



Molecular Mechanism of Tetrabromobisphenol A (TBBPA)-induced Target Organ Toxicity in Sprague-Dawley Male Rats

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Brominated flame retardants (BFRs) are present in many consumer products ranging from fabrics to plastics and electronics. Wide use of flame retardants can pose an environmental hazard, which makes it important to determine the mechanism of their toxicity. In the present study, dose-dependent toxicity of tetrabromobisphenol A (TBBPA), a flame retardant, was examined in male prepubertal rats (postnatal day 18) treated orally with TBBPA at 0, 125, 250 or 500 mg/kg for 30 days. There were no differences in body weight gain between the control and TBBPA-treated groups. However, absolute and relative liver weights were significantly increased in high dose of TBBPA-treated groups. TBBPA treatment led to significant induction of CYP2B1 and constitutive androstane receptor (CAR) expression in the liver. In addition, serum thyroxin (T4) concentration was significantly reduced in the TBBPA treated group. These results indicate that repeated exposure to TBBPA induces drug-metabolising enzymes in rats through the CAR signaling pathway. In particular, TBBPA efficiently produced reactive oxygen species (ROS) through CYP2B1 induction in rats. We measured 8-hydroxy-2'-deoxyguanosine (8-OHdG), a biomarker of DNA oxidative damage, in the kidney, liver and testes of rats following TBBPA treatment. As expected, TBBPA strongly induced the production of 8-OHdG in the testis and kidney. These observations suggest that TBBPA-induced target organ toxicity may be due to ROS produced by metabolism of TBBPA in Sprague-Dawley rats.

Key words: Tetrabromobisphenol A, Target organ, CAR, CYP2B1, Thyroid hormone

INTRODUCTION

Brominated flame retardants (BFRs) are ubiquitous chemicals with widespread and global industrial use, and many of them are still produced in large volumes. Among them, tetrabromobisphenol A (TBBPA) has the largest production volume (over 120,000 tons annually or 30% of all BFRs) among BFRs globally (WHO/ICPS, 1995). TBBPA is one of several brominated compounds which are widely used as flame retardants in consumer products, and several BFRs are found at quantifiable levels in animals as well as in

humans. TBBPA is used mostly as a reactive retardant and its primary application is in electrical components. From recent surveys, TBBPA has been detected in sewage sludges (Alaee *et al.*, 2003; Chu *et al.*, 2005), in several fish species along the North Sea and UK coasts (Morris *et al.*, 2004), in indoor environment and food (Tasaki *et al.*, 2004; Fernandes *et al.*, 2008) as well as in human blood (Jakobsen *et al.*, 2002).

TBBPA is readily absorbed up to 90% from the gastrointestinal tract, rapidly metabolized in the liver, and excreted mainly through the bile acid (Hakk and Letcher, 2003; Kang *et al.*, 2009). Exposure of the developing human fetus may be of particular concern because TBBPA appears to be readily transported over the placental barrier, and TBBPA was detected in umbilical blood in Japanese and French studies (Cariou *et al.*, 2008; Kawashiro *et al.*, 2008).

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After 28 days of TBBPA treatment, moderate hepatic microsomal enzyme induction was observed in rats (Germer *et al.*, 2006). In humans, free TBBPA is rapidly conjugated to give TBBPA-glucuronide and TBBPA-sulphate and systemic bioavailability is limited due to hepatic first pass metabolism (Schauer *et al.*, 2006). In rat, the half-life of TBBPA is also short, on average 3 days, with variations between approximately 10 hr in the liver and 70 hr in adipose tissue (Hakk and Letcher, 2003). The toxicological evaluation of TBBPA has been studied after oral administration for 90 days and toxic effects were not observed in doses up to 1000 mg/kg body weight (EU-Report, 2005). However, it was suggested that lower doses of TBBPA (200 mg/kg) resulted in slight renal impairment in newborn rats (Fukuda *et al.*, 2004). Although toxicity of TBBPA on experimental animal appeared to be low (Darnerud, 2003), the potential endocrine disruptive effect of TBBPA has raised concerns recently by the interaction of TBBPA with the thyroid hormone system, mainly through its strong competitive binding to transthyretin (TTR) (Meerts *et al.*, 2000; Legler and Brouwer, 2003). It was also reported that TBBPA showed antiestrogenic activity using the uterotrophic assay (Kitamura *et al.*, 2005). Generally, induction of drug metabolism may play a role in such effects by changing the body's homeostasis of certain hormones such as steroids and thyroid hormones. In particular, induction of drug-metabolising enzymes via the aryl hydrocarbon receptor (AhR), the pregnane X receptor (PXR) or the constitutive androstane receptor (CAR) can interfere with the homeostasis of thyroid hormones (Mikamo *et al.*, 2003; Xie *et al.*, 2003; Maglich *et al.*, 2004). In the pituitary cell line GH3, TBBPA exhibited thyroid hormone-like effects (Kitamura *et al.*, 2005), whereas it exhibited anti-thyroid hormone effects in thyroid hormone receptor-transfected Chinese hamster ovary (CHO) cells (Kitamura *et al.*, 2002). Furthermore, high affinity binding of TBBPA to the human thyroid hormone transport protein transthyretin was reported by Meerts *et al.* (2000). Although knowledge of TBBPA toxicology is increasing, our understanding is still quite limited.

