Debrominated, hydroxylated and methoxylated metabolism in maize (*Zea mays* L.) exposed to lesser polybrominated diphenyl ethers (PBDEs)

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HIGHLIGHTS

- Debromination, hydroxylation and methoxylation of PBDEs in plant occurred rapidly.
- The introduction of a hydroxyl/methoxy group at the *ortho*-positions was easier.
- PBDEs are more susceptible to metabolism in leaves and stems than in roots.
- Σ MeO-PBDEs concentration was significantly higher than Σ OH-PBDEs concentration.

ARTICLE INFO

Article history:

Received 7 February 2012

Received in revised form 14 May 2012

Accepted 15 May 2012

Available online 8 June 2012

Keywords:

PBDEs

Plant metabolism

Debromination

Hydroxylation

Methoxylation

ABSTRACT

A hydroponic experiment was conducted to investigate the debrominated, hydroxylated and methoxylated metabolism of polybrominated diphenyl ethers (PBDEs, BDE-15, -28 and -47) in maize. A total of six debrominated metabolites (de-PBDEs), seven hydroxylated PBDEs (OH-PBDEs, including two unidentified OH-di-PBDEs and one unidentified OH-tri-PBDE) and four methoxylated PBDEs (MeO-PBDEs) were determined in the exposed plants. The metabolic products were detected in maize only after 12 h of exposure to the PBDEs. However, the concentration of each type of the metabolites (de-PBDEs, OH-PBDEs or MeO-PBDEs) decreased at the later exposure time, possibly due to further metabolism. The removal of a bromine atom or the introduction of a hydroxyl/methoxy group was easier at the *ortho*-positions on the biphenyl structure than at the *para*-positions. Concentration ratios of the total debrominated, hydroxylated or methoxylated metabolites to the parent congener (BDE-28 or -47) generally followed the order of leaves > stems > roots, and MeO-PBDEs > de-PBDEs > OH-PBDEs. These results suggest that metabolism occurred preferentially in leaves and stems than in roots. Less transformation and shorter elimination half-life of OH-PBDEs would contribute to the lower concentrations of OH-PBDEs than of de-PBDEs or MeO-PBDEs in maize.

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1. Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used as new additive brominated flame retardants (de Wit, 2002). Owing to their high volume production, lipophilicity and persistence, PBDEs have become ubiquitous contaminants in the environment (UNEP, 2002; Hassanin et al., 2004; Hites, 2004). Since May 2009 commercial mixtures of penta- and octa-BDEs have been included on the Stockholm Convention list of priority persistent organic pollutants due to their high bioaccumulation and potential toxicity (California State Assembly, 2003; Directive EEC, 2003).

Degradation of PBDEs is an important process which determines their fate in the environment. PBDEs have been shown to break down into lower brominated congeners (de-PBDEs) with much higher toxicity by microbial (He et al., 2006; Betts, 2008; Robrock et al., 2008), photochemical (Eriksson et al., 2004; Shih and Wang, 2009), and plant degradation (Huang et al., 2010; Wang et al., 2011b). Hydroxylated PBDEs (OH-PBDEs) and methoxylated PBDEs (MeO-PBDEs) have been detected in marine organisms (algae, mussels, and fish), human blood, and abiotic samples (surface water, snow and rain) (Marsh et al., 2004; Sinkkonen et al., 2004; Malmvärn et al., 2005; Verreault et al., 2005; Ueno et al., 2008; Wang et al., 2011d). There are different opinions about the origin of OH-PBDEs and MeO-PBDEs in biota and abiotic environmental matrices. Some studies have demonstrated that

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OH-PBDEs are formed by hydroxylation of PBDEs or demethylation of MeO-PBDEs, and MeO-PBDEs are formed by methylation of the OH-PBDEs (Haglund et al., 1997; Teuten et al., 2005; Wan et al., 2009). Formation of OH-PBDEs and MeO-PBDEs in animals after exposure to PBDEs has also been observed in laboratory studies (Kierkegaard et al., 2001; Malmberg et al., 2005; Qiu et al., 2007; Munschy et al., 2010). On the other hand, OH-PBDEs and MeO-PBDEs have been identified as marine natural products (Wan et al., 2009). Plants are an important component of the terrestrial ecosystem and play an important role in the metabolism of organic contaminants in the environment (Collins et al., 2006). However, there have been few studies on the metabolic occurrence and fate of PBDEs in plants (Huang et al., 2010; Wang et al., 2011a).

There has been a considerable interest in investigating the behavior of OH-PBDEs in the environment owing to their greater toxicity than the parent PBDE congeners and the fact that they elicit a variety of effects on exposed organisms including disruption of thyroid hormone homeostasis, oxidative phosphorylation disruption, antiprogesterone potency, altered estradiol synthesis, and neurotoxic effects (Meerts et al., 2001; Qiu et al., 2007; Wan et al., 2009). Although the toxicity of MeO-PBDEs is low, the demethylation products of the corresponding OH-PBDEs may have high toxicity (Wan et al., 2009). Therefore, MeO-PBDEs and OH-PBDEs represent potential risks to human and animal health (Lacorte and Ionomou, 2009; Wan et al., 2009; Ren et al., 2010). Plant uptake and metabolism of organic chemicals are important and represent the first step of the food chain and it is very important to evaluate the potential exposure risks for the environment and human health. PBDEs have been detected in plants (Mueller et al., 2006; Huang et al., 2010; Wang et al., 2011b) and it is therefore essential to further study their metabolism in plants.

