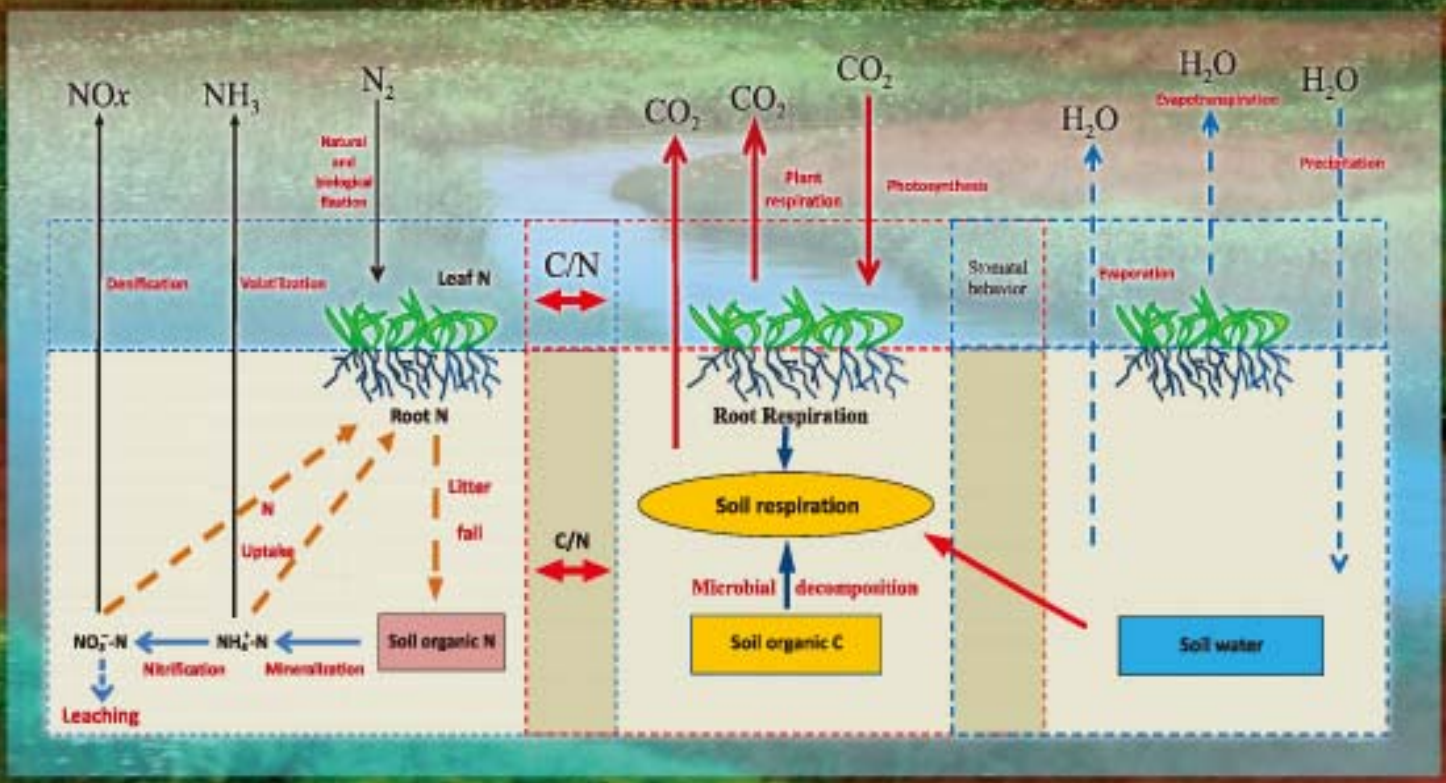
JES

JOURNAL OF ENVIRONMENTAL SCIENCES

ISSN 1001-0742
CN 11-2629/X

April 1, 2014 Volume 26 Number 4
www.jesc.ac.cn



Sponsored by
Research Center for Eco-Environmental Sciences
Chinese Academy of Sciences

ELSEVIER

Science Press

ISSN 1001–0742 **Journal of Environmental Sciences** Vol. 26 No. 4 2014

CONTENTS

Aquatic environment

Performance and microbial diversity of an expanded granular sludge bed reactor for high sulfate and nitrate waste brine treatment
Runhua Liao, Yan Li, Xuemin Yu, Peng Shi, Zhu Wang, Ke Shen, Qianqian Shi, Yu Miao, Wentao Li, Aimin Li ····· 717
Pollutant removal from municipal wastewater employing baffled subsurface flow and integrated surface flow-floating treatment wetlands
Tanveer Saeed, Abdullah Al-Muyeed, Rumana Afrin, Habibur Rahman, Guangzhi Sun ····· 726
Removal of polycyclic aromatic hydrocarbons from aqueous solution by raw and modified plant residue materials as biosorbents
Zemin Xi, Baoliang Chen ····· 737
Hybrid constructed wetlands for highly polluted river water treatment and comparison of surface- and subsurface-flow cells
Yucong Zheng, Xiaochang Wang, Jiaqing Xiong, Yongjun Liu, Yaqian Zhao ····· 749
Minimization of methabenzthiazuron residues in leaching water using amended soils and photocatalytic treatment with TiO_2 and ZnO
José Fenoll, Pilar Flores, Pilar Hellín, Joaquín Hernández, Simón Navarro ····· 757
Enhanced struvite recovery from wastewater using a novel cone-inserted fluidized bed reactor
Awoke Guadie, Siqing Xia, Wei Jiang, Lijie Zhou, Zhiqiang Zhang, Slawomir W. Hermanowicz, Xiaoyin Xu, Shuang Shen ····· 765
Evaluating the effectiveness of marine actinobacterial extract and its mediated titanium dioxide nanoparticles in the degradation of azo dyes
S Priyaragini, S Veena, D Swetha, L Karthik, G Kumar, K V Bhaskara Rao ····· 775
Effect of ozone on the performance of a hybrid ceramic membrane-biological activated carbon process
Jianning Guo, Jiangyong Hu, Yi Tao, Jia Zhu, Xihui Zhang ····· 783
Removal of perchlorate from aqueous solution by cross-linked Fe(III)-chitosan complex
Long Lv, Yanhua Xie, Guoming Liu, Guo Liu, Jing Yu ····· 792

Atmospheric environment

Origin of major ions in monthly rainfall events at the Bamenda Highlands, NorthWest Cameroon
Mengnjo J. Wirmvem, Takeshi Ohba, Wilson Y. Fantong, Samuel N. Ayonghe, Jonathan N. Hogarh, Justice Y. Suila, Asobo Nkengmatia E. Asaah, Seigo Ooki, Gregory Tanyileke, Joseph V. Hell ····· 801
Ionic composition of submicron particles ($PM_{1.0}$) during the long-lasting haze period in January 2013 in Wuhan, central China
Hairong Cheng, Wei Gong, Zuwu Wang, Fan Zhang, Xinming Wang, Xiaopu Lv, Jia Liu, Xiaoxin Fu, Gan Zhang ····· 810
Understanding the sources and composition of the incremental excess of fine particles across multiple sampling locations in one air shed
Jerome E. McGinnis, Jongbae Heo, Michael R. Olson, Andrew P. Rutter, James J. Schauer ····· 818
Characterization of particle size distribution of mainstream cigarette smoke generated by smoking machine with an electrical low pressure impactor
Xiang Li, Haohui Kong, Xinying Zhang, Bin Peng, Cong Nie, Guanglin Shen, Huimin Liu ····· 827

