TA92, TA98, TA100, TA1535, TA1537 and TA1538**.**

According to the RAR, all of the Ames tests were conducted in a way that was largely compatible with current regulatory guidelines.

- *number of replicates*
- *number of doses, justification of dose selection*
- *positive and negative control groups and treatment*
- *details on slide preparation*
- *number of metaphases analysed*
- *justification for choice of vehicle*
- *solubility and stability of the test substance in vehicle if known*
- *description of follow up repeat study*
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations)*

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* TBBPA
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity: N/A*
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number*

Administration/exposure

- Salmonella typhimurium TA 92, TA98, TA100, TA1535, TA1537 and TA1538
- *Type and composition of metabolic activation system:* with and without metabolic activation.
  - *species and cell type*
  - *quantity*
  - *induced or not induced*
  - *chemicals used for induction*
  - *co-factors used*
- *Test concentrations, and reasoning for selection of doses if applicable:* 5, 10, 50, 100, 500 and 1000 µg/plate. Toxicity observed at higher concentrartions

- *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water):* N/A
- *Statistical methods*

Results and discussion

- *Justification should be given for choice of tested dose levels (e.g. dose-finding studies).* Cytotoxicity seen at higher levels than the test levels
- *Cytotoxic concentrations with and without metabolic activation:* Yes, at levels higher than the tested concentrations
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation*

  No increase in the number of revertant colonies.
- *Concurrent negative (solvent/vehicle) and positive control data:* Positive and solvent controls gave the appropriate responses
- *Indicate test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they affect the selection of test concentrations or interpretation of the results*
- *Statistical results*
- *Provide information that may be needed to adequately assess data for reliability*
  - *frequency of reversions/mutations/aberrations, polyploidy*
  - *mean number of revertant colonies per plate and standard deviation, number of cells with chromosome aberrations and type of chromosome aberrations given separately for each treated and control culture,*
  - *precipitation concentration if applicable*
  - *mitotic index*

3.8.1.4 [Study 4] Velsicol Chemical Company, 1977, reported in EU RAR TBBPA, 2008

Study reference: **Supporting study 5 in the REACH registration**

Velsicol Chemical Company, 1977, summarized in RAR, 2008

Reliability indicated in the REACH registration: 2 (reliable with restrictions).

No GLP data; conducted according to generally accepted guidelines; no analytical verification of test compound concentrations.

Detailed study summary and results:

Test type

Bacterial reverse mutation test in Salmonella typhimurium TA 92, TA98, TA100, TA1535, TA1537 and TA1538.

According to the RAR, all of the Ames tests were conducted in a way that was largely compatible with current regulatory guidelines.

- *number of replicates*
- *number of doses, justification of dose selection*
- *positive and negative control groups and treatment*
- *details on slide preparation*
- *number of metaphases analysed*
- *justification for choice of vehicle: N/A*
- *solubility and stability of the test substance in vehicle if known*
- *description of follow up repeat study*
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations)*

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Yes Tetrabromobisphenol-A (BP4-A)
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity*
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number*

Administration/exposure

- Salmonella typhimurium TA 92, TA98, TA100, TA1535, TA1537 and TA1538
- *Type and composition of metabolic activation system* (not given in summary from RAR, but given in the REACH registration):
  - *species and cell type*: Rat liver homogenate
  - *quantity*
  - *induced or not induced*
  - *chemicals used for induction*
  - *co-factors used*
- *Test concentrations, and reasoning for selection of doses if applicable:* 0.1, 1, 19, 100 and 500 µg/plate

- *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water):* Dimethyl sulphoxide (DMSO)
- *Statistical methods*

Results and discussion

- *Justification should be given for choice of tested dose levels (e.g. dose-finding studies)*
- *Cytotoxic concentrations with and without metabolic activation:* Evidence of some chemically-induced effects at highest dose tested
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* No mutagenic response with or without metabolic activation
- *Concurrent negative (solvent/vehicle) and positive control data*
- *Indicate test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they affect the selection of test concentrations or interpretation of the results*
- *Statistical results*
- *Provide information that may be needed to adequately assess data for reliability*
  - *frequency of reversions/mutations/aberrations, polyploidy*
  - *mean number of revertant colonies per plate and standard deviation, number of cells with chromosome aberrations and type of chromosome aberrations given separately for each treated and control culture,*
  - *precipitation concentration if applicable*
  - *mitotic index*

3.8.1.5 [Study 5] Israel Institute for Biological Research, 1978, reported in EU RAR TBBPA, 2008

Study reference:

Key study 1 in the REACH registration.

Israel Institute for Biological Research, 1978, summarized in RAR, 2008, stated there to be briefly reported

Reliability indicated in the REACH registration: 2 (reliable with restrictions).

No GLP data; conducted according to generally accepted guidelines; no analytical verification of test compound concentrations.

Detailed study summary and results:

Test type

Bacterial reverse mutation test in Salmonella typhimurium TA98, TA100, TA1535 and TA1537

According to the RAR, all of the Ames tests were conducted in a way that was largely compatible with current regulatory guidelines (OECD TG 471).

- *number of replicates*: two
- *number of doses, justification of dose selection*
- *positive and negative control groups and treatment:* Solvent negative control, positive control: N-methyl-N-nitroso-N-nitrosoguanidine and acridine orange
- *details on slide preparation*
- *number of metaphases analysed*
- *justification for choice of vehicle*
- *solubility and stability of the test substance in vehicle if known*
- *description of follow up repeat study*
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations)*

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Yes, Tetrabromobisphenol-A, TBBA
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity:* N/A
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number:* Not specified, lot received from Dead Sea Bromine Co

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable:* see above
- *Type and composition of metabolic activation system:* Study was carried out with and without metabolic activation, but no details about this is given.
  - *species and cell type*
  - *quantity*
  - *induced or not induced:* Rat liver microsomes from animal injected with Arochlor 1254
  - *chemicals used for induction*
  - *co-factors used*

- *Test concentrations:* 1, 10, 100 µg/plate. Duration not reported. Higher concentrations were not tested due to poor solubility of the test substance andprecipitation on the plates at doses higher than 100 ug per plate.
- *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)* Dimethyl sulfoxide DMSO
- *Statistical methods*

Results and discussion

- *Justification should be given for choice of tested dose levels (e.g. dose-finding studies)*
- *Cytotoxic concentrations with and without metabolic activation:* based on relative total growth, but no toxicity observed
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* Negative with and without metabolic activation.
- *Concurrent negative (solvent/vehicle) and positive control data*
- *Indicate test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they affect the selection of test concentrations or interpretation of the results*
- *Statistical results:* No statistics available
- *Provide information that may be needed to adequately assess data for reliability*
    - *frequency of reversions/mutations/aberrations, polyploidy*
    - *mean number of revertant colonies per plate and standard deviation, number of cells with chromosome aberrations and type of chromosome aberrations given separately for each treated and control culture,*
    - *precipitation concentration if applicable*
    - *mitotic index*

Results presented in table from ECHA´s dissemination site:

			Number of relevant colonies per plate							
Type	Tissue	Dose	TA98		TA100		TA1535		TA1537	
Solvent control	–	DMSO	46	34	200	300	151	108	1	0
Solvent control	Liver	DMSO	79	64	240	300	44	34	7	9
Positive control	–	20µg AO	33	33						
Positive control	Liver	20µg AO	1270	1961	1208	1136			129	100
Positive control	–	10µg MNNG			10000		13000	15072		
TBBA	–	1µg	47	37	249	178	116	128	6	5
TBBA	Liver	1µg	86	84	245	209	42	32	8	6
TBBA	–	10µg	23	45	212	218	98	89	8	14
TBBA	Liver	10µg	82	72	287	306	40	60	15	8
TBBA	–	100µg	45	51	45	51	85	83	6	1
TBBA	Liver	100µg	83	96	150	211	29	33	7	11

3.8.1.6 [Study 6] Litton Bionetics Inc., 1976, reported in EU RAR TBBPA, 2008

Study reference:

Supporting study 4 in the REACH registration

Litton Bionetics Inc., 1976, summarized in RAR TBBPA, 2008, stated there to be briefly reported]

Reliability indicated in the REACH registration: 2 (reliable with restrictions).

No GLP data; conducted according to generally accepted guidelines; no analytical verification of test compound concentrations.

Detailed study summary and results:

Test type

Bacterial reverse mutation test in Salmonella typhimurium TA92, TA98, TA100, TA1535, TA1537 and TA1538

According to the RAR, all of the Ames tests were conducted in a way that was largely compatible with current regulatory guidelines (OECD TG 471)

- *number of replicates*
- *number of doses, justification of dose selection*
- *positive and negative control groups and treatment*
- *details on slide preparation*
- *number of metaphases analysed*
- *justification for choice of vehicle*

- *solubility and stability of the test substance in vehicle if known*
- *description of follow up repeat study*
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations)*

No GLP data; conducted according to generally accepted guidelines; no analytical verification of test compound concentrations

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Compound 279-117-2
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity:* N/A
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number:* Not reported, received from Great Lakes Chemical Corp

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable: see above*
- *Type and composition of metabolic activation system:* Study was carried out with and without metabolic activation, Rat liver homogenate
  - *species and cell type*
  - *quantity*
  - *induced or not induced*
  - *chemicals used for induction*
  - *co-factors used*
- *Test concentrations, and reasoning for selection of doses if applicable:* 0.25, 0.5, 5 and 50 µg/plate
- *Vehicle:* DMSO
- *Statistical methods*

Results and discussion

- *Justification should be given for choice of tested dose levels (e.g. dose-finding studies)*
- *Cytotoxic concentrations with and without metabolic activation:* Evidence of chemically-induced physiological effects at highest dose
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* Negative with and without metabolic activation.

- *Concurrent negative (solvent/vehicle) and positive control data:* Positive controls: MNNG, NF, QM, ANTH, AAF, AMQ, DMNA
- *Indicate test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they affect the selection of test concentrations or interpretation of the results*
- *Statistical results*
- *Provide information that may be needed to adequately assess data for reliability*
  - *frequency of reversions/mutations/aberrations, polyploidy*
  - *mean number of revertant colonies per plate and standard deviation, number of cells with chromosome aberrations and type of chromosome aberrations given separately for each treated and control culture,*
  - *precipitation concentration if applicable*
  - *mitotic index*

	Revertants per plate					
	TA1535	TA1537	TA1538	TA-98	TA-100	D4
Solvent control	36	15	19	42	113	66
Positive control	>10^3	>10^3	>10^3	420	>10^3	155
0.25µg	55	9	15	45	121	63
0.5µg	51	11	13	47	129	59
5µg	50	13	11	41	100	59
50µg	31	8	7	49	102	8

Table 2: Summary of Test Plate Results (with activation)

	Revertants per plate					
	TA1535	TA1537	TA1538	TA-98	TA-100	D4
Solvent control	28	17	24	20	156	37
Positive control	132	636	>10^3	>10^3	520	44
0.25µg	27	27	31	31	144	35
0.5µg	29	22	26	26	137	34
5µg	32	19	28	28	130	37
50µg	32	19	28	28	127	35

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3.8.1.7 [Study 7] Ethyl Corporation, 1981, reported in EU RAR TBBPA, 2008

Study reference:

Key study 2 in the REACH registration

Ethyl Corporation, 1981, summarized in RAR, 2008, two studies, the second stated in RAR to be briefly reported

Reliability indicated in the REACH registration: 2 (reliable with restrictions)

No GLP data; conducted according to generally accepted guidelines; no analytical verification of test compound concentrations

Detailed study summary and results:

Test type

Bacterial reverse mutation test in Salmonella typhimurium TA98, TA100, TA1535, TA1537 and TA1538

According to the RAR, all of the Ames tests were conducted in a way that was largely compatible with current regulatory guidelines (OECD TG 471)

- *number of replicates:* first study: triplicate
- *number of doses, justification of dose selection:* first study: 5
- *positive and negative control groups and treatment:* first study: Yes, but no details given. Positive control: 9-aminoacridine
- *details on slide preparation*
- *number of metaphases analysed*
- *justification for choice of vehicle*
- *solubility and stability of the test substance in vehicle if known*
- *description of follow up repeat study*
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations)*

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Saytex RB-100 (T1685) (equivalent, trade name)
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity:* Not reported
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number:* Saytex RB-100 (T1685): R6/FD2

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable*

- *Type and composition of metabolic activation system:* first study: Study was carried out with and without metabolic activation, but no details about this is given.
  - *species and cell type*
  - *quantity*
  - *induced or not induced*
  - *chemicals used for induction*
  - *co-factors used*
- *Test concentrations, and reasoning for selection of doses if applicable: 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
- *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)*
- *Statistical methods*

Results and discussion

- *Justification should be given for choice of tested dose levels (e.g. dose-finding studies)*
- *Cytotoxic concentrations with and without metabolic activation: First study:* Toxicity was apparent at the higher concentrations
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation: First study:* No significant increase in the number of revertant colonies observed
- *Concurrent negative (solvent/vehicle) and positive control data: First study:* within appropriate range
- *Indicate test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they affect the selection of test concentrations or interpretation of the results*
- *Statistical results*
- *Provide information that may be needed to adequately assess data for reliability*
  - *frequency of reversions/mutations/aberrations, polyploidy*
  - *mean number of revertant colonies per plate and standard deviation, number of cells with chromosome aberrations and type of chromosome aberrations given separately for each treated and control culture,*
  - *precipitation concentration if applicable*
  - *mitotic index*

		Number of relevant colonies per plate				
Type	Dose	TA1535	TA1537	TA1538	TA100	TA98
Negative control	Bacteria only	14.3 ± 5.1	15.0 ± 3.0	7.7 ± 2.5	86.0 ± 20.0	19 ± 4
Solvent control	100µL	10.7 ± 0.6	9.0 ± 2.6	7.0 ± 2.0	81.0 ± 17.3	10 ± 1
Sodium azide	30µg	1074 ± 126.2	NT	NT	1105.0 ± 77.8	NT
9-AA	10µg	NT	442.7 ± 84.7	NT	NT	NT
2-NF	5µg	NT	NT	451.7 ± 84.7	NT	374 ± 61
Saytex RB-100	0.005mg	8.0 ± 1.7	17.0 ± 6.0	10.0 ± 3.6	72.7 ± 9.3	12 ± 2
Saytex RB-100	0.015mg	14.7 ± 5.5	8.7 ± 1.5	8.0 ± 3.0	79.7± 9.3	12 ± 4
Saytex RB-100	0.05mg	11.0 ± 4.0	18.0 ± 3.0	7.7 ± 2.3	76.3 ± 2.5	9 ± 1
Saytex RB-100	0.15mg	7.7 ± 1.5	8.7 ± 2.1	8.3 ± 3.5	63. 3± 9.1	8 ± 3
Saytex RB-100	0.5mg	8.7 ± 4.0	8.0 ± 1.7	5.7 ± 1.5	67.3 ± 11.5	9 ± 2

NT= Not tested

Table 2: Results of Salmonella/Microsomal Assay with Metabolic Activation

		Number of relevant colonies per plate				
Type	Dose	TA1535	TA1537	TA1538	TA100	TA98
Negative control	Bacteria only	14.3± 5.1	15.0± 3.0	7.7± 2.5	86.0± 20.0	19± 4
Solvent control +S-9	100µL	13.7± 3.1	30.7± 8.6	11.7± 5.0	117.7± 8.3	25.3± 7.4
2-AA + S-9	5µg	350.3± 16.9	799.0± 47.7	1903.3± 12.6	4142.3±817.4	TNTC
Saytex RB-100	0.005mg	11.0± 1.0	15.0± 4.4	21.0± 3.0	119.3± 21.0	23.7± 4.0
Saytex RB-100	0.015mg	9.7± 3.2	18.7± 3.2	24.0± 9.6	114.0± 19.5	25.7± 2.9
Saytex RB-100	0.05mg	11.3± 5.1	21.7± 2.1	27.0 ± 7.8	141.0± 25.7	35.3± 9.3
Saytex RB-100	0.15mg	8.7± 2.1	10.3± 2.5	28.0± 6.1	102.7± 18.2	24.3± 1.5
Saytex RB-100	0.5mg	8.3± 2.5	10.3± 2.1	8.7± 4.0	105.0± 6.2	28.7± 5.0

TNTC: Too Numerous to Count

3.8.1.8 [Study 8] BioReliance, 2001, reported in EU RAR TBBPA 2008

Study reference:

Key study 3 in the REACH registration

BioReliance, 2001

Reliability indicated in the REACH registration: 1 (reliable without restriction).