In view of these concerns, the aim of this study is to investigate the influence of TBBPA on target organ toxicity under prolonged exposure, with special attention to oxidative stress-inducing activity of TBBPA in the Sprague-Dawley male rat. Moreover, this study was enhanced for endocrine endpoints, to obtain an improved profile of the endocrine activity of TBBPA.

MATERIALS AND METHODS

Chemicals. Tetrabromobisphenol A (TBBPA, 97%), sodium acetate and sodium hydroxide were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium carboxymethyl cellulose (CMC-Na) was obtained from Daejung Reagents Chemicals (Gyunggi-do, Korea).

Animals and housing. Sprague-Dawley CrI : CD female rats weighing 250 ± 15 g were obtained from the Charles River Laboratory Animal Resources (Seoul, Korea) and all animals were maintained under specific pathogen free (SPF)-conditioned room under a 12 hr light/dark cycle. Ambient air temperature was controlled at $23 \pm 2^\circ\text{C}$ and relative humidity was maintained at 55%. Prior to the experiment, all animals were checked for overt signs of illness and only healthy animals were selected for the study. Tap water and rodent chow were given *ad libitum*. Two females were placed with one male for 3 hr on 7 consecutive days. Daily vaginal smears were examined for the presence of sperm. The day of sperm detection was considered day 0 of gestation (GD 0). On the day of delivery (day of birth considered PND 1), all pups were counted and their sexes were determined. On PND 4, pups were sexed according to anogenital distance (AGD), and female pups were removed. All male litters of 8 pups were culled randomly by cross-fostering, and pups (9 animals/group) were subjected to the following treatment groups. TBBPA received daily oral gavage of vehicle or TBBPA at 125, 250 or 500 mg/kg body weight from postnatal day (PND) 18 to PND 48. The control group was administered corn oil in the same manner. The dosages used in the present study were based on previous reports concerning adverse effects on the kidney of newborn rats (Fukuda *et al.*, 2004). Body weights were recorded daily before applying the relevant dose. The animals were treated humanely, and care was taken to ease suffering. The experimental protocol was approved by the committee of Pusan National University in accordance with the Korea Food and Drug Administration Animal Protection.

Clinical signs and body weight changes. Throughout the study period, each animal was observed at least once daily for clinical signs of toxicity related to the chemical treatment. On working days, all cages were checked in the morning and afternoon for dead or moribund animals. All females were observed daily for clinical signs of toxicity.

Histological evaluation. For haematoxylin and eosin (H&E) staining, several consecutive 5 μm paraffin sections [one each for H&E and CAR] were de-paraffinized with xylene for 7 min, 3 times. After gradual rehydration of the slides in a graded series of alcohol and washing with deionized water, the sections were stained with H&E for 1 min, rinsed with deionized water and developed with tap water for 5 min. The tissue sections were destained by dipping the slide in acidified ethanol and rinsing in tap water. After washing with deionized water, the sections were stained with eosin for 30 sec, dehydrated and mounted. The histopathological findings of the testes were determined using a photonic microscope.

Western blot analysis. For Western blot analysis, total protein was extracted from the testis by homogenizing in a

PRO-PREPTM protein extract solution. Total protein was quantified using the protein assay reagent (BioRad, USA). Extracted proteins were denatured by boiling for 5 min in sample buffer. Thirty micrograms of total protein were run on 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) at 75 V for 15 min and then at 150 V for 40 min using a running buffer. The proteins were electrophoretically transferred to polyvinylidene difluoride (PVDF) membranes (NEN life Science) at 64 mA for 45 min in a blotting solution. The membranes were washed in TBS-T buffer (0.8% NaCl, 0.02 M Tris-base, and 0.3% Tween-20, pH 7.6) for 5 min and then blocked by TBS-T blocking buffer containing 5% skim milk. Following that, the membranes were incubated overnight at 4°C with the primary antibodies, anti-CAR (1:500), anti-CYP1A2 (1:200), anti-CYP2B1 (1:200), anti-CYP3A1 (1:200), anti-ERK (1:200), anti-p-ERK (1:200), anti-SOD (1:200), and β -actin (1:1000). The membranes were washed once every 5 min for 1 hr with TNT buffer containing 0.1% Tween-20 and incubated for 30 min with horseradish peroxidase-conjugated anti-rabbit or mouse immunoglobulin G (Santa Cruz, CA, USA). The membranes were washed again for 1 hr. Blots were developed using an Enhanced Chemiluminescence System (ECL, Amersham Corp., Cardiff, UK). Image Analyzer (Zeiss, Germany) was used to determine the relative band intensities.

