



ISSN 1001-0742
CN 11-2629/X

2012

Volume **24**
Number **7**

JOURNAL OF
**ENVIRONMENTAL
SCIENCES**



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

JOURNAL OF ENVIRONMENTAL SCIENCES

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Development of indirect competitive fluorescence immunoassay for 2,2',4,4'-tetrabromodiphenyl ether using DNA/dye conjugate as antibody multiple labels

Zi-Yan Fan¹, Young Soo Keum², Qing-Xiao Li³, Weilin L. Shelver⁴, Liang-Hong Guo^{1,*}

1. State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-environment Sciences, Chinese Academy of Sciences, Beijing 100085, China. E-mail: sumioo.van@gmail.com

2. College of Life and Environmental Sciences, Konkuk University in Korea, 1 Hwayang-dong, Gwangjin-gu, Seoul 143-701, Korea

3. Department of Molecular Bioscience and Bioengineering, University of Hawaii at Manoa, 1955 East-West Road, Honolulu, HI 96822, USA

4. USDA-ARS Bioscience Research Laboratory, 1605 Albrecht Boulevard, Fargo, ND 58105, USA

Received 29 September 2011; revised 30 November 2011; accepted 14 December 2011

Abstract

An indirect competitive fluorescence immunoassay using a DNA/dye conjugate as antibody multiple labels was developed on 96-well plates for the identification and quantification of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) in aqueous samples. A hapten, 2,4,2'-tribromodiphenyl ether-4'-aldehyde, was synthesized, and was conjugated to bovine serum albumin to form a coating antigen. Specific recognition of the antigen by anti-PBDE antiserum was confirmed by a surface plasmon resonance measurement. In the immunoassay, the coating antigen was adsorbed on a 96-well plate first, and a sample, antiserum and biotinylated goat anti-rabbit secondary antibody were then added and reacted sequentially. A biotinylated, double-stranded DNA with 219 base pairs was attached to the secondary antibody by using streptavidin as a molecular bridge. *In situ* multiple labeling of the antibody was accomplished after addition of a DNA-binding fluorescent dye, SYBR Green I. The working range of the immunoassay for the BDE-47 standard was 3.1–390 µg/L, with an IC₅₀ value of 15.6 µg/L. The calculated LOD of the immunoassay is 0.73 µg/L. The immunoassay demonstrated relatively high selectivity for BDE-47, showing very low cross-reactivity (< 3%) with BDE-15, BDE-153 and BDE-209. With a spiked river water sample containing 50 µg/L BDE-47, quantification by the immunoassay was 41.9 µg/L, which compared well with the standard GC-ECD method (45.7 µg/L). The developed immunoassay provides a rapid screening tool for polybrominated diphenyl ethers in environmental samples.

Key words: polybrominated diphenyl ethers; fluorescence immunoassay; microplate; multiple labeling

DOI: 10.1016/S1001-0742(11)60929-7

Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants with 209 congeners that have been widely used in many applications such as building materials, electronic products, plastics, and textiles (Hardy, 2002). There are three types of commercial PBDEs mixtures, namely “pentaBDEs”, “octaBDEs”, and “decaBDEs”. They were reported to be manufactured at a level of approximately 70,000 tons per year worldwide before they were regulated (de Wit, 2002).

As a result of their widespread usage as well as high stability and high bioaccumulation, PBDEs have been found in almost all environmental media and biological samples including air, soil, house dust, wildlife and humans (Lindberg et al., 2004; Sjödin et al., 2003; Zota et al., 2008). Even in Arctic air and biota, PBDEs have been found according to recent reports (de Wit et al., 2006; Muir

et al., 2006). Exposures to PBDEs have been shown to cause developmental and neurological effects, but many aspects of their toxicity are uncertain at this time. Because of their rapidly increasing presence in the environment and their potential health consequences, penta- and octa-BDEs were banned from all products in the European Union in 2004, and in ten states of the USA in 2007. In 2009, they were added to the list of persistent organic pollutants in the Stockholm Convention. In June 2008, the US EPA set a safe daily exposure level ranging from 0.1 to 7 µg/kg body weight per day for most common PBDEs. The European Union also set an upper limit of 1 g/kg body weight per day for the sum of polybrominated biphenyl and PBDEs in the RoHS directive. Therefore, there is a huge need for routine screening of PBDEs in the environment and food samples, especially for BDE-47 and BDE-99.