Detailed study summary and results:

The study was assessed as well conducted and well reported in the RAR: GLP study; conducted according to standard OECD guidelines; no analytical verification of test compound concentrations. Study perfomed equivalent or similar to OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)

Test type

In vitro mammalian chromosome aberration test in human peripheral blood lymphocytes

According to the RAR the study was well-conducted

- *number of replicates:* Duplicate cultures of human peripheral blood lymphocytes (HPBL)
- *number of doses, justification of dose selection*
- *positive and negative control groups and treatment:* Appropriate positive and solvent controls were used in all assays
- *details on slide preparation*
- *number of metaphases analysed:* A minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromatid and chromosome type aberrations
- *justification for choice of vehicle:* Solubility
- *solubility and stability of the test substance in vehicle if known:* Soluble in vehicle up to 500 mg/l
- *description of follow up repeat study*
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations)*

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Yes, Tetrabromobisphenol-A
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity:* 98.91%
- *Impurities (or a note that the impurities do not affect the classification):* o,p-tetrabromobisphenol-A: 0.05%, 2,4,6-Tribromophenol: <0.01%, Tribromobisphneol-A: 1.04%
- *Batch number:* AA47PV

Administration/exposure

- *Cell type:* Human peripheral blood lymphocytes (HPBL) exposed for 4 or 20 hours. All cells were harvested after 20 hours.
  Preliminary assay: Obtained from a healthy non-smoking 24-year-old male
  Definitive assay: Obtained from a healthy non-smoking 22-year-old male
- *Type and composition of metabolic activation system:*
  - *species and cell type*
  - *quantity*

  - *induced or not induced:* Cells exposed in presence and absence of the S9 activation system (rat liver homogenate)
  - *chemicals used for induction:* Aroclor-induced S9 activation system
  - *co-factors used*
- *Test concentrations:* preliminary toxicity test conducted to establish test concentrations in main study. Highest dose in main study induced at least 50% toxicity. 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. Positive control mitomycin C.
- *Vehicle:* DMSO (test substance soluble in DMSO up to 500 mg/l). For the 20 hour exposure assay doses of 0, 6.25, 25, and 75 µg/ml were used.
- *Statistical methods*

Results and discussion

- *Justification should be given for choice of tested dose levels (e.g. dose-finding studies)*
- *Cytotoxic concentrations with and without metabolic activation:* At doses greater than or equal to 150 ug/mL
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* At no concentration of TBBPA was the percentage of metaphases with structural and numerical aberrations statistically significantly greater than that of the solvent control
- *Concurrent negative (solvent/vehicle) and positive control data:* The solvent and positive controls gave the expected responses
- *Indicate test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they affect the selection of test concentrations or interpretation of the results*
- *Statistical result:s* No statistically significant increases in structural or numerical chromosome aberrations were observed in the non-activated or S9 activated 4 hour exposure groups relative to the solvent control group, regardless of dose level ($p>0.05$, Fisher's exact test). No statistically significant increases in structural or numerical chromosome aberrations were observed in the non-activated 20 hour continuous exposure group relative to the solevnt control group, regardless of dose level ($p>0.05$, Fisher's exact test).
- *Provide information that may be needed to adequately assess data for reliability*
  - *frequency of reversions/mutations/aberrations, polyploidy*
  - *mean number of revertant colonies per plate and standard deviation, number of cells with chromosome aberrations and type of chromosome aberrations given separately for each treated and control culture,*
  - *precipitation concentration if applicable*

– *mitotic index*

		Miotic Index (%)		Average aberrations per cell	
Treatment	Flask	4hr	20hr	4hr	20hr
DMSO	A	12.0	11.8	0.010	0.010
	B	11.0	12.6	0.000	0.000
6.25µg/mL TBBPA	A	10.4	11.0	0.000	0.000
	B	10.0	10.0	0.000	0.000
25µg/mL TBBPA	A	7.0	9.6	0.000	0.000
	B	7.2	8.8	0.000	0.000
100µg/mL TBBPA	A	5.0	4.8	0.000	0.000
	B	5.6	5.2	0.000	0.000
MMC 0.6µg/mL	A	7.0	5.8	0.150	0.120
	B	7.6	6.6	0.100	0.190

Table 2: Cytogenetic Analysis of Human Peripheral Blood Lymphoctyes Treated with TBBF

Treatment	Flask	Miotic Index (%)	Average aberrations per cell
DMSO	A	7.6	0.000
	B	8.4	0.000
6.25µg/mL TBBPA	A	7.0	0.000
	B	7.8	0.000
25µg/mL TBBPA	A	5.6	0.000
	B	6.0	0.000
100µg/mL TBBPA	A	3.0	0.000
	B	3.8	0.000
MMC 0.6µg/mL	A	2.0	0.070
	B	2.2	0.120

3.8.1.9 [Study 9] Helleday et al., 1999, reported in EU RAR TBBPA 2008

Study reference:

Helleday et al., 1999

Detailed study summary and results: *Study assumingly perfomed equivalent or similar to OECD Guideline 476 (In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes)*

Test type:

Intragenic Sp5/V79 and SPD8 recombination assays in mammalian cells (Chinese hamster cells)
Unconventional study.

- *number of replicates*
- *number of doses, justification of dose selection*
- *positive and negative control groups and treatment*
- *details on slide preparation*
- *number of metaphases analysed*
- *justification for choice of vehicle*
- *solubility and stability of the test substance in vehicle if known*
- *description of follow up repeat study*
- *criteria for evaluating results (e.g. cell evaluated per dose group, criteria for scoring aberrations)*

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* TBBPA
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity*
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number*

Administration/exposure

- *Strain or cell type or cell line, target gene if applicable:* Sp5/V79 and SPD8 (Chinese hamster cells). The clones in this assays have a duplication of the *hprt* gene which gives rise to a non-functional HGPRT protein. Both assays assess the ability of test substances to increase the reversion frequency of the mutant gene to the functional *hprt* gene phenotype and thus the ability of test substances to introduce intragenic recombinations. Cells were incubated for 24 hours with TBBPA
- *Type and composition of metabolic activation system:*
  - *species and cell type*
  - *quantity*
  - *induced or not induced*

  - *chemicals used for induction*
  - *co-factors used*
- *Test concentrations, and reasoning for selection of doses if applicable:* Dose levels were 0, 5, 10, 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.
- *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)*
- *Statistical methods*

Results and discussion

- *Justification should be given for choice of tested dose levels (e.g. dose-finding studies)*
- *Cytotoxic concentrations with and without metabolic activation:* Cloning efficiency and growth inhibition were assessed as a measure of cytotoxicity
- *Genotoxic effects (e.g. positive, negative, unconfirmed, dose-response, equivocal) with and without metabolic activation:* 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)
- *Concurrent negative (solvent/vehicle) and positive control data:* No data presented on the positive control (camptothecin 100nm)
- *Indicate test-specific confounding factors such as pH, osmolarity, whether substance is volatile, water soluble, precipitated, etc., particularly if they affect the selection of test concentrations or interpretation of the results*
- *Statistical results*
- *Provide information that may be needed to adequately assess data for reliability*
  - *frequency of reversions/mutations/aberrations, polyploidy*
  - *mean number of revertant colonies per plate and standard deviation, number of cells with chromosome aberrations and type of chromosome aberrations given separately for each treated and control culture,*
  - *precipitation concentration if applicable*
  - *mitotic index*

3.8.2 **Animal data**

3.8.2.1 [Study 1] NTP TR 587, 2014 p 140

National Toxicology Program. 2014; Toxicology studies of teterabromobisphenol A (Cas no. 79-94-7) in F344/NTac rats and B6C3F1/N mice and toxicology and carcinogeogenesis studies of tetrabromobisphenol A in Wistar Han [Crl:WI(Han)] rats and B6C3F1/N mice (gavage studies). NTP Technical Report 587

Detailed study summary and results:

Test type

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST (NTP TR 587, 2014)

The study was carried out on mice exposed for three months, details on this study is reported in the paragraph for STOT-RE (see section 3.12.1.1). The method/detailed protocol for the micronucleus test is described by MacGregor et al., 1990 reference given in the NTP-report (2014).

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Yes, the test material was TBBPA.
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity:* > 99%
- *Impurities (or a note that the impurities do not affect the classification):* N/A
- *Batch number:* Lot 25317K-1

Test animals

- *Species/strain/sex:* male or female B6C3F1/N mice
- *No. of animals per sex per dose:* see section 3.12.1.1
- *Age and weight at the study initiation*

Administration/exposure: *see details in section 3.12.1.1*

- *Doses:* 0, 10, 50, 100, 500 and 1000
- *Vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)*
- *Details on test system and conditions, and details on route of administration, exposure*
- *Actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable*
- *Duration of study, frequency of treatment, sampling times and number of samples:* At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice.

- *Control groups and treatment*
- *Positive and negative (vehicle/solvent) control data*
- *Methods of slide preparation:* Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of five animals per dose group.
- *Criteria for scoring and number of cells analysed per animal:* see above
- *Statistical methods:* One-tailed Cochran-Armitage trend test, followed by pairwise comparisons

Results and discussion

- *Effect on mitotic index or PCE/NCE (polychromatic erythrocyte/normochromatic erythrocyte) ratio by dose level by sex (if applicable):*
  The percentage of polychromatic (immature) erythrocytes (PCEs) in a population of 1,000 erythrocytes in the peripheral blood was scored for each dose group as a measure of bone marrow toxicity. No effect on micronucleated NCEs was oberserved. In addition, no significant changes in the percentage of circulating polychromatic (immature) erythrocytes (PCEs) were observed in dosed mice, suggesting that tetrabromobisphenol A did not induce bone marrow toxicity over the dose range tested.
- *Genotoxic effects (positive, negative, unconfirmed, dose-response, equivocal)*
  No increases in micronucleated NCEs were observed in male or female B6C3F1/N mice following 3 months of administration of tetrabromobisphenol A by gavage over a dose range of 10 to 1,000 mg/kg (Table E3).
- *Concurrent positive control data:* N/A
- *Statistical results:* Cochran-Armitage trend test: no significant findings.
- *Describe additional information that may be needed to adequately assess data for reliability and use, including the following, if available: Mortality at each dose level by sex:* No mortality observed.
  - *mutant/aberration/mPCE/polyploidy frequency*
  - *description, severity, time of onset and duration of clinical signs at each dose level and sex:* See section 3.12.1.1.
  - *body weight changes by dose and sex:* See section 3.12.1.1.
  - *food/water consumption changes by dose and sex:* See section 3.12.1.1.
- *Discuss if it can be verified that the test substance reached the general circulation or target tissue, if applicable:* Chemical-related effects on liver enzymes, organ weights, and kidney lesions indicate that the test substance reached the general circulation.

Copy of Table E3 from the NTP TR 587 (2014):

TABLE E3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Tetrabromobisphenol A by Gavage for 3 Months[a]

	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Corn oil[d]	0	5	1.70 ± 0.75		2.54 ± 0.31
Tetrabromobisphenol A	10	5	1.20 ± 0.30	0.7426	2.98 ± 0.38
	50	5	1.70 ± 0.82	0.5000	2.72 ± 0.25
	100	5	2.90 ± 0.68	0.1072	3.04 ± 0.32
	500	5	2.50 ± 0.76	0.1932	3.88 ± 0.40
	1,000	5	1.90 ± 0.24	0.4075	2.70 ± 0.37
			P=0.334[e]		
Female					
Corn oil	0	5	1.00 ± 0.27		3.16 ± 0.25
Tetrabromobisphenol A	10	5	1.60 ± 0.51	0.1195	2.90 ± 0.47
	50	5	1.20 ± 0.41	0.3348	3.34 ± 0.56
	100	5	1.10 ± 0.29	0.4136	2.84 ± 0.14
	500	5	1.60 ± 0.19	0.1195	2.98 ± 0.16
	1,000	5	1.20 ± 0.41	0.3348	2.30 ± 0.31
			P=0.431		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] Pairwise comparison with the vehicle control group; dosed group values are significant at P≤0.005
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at P≤0.025

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 [Study 1] NTP TR 587, 2014 and Dunnick et al., 2015 - 2 years carcinogenicity study in rats

National Toxicology Program. 2014; Toxicology studies of teterabromobisphenol A (Cas no. 79-94-7) in F344/NTac rats and B6C3F1/N mice and toxicology and carcinogeogenesis studies of tetrabromobisphenol A in Wistar Han [Crl:WI(Han)] rats and B6C3F1/N mice (gavage studies). NTP Technical Report 587

Dunnick et al., 2015, Environmental chemical exposure may contribute to uterine cancer development: studies with tetrabromobisphenol A, Toxicol Pathol. 2015 June; 43(4):464-473

Detailed study summary and results:

Test type

2 years carcinogenicity study in rats.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier
- *Degree of purity:* > 99%
- *Impurities:* 0.8-1.1% tribromo-BPA (not expected to affect the classification)
- *Batch number:* (25317K-1, C16263X, and 25337XX-8). Lot 25317K-1 was used during the 3-month studies; lots 25317K-1 and C16263X were combined into one lot and renamed lot M032607KA, which was used in the 2-year studies.

Test animals

- Wistar Han rats
- 60 male and 60 female rats per dose in dose group 0 and 1000 mg/kg bw (10 in each used for interim evaluation after 3 months. Interim evaluation included weighing of organs - heart, right kidney, liver, lung, right testis and thymus, and was done to compare with the 3-month endpoints in the F344/NTac rats, see section 3.12.1)
- 50 male and 50 female rats per dose in dose group 250 and 500 mg/kg bw
- Rats were 6 to 7 weeks old at the beginning of the study.

Administration/exposure

- *Route of administration* – By gavage in corn oil, 5 days per week for up to 104 (male rats) or 105 weeks duration of test/exposure period
- *doses/concentration levels, rationale for dose level selection:* In the 2-year study in rats the doses were 0, 250, 500 and 1000 mg/kg bw. The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to tetra-bromobisphenol A and to determine the appropriate doses to be used in the 2-year studies. TBBPA-related effects on organ weights, thyroid hormones, hematology parameters, and liver enzymes seen in the 3-month study were not considered to cause increased mortality in the 2-year study (see section 3.12.1 on STOT-RE studies).

- *frequency of treatment:* daily, 5 days per week
- *control group and treatment:* Corn oil
- *historical control data:* 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. The current 2-year rat study is the only one in Wistar Han rats using corn oil as a gavage vehicle in the historical control database; therefore, historical control incidences for all routes and all vehicles are used for Wistar Han rats in this Technical Report. The historical control database does not contain data for residual tissue evaluations or step sections of tissues.
- *post exposure observation period:* -
- *vehicle:* Corn oil
- *test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:* The determined concentration in the doses were close to the target concentration all through the 2-year study, and varied from -9 to +18% different from target dose on single days, for details see Appendix J in the NTP report.

Results and discussion *(for discussion of the study in mice, see section 3.9.1.2 below)*

Summary table from NTP TR 587, 2014 (page 10):

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Tetrabromobisphenol A

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice[a]	Female B6C3F1/N Mice[a]
Doses in corn oil by gavage	0, 250, 500, or 1,000 mg/kg	0, 250, 500, or 1,000 mg/kg	0, 250, 500, or 1,000 mg/kg	0, 250, 500, or 1,000 mg/kg
Body weights	500 and 1,000 mg/kg groups at least 10% less than the vehicle control group after week 25	Dosed groups within 10% of the vehicle control group	Dosed groups within 10% of the vehicle control group	1,000 mg/kg group at least 10% less than the vehicle control group after week 25
Survival rates	33/50, 28/50, 38/50, 39/50	35/50, 34/50, 29/50, 33/50	33/50, 26/50, 39/50, 12/50	40/50, 31/50, 36/50, 4/50
Nonneoplastic effects	None	Uterus: endometrium, hyperplasia, atypical (residual longitudinal review-2/50, 13/50, 11/50, 13/50) Ovary: rete ovarii cyst (1/50, 0/49, 6/50, 6/49)	Liver: clear cell focus (11/50, 10/50, 25/50); eosinophilic focus (20/50, 33/50, 40/50) Kidney: renal tubule, cytoplasmic alteration (0/50, 20/50, 47/50) Forestomach: ulcer (9/50, 9/49, 19/50); infiltration cellular, mononuclear cell (5/50, 8/49, 21/50); inflammation (9/50, 10/49, 20/50); epithelium, hyperplasia (10/50, 13/49, 27/50)	Forestomach: ulcer (2/50, 15/50, 40/50); infiltration cellular, mononuclear cell (2/50, 13/50, 33/50); inflammation (2/50, 14/50, 41/50); epithelium, hyperplasia (4/50, 16/50, 39/50)
Neoplastic effects	None	Uterus: adenoma (original transverse review-0/50, 0/50, 3/50, 4/50); adenocarcinoma (original transverse review-3/50, 3/50, 8/50, 9/50; original transverse and residual longitudinal reviews, combined-4/50, 10/50, 15/50, 16/50); malignant mixed Müllerian tumor (original transverse review-0/50, 4/50, 0/50, 2/50); adenoma, adenocarcinoma, or malignant mixed Müllerian tumor (original transverse review-3/50, 7/50, 11/50, 13/50; original transverse and residual longitudinal reviews, combined-6/50, 11/50, 16/50, 19/50)	Liver: hepatoblastoma (2/50, 11/50, 8/50)	None
Equivocal findings	Testis: interstitial cell, adenoma (0/50, 0/50, 1/50, 3/50)	None	Large intestine (cecum or colon): adenoma or carcinoma (0/50, 0/50, 3/50) Hemangiosarcoma (all organs): (1/50, 5/50, 8/50)	None
Level of evidence of carcinogenic activity	Equivocal evidence	Clear evidence	Some evidence	No evidence

- *mortality and time to death (indicate number died per sex per dose and time to death):* No effects. Survival rates were 33/50, 28/50, 38/50, 39/50 in male rats and 35/50, 34/50, 29/50, 33/50 in female rats
- *clinical signs:* No effects
- *body weight gain:* The mean body weight of male rats in the two highest dose groups were generally at least 10 % lower after 25 weeks than in the control group. This did not occur in females where the body weights were similar to the controls throughout the study.
- *food/water consumption:* N/A
- *ophthalmoscopic examination:* N/A
- *clinical chemistry:* None (see NTP report table 1, page 31)
- *haematology:* None (performed in the 3 months study)
- *urinalysis:* N/A
- *organ weights: :* 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.
- *necropsy findings: nature and severity.* Necropsy was performed on all animals. No treatment-related lesions occurred in the top dose rats at 3-month interim evaluation.
- *histopathological findings: nature and severity*

  Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, cervix, clitoral gland, esophagus, eyes, gallbladder (mice only), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina.

