Letter to the Editor

Toxic Effects of Tetrabromobisphenol A on Thyroid Hormones in SD Rats and the Derived-reference Dose*

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The present study determined the thyroid hormone interference of tetrabromobisphenol A (TBBPA) in Sprague-Dawley (SD) rats, and the derived-reference dose (RfD) of different endpoint effects on mammals based on experimental results and data collection. Based on repeated exposure toxicity tests on mammals and extensive research, the present study used BMDS240 Software to derive a benchmark dose, and analyzed the accuracy and uncertainty, and similarity with other studies. Test results on triiodothyronine (T_3), thyroxine (T_4), and thyroid stimulating hormone (TSH) demonstrated that all the indicators presented a non-monotonous dose-effect relationship clearly, except TSH in male rats exposed to 0-1000 mg/kg BW per day. Therefore, RfDs were derived from different critical effects. In summary, RfD for mammals in the present study was found to be 0.6 mg/kg per day.

TBBPA is an efficient, reliable, and effective flame retardant, and when applied to synthetic materials, it enhances flame retardancy, reduces smokiness, and has self-extinguishing properties. However, there is concern over the levels of TBBPA detected in the environment and the potential health consequences associated with exposure to this compound. TBBPA is an endocrine disruptor that potentially causes thyroid hormone interference, hepatotoxicity, renal toxicity neurotoxicity, and reproductive toxicity.

The chemical structure of TBBPA is similar to that of thyroid hormones. Many studies have shown that TBBPA, and not its metabolites, interferes with the normal functions of the thyroid gland in different ways. Chan et al. found in experiments on fishes that TBBPA exposure can reduce T_3 or T_4 and increase TSH expression in the serum[1]. Terasaki et al. found that TBBPA had a strong ability to combine with transthyretin (TTR), and potentially interfered with the transfer process of T_4 and interacted on a combination of T_3 and thyroid hormone receptor (TR) combining with TR in in vitro experiments[2].

Generally, dose-effect relationships are used in environmental health risk studies to determine the adverse effects of pollutants on organisms. For population health dose-effect relationship research, toxicity assessments of most of the compounds are determined by doses of reference (RfD) recommended by the United States Environmental Protection Agency (US EPA). Most studies use RfDs recommended by the Integrated Risk Information System (IRIS) as risk-reference doses, but the database of TBBPA-RfD values has not yet been fully exploited.

It is necessary to determine the scientifically acceptable range of RfD values in environmental risk research, so that the environment, ecology, and population health risk can be assessed and evaluated accurately. The purpose of the present study was to summarize the different toxic effects of TBBPA, primarily thyroid toxicity, from relevant research, and estimate the RfD values to provide a basis for the early warning of environmental risk.

The present study was conducted in accordance with the US EPA Office of Chemical Safety and Pollution Prevention (OCSPP) Guideline 890.1450 and 890.1500 (Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male/Female Rats Assay). The assay aims to assess the potential interaction of a chemical substance or mixture with the endocrine system in an *in vivo* mammalian system.

As shown in Figure 1, the levels of serum thyroid hormone (T_3, T_4, TSH) in rats tested in this study

doi: 10.3967/bes2016.038

*This work was supported by the National Natural Science Foundation of China (No. 21377045); and Joint Innovation Funding of Production and Research - a Prospective Joint Research Project (BY2015027-05).

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showed that all the indicators presented a non-monotonous dose-effect relationship clearly, except TSH in male rats. The serum T_4 and TSH hormone levels of female rats were all higher than that of male rats, whereas serum T_3 showed the opposite trend. The serum T_3 hormone level of female rats increased obviously at an exposure dose range of 0-50 mg/kg BW per day, and decreased from 50 to 250 mg/kg BW per day, then increased from 250 to 1000 mg/kg BW per day. The serum T_4 hormone level of female rats decreased from 0 to 250 mg/kg BW per day, and then increased from 250 to 1000 mg/kg BW per day. The serum TSH hormone level of female rats increased from 0 to 5 mg/kg BW per day, decreased from 5 to 250 mg/kg BW per day, and then increased from 250 to 1000 mg/kg BW per day. The serum T_3 hormone level of male rats increased considerably from 0 to 250 mg/kg BW per day, and decreased from 250 to 1000 mg/kg BW per day. The serum T_4 hormone level of male rats increased from 0 to 5 mg/kg BW per day, decreased from 5 to 50 mg/kg BW per day, and then increased from 50 to 250 mg/kg BW per day, and finally decreased again from 250 to 1000 mg/kg BW per day.