Terrestrial environment

Differential responses of short-term soil respiration dynamics to the experimental addition of nitrogen and water in the temperate semi-arid steppe of Inner Mongolia, China
Yuchun Qi, Xinchao Liu, Yunshe Dong, Qin Peng, Yating He, Liangjie Sun, Junqiang Jia, Congcong Cao ····· 834
Effects of bile salts and divalent cations on the adsorption of norfloxacin by agricultural soils
Xuesong Kong, Shixiang Feng, Xu Zhang, Yan Li ····· 846
Tannic acid and saponin for removing arsenic from brownfield soils: Mobilization, distribution and speciation
Zygmunt Mariusz Gusiatin ····· 855

Environmental biology

Molecular analysis of long-term biofilm formation on PVC and cast iron surfaces in drinking water distribution system
Ruyin Liu, Junge Zhu, Zhisheng Yu, DevRaj Joshi, Hongxun Zhang, Wenfang Lin, Min Yang ····· 865
Effect of a high strength chemical industry wastewater on microbial community dynamics and mesophilic methane generation
Harish Venkatakrishnan, Youming Tan, Maszenan bin Abdul Majid, Santosh Pathak, Antonius Yudi Sendjaja, Dongzhe Li, Jerry Jian Lin Liu, Yan Zhou, Wun Jern Ng ····· 875
Effects of cathode potentials and nitrate concentrations on dissimilatory nitrate reductions by *Pseudomonas alcaliphila* in bioelectrochemical systems
Wenjie Zhang, Yao Zhang, Wentao Su, Yong Jiang, Min Su, Ping Gao, Daping Li ····· 885
Arsenic dynamics in the rhizosphere and its sequestration on rice roots as affected by root oxidation
Weisong Pan, Chuan Wu, Shengguo Xue, William Hartley ····· 892

Environmental health and toxicology

Alterations of endogenous metabolites in urine of rats exposed to decabromodiphenyl ether using metabonomic approaches
Weijin Yang, Jianjie Fu, Thanh Wang, Hanxia Liu, Yawei Wang, Qunfang Zhou, Guibin Jiang ····· 900
Integrated biomarkers in wild crucian carp for early warning of water quality in Hun River, North China
Binghui Zheng, Kun Lei, Ruizhi Liu, Shuangshuang Song, Lihui An ····· 909
T-2 toxin induces developmental toxicity and apoptosis in zebrafish embryos
Guogang Yuan, Yimei Wang, Xiaoyan Yuan, Tingfen Zhang, Jun Zhao, Liuyu Huang, Shuangqing Peng ····· 917

Environmental analytical methods

Determining short chain fatty acids in sewage sludge hydrolysate: A comparison of three analytical methods and investigation of sample storage effects
Victor Ibrahim, Tobias Hey, Karin Jönsson ····· 926

Serial parameter: CN 11-2629/X*1989*m*217*en*P*24*2014-4

Journal of Environmental Sciences 26 (2014) 900–908

Available online at www.sciencedirect.com

Journal of Environmental Sciences

www.jesc.ac.cn

Alterations of endogenous metabolites in urine of rats exposed to decabromodiphenyl ether using metabonomic approaches

Weijin Yang, Jianjie Fu, Thanh Wang, Hanxia Liu, Yawei Wang, Qunfang Zhou*, Guibin Jiang

State Key Laboratory of Environmental Chemistry and Ecotoxicology Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China. E-mail: wjyang@cashq.ac.cn

ARTICLE INFO

Article history:
Received 15 July 2013
revised 16 September 2013
accepted 17 September 2013

Keywords:
decabromodiphenyl ether
metabonomic approaches
endogenous metabolites

DOI: 10.1016/S1001-0742(13)60533-1

ABSTRACT

There is large usage of polybrominated diphenyl ethers (PBDEs) especially for decabromodiphenyl ether (BDE-209, Deca-BDE) in controlling the risks of fire. The toxicological effects of PBDEs are worth being concerned about. Female SD rats were daily gavaged with BDE-209 ether at the dose of 100 mg/kg for 20 days. Histological observation was performed for the screening of the target organs for BDE-209 exposure. The distribution and metabolism of PBDEs in the exposed main organs were evidenced by HRGC-HRMS. Alterations of the endogenous metabolite concentrations in urine were investigated using metabonomic approaches based on ^{1}H NMR spectrum. Histopathological changes including serious edema in kidney, hepatocellular spotty necrosis and perivasculitis in liver indicated that BDE-209 caused potential influences on endogenous metabolism in the exposed liver and the kidney. BDE-209 was found to be highly accumulated in lipid, ovary, kidney and liver after 20 days' exposure. Occurrence of other lower brominated PBDEs in the rats demonstrated that reductive debromination process happened *in vivo*. Hydroxylated and methoxylated–BDEs, as metabolism products, were also detected in the rat tissues. A total of 12 different endogenous metabolites showed obvious alterations in urine from the exposed rats, indicating the disturbance of the corresponding internal biochemical processes induced by BDE-209 exposure. These findings *in vivo* suggested the potential health risk might be of concern due to the toxicological effects of BDE-209 as a ubiquitous compound in the environment.

Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of widely used flame retardant additives in plastics, electrical appliances, television sets and computer circuit boards and casings, and building materials (WHO, 1994). Among the PBDEs, BDE-209 is the congener which is produced in the highest quantities and its worldwide production was estimated at about 56,100 tons per year (BSEF, 2001). PBDEs can enter the environment during the production and disposal of materials containing PBDE flame-retardants, as well as during the lifetime of PBDE-containing products. PBDEs are not chemically bound to plastics, and can therefore evaporate into the indoor air or outdoor environment (Hutzinger and Thoma, 1987). Once released, elevated levels of PBDEs can be built up in different environment compartments, such as sediments, and living organisms (e.g. fish and other aquatic organisms), due to their high lipophilicity (Law et al., 2006).

PBDEs are very persistent in the environment and have a high potential for bioaccumulation. Available evidence suggests that PBDE congeners, that have bioaccumulated in living organisms probably disrupt thyroid hormones, cause neurobehavioral deficits and possibly induce cancer in laboratory animals, probably due to the structural similarity to thyroid hormones and polychlorinated diphenyls (PCBs) (Rahman et al., 2001). Early toxicological studies

* Corresponding author. E-mail: zhouqf@rcees.ac.cn

reported that BDE-209 exhibited low bioavailability and was rather innocuous (Hardy, 2002). However, according to the report of Mörck et al. (2003), it was found that at least 10% of the administered BDE-209 could be absorbed in the rat, and metabolites with guaiacol structure were detected. Changes in spontaneous behavior and altered response to nicotine were observed in the adult rats after neonatal exposure to BDE-209 (Viberg et al., 2007). The mice treated with BDE-209 initially had higher activity and an increased habituation (Rice et al., 2007). However, a weight-of-evidence analysis showed the developmental neurobehavioral effects of BDE-209 from different laboratories were in opposite directions (Goodman, 2009). In addition, several nona- and octa-BDEs due to debromination of BDE-209 (i.e. BDE-209) in rats were reported by Huwe and Smith (2007). Hydroxylated PBDE metabolites in rat blood were detected after exposure to a mixture of PBDE congeners including BDE-209 (Malmberg et al., 2004). Increasing evidences show that deca BDE can be degraded in the environment and metabolized to lower-brominated congeners that are persistent, bioaccumulative, and toxic chemicals (PBTs) (Rice, 2007).