Total CYP content in the liver. Hepatic microsomes were precipitated from post-mitochondrial supernatant using CaCl_2 . Total CYP content of hepatic microsomes was measured according to Omura and Sato (1964). Microsomal *O*-dealkylation of 7-ethoxy- (EROD), 7-methoxy- (MROD), 7-pentoxo- (PROD), and 7-benzyloxy-resorufin (BROD) were assayed fluorimetrically at 37°C using excitation and emission wavelengths of 530 and 585 nm, respectively.

Lipid peroxidation assay. The level of malondialdehyde (MDA) was determined in hepatic homogenates according to the method of Mihara and Uchiyama (1978). Homogenates were heated at 100°C in the presence of 1% orthophosphoric acid with 0.6% thiobarbituric acid (TBA) for 45 min. Lipid peroxidation products, especially MDA, reacted with TBA, and colored products of this reaction were extracted with *n*-butanol, and its extracts were measured spectrophotometrically at 532 nm. At the same time, a calibration curve was plotted with use of tetraepoxypropane, which in analytical conditions underwent hydrolysis, producing stoichiometric amounts of malonic dialdehyde.

Glutathione level. GSH was determined according to the method described by Sedlak and Lindsay (1968). After deproteinization of homogenates with 5-sulfosalicylic acid, non protein thiol groups were determined via the reaction with 5,5'-dithiobis(2-nitrobenzoic acid). The emerging yellow color was determined spectrophotometrically at 412

nm. The calibration curve was plotted using reduced GSH as the standard.

DNA isolation and enzymatic hydrolysis. DNA was isolated and purified as described by Lee and Santella (1988). Tissues (liver, kidney and testis) were homogenized using a tissue homogenizer at low speed in 10 ml for each gram of tissue with 10 mM Tris buffer containing 0.15 M NaCl, 10 mM EDTA and 1% SDS (pH 8.0). After homogenization, an equal volume of phenol/m-cresol/8-hydroxyquinoline/water (500 : 70 : 0.5 : 55, w/w/ w/w) was added and the mixture was stirred at room temperature for 30 min. The mixture was then centrifuged at 20,000 \times g for 30 min. DNA was precipitated from the upper aqueous phase by adding 2 vol of 95% ethanol containing 2% potassium acetate. The precipitated DNA was removed by centrifugation and/or spooled out into a glass rod and dissolved in phosphate buffered saline (PBS). The solution was incubated at 37°C with 10 $\mu\text{g}/\text{ml}$ RNase which had been heated at 80°C for 10 min to inactivate DNase. The sample was then incubated with 20 $\mu\text{g}/\text{ml}$ proteinase K for 1 hr at 37°C. The solution of DNA was deproteinized with chloroform/isoamyl alcohol (24 : 1, v/v), precipitated by adding 2 volumes of 95% ethanol and dissolved again in PBS. DNA concentration was determined by UV absorption spectroscopy. Extractions were repeated until the A_{260}/A_{280} ratios were between 1.8 and 1.9.

Determination of 8-OHdG in DNA. A marker of oxidative DNA damage, 8-OHdG was measured according to the method of Kasai *et al.* (1986). Briefly, each 50 μg of DNA sample was suspended in 50 μl of 20 mM sodium acetate buffer (pH 4.1) and denatured in boiling water for 10 min. The samples were digested to deoxynucleosides by incubation with 12 units of nuclease P1 at 37°C for 1 hr in 40 μl of 1 M Tris-HCl buffer (pH 7.5). The resulting mixture was injected into HPLC (Gilson, Middleton USA) equipped with both Gilson UV/Vis-151 detector (Gilson, Middleton USA) and an ESA Coulochem II System with ESA 5011 analytical cell electrochemical detector (Chelmsford, MA, USA) with the following features; column, Supelcosil ODS (250 \times 4.6 mm, 5 μm particle, Supelco, Bellefonte, PA, USA); eluent, 10% aqueous methanol containing 12.5 mM citric acid, 25 mM sodium acetate, 30 mM sodium hydroxide, and 10 mM acetic acid; flow rate, 0.8 ml/min. The molar ratio of 8-OHdG to deoxyguanosine in each DNA sample was determined, based on the peak area of authentic 8-OHdG using the electrochemical detector and the UV absorbance at A_{254} of dG.

Serum thyroid hormone concentration. The serum total T3 and T4 levels were measured using Coat-a-Count Total T3 and Total T4 radioimmunoassay kits (Amersham, IL, USA) according to the manufacturer's instructions.

Data analysis. All values were expressed as mean \pm SD ($n = 9$ animals). Absolute and relative organ weights were analysed using analysis of covariance (ANCOVA) with the body weight at necropsy as a covariate. When a significant treatment effect was present, Dunnett's test (control vs. treatment group) was used to compare treatment groups. If significant heterogeneity of variance was apparent, some data transformations were performed prior to the statistical analysis. The levels of statistical significance were set *a priori* at $\alpha = 0.05$.

RESULTS

Clinical signs, body, and organ weight change. TBBPA was administered orally at 0, 125, 250 or 500 mg/kg per day from age 18 days until age 48 days. During the study period, clinical signs were similar in every group (data not shown). The body weight gains of TBBPA 500 mg/kg treated groups were slightly lower than the control, but not significant (Fig. 1). In organ weight change, a high dose of TBBPA significantly increased absolute and relative liver weights, but there was no significant effect on kidney, testis, epididymes and adrenal glands in all treatment groups. In contrast, thyroid gland weights were significantly decreased in high dose TBBPA-treated groups compared to the control group (Table 1).