Current quantitative analytical methods for PBDEs detection are usually carried out by gas chromatography coupled with electron capture detection (GC-ECD) or gas chromatography combined with mass spectrometry (GC

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

MS) (Xu et al., 2009). These methods are very specific and accurate, but to some extent expensive and time-consuming due to complicated sample separation and pretreatment procedures. Alternatively, several enzyme-linked immunoassays have been developed in recent years as a rapid, low-cost and sensitive method for screening PBDEs in diverse environmental matrices. Targeting BDE-47 as the analyte, Shelver et al. (2005) synthesized four haptens with different bromine and linker substitution patterns. These haptens were then utilized to generate seven polyclonal antibodies, one of which exhibited the best results in competitive immunoassays for BDE-47, with an IC_{50} of 27.7 $\mu\text{g/mL}$. Using this antibody, a sensitive magnetic particle enzyme-linked immunoassay was developed to analyze PBDEs in water, milk, fish, and soil samples. The LOD of the assay was below 0.1 $\mu\text{g/L}$, with the cross-reactivity of BDE-28, BDE-100 and BDE-153 being less than 15% relative to BDE-47 (Shelver et al., 2008). More recently, Ahn et al. (2009) synthesized BDE-47 immunizing haptens with a rigid double-bonded hydrocarbon linker. With the screened antibody, indirect competitive ELISA immunoassay showed a linear detection range of 0.35–8.50 $\mu\text{g/L}$ with an IC_{50} value of 1.75 $\mu\text{g/L}$ for BDE-47. Little or no cross-reactivity (< 6%) was observed to related PBDE congeners containing the BDE-47 moiety and other halogenated compounds.

In our previous work, a method for attaching multiple fluorescent labels to an antibody was proposed and tested. In the method, a double-stranded DNA was first attached to the antibody. Fluorescent dyes were then allowed to bind to the DNA, forming a dye/DNA conjugate. Because the dye can bind to DNA at high ratios, the antibody is labeled with a large number of fluorescent dyes through the DNA (Zhang and Guo, 2007). Using this method, fluorescence immunoassays on 96-well plates showed a detection limit of 1.9 pg/mL for 17 β -estradiol, which is 200 fold lower than assays using conventionally labeled antibodies (Zhu et al., 2008). In the current work, a dye/DNA conjugate has been employed in competitive fluorescence immunoassays for PBDEs. Unlike ELISA, this format is compatible with

the current biochip technology, and thus can be readily developed into immunoassay biochips for the simultaneous detection of multiple analytes in a single sample.

1 Experimental

1.1 Reagents

N-Hydroxysuccinimide (NHS) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC) were purchased from Alfa Aesar (Ward Hill, USA). 4,4'-Dibromodiphenyl ether (BDE-15), 2,4,4'-tribromodiphenyl ether (BDE-28), 2,2',4,4'-tetra-bromodiphenyl ether (BDE-47), 2,2',4,4',5-penta-bromodiphenyl ether (BDE-99), 2,2',4,4',5,5'-hexa-bromodiphenyl ether (BDE-153), and decabromodiphenyl ether (BDE-209) were purchased from AccuStandard (New Haven, USA). Their structures are depicted in Fig. 1. Biotin-labeled goat anti-rabbit IgG was obtained from CW Biotech (Beijing, China). Taq DNA polymerase, DNA marker DL 2000 and DNA fragment purification kit were purchased from Takara Biotech Co. (Dalian, Liaoning, China). Streptavidin was obtained from Promega (Madison, USA). Fluorescein isothiocyanate (FITC) was obtained from Amresco (Solon, OH, USA) and SYBR Green I from Invitrogen (Carlsbad, USA). Rabbit anti-PBDEs serum was produced by immunizing a rabbit with 4-(2,4-dibromo-5-(2,4-dibromophenoxy) phenoxy) butyrate-BSA according to Shelver et al. (2005). A 219 bp biotinylated DNA was synthesized by PCR (see details in the supporting information). All buffers were prepared in high-purity water from a Millipore Milli-Q (Biocel) purification system (Billerica, USA). All other chemicals and solvents were purchase from Sigma-Aldrich (St. Louis, USA) unless specifically stated otherwise.

1.2 Hapten synthesis

2,4,2'-Tribromodiphenyl ether-4'-aldehyde was designed and synthesized as a hapten (Fig. 2). The main reactions to prepare the hapten containing the carboxaldehyde group were based on methods previously described (Marsh et al.,