Metabonomics is a method which is capable of simultaneously measuring a wide range of small molecule metabolites, thus can be used to fingerprint the response of biofluid and tissues to pathological stressors. Multivariate statistical analysis including principal component analysis (PCA) based on the complex nuclear magnetic resonance (NMR) spectral data can be used to visualize and characterize the biological changes. Preferred biological samples are biofluids such as urine which are obtained noninvasively. Metabonomics is a promising approach with successful applications in various fields such as clinical diagnosis (Brindle et al., 2002) and drug toxicity studies (Robosky et al., 2002). Metabonomics techniques may also have the potential to serve as a new attractive alternative for the studies of environmental toxicology, thus offering toxicological information on environmental pollutants like PBDEs.

We have conducted a simulative exposure system to characterize the effects of BDE-209 on endogenous metabolites in exposed rats using metabonomics techniques. The potential toxicological effects of PBDEs were screened in the main organs including liver and kidney by histopathological observations. Levels of PBDEs in various target organs of the rats were also analyzed to investigate their distribution and possible metabolism. A total of 30 endogenous metabolites in the rat urine were studied based on NMR spectral data to uncover the possible disturbances induced by BDE-209 exposure.

1 Materials and methods

1.1 Chemicals

BDE-209 ($C_6Br_5OC_6Br_5$, MW: 959.2, purity 97% or better, DE-83R) were obtained from Chemtura (Great Lakes Company, USA). A total of 5 g BDE-209 was evenly dispersed in 500 mL of peanut oil to make the stock solution (10 mg/mL) for the exposure experiment. Lower brominated PBDEs (BDE-3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 183) and the internal standards of ^{13}C-labeled BDE-MXC (BDE 47, 99 and 153) and BDE-139, were obtained from Wellington Laboratories for the analytical use.

1.2 Instrumentation

All ^{1}H NMR spectra were recorded on a Bruker Avance 600 NMR spectrometer equipped with a standard BBI probe (Rheinstetten, Germany).

The analysis of PBDEs was performed on an Agilent 6890 gas chromatograph coupled with an Autospec Ultima mass spectrometer operating with EI source in SIM mode. An aliquot of sample extract (2 μL) was injected with splitless mode into a DB-5 MS fused silica capillary column (60 m × 250 μm i.d. × 0.25 μm film thickness for low brominated PBDEs; 15 m × 250 μm i.d. × 0.25 μm film thickness for BDE-209) with helium (1.2 mL/min) as carrier gas. Oven temperature programs in the range of 90–340°C were used for the base-lined separation of the analytes. MS electron energy was 38 eV and the source temperature was controlled at 280°C (Liu et al., 2006).

The light microscope (Olympus BX41, Japan) was used for the observation of the cellular alterations in the exposed target organs.

1.3 Experimental protocol

A batch of 2-month-old adult female Sprague-Dawley rats (SPF grade, obtained from Experimental Animal Center, Peking University, China) with the mean weight of (200 ± 10) g were divided randomly into two groups with eight rats in each. The control group was gavaged with peanut oil, while the exposure group was gavaged with BDE-209 solution. As no obvious clinical signs of toxicity was observed in female Sprague-Dawley rats exposed to 1000 mg BDE-209 via gavage administration (Hardy et al., 2002), the exposure dosage of 100 mg/(kg·day) was thus selected for the screening of BDE-209's potential toxicities on endogenous biochemical metabolism in the animals without affecting their survival. The gavage volume was approximate 2 mL for each rat per day. In the morning, each rat was placed in an individual metabolism cage with access to food and water ad libitum. The metabolism cages facilitated the collection of urine in the sterile glass bottle which was placed on ice to inhibit bacterium growth. After 8 hr, around 2 mL of urine was obtained from

each rat. The urine was marked with identification number and immediately stored at –20°C until analysis. The rat daily activities including movement, ingestion, excretion, psychosis and mortality were observed 8 hr after dosing. If death occured, the rat would be removed from the test. In the evening, the rats were returned to their group cages to ease the tension induced by the separation in daytime. All the experimental animals were pre-adapted to gavage of blank oil (about 2 mL/day) and daytime life in metabolism cages for 5 days prior to the subsequent 20-day exposure experiment. The rats were finally sacrificed for the analysis of PBDE accumulation and the histological observation in the target organs including liver and kidney. All experiments were performed humanely in accordance with the regulations of and following the approval of the Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College Animal Use and Care Committee.

1.4 Experimental procedure

The trimmed organ specimen including liver and kidney from the control and treated groups (n = 3 for each group) was embedded in paraffin blocks after dehydration with a graded ethanol series. The 4–5 μm sections were stained with hematoxylin and eosin, then observed and photographed under light microscope.

The pretreatment procedure for the detection of tissue PBDEs concentrations was performed as follows: the freeze-dried samples from the control and treated groups (n = 5 for each group) were pulverized with anhydrous sodium sulphate and spiked with ^{13}C-labeled surrogate standards (BDE-MXC). After a 24-hour Soxhlet extraction with 1:1 dichloromethane: hexane, the acidic silica (30%, W/W) was added to remove lipid impurities. The organic layer was transferred to a complex silica column after concentration, and then followed by further purification with gel permeation chromatography (packed with 30 g SX-3 bio-beams using 1:1 DCM: hexane as the elution solvent). The eluted sample was finally analyzed by HRGC-HRMS after the addition of ^{13}C-labeled internal standard (BDE 139) (Liu et al., 2006). A total of 20 PBDE congeners (BDE-3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 183 and 209) were measured.

For metabonomics analysis, 400 μL of SD-rat urine was mixed with 200 μL of 0.2 mol/L phosphate buffer (0.2 mol/L Na_2HPO_4-NaH_2PO_4 D_2O aquatic solution containing 1 mmol/L TSP as chemical shift reference, pH 7.4). Samples were centrifuged (RCF 11,000 ×g, temperature: 10°C) for 8 min to remove insoluble materials. Approximate 550 μL of supernatant liquid was placed in a 5 mm outer diameter NMR tube for ^{1}H NMR spectroscopic analyses. We used a standard 1D pulse sequence (recycle delay-90°-t_1-90°-t_m-90°-acquisition) and an irradiation on the water peak was performed to achieve water suppression during the recycle delay (2 sec) and mixing time, t_m of 100 msec. The t_1 parameter was set to 3 μsec. All free induction decays were multiplied by an exponential function equivalent to a 0.3-Hz line-broadening factor before Fourier transformation. For assignment purposes, 2D correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), 2D HSQC and 2D HMBC NMR spectra were also acquired for the urine samples.

1.5 Statistical analysis

The analytical data are expressed as mean ± SD. For ^{1}HNMR data, all multivariate data analyses (MVAs), PCA and partial least squares–discriminant analyses (PLS-DAS) were performed by using a mean-centered approach with the software SIMCA-p 10.0 (Umetrics, Umea, Sweden). For all MVAs, a significance level of 0.05 was used, and centered and scaled data (to variance 1) were fitted to a principal component model. Determination of the significant number of components was made by cross validation. The principal component (PC) score and loading plots were used to visualize data. Each point on the score plot represents an individual sample, and each point on the loadings plot represents a single NMR spectral region.

2 Results and discussion

2.1 Living behavior

During the whole exposure procedure, no significant abnormal behavior alterations were observed between the control and the exposure groups. No death was caused by the chemical perfusion. Similar body weight gain at approximate 2 g/day was observed in both control and exposed rats. Exposure to 100 mg/(kg·day) of BDE-209 for 20 days caused no obvious alteration of hepatosomatic index (100 × liver weight (g)/rat body weight (g)) or nephrosomatic index (100 × gonad weight (g)/rat body weight (g)) in the rats. The observation of the tested animals during the whole exposure procedure indicated that the given dosage of BDE-209 did not induce acute toxicity in rats.