RfD is derived from many sources of data on health effects; data from repeated toxicity experiments on mammals are especially important. The neurotoxicity, nephrotoxicity, hepatotoxicity, reproductive toxicity, and thyroid hormone interference caused by exposure to TBBPA are of increasing concern, and the no observed adverse effect level/lowest observed adverse effect level (NOAEL/LOAEL) or benchmark dose/benchmark dose level (BMD/BMDL) according to different effect endpoints have also been derived (Table 1). Van de Ven et al.[3] studied the neurotoxicity and thyroid hormone interference of TBBPA in Wistar rats by oral gavage for 28 days, and the BMDLs value derived were 48 (T_4), 124 (T_3), and 0.6 (neurotoxicity) mg/kg. Lilienthal et al.[4] also analyzed the neurotoxicity of TBBPA in Wistar rats through continuously infected rats (female: 2 weeks; male: 10 weeks) and derived the BMDL value at 8 mg/kg. The benchmark doses for effects on the brainstem auditory evoked potentials were similar to values for decreased circulating thyroid hormones. Cope et al.[5] found that the BMDLs of neurotoxicity in F_2 generation rats were 160 mg/kg in female rats and 73 mg/kg in male rats. Strain et al.[6] researched neurotoxicity of TBBPA in Wistar rats and derived a BMDL of 0.9 mg/kg. Tada et al.[7] set up four exposure dose groups using dietary intake; dams were given 0.01, 0.1, or 1.0% TBBPA in diets resulting in daily doses of approximately 15.7, 140.5, or 1639.7 mg/kg for the gestational period (GD0-17),

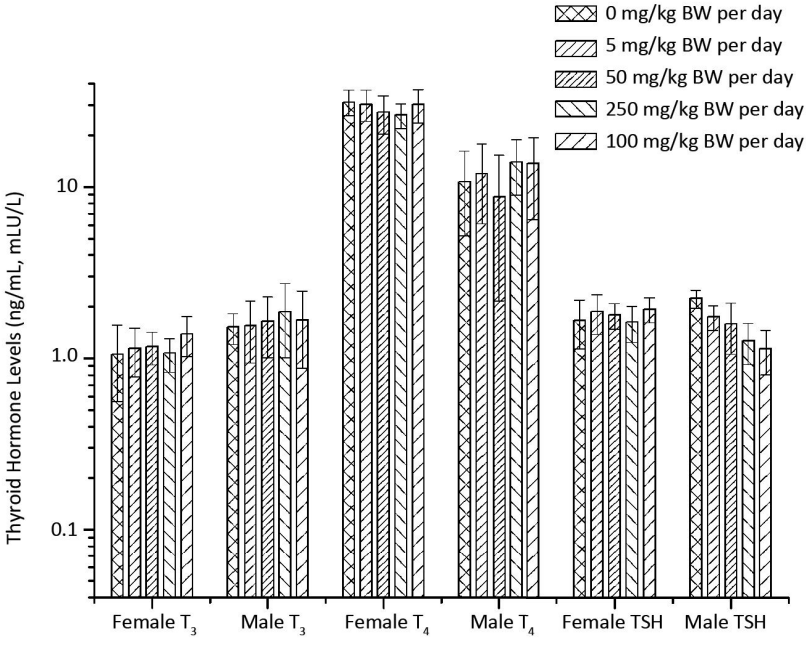

Figure 1. Test results of thyroid hormone levels in SD rats.

and 42.1, 379.9, or 4155.9 mg/kg for the lactational period (PND0-21). Histological findings in treated dams or offspring showed an increase of focal necrosis of hepatocytes and inflammatory cell infiltration in the liver, and dilation or atrophy of renal tubules and cysts in the kidney; NOAEL was derived as 42 mg/kg. Fukuda et al.[8] found polycystic lesions associated with tubule dilation in the kidneys and derived the NOAEL as 40 mg/kg. In 2004, the Committee on Toxicology (COT) in the UK[9] found that the NOAEL of reproductive toxicity was 1000 mg/kg. Saegusa found that sperm number, number of offspring, and other reproductive outcome parameters showed no obvious change, and NOAEL was derived as 2129 mg/kg[10].

To explore the impact on containments is the key step to determining the RfD values in non-carcinogenic risk assessments. Estimation of the benchmark dose and derivation of RfD by dose-effect relationships to the biological toxicity in animal experiments are currently the most widely used methods globally.

The present study investigated thyroid hormone interference in SD rats and derived benchmark doses using US EPA BMDS240 Software. The BMDLs were 220.01, 948.32, and 618.78 mg/kg in female rats, and 5.62, 42.99, 28.35 mg/kg in male rats. There was a significant difference in the BMDL of the thyroid hormone interference between Reference 3 and the present study. In contrast, the BMDL value in female rats was higher in the present study. However, the BMDL value in male rats was lower. Furthermore, compared with References 3, 4, 5, 6, 9, and 10, the values derived in the present study were not identical even if the effective endpoint was consistent.

