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Absorption and excretion of Tetrabromobisphenol A in male Wistar rats following subchronic dermal exposure

Yunjiang Yu [a, b, *], Mingdeng Xiang [a, b], Dandan Gao [c], Hao Ye [c], Qiong Wang [d], Yanping Zhang [c], Liangzhong Li [b], Hui Li [e]

[a] State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China
[b] Center for Environmental Health Research, South China Institute of Environmental Sciences, MEP, Guangzhou 510535, China
[c] State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China
[d] Institute of Environmental Health and Related Product Safety, China CDC, Beijing 100021, China
[e] State Environmental Protection Key Laboratory of Environmental Risk Assessment and Control on Chemical Process, School of Resource and Environmental Engineering, East China University of Science and Technology, Shanghai 200237, China

HIGHLIGHTS

- TBBPA in serum, urine and feces after subchronic dermal exposure were determined.
- About 3.31–11.21% TBBPA was absorbed dermally under different dosing regimens.
- The majority of TBBPA was excreted in feces, but the minority in urine.

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ABSTRACT

Tetrabromobisphenol A (TBBPA) is widely used as a flame retardant and frequently detected in environmental and biological samples. In this study, male Wistar rats were repeatedly exposed by dermal application to 20, 60, 200 and 600 mg TBBPA/kg body weight during 90 days. Concentrations of TBBPA in serum, urine and feces after dermal exposure were determined. TBBPA concentrations in serum ranged from 19.04 to 427.20 g/g lipids. The percentage of TBBPA dose recovered in serum on the 90th day varied from 0.002 ± 0.002% to 0.013 ± 0.008%, and the percentage of dose excreted in urine varied from 0.004 ± 0.002% to 0.072 ± 0.027%, while the percentage of dose recovered in feces were 3.30 ± 0.61% to 11.13 ± 3.16%. The results showed that about 3.31–11.21% TBBPA was absorbed dermally under different dosing regimens. TBBPA was excreted mainly in feces and small only amounts were recovered in urine.

1. Introduction

Tetrabromobisphenol A (TBBPA) is one of the most widely used brominated flame retardants (BFRs) due to its flame retardant efficiency and superior thermal stability and is present in plastic compounds, printed circuit boards and finished electronic equipment (Lévy-Bimbot et al., 2012). More attention was paid to health effects resulting from TBBPA exposure in humans since it was detected frequently in environmental and biological samples. Due to the heavy usage and potential consumer exposure, the toxicity of TBBPA has been extensively investigated in many experiment animal studies mainly by oral administration. Several oral studies have shown that TBBPA could induce endocrine disrupting effects (Grasselli et al., 2014; Meerts et al., 2000, 2001; Van der Ven et al., 2008), hepatotoxicity and nephrotoxicity (Szymańska et al., 2000; Tada et al., 2006, 2007; Nakagawa et al., 2007; Fukuda et al., 2004), immunotoxicity (Pullen et al., 2003) and neurotoxicity (Fukuda et al., 2004; Lilienthal et al., 2008). Researches on TBBPA after oral administration suggested that TBBPA was absorbed by gastrointestinal tract and excreted rapidly via feces and urine mainly as parent compound. Within 72 h after oral administration,

* Corresponding author. State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China.
E-mail address: yuyunjiang@scies.org (Y. Yu).

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most of TBBPA were detected in the feces and little were associated with tissue retention and bioaccumulation (Hakk et al., 2000; Knudsen et al., 2014; Colnot et al., 2014; Kuester et al., 2007).

However, for non-occupational human exposure to BFRs (e.g. HBCD, TBBPA, and PBDEs), the current understanding is that the exposure mainly occurs via a combination of diet, ingestion of indoor dust, dermal contact with dust/consumer products, and inhalation of indoor air (Roper et al., 2006; Abdallah et al., 2008; Frederiksen et al., 2009; Watkins et al., 2011). Several studies have reported that indoor dust (via ingestion or dermal contact) was the major exposure pathway to PBDEs for all age groups contributing 70–80% to the average overall daily exposure (Lorber, 2008; Trudel et al., 2011). In contrast to PBDEs, only a few studies are available that address dermal exposure to TBBPA and other novel brominated flame retardants (Abdallah et al., 2015). Dermal absorption is a potential pathway contributing to the total exposure burden in human health risk assessments (Trudel et al., 2011; Abdallah et al., 2015). Several investigators have discussed the absence of experimental data on dermal absorption of various BFRs, and highlighted the potential inaccuracies of the current estimates of human exposure to these BFRs owing to a general lack of knowledge on the dermal route (Boyce et al., 2009; Trudel et al., 2011; U.S.EPA, 1992).

This paucity of information was evident in the EU risk assessment reports on TBBPA (EU Risk Assessment Report, 2006) where the lack of experimental data has led to the assumption of dermal absorption efficiencies based on consideration of compound-specific physicochemical properties and extrapolation from data available for PCBs (Abdallah et al., 2015).