2.2 Histopathological observation

For screening of the possible target organs for BDE-209 exposure, histopathological observation was carried out after the conventional pretreatment procedure for main organ samples in the rats. Compared to the normal renal corpuscle, renal cortex labyrinth and medullary ray in the control kidney (**Fig. 1a**), the obvious histopathological alteration found in the exposed kidney was serious edema (**Fig. 1b**). The liver tissues from the control group had normal intact structures with obvious lobules. The polygonal hepatocytes with homogenous cytoplasm and perfect round nucleus and nucleous regularly arrayed (**Fig. 1c**). However, typical marked histopathological changes in-

cluding mild hepatic necrosis (**Fig. 1d**) and hepatocellular inflammation (**Fig. 1e**) were observed in the treated liver tissues. The histopathological alterations including renal edema, mild hepatic necrosis and hepatocellular inflammation provided evidence on the toxicological effects of PBDEs on the target organs in tested rats. These histological alterations may suggest the possible occurrence of the metabolism for exogenous chemical and abnormal metabolism for endogenous metabolites in these BDE-209 exposed organs. Possible disturbance of the normal function may be induced in the injured organs. Previous subacute/subchronic oral toxicity studies with deca-, octa-, and penta-BDEs also proved that target organs were liver, kidney, and thyroid gland in rats (Norris et al., 1975; Rahman et al., 2001). Series of histological alterations including the occurrence of inflammation and mild hepatic necrosis in the liver could be directly induced by the accumulated BDE-209 and its potential metabolites. Hepatic necrosis was the most serious depravation condition after the cease of the metabolism in the hepacytes, which might lead to the complete loss of the liver's normal functions. A period of 20 days exposure to BDE-209 could disturb the endogenous metabolism of the cell and tissue, thus leading to the observed necrosis. The correlative deleterious effects occurring in the liver and the kidney might show the potential damage in their normal functions, thus leading to the alterations of the endogenous metabolites in the urine samples.

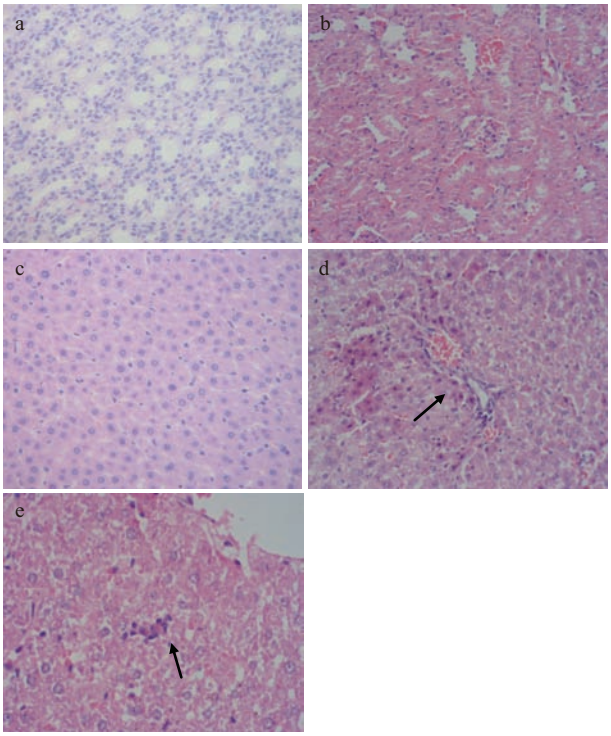


Fig. 1 Histopathological observation of the target organs from the control and BDE-209 treated groups. (a) Normal renal corpuscle, renal cortex labyrinth and medullary ray in the control kidney; (b) serious edema in the exposed kidney; (c) polygonal hepatocytes with homogenous cytoplasm and perfect round nucleus and nucleous in the control liver; (d): mild hepatic necrosis; (e) hepatocellular inflammation.

2.3 PBDEs analysis

Chemical analysis of PBDEs in various organs of the control and exposed rats can offer the information on the accumulation of PBDEs in target tissues. The analytical results of BDE-209 and its congeners were listed in **Table 1**. Because of the possible background exposures (e.g. feed or air-born dust) or low-level laboratory contamination, the control tissues contained low amount of BDE-209 (0.04–1.07 ng/g) and the lower brominated diphenyl ethers (N.D.–0.82 pg/g). However, such trace levels of contam-

Table 1 BDE-209 and its congeners in the rat tissues after 20 days exposure to BDE-209 (mean ± SD, n = 5)

PBDE congeners		BDE- 209	BDE-183	BDE-154	BDE-153	BDE-100	BDE-99	BDE-47	BDE-28
Control	Kidney	0.62 ± 0.04	0.57 ± 0.05	0.42 ± 0.12	0.55 ± 0.04	0.03 ± 0.01	N.D.	0.08 ± 0.05	N.D.
	Lipid	1.07 ± 0.32	0.81 ± 0.02	0.07 ± 0.02	0.82 ± 0.26	N.D.	0.31 ± 0.09	0.07 ± 0.03	N.D.
	Spleen	0.71 ± 0.11	N.D.	N.D.	0.56 ± 0.36	0.01 ± 0.01	N.D.	N.D.	N.D.
	Ovary	0.74 ± 0.10	0.69 ± 0.04	0.67 ± 0.04	N.D.	0.03 ± 0.02	0.63 ± 0.13	0.13 ± 0.05	0.01 ± 0.01
	Liver	0.64 ± 0.05	0.71 ± 0.13	0.08 ± 0.03	0.19 ± 0.06	N.D.	N.D.	N.D.	0.03 ± 0.02
	Cerebra	0.13 ± 0.01	N.D.	N.D.	0.23 ± 0.09	0.05 ± 0.02	0.02 ± 0.01	N.D.	N.D.
	Urine	0.04 ± 0.01	N.D.	N.D.	N.D.	0.23 ± 0.08	N.D.	0.07 ± 0.12	N.D.
	Muscle	0.42 ± 0.03	N.D.	0.16 ± 0.12	0.59 ± 0.34	0.56 ± 0.11	0.18 ± 0.10	N.D.	0.07 ± 0.04
Exposed	Kidney	3510.89 ± 260.24	19768.61 ± 359.71	221.91 ± 25.61	2321.02 ± 96.58	2.08 ± 0.32	N.D.	N.D.	N.D.
	Lipid	3295.89 ± 136.21	55605.25 ± 862.35	649.89 ± 32.54	16895.22 ± 628.31	N.D.	460.70 ± 22.42	N.D.	N.D.
	Spleen	2934.25 ± 112.11	3149.61 ± 125.67	23.42 ± 5.67	465.80 ± 48.73	N.D.	N.D.	N.D.	N.D.
	Ovary	2676.12 ± 89.52	4369.26 ± 113.24	14.41 ± 2.36	483.37 ± 85.12	2.53 ± 0.16	70.46 ± 2.33	37.97 ± 5.82	1.17 ± 0.23
	Liver	1432.19 ± 130.21	13744.53 ± 769.46	N.D.	1743.22 ± 125.30	N.D.	N.D.	N.D.	N.D.
	Cerebra	452.52 ± 53.68	1726.58 ± 321.72	26.56 ± 4.67	508.72 ± 60.44	3.99 ± 0.32	N.D.	N.D.	N.D.
	Urine	335.53 ± 26.43	126.88 ± 36.15	0.63 ± 0.05	N.D.	0.36 ± 0.08	4.03 ± 0.58	4.40 ± 0.56	10.51 ± 4.33
	Muscle	18.77 ± 2.14	6802.6 ± 226.57	N.D.	1371.75 ± 231.55	N.D.	23.23 ± 5.61	N.D.	N.D.