Histopathology. There were no exposure related histopathological changes observed in any of the organs exam-

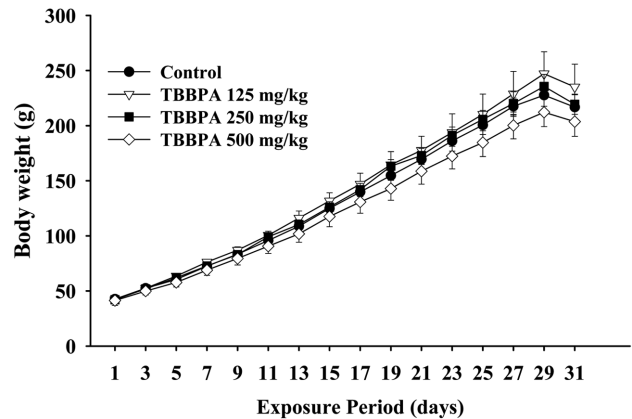


Fig. 1. Body weights changes of Sprague-Dawley male rats treated with tetrabromobisphenol A (TBBPA) over a 30-day period. Each point represents the mean \pm SD for 9 animals/group. Prepubertal male rats given TBBPA orally at 0, 125, 250 or 500 mg/kg from postnatal day (PND) 18 to PND 48.

ined in Sprague-Dawley rats treated with TBBPA (Fig. 2). Notably, there were no observed histopathological effects in the liver to explain the increased weight of this organ. In addition, there were no histopathological findings of the kidney such as polycystic lesions associated with the dilation of the tubules as previously reported (Fukuda *et al.*, 2004). Similarly, there were no TBBPA-induced histopathological changes in the thyroid gland, based on follicle size and cell activation (cell and nuclear hypertrophy and cell

Table 1. Comparisons of the absolute or relative organ weights in the Sprague-Dawley rats treated with tetrabromobisphenol A^a

Organ weight	Groups	Vehicle control ^b 0 mg/kg	TBBPA 125 mg/kg	TBBPA 250 mg/kg	TBBPA 500 mg/kg
Absolute weight (g)					
Initial B.W.		37.42 \pm 0.98 ^c	39.25 \pm 1.17	39.27 \pm 1.11	40.43 \pm 1.01
Final B.W.		233.32 \pm 9.57	237.61 \pm 12.63	231.91 \pm 9.19	235.84 \pm 18.69
Liver		8.26 \pm 0.68	8.44 \pm 0.51	8.50 \pm 0.83	9.21 \pm 1.07*
Kidney		2.06 \pm 0.17	2.14 \pm 0.27	2.21 \pm 0.29	2.04 \pm 0.24
Spleen		0.77 \pm 0.13	0.74 \pm 0.11	0.81 \pm 0.10	0.78 \pm 0.08
Testes		2.05 \pm 0.26	2.01 \pm 0.34	2.11 \pm 0.24	1.95 \pm 0.26
Epididymis		0.38 \pm 0.05	0.35 \pm 0.04	0.30 \pm 0.06	0.32 \pm 0.04
Adrenal glands (mg)		42.70 \pm 7.91	44.01 \pm 5.69	40.21 \pm 7.84	42.82 \pm 7.15
Thyroid glands (mg)		12.12 \pm 2.76	11.54 \pm 3.72	11.48 \pm 2.84	9.25 \pm 3.24*
Relative weight (mg/ g b.w.)					
Liver		35.45 \pm 1.30	35.61 \pm 1.54	36.65 \pm 1.52	39.19 \pm 2.36*
Kidney		8.82 \pm 0.74	9.04 \pm 0.51	9.24 \pm 0.48	8.76 \pm 0.34
Spleen		3.32 \pm 0.52	3.13 \pm 0.34	3.46 \pm 0.39	3.35 \pm 0.50
Testes		9.63 \pm 0.61	9.26 \pm 0.84	9.62 \pm 0.54	9.50 \pm 0.63
Epididymis		1.39 \pm 0.15	1.32 \pm 0.16	1.29 \pm 0.04	1.32 \pm 0.16
Adrenal glands		0.183 \pm 0.031	0.185 \pm 0.028	0.175 \pm 0.029	0.182 \pm 0.024
Thyroid glands		0.052 \pm 0.026	0.048 \pm 0.023	0.049 \pm 0.010	0.044 \pm 0.011*

^aMale rats were administered with tetrabromobisphenol A (250, 500 or 1000 mg/kg/day) by oral gavage for 30 days.

^bVehicle control received corn oil.

^cData are presented as mean \pm SD ($n = 9$).

*Significantly different from vehicle control ($p < 0.05$).

