Toxic Effects of Tetrabromobisphenol A: Focus on Endocrine Disruption

Didem Oral,[a] Aylin Balcı,[a] Ming-Wei-Chao,[b,c] & Pinar Erkekoglu[a,d,*]

[a]Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkey; [b]Department of Bioscience Technology, Chung Yuan Christian University, Zhongli District, Taoyuan 32023, Taiwan; [c]Center for Nanotechnology, Chung Yuan Christian University, Taoyuan 32023, Taiwan; [d]Hacettepe University, Vaccine Institute, Ankara, Turkey

*Address all correspondence to: Pinar Erkekoglu, Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, Ankara, Turkey; Tel.: +903123052178; Fax: +90 312 3092958, E-mail: erkekp@yahoo.com

ABSTRACT: Tetrabromobisphenol A (TBBPA) is a brominated flame retardant that is used in a variety of consumer products such as electronic equipment, fire extinguishers, furniture, plastics, textiles, and kitchen hoods. Most studies show that the TBBPA production process and TBBPA in industrial and urban sewage waste result in extensive human exposure and environmental contamination. TBBPA can accumulate in organisms, particularly aquatic life, and is classified as a group 2A carcinogen (likely carcinogenic to humans) by the International Agency for Research on Cancer. This compound produces low acute toxicity, but chronic exposure may produce serious consequences. In this review, we focus on TBBPA toxicity by discussing results of various studies that were published in the last two decades. Studies show that TBBPA acts as an endocrine disruptor, causing neurobehavioral and immunotoxic effects, oxidative stress, and apoptosis. Although several experiments were performed *in vitro* and *in vivo*, human data are lacking, and thus, chronic toxic effects of TBBPA on humans are not well known, particularly in sensitive populations including pregnant women, newborns, children, and the elderly. Epidemiological studies that comprehensively assess TBBPA levels in biological fluids of different populations and in different pathological conditions are needed. Research on the impact of TBBPA, particularly regarding endocrine disorders and cancer, must also be performed.

KEY WORDS: tetrabromobisphenol A, TBBPA, toxicity, oxidative stress, endocrine disruption, endocrine system disorders

I. INTRODUCTION

Tetrabromobisphenol A (TBBPA) is an efficacious brominated flame retardant that is mainly used in epoxy resin circuit boards, polycarbonate ether, and polyester production. Polycarbonates and polyesters are present in a variety of consumer products such as electronic equipment, furniture, plastics, textiles, and kitchen hoods.[1–3] It is estimated that 70%–90% of TBBPA is used as a reactive flame retardant in epoxy, phenolic, and polycarbonate resins. The remaining 10%–20% is found in additive flame retardants for plastics and phenolic resins.[4] The chemical structure of TBBPA is given in Fig. 1.[5]

TBBPA is involved in more than 200,000 metric tons of annual global production. In descending order, China, Israel, the United States, Jordan, and Japan are the highest TBBPA-producing countries in the world.[6,7] In 1999, the global market demand for TBBPA was 121,300 tons, and in 2004 production reached 170,000 tons. This number grows by 8%–9% every year.[8,9] During the next 5 yr, the TBBPA market will register a 3.1% compound annual growth rate in terms of revenue, and global market size will reach $1080.2 million by 2024, from $956.4 million in 2019.[10] No current restrictions on production of TBBPA or its derivatives are in effect in the European Union or worldwide.[11] The United Kingdom Committee on Toxicology set the only exposure limit value for TBBPA to be a tolerable intake of 1 mg/kg of body weight (bw)/d.[12] According to the Globally Harmonized System of Classification and Labelling of Chemicals, TBBPA is very toxic to aquatic life (H400), producing very toxic to aquatic life with long lasting effects (H410).[13]

In 2006, the European Chemicals Agency published a risk assessment report on TBBPA and concluded that exposure does not cause pronounced effects in humans.[13] However, in 2018 the International Agency for Research on Cancer (IARC)

0731-8898/21/$35.00

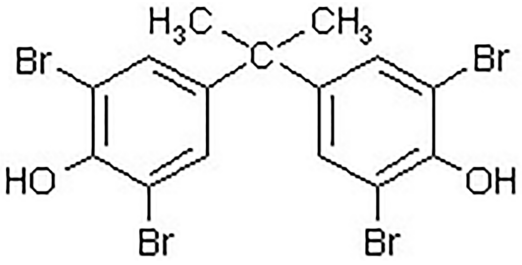

FIG. 1: TBBPA chemical structure

classified TBBPA as a group-2A carcinogen (likely carcinogenic to humans).[11] *In vivo* and *in vitro* studies show clear evidence of carcinogenicity in laboratory animals and strong mechanistic evidence in humans.[7]

In this review, we discuss possible toxic effects of TBBPA and its relevance to human health and other organisms. We also focus on reprotoxicity and the endocrine disruption that is caused by this particular bisphenol derivative. We cite both *in vitro* and *in vivo* studies that have been published in recent years.

II. TBBPA ENVIRONMENTAL DISTRIBUTION

TBBPA includes two phenolic hydroxyl groups. Because of these reactive groups, TBBPA can act as an alternative material for polymers, such as polypropylene, high- and low-density polyethylene, and high-impact polystyrene. These hydroxyl groups are estimated to have pKa_1 and pKa_2 values of 7.5 and 8.5, respectively. TBBPA solubility in water enhances with increasing pH.[7]

Some reactive flame retardants (polybrominated biphenyls, polybrominated diphenyl ethers, and hexabromocyclododecane) are not chemically bound to polymers.[14] Although TBBPA has a greater binding capacity to the polymer matrix compared to other flame retardants, it can also leach out of the matrix, like bisphenol A (BPA), and can be easily released into the environment.[15] TBBPA is a highly lipophilic substance (log K_{ow} = 4.5–5) with environmental stability. Therefore, it can spread through various environmental areas such as soil, dust, river marine sediment, and wastewaters.[16,17]

Most studies show that TBBPA production as well as waste processes (industrial and urban sewage) cause leaks into the aquatic environment. Additionally, TBBPA can be released as a dissolution substance into overlying water and become absorbed as suspended particulate material. Studies showed that due to its low water solubility, TBBPA is stored in sediment, can instantly release into water, and relocate over long ranges.[8,14] Therefore, TBBPA can accumulate in organisms.[17,18]

Recent studies have shown that TBBPA is present in wastewater treatment plants, air, soil, rivers, marine sediment, and dust.[17] TBBPA has been found at 0.3–0.54 ng/L concentrations in landfill leachate in Japan.[19] In terms of TBBPA concentrations in soil, dust, and water, China has higher levels of TBBPA compared to other countries.[4] A study conducted in China showed that TBBPA was present from 1110 to 2830 pg/L in river water.[20] Xiong et al.[21] estimated that TBBPA concentrations in water and sediment samples ranged from 20 to 270 pg/L and from 20 to 600 pg/g in the Beijing River (South China), which had a 4.82×10^{10} m^3 annual runoff volume. Two electronic waste separation regions are located upstream of Beijing River, and the river runs through urban, industrialized, less industrialized, and rural areas.[21] Another study conducted in China demonstrated that TBBPA concentrations in Taihu Lake's sediment ranged from 0.168 to 2.66 ng/g.[15] In a study by Gorga et al.[22] conducted in Spain, determined that TBBPA accumulated in sewage sludge in northeastern Spain (13.5 kg/yr).[22]

III. TBBPA EXPOSURE ROUTES IN HUMANS

It has been suggested that the general population may be exposed to TBBPA due to inhalation of ambient/indoor air and air dust. Moreover, dermal contact, ingestion from foods (particularly seafood), breast milk, and drinking water are other routes of exposure. TBBPA has been detected at parts per billion levels in human serum/plasma/whole blood. A 2004 study by the World Wildlife Fund[23] found that TBBPA was present at 0.002–0.3333 μg/L concentrations in whole blood samples of the European population (33%; $n = 47$).[23] In the same year, Peters[24] conducted a study in The Netherlands and found that

TBBPA serum concentrations varied from 0.056 to 0.787 μg/L in the Dutch population (50%; $n = 91$).[24] Later, the same author conducted another study and observed TBBPA maternal serum concentrations to be between 0.06 and 0.19 μg/L (21%; $n = 42$).[25] Thomsen et al.[26] performed a study in Norway and found that serum TBBPA levels ranged from 0.002 to 0.005 μg/L (100%; $n = 20$).[26] Kiciński et al.[27] conducted a study in Belgium on 515 adolescents and found serum concentrations of TBBPA to be < 0.01–0.186 μg/L.[27] In a study conducted in Belgium on 274 nonoccupationally exposed individuals between ages 18 and 76 yr, who lived in Liege (Belgium) and the surrounding area, TBBPA was positively detected in 31% of samples, which was higher than the detection rate reported by Dirtu et al.[28] in another Belgian population (5%; $n = 20$) but similar to the detection rate reported in Romania in the same study (36%; $n = 53$).[28] Other studies highlighted the presence of TBBPA in human serum samples with similar detection rates, notably in France (32%; $n = 91$), Japan (28%; $n = 60$), and China (36%; $n = 42$).[29–31] However, small sizes of human biological samples and wide differences among sensitivities of analytical methods (e.g., differing limits of quantifications) made comparisons difficult.

Several studies determined that TBBPA concentrations in breast milk could range from 0.062 to 37.4 ng/g. It was also suggested that 70% of all food samples contain TBBPA (range: 0.19 to 0.74 ng/g). Aquatic food holds the highest levels, followed by meat, milk, and eggs, respectively.[6] Ni and Zeng[32] indicated that contaminated dust in office buildings may contribute to > 80% of human exposure, 76% from ingestion, and 4% from inhalation in Shenzen, China.[32]

Studies have shown that occupational exposure to TBBPA is of particular importance. Occupational exposure can arise from dust inhalation, dermal contacts, or ingestion at work sites where TBBPA is used or produced.[6,32]

IV. TBBPA BIOTRANSFORMATION

Oral intake (via diet and/or dust in mucociliary transport) is the major exposure route for TBBPA. TBBPA bioavailability and excretion kinetics are suggested to be similar among rats and humans. After oral administration, 71% to 100% of TBBPA is absorbed from the gastrointestinal tract in rats.[33] A study by Kuester et al.[34] reported that TBBPA had low bioavailability due to first-pass elimination and conjugation (by glucuronic acid and sulfate) in male Fischer 344 (F344) rats. Using C^{14}-TBBPA, the researchers observed that following a single oral administration (at 2, 20, or 200 mg/kg doses), TBBPA was eliminated in the first 24 h. With feces the main TBBPA elimination route, 90% of the C^{14}-TBBPA was found there. Following oral and intravenous administration, TBBPA has a meaningful positive linear excretion with exposure dose.[34] However, after inhalation, TBBPA was detected in < 0.1% of the inhaled dose in serum and the eliminated amount of TBBPA in urine was found to be less than that of the inhaled dose.[35] Studies indicate that TBBPA can also be detected in adipose tissue; therefore, TBBPA may accumulate in fat.[29,36]

Quantitative studies demonstrated that after oral administration of TBBPA, highest TBBPA levels and metabolites were observed in blood within 4–6 h, and then levels rapidly decreased. Schauer et al.[37] studied TBBPA systemic bioavailability in rats and humans after oral administration. TBBPA was administrated in a single oral dose of 0.1 mg/kg within a gel capsule to five healthy male and five healthy female subjects. Urine and blood samples were collected at predetermined intervals from 0–178 h. For the animal study, six male Sprague–Dawley (SD) rats were administered a single oral dose of 300 mg/kg TBBPA. Urine and blood samples were collected 100 h after dosing. All samples were quantified by liquid chromatography tandem mass spectrometry. In human blood samples, TBBPA, TBBPA-glucuronide, and TBBPA-sulfate were detected. TBBPA-sulfate was found to be the major metabolite of TBBPA in plasma and reached peak concentrations of ~ 700 nmol/L 6 h after oral treatment. TBBPA-glucuronide concentrations reached peak concentrations of 16 nmol/L 2–6 h after oral exposure. In urine samples, TBBPA-glucuronide concentrations peaked at 4 nmol/L after 63 h in rats after a single oral dose of 300 mg/kg, and peak concentration reached 103 μmol/L within 3 h.[37] *In vitro* and *in vivo* TBBPA metabolism is given in Fig. 2.[29,33,34,36–38]

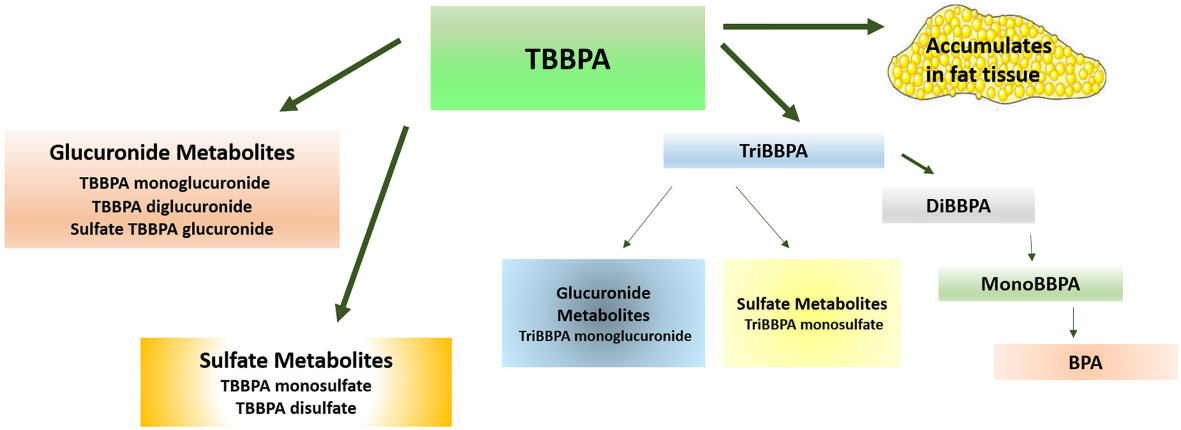

FIG. 2: *In vitro* and *in vivo* TBBPA metabolism. BPA, Bisphenol A; DiBBPA, dibromobisphenol A; monoBBPA, monobromobisphenol A; TBBPA, tetrabromobisphenol A; TriBBPA, tribromobisphenol A.