The unit for BDE-209 was ng/g or ng/mL; the unit for the other lower brominated diphenyl ethers was pg/g or pg/mL. N.D.: not detected.

ination could be neglected when compared to the high accumulation of PBDEs in the treated rats. Based on the data depicted in **Table 1**, BDE-209 at microgram per gram level was found in the exposed samples, wherein the kidney contained the highest level of 3.5 μg/g. The lipid (i.e. fat tissues dissected from the abdomen of the rats), spleen, ovary and liver tissues also contained high levels of BDE-209 level. Interestingly, total 7 lower brominated congeners including tri-, tetra-, penta-, hexa-, and hepta-BDEs at the level of nano-gram per gram were detected in the treated tissue samples. Lipid samples contained the highest level of total lower brominated PBDEs (73.6 ng/g), taking the proportion of approximate 56%, while the levels of lower brominated PBDEs in the kidney (22.3 ng/g) and the liver (15.5 ng/g) were the second and third highest, respectively. BDE-183 and BDE-153 were the dominant species. The levels of BDE-183 and BDE-153 in lipid reached 55605.25 pg/g and 16895.22 pg/g, separately. The profiles of PBDEs bioaccumulation might be related with the lipid contents of various tissues (Williams et al., 1945). In addition, several metabolites of PBDEs including CH_3O-TeBDE and HO-NoBDEs were found in the liver sample, indicating the occurrence of oxidative metabolism for BDE-209 in the rats as previously reported (Hakk et al., 2002).

The distribution of BDE-209 among different tissues indicated that kidney and lipid displayed the highest accumulation of this compound. These results were in agreement with the fact that BDE-209 was not most readily distributed to adipose tissue like many halogenated aromatic compounds as previously reported by Mörck et al. (2003). The highest concentration of BDE-209 existed in highly perfused organs such as liver, kidney, spleen and ovary. A possible explanation might be a high plasma protein binding (Mörck et al., 2003). In addition, BDE-209 levels in kidney, lipid, spleen and ovary were higher than that in liver, which was rather different from the results obtained from the exposed juvenile rainbow trout and common carp (Stapleton et al., 2006) where the fish livers acted as a sink for BDE-209. Lower brominated diphenyl ethers are highly accumulated in the adipose tissues like lipid. Based on the comparison of BDE-209 and lower brominated congeners in muscle tissues with those in the other treated tissues, it could be found that the disposition pattern of the higher brominated congener (BDE-209) was different from that of lower brominated PBDEs (Huwe and Smith, 2007). Due to the extremely low solubility of PBDEs in water, the levels of both BDE-209 and lower brominated PBDEs in the tested urine samples were correspondingly very low.

The congener profiles showed 7 lower brominated diphenyl ethers occurred in the exposed rat tissues, especially for BDE-183 and BDE-153. The presence of the lower brominated diphenyl ethers could be attributed to metabolic debromination of BDE-209. The formation of BDE-183 might result from ortho- and meta-debrominations in the same phenyl ring of BDE-207. The debromination of BDE-209 to form BDE-153 could be produced by the removal of four bromine atoms in both ortho and meta positions possibly through the intermediate product of BDE-197. The formation of nona-BDE (e.g. BDE-207) and octa-BDE (e.g. BDE-197) from *meta*- and *para*-debromination were found in male Sprague-Dawley rats (Huwe and Smith, 2007). The further reductive debromination to lower brominated diphenyl ethers could be induced under photolytic conditions (Söderström et al., 2004; Ahn et al., 2006). The debromination of BDE-183 to form BDE-154 in fish resulted from the removal of one bromine atom in the meta position (Stapleton et al., 2004; Tomy et al., 2004). Higher levels of lower PBDEs in the exposed rats indicated that reductive debromination was a probable degradation process in rats, which might be similar to photodegradation of BDE-209. Besides BDE-209, approximate 0.3%–3.0% of other brominated diphenyl ethers, mainly nona-BDE in the dose (European Chemicals Bureau, 2002) might also contribute to the occurrence of lower brominated diphenyl ethers to a minimal extent.

2.4 Metabonomic analysis

Based on ^{1}H NMR spectrum, total of 30 endogenous metabolites was identified which is shown in **Fig. 2**. Resonance assignments were conducted and further confirmed by 2D COSY, 2D TOCSY, 2D HSQC, and 2D HMBC analysis. Visual inspection of the spectra revealed differences in overall composition between urine samples obtained from the control and BDE-209-treated rats. Using the pattern recognition method, comparison was carried out on the 2, 2, 3, 3-D4-3-(trimethylsilyl)propinoic acid sodium salt (TSP) normalized NMR dataset by mean-centered data of the endogenous metabolites in urine between the control and BDE-209 exposed rats. **Figure 3** shows the comparison of the samples obtained after 6, 11 and 19 days exposure. Slight difference occurred based on PCA plot after 6 days of exposure as shown in **Fig. 3a**. After 11 days of exposure, obvious difference appeared as shown in **Fig. 3b**, which indicated that the ingested BDE-209 induced alterations of the biochemical process in the rats, resulting in the great changes in the levels of endogenous metabolites in urine samples. However, the results obtained from the samples collected after 19 days' exposure (**Fig. 3c**) showed that the difference between the control and the exposed rats was less obvious when compared with the results of 11-days' exposure.

After clustering the related data to BDE-209 exposure by PCA, PLS-DA was subsequently used to disclose the variations of the levels of endogenous metabolites in urine from the exposed rats. As depicted in **Table 2**, a total of 13 endogenous metabolites showed significant increase or decrease in their levels after 6 days of exposure ($P < 0.05$). For example, depletion of taurine and urea, increase of

Table 2 Alterations of endogenous metabolites in the urine samples from the rats exposed to BDE-209 for 6, 11 and 19 days

Metabolites	Chemical shift δ (ppm)	Changes			Importance of contribution (rank)		
		6 days	11 days	19 days	6 days	11 days	19 days
Taurine	3.45, 3.29	Decrease	Decrease	Decrease	4.9, 5.38	4.97, 5.08	4.1
Urea	5.98–5.62	Decrease	Decrease	Decrease	14.6 (total)	5.32 (δ5.82–5.78)	17.6 (δ5.98–5.62)
TMAO	3.54	\	\	Increase	\	\	4.82 (δ3.29)
Allantoin	5.45, 6.06	Increase	Increase	Increase	6.33	6.09	5.22
Acetate	1.93	Decrease	Decrease	Decrease	2.55	0.40	1.32
Hippurate	7.86, 7.66, 7.58	Decrease	Decrease	Decrease	1.48, 0.68, 1.38	0.98, 0.49, 0.88	0.85, 0.91
Throenine	1.35	Decrease	Decrease	Decrease	0.55	0.86	0.96
Succinate	2.41	Decrease	Decrease	Decrease	1.50	0.79	0.38 (δ2.37)
Citric acid	2.73, 2.57	Increase	Increase	Increase	2.00, 1.57	1.58, 1.83	1.03, 1.28
Creatinine	3.05	Increase	Increase	Increase	3.24	1.69	2.01
α-Ketoglutarate	2.45, 3.01	Increase	Increase	Increase	1.41, 0.54	2.38, 0.37	3.46 (δ2.44)
Phenylacetylglycine	7.44, 7.38, 3.69	Increase	Increase	Increase	0.61, 1.73	0.60, 1.84, 1.60	1.82(δ7.38)
N-acetylglutamine	2.04, 1.94, 2.12, 2.33	Increase	Increase	Increase	0.90 (2.04)	0.90 (δ2.05–2.01)	0.7 (δ2.05)
Tryptophane	7.7, 7.50, 7.28, 7.20	\	Increase	Increase	\	0.17, 0.20, 0.42, 0.56	0.65 (δ7.22)
4-Hydroxyphenylacetic acid	7.18, 6.86	\	Increase	Increase	\	0.19 (δ6.86)	0.24 (δ6.86)
Carbohydrate	4.49–3.61	Increase	Increase	Increase	Total >10		

"\": no change.