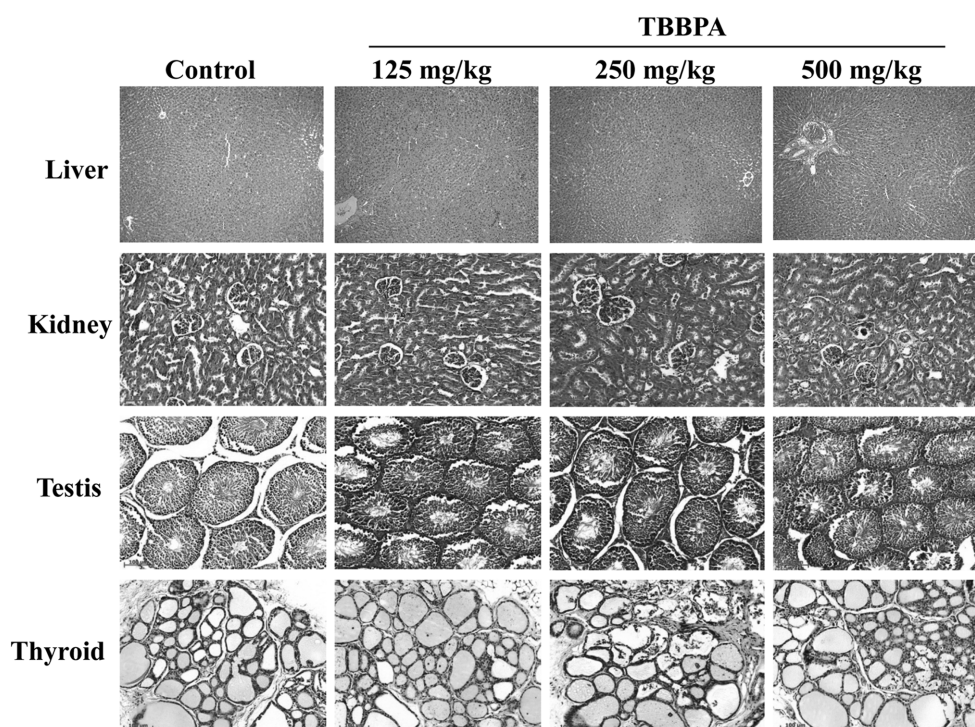


Fig. 2. Histological findings in the liver, kidney, testis and thyroid glands of Sprague-Dawley rat treated with tetrabromobisphenol A (TBBPA). Animals were treated with TBBPA (125, 250 or 500 mg/kg) by oral intubation for 30 days. The photomicrographs were taken at a 200 \times magnification after hematoxylin & eosin (H&E) staining. The scale bar represents 100 μ m.

vacuolization), in both studies.

Expression of CAR1/2, CYPs, ERK, and SOD. The expression of CAR and CYP protein were measured in the liver from TBBPA-treated rats. TBBPA led to a slightly increase in CYP2B1 at high dose of TBBPA, whereas the levels of CYP1A2 and CYP3A1 were not changed. CAR plays an important role in transcriptional activation of CYP2B genes. TBBPA also significantly increased the expression of CAR in the nuclear extracts of the liver from TBBPA-treated rats (Fig. 3A). In order to assess the involvement of ERK1/2 in the intracellular pathways underlying TBBPA-induced CYP2B1 expression, we tested using specific antibodies against the phosphorylated ERK. Phosphorylation of ERK1/2 increased in the liver of TBBPA-treated animals in a dose-dependent manner. Moreover, expression levels of SOD were significantly increased only at a high dose of TBBPA (Fig. 3B).

CYPs enzyme activity. CYPs subfamily enzyme activity was measured in the liver microsomal fraction. As showed in Table 2, TBBPA (250 and 500 mg/kg) significantly increased the activity of CYP2B1 in the liver, but there was no significant effect on CYP1A2, CYP2E1, and CYP3A1 enzyme activity in response to TBBPA (Table 2). These results were similar to the expression levels of

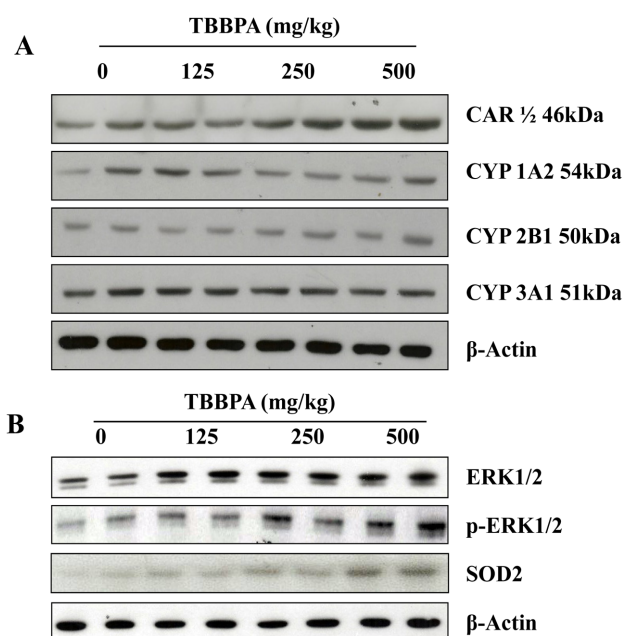


Fig. 3. Effect of tetrabromobisphenol A (TBBPA) on the expression of drug metabolism signaling in the liver. (A) CAR1/2 and CYPs expression levels were determined by Western blot analysis. (B) Expression levels of ERK and p-ERK were determined by Western blot analysis. Proteins were isolated from liver of rats treated with control or TBBPA (0, 125, 250 or 500 mg/kg).