  The incidence of atypical endometrium hyperplasia increased significantly in dosed female rats (2/50, 13/50, 11/50, 13/50 in the control and three dose groups, respectively).
- *tumour incidence data by sex, dose and tumour type*

  In female rats there were significant increases in the incidences of malignant tumors of the uterus in the dose groups 500 and 1000 mg/kg bw (details given below), and increased incidence of epithelial atypical hyperplasia at 250 mg/kg bw. Three male rats receiving 1,000 mg/kg of tetrabromobisphenol A had uncommon adenomas of the testis. No other tumour types were significantly increased in dosed animals.

Uterus: Transverse sections were made through each uterine horn, without investigating the cervix/vagina unless there were gross lesions. To determine the primary location for adenocarcinomas in the cervix and vagina, and to review all the cervices for hyperplasia and fibrosis, residual longitudinal tissue review (i.e. longitudinal sectioning) was carried out on remaining formalin fixed cervix, vagina and uterine remnants.

Table 5: 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 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)

Table 6: Neoplasms of the uterus in female Wistar Han rats in the 2 year gavage study, based on overall rates:

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**
Adenoma	0	0	3	4
Adenocarcinoma	3	3	8	9
Malignant Mixed Müllerian Tumor	0	4	0	2
Residual longitudinal review				
Adenoma, Adenocarcinoma, or Malignant Mixed Müllerian Tumor	6**	10	16**	16**
Adenoma	3	2	1	3
Adenocarcinoma	4	9	15	15
Malignant Mixed Müllerian Tumor	0	0	0	1
Atypical hyperplasia	2	13**	11**	13**
Combined original transverse and residual longitudinal reviews				
Adenoma, Adenocarcinoma, or Malignant Mixed Müllerian Tumor	6**	11	16**	19**
Adenoma	3	2	4	6
Adenocarcinoma	4	10	15	16
Malignant Mixed Müllerian Tumor	0	4	0	2
Atypical 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

Historical control data:

Historical control incidence for uterine adenoma in original transverse review for 2-year studies (all routes): 0/150. In this study the incidence was 0/50 in the control group.

Historical control incidence for uterine adenocarcinoma in original transverse review for 2-year studies (all routes): 7/150 (includes one endometrium carcinoma). In this study the incidence was 3/50 in the control group.

Testis and ovary:

male rats, there was a significant trend in the incidence of interstitial cell adenoma (includes bilateral) (0/50, 0/50, 1/50, 3/50, trend), as three male rats receiving 1,000 mg/kg of tetrabromobisphenol A had uncommon adenomas of the testis. The incidence exceeded the historical control incidence (4/150), but

was not statistically significant with pairwise comparison. Atrophy in germinal epithelium of the testes occurred in 0, 4, 1 and 2 animals in the groups, corresponding to 0, 8, 2 and 4% of the animals in the control, low, medium and high dose group. Severity of the lesion increased with increasing dose, but was not significantly different from controls.

In female rats, the incidences of rete ovarii cyst were significantly increased in the two highest dose groups (6 cases in both groups compared to 1 and 0 in the control and low dose group, respectively).

- *local or multi-site responses:* Clear evidence of carcinogenicity from TBBPA was only found in female rats uteri.
- *progression of lesions to malignancy:* Yes. Uterine tumour metastases were found in several organs in female rats. Uterine tumor metastases were found in the intestine, liver, mesentery, pancreas, glandular stomach, adrenal cortex, lymph nodes, spleen, thymus, skeletal muscle, lung, kidney, and urinary bladder. Also malignant mixed Müllerian cell tumours were seen in female rats.
- *gender and/or species-specific responses:* Female rats
- *tumour incidence data by sex, dose and tumour type:* see above
- *mode of action (genotoxic, non-genotoxic):* There was a significantly higher incidence of mutations in the tumour suppressor gene *Tp53* in uterine adenocarcinomas from rats treated with TBBPA compared to spontaneous tumours in controls. This suggest that the mode of action for the uterine tumours are at least partly driven by alterations in the *Tp53* signaling pathway.
- *toxic response data by sex and dose:* The incidence of atypical endometrium hyperplasia increased significantly in dosed female rats, see numbers above
- *tumour latency:* Reduced latency based on days of onset.
- *statistical methods and results (unless already described with specific test results above): Text copied from Dunnick et al., 2015: "*Survival was compared among dose groups using Tarone's life table test to test for dose-related trends (Tarone, 1975) and Cox's proportional hazards method for pairwise comparisons of each dose group to the control group (Cox, 1972). The poly-3 test, which takes survival differences into account, was used to assess neoplastic and nonneoplastic lesion incidences (Bailer and Portier, 1988, Piegorsch and Bailer, 1997, Portier and Bailer, 1989). When applied to all exposure groups, this test evaluated the significance of a dose-related trend in lesions; when applied to the control group and one exposure group, the test evaluated the significance of the pairwise difference of lesion incidence in the exposed group compared to the control group without correction for multiple testing. Historical control data for the mice were taken from the NTP Historical Control Report (National Toxicology Program, 2013).

3.9.1.2 [Study 2] NTP TR 587, 2014 and Dunnick et al., 2015 - 2 years carcinogenicity study in mice

National Toxicology Program. 2014; Toxicology studies of teterabromobisphenol A (Cas no. 79-94-7) in F344/NTac rats and B6C3F1/N mice and toxicology and carcinogeogenesis studies of tetrabromobisphenol A in Wistar Han [Crl:WI(Han)] rats and B6C3F1/N mice (gavage studies). NTP Technical Report 587

Dunnick et al., 2015, Environmental chemical exposure may contribute to uterine cancer development: studies with tetrabromobisphenol A, Toxicol Pathol. 2015 June; 43(4):464-473

Detailed study summary and results:

Test type

2 years carcinogenicity study in mice.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier
- *Degree of purity* > 99%
- *Impurities:* 0.8-1.1% tribromo-BPA (not expected to affect the classification)
- *Batch number:* (25317K-1, C16263X, and 25337XX-8). Lot 25317K-1 was used during the 3-month studies; lots 25317K-1 and C16263X were combined into one lot and renamed lot M032607KA, which was used in the 2-year studies.

Test animals

- B6C3F1/N mice
- No. of animals per sex per dose: 50
- Mice were 5 to 6 weeks old at the beginning of the study.

Administration/exposure

- *Route of administration* – By gavage oral in corn oil
- *duration of test/exposure period: 2 years* By gavage in corn oil, 5 days per week for 105 weeks

- *doses/concentration levels, rationale for dose level selection:* 50 male and 50 female mice were administered 0, 250, 500, or 1,000 mg/kg in corn oil by gavage, 5 days per week for up to 105 weeks. The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to tetra-bromobisphenol A and to determine the appropriate doses to be used in the 2-year studies (see section on STOT-RE)
- *frequency of treatment* - daily, 5 days per week
- *control group and treatment* - Corn oil
- *historical control data* 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, including the current mouse study.
- *post exposure observation period:* -
- *vehicle:* Corn oil
- *test substance formulation/diet preparation, achieved concentration, stability and homogeneity of the preparation:* The determined concentration in the doses were close to the target concentration all through the 2-year study, and varied from -11 to +1% different from target dose, for details see Appendix J in the NTP report.

Results and discussion

Summary table from NTP TR 587, 2014:

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Tetrabromobisphenol A

	Male Wistar Han Rats	Female Wistar Han Rats	Male B6C3F1/N Mice[a]	Female B6C3F1/N Mice[a]
Doses in corn oil by gavage	0, 250, 500, or 1,000 mg/kg	0, 250, 500, or 1,000 mg/kg	0, 250, 500, or 1,000 mg/kg	0, 250, 500, or 1,000 mg/kg
Body weights	500 and 1,000 mg/kg groups at least 10% less than the vehicle control group after week 25	Dosed groups within 10% of the vehicle control group	Dosed groups within 10% of the vehicle control group	1,000 mg/kg group at least 10% less than the vehicle control group after week 25
Survival rates	33/50, 28/50, 38/50, 39/50	35/50, 34/50, 29/50, 33/50	33/50, 26/50, 39/50, 12/50	40/50, 31/50, 36/50, 4/50
Nonneoplastic effects	None	Uterus: endometrium, hyperplasia, atypical (residual longitudinal review-2/50, 13/50, 11/50, 13/50) Ovary: rete ovarii cyst (1/50, 0/49, 6/50, 6/49)	Liver: clear cell focus (11/50, 10/50, 25/50); eosinophilic focus (20/50, 33/50, 40/50) Kidney: renal tubule, cytoplasmic alteration (0/50, 20/50, 47/50) Forestomach: ulcer (9/50, 9/49, 19/50); infiltration cellular, mononuclear cell (5/50, 8/49, 21/50); inflammation (9/50, 10/49, 20/50); epithelium, hyperplasia (10/50, 13/49, 27/50)	Forestomach: ulcer (2/50, 15/50, 40/50); infiltration cellular, mononuclear cell (2/50, 13/50, 33/50); inflammation (2/50, 14/50, 41/50); epithelium, hyperplasia (4/50, 16/50, 39/50)
Neoplastic effects	None	Uterus: adenoma (original transverse review-0/50, 0/50, 3/50, 4/50); adenocarcinoma (original transverse review-3/50, 3/50, 8/50, 9/50; original transverse and residual longitudinal reviews, combined-4/50, 10/50, 15/50, 16/50); malignant mixed Müllerian tumor (original transverse review-0/50, 4/50, 0/50, 2/50); adenoma, adenocarcinoma, or malignant mixed Müllerian tumor (original transverse review-3/50, 7/50, 11/50, 13/50; original transverse and residual longitudinal reviews, combined-6/50, 11/50, 16/50, 19/50)	Liver: hepatoblastoma (2/50, 11/50, 8/50)	None
Equivocal findings	Testis: interstitial cell, adenoma (0/50, 0/50, 1/50, 3/50)	None	Large intestine (cecum or colon): adenoma or carcinoma (0/50, 0/50, 3/50) Hemangiosarcoma (all organs): (1/50, 5/50, 8/50)	None
Level of evidence of carcinogenic activity	Equivocal evidence	Clear evidence	Some evidence	No evidence

Clinical pathology, liver toxicity, histopathology and sperm motility and vaginal cytology was carried out in the NTP 3-months study, but only histopathology was carried out in the NTP 2-y study

- *mortality and time to death (indicate number died per sex per dose and time to death):* Increased mortality was seen in male and female mice 6 months into the study and was possibly due to gastrointenstinal toxicity
- Survival of top dose males and females was significantly reduced. Survival of low and medium dose groups was similar to that of the vehicle control group. Survival rate was 33/50, 26/50, 39/50, **12/50** in male mice and 40/50, 31/50, 36/50, **4/50** in female mice
- *clinical signs:* N/A
- *body weight gain:* Reduced body weight was seen in top dose females. The body weights were 10-25% of vehicle controls after week 25
- *food/water consumption:* N/A
- *ophthalmoscopic examination:* N/A
- *clinical chemistry:* None (see NTP report table 1, page 31)
- *haematology:* None
- *urinalysis:* None
- *organ weights:* None, only reported for 3 months study, see section STOT-RE
- *necropsy findings: nature and severity: -*
- *histopathological findings: nature and severity:* In the liver, the incidence of clear cell focus (liver foci) were significantly increased in 500 mg/kg bw males (25 vs. 11 and 10 in control and low dose, respectively). Also the incidence of eosinophilic focus were significantly increased in the 250 and 500 mg/kg bw males (33 and 40 vs. 20 in the controls).
- *tumour incidence data by sex, dose and tumour type*
  There was no treatment-related tumorigenic effects in female mice. In male mice there was a significant increase in the incidence of a rare type of liver tumor (hepatoblastoma) and also occurrences of hemagiosarcomas (1/50, 5/50, **8/50**) and three cases of rare tumors of the large intestine (adenoma or carcinoma (combined), 0/50, 0/50, **3/50**, trend). The incidence of multiple hepatocellular adenoma was significantly increased in 500 mg/kg males (12/50, 20/50, **28/50** cases in control, low and medium dose group). The incidence of hepatocellular adenoma (includes multiple) was not increased (32/50, 33/50, 38/50). The incidence of hepatocellular carcinoma was not significantly increased in male mice (11/50, 15/50, 17/50 cases in control, low and medium dose group). The incidences of hepatoblastoma and of hepatocellular carcinoma or hepatoblastoma (combined) were significantly increased in 250 mg/kg males (**24/50**); the incidences of hemangiosarcoma were slightly increased in 250 and 500 mg/kg males. The incidence of hepatoblastoma in the 250 and 500 mg/kg male mice exceeded the historical controls. The

incidences of adenoma or carcinoma (combined) of the cecum or colon occurred with a significant positive trend in males and the incidence in the 500 mg/kg group exceeded the historical control ranges for corn oil gavage studies and for all routes of administration. The incidence of hepatocellular adenoma was not significantly increased (32/50, 33/50, 38/50).

Table copied from NTP (2014) (due to early mortality, data for the 1000 mg/kg group is not presented):

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Male Mice in the 2-Year Gavage Study of Tetrabromobisphenol A[a]

	Vehicle Control	250 mg/kg	500 mg/kg
Number Examined Microscopically	50	50	50
Clear Cell Focus[b]	11	10	25**
Eosinophilic Focus	20	33**	40**
Mixed Cell Focus	7	8	12
Hemangiosarcoma	0	4	3
Hepatocellular Adenoma, Multiple	12	20	28*
Hepatocellular Adenoma (includes multiple)[c]	32	33	38
Hepatocellular Carcinoma, Multiple	2	4	5
Hepatocellular Carcinoma (includes multiple)[d]	11	15	17
Hepatocellular Adenoma or Hepatocellular Carcinoma	39	39	43
Hepatoblastoma[e]			
Overall rate[f]	2/50 (4%)	11/50 (22%)	8/50 (16%)
Adjusted rate[g]	4.6%	25.6%	17.6%
Terminal rate[h]	1/33 (3%)	7/25 (28%)	7/39 (18%)
First incidence (days)	619	535	722
Poly-3 test[i]	P=0.065	P=0.006	P=0.052
Hepatocellular Carcinoma or Hepatoblastoma[j]			
Overall rate	12/50 (24%)	24/50 (48%)	20/50 (40%)
Adjusted rate	26.8%	52.8%	41.5%
Terminal rate	7/33 (21%)	12/25 (48%)	12/39 (31%)
First incidence (days)	521	535	513
Poly-3 test	P=0.099	P=0.008	P=0.099
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma	39	42	43

* Significantly different (P≤0.05) from the vehicle control group by the Poly-3 test
** P≤0.01
[a] Due to early mortality, data for the 1,000 mg/kg group are not presented.
[b] Number of animals with lesion
[c] Historical incidence for 2-year gavage studies with corn oil vehicle control groups (mean ± standard deviation): 145/250 (58.0% ± 5.1%), range 52%-64%; all routes: 594/949 (62.6% ± 9.1%), range 48%-78%
[d] Historical incidence for corn oil gavage studies: 87/250 (34.8% ± 10.9%), range 22%-44%; all routes: 348/949 (36.7% ± 11.4%), range 22%-56%
[e] Historical incidence for corn oil gavage studies: 9/250 (3.6% ± 2.6%), range 0%-6%; all routes: 40/949 (4.2% ± 3.5%), range 0%-12%
[f] Number of animals with neoplasm per number of animals with liver examined microscopically
[g] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[h] Observed incidence at terminal kill
[i] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[j] Historical incidence for corn oil gavage studies: 93/250 (37.2% ± 10.0%), range 24%-48%; all routes: 371/949 (39.1% ± 11.6%), range 22%-54%

- *local or multi-site responses:* Liver and large intestine
- *progression of lesions to malignancy:* Yes, hepatoblastoma is a rare malignant liver cancer
- *gender and/or species-specific responses:* Male mice
- *tumour incidence data by sex, dose and tumour type:* In male mice there was a significant increase in the incidence of a rare type of liver tumor (hepatoblastoma) in the 250 and 500 mg/kg groups and also occurrences of hemagiosarcomas and three rare tumors of the large intestine. Data for neoplasms was not available for the top dose group due to early mortality
- *mode of action (genotoxic, non-genotoxic):* In the liver, altered hepatocellular foci (AHF) developes first, followed subsequently by hepatocellular adenomas, and then carcinomas
- *toxic response data by sex and dose:* High mortality in the top dose, only 4 of 50 female and 12 of 50 male mice survived until study termination. Dose-related effects in kidney in male mice (cytoplasmic alteration in renal tubule) and dose-related effects in forestomach (ulceration, and robust immune response in underlying areas) in male and female mice observed.
- *tumour latency:* N/A
- *statistical methods and results (unless already described with specific test results above):* See in the table above

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 [Study 1] Unnamed, 2002, Cope et al., 2015 and EU RAR TBBPA, 2008

Study reference:

- Unnamed (2002)
- Cope, R.B., Kacew, S. and Dourson, M. A reproductive, developmental and neurobehavioral study following oral exposure of tetrabromobisphenol A on Sprague-Dawley rats. Toxicology 329 (2015) 49-59
- EU risk assessment (United Kingdom) TBBPA, 2008.