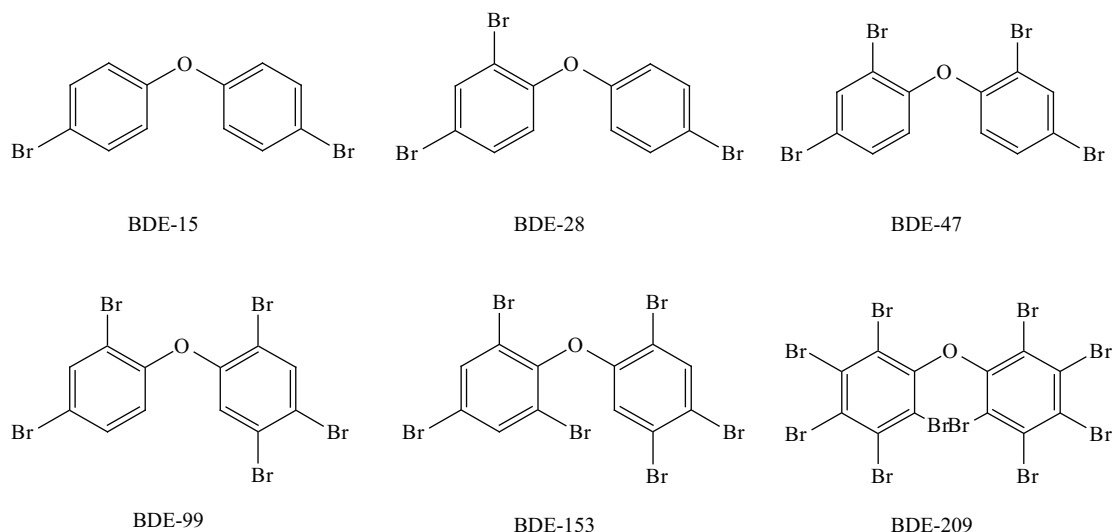


Fig. 1 Structure of the six PBDE congeners investigated in this study.

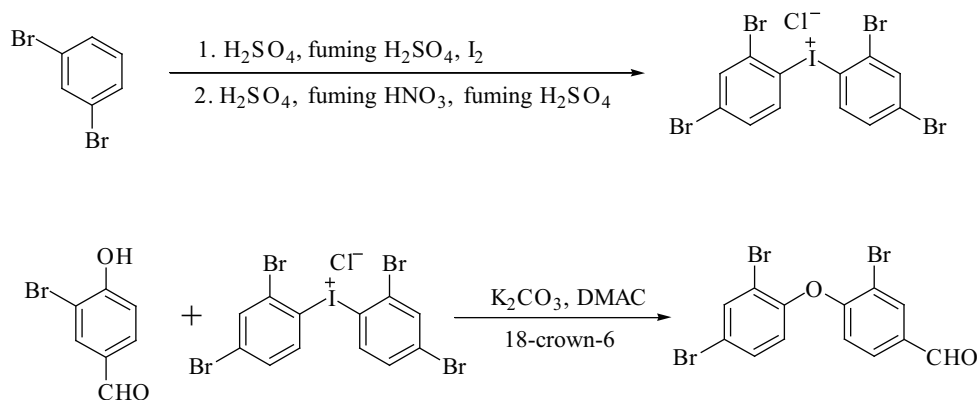


Fig. 2 Synthesis scheme for the hapten 2,4,2'-tribromodiphenyl ether-4'-aldehyde.

2003). There are two main steps: synthesis of iodonium chloride and coupling of iodonium salt with benzaldehyde. All the synthetic steps are detailed as follows.

1.2.1 Synthesis of iodonium chlorides

A mixture of sulfuric acid (10 mL) and fuming sulfuric acid (40% SO₃, 10 mL) was added dropwise to iodine (5 g) at room temperature. Then a mixture of sulfuric acid (2 mL), fuming sulfuric acid (40% SO₃, 1 mL) and fuming nitric acid (85%, 3 mL) was added over 20 min followed by stirring at 80°C for 3 hr. The reaction mixture was diluted with sulfuric acid (20 mL), and 1,3-dibromobenzene (25 g) was added over 30 min at 10°C. The mixture was stirred at 40°C overnight. Water (100 mL) was gently added into the mixture in an ice bath. After standing in the refrigerator for 1 day, the clear aqueous solution was decanted off. The crude product was dissolved in methanol (200 mL). Then, HCl (5 mL) in methanol (10 mL) was added dropwise. The crystalline product was filtered off, washed with methanol and water, and dried under vacuum for 1 day.

1.2.2 Coupling of iodonium salt with benzaldehyde

Iodonium chloride synthesized in the first step was added to a mixture of 3-bromo-4-hydroxybenzaldehyde (2.5 mmol), 18-crown-6 (0.1 g), and K₂CO₃ (0.7 g) in N,N-dimethylacetamide (40 mL) with stirring at 80°C for 1 hr. Then, the mixture was diluted with CH₂Cl₂ (40 mL) and water (80 mL). The organic layer was separated, and the water layer was extracted with CH₂Cl₂ (30 mL). After extraction, the organic layer was washed sequentially with aqueous NaHSO₃ (5%, 50 mL), aqueous NaOH (1 mol/L, 2×100 mL), and water (2×100 mL). Finally, the organic solvent was removed, and the residues were further purified by silica gel column chromatography with a mixture of chloroform-hexane as the eluent.