Table 2. The levels of MDA, GSH, CYP activities in the microsomes of liver isolated from tetrabromobisphenol A-treated Sprague-Dawley rats

Parameters	Control	TBBPA 125 mg/kg	TBBPA 250 mg/kg	TBBPA 500 mg/kg
CYP1A1	n.d.	n.d.	n.d.	n.d.
CYP1A2	100 ± 29	145 ± 37	162 ± 17	159 ± 43
CYP2B1	100 ± 13	193 ± 62	424 ± 140**	402 ± 115*
CYP3A1	100 ± 17	139 ± 21	91 ± 21	66 ± 11
Total CYP content (nmol/mg protein)	0.84 ± 0.03	0.92 ± 0.13	1.01 ± 0.04	0.92 ± 0.06
Cytochrome b5 content (nmol/mg protein)	0.25 ± 0.04	0.32 ± 0.05	0.33 ± 0.06	0.26 ± 0.03
Total GSH (µmol/g liver)	3.4 ± 0.7	4.2 ± 0.4	6.5 ± 0.6**	4.1 ± 0.9
GSSG (nmol/g liver)	45 ± 10	54 ± 18	59 ± 5	42 ± 10
Malondialdehyde (MDA) (nmol/g liver)	39.5 ± 4.9	37.4 ± 3.4	38.4 ± 4.6	35.3 ± 2.4

Male rats were administered with tetrabromobisphenol A (250, 500 or 1000 mg/kg/day) by oral gavage for 30 days.

Vehicle control received corn oil.

Data are presented as mean ± SD (n = 9).

Significantly different from vehicle control (* $p < 0.05$ and ** $p < 0.01$).

CYP2B1 demonstrated by Western blot analysis.

Hepatic GSH and MDA levels. Following the treatment of rats with 125, 250 or 500 mg/kg of TBBPA for 30 days, their livers were analyzed for determining hepatic levels of total GSH, GSSG and MDA. Changes in the total GSH level (µmol/g liver) was significantly increased only at a dose of 250 mg/kg TBBPA, but GSSG levels were not changed for any of the treatment groups. Furthermore, hepatic MDA levels were not changed by TBBPA treatment (Table 2).

8-OHdG levels. Sprague-Dawley male rats were orally treated with TBBPA to investigate oxidative stress to DNA. 8-OHdG levels were determined by an HPLC-ECD system and typical chromatographic profiles of 8-OHdG are shown in Fig. 4A. The level of 8-OHdG in the testis DNA was significantly increased in 500 mg/kg dose of TBBPA. In the kidney, 8-OHdG was also increased in the 250 and 500 mg/kg doses of TBBPA. However, 8-OHdG did not increase in the liver following TBBPA treatment (Fig. 4B).

Serum thyroid hormone levels. Serum total T4 concentration was dramatically reduced in 250 and 500 mg/kg TBBPA treatment groups. Effects of TBBPA on serum total T3 concentrations were much lower than effects on T4. Serum total thyroid-stimulating hormone (TSH) concentrations were not changed by any of the TBBPA treatments (Fig. 5).

DISCUSSION

TBBPA is detected in environmental and human samples because TBBPA is produced in large amounts (de Wit, 2002). It was evident that possible toxic effects were caused by BBPA exposure (Alaee *et al.*, 2003; Sjödin *et al.*, 2003). A previous study demonstrated that marked nephrotoxicity,

characterized by the formation of polycystic lesions, was observed in newborn rats treated with 200 and 600 mg/kg TBBPA (Fukuda *et al.*, 2004). However, we did not find any toxicological effects of TBBPA on kidney. We clearly observed that TBBPA treatment led to induction of CYP2B1 enzyme activity and nuclear protein levels of CAR markedly increased in rat livers. Nuclear accumulation of CAR is the general step regulating the induction of CYP2B genes (Kawamoto *et al.*, 1999). The results are consistent with a previous report of hepatic biochemical changes on repeated oral TBBPA administration for 1 week in rats (Szymanska *et al.*, 2000).

CYPs are involved in biotransformation of many xenobiotics (Nelson *et al.*, 1996). In a previous study, no effects of TBBPA (30, 100, or 300 mg/kg/day) were observed on CYP-catalysed EROD, PROD or LBD activities after 28 days treatment (Germer *et al.*, 2006). However, we find that 500 mg/kg TBBPA treatment significantly increased hepatic CYP2B1 enzyme activity and nuclear protein levels of CAR in rat. In normal untreated hepatic cells, CAR is located in the cytoplasm in a complex with CAR cytoplasmic retention protein (CCRP) and heat shock protein 90 (Hsp90), CAR is translocated to the nucleus where it forms a functional heterodimer with the retinoid X receptor (RXR). The CAR:RXR heterodimer recruits p160/SRC-1 and other coactivator proteins (Xia *et al.*, 2007). Binding of the CAR:RXR heterodimer to NR1-binding sites (DR-4 motifs) in the CYP2B gene results in activation of the distal promoter (Swales and Negishi, 2004).