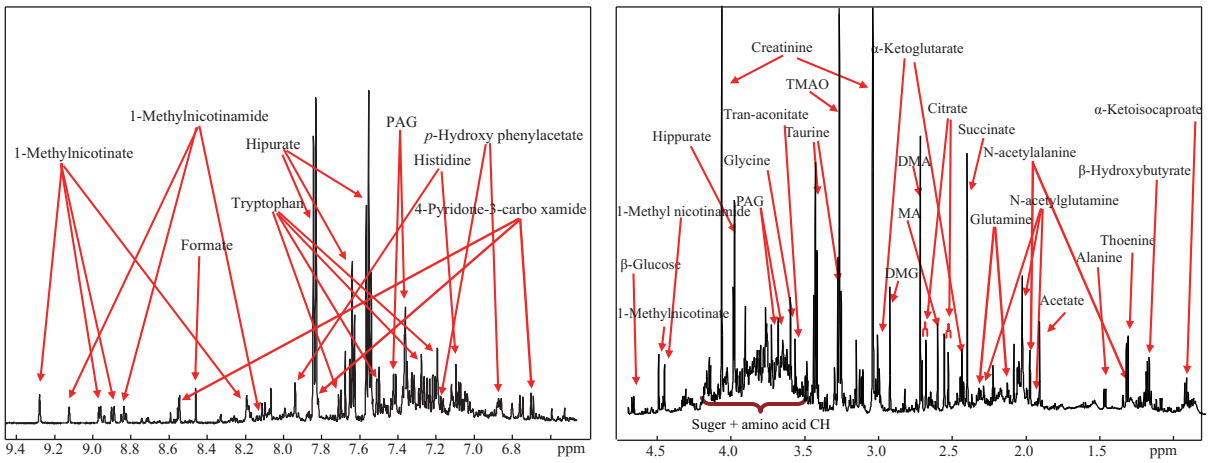


Fig. 2 Identification of 30 endogenous metabolites from rat urine. PAG: phenylacetylglycine; TMAO: trimethylamine N-oxide; DMA: dimethylamine; MA: methylamine; DMG: N, N-dimethylglycine.

allantoin, slight decrease of acetate, hippurate, threonine, succinate and elevated level of α-ketoglutarate, phenylacetylglycine, N-acetylglutamine and carbohydrates were observed in urine of the exposed rats. The alterations of endogenous metabolites in urine after 11-day exposure were similar to the results described above, except that the alteration of acetate decreased and that tryptophane and 4-hydroxyphenylacetic acid levels increased. However, the decrease extent in taurine level observed on exposure day 19 was much less than those found on exposure day 6 and 11. The difference in the endogenous metabolite levels between the control and the exposure group on exposure day 19 was thus not so obvious as those obtained on exposure day 6 and 9, as shown in **Fig. 3**. Additionally, it was also found that the excretion of TMAO increased after 19 days' exposure (**Table 2**).

Information from **Table 2** clearly shows that some of the biochemical processes were affected by BDE-209 exposure, leading to an increase or decrease of some endogenous metabolite levels. As the excretion of BDE-209 in the feces of rats was 90% of the oral dose (Mörck et al., 2003), high levels of BDE-209 and its metabolites might disrupt intestine microorganisms, thus leading to the disturbance of their secretion such as the intermittence decrease of acetic acid, the increase of phenylacetylglycine and slight decrease of hippurate. Urinary taurine, as an important component which can be conjugated with bile acid (Ijare et al., 2005), is derived from the decarboxylation of cysteine. Disturbance of amino acid metabolism by BDE-209 exposure might explain the observed depletion

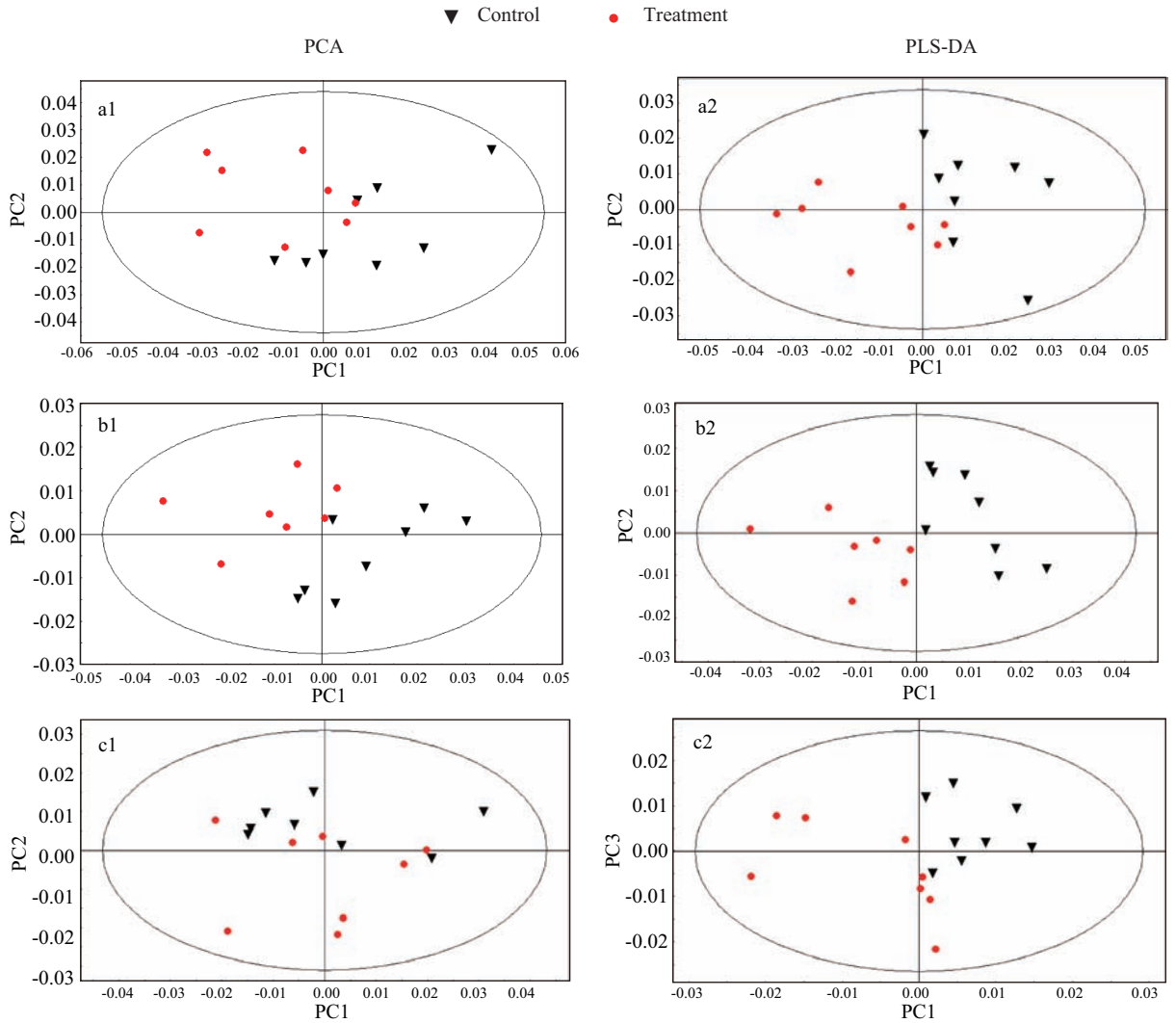


Fig. 3 PCA (a1–c1) and PLS-DA score plots (a2–c2) for the analysis of endogenous metabolites in the urine from the control and treated rats after 6 (a), 11 (b) and 19 (c) days' exposure. Four components were calculated and modelled corresponding to the first four PCs explained 88% (a), 89% (b), 84% (c) of the total variance, respectiely.