In general, the reaction yield of this procedure was 50%–60%, giving a pale yellow oil. GC/MS (Agilent Technologies 6890/5973N GC-MS, Santa Clara, USA) analysis indicated greater than 95% purity. The identity of the product was confirmed by NMR (Bruker Avance 400 NMR spectrometer, Billerica, USA). ¹H NMR (DMSO), δ: 9.90 (s, CHO), 8.18 (s, Ar), 7.84 (s, Ar), 7.74 (d, J = 8.1 Hz, Ar), 7.49 (d, J = 8.7 Hz, Ar), 6.97 (d, J = 9.0 Hz, Ar), and 6.76 (d, J = 8.4 Hz, Ar). ¹³C NMR (DMSO), δ: 191.17 (CHO), 157.62, 151.03, 136.33, 135.46, 133.40, 133.12,

131.07, 123.88, 118.67, 117.96, 116.10, and 113.10 (Ar). Electrospray ionization-mass spectrometry (Agilent Technologies 5973N, Santa Clara, USA): *m/z* 435 [M+H]⁺, 247 [M-COBr₂]⁺.

1.3 Preparation of Coating Antigen

2,4,2'-Tribromodiphenyl ether-4'-aldehyde was conjugated to bovine serum albumin (BSA) according to the method of Guar et al. with modification (Gaur et al., 1981; Li et al., 1991). A BSA solution (50 mg in 4 mL of 0.2 mol/L PBS, pH 7.4) was added to 50 μmol 2,4,2'-tribromodiphenyl ether-4'-aldehyde dissolved in 1 mL methanol and stirred for 1 hr at 37°C. Then 30 μmol NaBH₄ dissolved in methanol was added into the mixture. After stirring for another hour at 4°C, 1 mL of 0.1 mol/L HCl was added to destroy the excess NaBH₄. The mixture was then transferred into a Pierce D-Salt™ polyacrylamide 6000 desalting column (Rockford, USA) for purification. The concentration of the purified BSA conjugate (PBDE-BSA) was determined using an Agilent 8453 UV-Visible spectrophotometer at 280 nm (Santa Clara, USA).

1.4 Surface plasmon resonance measurement

Surface plasmon resonance measurements were carried out on a Biosensing Instruments SPR-1000 System (Tempe, USA). In the measurement, PBDE-BSA was immobilized *in situ* on a bare gold chip by injecting 50 μL of PBDE-BSA solution (100 mg/L in PBS pH 7.4) into the flow cell at a flow rate of 20 μL/min. After washing the gold surface with PBS and blocking it with 1% BSA, the anti-PBDE antiserum (1/100 dilution in PBS, pH 7.4) was injected into the flow cell at the same flow rate, and the angular shift due to antibody binding was recorded over time. In the reference measurement, BSA and anti-estradiol antibody (10 mg/L) was applied instead of the anti-PBDE antiserum, and the angular shift was recorded over time.

1.5 Indirect competitive fluorescence immunoassay on 96-well plate

A 96-well plate was coated with PBDE-BSA antigen by incubating with a 100 μL solution (10 mg/L in 50 mmol/L NaHCO₃, 50 mmol/L Na₂CO₃, pH 9.15) per well overnight at 4°C. The plate was washed three times

with phosphate-buffered saline containing 0.1% Tween 20 (PBST). It was then blocked overnight at 4°C with 100 µL of 1% BSA (in PBS) per well and washed. Then 50 µL/well of a BDE standard or sample was added into the plate, followed by the addition of 50 µL/well PBDE antiserum, and incubated at 37°C for 2 hr with shaking. After incubation, 100 µL of biotin-labeled goat anti-rabbit antibody (1/400 dilution) was added into the wells and reacted at 37°C for 2 hr with shaking, followed by the addition of 100 µL of 5 mg/L streptavidin. After shaking for 2 hr at 37°C, biotin-labeled 219 bp DNA was added into the plate and reacted at 37°C for 1 hr with shaking, and washed. Finally, SYBR Green I (600 nmol/L) was added into the plate and incubated for 7 min at room temperature. After washing to move unbound dyes, the fluorescence intensity was measured on a Perkin Elmer LS-55 fluorescence spectrometer (Waltham, USA) with 498 nm excitation, 525 nm emission, 5 nm slit width, and 515 nm cutoff.