When there are multiple BMDLs or NOAELs in experimental calculations, choosing suitable benchmark doses for accurate RfD estimations is particularly important. In practical applications, the use of models and inconsistent endpoints resulted in a variation of benchmark doses. For the derivation of

Table 1. Effect Endpoints of NOAEL/LOAEL and BMDL/BMD in Different Rats

Animal Type	Exposure Dose (mg/kg BW per day; ppm)	Toxic Type	BMDL/NOAEL (mg/kg)	Study Reference
SD rats	0、5、50、250、1000	Thyroid hormone interference	T_3 (BMDL, female): 220.01 T_3 (BMDL, male): 5.62 T_4 (BMDL, female): 948.32 T_4 (BMDL, male): 42.99 TSH (BMDL, female): 618.78 TSH (BMDL, male): 28.35	This study
Wistar rats	0、3、10、30、100、300、1000、3000	Thyroid hormone interference	T_4 (BMDL): 48 T_3 (BMDL): 124	Van der Ven et al. (2008)[3]
		Neurotoxicity	BMDL: 0.6	
Wistar rats	0、3、10、30、100、300、1000、3000	Neurotoxicity	BMDL: 8	Lilienthal et al. (2008)[4]
SD rats	0、100、300、1000	Neurotoxicity	BMDL (female): 160 BMDL (male): 73	Cope et al. (2015)[5]
Wistar rats	0、3、10、30、100、300、1000、3000	Neurotoxicity	BMDL: 0.9	Strain et al. (2009)[6]
ICR mice	0、0.01%, 0.1% or 1.0% TBBPA in diet	Hepatotoxicity	NOAEL: 42	Tada et al. (2006)[7]
SD rats	0, 40, 200, 600	Nephrotoxicity	NOAEL: 40	Fukuda et al. (2004)[8]
SD rats	—	Reproductive toxicity	NOAEL: 1000	COT 2004[9]
SD rats	0、100、1000、10000	Reproductive toxicity	NOAEL: 2129	Saegusa et al. (2009)[10]

Note. Units of BMDL/NOAEL were mg/kg uniformly.

Table 2. Summary of RfD Estimates for TBBPA

Toxic Type	RfD Value (mg/kg)	Study Reference
Thyroid hormone interference	T_3 (female): 220.01 T_3 (male): 5.62 T_4 (female): 948.32 T_4 (male): 42.99 TSH (female): 618.78 TSH (male): 28.35	This study
Thyroid hormone interference	T_4: 48 T_3: 124	Van der Ven et al. (2008)[3]
Neurotoxicity	0.6	
Neurotoxicity	8	Lilienthal et al. (2008)[4]
Neurotoxicity	160 (female) 73 (male)	Cope et al. (2015)[5]
Neurotoxicity	0.9	Strain et al. (2009)[6]
Hepatotoxicity	4.2	Tada et al. (2006)[7]
Nephrotoxicity	4	Fukuda et al. (2004)[8]
Reproductive toxicity	100	COT 2004[9]
Reproductive toxicity	212.9	Saegusa et al. (2009)[10]

Note. Units of RfDs were mg/kg uniformly.

compound RfD values, the minimum benchmark dose or geometric average of the baseline dose was used.

When using the benchmark dose for RfD derivation, selection of the appropriate uncertain factor (UF) and modifying factor (MF) is needed. The factors affecting UF include five aspects: UF_H, UF_A, UF_S, UF_L, and UF_D; every factor has a coefficient, and the default value is 10. Moreover, some of the aspects of the MF are generally used to indicate uncertainty; however, there is no clear consideration when using the standard UFs. Generally, the MF is chosen as 1.

In the present study, different RfDs from the effect of TBBPA were derived from the results of the experiment and relevant literature. For each toxic effect, a minimum benchmark dose for the same index was taken as a critical value. As shown in Table 2, RfDs of thyroid hormone interference, neurotoxicity, hepatotoxicity, reproductive toxicity, and nephrotoxicity were 5.62, 0.6, 4.2, 100, and 4 mg/kg, respectively. Presently, the RfD is usually the minimum recommended value; therefore, the RfD in the present study was 0.6 mg/kg. Many researchers derived RfDs of TBBPA; data from NTP 2-year and 13-week studies[11] were selected to develop toxicity values. The RfD of reproductive toxicity was 0.6 mg/kg, which was the same as that observed in the present study.

The present study investigated the interfering effect of TBBPA on thyroid hormone in SD rats. Test results of T_3, T_4, and TSH showed that all the indicators clearly presented a non-monotonous dose-effect relationship, except TSH in male rats in an exposure dose range of 0-1000 mg/kg BW per day. Based on results of previous studies of TBBPA, RfDs derived from different critical effects were different. Furthermore, recommended reference dose of TBBPA in mammals was 0.6 mg/kg.

The authors sincerely thank for prof. YU for his valuable advice and also thankful to the all participants in the study.

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Received: November 10, 2015;
Accepted: April 5, 2016

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