V. TOXIC EFFECTS OF TBBPA

In recent years, most studies that were conducted on animal models and human volunteers as well as *in vitro* studies focused on TBBPA's toxic effects. Studies showed that TBBPA could act as an endocrine-disrupting chemical and cause neurobehavioral effects, oxidative stress, and apoptosis. Additionally, it was suggested to have immunotoxic effects.[39] On the other hand, studies showed that TBBPA had low acute toxicity but may result in considerable adverse effects after repeated doses.[33] All possible toxic effects of TBBPA are summarized in Fig. 3.[33,39]

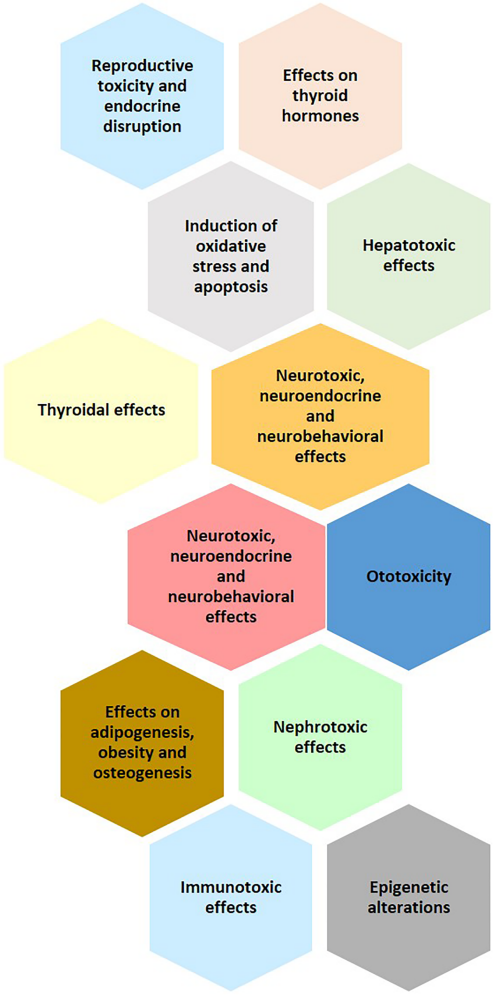

FIG. 3: All possible toxic effects of TBBPA

VI. TBBPA EFFECTS ON OXIDATIVE STRESS AND APOPTOSIS

A. *In Vitro* Studies

Studies show that one of the main underlying mechanisms of hepatotoxicity is oxidative stress, which may lead to hepatocyte apoptosis. Additionally, nuclear factor erythroid 2–related factor 2 (Nrf2) expression plays a key part in hepatocyte sensitivity to oxidative stress by regulating antioxidant enzymes and detoxification pathways. TBBPA was suggested to induce oxidative stress by affecting the Nrf2 pathway. Zhang et al.[40] studied the effects of TBBPA on oxidative stress, mitochondrial apoptosis, and the

Nrf2 signaling pathway. These researchers exposed L02 cells (human hepatocytes) to different doses (0, 5, 10, 20, and 40 μM) of TBBPA. Results showed that TBBPA significantly enhanced intracellular reactive oxygen species (ROS) production, increased malondialdehyde (MDA) levels, and augmented the ratio of oxidized/decreased glutathione (GSH) in a dose-dependent manner. Additionally, TBBPA reduced cell mitochondrial membrane potential, causing cytochrome C to release into the cytoplasm and triggering expression of caspase 9 and 3, which eventually led to apoptosis. Moreover, TBBPA induced expression of antioxidant genes related to Nrf2, such as catalase (CAT), heme oxygenase, and quinone oxidoreductase 1.[40]

Jarosiewicz et al.[41] evaluated the effects of TBBPA and other brominated flame retardants (tetrabromobisphenol S [TBBPS]; 2,4-dibromophenol [2,4-DBP]; 2,4,6-tribromophenol [2,4,6-TBP]; and pentabromophenol) on hemolysis induction and hemoglobin oxidation in human erythrocytes. Human erythrocytes were exposed to brominated flame retardants in different doses (0.01 to 100 μg/mL) with different incubation periods (24, 48, and 72 h). All of the brominated flame retardants had hemolytic potential and caused induction of methemoglobin formation in dose- and time-dependent manners. The numbers of aromatic rings and bromine atoms in the brominated flame retardants positively impacted hemoglobin oxidation and cellular membrane damage. 2,4-DBP was found to be the most potentially toxic compound, because it led to marked changes at the lowest concentration. However, highest toxicity was observed at the highest TBBPA concentration.[41] In another study, Jarosiewicz et al.[42] demonstrated that TBBPA could disturb redox balance in human erythrocytes. Human erythrocyte cells were incubated with TBBPA at varying concentrations (1–100 μg/mL) for 48 h. Later, superoxide dismutase (SOD), CAT, and GSH peroxidase (GPx) activities and GSH levels were measured. Results showed that TBBPA exposure caused decreases in SOD, CAT, and GPx activities and in GSH levels in a dose-dependent manner.[42] The same working group also investigated the effect of TBBPA; TBBPS; 2,4-DBP; and 2,4,6-TBP on apoptosis of human erythrocytes and assessed eryptotic potential of these substances by determining changes in phosphatidylserine (PS) translocation, alterations in intracellular ROS and calcium ion levels, and caspase 3 and calpain activation. All brominated flame retardants caused increases in intracellular ROS production, even at lowest concentrations applied (0.001 μg/mL), and all led to apoptosis by PS externalization and caspase 3 activation in erythrocytes. However, the researchers suggested that both calcium ions and calpain did not have marked roles in eryptosis induction by brominated flame retardants.[43]

Matrix metalloproteinase-9 (MMP-9) has a key role in angiogenesis, wound healing, and inflammatory response. Elevated MMP-9 levels may induce carcinogenesis and metastasis.[44] MMP-9 production has two major critical regulation pathways: mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt). Activated MAPKs and Akt can induce transcription factors such as nuclear factor (NF)-κB and activator protein–1 (AP-1) that in turn regulate MMP-9 expression. It has been suggested that nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX)-derived ROS can trigger various signaling pathways such as Akt and MAPK and may activate transcription factors such as NF-κB and AP-1.[44] Recently, Lee et al.[45] reported that TBBPA has immunotoxic potential and can induce MMP-9 expression. After Michigan Cancer Foundation (MCF) cells were treated for 24 h with different concentrations of TBBPA (ranging from 1 to 10 μM), TBBPA exposure induced MMP-9 messenger (m)RNA expression and MMP-9 luciferase activity in dose-dependent manners. Additionally, TBBPA caused concentration-dependent increases in MMP-9 protein expression. This particular BPA derivative significantly increased NF-κB– and AP-1–responsive luciferase activity dose dependently. Additionally, TBBPA induced production of NOX-derived ROS by activating both Akt and MAPK signaling pathways. These findings suggest that TBBPA increases MMP-9 expression by NF-κB and AP-1 activation and activates Akt/MAPK signaling pathways by increasing intracellular ROS production in MCF-7 cells.[45]

Studies show that TBBPA exposure could lead to metabolic perturbation through high levels of

ROS production. Zhao et al.[46] showed that micromolar levels (0–10 μM) of TBBPA triggered cell proliferation and activated glycolysis and amino acid metabolism in human breast cancer cells. Moreover, TBBPA caused an imbalance between intracellular ROS production and antioxidant defense by down-regulating GSH biosynthesis and up-regulating nucleotide metabolism.[46]

After ingestion and dermal contact, inhalation is the most important route of exposure to different chemicals. Studies showed that TBBPA concentrations in indoor air were significantly higher than those of outdoor air. Inhalation of indoor dust is responsible for 70%–80% of TBBPA exposure in all age groups.[47] Lungs are directly exposed to contaminated air particles after inhalation, and they are one of the main target organs of TBBPA. TBBPA can easily be distributed to the whole respiratory tract and may reach pulmonary alveoli. Wu et al.[48] investigated the cellular response including intracellular ROS production, MDA levels, caspase 3 activation, and ultrastructural changes of the lung epithelial-like cell line (A549 cells) to TBBPA. When A549 cells were incubated for 12, 24, and 48 h with various concentrations of TBBPA (8, 16, 32, and 64 μg/mL), TBBPA was found to induce cytotoxicity. Both MDA levels and caspase 3 activities significantly increased in dose- and time-dependent manners. After 24 h of exposure, significant increases in intracellular ROS production were found at 32 and 64 μg/mL. After 48 h, TBBPA caused marked increases in ROS production at 16, 32, and 64 μg/mL. The cells showed ultrastructural changes, including mitochondrial damage and smooth endoplasmic reticulum dilatations, after 48 h of TBBPA exposure at 64 μg/mL. Increasing TBBPA concentrations also caused vacuolization in the mitochondrial inner membrane. These investigators suggested that both TBBPA exposure period and dose significantly affected cell viability, ROS and MDA levels, and caspase 3 activity.[48]

B. Aquatic Animal Studies

Toxicity studies have shown that TBBPA is especially destructive to aquatic animals. We know that TBBPA can leach from landfills to surface waters and latent risks exist for multiple aquatic species.[49] In fish toxicity studies, TBBPA led to acute toxicity in aquatic life even at low concentrations. Tests conducted on zebrafish (*Danio rerio*) indicated that during zebrafish development, TBBPA could be more toxic than BPA and TBBPA–dimethyl ether.[50]

Wu et al.[39] demonstrated that TBBPA could lead to oxidative stress and apoptosis in zebrafish. The researchers exposed embryos to TBBPA at different concentrations (0.10, 0.40, 0.70, and 1 mg/L). After 72 h of exposure, a significant decrease in serum Cu/Zn SOD activity was detected at 0.40, 0.70, and 1 mg/L concentrations versus in controls. In addition, marked decreases in serum CAT and GPx activities occurred at different doses (0.40, 0.70, and 1 mg/L) compared to controls. TBBPA significantly induced apoptosis in zebrafish embryos and larvae at all concentrations.[39]

Sharma et al.[51] demonstrated that TBBPA has genotoxic potential and can induce oxidative stress in the freshwater fish *Channa punctatus*. In this study, the median lethal concentration (LC_{50}) value for TBBPA was found to be 10.18 mg/L. Fish were exposed to 5.09 mg/L TBBPA (half of LC_{50}). Acetone and water were used as positive and negative groups, respectively. Blood samples were collected at 24, 48, 72, and 96 h, and MDA levels were found to significantly increase in the TBBPA-exposed group compared to both positive and negative controls at all exposure periods. Moreover, TBBPA induced significant DNA damage after 96 h, as evidenced by both tail intensity and tail moment increases using the Comet assay (versus both positive and negative controls; $p < 0.05$ for both).[51]

VII. EFFECTS OF TBBPA ON THYROID HORMONES

A. *In Silico* Studies

In 2006, Hamers et al. showed TBBPA to be a potent competitor of thyroxine (T_4) when binding to transthyretin (TTR) in a TTR-binding assay. This bisphenol derivative showed even higher TTR-binding potency compared to the natural ligand T_4.[52]

B. *In Vitro* Studies

In vitro studies indicate that thyroid hormone functions could be greatly affected after TBBPA exposure. As mentioned above, TBBPA competes in binding T_4 to TTR, a plasma transport protein. Additionally, high TBBPA doses can increase thyroid hormone–dependent growth hormone (GH) in rat pituitary tumor cells (GH3 cells).[53] Kitamura et al.[53] reported increased GH3 cell proliferation when cells were treated with TBBPA at 1×10^{-10} to 1×10^{-6} M concentrations. After exposure to TBBPA, the cells showed dose-dependent GH release activity. When the cells were exposed to triiodothyronine (T_3) at a wide range of doses (1×10^{-9} to 1×10^{-1} M), they also showed dose-dependent GH release. Therefore, due to TBBPA's structural resemblance to thyroid hormones (due to phenyl groups that include the 4-hydroxyl group and two adjacent 3,5-halogen substituents), TBBPA can stimulate GH release and production in GH3 cells and may be as potent as T_3.[53]