1.6 GC-ECD analysis

River water samples were collected from the Tonghui River located in the southeastern portion of Beijing, which serves as a main urban drainage river for Beijing. The samples were spiked with a proper volume of BDE-47 (50 mg/L, in isooctane) to a final concentration of 50 µg/L. The pretreatment procedures were as follows. The sample (2 mL) was extracted with 8 mL hexane/dichloromethane (1:3, V/V) by sonication for 10 min, followed by centrifugation. The supernatant was collected. This process was repeated twice. Then the extract was condensed to approximately 2 mL using a rotary evaporator, and passed through a 15-mm i.d. column packed with 3 cm height anhydrous sodium sulfate. The sample was then transferred into the GC inlet after concentrating to approximately 0.1 mL by evaporation with a nitrogen stream.

The GC-ECD setup consisted of an Agilent 6890 gas chromatography (GC) coupled with an electron capture detector (ECD) (Santa Clara, USA). The separation was accomplished on a DB-5 fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness). High purity helium (≥ 99.999%) was used as carrier gas. The injector temperature was set at 250°C, and a splitless mode was used. The oven temperature program was as follows: 80°C for 2 min, ramped at 10°C/min to a final temperature of 300°C, held for 5 min. The temperature of the detector was held constant at 300°C. BDE-47 was identified by the retention time.

2 Results and discussion

2.1 Antibody multiple labeling strategy

The PBDE immunoassay we aimed to develop in this study is an indirect competitive fluorescence immunoassay using a DNA/dye conjugate as antibody multiple labels. The DNA/dye multiple labeling strategy was proposed and tested in our previous work (Zhang and Guo, 2007; Zhu et al., 2008; Zhang et al., 2011). The same strategy

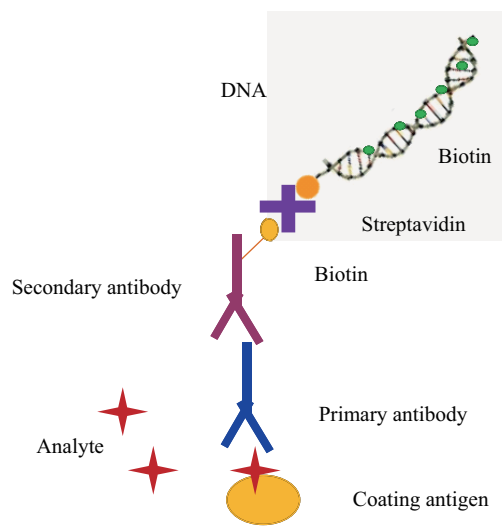


Fig. 3 Illustration of indirect competitive fluorescence immunoassay for BDE-47 using DNA/dye conjugate as antibody multiple labels.

was employed in the PBDE fluorescence immunoassay in the current work with slight modification. Anti BDE-47 antiserum and biotinylated goat anti-rabbit secondary antibody were used in the current assay, and the latter was labeled with the DNA/dye conjugate, as illustrated in Fig. 3.

2.2 Antigen preparation and antibody recognition

Among all the 209 PBDE congeners, 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) has been identified as the one with the highest body burden, especially in human milk, serum, and fat tissues (Hites, 2004; Petreas et al., 2003). According to our experimental design, an indirect competitive immunoassay format was selected for BDE-47 detection. In this format, BDE-47 needs to be immobilized on the bottom of a microplate well. Since BDE-47 is a small molecule, it requires conjugation to a protein carrier to realize immobilization. Therefore, the hapten 2,4,2'-tribromodiphenyl ether-4'-aldehyde was designed and synthesized as described above in the Experimental Section. After hapten characterization by GC-MS and NMR, it was conjugated to BSA to form the coating antigen PBDE-BSA. Binding of PBDE-BSA with anti-PBDE antiserum was then investigated by SPR measurement to make sure this coating antigen could be recognized by the antibody. In the measurement, PBDE-BSA was immobilized on a bare gold sensor chip by passive adsorption. A solution of the anti-PBDE antibody was then passed over the chip surface while the SPR angular shift was monitored. As shown in Fig. 4, about 30 mDeg angular shift was observed after the antiserum was injected into the SPR flow cell, suggesting that the antibody had bound to PBDE-BSA on the sensor surface. In contrast, when a high concentration solution of either BSA or anti-E2 antibody was passed over the PBDE-BSA coated sensor surface, there was no change in angular shift. This demonstrates the high selectivity in the binding of PBDE-BSA with the corresponding antiserum.