Detailed study summary and results:

Test type

OECD TG 416 – Two Generation Reproduction Toxicity Study with a developmental neurotoxicity component in the F2 generation. Conducted according to standardized guideline. GLP-study.

Test substance

- *Test material used in the study is equivalent to the substance identified in the CLH dossier*. The study was conducted using a composite of three commercial TBBPA products.
- *Degree of purity*: Analytic purity 98.91 %
- *Impurities (or a note that the impurities do not affect the classification)*: o,p-Tetrabromobishenol-A (0.05 %), 2,4,6-Tribromophenol (<0.01 %), Tribromobisphenol-A (1.04 %)
- *Batch number*: Composite of 5381B and 5381

Test animals

- *Species/strain/sex:*Rat, Sprague-Dawley, male/female
- *No. of animals per sex per dose*: 30 male and 30 female
- *Age and weight at the study initiation*: Parental animals were 6 weeks of age, F1 animals were 3 weeks of age. Parental animals were treated at least 10 weeks before mating. Age at mating of the mated animals was 14 weeks. Weight at study initiation was 258-338 g for P males and 190-248 g for P females.

Administration/exposure

- *Route of administration* – Oral (gavage)
- *duration and frequency of test/exposure period*: 36 weeks of daily exposure (once per day, seven days per week throughout the study).
- *doses/concentration levels, rationale for dose level selection:* Dose selection rationale from results of previous studies.
- *control group and treatment:* vehicle control group consisted of 30 animals/sex/generation
- *vehicle:* Corn oil
- *actual doses (mg/kg bw/day) and conversion factor from diet/drinking water test:* 10, 100 and 1000 mg/kg/day (actual ingested)

Description of test design:

- *details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy)*: The male and female ratios were reported as 1:1 per cage. The length of cohabitation was 14 days. Vaginal plug/sperm in vaginal smear was considered proof of pregnancy.
- *premating exposure period for males and females (P and F1)*
- *dosing schedules and pre and post dosing observation periods for P, F1 and F2, as appropriate:* F1 parental generation (30 animals/sex/group) started treatment at PND 22.
- *standardization of litters (yes/no and if yes, how and when)*: performed on day 4 postpartum. A maximum of 8 excess pups/litter were killed and discarded. Offspring parameters examined were

number and sex of pups, stillbirths, live births, presence of gross abnormalities, weight gain, physical or behavioural abnormalities.

- *parameters assessed for P and F1*: For P-animals cage side observations twice per day, seven days per week. Observations included was morbidity, mortality and signs of injury. Body weight examinations were performed once per week. After sacrifice, P and F1 animals were subjected to macroscopic and microscopic examinations. The organs examined were adrenals, brain, gonads (ovary, testis, epididymis), kidney, liver, pituitary, prostate, seminal vesicle with coagulating glands, spleen, thymus, tissue masses, uterus (both horns) with oviducts and cervix and vagina. Serum T3, T4 and TSH consentrations were determined in 10 animals/sex/group in the parental (P) and retained F1 animals several days prior to sacrifice.
- *estrous cycle length and pattern, sperm examination, clinical observations performed and frequency*: Vaginal smears (lavage) were performed daily beginning three weeks prior to pairing in all parental females (P and F1 to establish estrous cyclicity). In males the parameters examined was daily sperm production, sperm motility, epididymal sperm count and morphology.
- *parameters assessed for F1 and F2:* Postmortem examination included were gross pathology, histopathology organ weights. The tissues from organ weights were prepared for microscopic examination and weight. F1 offspring that were not selected as parental animals and all F2 offspring were sacrified at weaning.
- *clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance*): For P-animals more detailed clinical observations were performed once a week. Postmortem examination of the P-animals were gross necropsy consisting of external and internal examinations including the cervical, thoracic and abdominal viscera. Male animals were sacrificed at completion of parturition. Maternal animals were sacrified after the last litter of each generation was weaned.
- For neurobehavioral studies 40 animals per sex and dose group were randomly selected from the F2 generation. Additional 20 animals per sex and dose group were retained for neuropathological studies (such as brain weight and neuropathological evaluation of the brain, spinal cord and peripheral nerves). A unique sets of animals [10 pups/sex/group] were randomly selected for each assessment of neuropathological or neurobehavioral examinations: at PND 60 brain weights, perfusion and neuropathology. Furthermore, special detailed clinical examinations (PND 4, 11, 21, 35, 45, and 60), motor activity [MA] (PND 13, 17, 21, and 60), auditory startle habituation [ASH] (PND 22 and 60), and learning and memory [L&M] (PND 22, 60 and 110) were reported. Additionally, 10 F2 pups/sex/group were selected randomly on PND 11 for collecting, weighing, and preserving of the brains.
    - Motor activity was assessed in 10 pups/sex/group by using a Digiscan Activity Monitor equipped with an electronic analyser-recorder. Animals were placed in an activity chamber for 20 minutes before activity was recorded. The activity recorded was horizontal and vertical

activity counts, total distance travelled and emotionality assessment by recording defeciation, urination, rearing, grooming and backing.

- Learning and memory was studied by the passive avoidance test and the water M-maze test and was assessed in 10 pups/sex/group. The same animals were used in both tests. For the passive avoidance test the test apparatus consisted of light and dark compartments separated by a mechanical door. Each animal was tested once a day for 3 consecutive days. Each animal was initially placed in the light side and allowed to acclimatise for 30 seconds. Then the barrier was removed allowing the animal to move to the dark side for a 3 minute period. The duration spent in the light was recorded for each session. On day one of the test any rat that moved from the light to the dark side was administrated an electric shock for 3 seconds. The animal was not shocked on day 2 and three of the test if they moved from the light to the dark side within the 3 minutes period. The time spent in the light was recorded for each time when the animals moved to the dark side. The animals that did not move to the dark side within the 3 minute time periode was returned to their cage. The same animals were also subjected to the water M-maze test starting on PND 110. The test consisted of 10 trials per day for 4 consecutive days to assess short-term memory. The animals were tested again 5 days later (over 10 trials) to assess long-term memory. The animals had 60 seconds to complete the maze and was analysed in regard to if they passed or fail, time to complete and number of errors made.
- 10 F2 pups/sex/group were randomly selected and subjected to investigation of brain weight and neuropathological evaluation og the brain, spinal cord and peripheral nerves at PND 60. In addition the thickness of the parietal cortex measured in 10 males of the 0 and 1000 mg/kg bw/day groups and 10 and 9 females of the 0 and 1000 mg/kg bw/day groups. 10 F2 pups/sex/group were also selected randomly for sacrifice at PND 11 for neuropathological evaluation and morphometric measurements, the latter comprising of measurements of the thickness of the parietal cortex, hippocampus, the external granular, molecular and Prukinje/internal granular layers of the cerebellum, and thalamus.

Results and discussion

In the P generation there were no general toxicity effects observed in clinical signs, food consumption and compound intake, organ weight findings including organ/body weight ratios and non-neoplastic histopathological findings. There were no effects observed on reproductive function (oestrous cycle and sperm measures) and reproductive performance.

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

Thyroid effects in P and F1-generation

Treatment related reduced levels on total thyroxine (T4) in TBBPA treated groups for both male and female rats in the P and F1 parental generation. 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 tables 7-10. Significant changes are marked in bold.

Table 7: Summary of P Male Mean Thyroid Hormone Values

Endpoint	0 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day
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***

* significantly different from controls p<0.05

Table 8: Summary of P Female Mean Thyroid Hormone Values

Endpoint	0 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day
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

* significantly differrent from controls p<0.05

Table 9: Summary of F1 Male Mean Thyroid Hormone Values

Endpoint	0 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day
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

* significantly different from controls, p<0.05

Table 10: Summary of F1 Female Mean Thyroid Hormon Values

Endpoint	0 mg/kg/day	10 mg/kg/day	100 mg/kg/day	1000 mg/kg/day

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

* significantly different from controls, $p<0.05$

Body weight

Lower body weights and body weights gain was observed in F1 males at 1000 mg/kg bw/day. The F1 males had lower body weights for several weekly intervals during the study and lower weight gain (7%) over week 1-11 premating period. For the P animals and F1 parental females the weight and weight gain were not changed. No other treatment related effects were evident for the P and F1 animals. This was based on clinical signs of toxicity, estrous cyclicity, reproductive performance, body weight gain, gestation/lactation body weights or food consumtion, gestation length, litter data, or on the macroscopic and microscopic evaluations, organ weights, sperm evaluations and primordial follicle counts.

There were no changes in bodyweight, clinical finding, sex ratio, survival to weaning, macroscopic findings or organ weight data for the F1 and F2 pups.

Neurobehavioral toxicity -motor activity

The treated animals had no statistical significant differences from controls in terms of activity and emotionality at PND 13. At PND 17, females had significant decrease in horizontal activity in the 15-20 min segment of the test in the 10 mg/kg bw/day group. And also in the 20 min period in the 100 mg/kg bw/day group (no difference in the 5 min segments). At PND 21 females exposed to 100 mg/kg bw/day showed in the 5-10 min segment and over the 20 min test period as a whole, horizontal activity and distance travelled that were significant reduced compared to control. At PND 60 no data on distance travelled are presented for male and females. For males there were significant reductions horizontal activity in the 0-5 min segment of the test at both 100 and 1000 mg/kg bw/day groups and during the 5-10 min segment in the 1000 mg/kg bw/day group. No other significant changes were seen and the EU RAR TBBPA (2008) consideres that the overall results have a lack of consistent pattern observed and that the significant changes are most likely by chance and unrelated to exposure.

Neurobehavioural toxicity – Learning and memory

passive avoidance test

On day one of testing there were no differences in time spent in the light (which was relatively short) between the control and exposed group. On day 2 the males spent longer time in the light, this was expected. However, for the highest exposed males (1000 mg/kg bw/day) they spent significantly shorter time in the light. On day 3 there were no difference between control and exposed groups. There were no differences between female

controls and exposed groups. EU RAR TBBPA (2008) consideres that the overall results have a lack of consistency and that the significant changes in males at day 2 are most likely by chance and unrelated to exposure.

Water M-maze

Very few animals failed to complete the maze and effectively there was no difference between control and treated groups. There were no difference between controls and treated animals in terms of mean times to complete the maze. On each day on which trials took place the mean time to completion was similar across the groups. There were no overall differences between control and treated groups in mean number of errors in the trials on each day of the trial. The data on day 9 were similar to those on day 4 indicating that like the short-term memory there was no treatment-related effect on long-term memory.

Neuropathology

For the morphometric measurements there was observed a significant decrease of the parietal cortex thickness of the 1000 mg/kg bw/day pups sacrified at PND 11. In males the thickness was 1.61, 1.56, 1.49 and 1.23 mm, for females the thickness was 1.60, 1.46, 1.56, 1.33 mm at 0, 10, 100 and 1000 mg/kg, respectively. Reductions were also seen at the 10 and 100 mg/kg groups, however these changes were not significant. There were no histological changes evident in the parietal cortex (including degeneration, necrosis, cell loss, demyelination, proliferative changes or changes in neuronal cell density). The thickness of the parietal cortex was also measured for the control and 1000 mg/kg bw/day F2 pups at PND 60. There were no differences in the thickness in males (2.13 and 2.09 mm, respectivly) or females (2.10 and 2.06 mm, respectively). There were no treatment related effects on PND 60 brain weights and no microscopic alterations were observed in the brain, spinal cord, nerves and ganglia in PND 60 F2 animals.

3.10.1.2 [Study 2] Lilienthal et al., 2008

Study reference:

Lilienthal, H., Verwer, C.M., van der Ven, L.T.M., Piersma, A.H. and Vos, J.G. Exposure to tetrabromobisphenol A (TBBPA) in Wistar rats: Neurobehavioral effects in offspring from one-generation reproduction study. Toxicology 246 (2008) 45-54. The testresults were published in two different publications, more information described in Van der Ven et al 2008 (see 3.10.1.3, study 3)

Detailed study summary and results:

Test type

OECD TG 415 One generation reproduction toxicity study with enhancements for endocrine and immunological endpoints and additional analyses for bone and neurophysiological parameters. Differences in regard to dosing (8 dose groups) and number of animals (10 animals per group).

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* TBBPA. TBBPA was obtained from the Bromine Science and Environmental Forum (BSEF).
- *Degree of purity:*Mixture of products from several manufacturers. The purity was 98% with tribromobisphenol A.
- *Impurities (or a note that the impurities do not affect the classification):* tribromobisphenol A and *o,p'*TBBPA were the major contaminants.
- *Batch number*

Test animals

- *Species/strain/sex:* Wistar rats (HsdCpb:WU), male and female
- *No. of animals per sex per dose:* 10 parental animals per sex per dose group
- *Age and weight at the study initiation:* 7 weeks when purchased. Acclimatized at least one week before beginning exposures.

Administration/exposure

- *Route of administration* – Oral, mixed with standard rat feed without soy
- *duration and frequency of test/exposure period:* The Wistar rats were housed 2/cage. Paternal and maternal exposure started at 10 and 2 weeks premating, respectively, because of the different development of germ cells in both sexes. A full spermatogenic cycle should be covered by premating exposure in males. Exposure was continued throughout mating, gestation and lactation. After weaning on postnatal day 21. The offspring were housed in group cages (2/cage) and fed the same diets as their respective mothers throughout life.
- *doses/concentration levels, rationale for dose level selection:* 8 dose group fed diets with 0, 37.5, 125, 375, 1250, 3750, 12500 or 37500 mg/kg diet, resulting in an average daily intake of 0, 3, 10, 30, 100, 300, 1000, 3000 mg TBBPA/kg bw for daily consumption of 10-25g depending on age.
- *vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water):* TBBPA was added to a soy-free standard laboratory diet

Description of test design:

- *parameters assessed for F1:* Brainstem auditory evoked potentials (BAEPs), conditional fear and sweet preference. The BAEP is an electrophysiologic response elicited by auditory stimuli and

electric activity recorded from the scalp or brain surface using electrodes, measuring the integrity of auditory neuronal pathway. BAEP were examined at approximately 50-100 days of age.

Results and discussion

Statistical analysis

Reported in Van der Ven et al. 2008 (see 3.10.1.3). Additional statistical analysis as reported by Lillenthal et al. For comparison, trend analyses were performed by calculating linear contrasts for dose, according to an ANOVA model. The general linear models (GLM) procedure of the SAS statistical software was used for these evaluations (Version 9.1, SAS Institute, Cary, NC). Also, analyses of covariance (ANCOVA) were calculated with age as the covariate to check for any influences of age at testing on the BAEP results. The percentage of consumed saccharin solution to total fluid intake was calculated to determine sweet preference. In addition, relative intakes of saccharin solution and water were determined by standardization to body weight. Due to a technical failure, data from days 3 to 5 were lost, so that only the first 2 days could be evaluated. The percentage of freezing duration and the percentage of activity duration were determined for the evaluation of conditional fear (CCF) data. Data are presented as percentage of activity. Results of sweet preference and CCF were analysed by ANOVA model with group and sex as between factors and repeated measures on the factor testing time (within factor). These analyses were followed by preplanned separate analyses for each sex. Litter was the statistical unit in all analyses and probabilities <0.05 were considered significant.

Brainstem auditory evoked potentials (BAEPs)

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. Younger and older rats were randomly distributed within the testing period. 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-group compared to controls. Benchmark analysis was performed, significant fits to dose-response curves were obtained at 0.5 and 2 kHz. 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 vurves at 0.5kHz in female rats. With and absolute increase of 0.14 ms at the highest exposure level compared to controls, corresponding critical effect dose and benchmark dose level was 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 iin 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.

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.

Conditional fear testing (CCF)

Results of CCF testing revealed no exposure-related effects on cue or context conditioned fear in male and female rats.

3.10.1.3 [Study 3] Van der Ven et al., 2008

Study reference:

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

Detailed study summary and results:

Test type

One generation reproduction toxicity study with enhancements for endocrine and immunological endpoints and additional analyses for bone and neurophysiological parameters. Conducted according to OECD TG 415. Differences in regard to dosing (8 dose groups) and number of animals (10 animals per group).

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* TBBPA. TBBPA was obtained from the Bromine Science and Environmental Forum (BSEF).

- *Degree of purity:*Mixture of products from several manufacturers. The purity was 98% with tribromobisphenol A.
- *Impurities (or a note that the impurities do not affect the classification):* tribromobisphenol A and *o,p'*TBBPA were the major contaminants.

Test animals

- *Species/strain/sex:* Wistar rats (HsdCpb:WU), male and female
- *No. of animals per sex per dose:* 10 parental animals per sex per dose group
- *Age and weight at the study initiation:* 7 weeks when purchased. Acclimatized at least one week before beginning exposures.

Administration/exposure

- *Route of administration* – Oral, mixed with standard rat feed without soy
- *duration and frequency of test/exposure period:* The Wistar rats were housed 2/cage. Paternal and maternal exposure started at 10 and 2 weeks premating, respectively, because of the different development of germ cells in both sexes. A full spermatogenic cycle should be covered by premating exposure in males. Exposure was continued throughout mating, gestation and lactation. After weaning on postnatal day 21. The offspring were housed in group cages (2/cage) and fed the same diets as their respective mothers throughout life.
- *doses/concentration levels, rationale for dose level selection:* 8 dose groups 0, 3, 10, 30, 100, 300, 1000, 3000 mg TBBPA/kg bw/day. 8 doses were chosen to be able to perform dose-response analysis and calculate the benchmark doses (BMDL)
- *vehicle:* TBBPA was added to a soy-free standard laboratory diet

Description of test design:

Parameters assessed for the parental animals (P): The parental animals were euthanized after mating (males) or after weaning on postnatal day (PND) 21 (females). Number of implantation sites in the uterus was recorded after the female rats were euthanized.