Generally, CAR has been implicated in regulation of circulating thyroid hormone concentrations. Phenobarbital treatment induced thyroid hormone conjugation pathways in a CAR-dependent manner resulting in diminished serum T4. Although toxicity and tetranogenicity of TBBPA in experimental *in vivo* studies appeared to be low (Darnierud, 2003), concern was expressed recently for the potential endocrine

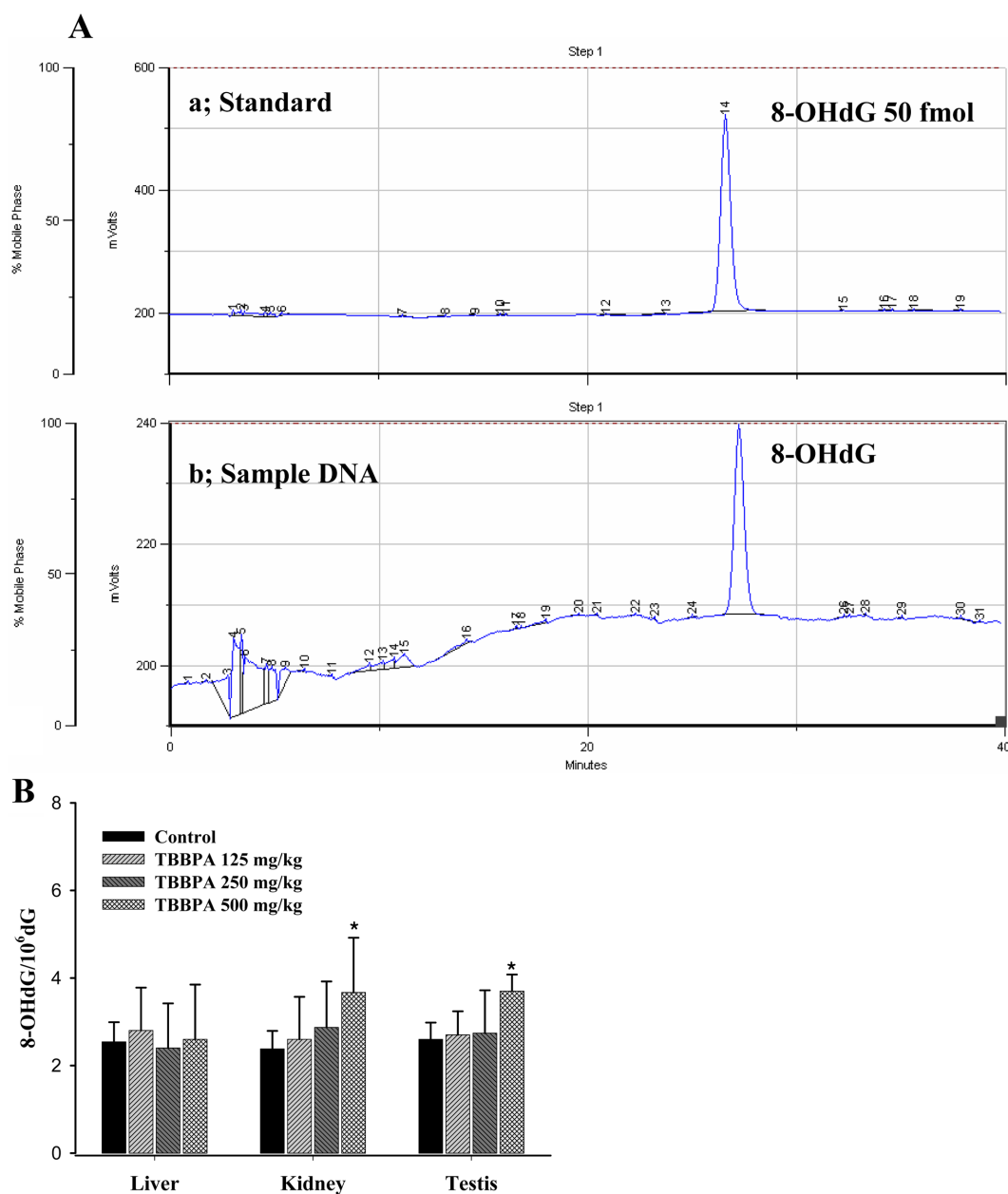


Fig. 4. Detection of 8-OHdG in the testes DNA of male rats 30 days after treatment with tetrabromobisphenol A (TBBPA). (A) The HPLC-ECD profile of standard 8-hydroxy-2'-deoxyguanosine (8-OHdG) (a) and 500 mg/kg of TBBPA-treated rat testes (b). (B) Levels of 8-OHdG in liver, kidney and testis of rats treated with TBBPA. Genomic DNA was isolated and 2-dG and 8-OHdG were measured by HPLC-UV or HPLC-ECD systems, simultaneously. The asterisk indicates significantly different from each control (* $p < 0.05$).