C. Aquatic Animal Studies

TBBPA has structural similarities to thyroid hormones T_3 and T_4. Therefore, this bisphenol derivative may interact with thyroid hormones and affect several components of the thyroid system.[54] Kuiper et al.[55] studied long-term exposure of European flounder (*Platichthys flesus*) to flame retardants. The researchers found that exposure to TBBPA could increase plasma T_4 levels in this fish, possibly by competing for plasma binding protein. They demonstrated that exposure to TBBPA at nominal concentrations (0, 0.001, 0.01, 0.1, 0.2, 0.4, and 0.8 μM) for a period of 105 d resulted in constant plasma T_3 levels but increased plasma T_4 levels compared controls.[55]

Parsons et al.[56] examined TBBPA effects on gene suite expression in the hypothalamic–pituitary–thyroid (HPT) axis of zebrafish during early development. To determine LCs and effective concentrations (ECs), the team also investigated TBBPA acute toxicity on zebrafish larvae with regard to induced mortalities and deformities. In this study, zebrafish embryo and larvae were exposed to 0.9 μM TBBPA (the LC_{50} of this compound) for 96 h beginning at fertilization. In the past, it was demonstrated that TBBPA exposure results in higher mRNA levels for genes that encode transport proteins (e.g., TTR), thyroid follicle synthesis protein, paired box 8 (pax8), and deiodinases (DIO1) in whole-body extracts. However, lower levels of dio3b mRNA were detected in whole-body extracts. These researchers suggested that TBBPA could disturb the thyroid hormone system at multiple tiers, such as increasing both thyroid hormone conjugation and clearance, altering thyroid hormone transport, and damaging thyroid follicle development.[56]

In another study, Chan and Chan[57] investigated TBBPA effects on the HPT axis in zebrafish embryo and larvae. After 96 h, TBBPA LC_{50} was 5.27 mg/L, whereas EC_{50} at 96 h was 1.09 mg/L. Sub-LCs for larvae were 0% (control), 10% (0.53 mg/L), 25% (1.32 mg/L), 50% (2.64 mg/L), and 75% (3.95 mg/L) of the 96-h LC_{50} value. Sub-LCs for embryo were 0% (control), 10% (0.11 mg/L), 25% (0.27 mg/L), 50% (0.55 mg/L), and 75% (0.82 mg/L) of the 96-h EC_{50} value. TBBPA demonstrated significant up-regulations in TTR mRNA levels, thyroid receptor α (TRα), and TRβ genes in larvae and significant down-regulation of thyroid-stimulating hormone (TSH)β protein in embryos with all applied doses. The researchers concluded that zebrafish larvae were predominantly affected by TBBPA. In contrast, the zebrafish embryos showed very little response. Chorion was suggested to provide the embryo with a protective barrier against the environment but is sometimes removed to safeguard against chemical uptake inhibition.[57]

Zhu et al.[58] studied TBBPA thyroid-disrupting potency and neurodevelopmental toxicity after exposing zebrafish embryos and larvae to a series of concentrations with or without T_3. Zebrafish embryos (2 h postfertilization) were exposed to different concentrations of TBBPA (50, 100, 200, and 400 mg/L) alone or in combination (T_3 at 20 mg/L and TBBPA at 200 mg/L). Results showed that TBBPA could lead to thyroid toxicity by increasing T_4 and decreasing T_3 levels. TBBPA led to up-regulation of TSHβ and thyroglobulin mRNA and down-regulation of TTR and TRβ mRNA levels in zebrafish larvae. Moreover, TBBPA also induced

neurodevelopmental toxicity by down-regulating transcription of genes that were related to central nervous system development (e.g., α1-tubulin, myelin basic protein, and sonic hedgehog A protein). TBBPA decreased locomotor activity and average swimming speed. These researchers suggested that treatment using T_3 could reverse or eliminate TBBPA-induced thyroidal effects as well as neurodevelopmental toxicity.[58]

D. Other Animal Studies

Animal studies demonstrated that TBBPA primarily decreased serum T_4 levels and reduced the circulating thyroid hormone reserve pool but did not affect the circulating pool of ultimate active T_3 hormone. Recently, it was demonstrated that oral TBBPA (100, 300, or 1000 mg/kg) administration to SD rats for 90 d caused significant decreased serum T_4 levels but did not lead to any alteration in serum T_3 and TSH levels. On the other hand, none of the applied concentrations impacted mortality rate, thyroid morphology, and clinical parameters.[59] Another study showed that 28-d targeted oral TBBPA doses (0, 3, 10, 30, 100, 300, 1000, and 3000 mg/kg bw/d) decreased circulating T_4 levels and increased T_3 levels in Wistar rats in a dose-dependent manner. However, TBBPA did not stimulate any histopathological changes in rat thyroid.[60] Meerts et al.[61] showed that after oral treatment at 5 mg/kg, TBBPA had little effect on thyroid weight and histology in pregnant or fetal rats.[61]

Jagnytsch et al.[54] investigated TBBPA effects on amphibian metamorphosis using *Xenopus laevis* because *X. laevis* is the most sensitive model for detecting thyroid disruption. The authors used the *X. laevis* metamorphosis assay to measure long- and short-term TBBPA effects. The assay also detected effects on thyroid hormone–dependent morphological development. Different concentrations of TBBPA (2.5–500 μg/L) were applied to tadpoles for 21 d. Short-term TBBPA effects were analyzed on TRβ and basic leucine zipper domain (β/ZIP), thyroid hormone–induced genes. TBBPA showed inhibitory effects on thyroid hormone–induced gene expressions at low concentrations. In addition, TBBPA also inhibited T_3-induced TRβ-mRNA expression and β/ZIP–mRNA expression in a dose-dependent manner at all tested concentrations. Study results showed that TBBPA could actually interfere with the thyroid hormone–induced cell-signaling pathway in *X. laevis*. It was suggested that TBBPA could mimic thyroid hormone actions slightly at pharmacological concentrations, probably as a result of binding to thyroid receptors.[54]

VIII. TBBPA REPRODUCTIVE TOXICITY AND ENDOCRINE DISRUPTION EFFECTS

A. *In Vitro* Studies

As mentioned above, a study on MCF-7 cells investigated TBBPA concentration-dependent toxicity and underlying toxicity mechanisms.[46] Low levels of TBBPA (0–10 μM) induced cell proliferation and activated energy metabolism in MCF-7 cells. On the other hand, higher concentrations (10–50 μM) perturbed the balance between ROS and antioxidant defense mechanisms, down-regulated GSH biosynthesis, and up-regulated nucleotide metabolism.[46]

B. Animal Studies

TBBPA may lead to reproductive toxicity in some organisms. It was demonstrated TBBPA had weak estrogenic activity and could induce testicular cell apoptosis and decrease sperm quality.[55,62] Linhartova et al.[63] showed that TBBPA exposure can cause epididymal sperm DNA damage, protein distribution anomalies, decreased sperm count, and increased abnormal sperm.[63]

Zhang et al.[64] determined TBBPA reprotoxic effects in male *Rana nigromaculatus*. For 14 d, animals were treated with TBBPA at 0, 0.001, 0.01, 0.1, and 1 mg/L concentrations. After d 14, the researchers collected blood, sperm, and tissue samples and determined testosterone (T) and 17-β estradiol (E2) levels as well as luteinizing hormone (LH) and follicle-stimulating hormone (FSH) expression. Sperm counts and motility were found to be significantly decreased, and abnormal sperm were markedly higher in TBBPA-exposed animals versus controls. Effects on sperm count, motility, and morphology were dose dependent. Serum T content significantly

increased in 0.01, 0.1, and 1 mg/L TBBPA groups compared to controls, whereas E2 content was significantly higher in only 1 mg/L TBBPA–exposed animals. LH and FSH expression significantly decreased in the 1 mg/L TBBPA–exposed group versus controls. Gene analysis showed that relative levels of androgen receptor (AR) mRNA increased significantly after exposure to 0.001, 0.01, and 1 mg/L TBBPA in a dose-dependent manner. The researchers concluded that TBBPA could lead to endocrinopathy by disrupting spermatogenesis and altering AR, LH, and FSH gene expression and sex hormone levels.[64]

Available data support that TBBPA competes with estrogen in conjugation reactions (i.e., glucuronidation and sulfation). It was suggested that high TBBPA doses could decrease estrogen elimination indirectly and induce higher serum estrogen levels in rats. High doses of circulating estrogens can cause mutations in hormone-responsive genes and may lead to cell proliferation and, finally, uterine tumorigenesis.[65] Dunnick et al.[66] conducted a study of male and female Winstar Han rats that were treated with 0, 250, 500, and 1000 mg/kg TBBPA for 2 yr. TBBPA was found to induce cell proliferation and uterine epithelial tumors such as adenomas, adenocarcinomas, atypical endometrial hyperplasia, and malignant mixed Müllerian tumors in female rats in a dose-dependent manner. The researchers suggested that TBBPA or TBBPA–metabolite-mediated disruption in estrogen homeostasis might lead to uterine cancers. Additionally, TBBPA induced atrophy of the germinal epithelium and testicular interstitial cell adenoma in male rats dose dependently.[66]

Sanders at al.[67] investigated estrogen homeostasis disruption in Wistar female rats (TBBPA: n = 10; control: n = 10; both groups 9 wk old). In the TBBPA group, rats were exposed to five daily oral doses of 250 mg/kg TBBPA. Each rat's estrous cycle was monitored daily using a vaginal cytology assay. Following TBBPA exposure for 5 d, the researchers showed that expression of estrogen receptor (ER) α and ERβ genes were up-regulated in the TBBPA group but not in controls.[67]

In the study by van der Ven et al.,[60] reprotoxic effects of TBBPA were also studied in rats (at doses of 0, 3, 10, 30, 100, 300, 1000, and 3000 mg/kg bw/d, administered orally). Ten parental animals per sex were used in each dose group. Exposures started 70 d before mating for males and 14 d before mating for females. The investigation thus lasted for at least one full spermatogenic cycle or two estrogenic cycles. Additionally, this process continued during mating, pregnancy, and during lactation in dams. TBBPA caused significant dose-dependent increases in testes weights in adulthood. However, no histopathological effects occurred on testes of both adult F_1 males and littermates. On the other hand, plasma T levels of F_1 males were positively correlated with the increased testes weights. The authors concluded that TBBPA might increase testis weight and plasma T levels in male rats.[60]

Zatecka et al.[68] focused on multigenerational reprotoxic TBBPA effects in mice after long-term exposure. The researchers examined TBBPA on reproductive parameters in two generations of outbred mice. Experimental groups were comprised of the parental generation and F_1 and F_2 generations. TBBPA was introduced in water (200 µg/L), so that the daily dose of water consumed by mice was calculated as 5 mL to expose the animals to 1 µg TBBPA/d, which is equivalent to 35 µg/kg. The parental group that was comprised of only females were exposed to TBBPA during gestation. The F_1 generation group had males and females that were exposed to TBBPA during gestation, lactation, prepuberty, puberty, and adulthood. The F_2 generation group was comprised of males. To evaluate TBBPA effects on the male reproductive tract, the researchers measured body and organ weights, number, sex ratio of progeny, and anogenital distances (AGDs) and compared them with controls. They found that TBBPA has no effect on progeny number, AGDs, and sex ratio in parental and F_1 generations. But testis, prostate, epididymis, and seminal vesicle weights were significantly decreased in F_2 generation. No significant differences were found in sperm morphology, viability, and state of acrosomes in all groups versus controls. The authors observed higher levels of apoptosis in testes and changes in morphometry of seminiferous tubules in all F_2 generation treatment groups. Additionally, expression pattern of selected genes such as those encoding acrosomal proteins, androgen-responsible genes, heat shock protein

(HSP) genes, apoptosis regulation genes, and Sertoli cell–specific genes that have important roles during spermatogenesis were markedly changed. The authors concluded that TBBPA might induce testicular apoptosis and changes in seminiferous tubule morphometry in cluster of differentiation (CD)1 mice, and the effect was multigenerational.[68]

Research has suggested that TBBPA could act as an estrogenic agonist. Carcinogenicity studies showed that TBBPA exposure led to uterine tumor development when increasing estrogen levels and promoting tumor protein (TP)53 mutations. Harvey et al.[69] showed that TBBPA exposure (at 0, 250, 500, and 1000 mg/kg for 2 yr) was associated with significant increases in uterine tumors in Wistar Han rats, remarkable increases in tumor p53 mutations, and overexpression of human epidermal growth factor (EGF) receptor 2 in a dose-dependent manner. Moreover, uterine carcinomas in TBBPA-exposed rats were ERα positive and progesterone receptor negative. The authors suggested that morphologic and molecular features of uterine carcinomas after TBBPA exposure resembled high-grade type-I tumors in women, and TBBPA exposure could lead to increased uterine cancer risk.[69] Moreover, TBBPA was shown to induce proliferation of breast cancer cells.[46] TBBPA effects on reproduction and the endocrine system are summarized in Fig. 4.[46,60,65–69]

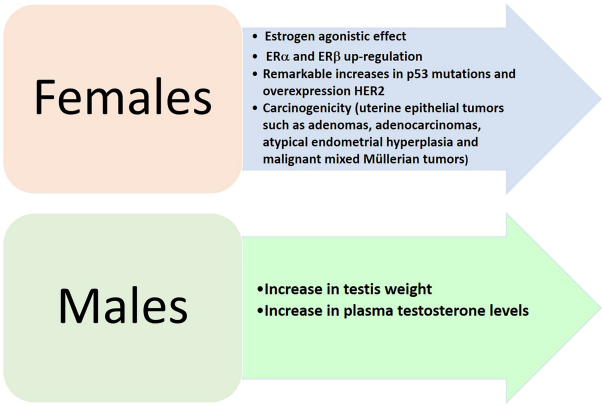

FIG. 4: TBBPA effects on reproduction and the endocrine system. ERα, Estrogen receptor α; ERβ, estrogen receptor β; HER2, human epidermal growth factor receptor 2.