Parameters assessed for the F1 animals: At the day of birth, number of living and dead pups, weights, sex, anogenital distance and macroscopic pathology were recorded for the F1 generation. Litter size was not standardized. During lactation mortality and the time to vaginal opening or balano-preputial separation were monitored. Two males and to females were euthanized at the time of weaning (PND 21) and their reproductive organs were inspected. The remaining animals received the same doses as the P-generation. The male animals were hosted in small groups (3-4 animals). Animals were marked for either neurobehavioral tests, immunisation assay or necropsy at week 10, the tests were carried out in the course of 7 weeks. Necropsies

were carried out for five animals per sex per dose group at the average age of 14 weeks (+/- 1 week). Sperm from the cauda epididymis was analysed and the full range of organs (including samples of whole blood, bone marrow of one femur and spleen) were dissected during necropsy. All organs were inspected visually and weighed. Biochemical analysis was performed on tissue from liver, intestines, brain, adrenals, testes and ovaries and muscle and fat. Thyroid and sex hormones (only males) were analysed from plasma.

Results and discussion

Statistical treatment of the results (as described by Van der Ven et al)

The study was performed with 8 dose groups to enable dose-response analysis and calculation of benchmark doses. Dose-response analysis of effects, based on external dosing (mg/kg bw) was done using a nested family of purely descriptive (exponential) models with the PROAST software. The model enables integrated evaluation of the complete data set. From the best fitted curve, indicated by significance at the 5% level, a critical effect dose (CED) was calculated at a default critical effect size (CES) of 10%. For testis weight and bone parameters a CES of 5% were used. The analysis was completed with the calculation of 90% confidence interval (two-sided), thus enabling the calculation of a 5% lower confidence bound of the CED estimate. This value was considered as BMDL (bench mark dose lower confidence bound) for continuous data. The CED/BMDL was used as a measure for the statistical uncertainty in a data set; a 10-fold difference between CED and BMDL was used as a practical limit for informative value. The controls were included as zero value input for the modelling calculations, although for graphical representationon a log-scale, an arbitrary value (but lower than the lowest dose) is used. BMDL values expressed as external doses were converted to internal doses (liver concentrations) using regression equation that was fitted to external versus internal dose. For the most sensisitive effects in the study, i.e. effects with the lowest BMDLs, the benchmark analysis was repeated based on internal doses.

Parameters from the study which showed sensitive effects, i.e. at BMDLs in the low- to mid-dose range, were used for correlation testing against all other parameters. This collection of univariate responses was screened for classes of correlation coefficients as defined in table 11, and all responses that fitted to those classes were reported in that table in a clustered manner. The results from the neurophysiologic tests (described in Lilienthal et al, 2008, see 3.10.1.2, study 2) were also included in the analysis. The correlation coefficient were based on group averages rather than comparison by individual, to allow comparisons across age cohorts and across sexes; this method ignores variability within groups, and these correlations should therefore be considered as indicative for clustering.

Table 11:

Correlations of TBBPA induced effects in F1 animals

effect	correlated parameters																	
	TT4 f	TT4 m	sr m	bwr f	agd7 f	surv m+f	dvo	E2 m	bwr m	pit f	gon21 f	gon21 m	testis	uterus	em	sr f	TSN m	TT3 f
survival m+f	-3	-1	2															
threshold 0.5 kHz f	-1	-3	2															1
threshold 2 kHz f	-1	-2					2											2
latency peak 2, 0.5 kHz f	-2	-2	2	1	-1			1										
latency peak 4, 0.5 kHz f	-2	-2	2	1					1	1								2
latency peak 4, 0.5 kHz m	-2	-2	1	1	-2			1			1							2
latency peak 4, 2 kHz m	-2	-2	1		-1	1	2											1
TT4 f		2	-3		1	-3												-1
TT4 m	2		-3	1		-1												-2
TT3 f	-1	-2	1					1			2	2	1					
gonads d21 m											1		1			1	1	2
testis weight												1		1	2	2	1	1
pituitary m				1									2			1		

Only effects at BMDLs in the low- to mid-dose range were included for correlation testing (left column). These were tested against parameters (top row) with significant d responses, or of interest because of mechanistic considerations. Correlation coefficients are staged in three equidistant classes, 0.56–0.71 (1), 0.72–0.86 (2), 0.87–1.0 (3). defined by the correlation coefficient of TT3 in females to external dose (not in the table). Open cells represent correlations below 0.56; auto-correlations of variables that are i provided (hatched cells). Variables that tested below the lowest correlation class were excluded from the table. The correlation coefficients were based on group averages ra allow comparisons across age cohorts and across sexes; this method ignores variability within groups, and these correlations should therefore be considered as an indicative of analysis. TT4/TT3, total thyroxin/triiodothyronine; sr, total saccharine intake, and bwr, basal water intake in the sweet preference test, both related to body weight; surv, postnatal day 7; dvo, postnatal day of opening of the vagina; gon21, gonad weight on postnatal day 21; testis, testis weight; uterus, uterus weight; em, endometrium thickness; E2, plasma estradiol; TSN, plasma testosterone; pit, pituitary weight; f, female; m, male; thresholds and latencies are variables from the brainstem auditory evoked potentials

Food intake:

In the P animals food intake was temporarily reduced in the early weeks (week 1 and 2) of exposure to high doses of TBBPA for both sexes. Females also had reduced food intake at the highest doses the first two week after gestation, with a benchmark dose of 207 mg/kg bw/day. The reduced food intake also affected the body weights in females before mating, but the benchmark dose was close to the highest concentration given (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 with benchmark doses of 94 and 298 mg/kg bw/day, respectively. Exceptions were for liver weight and immune parameters, where a CES of 20% were used.

Reproduction effects:

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 and a delayed time to vaginal opening, benchmark doses was around the highest concentration. Male pups showed increased weight of the reproductive organs at weaning with a benchmark dose of 0.5 mg/kg bw/day. There was a dose dependent decrease in mortality during lactation with a benchmark dose of 4.8 mg/kg bw/day. And a decrease in rate of litters with mortality (benchmark dose 33 mg/kg bw/day). 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% with benchmark dose around the highest concentration.

Concentration of TBBPA in different tissues in the F1 animals

Liver: Dose dependent increase of TBBPA concentration with averages around 25 µg TBBPA per g wet weight liver tissue in both sexes at the highest concentration. Male and female rats had similar concentration of TBBPA in the liver. Low concentration of TBBPA was detected in 5 of 9 control animals (both sexes)

Plasma: There was also an increase in plasma TBBPA, TBBPA-sulphate and TBBPA-glucuronide with exposure dose. Males had higher plasma concentration of TBBPA then females. TBBPA, TBBPA-sulphate and TBBPA-glucuronide was detected in one female control, however there was no detectable TBBPA in the liver.

Organ weights:

F1 animals: Male rats showed a significant dose-dependent increase in liver weight (maximum increase 11.4%). There was a significant dose-dependent increase in adult testis weight with benchmark dose 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 (see study 2), 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.

Endocrinology:

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.

Thyroid: Plasma T4 levels was decreased in both sexes with a benchmark dose 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 with a benchmarkdose of 2.3 mg/kg bw/day. The levels of T4, T3, 17 betaestradiol and CYP19 are shown in table 12.

Table 12: Endocrine parameters assessed for the F1 animals

		females			*males*			*males*			*females*
TBBPA dose mg/kg bw	n	TT4 nmol/L	TT3 nmol/L	n	TT4 nmol/L	TT3 nmol/L	n	E2 pmol/L	TSN nmol/L	n	CYP19 pmol/h/mg protein
0	4	34.3 ± 2.2	0.7 ± 0.1	4	53.4 ± 6.9	1.0 ± 0.1	4	51.5 ± 9.0	3.9 ± 0.9	4	2.2 ± 2.1
3	5	33.5 ± 7.7	0.8 ± 0.1	5	40.7 ± 3.1	0.8 ± 0.1	5	46.6 ± 9.8	8.4 ± 3.8	3	5.4 ± 3.4 [a]
10	5	38.0 ± 6.9	0.8 ± 0.1	5	45.7 ± 2.6	0.9 ± 0.1	5	47.2 ± 13.9	6.9 ± 4.7	4	5.5 ± 2.5 [a]
30	5	41.2 ± 10.1	0.9 ± 0.1	5	47.6 ± 7.8	1.0 ± 0.1	5	54.4 ± 6.8	8.1 ± 4.4	5	6.3 ± 3.6
100	5	27.1 ± 10.1	1.0 ± 0.1	5	43.0 ± 13.5	0.9 ± 0.1	5	60.2 ± 9.9	7.0 ± 2.7	5	6.5 ± 3.3
300	5	23.2 ± 7.5	0.9 ± 0.1	4	31.5 ± 5.9	1.0 ± 0.2	4	55.8 ± 18.3	7.0 ± 3.6	5	2.9 ± 0.6
1000	5	22.2 ± 4.7	1.0 ± 0.2	4/5	26.5 ± 4.4	0.8 ± 0.2	5	60.4 ± 8.4	6.0 ± 3.9	6	3.3 ± 2.2
3000	5	18.4 ± 3.8	1.0 ± 0.2	5	27.9 ± 14.3	1.0 ± 0.2	5	51.6 ± 9.4	5.9 ± 2.0	3	3.7 ± 2.7 [b]
dose response		+	+		+	-		-	-		-

Figures are average ± standard deviation of n animals per dose group; +, Significant dose response for results see Table 1 in the text; -, no significant dose response. [a] One or [b] two outliers (>20) excluded.

Immunotoxic and hematologic effects in the F1 animals

No immunisation response against Sheep Red Blood Cells in male. No effects on NK activity in spleen cells. Increase in total spleen cell counts. The splenocyte count 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.

Development:

3.10.1.4 [Study 4] Unnamed, 2001, Cope et al., 2015 and EU RAR TBBPA, 2008

Study reference:

- Unnamed, 2001
- Cope, R.B., Kacew, S. and Dourson, M. A reproductive, developmental and neurobehavioral study following oral exposure of tetrabromobisphenol A on Sprague-Dawley rats. Toxicology 329 (2015) 49-59
- EU risk assessment (United Kingdom) TBBPA, 2008.

Detailed study summary and results:

Test type

OECD TG 414 (Prenatal Developmental Toxicity Study), EPA OPPTS 870.3700 (Prenatal Developmental Toxicity Study). GLP-study

Test substance

- *The test material used in the study is equivalent to the substance identified in the CLH dossier:* Tetrabromobisphenol-A

- *Degree of purity:* Analytic purity of 98.91%
- *Impurities (or a note that the impurities do not affect the classification):*o,p-tetrabromobisphenol-A (0.05%), 2,4,6-Tribromobisphenol-A (<0.01%), Tribromobisphenol-A (1.94%)
- *Batch number:* 5381

Test animals

- *Species/strain/sex:* Rat, Sprague-Dawley
- *No. of animals per sex per dose:* 25 females per dose plus control
- *Age and weight at the study initiation:* Age of animals were 10 weeks and their weights between 203-274 g at study initiation.

Administration/exposure

- *Route of administration:* oral (gavage)
- *duration and frequency of test/exposure period*: Exposed daily for 20 days from Day 0 to gestation Day 19.
- *doses/concentration levels, rationale for dose level selection:* Exposed to 100, 300, and 1000 mg/kg/day (actual ingested). The test article was administrated daily as a single dose. Dose selection rationale based on preliminary repeated-dose-sub-chronic and chronic studies. Maximum dose did not exceed the maximum tolerated repeated dose exposure for TBBPA for the study durations.
- *control group and treatment:* Control group was treated with vehicle
- *vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water):* Corn oil, the amount of vehicle used was 5 mL/kg. Vehicle batch nr: PU0041 and PO0173. There were no justification for use of vehicle other than being a standard vehicle.
- *Details on analytical verification of doses:* Prior to initiation oftest article administration, test batches of the test article suspensions at the low and high concentrations used in the study (20 and 200 mg/mL, respectively) were prepared to assess the homogeneity of the test preparations employing the same method and batch size to be used during the study. Validation was performed by HPLC with UV detection, with an internal standard operating procedure. LLOQ = 0.0100 mg/mL

Description of test design:

- *details on mating procedure (M/F ratios per cage, length of cohabitation, proof of pregnancy):* the animals were cohoused in the impregnation period. The M/F ratio per cage was 1:1. The length of the cohabitation and if further matings after more then two attempts was performed was not reported. Proof of pregnancy was not reported. No deviations from standard protocol was reported.
- *Maternal examinations:* Cage side observations were performed twice daily, seven days a week for morbidity, mortality, and signs of injury. Detailed clinical observations were performed daily. Body

weight examinations were performed on days 0, 3, 6, 9, 12, 15, 18 and 20 of gestation. Food consumption for each animal was determined as mean diet consumption calculated as g food/kg bw/day (no data). Compound intake was calculated as time-weighted averages from the consumption and body weight gain data (no data).

- *clinical observations performed and frequency, organs examined at necropsy, others (e.g. anogenital distance):*

  Maternal examinations: Post mortem examinations were performed in maternal animals. Animals were sacrified on gestation day 20. Uterus, placenta, standard gross necroscopy on maternal rats and fetal examinations were were reported as organs examined. After termination the ovaries and uterine was examined and included gravid uterous weight, number of corpora lutea, number of implantations, number of early resorptions and number of late resorptions.

  Fetal examinations: Fetal examinations included external examinations (all per litter), viable and non-viable foetuses and fetal sex and weight. Soft tissue examinations (half by litter) and skeletal examinations (half per litter) and visceral examination and/or malformations. Approximately one-half of the foetuses in each litter were fixed in Bouin's solution and the remaining foetuses were skinned and preserved in alcohol.
- *post exposure observation period*

Results and discussion

Describe the relevant findings. If no effects occurred, explicitly note "No effects".

- *statistical treatment of results, where appropriate:* As reported by Cope et al, group pair-wise comparisons were used for the following endpoints: gestation body weights, gestation food consumption, absolute and relative liver weight, gravid uterine weights, corpora lutea counts, total implantation counts, viable foetus count and early resorption counts. Group pair-wise comparisons involved examination for homogeneity of group variances using Levene's test. If homoscedasticity was established by Levene's test ($p \leq 0.01$), Dunnett's test was used to compare each treatment group with the control group. If Levene's test was significant ($p < 0.01$), comparisons with the control group were made using Welch's t-test with a Bonferroni correction. Two tailed tests were used when relevant. Arcsin-square-root transformation followed by group pair-wise comparisons was used for the following endpoints: foetal sex ratio, % viable foetuses/implants, % early resorptions/implants, preimplantation loss, and post-implantation loss. Data comprised % values were transformed using the arcsin of the square root. Data were then subjected to the group pair-wise comparison techniques described above. Fisher's exact test was used for the following endpoints: malformation by type and total (litter was the experimental unit), developmental variations by

type and total (litter was the experimental unit), and pregnancy index. For binomial endpoints (excluding sex ratios), each treatment group was compared with the control using a Fisher's exact test with a Bonferroni correction. Two-tailed tests were used when relevant. For foetal body weights, litter size was included as a covariate in the model used to conduct the Dunnett's test. Each treatment group was compared with the control group. Two-tailed tests were used as appropriate.

- *Results maternal animals:* No maternal toxic effects were reported. One mortality in the 300 mg/kg/day group on gestation day 5 was attributed to intubation injury. All other animals survived to scheduled euthanasia. Salivation effects determined in the treated animals and occurred most frequently in the 300 and 1000 mg/kg/day groups. Registrant reports this effect to be occurring sporadic and was not considered to represent a direct effects, but more likey in response to the taste of residual amounts of test article on the dosing catheter. There was a slight statistically significantly lower maternal liver weight in the 100 mg/kg/day group, but in the absence of similar findings in the 300 and 1000 mg/kg/day groups, it was considered to be unrelated to treatment. No other effects of treatment were seen from the clinical observations and no effects of treatment was evident from gestational parameters (body weight, body weight gain, or food consumption) and uterine implantation data. Results were summarized by Cope et al (2015) and shown in table 13.
- *Results fetuses:* There were no embyotoxic/teratogenic effects reported, no effects of treatment was evident fetal body weight, from fetal sex distribution, or from fetal external observations and visceral, and skeletal examinations. The few external findings (malformation and variations) seen in the treated groups occurred with low incidence in fetuses and litters and were considered unrelated to treatment. The litter incidences for these findings for the treated groups did not differ statistically from controls. Results were summarized by Cope et al (2015) and shown in table 13.

Table 13: Treatment related effects of TBBPA

Influence of TBBPA on reproductive and pre-natal developmental parameters.