of urinary taurine and threonine in the rat urine. The large decrease of urea was confirmed by the combination of the characteristic broad peaks of urea in ^{1}H NMR spectrum and using PCA analysis. As urea is produced mostly in the liver, the hypohepatia caused by BDE-209 exposure (**Fig. 1d, e**) could possibly block the production of urea, leading to the depletion of urea in urine. As a product of amino acid metabolism, the decrease of urea further indicated the possibility of the disturbance of amino acid metabolic process posed by BDE-209 exposure. The increase of α-ketoglutaric acid (α-KG) and decrease of succinic acid might suggest the inhibition effect of BDE-209 on α-KG dehydrogenase in tricarboxylic acid cycle (TCA). When compared to the importance of other contribution, it could be concluded that the effects of BDE-209 on TCA process was less obvious than those on amino acid metabolism. As known, purine can ultimately be transformed to uric acid though several steps, while uric acid can be further oxidized to allantoin under the catalysis of uricase (Alvares et al., 1992). The high increase of allantoin in urine from the exposed rats might show that the possible disturbance in the nucleic acid metabolism induced by BDE-209 exposure. It is known that allantoin can be transformed to urea in some animals such as amphibians. The noticeable decrease of urea and increase of allantoin in BDE-209 exposed rat urine might indicate the blockage of the transformation process from allantoin to urea due to the possible inhibition of related enzyme like allantoinase. The existence of ureidoglycollate lyase discovered by Fujiwara et al. (1995) suggested the possibility for biotransformation of allantoin to urea in rats, which could be well used for the correlative alterations of urea and allantoin. Creatinine is the final metabolic product of creatine and creatine phosphate, which plays key roles in energy storage. Creatine which is widely distributed in tissues including muscle, cardiac muscle and brain for ATP production, is originally

produced in the liver and the kidney. As a biomarker for tubular damage (Holmes et al., 1998) and renal toxicity (Gartland et al., 1989), the increase of creatinine indicated that the kidney was seriously affected in the exposed rats. Abumrad et al. (1989) pointed out that the deacetylation process of N-acetyl glutamine only occurred in the kidney. The increase of N-acetyl glutamine in urine further confirmed the adverse effects induced by BDE-209 on the kidney. Relatively high levels of carbohydrates in urine after BDE-209 exposure might indicate that the carbohydrate metabolism was interfered, reducing their corresponding transformation and utilization in the rat. The alterations of some endogenous metabolites, such as the obvious decrease in the changes of taurine levels after 19 days' exposure, might indicate that the repair capacity in certain biochemical process of the rats may withstand the external stress. Alternatively, further disturbance might be induced in the exposed rats as shown by the increase of TMAO in urine during the later exposure stage (19 days) due to the possible nephrotoxicity, as confirmed by histological observation. The papillary dysfunction might thus occur in the kidney (Bairaktari et al., 2002).

3 Conclusions

After 20 days exposure to BDE-209 by daily gavage, the rats showed histopathological alterations including edema, spotty necrosis and perivasculitis in target tissues such as the liver and the kidney. High levels of BDE-209 and the lower brominated diphenyl ethers were accumulated in adipose tissues. The alteration of endogenous metabolites in urine from treated rats confirmed that the internal biochemical processes were disrupted in the liver and the kidney. In view of the wide occurrence of BDE-209 in the environment, risk assessment for its potential hazards to environment and human health should be concerned.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 20621703, 40590392), the Chinese Academy of Sciences (No. KZCX2-YW-420-21) and the National Science and Technology Supporting Item (No. 2007BAC27B02-1a).

REFERENCES

Abumrad, N. N., Morse, E. L., Lochs, H., Williams, P. E., Adibi, S. A., 1989. Possible sources of glutamine for parenteral nutrition: impact on glutamine metabolism. Am. J. Physiol. 257: E228–E234.

Ahn, M. Y., Filley, T. R., Jafvert, C. T., Nies, L., Hua, I., Bezares-cruz, J., 2006. Photodegradation of decabromodiphenyl ether adsorbed onto clay minerals, metal oxides, and sediment. Environ. Sci. Technol. 40(1), 215–220.

Alvares, K., Widrow, R. J., Abu-Jawdeh, G. M., Schmidt, J. V., Yeldandi, A. V., Rao, M. S. et al., 1992. Rat urate oxidase produced by recombinant baculovirus expression: formation of peroxisome crystalloid core-like structures. Proc. Natl. Acad. Sci. USA 89, 4908–4912.

Bairaktari, E., Seferiadis, K., Liamis, G., Psihogios, N., Tsolas, O., Elisaf, M., 2002. Rhabdomyolysis-related renal tubular damage studied by proton nuclear magnetic resonance spectroscopy of urine. Clin. Chem. 48, 1106–1109.

Brindle, J. T., Antti, H., Holmes, E., Tranter, G., Nicholoson, J. K., Bethell, H. W. L. et al., 2002. Rapid and noninvasive diagnosis of the presence and severity of coronary heart disease using 1H-NMR-based metabonomics. Nature 8(12), 1439–1445.

Bromine Science, B. S. E. F., Environmental Forum (BSEF), 2001. Available from http://www. bsef. com [accessed 10 March, 2006].

European Chemicals Bureau, 2002. European Union Risk Assessment Report. Bis(pentabromophenyl) ether. http://ecb. jrc. it/DOCUMENTS/Existing-Chemicals/RISK ASSESSMENT/REPORT/decabromodiphenyletherreport013.pdf.

Fujiwara, S., Noguchi, T., 1995. Degradation of purines: Only Ureidoglycillate Lyase out of four allantoin degrading enzymes is present in mammals. Biochem. J. 312, 315–318.

Gartland, K. P., Bonner, F. W., Timbrell, J. A., Nicholson, J. K., 1989. Biochemical characterisation of *para*-aminophenol-induced nephrotoxic lesions in the F344 rat. Arch. Toxicol. 63(2), 97–106.

Goodman, J. E., 2009. Neurodevelopmental effects of decabromodiphenyl ether (BDE-209) and implications for the reference dose. Regulat. Toxicol. Pharmacol. 54(1), 91–104.

Hakk, H., Larsen, G., Klasson-Wehler, E., 2002. Tissue disposition, excretion and metabolism of 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) in the male sprague-dawley rat. Xenobiotica 32(5), 369–382.

Hardy, M. L., Schroeder, R., Biesemeier, J., Manor, O., 2002. Prenatal oral (gavage) developmental toxicity study of decabromodiphenyl oxide in rats. Inter. J. Toxicol. 21(2), 83–91.

Holmes, E., Connor, S. C., Nicholls, A. W., Polley, S., Nicholson, J. K., Lindon, J. C. et al., 1998. The identification of novel biomarkers of renal toxicity using automatic data reduction techniques and PCA of proton NMR spectra of urine. Chemometri. Intell. Labor. Sys. 44(1-2), 245–255.