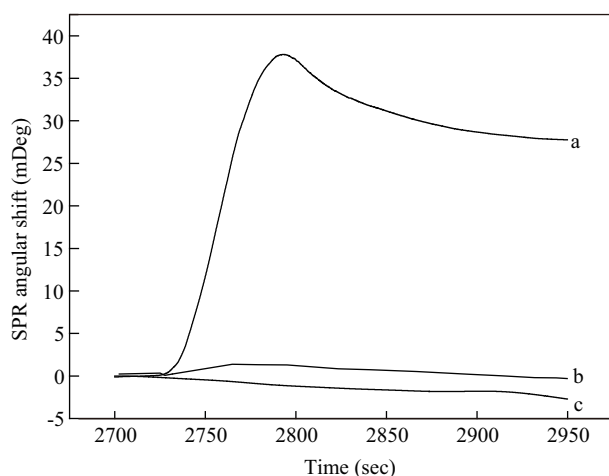


Fig. 4 Surface plasma resonance (SPR) angular shift of PBDE-BSA coated gold chip after injection of (line a) 1/100 dilution anti-PBDE antiserum, (line b) 10 mg/L BSA solution, (line c) 10 mg/L anti-estradiol antibody. Flow rate: 20 $\mu\text{L}/\text{min}$.

2.3 Competitive immunoassay for BDE-47

In general, the assay consists of the following steps: (1) the immobilization of the coating antigen on 96-well plates; (2) the addition of antiserum and BDE-47 standard or sample, and immuno-reaction; (3) the addition of the secondary antibody; (4) the attachment of DNA to the secondary antibody through streptavidin/biotin interaction; (5) the binding of fluorescent dyes to DNA; (6) the fluorescence detection. The detailed assay protocol is described in the Experimental Section. The assay conditions (reagent concentration, reaction time, reaction temperature) are very similar to those optimized for E2 in our previous work (Zhu et al., 2008). Figure 5 shows the results of the competitive immunoassay for BDE-47. In the range of 0.125–390 $\mu\text{g}/\text{L}$ BDE-47, the change of the fluorescence signal with BDE-47 concentration exhibited a typical sigmoidal shaped competition curve. The measured limit of detection for BDE-47 (the lowest BDE-47 concentration which produced a signal reduction in the assay that is more than three times the standard deviation of the blank) was 3.1 $\mu\text{g}/\text{L}$. The log-logit fit of the curve was reasonably linear in the range of 3.1–390 $\mu\text{g}/\text{L}$ BDE-47, with an R^2

of 0.9924. From the fitting equation, the calculated LOD is 0.73 $\mu\text{g}/\text{L}$. Therefore, the working range of the assay spans 2 orders of magnitude. Unlike E2 assays, use of the DNA/dye conjugate label did not improve the detection limit of BDE-47 significantly when compared with previous ELISA assays (Ahn et al., 2009). As we know, assay sensitivity is determined not only by the signal detection method, but also by antibody affinity. Presumably, the affinity of the BDE-47 polyclonal antibody is not as high as the E2 monoclonal antibody, which is in the range of 10^{10} L/mol. The low affinity of the BDE-47 antibody would put a limit on the BDE-47 assay sensitivity. To further improve BDE-47 immunoassay sensitivity, new antibodies with high affinity need to be developed.

2.4 PBDE congener selectivity of the immunoassay

To investigate the selectivity of the new immunoassay, several PBDE congeners including BDE-15, -28, -99, -153 and -209 were evaluated for their potential cross-reactivity (CR) with the anti-PBDE antiserum. CR is often employed as a parameter for evaluating immunoassay selectivity, and is usually calculated from the IC_{50} value in a competition curve for each congener. However, many of the listed PBDEs have poor water solubility, and it is not possible to obtain an IC_{50} value for them. To overcome this problem, all the congeners were assayed at a fixed concentration (500 $\mu\text{g}/\text{L}$) instead of a full range competition curve. Relative CR of a particular congener was then obtained by dividing the fluorescence signal of BDE-47 by that of the congener. The results are summarized in Table 1. From the table, BDE-99 had the highest CR relative to BDE-47, followed by BDE-28, whereas BDE-15, BDE-153 and BDE-209 exhibited insignificant CR. The data suggest that the antibody's selectivity is based on the number of bromines of a congener. It recognizes PBDEs with 3, 4, and 5 bromines effectively, but discriminates against low and high bromination PBDEs. All the CR values are below 100%, indicating that the antibody binds to BDE-47 with the highest affinity. The results are in good agreement with ELISA measurements (Shelver et al., 2005).