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

3.10.1.5 [Study 5] Hass et al., 2003 unpublished, reviewed in EU RAR TBBPA, 2008

Hass U, Wamberg C, Ladefoged O, Dalgaard M, Lan HR, Vinggaard AM (2003). Developmental neurotoxicity of tetrabromobisphenol A in rats (reference in EU RAR TBBPA, 2008). The study was presented in a conference in 2002, but never published: *Hass, U., & Wamberg, C. (2002). Developmental neurotoxicity study of the brominated flame retardant tetrabromobisphenol A in rats. Poster session presented at 30th Conference of European Teratology Society, Hannover, Germany.*

Detailed study summary and results:

Test type

The study design was based on OECD TG 426 (developmental neurotoxicity study)

Test substance

- The test material used in the study is equivalent to the substance identified in the CLH dossier
- Degree of purity: N/A
- Impurities: N/A

Test animals

- *Strain:* Wistar rats

- *No. of animals per sex per dose:* n=20 pregnant rats per group (0, 50, 250 mg/kg bw/d), behavioural testing was performed using one male and one female per litter
- *Age and weight at the study initiation:*

Administration/exposure

- *Route of administration:* oral (gavage)
- *Dosing* daily from gestation day (GD) 7 to postnatal day (PND) 17
- *Doses/concentration levels, rationale for dose level selection:* Two dose groups 50 or 250 mg/kg bw/day. No rationale for the doses given.
- *Control group* received only peanut oil, daily by oral (gavage) from gestation day (GD) 7 to postnatal day (PND) 17
- *historical control data if available: N/A*
- *vehicle:* peanut oil

Description of test design

- Three groups (n=20) of pregnant Wistar rats were included in this developmental study
- Dosing was from gestation day (GD) 7 to postnatal day (PND) 17
- The dams were monitored daily for signs of toxicity;
- Body weights were monitored throughout the dosing period.
- Following delivery, pups were counted, sexed and checked for anomalies; Anogenital distance was measured at birth. Pups were examined for the presence of either areola or nipples on PND 13 and 14. The age and bodyweight of animals reaching sexual maturation was recorded.
- Thyroid hormone levels were measured on PND 15.
- Gross and histopathological examination was performed on 5 pups per sex per group (from 5 litters) on PND 15, on 10 pups/sex/group on PND 22 and on all weaned adult animals(1 male and 1 female per litter).
- The following reproductive organs were excised and weighed: the left and right testes and the thyroid of PND 15 and 22 males and adults; the brain, left and right epididymes, ventral prostate and seminal vesicles of PND 22 males and adults.
- Histopathological examination was performed on the testis and thyroid of PND 15 males; the left testis, the left epididymes, thyroid, ventral prostate and seminal vesicles of PND 22 males; and the brain of PND 22 males and females.
- Serum T3 and T4 levels were analysed in animals sacrificed on PND 22 (8 – 10/group;1/litter). Also in the animals sacrificed on PND 22, brain homogenates were analysed for neuro-transmitter levels. These included 5-hydroxytryptamine (5-HT), noradrenaline (NA) and dopamine (DA).

- Pups were weaned on PND 21 and one male and one female from each litter were randomly selected for the behavioural testing and neurochemistry, as described in the section on developmental toxicity.

Behavioural tests:

- A number of different behavioural tests were undertaken to evaluate developmental neurotoxicity.
- Motor activity of dams and offspring was recorded for 30 minutes in activity boxes in adult animals at 12 weeks and in offspring on PNDs 21 and 27. Assessment of offspring play behaviour was performed on PND 31.
- Learning and memory was evaluated using both a Morris water maze and a radial arm maze. In the Morris maze the animals were tested at ages 9, 13 and 17 weeks. The animals were tested in 4 daily trials starting from 4 different points, the trial being completed when the animals swam to and climbed onto a submerged platform. The animals were trained for 5 consecutive days (4 trials/day). Four weeks after this learning period, the animals were tested again for 2 consecutive days to assess memory, and after a further 4 weeks for 1 day only. The day after this last "memory" test, the platform was placed opposite the original location to test "reversal learning". The following day the platform was moved to the center of the pool and the animals were tested over 4 trials ("new learning").
- When the animals were 5 months old they were investigated in the sweet preference test and given the choice between normal water and water sweetened with 0.25% saccharin for 3 days.
- At the age of 6 – 7 months the animals were investigated in an 8 arm radial maze. The arms of the maze were baited with rewards in the form of peanut chops. The animals were tested in 15 daily sessions over 3 weeks (5 sessions/week). The rats were placed in the center of the maze and allowed to explore until all arms were visited or 10 minutes had passed. Latency to visit all arms was recorded. In addition the number of errors as defined by visiting an arm on more than one occasion was also recorded.

Results and discussion (copied from EU RAR TBBPA, 2008)

- Maternal bodyweight gains during pregnancy, gestation lengths, litter sizes, frequency of neonatal death and birth weights were similar between control and treated animals.
- No treatment-related effects of exposure were induced on anogenital distances at birth, areolas/nipples on PND 13, or the timing of sexual maturation.
- At weaning (PND 21), the body weights of the pups were statistically significantly lower in males and females (89 and 88% of control values, respectively) exposed to 50 mg/kg/day but not at 250 mg/kg/day compared to control offspring. At PND 92 a similar finding is reported with the bodyweights of males and females (92 and 92% of control values, respectively) being statistically significant lower than controls in the 50 mg/kg/day group but not in the 250 mg/kg/day group. Given that statistically significant findings only occur at the lowest dose, and on PND 92 they were reduced

by less than 10% of control values they are not considered to be a toxicologically significant treatment-related effect.

- The latency to initiate play behaviour and the number of pinnings did not differ significantly between the groups. Also, there was no significant difference between controls and treated animals in the sweet preference test.
- Motor activity during the entire 30 minute observation period on PND 21 did not differ significantly between treated and control groups in either males or females. However, when the pattern of activity was analysed as two 15-minute segments statistically significant changes are reported in females. During the first segment, there were no statistically significant differences in activity between control and treated animals. During the second segment, activity in the control and 50 mg/kg/day decreased to 17% and 32%, respectively, of that observed in the first segment, while activity in the top dose group remained similar to that in the first segment and was statistically significantly greater than control activity. This indicates a decreased habituation activity in females exposed to 250 mg/kg/day. For males, the 15 minute segment activity analysis provided no evidence of an effect on habituation. Motor activity during the entire 30 minute period on PND 28 again did not differ significantly between treated and control groups in either males or females. In females, no difference in activity was reported during the first segment of the test. However, during the second segment, there was higher activity in the 250 mg/kg/day group and the 50 mg/kg/day groups compared with controls. The second segment control activity was about 6% of that observed in the first segment, apparently due to the majority of the females having no activity during this period. For the 50 and 250 mg/kg/day groups, the second segment activity was 35% and 23% of that observed during the first period. The differences from controls were not significant using ANOVA, but were significant using the non-parametric Fisher Chi-square test (the latter test is probably the most appropriate given the obviously non-normal distribution of the results for the controls). This shows reduced habituation activity in females at 50 mg/kg/day and 250 mg/kg/day groups compared with controls, but the absence of dose-response relationship raises the possibility that this may be a chance finding. For males, the 15 minute segment activity analysis provided no evidence of an effect on habituation.
- For the adults tested at 12 weeks of age, again no differences were reported between treated and control groups in males, and over the entire 30 minute period no differences between treated and control females. For the females during segment 1, activity in the 50 mg/kg/day group was reduced compared to controls but this did not reach statistical significance, whereas activity in the 250 mg/kg/day group was similar to controls. For the second segment, activity in the 250 mg/kg/day group was higher than that in the control group; mean activity counts (± SEM) were 368 (± 77), 399 (± 49) and 463 (± 38) in the control, 50 and 250 mg/kg/day groups, respectively. The differences between the activity counts for control and 250 mg/kg/day groups was not significant by ANOVA, but significant by Fisher's test. It is not possible to judge from the information currently available which is the more appropriate test. A justification for favouring the Fisher's test is not available in the report. Comparing the changes in

activity between the two segments, activities during the second segment were 42%, 61% and 53% of the first segment in the respective groups; this comparison suggests that the 50 mg/kg/day group (rather than the 250 mg/kg/day group) may have displayed the lowest habituation activity. It can be concluded that the habituation capability on PND 21 in the 250 mg/kg/day females was different to that of the control group. However, for the females there was no firm evidence of an effect on habituation on PND 28 and only weak evidence of an effect in the adults. Also, habituation of the males was not affected at any of the time points.

- During the first 5 days of testing using the Morris water maze, statistically significant differences between the control and treated groups in terms of swim length and latency occurred twice. Firstly on day 1, females of the 250 mg/kg/day group showed a statistically significant decrease in swim length and latency to find the platform compared with control animals. Secondly on day 5, males of the 250 mg/kg/day groups showed a statistically significant increase in swim length and latency to find the platform compared with control animals. The swim speed of the animals did not differ between groups of either sex or exposure. The absence of a consistent pattern of differences between the controls and exposure groups indicated that the occasional statistically significant differences arose by chance. When the trials were undertaken once again to assess memory, a statistically significant increase in swimming length was reported in males of the 250 mg/kg/day group in the first and second of the 4 trials conducted on day 1 of the testing; also latency to find the platform was statistically significantly increased in the second trial. Mean swim length over the 4 trials on day 1 was not significantly different between treated and control animals. No differences were found in females over the 3 days of trials or in males on the second and third day of the trials. Since significant differences were observed only very occasionally, and there was no consistent pattern of changes across the 12 trials it is considered unlikely that these results indicate a treatment-related effect on memory.
- No significant treatment-related differences were reported in the “reversal learning” part of this study. During the “new learning” phase of the testing a statistically significant increase in swim length was reported in females of the 250 mg/kg/day group and in males of the 50 mg/kg/day group in trial 1, but no other differences were reported in trials 2 – 4. These occasional differences did not form part of a consistent pattern and are considered to have arisen by chance. Consequently it can be concluded that there is no clear treatment-related effect on ‘new learning’. When the animals were tested using the radial arm maze there were no statistically significant differences in mean latency between treated and control groups during the trials in week 1, 2 or 3. The mean number of errors decreased over the 3 weeks of the study. Male animals of the 250 mg/kg/day group showed a statistically significant increase in the number of errors compared with controls in weeks 1 (mean ± SD: 4.09 ± 1.34 and 5.13 ± 1.76 for control and 250 mg/kg/day groups, respectively) and 2 (1.13 ± 1.13 and 2.60 ± 1.90) but not week 3. The authors declared these differences to be statistically significant, although the overlapping SDs of the means suggests that this may not be the case if routine parametric statistical tests are used. No significant differences were reported in females. In terms of choosing adjacent arms,

there was a statistically significantly lower frequency in males of the 250 mg/kg/day group in week 1 but not among males on weeks 2 and 3 and females throughout the test. Overall, this study provides evidence of a marginal effect on the learning ability and memory of top dose male rats.

- 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.

3.10.1.6 [Study 6] Saegusa et al., 2009

Study reference:

Saegusa Y, Fujimoto H, Woo GH, Inoue K, Takahashi M, Mitsumori K, Hirose M, Nishikawa A and Shibutani M. Developmental toxicity of brominated flame retardants, tetrabromobisphenol A and 1,2,5,6,9,10-hexabromocyclododecane, in rat offspring after maternal exposure from mid-gestation through lactation. Reproductive Toxicology 28 (2009) 456-467.

Detailed study summary and results:

Test type

None guideline study. Developmental study.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* TBBPA
- *Degree of purity:* >98%
- *Batch number:* Catalog No. T0032, Lot No. GH01 from Tokyo Kasei Koguo Co. Ltd. (Tokyo, Japan).

Test animals

- *Species/strain/sex:* Rat, Cjr:CD®(SD)IGS
- *No. of animals per sex per dose:* 8 dams per group
- *Age and weight at the study initiation:* Pregnant rats at gestation day (GD) 3. GD 0 was the day when vaginal plugs were observed. Exposure from GD 10 after 1 week acclimation

Administration/exposure

- *Route of administration* – Oral, through a soy free diet.
- *duration and frequency of test/exposure period:* Exposure from GD 10 until GD 20 after delivery (day after weaning).
- *doses/concentration levels, rationale for dose level selection:* 0, 100, 1000 and 10 000 ppm. A preliminary dose range finding study was performed with the same dose settings from GD 10 to 20 after delivery. The highest dose chosen had a weak positive response in dams.

Description of test design:

Dams were weighed and food consumption was measured throughout the experimental period. On PND 1 the numer, weights and anogenital distance (AGD) of neonates were recorded. On PND 2, litters were culled randomly to adjust to eight offspring per dam (four males and four females). Dosing was terminated on day 20 after delivery and all dams were euthenized. 20 males and 20 females offspring per group were subjected to prepubertal necropsy for histopathological assessment (10 females and 10 males per group) and for other experimental purposes (10 males and 10 females per group). The remaining offspring were housed four animals in each cage and given a regular CRF-1 basal diet and water ad libitum, and maintained further for adult examination when they were 11 weeks old. For the animals subjected to prepubertal necroscopsy, the organs were removed and weighed and immunehistopathological assessment performed. This was also performed on the thyroid gland in dams as well as number of implantations. The female offspring were monitored daily for vaginal opening from PND 26 and the male pups examined for preputial separation from PND 34 until each animal acquired this. Age onset for puberty was recorded for the offspring. Estrous cycles for females were examined by daily microscopy observation of vaginal smears from postnatal week (PNW) 8-11. Offsprings were sacrified at PNW 11 and tissues were subjected to immunohistopathological assessment and thyroid-related hormone measurement.

The study included thyroid-related hormone (TSH, T4 and T3) measurement in serum at PND 20 and PNW 11 in males.

Immunihistochemistry was performed on brains of male offspring at PNW 11 for 2,3-cyclic nucleotide 3-phosphodiestrase (CNPase) and neuron-specific nuclear protein (NeuN) for staining of oligodendrocytes and neurons, respectively. This was followed by morphometric assessment. Quantitative measurement of the variability in the distribution of neurons located within and latteral to the pyramidal cell layer of the hippocampal CA1 region was performed at PNW 11 by using the NeuN stained brain section from males.

Oligodendroglial development, areas of the white matter tract immunoreactive for CNPase and the number of CNPase positive oligodendrocytes surrounding myelinated acons distributed in the cerebral cortical area were measured.

Results and discussion

Describe the relevant findings. If no effects occurred, explicitly note "No effects".

- statistical treatment of results, where appropriate: Numerical data was analysed for homogeneity of variance using Bartlett's test. When the variance was homogeneous among the groups, a one-way ANOVA was carried out. If the results were significant then the mean value for each exposure was compered with the control group using Dunnett's test. If the Bartlett's test showed heterogenous variance, then the Kruskal-Wallis's H-test was used. Upon significant different results, then Dunnet-type rank -sum test was performed. Incidences of histopathological lesions and estrus cycles were compared statistically by using the Fisher's exact probability test. Severity of the lesions by grading was compared using Mann-whitney's U-test.
- Effects in dams: increased body weight gain from day 9-20 after delivery at 10 000 ppm. At day 20 after delivery the weight was unchanged when compared to controls. Food consumption was not affected during gestation and lactation. Duration of pregnancy was not affected. Dams did not show any change in relative thyroid weights compared to controls at day 20 after delivery. However, there were dose-unrelated increasing tendency noted for the relative thyroid weight of all treatment groups. Inscidence of diffuse thyroid follicular cell hypertrophy showed a marginal increase from 1000 ppm (although, not significant).
- Effects in offspring: No offspring parameters showed any abnormalities in clinical observations, number of implantation sites, number of live offspring, male ratio and body weight and AGD at PND 1 in all groups. Body weights from PND 1 through weaining was not affected. No obvious body and organ weight changes at any dose for both sexes. There were no effects on onset of puberty in either sex after weaning, while a higher body weight was observed in males at 10 000 ppm as compared with the untreated controls at the onset of puberty. No significant irregularities in female offspring estrus cycle. For male offspring there was a dose-unrelated decrease of T3 concentration observed at 100 and 1000 ppm on PND 20, however, T3 levels were not significantly changed in the 10 000 ppm exposed group. No significant change in serum T4 and TSH concentrations at any dose. No exposure related changes in serum concentration of any thyroid hormones at PNW 11. At adult stage of the offspring there were no change in body and organ weights in males on PNW 11. In females decreased in relative kidney and uterus weights were observed at 1000 and 10 000 ppm, respectively. No treatment-related effects were observed in the histopathological assessment at PND 20 or PNW 11. The were no findings from the brain morphometric assessments in terms of neuronal migration and oligodendroglial development in male offspring at adult stage.

3.11 Specific target organ toxicity – repeated exposure

3.11.1 Animal data

3.11.1.1 [Study 1] NTP TR 587, 2014

Study reference:

National Toxicology Program. 2014; Toxicology studies of teterabromobisphenol A (Cas no. 79-94-7) in F344/NTac rats and B6C3F1/N mice and toxicology and carcinogeogenesis studies of tetrabromobisphenol A in Wistar Han [Crl:WI(Han)] rats and B6C3F1/N mice (gavage studies). NTP Technical Report 587

Detailed study summary and results:

Test type

3 months oral gavage study in rats and mice

Test substance

- Test material used in the study is equivalent to the substance identified in the CLH dossier
- Degree of purity: > 99%
- The impurities do not affect the classification
- Batch number 25317K-1

Test animals

- Male and female F344/NTac rats; male and female B6C3F1/N mice
- *No. of animals per sex per dose:* 10 male and 10 female rats and mice
- *Age and weight at the study initiation:* On receipt, the rats were 3 to 4 weeks old, and mice were 4 to 5 weeks old. rats were 5 to 6 weeks old and mice were 6 to 7 weeks old on the first day of the studies.