IX. NEUROTOXIC, NEUROENDOCRINE, AND NEUROBEHAVIORAL TBBPA EFFECTS

A. *In Vitro* Studies

Liang et al.[70] evaluated TBBPA neurotoxic effects on human neuronal stem cells (hNSCs) at environmental and human exposure–relevant concentrations ranging from 1 to 1000 nM in two different media (E6 medium [Dulbecco's Modified Eagle Medium/F-12 supplemented with sodium selenite, L-ascorbic acid 2–phosphate sesquimagnesium salt hydrate, human holo-transferrin, and insulin] and N2B27 medium [hNSC maintenance medium that is deprived of basic fibroblast growth factor]). TBBPA did not affect hNSC viability and proliferation at concentrations as high as 1 μM in N2B27 medium. In this study, the authors also assessed TBBPA exposure effects on expression of typical hNSC markers (PAX protein 6 [PAX6], nestin [NES], Sry box transcription factors 1 and 2 [SOX1/SOX2], and the tubulin β 3 [TUBB3] chain) for 6 d. They also studied Notch 1 protein (NOTCH) signaling target proteins, such as fatty acid–binding protein 7 (FABP7) in brain and Hes Family BHLH Transcription Factor 5 (HES5). SOX2 expression was slightly down-regulated, NES expression was marginally repressed, and FABP7 expression was up-regulated at 10 and 100 nM TBBPA in N2B27 medium. PAX6 and SOX1 expressions were unaffected, FABP7 was down-regulated, and HES5 was up-regulated. In N2B27 medium, SOX3 expression decreased dose dependently, whereas in E6 medium TBBPA caused decreased SOX3 expression. TUBB3 expression increased in N2B27 medium, but in E6 medium TUBB3 expression was significantly lower than controls. Because hNSCs are multipotent and able to generate neurons and glial cells, the authors also investigated TBBPA effects on nerve cell differentiation. Glycogen synthase kinase (GSK)3β is a major regulator of neural development and its high levels maintain the NSC undifferentiated state, so this research group used CHIR99021 as an GSK3β inhibitor and Wingless–Int-1 (WNT) agonist. In TBBPA-exposed cells, SOX repression was rescued after CHIR treatment. Additionally, after the researchers differentiated hNSCs by using N2B27

and E6 media, they investigated TBBPA effects on hNSC differentiation that could also be modulated by T_3. They detected that TBBPA increased SOX3 expression in the absence of T_3. Liang and colleagues suggested that TBBPA may potentially change hNSC identity and neurogenesis by interfering with GSK3β, NOTCH, and T_3 signaling.[70]

In another study, Zieminska et al.[71] demonstrated the role of Ca^{+2} transients that were triggered by TBBPA in rat cerebellar granule cells (CGCs). The CGCs were treated with TBBPA at 10 or 25 μM concentrations for 30 min. The authors measured intracellular Ca^{+2} concentrations, ROS and GSH levels, CAT activity, and mitochondria potential. TBBPA caused significant increases in Ca^{+2} and ROS production and marked decreases in GSH levels and CAT activity. Moreover, TBBPA administration led to decreased mitochondria potential and neuronal activity.[71]

Oxidative stress and Ca^{+2} imbalance are known to induce Zn^{+2} release from intracellular stores. It was shown that Zn^{+2} is a mediator in the excitotoxicity cascade that includes *N*-methyl-D–aspartate receptor (NMDAR)-mediated oxidative stress, mitochondrial membrane potential failure, Ca^{+2} deregulation, and neuronal cell damage. We know that Zn^{+2} ions confer both protective and cytotoxic effects on nerve cells. A study by Zieminska et al.[72] showed that TBBPA exposure caused Zn^{+2} release from intracellular stores and disturbed Zn^{+2} homeostasis in primary rat CGCs. Using Zn^{+2}-sensitive fluorescent probes such as FluoZin-3 (measures changes in intracellular Zn^{+2} concentration) and FluoZin-1 (evaluates total zinc levels in CGC culture lysates) as well as zinc chelator N,N,N′,N′-tetrakis[2-pyridinylmethyl]-1,2-ethanediamine (TPEN), the authors also investigated Zn^{+2} effects on NMDAR channel activity. CGCs were incubated with TBBPA at 10 and 25 μM for 1 and 5 min. Changes in intracellular Ca^{+2} concentration in CGCs were monitored with the fluorescent Ca^{+2}-sensitive probe Fluo-3. TBBPA altered cellular Zn^{+2} homeostasis, increased intracellular Zn^{+2} concentration, and promoted Zn^{+2} redistribution from cells to the intracellular compartment in CGC cultures. Endogenous Zn^{+2} inhibited NMDARs. Ca^{+2} and ROS partially mediated TBBPA-evoked Zn^{+2} transients. TPEN did not aggravate TBBPA neurotoxicity and even slightly decreased neuronal death that TBBPA induced at 25 μM. The researchers concluded that TBBPA exposure may induce Zn^{+2} imbalance, disrupt cellular Zn^{+2} homeostasis, and promote Zn^{+2} redistribution from neuronal cell cultures to the extracellular compartment. It was suggested that mechanisms related to Zn^{+2} homeostasis might modify TBBPA toxicity.[72]

Szychowski and Wójtowicz[73] showed that TBBPA concentrations ranging from 100 nM to 100 μM (for 30 min and 3, 6, and 24 h) could cause dose-dependent cytotoxicity, intracellular ROS production, caspase 3 activation, apoptosis-like nuclear contraction, DNA fragmentation, chromatin condensation, and apoptotic body formation in mouse primary hippocampal neuron cultures.[16,73] In another study, TBBPA (2–20 μM) increased intracellular free Ca^{+2} levels in a dose-dependent manner and high extracellular glutamate levels in a time-dependent manner in CGCs.[74]

Yin et al.[75] investigated TBBPA cytotoxicity and neural developmental toxicity in mouse embryonic stem cells (ESCs) at environmental- and human exposure–relevant doses (0, 1, 10, 100, 150, and 200 μM) after 72 h. TBBPA half-maximal inhibitory concentration measured 102 ± 6 μM. TBBPA caused perturbations in intracellular Ca^{+2} levels and stimulated ROS formation; however, these changes were not directly dose dependent, although at higher concentrations the effects were more pronounced than those of controls. At 10 μM, TBBPA provoked an increase in intracellular Ca^{+2} ions in ESCs after exposure time of 8 min, but those perturbations returned to normal levels after ~ 35 min. The researchers suggested that 10 μM TBBPA could perturb cellular Ca^{+2} levels, but cells recovered due to the antistress machinery. However, at 100 and 200 μM, TBBPA significantly increased free Ca^{+2} levels. This group also analyzed changes in specific marker gene expressions. They found TBBPA to enhance expression of neural progenitor markers including PAX6, SOX1, and SOX3 and neurogenesis genes such as microtubule-associated protein 2 and neurogenic differentiation 1 gene. Additionally, NOTCH effectors such as transcription factors HES1 and HES5 and WNT target genes such as Lef1 and Axin2 were slightly up-regulated at all TBBPA concentrations; however, those effects were not directly dose dependent. The

authors suggested that TBBPA could cause cytotoxicity, neural developmental toxicity, and alteration in neural specification due to the compound's effects on NOTCH and canonical WNT pathways.[75]

Diamandakis et al.[76] investigated TBBPA effects on plasma membrane potential as well as its interactions with NMDAR and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) in CGCs. CGC plasma membrane potential was measured using cell current-clamp recordings and the fluorescent probe oxonol VI. Effects of NMDARs and AMPARs, voltage-gated sodium channels, and intracellular Ca^{+2} mobilization were tested via their selective antagonists and inhibitors. Additionally, TBBPA with NMDAR direct interactions were measured using specific binding of radiolabeled NMDA ligands. The researchers observed that TBBPA caused CGC plasma membrane depolarization in a dose-dependent manner and suggested that TBBPA could activate NMDARs, perhaps leading to depolarization CGC plasma membranes.[76]

B. Aquatic Animal Studies

We have learned that chronic exposure to TBBPA can cause sex-specific neurobehavioral changes and alterations in animal social interaction. Chen et al.[8] studied developmental and neurobehavioral effects in zebrafish that were exposed to 0, 0.5, 5, and 50 nM TBBPA for 1 to 120 d postfertilization. Exposure at 0.5 nM TBBPA was not associated with malformations in embryos and did not alter sex ratio but resulted in reduced zebrafish bw and length. At exposures of both 5 and 50 nM TBBPA in adult females, average swim speeds were consistently and significantly higher than those of controls in the visible light period but not in the dark period. In adult males, average swim speeds were consistent and significant versus those of controls in light and dark periods at TBBPA exposures of both 5 and 50 nM. For aggression assessment, the researchers used a mirror-image attack behavior test. In the surrounding four-mirror test, no differences occurred between females and males in both exposure groups concerning mirror attack frequency. In males, mirror attack frequency in fish that received at 5 nM TBBPA was the same as that of controls. However, males exposed to 50 nM TBBPA were significantly more aggressive compared to nonexposed fish. Time spent in the defined mirror zone by females did not differ in nonexposed females and females exposed to 5 nM TBBPA. Increases in time spent in the defined mirror zone occurred for both males and females that were exposed to 50 nM TBBPA (versus controls; $p < 0.05$). The authors concluded that chronic exposure to TBBPA caused more pronounced behavioral changes and hyperactivity in male compared to female zebrafish.[8]

C. Other Animal Studies

In vivo studies indicate that TBBPA could accumulate in different brain regions. Animals that were chronically exposed to TBBPA might have higher concentrations of brain TBBPA compared to concentrations in other body compartments. Nakajima et al.[77] conducted a study on mice that were exposed to one oral acute TBBPA dose (0.1, 5, and 250 mg/kg). The researchers found high amounts of TBBPA in striatum after mice were treated with 0.1 and 5 mg/kg TBBPA. But after treatment with 250 mg/kg TBBPA, a nonspecific accumulation was found in brain. Effects of acute TBBPA exposure in mice were also investigated by open-field activity, contextual memory, the Y-maze test, and spatial working memory tests. These investigators indicated that a 0.1 mg/kg oral TBBPA dose resulted in memory increase in the contextual fear conditioning paradigm and Y-maze test. Additionally, a 5 mg/kg oral dose increased locomotor stimulating effects in the open-field test. Conversely, 250 mg/kg TBBPA did not result in any neurobehavioral effects. These results show that at low and medium doses, TBBPA could accumulate in brain regions including striatum and possibly induce behavioral alterations.[77]

Another study on mice suggested that neonatal exposure to TBBPA (oral dose at 11.5 mg/kg bw) could reduce binding sites of the nicotinic ligand cytisine in frontal cortex.[78] An *ex vivo* study by Mariussen and Fonnum[79] showed that TBBPA was incubated with synaptosomes at different concentrations (2–20 μM). TBBPA was shown to inhibit neurotransmitter uptake and produced concentration-dependent inhibition of dopamine, glutamate,

and γ-aminobutyric acid uptake in rat brain. Kinetic studies with TBBPA and dopamine uptake indicated that TBBPA showed mixed competitive and non-competitive inhibition, depending to a certain extent to dopamine concentrations in brain. Additionally, TBBPA effects on tetraphenylphosphonium uptake correlated with dopamine and glutamate inhibition, and TBBPA could affect synaptosome membrane properties by decreasing membrane potential.[79]

Thyroid hormones may interfere with neurodevelopment. In Wistar rats fed different diets containing TBBPA (diets with 0; 37.5; 125; 375; 1250; 3750; 12,500; or 37,500 mg TBBPA/kg), Lilienthal et al.[1] studied effects of prenatal and postnatal TBBPA exposure on the auditory system and conditional fear, which are modulated by thyroid hormones. The researchers demonstrated that brainstem auditory–evoked potentials increased in female rat offspring at a low-frequency range in a dose-dependent manner. No effects were observed in male rats.[1]