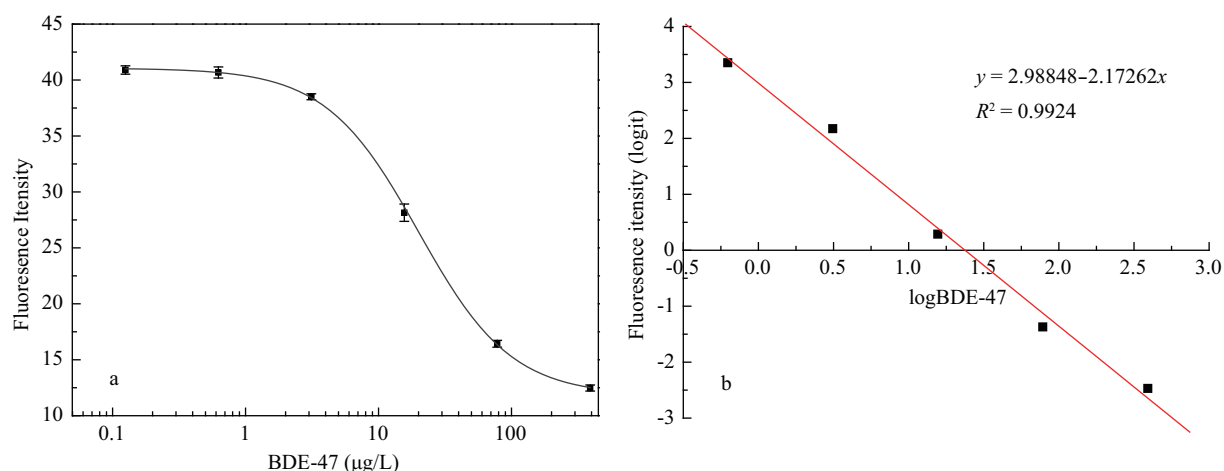


Fig. 5 Competitive fluorescence immunoassay for the detection of BDE-47 on PBDE-BSA coated 96-well plates using the DNA/dye multiple label format (a) and log-logit curve fit of the experiment results (b).

Table 1 Cross-reactivity of some PBDE congeners

Congener	Cross-reactivity (%)
BDE-15	1
BDE-28	36
BDE-47	100
BDE-99	90
BDE-153	3
BDE-209	<1

2.5 Immunoassay accuracy

To evaluate the accuracy of our method, GC-ECD was introduced as the reference method. In this evaluation, 50 µg/L BDE-47 was spiked into a river water sample. After the sample treatment described in the Experimental Section, the samples were measured both by our immunoassay and by the GC-ECD method, and the recovery was calculated. As shown in Table 2, the measured concentration and recovery of BDE-47 in the spiked river water sample by the immunoassay were in accordance with the GC-ECD method. These results demonstrate that the developed fluorescence immunoassay can be used to detect PBDEs in real environmental samples.

Table 2 Concentration (µg/L) and recovery of BDE-47 in spiked river water sample measured by immunoassay and GC-ECD

Chemical	Spiked	Immunoassay	Recovery	GC-ECD	Recovery
BDE-47	50	41.9 ± 1.1	83.7%	45.7 ± 3.4	91.4%

3 Conclusions

We have developed a competitive fluorescence immunoassay of PBDEs, using a DNA/dye conjugate as antibody multiple labels. Under the currently optimized conditions, the immunoassay yielded a detection limit of 726 ng/L for BDE-47, with a working range spanning 2 orders of magnitude. The assay showed high selectivity for 3-, 4-, and 5-brominated PBDEs, and discriminated against lower and higher bromination PBDEs. The assay was also certified by the GC-ECD method in the detection of spiked BDE-47 in river water samples. The assay uses common biochemical reagents (such as biotin, streptavidin, oligonucleotide, fluorescent dye) for *in situ* antibody labeling, and thus can be implemented easily by others. Due to the existence of a large variety of organic contaminants in the environment, simultaneous detection of multiple contaminants in a single sample is highly desirable. Because this assay is based on fluorescence detection, it can be readily configured into the current microarray technology platform. Research work in this direction is currently underway in our lab.

Acknowledgments

This work was supported by the Chinese Academy of Sciences (No. KSSCX2-YW-G-059), the National Hi-Tech Research and Development Program of China (No. 2007AA06A407) and the National Natural Science Foundation of China (No. 20825519, 20890112, 20921063).

We thank Yaxian Zhao for skillful technical support and Zongyan Cui for GC-ECD operation.