Hutzinger, O., Thoma, H., 1987. Polybrominated dibenzo-*p*-dioxins and dibenzofurans: the flame retardant issue. Chemosphere 16(8-9), 1877–1880.

Huwe, J. K., Smith, D. J., 2007. Accumulation, whole-body depletion, and debromination of decabromodiphenyl ether in male Sprague-Dawley rats following dietary exposure. Environ. Sci. Technol. 41(7), 2371–2377.

Ijar, O. B., Somashekar, B. S., Gowda, G. A., Sharma, A., Kapoor, V. K., Khetrapal, C. L., 2005. Quantification of glycine and taurine conjugated bile acids in human bile using 1H NMR spectroscopy. Mag. Reson. Med. 53(6), 1441–1446.

Law, R. J., Allchin, C. R., de Boer, J., Covaci, A., Herzke, D., Lepom, P. et al., 2006. Levels and trends of brominated flame retardants in the European environment. Chemosphere 64(2), 187–208.

Liu, H. X., Zhang, Q. H., Cai, Z. W., Li, A., Wang, Y. W., Jiang, G. B., 2006. Separation of polybrominated diphenyl ethers, polychlorinated diphenyls, polychlorinated dibenzo-*p*-dioxins and dibenzo-furans in environmental samples using silica gel and

florisil fractionation chromatography. Analy. Chim. Acta, 557(1-2), 314–320.

Malmberg, T., Athanasiadou, M., Marsh, G., Brandt, I., Bergman, A., 2004. Hydroxylated PBDE metabolites in rat blood after exposure to a mixture of PBDE congeners. Organoh. Comp. 66, 3836–3841.

Mörck, A., Hakk, H., Örn, U., Klasson-Wehler, E., 2003. Decabromodiphenyl ether in the rat: absorption, distribution, metabolism, and excretion. Drug Metab. Dispos. 31(7), 900–907.

Norris, J. M., Kociba, R. J., Schwetz, B. A., Rose, J. Q., Humiston, C. G., Jewett, G. L. et al., 1975. Toxicology of octabromodiphenyl and decabromodiphenyl oxide. Environ. Health Perspect. 11, 153–161.

Rahman, F., Langford, K. H., Scrimshaw, M. D., Lester, J. N., 2001. Polybrominated diphenyl ether (PBDE) flame retardants. Sci. Total Environ. 275(1-3), 1–17.

Rice, D. C., 2007. Risk assessment and regulation of PBDEs. Neurotoxicol. Teratol. 29, 413–414.

Rice, D. C., Reeve, E. A., Herlihy, A., Zoeller, R. T., Thompson, W. D., Markowski, V. P., 2007. Developmental delays and locomotor activity in the C57BL6/J mouse following neonatal exposure to the fully-brominated PBDE, decabromodiphenyl ether. Neurotoxicol. Teratol. 29(4), 511–520.

Robosky, L. C., Robertson, D. G., Baker, J. D., Rane, S., Reily, M. D., 2002. *In vivo* toxicity screening programs using metabonomics. Combin. Chem. High Throug. Scre. 5, 651–662.

Söderström, G., Sellström, U., De Wit, C. A., Tysklind, M., 2004. Photolytic debromination of decabromodiphenyl ether (BDE-209). Environ. Sci. Technol. 38(1), 127–132.

Stapleton, H. M., Alaee, M., Letcher, R. J., Baker, J. E., 2004. Debromination of the flame retardant decabromodiphenyl ether by juvenile carp (*Cyprinus carpio*) following dietary exposure. Environ. Sci. Technol. 38(1), 112–119.

Stapleton, H. M., Brazil, B., Holbrook, R. D., Mitchelmore, C. L., Benedict, R., Konstantinov, A. et al., 2006. *In vivo* and *in vitro* debromination of decabromodiphenyl ether (BDE-209) by juvenile rainbow trout and common carp. Environ. Sci. Technol. 40(15), 4653–4658.

Tomy, G. T., Palace, V. P., Halldorson, T., Braekevelt, E., Danell, R., Wautier, K. et al., 2004. Bioaccumulation, biotransformation, and biochemical effects of brominated diphenyl ethers in juvenile lake trout (*Salvelinus namaycush*). Environ. Sci. Technol. 38(5), 1496–1504.

Viberg, H., Fredriksson, A., Eriksson, P., 2007. Changes in spontaneous behaviour and altered response to nicotine in the adult rat, after neonatal exposure to the brominated flame retardant, decabrominated diphenyl ether (PBDE 209). Neurotoxicology 28(1), 136–142.

WHO/ICPS, 1994. Environmental Health Criteria 162: Brominated diphenyl ethers. World Health Organization, Geneva.

Williams, H. H., Galbraith, H., Kaucher, M., Moyer, E. Z., Richards, A. J., Macy, I. G., 1945. The effect of growth on the lipid composition of rat tissues. J. Biol. Chem. 161, 475–484.

Editorial Board of Journal of Environmental Sciences

JOURNAL OF ENVIRONMENTAL SCIENCES

环境科学学报(英文版)

(http://www.jesc.ac.cn)

Aims and scope

Journal of Environmental Sciences is an international academic journal supervised by Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences. The journal publishes original, peer-reviewed innovative research and valuable findings in environmental sciences. The types of articles published are research article, critical review, rapid communications, and special issues.

The scope of the journal embraces the treatment processes for natural groundwater, municipal, agricultural and industrial water and wastewaters; physical and chemical methods for limitation of pollutants emission into the atmospheric environment; chemical and biological and phytoremediation of contaminated soil; fate and transport of pollutants in environments; toxicological effects of terrorist chemical release on the natural environment and human health; development of environmental catalysts and materials.

For subscription to electronic edition

Elsevier is responsible for subscription of the journal. Please subscribe to the journal via http://www.elsevier.com/locate/jes.

For subscription to print edition

China: Please contact the customer service, Science Press, 16 Donghuangchenggen North Street, Beijing 100717, China. Tel: +86-10-64017032; E-mail: journal@mail.sciencep.com, or the local post office throughout China (domestic postcode: 2-580).

Outside China: Please order the journal from the Elsevier Customer Service Department at the Regional Sales Office nearest you.

Submission declaration

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere. The submission should be approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out. If the manuscript accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Submission declaration

Submission of the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere. The publication should be approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out. If the manuscript accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Editorial

Authors should submit manuscript online at http://www.jesc.ac.cn. In case of queries, please contact editorial office, Tel: +86-10-62920553, E-mail: jesc@263.net, jesc@rcees.ac.cn. Instruction to authors is available at http://www.jesc.ac.cn.

Journal of Environmental Sciences (Established in 1989)

Vol. 26 No. 4 2014

Supervised by	Chinese Academy of Sciences	**Published by**	Science Press, Beijing, China
Sponsored by	Research Center for Eco-Environmental		Elsevier Limited, The Netherlands
	Sciences, Chinese Academy of Sciences	**Distributed by**	
Edited by	Editorial Office of Journal of	Domestic	Science Press, 16 Donghuangchenggen
	Environmental Sciences		North Street, Beijing 100717, China
	P. O. Box 2871, Beijing 100085, China		Local Post Offices through China
	Tel: 86-10-62920553; http://www.jesc.ac.cn	Foreign	Elsevier Limited
	E-mail: jesc@263.net, jesc@rcees.ac.cn		http://www.elsevier.com/locate/jes
Editor-in-chief	Hongxiao Tang	**Printed by**	Beijing Beilin Printing House, 100083, China

CN 11-2629/X **Domestic postcode: 2-580** **Domestic price per issue RMB ¥ 110.00**

ISSN 1001-0742