disruptive action of BFRs (Legler and Brouwer, 2003). Reports on interaction of TBBPA with the thyroid hormone receptor (TR) led to concerns about possible interactions of this compound with TH homeostasis. In this study, serum total T4 concentration was dramatically reduced in 250 and 500 mg/kg dose of TBBPA. Decreased circulating T4 levels were also observed in previous studies (Vranckx *et al.*, 1990; Schussler, 2000), which is supported by the increased T3 that accompanied the low T4 to various extents. Dis-

placing mechanisms like these have been observed in *in vitro* bioassays (Meerts *et al.*, 2000). However, here we observed that effects of TBBPA on serum T3 concentrations were much smaller than effects on T4. Serum total TSH concentrations were dose-dependently increased by TBBPA treatment, but not significant. Apparently, interactions of TBBPA with the TH system and mechanisms of feedback and adaptation can lead to low T4, which appears to be consistent between studies and between sexes, result-

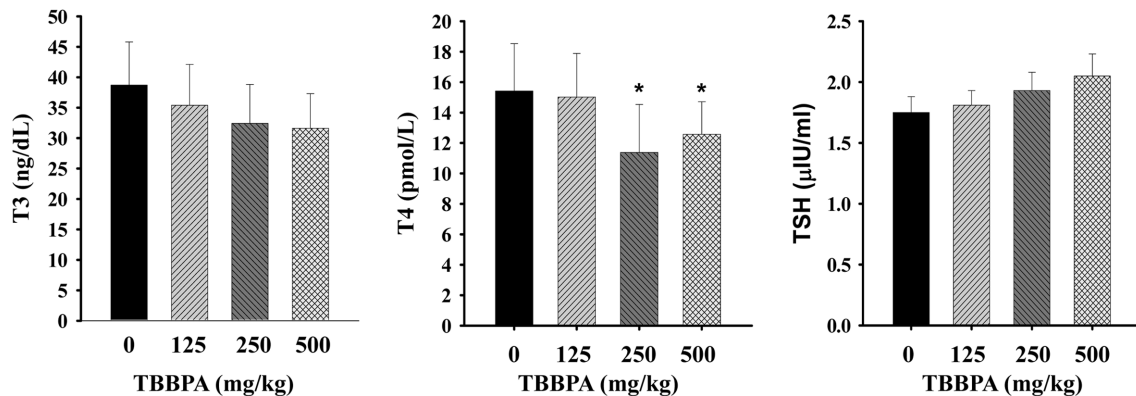


Fig. 5. Effect of tetrabromobisphenol A (0, 125, 250 or 500 mg/kg) on serum thyroid hormone levels. Data reported as the mean \pm SD for 9 animals/group. The asterisk indicates significantly different from each control ($*p < 0.05$).

ing in variable outcomes of T3 levels. These results suggest that prolonged exposure to TBBPA can result in the accumulation of TBBPA or its metabolites in rats. Induction of hepatic enzymes and disruption of TH may occur following longer exposures.

Recent studies suggested that TBBPA might induce oxidative stress in aquatic organisms through the generation of ROS (Ronisz *et al.*, 2004; Shi *et al.*, 2005). TBBPA-treated rats showed a decrease of GSH level and an increase of MDA, a product of lipid peroxidation (Szymanska *et al.*, 2000). However, we showed that TBBPA caused little affect on decrease of GSH level and no effect on increase of MDA level. Interestingly, in rat liver, the expression of the antioxidant enzymes, SOD2 showed an increase in a dose-dependent manner. These data indicate that ROS or other stresses which are generated by TBBPA may be via pERK1/2 activation. Therefore, ERK activation by TBBPA-induced oxidative stress may be a candidate biomarker for target organ toxicity. The 8-OHdG is a major oxidative DNA damage product *in vivo* and is produced by either the hydroxyl radical or singlet oxygen (Hattori-Nakakuki *et al.*, 1994). Hydroxyl radicals destroy DNA and cause cell apoptosis and tumors (Shen *et al.*, 2001). The level of 8-OHdG in the kidney and testis was significantly increased in 500 mg/kg/day TBBPA-treated group. However, 8-OHdG was not significantly changed in the liver, and was close to the control groups. In a previous study, phenobarbital, an inducer of CYP2B1, produced hydroxy radicals (Imaoka *et al.*, 2004). This study suggests that the induction of CYPs, especially CYP2B1/2B2, by TBBPA-induced production of ROS also induced oxidative DNA damage.

In conclusion, long term exposure to TBBPA (500 mg/kg) led to an induction of CYP2B1 and nuclear protein levels of CAR increased in the rat liver. Thus, serum total T4 concentrations were reduced under a high dose of TBBPA. In particular, TBBPA strongly induced the production of 8-OHdG in the rat testis and kidney. These results suggest that

active oxygen produced by CYP2B1-induced oxidative stress may contribute to oxidative DNA damage in the testis and kidney.

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