References

- Ahn K C, Gee S J, Tsai H J, Bennett D, Nishioka M G, Blum A et al., 2009. Immunoassay for monitoring environmental and human exposure to the polybrominated diphenyl ether BDE-47. *Environmental Science & Technology*, 43(20): 7784–7790.
- de Wit C A, 2002. An overview of brominated flame retardants in the environment. *Chemosphere*, 46(5): 583–624.
- de Wit C A, Alaee M, Muir D C G, 2006. Levels and trends of brominated flame retardants in the Arctic. *Chemosphere*, 64(2): 209–233.
- Gaur P K, Lau H P, Pestka J J, Chu F S, 1981. Production and characterization of aflatoxin B₂ antiserum. *Applied Environmental Microbiology*, 41(2): 478–482.
- Hardy M L, 2002. The toxicology of the three commercial polybrominated diphenyl oxide (ether) flame retardants. *Chemosphere*, 46(5): 757–777.
- Hites R A, 2004. Polybrominated diphenyl ethers in the environment and in people: A meta-analysis of concentrations. *Environmental Science & Technology*, 38(4): 945–956.
- Li Q X, Zhao M S, Gee S J, Kurth M J, Seiber J N, Hammock B D, 1991. Development of enzyme-linked immunosorbent assays for 4-nitrophenol and substituted 4-nitrophenols. *Journal of Agriculture and Food Chemistry*, 39(9): 1685–1692.
- Lindberg P, Sellström U, Häggberg L, de Wit C A, 2004. Higher brominated diphenyl ethers and hexabromocyclododecane found in eggs of peregrine falcons (*Falco peregrinus*) breeding in Sweden. *Environmental Science & Technology*, 38(1): 93–96.
- Marsh G, Stenutz R, Bergman Å, 2003. Synthesis of hydroxylated and methoxylated polybrominated diphenyl ethers – natural products and potential polybrominated diphenyl ether metabolites. *European Journal of Organic Chemistry*, 2003(14): 2566–2576.
- Mazdai A, Dodder N G, Abernathy M P, Hites R A, Bigsby R M, 2003. Polybrominated diphenyl ethers in maternal and fetal blood samples. *Environmental Health Perspectives*, 111(9): 1249–1252.
- Muir D C G, Backus S, Derocher A E, Dietz R, Evans T J, Gabrielsen G W et al., 2006. Brominated flame retardants in polar bears (*Ursus maritimus*) from Alaska, the Canadian Arctic, East Greenland, and Svalbard. *Environmental Science & Technology*, 40(2): 449–455.
- Petreas M, She J W, Brown F R, Winkler J, Windham G, Rogers E et al., 2003. High body burdens of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47) in California women. *Environmental Health Perspectives*, 111(9): 1175–1179.
- Shelver W L, Keum Y S, Kim H J, Rutherford D, Hakk H H, Bergman Å et al., 2005. Hapten syntheses and antibody generation for the development of a polybrominated flame retardant ELISA. *Journal of Agricultural and Food Chemistry*, 53(10): 3840–3847.
- Shelver W L, Parrotta C D, Slawecki R, Li Q X, Ikonomou M G, Barcelo D et al., 2008. Development of a magnetic particle immunoassay for polybrominated diphenyl ethers and application to environmental and food matrices. *Chemosphere*, 73(1): S18–S23.
- Sjödin A, Patterson D G Jr, Bergman Å, 2003. A review on human exposure to brominated flame retardants – particularly

- polybrominated diphenyl ethers. *Environment International*, 29(6): 829–839.
- Xu T, Cho I K, Wang D L, Rubio F M, Shelver W L, Gasc A M E et al., 2009. Suitability of a magnetic particle immunoassay for the analysis of PBDEs in Hawaiian euryhaline fish and crabs in comparison with gas chromatography/electron capture detection-ion trap mass spectrometry. *Environmental Pollution*, 157(2): 417–422.
- Zhang Q, Guo L H, 2007. Multiple labeling of antibodies with Dye/DNA conjugate for sensitivity improvement in fluorescence immunoassay. *Bioconjugate Chemistry*, 18(5): 1668–1672.
- Zhang Q, Zhu S C, Guo L H, 2011. Dye/DNA conjugates as multiple labels for antibodies in sensitive fluorescence immunoassays. In: *Bioconjugation Protocols: Strategies and Methods* (2nd ed.) (Sonny, S. Mark ed.). Pacific Biosciences, USA. 43–54.
- Zhu S C, Zhang Q, Guo L H, 2008. Part-per-trillion level detection of estradiol by competitive fluorescence immunoassay using DNA/dye conjugate as antibody multiple labels. *Analytica Chimica Acta*, 624(1): 141–146.
- Zota A R, Rudel R A, Morello-Frosch R A, Brody J G, 2008. Elevated house dust and serum concentrations of PBDEs in California: unintended consequences of furniture flammability standards? *Environmental Science & Technology*, 42(21): 8158–8164.

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Journal of Environmental Sciences (Established in 1989)

Vol. 24 No. 7 2012

Supervised by	Chinese Academy of Sciences	Published by	Science Press, Beijing, China
Sponsored by	Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences		Elsevier Limited, The Netherlands
Edited by	Editorial Office of Journal of Environmental Sciences (JES) P. O. Box 2871, Beijing 100085, China Tel: 86-10-62920553; http://www.jesc.ac.cn E-mail: jesc@263.net , jesc@rcees.ac.cn	Distributed by	Domestic Science Press, 16 Donghuangchenggen North Street, Beijing 100717, China Local Post Offices through China Foreign Elsevier Limited 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



9 771001 074123