Therefore, the lack of experimental information on dermal exposure of TBBPA, represents an important research gap that hampers accurate health risk assessment of human exposure to BFRs. The primary objective of this study was to determine the absorption and excretion of TBBPA following subchronic dermal exposure. TBBPA was administrated to Wistar rats via dermal exposure. The concentrations of TBBPA in serum, urine and feces were determined.

2. Materials and methods

2.1. Animal and general housing condition

Thirty-six male Wistar rats (180 ± 20 g) were obtained from Academy of Military Medical Science at six weeks of age and housed in groups of six individuals. Food and water were given ad libitum and animals maintained on a 12 h light/dark cycle. Temperature and relative humidity were continually monitored, with daily means in the range of 22 °C and 40–60%, respectively.

2.2. Animal treatment

Animals were acclimatized for 7 days prior to treatment. At the end of the acclimatization period the rats were randomly assigned to the respective control or exposure groups. The test material was slightly moistened with physiological saline and applied to an (6*6)-cm^2 area of the skin in the back region which was clipped 24 h prior to treatment. The hair in this area was clipped as required for the duration of the study. The application area was then covered with medical gauze and tape to prevent loss of compound by being rubbed off or being licked. The cover allowed air circulation over the application site to allow normal evaporation of surface water from the skin. The test preparation remained on the skin for 6 h, relating to potential human exposure. TBBPA was then removed by banister brush and plenty of warm water. Finally, paper towel was used to dry the skin and the hair. During exposure, rats were housed individually in rearing cages or in metabolism cages to collect their feces and urine samples. All toxicological procedures described were performed in compliance with Good Laboratory Practice (GLP) requirements (OECD, 2004), according to the animal welfare regulations.

2.3. Dosing

The rats were exposed by 6 h/d dermal application to 20, 60, 200 and 600 mg TBBPA/kg body weight (bw) during 90 days. Dose levels selected in the present study were based on experiment animal strains, the half lethal dose (LD50) of TBBPA, duration of exposure and related literature (IPCS, 1995; EU Risk Assessment Report, 2006). A vehicle control group received normal saline, while the control group was only shaved. Clinical observations of the rats were made every day.

2.4. Sample collection

During the first 24 h administration, the urine and feces were sampled at 0 and 24 h. Afterward all excreta were sampled at intervals of 10 days during the 90 d experiment. Urine and feces were collected overnight in metabolism cages (equipped with water bottles and powdered feed ad libitum). The urine collection equipment prevented contamination of the urine with water, chow or feces. At the completion of the 90-day treatment period, all rats were anesthetized with pentobarbital. 12 mL blood sample were collected by cardiac puncture in deeply anesthetized rats before sacrifice. Serum was isolated from blood by centrifugation (10 min at 3000 RPM).

2.5. Extraction and determination of TBBPA

2.5.1. Serum samples

Two milliliter HCl (6 M) and 6 mL Dimethylcarbinol were added to 2 mL serum, then the serum samples were ultrasonic extracted for three times with 15 mL Hexane/Methyl Tertiary Butyl Ether (MTBE) (1:1) using Sonifier Cell Disrupter (SCIENTZ-JY92-II N, Ningbo Scientz Biotechnology Co). The mixtures were centrifuged and the supernatants were pooled and mixed with 20 mL KCl to precipitate the protein. 20 mL distilled water were added to remove impurities. Water in extracting solution was removed with sodium sulfate (baked for 6 h at 450 °C). The solvent was then concentrated to 10 mL by rotary evaporation. 1 mL concentrate was used to determine the fat contents of serum. The remaining 9 mL concentrate were dried under nitrogen and eluted using 1.5 mL methanol. A Hybrid sep-pak cartridge (Supelclean, HybridSPE 30 mg/mL) was then used to remove proteins and phospholipids in serum and analyzed by HPLC-MS/MS.

2.5.2. Urine samples

Urine samples were filtered to remove large particles and impurities. An aliquot (2 mL) of 24 h urine sample was adjusted to pH 5.5 with HCl (6 M), buffered with 0.5 mL acetate buffer (pH 5.5) and incubated 4 h with 10 μL β-glucuronidase/arylsulfatase at 37 °C away from light. A sep-pak C18 cartridge (Supelclean ENVI-18, 500 mg/3 mL) was used for the solid phase extraction of TBBPA. After priming the cartridge with 9 mL methanol and 3 mL distilled water, respectively, the hydrolyzed sample was passed through the cartridge at a rate of approximately 10 mL/min. Subsequently, the cartridge was washed with 3 mL methanol/water (5:100). Retained solutes were eluted using 2 mL dichloromethane and 3 mL methanol respectively. Then 10 mL dichloromethane and 10 HCl/water (1:100) were added into the solution to remove the impurities. The solution was vortexed and centrifuged at 3000 r/min for 5 min, and