Administration/exposure

- *route of administration* – oral (gavage)
- *duration and frequency of test/exposure period:* Three months
- *doses/concentration levels, rationale for dose level selection:* Doses were 0, 10, 50, 100, 500, or 1,000 mg/kg body weight, 5 days per week for 14 weeks. Highest dose was 1,000 mg/kg for both rats and mice. Dosing volumes were 5 mL/kg for rats and 10 mL/kg for mice. Additional special study groups of 10 male and 10 female rats were administered the same doses for 23 days. Doses based on previous studies by WHO (IPCS, 1995) and NTP chemistry.
- *post exposure observation period:* N/A

- *vehicle:* Corn oil.
- *control group and treatment:* Corn oil alone
- *test substance formulation/diet preparation, achieved concentration by sex and dose level, stability and homogeneity of the preparation*
- *actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable*
- *statistical methods*

Results and discussion

Describe the relevant findings and toxic response/effects by sex and dose level. If no effects occurred, explicitly note "No effects".

- *body weight and body weight changes:* No treatment-related effect in rats.
- *food/water consumption*
- *description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed)*
- *sensory activity, grip strength and motor activity assessments (when available)*
- *ophthalmologic findings: incidence and severity*
- *haematological findings: incidence and severity:* Small effects observed in rats
- Clinical pathology and liver toxicity investigated (see page 31 in the NTP report)
  Low effect on hepatic function/injury and cholestasis in rats
- *clinical biochemistry findings: incidence and severity*
- *gross pathology findings: incidence and severity:* There were significant increases in the absolute and relative liver weights of 500 and 1,000 mg/kg male rats and female rats. 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.
- *histopathology findings: incidence and severity*
- *mortality and time to death (if occurring):* No mortality observed.
- At the end of the studies, spermatid and sperm samples were collected from male rats and mice in the vehicle control, 100, 500, and 1,000 mg/kg groups. The following parameters were evaluated: spermatid heads per testis and per gram testis, sperm motility, and sperm per cauda epididymis and per gram cauda epididymis. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from female rats and mice in the vehicle control, 100, 500, and 1,000 mg/kg groups.
- Consistent, progressive, and dose-related decreases in total thyroxine (T4) concentrations occurred in 500 and 1,000 mg/kg male and female rats; this effect was observed with with less consistency in the 100 mg/kg groups.

3.11.1.2 [Study 2] Dunnick et al., 2017

13 weeks oral gavage study in female Wistar Han rats

Study reference:

Dunnick et al., 2017, Tetrabromobisphenol A activates the hepatic interferon pathway in rats, Toxicol Lett. 2017 January 15; 266: 32–41

Detailed study summary and results:

Test type

Academic study to gain further insight into early gene and pathway changes leading to cancer in the 2-year studies in rats and mice (NTP, 2014). TBBPA-induced transcritomic changes were investigated in uterus (target site for tumours) and liver (primary site for metabolism). *[Guideline followed and any significant deviations from the guideline if applicable. If no guideline was followed, include a description of the test design. Please state if the study is GLP compliant or not.*

In addition to standard 28-day, 90-day and 2-year animal studies, other long-term exposure studies such as carcinogenicity, neurotoxicity and reproductive toxicity studies may provide evidence on specific target organ toxicity following repeated exposure.]

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Yes, TBBPA
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity:* > 99%
- *Impurities (or a note that the impurities do not affect the classification):* 0.8-1.1% tribromo-BPA (not expected to affect the classification)
- *Batch number:* M032607 K

Test animals

- *Species/strain/sex:* female Wistar Han rats
- *No. of animals per sex per dose:* 25
- *Age and weight at the study initiation:* 5-6 weeks

Administration/exposure

- *route of administration – oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapour, gas, particulate), other:* Oral gavage in corn oil

- *duration and frequency of test/exposure period*
- *doses/concentration levels, rationale for dose level selection*; 0, 25, 250, or 1000mg/kg, 5 times per week for 13 weeks
- *post exposure observation period*
- *vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water):* Corn oil, 5 ml/kg bw
- *control group and treatment*
- *test substance formulation/diet preparation, achieved concentration by sex and dose level, stability and homogeneity of the preparation*
- *actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable*
- *statistical methods:* bootstrap *t*-test approach (gene expression), rank-based statistical procedures (Ingenuity Pathway Analysis database), Ingenuity knowledge base, and the interferon database

Results and discussion

Describe the relevant findings and toxic response/effects by sex and dose level. If no effects occurred, explicitly note "No effects".

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.
Gene expression was evaluated between controls and dosed animals, and the Ingenuity Pathway Analysis database and the interferome database were used to analyse the results. See details in Dunnick et al., 2017.
No treatment-related microscopic lesions in the liver or uetrus.
The interferon (IFN) pathway was the most significantly affected pathway by TBBPA. This included transcripts associated with IFN pathway regulation (e.g. Stat1, Stat2, Ilf7, Irf9, Pml), antiviral activity (e.g. Mx1, Mx2, Ifit3, Isg15), and regulation of immune response. Also the TBBPA transcripts involved in liver xenobioticand fatty acid metabolism was upregulated. There were few changes in the uterine transciptome after TBBPA exposure. 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 also increased levels of the Cyp2b6, a transcript induced by phenobarbital and in xenobiotic metabolism.

- *body weight and body weight changes:* No effects
- *food/water consumption*
- *description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed)*
- *sensory activity, grip strength and motor activity assessments (when available)*

- *ophthalmologic findings: incidence and severity*
- *haematological findings: incidence and severity*
- *clinical biochemistry findings: incidence and severity*
- *gross pathology findings: incidence and severity*
- *histopathology findings: incidence and severity:* No treatment-related microscopic lesions in the liver or uetrus.
- *mortality and time to death (if occurring):* No deaths

3.11.1.3 [Study 3] Osimitz et al., 2016

Study reference:

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

Detailed study summary and results:

Test type

OECD TG 408 Repeated Dose 90-day Oral Toxicity Study in Rodents

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Yes, TBBPA. TBBPA was a composite of three commercial lots produced by Albemarle Corporation (Baton Rouge, Los Angeles, USA), Chemtura Corporation (Lafayette, Indiana, USA), and Israeli Chemical (Tel Aviv, Israel).
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity:* approximately 99%
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number*

Test animals

- *Species/strain/sex:* Male and female CD1 (Crl: CD (SD) IGS BR) rats from Charles River Laboratories (Portage, Michigan, USA)
- *No. of animals per sex per dose*
- *Age and weight at the study initiation:* 6-weeks old on arrival; body weight of males 128– 156 g and 110–151 g for females

Administration/exposure

- *route of administration – oral (gavage, drinking water, feed), dermal, inhalation (aerosol,*

vapour, gas, particulate), other: oral gavage

- *duration and frequency of test/exposure period:* Animals were treated for 13 consecutive weeks (once/day, 7 days/week). Recovery animals in the control and high-dose groups were not treated during a 6-week post-treatment recovery period.
- *doses/concentration levels, rationale for dose level selection:* Doses of 0, 100, 300, and 1000 mg/kg/day (gavage) were based on data from previous studies that indicated that the limit dose of 1000 mg/kg/day would not produce excessive toxicity. Two recovery groups were included (control, 1000 mg/kg/day).
- *post exposure observation period*
- *vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water):* corn oil
- *control group and treatment*
- *test substance formulation/diet preparation, achieved concentration by sex and dose level, stability and homogeneity of the preparation:* Homogeneity was assessed on test article suspensions at the low and high concentrations used during the first week of the study (20 and 200 mg/mL, respectively). Six samples (two top, two middle, and two bottom) were collected from each mix while stirring to assess for homogeneity. Refrigerated formulations of TBBPA in corn oil were determined to be stable for at least 14 days. Dosing formulations were analyzed for concentration and adjusted throughout the study to ensure the correct concentrations based on body weight.
- *actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable*
- *statistical methods:* Group pairwise comparisons (all end points except FOB (except continuous data), leukocyte counts, and urinalysis)

Results and discussion

Describe the relevant findings and toxic response/effects by sex and dose level. If no effects occurred, explicitly note "No effects".

- *body weight and body weight changes:* TBBPA had no effect on body weights
- *food/water consumption:* TBBPA had no effect on feed consumption
- *description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed):* The low occurrence of salivation in the 300 and 1000 mg/kg/day groups, possibly related to residual test article in the oral cavity, was not considered toxicologically meaningful because salivation was not seen in the 1000 mg/kg/day animals during the recovery period. Other clinical findings in the TBBPA-treated groups occurred at low incidence and were considered unrelated to treatment because they were spurious and not dose related.
- *sensory activity, grip strength and motor activity assessments (when available):*

- *ophthalmologic findings: incidence and severity:* Ophthalmoscopic examination revealed no TBBPA treatment-related effects.
- *haematological findings: incidence and severity:* A few spurious results were seen, but overall, there were no hematological alterations attributed to TBBPA treatment.
- *clinical biochemistry findings: incidence and severity:* With the exception of total bilirubin and serum alkaline phosphatase (ALP), all clinical chemistry values were within normal ranges for this species and not different from concurrent control values. After 13 weeks of dosing, total bilirubin values (mg/dL) were statistically higher than control mean values (males: 0.14 + 0.05; females: 0.13 + 0.05) in males in the 1000 mg/kg/day dose (0.34 + 0.024; $p < 0.01$) and in females in the 300 (0.19 + 0.03; 1218 Human and Experimental Toxicology 35(11) $p < 0.05$) and 1000 mg/kg/day (0.2 + 0.06; $p < 0.01$) groups. Mean serum ALP levels (U/L) after 90 days of dosing in the female 1000 mg/kg/day (98.9 + 49.47) group were statistically higher than that of the control mean values (58.4 + 28.46; $p < 0.05$; females: 47.7 + 8.15 in the 100 mg/kg/day group; 62.6 + 20.92 in the 300 mg/kg/day group). Serum bilirubin and ALP levels in control and treated groups of both sexes were comparable after the recovery period. In the absence of any corresponding elevation in liver weights, liver weight/body weight, or liver weight/ brain weight, as well as no histopathological changes, these clinical chemistry elevations were not considered to be toxicologically significant.

  No treatment-related changes in mean plasma T3 or TSH were seen at any dose or time in either males or females. In contrast, mean plasma T4 levels were decreased at all dose levels in both males and females at termination of the study (Figure 1 for males; Figure 2 for females, see below). Mean T4 levels (ng/dL) were statistically lower than control (day 33: 4.96 + 0.84; terminal: 5.09 + 0.80) in the 100 (day 33: 3.66 + 0.88; terminal: 3.27 + 0.67), 300 (day 33: 3.42 + 0.71; terminal: 2.61 + 0.87), and 1000 (day 33: 3.39 + 0.55; terminal: 3.09 + 0.91) mg/kg/day male dose groups at days 33 and 90 ($p < 0.01$), respectively. Mean T4 levels were also statistically lower than control values (4.27 + 0.96) in females in the 100 (3.31 + 1.08), 300 (3.24 + 0.85), and 1000 (3.33 + 0.84) mg/kg/day dose groups at day 33 ($p < 0.01$). Mean T4 levels in all female dose groups were unchanged relative to the control mean values at day 90. Following the recovery period, T4 levels in males at 1000 mg/kg recovered to levels comparable to controls, whereas the T4 levels in females at 1000 mg/kg were lower relative to controls, although they were comparable to controls at the 90-day time point. Table 6 (male rats) and Table 7 (female rats) below summarize the thyroid-related parameters.

Figures and tables from Osimitz et al., 2016:

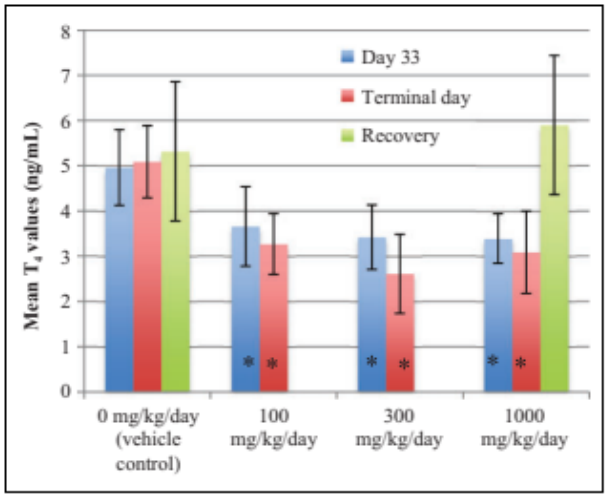

Figure 1. Mean T_4 levels in male animals (+/− SD). *$p < 0.01$: Significantly different from control.

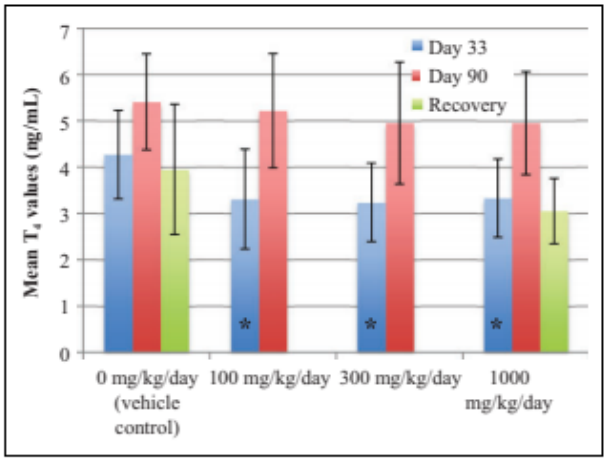

Figure 2. Mean T_4 levels in female animals (+/− SD). *$p < 0.01$: Significantly different from control.

Table 6. Treatment-related thyroid responses in male rats.

Dose (mg/kg/day)	Study day	Mean T_4 (ng/dL)	SD	*N* (number of measures used to calculate mean)	T_4 Percentage Change in Serum Level Compared to Controls (at Study day 33, 90, or recovery, as appropriate)	Mean thyroid/ parathyroid weight (mg)[a]	SD	*N* (number of measures used to calculate mean)	Thyroid Histopathology
0	33	4.96	0.837	15	NA	ND	ND	ND	ND
	90	5.09	0.797	15	NA	33	5.7	10	WNL
	Recovery	5.32	0.944	5	NA	35	3.5	5	ND
100	33	3.66[b]	0.878	10	−35.5%	ND	ND	ND	ND
	90	3.27[b]	0.672	10	−55.6%	30	7.6	10	ND
300	33	3.42[b]	0.713	10	−45.0%	ND	ND	ND	ND
	90	2.61[b]	0.874	10	−95.0%	32	3.6	10	ND
1000	33	3.39[b]	0.548	15	−46.3%	ND	ND	ND	ND
	90	3.09[b]	0.910	15	−64.7%	33	4.6	10	ND
	Recovery	5.90[c]	1.538	5	+10.9%	36	4.4	5	WNL

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

Table 7. Treatment-related thyroid responses in female rats.

Dose (mg/kg/day)	Study day	Mean T_4 (ng/dL)	SD	*N* (number of measures used to calculate mean)	T_4 percentage change in serum level compared to controls (at study day 33, 90, or recovery, as appropriate)	Mean thyroid/ parathyroid weight (mg)[a]	SD	*N* (number of measures used to calculate mean)	Thyroid histopathology
0	33	4.27	0.957	15	NA	ND	ND	ND	ND
	90	5.41	1.036	12	NA	26.0	4.2	9	WNL
	Recovery	3.95	1.406	4	NA	28.0	2.4	4	ND
100	33	3.31[b]	1.079	10	−29.0%	ND	ND	ND	ND
	90	5.22	1.234	10	−3.6%	29.0	4.9	10	ND
300	33	3.24[b]	0.846	10	−31.8%	ND	ND	ND	ND
	90	4.95	1.316	10	−9.3%	26.0	3.0	10	ND
1000	33	3.33[b]	0.844	15	−28.2%	ND	ND	ND	ND
	90	4.95	1.111	11	−9.3%	29.0	3.9	7	WNL
	Recovery	3.05[c]	0.705	4	−29.5%	27.0	3.1	4	ND

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

- *gross pathology findings: incidence and severity:* There were no test article-related macroscopic observations noted in either sex at terminal or recovery necropsy. There were no test article-related microscopic findings either in male or female animals at the high dose (1000 mg/kg/day) at terminal

euthanasia. Thus, tissues from the mid and low dose terminal euthanasia animals and from recovery animals were not evaluated.

- *histopathology findings: incidence and severity*
- *mortality and time to death (if occurring):* Six females (two control animals and four rats in the 1000 mg/kg/day group) died or were euthanized in extremis. The mortality/morbidity was related to dosing and was not treatment related. On the first day of treatment, one male in the 300 mg/kg/day group was euthanized due to excessive weight loss; this animal was replaced. No mortality occurred as a result of treatment with TBBPA.