Different studies suggest that TBBPA exposure could cause neuropathological changes. Cope et al.[3] examined TBBPA neuropathological, behavioral, and neurological effects at oral doses of 10, 100, and 1000 mg/kg bw/d in two generations of SD rats. In a separate study, TBBPA (oral 0, 100, 300, or 1000 mg/kg bw/d) effects in rats were examined on embryonic/fetal development between gestation d 1 and 19. In the reproductive study, exposure to ≥ 100 mg/kg bw/d TBBPA caused decreases in circulating T_4 levels without any significant alterations in T_3 and TSH. However, the findings could not be extrapolated to humans due to differences in TBBPA catabolism in both. At up to 1000 mg/kg bw/d, TBBPA did not lead to with any marked effects on reproduction, growth, and development. A subtle reduction in parietal cortex thickness and microanatomic changes in 11-d-old F_2 pups in the 1000 mg/kg bw/d group were noted; but the researchers noted that the biological relevance of these effects in humans could not be clearly known. Estrogenic effects strong enough to cause macroanatomic features, fertility, reproduction, development, developmental neurotoxicity/neuropathology, survival, or behavior were not detected in the embryo–fetal development study nor the multigenerational study. The no-observed-effect level for maternal and developmental toxicity was suggested to be 1000 mg/kg bw/d for TBBPA.[3]

TBBPA derivatives' potential neurotoxicity was demonstrated using *in vitro* and *in vivo* studies. Liu et al.[80] investigated neurotoxic effects of TBBPA–bis(2-hydoxy ethyl ether) (TBBPA-BHEE) on neonatal Sprague–Dawley (SD) rats that were exposed to the chemical using long-term intranasal instillation 1×/d at 1 μg/g doses for 21 d. Neurobehavioral and histopathological changes and gene expressions were evaluated. Results showed that TBBPA-BHEE decreased motor coordination performance and changed locomotor behavior. Significant histopathological changes occurred in both cerebrum and cerebellum tissues and neural cell swelling, microglial activation, and proliferation were also observed. Additionally, 911 genes were found to be up-regulated, and 433 genes were down-regulated. Gene set enrichment analysis showed that multiple signaling pathways (such as ubiquitin-mediated proteolysis and WNT signaling pathways) were affected by TBBPA-BHEE exposure. Gene ontology enrichment analysis demonstrated that TBBPA-BHEE also altered basic cellular functions and neurological processes including synaptic transmission.[80]

TBBPA neurotoxic, neuroendocrine, and neurobehavioral effects are summarized in Fig. 5.[16,70–73,75,76]

X. HEPATOTOXIC EFFECTS OF TBBPA

A. *In Vitro* Studies

As indicated above, due to the structural resemblance of TBBPA to thyroid hormones, TBBPA can have similar effects with T_3 and T_4 on lipid metabolism and a direct antisteatotic effect on hepatocytes. Grasselli et al.[81] investigated TBBPA thyromimetic and receptor-independent effects on lipid homeostasis in steatotic rat hepatoma cells (FaO cell line), a defective liver cell line for functional thyroid hormones. Cells were exposed for 24 h to TBBPA at 10^{-8}–10^{-5} M concentrations. A maximum TBBPA effect was obtained at 10^{-6} M for the 24 h. Similar to T_3, TBBPA enhanced triglyceride content and increased lipid droplet size within FaO cells. Substantial modifications in transcription of peroxisome proliferator–activated receptor (PPAR)-β/-γ and -α

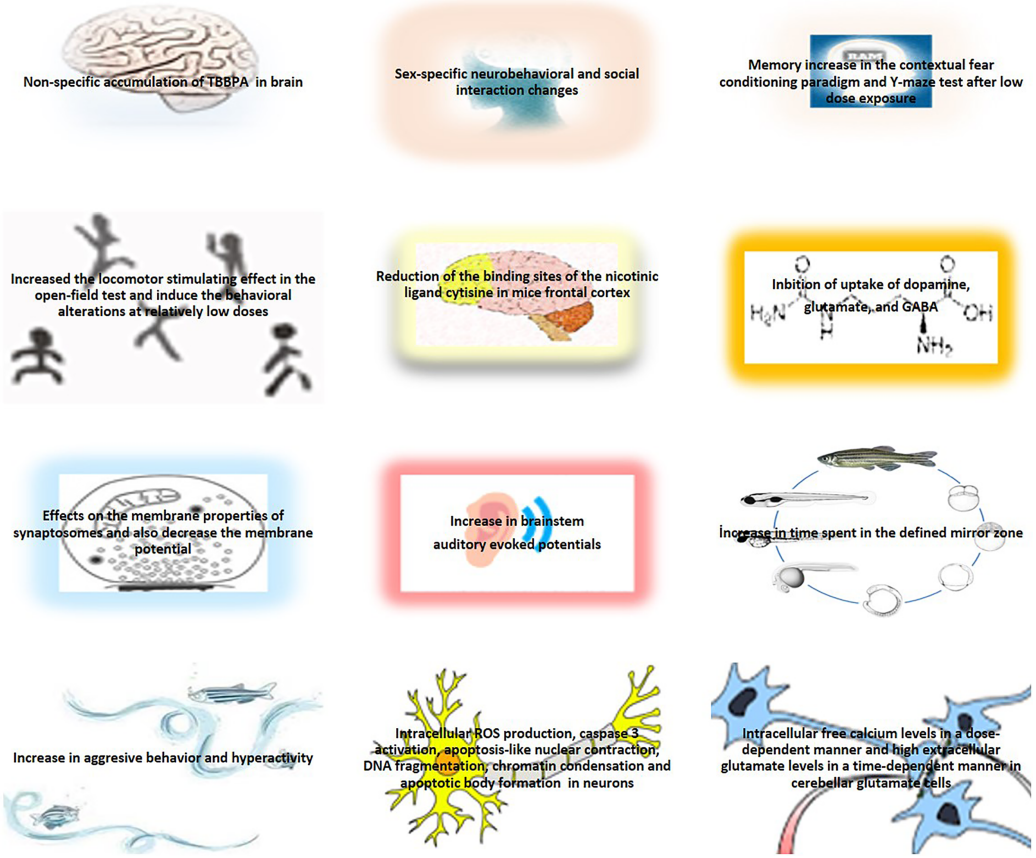

FIG. 5: Neurotoxic, neuroendocrine, and neurobehavioral effects of TBBPA. GABA, γ-Aminobutyric acid; ROS, reactive oxygen species; TBBPA, tetrabromobisphenol A.

isoforms occurred due to PPAR-β/-γ down-regulation and PPAR-α up-regulation. Additionally, cytochrome P450, family 4, subfamily a, polypeptide 1 mRNA levels increased in steatotic cells, initiating fatty acid ω oxidation in microsomes.[81]

In another study, researchers reported that TBBPA exposure at 0.25–1.0 mM doses for 0–3 h can cause cytotoxicity, loss of cellular adenosine triphosphate, and adenine nucleotide pools; decreased GSH and protein thiols; and GSH disulfide and MDA accumulation in F344/Jcl rat hepatocytes. The authors suggested that the cytotoxicity might be due to mitochondrial dysfunction induced by TBBPA in hepatocytes.[82]

B. Aquatic Animal Studies

TBBPA may induce oxidative stress that decelerates glycolysis and enhances gluconeogenesis to protect cellular glucose, glucose-6-phosphate, and NADPH production.[39] Proteomic studies on liver in aquatic animals suggest that exposure to TBBPA causes enhanced production of gluconeogenic proteins, related to oxidative stress. Kling and Förlin[83] investigated TBBPA hepatic effects in zebrafish liver cells. In this proteomic study, cells were exposed for 24 and 72 h to TBBPA (0, 0.5, 5, and 220 μM). Peptide mapping with advanced techniques showed that TBBPA caused dose-dependent increases in proteins Hsp70 and transketolase, important in the protein folding process and NADPH production. Additionally, following TBBPA exposure, overlapping occurred in protein responses in glyceraldehyde 3-phosphate dehydrogenase (GAPDH), methylcrotonoyl Co-A carboxylase 2 (MCCC2), and Aldolase A (ALDOA), enzymes responsible for gluconeogenesis (GADPH and ALDOA) and ligase activity

(MCCC2). Results found that high expression of GAPDH and ALDOA caused by TBBPA exposure might cause adverse effects associated with apoptosis and hepatocellular carcinoma.[83]

Several *in vivo* studies were conducted measuring TBBPA effects on hepatic enzymes, suggesting that TBBPA can affect microsomal and cytosolic drug–metabolizing hepatic enzymes in aquatic animals. Ronisz et al.[84] measured changes in cytochrome P450 1A (CYP1A; measured as ethoxyresorufin-*O*-deethylase), GSH reductase (GR), GSH-*S*-transferase (GST), and CAT in rainbow trout (*Oncorhynchus mykiss*). Additionally, the authors evaluated vitellogenin (VTG) induction, DNA adduct formation, and liver somatic index (LSI). Fish received TBBPA doses of 0, 1, 1, 10, or 100 mg/kg intraperitoneally for 1 d; 1, 10, 50, 100, and 500 mg/kg for 4 d, and 100 mg/kg for 14 and 28 d, respectively. Results indicated that TBBPA induced GR activity at the 100 mg/kg dose after 4, 14, and 28 d. TBBPA also induced activity in CYP1A protein levels at the highest applied dose. However, the authors suggested that TBBPA is not a CYP1A inducer but rather a substrate for CYP1A and can disturb CYP1A-mediated metabolism. Additionally, the study showed that TBBPA decreased CAT activity and LSI at 50 mg/kg doses after 4 d of exposure. TBBPA did not cause formation of DNA adducts at any of the applied doses or periods.[84]

C. Other Animal Studies

The liver is the main organ that metabolizes hormones and chemicals. TBBPA exposure may lead to a series of events that can eventually cause hepatocarcinogenesis. Dunnick et al.[85] conducted a study on female Wistar Han rats that were exposed for 13 weeks to TBBPA (doses at 0, 25, 250, and 1000 mg/kg by oral gavage). No changes occurred in bw and organ (liver, uterus) weights at any applied dose. However, hepatic and uterine transcriptome analyses showed that TBBPA exposure at 1000 mg/kg induced changes primarily in the liver, with 159 transcripts corresponding to 132 genes differentially expressed compared to controls. The authors found that the interferon (INF) pathway in the liver was activated after 13 wk of TBBPA exposure and thus suggested that long-term TBBPA exposure might lead to immunomodulatory changes that could contribute to a hepatocarcinogenic process.[85]

Studies show that TBBPA could alter serum concentrations of total cholesterol and liver weights and cause histopathological changes in the liver. Tada et al.[86] examined TBBPA effects on Institute for Cancer Research mice after prenatal and postnatal exposure. TBBPA was administered in the diet (0%, 0.01%, 0.1%, and 1.0%) from gestational d 0 to weaning at postnatal d 27. Serum concentrations of total cholesterol were significantly higher in high-dose–group dams, middle- and high-dose–groups males, and female offspring of the high-dose group versus controls ($p < 0.05$; all). Serum triglyceride concentrations in high-dose–group dams was considerably higher than controls; however, in high-dose–group female and male offspring, triglyceride concentrations were dramatically lower than those of controls. Histological findings in treated dams or offspring showed increases in hepatocyte focal necrosis, inflammatory liver cell infiltration, and kidney cysts. Moreover, mild expansiveness of hepatocytes and thin granular changes in cytoplasm in treated dams and offspring groups were present versus controls.[86] Hepatotoxic TBBPA effects are summarized in Fig. 6.[39,81–86]

XI. NEPHROTOXIC EFFECTS OF TBBPA

A. Animal Studies

Few studies investigated TBBPA adverse effects in animal kidneys, but it was suggested that TBBPA could cause oxidative stress in kidney at high concentrations. To evaluate TBBPA nephrotoxic potential, Kang et al.[87] performed single-dose and 14-day repeated-dose studies by applying 200, 500, and 1000 mg/kg to adult rats. At 1000 mg/kg, TBBPA significantly elevated renal lipid peroxidation levels, and SOD activity increased at all administered doses. No changes in renal CAT activities occurred in all TBBPA-receiving groups. The researchers indicated that acute administration of TBBPA at 1000 mg/kg produced transient renal changes at 5 h. Moreover, results showed that limited TBBPA amounts could remain in kidney compared to serum or urine.[87]

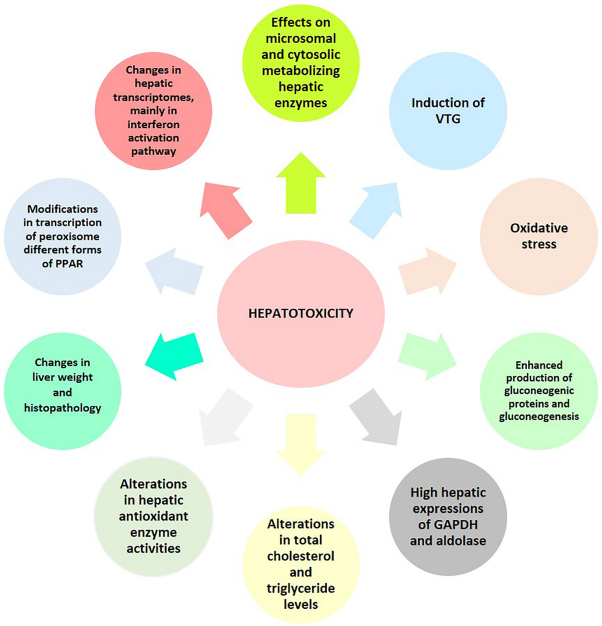

FIG. 6: Hepatotoxic effects of TBBPA. GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PPAR, peroxisome proliferator–activated receptor; VTG, vitellogenin.