3.11.1.4 [Study 4] Borghoff et al., 2016

Study reference:

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

Detailed study summary and results:

Test type

Similar to OECD TG 407 study, but only females and 6 animals per dose.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Yes, TBBPA
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity:* 98.83%
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number*

Test animals

Species/strain/sex: Female Wistar Han rats

- *No. of animals per sex per dose:* 6 per dose group
- *Age and weight at the study initiation:* 10 weeks of age

Administration/exposure

- *route of administration – oral (gavage, drinking water, feed), dermal, inhalation (aerosol, vapour, gas, particulate), other:* oral gavage
- *duration and frequency of test/exposure period:* 28 days
- *doses/concentration levels, rationale for dose level selection:* 0, 50, 100, 250, 500 and 1000 mg/kg
- *post exposure observation period*
- *vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water):* corn oil
- *control group and treatment*
- *test substance formulation/diet preparation, achieved concentration by sex and dose level, stability and homogeneity of the preparation*
- *actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable*
- *statistical methods*

Results and discussion

- *body weight and body weight changes:* Body weight gain did not change with dose of TBBPA (data not shown), nor was there any significant difference in the final terminal body weight on day 28 at any TBBPA dose level compared to the vehicle control group. There were no significant changes noted in the 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.
- *food/water consumption*
- *description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed)*
- *sensory activity, grip strength and motor activity assessments (when available)*
- *ophthalmologic findings: incidence and severity*
- *haematological findings: incidence and severity*
- *clinical biochemistry findings: incidence and severity:*

  Liver: The concentrations of TBBPA, TBBPA-GA and TBBPA-S measured in liver tissue collected from rats administered TBBPA for 28 consecutive days increased with dose of TBBPA (trend test; $p < 0.01$) at 4- and 8-h following the last dose. The concentration of TBBPA at 8-h was significantly lower than at 4-h at the two highest dose levels (t-test, $p < 0.05$). The concentration of TBBPAS was significantly higher (t-test, $p < 0.05$) than the concentration of TBBPA-GA at all dose levels.

  Plasma: The plasma concentration of TBBPA in rats administered TBBPA for 7, 14, and 28 days increased with TBBPA dose level (trend test, $p < 0.01$) at both 4- and 8-h following the last dose on each respective day, with lower levels measured at 8 h compared to the 4-h time point at the higher dose levels (t-test, $p < 0.05$). Similar to the parent TBBPA, the conjugates increased with TBBPA dose

level(trend test, $p < 0.01$) at both 4- (Fig. 7) and 8-h(data not shown) following the last dose on each respective day. TBBPA-GA plasma levels were higher compared to levels of TBBPA-S at dose levels >250 mg/kg (t-test, $p < 0.05$) at 4-h post dose. TBBPA-GA and TBBPA-S plasma levels were significantly higher following 28-days of dosing compared to either 7 or 14 days at dose levels above 50 mg/kg ($p < 0.05$). Similar to what was observed in the liver, the GA/TBBPA ratio evaluated in plasma increased with dose (trend test, $p < 0.01$) with no change in the S/TBBPA ratio at either 4- or 8-h following dosing on day 28.

Uterine: Similar to liver and plasma,the concentration of TBBPA and conjugates in the uterus increased with dose of TBBPA at both 4- and 8-h following dosing on day 28 (trend test, $p < 0.01$). Again, higher levels of TBBPA were measured at 4-h compared to 8-h post dose at dose levels of 250 mg/kg and above (t-test, $p < 0.05$). The concentration of TBBPA-GA was higher at 4-h compared to 8-h but only statistically significant following administration of the highest dose level of 1000 mg/kg dose (Fig. 10). Similar to plasma, the concentrations of TBBPA-GA in the uterus were higher compared to TBBPA-S at both 4- and 8-h post dosing, however, statistical significant in this tissue was only detected at the highest dose of 1000 mg/kg (t-test, $p \leq 0.05$).

The overall dose-related changes in the concentrations of TBBPA, TBBPA-GA and TBBPA-S, measured in all three tissues at 4 h following dosing on study day 28, are shown in Fig. 13 below.

Figure from Borghoff et al., 2016:

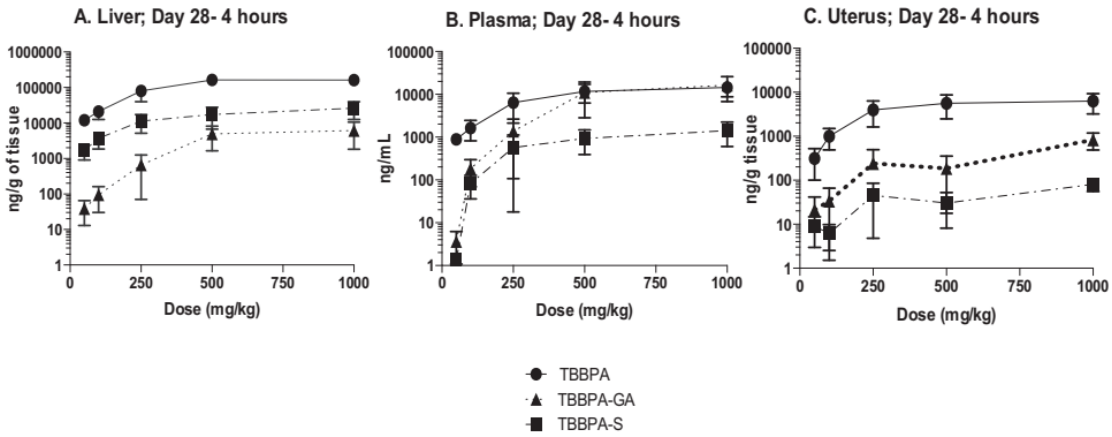

Fig. 13. The concentration of TBBPA, TBBPA-GA, and TBBPA-S in A liver, B plasma, and C uterus tissue 4 h post dosing on day 28. Each symbol is the mean ± SD (n = 4–6 per group).

- *gross pathology findings: incidence and severity*
- *histopathology findings: incidence and severity*

- *mortality and time to death (if occurring):* One animal in the 500 mg/kg group was found dead following dose administration on study day 21 due to suspected dosing error. No other instances of mortality or morbundity were observed.

3.11.1.5 [Study 5] Van der Ven et al., 2008

Study reference:

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

Detailed study summary and results:

Test type

Subacute toxicity study conducted as a repeated dose 28-day oral toxicity study in rodents. The study was conducted according to OECD TG 407 and enhanced for endocrine and immune parameters

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* TBBPA. TBBPA was obtained from the Bromine Science and Environmental Forum (BSEF).
- *Degree of purity:* The purity was 98% with tribromobisphenol A.
- *Impurities (or a note that the impurities do not affect the classification):* tribromobisphenol A and *o,p'*TBBPA were the major contaminants.

Test animals

- *Species/strain/sex:* Wistar rats (HsdCpb:WU)
- *No. of animals per sex per dose:* Each dose group and the control had 10 animals per per sex
- *Age and weight at the study initiation:* 7 weeks when purchased. Acclimatized at least one week before beginning exposures.

Administration/exposure

- *route of administration* – Oral, mixed with standard rat feed without soy
- *doses/concentration levels, rationale for dose level selection:* 4 dose groups 0, 30, 100 and 300 mg TBBPA/kg bw
- *statistical methods* (as reported by Van der Ven et al., 2008): Statistical analysis was performed wuth the same method as the reproduction study (see 3.10.1.3) except that there were three dose groups. Dose–response analysis of effects, based on external dosing (mg/kg bw) was done using a nested

family of purely descriptive (exponential) models with the PROAST software. This method enables integrated evaluation of the complete data set. From the best fitted curve, indicated by significance at the 5% level, a critical effect dose (CED, also referred to as Benchmark Dose) was calculated at a default critical effect size (CES) of 10%. Notable exceptions were liver weight and immune parameters, with a CES of 20%. A CES of 5% was used for testis weight and bone parameters. The analysis was completed with the calculation of a 90% confidence interval (two-sided), thus enabling the calculation of a 5% lower confidence bound of the CED estimate. This value may be considered as a BMDL (the lower confidence bound) for continuous data. The CED/BMDL ratio was used as a measure for the (statistical) uncertainty in a data set; a 10-fold difference between CED and BMDL was used as a practical limit for informative value. The controls were included as zero value input for the modelling calculations, although for graphical representation on a log-scale, an arbitrary value (but lower than the lowest dose) is used. BMDL values expressed as external doses were converted to internal doses (liver concentrations) using the regression equation that was fitted to external versus internal dose. For the most sensitive effects in this study, i.e. effects with the lowest BMDLs, the benchmark analysis was repeated based on internal doses.

Results and discussion

- TBBPA concentration in liver and plasma: Liver concentrations of TBBPA increased in a dose-dependent way, when compared to the concentrations measured in the reproductive study (see 3.10.1.3) it was reported 12–55 times lower concentrations. In contrast the plasma concentrations were higher in comparison to what was reported in the reproduction study (see 3.10.1.3) in both sexes. This was the similar for all dose groups except 300 mkd in females. Plasma concentrations of all three analytes correlated well with external dose in males, although not in females due to incongruity in the 300 mg/kg bw dose group. In controls female samples it was determined relative high concentrations in a limited number of samples in the, but the majority was blank.
- Organ weight: No dose related effects in any of the organ weights, notably not of the testis and male pituitary
- Histopathology: No TBBPA related effects in the histopathology parameters.
- Tyroid Hormones: Significant decrease of T4 (BMDL of 48 mg/kg bw) and increase of T3 (BMDL of 123.8 mg/kg bw) in males. In females non-significant parallel trends were reported
- Bone parameters: No dose related effects in trabecular and cortical bone parameters of tibia
- Immunotoxicology and haematology: No important TBBPA related effects in the immune and haematological parameters

No effects on food intake, body weight, or organ weights in both sexes. No TBBPA related effects in the histopathological parameters. No effects on trabecular and cortical bone parameters of tibia or immune and haematological parameters.

Liver concentrations of TBBPA increased in a dose-dependent manner, but they were 12-55 times lower compared to the same dose groups in the reproduction study (data reported in 3.10.1.3. Study 3 Van der Ven et al, 2008). Furthermore, plasma concentrations of TBBPA were higher then in the reproduction study for both sexes in all dose groups except females exposed to 300 mg/kg bw/day).

There were changes in 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).

3.11.1.6 [Study 6] Unnamed study report 2001

Study reference:

Unnamed study report, 2001

(Registrant: Reliable without restriction: 1)

Detailed study summary and results:

Test type

Repeated Dose 90-Day Oral Toxicity in Rodents (OECD TG 408). GLP compliant.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier*
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity:* 98.71 - 98.87 %
- *Impurities:* o,p-Tetrabromobisphenol-A (0.04%) and Tribromobisphenol-A (1.25%)
- *Batch number:* 5381A

Test animals

- *Species/strain/sex:* Rat, Sprague-Dawley, males and females
- *No. of animals per sex per dose:* 100 and 300 mg/kg/day: 10m/10f. Controls and 1000 mg/kg/day: 15m/15f.
- *Age and weight at the study initiation*: 8 weeks, male weight: 128-156 g, female weight: 100-151 g

Administration/exposure

- *route of administration* – oral (gavage)
- *duration and frequency of test/exposure period*
- *doses/concentration levels, rationale for dose level selection:* 0, 100, 300 and 1000 mg/kg/day. Based on available data from previous studues
- *post exposure observation period:* 5f/5m from the control and 5f/5m from the 1000 mg/kg/day group where evaluated over a 6 week post treatment period.
- *vehicle:* Corn oil
- *control group and treatment:* treated with vehicle
- *test substance formulation/diet preparation, achieved concentration by sex and dose level, stability and homogeneity of the preparation:* Diet preparations: weekly, mixing appropriate amounts with corn oil for gavage.
- *actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable*
- *statistical methods*

Results and discussion

Describe the relevant findings and toxic response/effects by sex and dose level. If no effects occurred, explicitly note "No effects".

- *body weight and body weight changes:* no effects observed
- *organ weight:* no effects observed
- *food/water consumption:* no consistent effects observed
- *description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed)*
- *motor activity: no effects observed*
- *ophthalmologic findings:* no effects observed
- *haematological findings:* no effects observed, except for transient decrease in platelet counts in top dose males
- *clinical biochemistry findings:*

  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 the table below. 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).

Table 14: Treatment related serum T4 levels (ng/dL) in male and female rats

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 (*)

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.

- *gross pathology findings: no effects observed*
- *histopathology findings: no effects observedy*
- *mortality and time to death: no effects observed.* 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.

3.11.1.7 [Study 7] Unnamed, Study report 1975

Study reference:

Unnamed, Study report 1975.

(Registrant: reliable with restrictions: 2)

Detailed study summary and results:

Test type

2 weeks study, similar to OECD TG 412, (Subacute Inhalation Toxicity: 28-Day Study). GLP not specified.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Constituent
- *EC number (if different from the substance identified in the CLH dossier)*

- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity*
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number*

Test animals

- *Species/strain/sex:* Rat, Crj: CD(SD), male and female
- *No. of animals per sex per dose:* 5m/5f/dose
- *Age and weight at the study initiation*

Administration/exposure

- *route of administration* –inhalation (dust)
- *duration and frequency of test/exposure period*: 4 h daily, 5 d/wk for 2 weeks
- *doses/concentration levels, rationale for dose level selection:*
  Doses / Concentrations: 0, 2, 6, 18 mg/L
  Basis: nominal conc.
- *post exposure observation period*
- *vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water)*
- *control group and treatment:* yes concurrent, no treatment
- *test substance formulation/diet preparation, achieved concentration by sex and dose level, stability and homogeneity of the preparation*
- *actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable*
- *statistical methods*

For inhalation studies:

- *type of inhalation exposure and test conditions (e.g.: exposure apparatus)*
- *method of exposure (“whole body”, “oro-nasal”, or “head only”), exposure data*
- *analytical verification of test atmosphere concentrations*
- *particle size (for studies with aerosols, indicate mass median aerodynamic diameter and geometric standard deviation or give other specifications)*
- *type or preparation of particles (for studies with aerosols)*

Results and discussion

Describe the relevant findings and toxic response/effects by sex and dose level. If no effects occurred, explicitly note "No effects".

- *body weight and body weight changes:* No effects observed
- *food/water consumption:* No effects observed
- *description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed):* excessive salivation, red or clear nasal discharge
- *sensory activity, grip strength and motor activity assessments (when available)*
- *ophthalmologic findings: incidence and severity:* effects observed, treatment-related, excessive tearing
- *haematological findings: incidence and severity:* No effects observed
- *clinical biochemistry findings: incidence and severity:* No effects observed
- *gross pathology findings: incidence and severity:* No effects observed
- *histopathology findings: incidence and severity:* No effects observed
- *mortality and time to death (if occurring):* Mortality observed, treatment-related

3.11.1.8 [Study 8] Unnamed, Study report 1979

Study reference:

Unnamed, Study report 1979.

(Registrant: reliable with restrictions: 2)

Detailed study summary and results:

Test type

3 weeks study, Short-term repeated dose toxicity: dermal, no guideline available. GLP not specified. No analytical verification of test compound concentrations.

Tetrabromobisphenol-A was applied to the shaved skin of male and female rabbits 5 days a week for three weeks at dose levels of 100, 500, and 2500 mg/kg/day. The rabbits were observed daily for signs of overt toxicity, dermal irritation, moribundity and mortality . Body weights were recorded weekly. Hematologic and biochemical studies and urinalyses were conducted during the pretest period and at 3 weeks of study.

Test substance

- *Indicate if the test material used in the study is equivalent to the substance identified in the CLH dossier:* Constituent
- *EC number (if different from the substance identified in the CLH dossier)*
- *CAS number (if different from the substance identified in the CLH dossier)*
- *Degree of purity*
- *Impurities (or a note that the impurities do not affect the classification)*
- *Batch number:* 1021-85, received from Velsicol Chemical Corp

Test animals

- *Species/strain/sex:* New Zealand White rabbits
- *No. of animals per sex per dose:* 4 male, 4 female
- *Age and weight at the study initiation:* Age not reported. Male 1886 to 2284g; Female 2030 to 2311g

Administration/exposure

- *route of administration:* dermal
- *duration and frequency of test/exposure period:* 6 h daily, 5 d/wk for 3 weeks,
- *doses/concentration levels, rationale for dose level selection:* Doses / Concentrations: 0, 100, 500 and 2500 mg/kg/day. Increase of 5x.
- *post exposure observation period*
- *vehicle: identification, concentration and volume used, justification of choice of vehicle (if other than water):* 0.9% physiological saline to form paste
- *control group and treatment:* yes, concurrent vehicle
- *test substance formulation/diet preparation, achieved concentration by sex and dose level, stability and homogeneity of the preparation*
- *actual dose (mg/kg bw/day) and conversion factor from diet/drinking water test substance concentration (ppm) to the actual dose, if applicable*
- *statistical methods:* One-way ANOVA, Bartlett's test, Dunnett's

For dermal studies:

- *area covered (e.g. 10% of body surface):* 10% of body area
- *occlusion (e.g. semi-occlusive)*
- *total volume applied:* 1.5 mL/kg
- *removal of test substance (e.g. water or solvent)*

Results and discussion

Describe the relevant findings and toxic response/effects by sex and dose level. If no effects occurred, explicitly note "No effects".

- *body weight and body weight changes:* no effects observed
- *food/water consumption:* not examined
- *description, severity, time of onset and duration of clinical signs (reversible, irreversible, immediate, delayed):* no effects observed
- *sensory activity, grip strength and motor activity assessments (when available)*
- *ophthalmologic findings:* not examined
- *haematological findings:* no effects observed
- *clinical biochemistry findings:* no effects observed

- *gross pathology findings:* no effects observed
- *histopathology findings:* no effects observed
- *mortality and time to death (if occurring:* no mortality observed
- *organ weights:* there was a statistically significant increase in mean absolute brain weight for males in the 500-mg/kg/day group. The biological significance of this variation is not known.
- *erythema:* 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.

Table 15: Description of dermal effects observed in rabbits exposed to TBBPA

Treatment level	**Effect**	**Degree**	**Number affected**	**Duration**
100 mg/kg/day	Erythema	Very slight	2	Short –term, not specified
500 mg/kg/day	Erythema	Very slight	8	1 to 3 days
2500 mg/kg/day	Erythema	Very slight	6	3+ days