Fukuda et al.[88] examined effects of oral TBBPA exposure to newborn female and male rats. For 18 d (postnatal d 4 to weaning at 21 d) rats received 0, 40, 200, and 600 mg/kg/d doses. Treatment with 200 or 600 mg/kg TBBPA induced nephrotoxicity that was characterized by polycystic lesion formation, and some deaths occurred in the highest TBBPA-exposed group. The researchers did not observe gender differences regarding nephrotoxic TBBPA effects. After 85 d, nephrotoxic lesions were still present in the 200 and 600 mg/kg groups. To compare TBBPA nephrotoxic effects in newborn rats versus young rats, young rats were treated with 2000 and 6000 mg/kg TBBPA doses for 18 d. No histopathological changes nor abnormalities in young rats occurred even though higher doses were applied. The authors suggested that TBBPA renal toxicity can be age related, and newborn susceptibility to TBBPA can result from immature renal functions, an imbalance between cellular proliferation and stimulation of apoptosis, and interaction of multiple growth factors and cytokines.[88] Nephrotoxic effects of TBBPA are given in Fig. 7.[87,88]

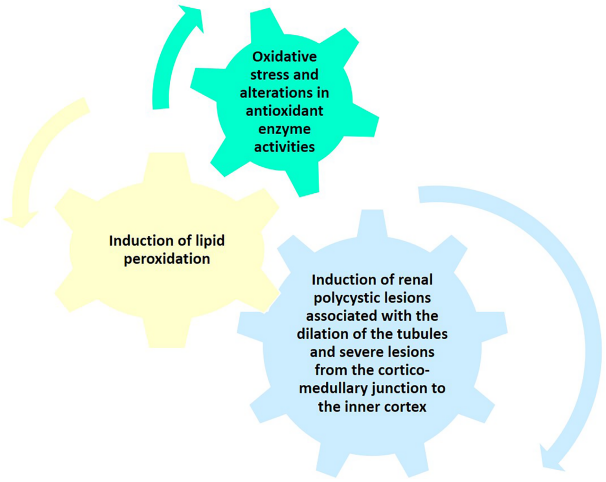

FIG. 7: Nephrotoxic effects of TBBPA

XII. OTHER TBBPA TOXIC EFFECTS

A. *In Vitro* Studies

Administration of TBBPA to immune-system cells was shown to affect their specific properties as well as cell surface protein expression. Kibakaya et al.[89] demonstrated that exposure to TBBPA inhibited natural killer (NK) cells ability to bind and lyse tumor cells and diminished expression of several cell proteins that are necessary for NK lytic function. It was suggested that TBBPA can activate lytic signaling pathway components such as p44/42, which can cause NK lytic function loss.[89] On the basis of this data, Cato et al.[90] indicated that TBBPA increases p44/42 and p38 activation in dose- and time-dependent manners.[90]

Limited inhalation exposure studies performed on TBBPA show that long-term TBBPA exposure could trigger chronic obstructive pulmonary disease, asthma, pulmonary fibrosis, and pulmonary tumors. It is well known that lung is directly exposed to contaminated air particles after inhalation, and TBBPA may easily and completely distribute in the respiratory tract and pulmonary alveoli. Wu et al.[91] studied TBBPA-induced toxic effects and gene expression alterations in bronchial epithelial (BEAS-2B) cells. Cells were exposed to TBBPA at 0 (control), 8, 16, 32, and 64 µg/mL concentrations for 12, 24, and 48 h. Cell viability, cellular morphological changes,

cytokine release, and gene expression alterations were evaluated. When compared to controls, TBBPA caused significant decreases in cell viability in dose- and time-dependent manners. In the morphological analysis, cellular swelling, cell membrane lysis, homogenized unstructured areas, and chromatin changes, as well as the severity of these alterations, were dose dependent. Additionally, cellular morphological change (irregularly condensed mitochondria and lysosomal vacuoles and abnormally shaped nuclei) severity was also observed in a dose-dependent manner. After treatment with TBBPA, 87 genes exhibited ≥ 1.5-fold changes in expression. The researchers also observed that the NF-κB pathway, tumor necrosis factor (TNF)α signaling, the Toll-like receptor pathway, MAPK signaling, and B-cell receptors were affected by TBBPA exposure.[91]

Various types of stressors including noise, aging, infections, and drugs can cause hair-cell damage in the cochlea and lead to hearing impairment. Ototoxic compounds can emerge as free radicals, such as ROS and reactive nitrogen species, and eventually cause apoptosis. Park et al.[92] demonstrated that TBBPA could induce hair-cell loss in zebrafish neuromasts and rat cochlea. TBBPA was also responsible for apoptotic cell death in the organ of Corti and sensory cell lines in dose- and time-dependent manners, most probably due to overproduction of ROS and the proinflammatory cytokine interleukin 6 (IL-6).[92]

Studies also reported that TBBPA activated inflammatory pathways, such as cytokine and prostaglandin production. Park et al.[93] studied TBBPA effects on inflammatory responses in gestational tissues. It was shown that TBBPA increased IL-6, IL-8, and prostaglandin E2 release and suppressed transforming growth factor β release in human transfected trophoblast cells (HTR-8/SVneo) cells in dose- and time- dependent manners.[93]

TBBPA was shown to change the tumor killing function of NK cells and secretion of inflammatory cytokines such as INF-γ, IL-1β, and TNFα. Produced by lymphocytes and monocytes/macrophages, TNFα is a key proinflammatory cytokine that is crucial to the immune response. Yasmin and Whalen[94] focused on TBBPA effects on TNFα in human immune cells. In their study, NK cells, monocyte-depleted peripheral blood mononuclear cells (predominantly T and NK lymphocytes), and peripheral blood mononuclear cell lymphocytes/monocytes, were treated for 24 and 48 h and 6 d with TBBPA at 0.005–0.5 μM concentrations. TNFα secretion decreased in a dose-dependent manner in all cells. The authors suggested that TBBPA can disrupt inflammatory response and that this phenomenon could cause serious immunological problems.[94]

Exposure to TBBPA can activate PPAR-γ, which is important for regulating the adipogenesis and osteogenesis differentiation balance and osteoporosis. Watt and Schlezinger[95] used a primary mouse bone-marrow culture model and tested the effects of TBBPA (10–20 μM) on PPAR-γ activation. The investigators found that TBBPA activated PPAR-γ1 and PPAR-γ2, leading to increased adipogenesis and suppressed osteogenesis in mouse primary bone-marrow cultures. In addition, TBBPA caused significant increases in lipid accumulation and PPAR-γ target gene expression. Like the primary metabolite of di(2-ethylhexyl) phthalate, namely, mono(2-ethylhexyl) phthalate, TBBPA was found to be a partial and low-potency ligand of PPAR-γ, with moderate but not maximal efficacy in inducing adipocyte differentiation.[95] These results were in accordance with other studies performed on human PPAR-γ–based reporter cells, where tribrominated BPA, and TBBPA produced greatest potency and efficacy in activating PPAR-γ. With decreasing BPA substitution, activity significantly decreased.[96] The authors concluded that TBBPA could induce adipogenesis, along with marked increases in FABP4 and perilipin 1 expression. In addition, they found that TBBPA may be a low-potency ligand and have moderate efficacy for PPAR-γ in bone-marrow cells.[95]

Honkisz and Wójtowicz[97] found that TBBPA increased PPAR-γ and the β subunit of β human chorionic gonadotropin (β-hCG) in human choriocarcinoma (JEG-3) cells. The authors also evaluated the action of TBBPA on placental progesterone secretion, cytotoxicity, and apoptosis. After TBBPA treatment at 10 nM and 10 μM doses, PPAR-γ protein expression elevated in a time-dependent manner until 48 h and then slightly decreased at 72 h, still remaining above that of controls. This phenomenon was accompanied by decreases in β-hCG levels. On the other hand,

when JEG-3 cells were exposed to 2-chloro-5-nitrobenzanilide (GW9662) (a PPAR-γ antagonist) along with TBBPA, GW9662 reversed changes in PPAR-γ protein expression that were caused by TBBPA and potentiated the inhibitory effect of TBBPA on β-hCG secretion. This unexpected GW9662 effect indicated that the underlying alteration mechanism in β-hCG secretion after TBBPA exposure is likely PPAR-γ independent. At 10 and 50 μM doses, TBBPA increased progesterone concentrations but decreased progesterone levels at 100 μM doses, probably due to the high cytotoxic effect at this concentration. TBBPA significantly increased apoptosis, as evidenced by increases in caspase 3 activity and immunostaining.[97]

Woeller et al.[98] conducted a study of TBBPA effects on Thy-1 cell surface antigen (THY1) and CD90, a glycophosphatidylinositol-anchored membrane protein that serves as a marker for stem cells and also has an important role in regulating adipogenesis and obesity. In this study, TBBPA reduced THY1, CD90 in both human and mouse cells, via expression for promoting adipogenesis after microRNA-103/-107 induction.[98] The authors suggested that Thy-1 was a TBBPA target and Thy-1 could be a key protein that is targeted by different endocrine disruptors, leading to adipogenesis and obesity.

B. Animal Studies

Yu et al.[35] observed diminished motor activity, eye squint, mild dyspnea, and erythema in an acute TBBPA inhalation study in rats. Additionally, local irritation occurred in rat upper respiratory tracts after 14 d inhalation.[35] Koike et al.[99] showed that TBBPA exposure could stimulate proinflammatory protein expression, intracellular adhesion protein 1 (ICAM-1), and IL-6 in normal BEAS-2B cells by disrupting intracellular signaling. Exposure to TBBPA at 1 mg/mL concentration led to increased ICAM-1 expression and IL-6 production; however, exposure to TBBPA at 10 mg/mL caused decreased ICAM-1 expression and IL-6 production, most probably due to cytotoxic effects of high-dose TBBPA. Furthermore, exposure to TBBPA at 0.1–10 mg/mL led to decreased IL-8 production in BEAS-2B cells in a dose-dependent manner. TBBPA exposure also significantly induced EGF production.[99]

XIII. SUMMARY

TBBPA, a bisphenol derivative, is a brominated flame retardant, widely used in a variety of industrial polymers, such as polyester and epoxy resins, and in polycarbonates. These compounds are found in a variety of consumer products such as circuit boards, electronics equipment, furniture, plastics, textiles, kitchen hoods, and baby products. TBBPA can seep out from consumer products, and due to its environmental stability, spread into various environmental areas to contaminate air, soil, water, marine sediments, and food. Humans are exposed to TBBPA by inhaling ambient air, dermal contact, and ingesting foods (particularly seafood, milk, and egg) and drinking water. Oral intake is the major exposure route for TBBPA. Occupational exposure is of particular concern because it is responsible for high exposure through inhaling workplace dust. Workers may also be exposed through dermal contact and ingestion.

TBBPA undergoes first-pass metabolism and is conjugated by glucuronic acid or sulfate. TBBPA has low bioavailability, and the main elimination route is feces. *In vivo* experiments indicate that high TBBPA exposure can cause cancer in laboratory animals, particularly uterine, testis, and liver cancer. In 2018, the IARC declared that TBBPA is a group-2A carcinogen.

During the past 10 yr, studies on different cells in animal models and human volunteers indicate that TBBPA has low acute toxicity, but chronic exposure to this compound may have serious consequences. TBBPA can act as an endocrine disruptor and cause neurobehavioral and immunotoxic effects, oxidative stress, and apoptosis. *In vivo* studies report that liver may be a target organ for TBBPA, and TBBPA-induced cytotoxicity can result from liver mitochondrial dysfunction. TBBPA can also affect glycolysis, gluconeogenesis, adipogenesis, and osteogenesis. Due to its structural resemblance to thyroid hormones, TBBPA can affect several components of the thyroid system and lead to alterations in thyroid hormone levels, such as increased plasma T_4.

Although several experiments have been performed *in vitro* and *in vivo*, a lack of human data still

exists and chronic toxic TBBPA effects on humans, particularly sensitive populations such as pregnant women, newborns, children, and elderly, are not well known. Therefore, epidemiological studies are needed to comprehensively investigate TBBPA levels in biological fluids. Moreover, lack of data exists for TBBPA levels in different pathological conditions. Thus, studies on TBBPA impact, particularly on endocrine disorders and cancer, must also be performed.

REFERENCES

1. Lilienthal H, Verwer CM, van der Ven LT, Piersma AH, Vos JG. Exposure to tetrabromobisphenol A (TBBPA) in Wistar rats: Neurobehavioral effects in offspring from a one-generation reproduction study. Toxicology. 2008;246(1):45–54.
2. Guyot R, Chatonnet F, Gillet B, Hughes S, Flamant F. Toxicogenomic analysis of the ability of brominated flame retardants TBBPA and BDE-209 to disrupt thyroid hormone signaling in neural cells. Toxicology. 2014;325:125–32.
3. Cope RB, Kacew S, Dourson M. A reproductive, developmental and neurobehavioral study following oral exposure of tetrabromobisphenol A on Sprague–Dawley rats. Toxicology. 2015;329:49–59.
4. Liu K, Li J, Yan S, Zhang W, Li Y, Han D. A review of status of tetrabromobisphenol A (TBBPA) in China. Chemosphere. 2016;148:8–20.
5. Tetrabromobisphenol A compound summary [database on the Internet]. Bethesda, MD: National Institutes of Health, National Library of Medicine, National Center for Biotechnology Information, PubChem. c2020 [cited 2020 Mar 16]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Tetrabromobisphenol-A.
6. Malkoske T, Tang Y, Xu W, Yu S, Wang H. A review of the environmental distribution, fate, and control of tetrabromobisphenol A released from sources. Sci Total Environ. 2016;569-570:1608–17.
7. Abou-Elwafa Abdallah M. Environmental occurrence, analysis and human exposure to the flame retardant tetrabromobisphenol-A (TBBP-A): A review. Environ Int. 2016;94:235–50.
8. Chen J, Tanguay RL, Simonich M, Nie S, Zhao Y, Li L, Bai C, Dong Q, Huang C, Lin K. TBBPA chronic exposure produces sex-specific neurobehavioral and social interaction changes in adult zebrafish. Neurotoxicol Teratol. 2016;56:9–15.
9. EFSA Panel on CONTAM. Scientific opinion on tetrabromobisphenol A (TBBPA) and its derivatives in food. EFSA J. 2011;9(12):2477.
10. Decision Databases [homepage on the Internet]. Global tetrabromobisphenol-A (TBBA) (CAS 79-94-7) Market Growth 2019–2024 [cited 2020 Mar 15]. Available from: https://www.decisiondatabases.com/ip/41191-tetrabromobisphenol-a-tbba-market-analysis-report.
11. iarc.who.int [homepage on the Internet]. IARC Monographs Volume 115: Some industrial chemicals. Lyon, France: International Agency for Research on Cancer. c2018 [cited 2019 Dec 10]. Available from: https://www.iarc.fr/news-events/iarc-monographs-volume-115-some-industrial-chemicals/.
12. Committee on Toxicity [homepage on the Internet]. London, UK: Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment. 2004 COT statement on tetrabromobisphenol A. Review of toxicological data. c2004 [cited 2020 Jan 10]. Available from: http://cot.food.gov.uk/sites/default/files/cot/cotstatements04tbbpa.pdf.
13. European Union Risk Assessment Report. Environmental Addendum of 2008 in conjunction with EU RAR of BPA, 2003. ECHA (European Chemicals Agency). 2,2′,6,6′-Tetrabromo-4,4′-isopropylidenediphenol (tetrabromobisphenol-A or TBBP-A) [cited 2020 Jan 10]. Available from: https://echa.europa.eu/documents/10162/d1d9e186-4385-4595-b6cb-5a1a7a160f07.
14. de Wit CA. An overview of brominated flame retardants in the environment. Chemosphere. 2002;46(5):583–624.
15. Liu D, Liu J, Guo M, Xu H, Zhang S, Shi L, Yao C. Occurrence, distribution, and risk assessment of alkylphenols, bisphenol A, and tetrabromobisphenol A in surface water, suspended particulate matter, and sediment in Taihu Lake and its tributaries. Mar Pollut Bull. 2016;112(1-2):142–50.
16. Szychowski KA, Rybczyńska-Tkaczyk K, Leja ML, Wójtowicz AK, Gmiński J. Tetrabromobisphenol A (TBBPA)-stimulated reactive oxygen species (ROS) production in cell-free model using the 2′,7′-dichlorodihydrofluorescein diacetate (H2DCFDA) assay: Limitations of method. Environ Sci Pollut Res Int. 2016;23(12):12246–52.
17. Wang S, Cao S, Wang Y, Jiang B, Wang L, Sun F, Ji R. Fate and metabolism of the brominated flame retardant tetrabromobisphenol A (TBBPA) in rice cell suspension culture. Environ Pollut. 2016;214:299–306.
18. Kuramochi H, Kawamoto K, Miyazaki K, Nagahama K, Maeda K, Li XW, Shibata E, Nakamura T, Sakai S. Determination of physicochemical properties of tetrabromobisphenol A. Environ Toxicol Chem. 2008;27(12):2413–8.
19. Suzuki S, Hasegawa A. Determination of hexabromocyclododecane diastereoisomers and tetrabromobisphenol A in water and sediment by liquid chromatography/mass spectrometry. Anal Sci. 2006;22(3):469–74.
20. Kim UJ, Lee IS, Oh JE. Occurrence, removal and release characteristics of dissolved brominated flame retardants and their potential metabolites in various kinds of wastewater. Environ Pollut. 2016;218:551–7.
21. Xiong J, Li G, An T, Zhang C, Wei C. Emission patterns and risk assessment of polybrominated diphenyl ethers and bromophenols in water and sediments from the Beijiang River, South China. Environ Pollut. 2016;219:596–603.
22. Gorga M, Martínez E, Ginebreda A, Eljarrat E, Barceló D.

Determination of PBDEs, HBB, PBEB, DBDPE, HBCD, TBBPA and related compounds in sewage sludge from Catalonia (Spain). Sci Total Environ. 2013;444:51–9.
23. World Wildlife Fund. WWF DetoX campaign: An analysis of chemicals in the blood of the European Parliament [monograph on the Internet]. Washington, DC: World Wildlife Fund; 2004 [cited 2020 Mar 16]. Available from: https://wwfeu.awsassets.panda.org/downloads/checkup-main.pdf.
24. Peters R. Man-made chemicals in human blood. TNO Rep R. 2004;493:1–33.
25. Peters RJB. Man-made chemicals in maternal and cord blood. TNO Rep R. 2005;129:1–39.
26. Thomsen C, Lundanes E, Becher G. Brominated flame retardants in plasma samples from three different occupational groups in Norway. J Environ Monit. 2001;3(4):366–70.
27. Kiciński M, Viaene MK, Den Hond E, Schoeters G, Covaci A, Dirtu AC, Nelen V, Bruckers L, Croes K, Sioen I, Baeyens W, Van Larebeke N, Nawrot TS. Neurobehavioral function and low-level exposure to brominated flame retardants in adolescents: A cross-sectional study. Environ Health. 2012;11:86.
28. Dirtu AC, Jaspers VL, Cernat R, Neels H, Covaci A. Distribution of PCBs, their hydroxylated metabolites, and other phenolic contaminants in human serum from two European countries. Environ Sci Technol. 2010;44(8):2876–83.
29. Cariou R, Antignac JP, Zalko D, Berrebi A, Cravedi JP, Maume D, Marchand P, Monteau F, Riu A, Andre F, Le Bizec B. Exposure assessment of French women and their newborns to tetrabromobisphenol-A: Occurrence measurements in maternal adipose tissue, serum, breast milk and cord serum. Chemosphere. 2008;73(7):1036–41.
30. Fujii Y, Harada KH, Hitomi T, Kobayashi H, Koizumi A, Haraguchi K. Temporal trend and age-dependent serum concentration of phenolic organohalogen contaminants in Japanese men during 1989–2010. Environ Pollut. 2014;185:228–33.
31. Shi Z, Wang Y, Niu P, Wang J, Sun Z, Zhang S, Wu Y. Concurrent extraction, clean-up, and analysis of polybrominated diphenyl ethers, hexabromocyclododecane isomers, and tetrabromobisphenol A in human milk and serum. J Sep Sci. 2013;36(20):3402–10.
32. Ni HG, Zeng H. HBCD and TBBPA in particulate phase of indoor air in Shenzhen, China. Sci Total Environ. 2013;458–460:15–9.
33. Colnot T, Kacew S, Dekant W. Mammalian toxicology and human exposures to the flame retardant 2,2′,6,6′-tetrabromo-4,4′-isopropylidenediphenol (TBBPA): Implications for risk assessment. Arch Toxicol. 2014;88(3):553–73.
34. Kuester RK, Sólyom AM, Rodriguez VP, Sipes IG. The effects of dose, route, and repeated dosing on the disposition and kinetics of tetrabromobisphenol A in male F-344 rats. Toxicol Sci. 2007;96(2):237–45.
35. Yu Y, Wang Z, Wang Q, Xiang M, Zhang Y, Ge Q, Li L, Li H, Ma R. Excretion characteristics of tetrabromobisphenol-A in Wistar rats following mouth and nose inhalation exposure. Chemosphere. 2017;175:147–52.
36. Johnson-Restrepo B, Adams DH, Kannan K. Tetrabromobisphenol A (TBBPA) and hexabromocyclododecanes (HBCDs) in tissues of humans, dolphins, and sharks from the United States. Chemosphere. 2008;70(11):1935–44.
37. Schauer UM, Völkel W, Dekant W. Toxicokinetics of tetrabromobisphenol A in humans and rats after oral administration. Toxicol Sci. 2006;91(1):49–58.
38. Yu Y, Li L, Li H, Yu X, Zhang Y, Wang Q, Zhou Z, Gao D, Ye H, Lin B, Ma R. In vivo assessment of dermal adhesion, penetration, and bioavailability of tetrabromobisphenol A. Environ Pollut. 2017;228:305–10.
39. Wu S, Ji G, Liu J, Zhang S, Gong Y, Shi L. TBBPA Induces developmental toxicity, oxidative stress, and apoptosis in embryos and zebrafish larvae (Danio rerio). Environ Toxicol. 2016;31(10):1241–9.
40. Zhang Y, Wang X, Chen C, An J, Shang Y, Li H, Xia H, Yu J, Wang C, Liu Y, Guo S. Regulation of TBBPA-induced oxidative stress on mitochondrial apoptosis in L02 cells through the Nrf2 signaling pathway. Chemosphere. 2019;226:463–71.
41. Jarosiewicz M, Duchnowicz P, Włuka A, Bukowska B. Evaluation of the effect of brominated flame retardants on hemoglobin oxidation and hemolysis in human erythrocytes. Food Chem Toxicol. 2017;109(Pt 1):264–71.
42. Jarosiewicz M, Krokosz A, Marczak A, Bukowska B. Changes in the activities of antioxidant enzymes and reduced glutathione level in human erythrocytes exposed to selected brominated flame retardants. Chemosphere. 2019;227:93–9.
43. Jarosiewicz M, Michałowicz J, Bukowska B. In vitro assessment of eryptotic potential of tetrabromobisphenol A and other bromophenolic flame retardants. Chemosphere. 2019;215:404–12.
44. Roy R, Yang J, Moses MA. Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J Clin Oncol. 2009;27(31):5287–97.
45. Lee GH, Jin SW, Kim SJ, Pham TH, Choi JH, Jeong HG. Tetrabromobisphenol A induces MMP-9 expression via NADPH oxidase and the activation of ROS, MAPK, and Akt pathways in human breast cancer MCF-7 cells. Toxicol Res. 2019;35(1):93–101.
46. Zhao C, Tang Z, Chung ACK, Wang H, Cai Z. Metabolic perturbation, proliferation and reactive oxygen species jointly contribute to cytotoxicity of human breast cancer cell induced by tetrabromo and tetrachloro bisphenol A. Ecotoxicol Environ Saf. 2019;170:495–501.
47. Abdallah MA, Pawar G, Harrad S. Evaluation of 3D-human skin equivalents for assessment of human dermal absorption of some brominated flame retardants. Environ Int. 2015;84:64–70.
48. Wu S, Wu M, Qi M, Zhong L, Qiu L. Effects of novel brominated flame retardant TBBPA on human airway

epithelial cell (A549) in vitro and proteome profiling. Environ Toxicol. 2018;33(12):1245–53.
49. Hu J, Liang Y, Chen M, Wang X. Assessing the toxicity of TBBPA and HBCD by zebrafish embryo toxicity assay and biomarker analysis. Environ Toxicol. 2009;24(4):334–42.
50. McCormick JM, Paiva MS, Häggblom MM, Cooper KR, White LA. Embryonic exposure to tetrabromobisphenol A and its metabolites, bisphenol A and tetrabromobisphenol A dimethyl ether, disrupts normal zebrafish (Danio rerio) development and matrix metalloproteinase expression. Aquat Toxicol. 2010;100(3):255–62.
51. Sharma P, Chadha P, Saini HS. Tetrabromobisphenol A induced oxidative stress and genotoxicity in fish Channa punctatus. Drug Chem Toxicol. 2019;42(6):559–64.
52. Hamers T, Kamstra JH, Sonneveld E, Murk AJ, Kester MH, Andersson PL, Legler J, Brouwer A. In vitro profiling of the endocrine-disrupting potency of brominated flame retardants. Toxicol Sci. 2006;92(1):157–73.
53. Kitamura S, Jinno N, Ohta S, Kuroki H, Fujimoto N. Thyroid hormonal activity of the flame retardants tetrabromobisphenol A and tetrachlorobisphenol A. Biochem Biophys Res Commun. 2002;293(1):554–9.
54. Jagnytsch O, Opitz R, Lutz I, Kloas W. Effects of tetrabromobisphenol A on larval development and thyroid hormone-regulated biomarkers of the amphibian Xenopus laevis. Environ Res. 2006;101(3):340–8.
55. Kuiper RV, Cantón RF, Leonards PE, Jenssen BM, Dubbeldam M, Wester PW, van den Berg M, Vos JG, Vethaak AD. Long-term exposure of European flounder (Platichthys flesus) to the flame-retardants tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCD). Ecotoxicol Environ Saf. 2007;67(3):349–60.
56. Parsons A, Lange A, Hutchinson TH, Miyagawa S, Iguchi T, Kudoh T, Tyler CR. Molecular mechanisms and tissue targets of brominated flame retardants, BDE-47 and TBBPA, in embryo-larval life stages of zebrafish (Danio rerio). Aquat Toxicol. 2019;209:99–112.
57. Chan WK, Chan KM. Disruption of the hypothalamic-pituitary-thyroid axis in zebrafish embryo-larvae following waterborne exposure to BDE-47, TBBPA and BPA. Aquat Toxicol. 2012;108:106–11.
58. Zhu B, Zhao G, Yang L, Zhou B. Tetrabromobisphenol A caused neurodevelopmental toxicity via disrupting thyroid hormones in zebrafish larvae. Chemosphere. 2018;197:353–61.
59. Osimitz TG, Droege W, Hayes AW. Subchronic toxicology of tetrabromobisphenol A in rats. Hum Exp Toxicol. 2016;35(11):1214–26.
60. 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;245(1-2):76–89.
61. Meerts IA, van Zanden JJ, Luijks EA, van Leeuwen-Bol I, Marsh G, Jakobsson E, Bergman A, Brouwer A. Potent competitive interactions of some brominated flame retardants and related compounds with human transthyretin in vitro. Toxicol Sci. 2000;56(1):95–104.
62. Ogunbayo OA, Lai PF, Connolly TJ, Michelangeli F. Tetrabromobisphenol A (TBBPA) induces cell death in TM4 Sertoli cells by modulating Ca^{2+} transport proteins and causing dysregulation of Ca^{2+} homeostasis. Toxicol In Vitro. 2008;22(4):943–52.
63. Linhartova P, Gazo I, Shaliutina-Kolesova A, Hulak M, Kaspar V. Effects of tetrabrombisphenol A on DNA integrity, oxidative stress, and sterlet (Acipenser ruthenus) spermatozoa quality variables. Environ Toxicol. 2015;30(7):735–45.
64. Zhang H, Liu W, Chen B, He J, Chen F, Shan X, Du Q, Li N, Jia X, Tang J. Differences in reproductive toxicity of TBBPA and TCBPA exposure in male Rana nigromaculata. Environ Pollut. 2018;243(Pt A):394–403.
65. Lai DY, Kacew S, Dekant W. Tetrabromobisphenol A (TBBPA): Possible modes of action of toxicity and carcinogenicity in rodents. Food Chem Toxicol. 2015;80:206–14.
66. Dunnick JK, Sanders JM, Kissling GE, Johnson CL, Boyle MH, Elmore SA. Environmental chemical exposure may contribute to uterine cancer development: Studies with tetrabromobisphenol A. Toxicol Pathol. 2015;43(4):464–73.
67. Sanders JM, Coulter SJ, Knudsen GA, Dunnick JK, Kissling GE, Birnbaum LS. Disruption of estrogen homeostasis as a mechanism for uterine toxicity in Wistar Han rats treated with tetrabromobisphenol A. Toxicol Appl Pharmacol. 2016;298:31–9.
68. Zatecka E, Ded L, Elzeinova F, Kubatova A, Dorosh A, Margaryan H, Dostalova P, Peknicova J. Effect of tetrabrombisphenol A on induction of apoptosis in the testes and changes in expression of selected testicular genes in CD1 mice. Reprod Toxicol. 2013;35:32–9.
69. Harvey JB, Osborne TS, Hong HH, Bhusari S, Ton TV, Pandiri AR, Masinde T, Dunnick J, Peddada S, Elmore S, Hoenerhoff MJ. Uterine carcinomas in tetrabromobisphenol A-exposed Wistar Han rats harbor increased Tp53 mutations and mimic high-grade type I endometrial carcinomas in women. Toxicol Pathol. 2015;43(8):1103–13.
70. Liang S, Liang S, Zhou H, Yin N, Faiola F. Typical halogenated flame retardants affect human neural stem cell gene expression during proliferation and differentiation via glycogen synthase kinase 3 β and T3 signaling. Ecotoxicol Environ Saf. 2019;183:109498.
71. Zieminska E, Lenart J, Diamandakis D, Lazarewicz JW. The role of Ca^{2+} imbalance in the induction of acute oxidative stress and cytotoxicity in cultured rat cerebellar granule cells challenged with tetrabromobisphenol A. Neurochem Res. 2017;42(3):777–87.
72. Zieminska E, Ruszczynska A, Lazarewicz JW. Tetrabromobisphenol A disturbs zinc homeostasis in cultured

cerebellar granule cells: A dual role in neurotoxicity. Food Chem Toxicol. 2017;109(Pt 1):363–75.
73. Szychowski KA, Wójtowicz AK. TBBPA causes neurotoxic and the apoptotic responses in cultured mouse hippocampal neurons in vitro. Pharmacol Rep. 2016;68(1):20–6.
74. Reistad T, Mariussen E, Ring A, Fonnum F. In vitro toxicity of tetrabromobisphenol-A on cerebellar granule cells: Cell death, free radical formation, calcium influx and extracellular glutamate. Toxicol Sci. 2007;96(2):268–78.
75. Yin N, Liang S, Liang S, Yang R, Hu B, Qin Z, Liu A, Faiola F. TBBPA and its alternatives disturb the early stages of neural development by interfering with the NOTCH and WNT pathways. Environ Sci Technol. 2018;52(9):5459–68.
76. Diamandakis D, Zieminska E, Siwiec M, Tokarski K, Salinska E, Lenart J, Hess G, Lazarewicz JW. Tetrabromobisphenol A-induced depolarization of rat cerebellar granule cells: Ex vivo and in vitro studies. Chemosphere. 2019;223:64–73.
77. Nakajima A, Saigusa D, Tetsu N, Yamakuni T, Tomioka Y, Hishinuma T. Neurobehavioral effects of tetrabromobisphenol A, a brominated flame retardant, in mice. Toxicol Lett. 2009;189(1):78–83.
78. Viberg H, Eriksson P. Differences in neonatal neurotoxicity of brominated flame retardants, PBDE 99 and TBBPA, in mice. Toxicology. 2011;289(1):59–65.
79. Mariussen E, Fonnum F. The effect of brominated flame retardants on neurotransmitter uptake into rat brain synaptosomes and vesicles. Neurochem Int. 2003; 43(4-5):533–42.
80. Liu QS, Liu N, Sun Z, Zhou Q, Jiang G. Intranasal administration of tetrabromobisphenol A bis(2-hydroxyethyl ether) induces neurobehavioral changes in neonatal Sprague Dawley rats. J Environ Sci. 2018;63:76–86.
81. Grasselli E, Cortese K, Fabbri R, Smerilli A, Vergani L, Voci A, Gallo G, Canesi L. Thyromimetic actions of tetrabromobisphenol A (TBBPA) in steatotic FaO rat hepatoma cells. Chemosphere. 2014;112:511–8.
82. Nakagawa Y, Suzuki T, Ishii H, Ogata A. Biotransformation and cytotoxicity of a brominated flame retardant, tetrabromobisphenol A, and its analogues in rat hepatocytes. Xenobiotica. 2007;37(7):693–708.
83. Kling P, Förlin L. Proteomic studies in zebrafish liver cells exposed to the brominated flame retardants HBCD and TBBPA. Ecotoxicol Environ Saf. 2009;72(7):1985–93.
84. Ronisz D, Finne EF, Karlsson H, Förlin L. Effects of the brominated flame retardants hexabromocyclododecane (HBCDD), and tetrabromobisphenol A (TBBPA), on hepatic enzymes and other biomarkers in juvenile rainbow trout and feral eelpout. Aquat Toxicol. 2004;69(3):229–45.
85. Dunnick JK, Morgan DL, Elmore SA, Gerrish K, Pandiri A, Ton TV, Shockley KR, Merrick BA. Tetrabromobisphenol A activates the hepatic interferon pathway in rats. Toxicol Lett. 2017;266:32–41.
86. Tada Y, Fujitani T, Ogata A, Kamimura H. Flame retardant tetrabromobisphenol A induced hepatic changes in ICR male mice. Environ Toxicol Pharmacol. 2007;23(2):174–8.
87. Kang MJ, Kim JH, Shin S, Choi JH, Lee SK, Kim HS, Kim ND, Kang GW, Jeong HG, Kang W, Chun YJ, Jeong TC. Nephrotoxic potential and toxicokinetics of tetrabromobisphenol A in rat for risk assessment. J Toxicol Environ Health A. 2009;72(21-22):1439–45.
88. Fukuda N, Ito Y, Yamaguchi M, Mitumori K, Koizumi M, Hasegawa R, Kamata E, Ema M. Unexpected nephrotoxicity induced by tetrabromobisphenol A in newborn rats. Toxicol Lett. 2004;150(2):145–55.
89. Kibakaya EC, Stephen K, Whalen MM. Tetrabromobisphenol A has immunosuppressive effects on human natural killer cells. J Immunotoxicol. 2009;6(4): 285–92.
90. Cato A, Celada L, Kibakaya EC, Simmons N, Whalen MM. Brominated flame retardants, tetrabromobisphenol A and hexabromocyclododecane, activate mitogen-activated protein kinases (MAPKs) in human natural killer cells. Cell Biol Toxicol. 2014;30(6):345–60.
91. Wu S, Zhu Z, Chen J, Wu M, Qiu L. Transcriptomic analyses of human bronchial epithelial cells BEAS-2B exposed to brominated flame retardant (tetrabromobisphenol A). Environ Toxicol. 2019;34(6):742–52.
92. Park C, Kim SJ, Lee WK, Moon SK, Kwak S, Choe SK, Park R. Tetrabromobisphenol-A induces apoptotic death of auditory cells and hearing loss. Biochem Biophys Res Commun. 2016;478(4):1667–73.
93. Park HR, Kamau PW, Korte C, Loch-Caruso R. Tetrabromobisphenol A activates inflammatory pathways in human first trimester extravillous trophoblasts in vitro. Reprod Toxicol. 2014 Dec;50:154–62.
94. Yasmin S, Whalen M. Flame retardants, hexabromocyclododecane (HBCD) and tetrabromobisphenol A (TBBPA), alter secretion of tumor necrosis factor alpha (TNFα) from human immune cells. Arch Toxicol. 2018;92(4):1483–94.
95. Watt J, Schlezinger JJ. Structurally diverse, PPARγ-activating environmental toxicants induce adipogenesis and suppress osteogenesis in bone marrow mesenchymal stromal cells. Toxicology. 2015;331:66–77.
96. Riu A, Grimaldi M, le Maire A, Bey G, Phillips K, Boulahtouf A, Perdu E, Zalko D, Bourguet W, Balaguer P. Peroxisome proliferator-activated receptor γ is a target for halogenated analogs of bisphenol A. Environ Health Perspect. 2011;119(9):1227–32.
97. Honkisz E, Wójtowicz AK. The role of PPARγ in TBBPA-mediated endocrine disrupting effects in human choriocarcinoma JEG-3 cells. Mol Cell Biochem. 2015;409(1-2):81–91.
98. Woeller CF, Flores E, Pollock SJ, Phipps RP. Editor's highlight: Thy1 (CD90) expression is reduced by the environmental chemical tetrabromobisphenol-A to promote adipogenesis through induction of microRNA-103. Toxicol Sci. 2017;157(2):305–19.
99. Koike E, Yanagisawa R, Takano H. Brominated flame

retardants, hexabromocyclododecane and tetrabromobisphenol A, affect proinflammatory protein expression in human bronchial epithelial cells via disruption of intracellular signaling. Toxicol In Vitro. 2016;32:212–9.