In the present work a hydroponic experiment was conducted to investigate the metabolism of lesser PBDEs (BDE-15, -28 and -47) in maize. De-PBDEs, OH-PBDEs and MeO-PBDEs in different parts of maize plants were analyzed after different exposure times in order to elucidate the metabolism of PBDEs in plants.

2. Materials and methods

2.1. Chemicals and reagents

The following standards were purchased from AccuStandard (AccuStandard, New Haven, CT, USA): BDE-15, -28 and -47, a standard solution of PBDEs containing 39 native congeners (mono- through hepta-PBDEs), OH-PBDE standards (2'-OH-BDE3, 3'-OH-BDE7, 4'-OH-BDE17, 3'-OH-BDE28, 3-OH-BDE47, 5-OH-BDE47 and 6-OH-BDE47) and MeO-PBDE standards (2'-MeO-BDE3, 3'-MeO-BDE7, 4'-MeO-BDE17, 3'-MeO-BDE28, 3-MeO-BDE47, 5-MeO-BDE47 and 6-MeO-BDE47). Their molecular structures are shown in Table S1 in the Supplementary material. Standards of ^{13}C -PCB-141 and ^{13}C -PCB-208 were purchased from Cambridge Isotope Laboratory (Andover, MA, USA). Anhydrous sodium sulfate (Na_2SO_4), silica gel and alumina (100–200 mesh) were washed with hexane and used after heating overnight at 150 °C. Florisil (60–100 mesh, Acros Organics, Geel, Belgium) was activated at 450 °C for 12 h and deactivated by adding 1% (w/w) water. All solvents used, i.e., methyl *tert*-butyl ether (MTBE), dichloromethane (DCM), hexane, methanol, acetonitrile, acetone, were of HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ultrapure water (18.2 M Ω) used was obtained by using a Milli-Q system (Milli-Q Advantage A10, Millipore Corporation, Billerica, MA, USA). All other chemicals and reagents used were of reagent grade.

2.2. Exposure experiment

Maize (*Zea mays* L.) seeds were purchased from the Chinese Academy of Agricultural Sciences, Beijing, China. They were surface sterilized by soaking in a 3% (v/v) H_2O_2 solution for 30 min, rinsed with autoclaved deionized water and pre-germinated on moist filter paper in the dark for 4 d. Seedlings were then selected and transplanted in containers of half Hoagland nutrient solution and cultivated under a controlled environment growth chamber at a light intensity of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$ provided by supplementary illumination with a photoperiod of 14 h d^{-1} , a 25/20 °C day/night temperature regime, and a relative humidity of 60–70%. Autoclaved deionized water was used to prepare Hoagland nutrient solution which was saturated with oxygen to minimize the metabolism of anaerobic microorganisms.

After growth for 9 d, every three selected plant seedlings were transferred to an exposure container, a 150-mL glass-stoppered flask enclosed in aluminum foil to eliminate photolysis of PBDEs. Exposure experiments with BDE-15, -28 and -47 were conducted separately with their concentrations at 19.5, 21.6 and 15.5 $\mu\text{g L}^{-1}$, respectively, lower than their water solubility. Fresh exposure solutions were prepared with the standard solutions of BDE-15, -28 and -47, dissolved in acetone (lower than 1% v/v, in the exposure solution). All these were conducted in a laminar flow hood. During plant exposure, approximately 10 mL d^{-1} of autoclaved deionized water saturated with oxygen was injected into the containers to compensate for the transpiration losses. Blank (without PBDE exposure) and unplanted containers were included as controls and all the treatments were set up in triplicate. The containers were positioned randomly and re-randomized every day.

2.3. Sample preparation

Maize plants were harvested at intervals of 12, 24, 48, 96, 144, and 216 h. Root samples were first carefully rinsed with deionized water to remove any adhering PBDEs, and rinses were collected and combined into the exposure solutions. Then maize leaves, stems and roots were washed thoroughly with deionized water, blotted with filter paper and weighed. All the samples were then frozen at –50 °C overnight, freeze-dried for 48 h in a lyophilizer (FD-1, Beijing Boyikang Instrument Ltd, Beijing, China), and weighed to determine their dry weights. The dried samples were finely chopped and stored in glass containers at –20 °C before chemical analysis.

2.4. Extraction and analysis

Extraction and cleanup of PBDEs and MeO-PBDEs were based on the methods of López et al. (2009) and Wang et al. (2011b), and the detail method is provided in the Supplementary material. ^{13}C -PCB-141 was added as surrogate standard to the samples prior to extraction and ^{13}C -PCB-208 was added to the final solutions as an internal standard. An Agilent 7890 GC–MS (5975 inert) (Agilent, Palo Alto, CA, USA) and a HP-5MS column (30 m $\times$ 0.32 mm i.d., 0.25 μm film thickness) (J & W Scientific, Folsom, CA) were used for the analysis of PBDEs and MeO-PBDEs (Wang et al., 2011b). The GC oven temperature program for the determination of PBDEs was as follows: 1 min at 110 °C, first ramp at 30 °C min^{-1} to 200 °C (1 min hold), second ramp at 3 °C min^{-1} to 260 °C (12.5 min hold), and third ramp at 20 °C min^{-1} to 295 °C (20 min hold). For the determination of MeO-PBDEs, the oven temperature program had the following parameters: 110 °C (1 min hold), raised to 215 °C at 15 °C min^{-1} (1 min hold), finally at 270 °C at 2 °C min^{-1} (16 min hold). The carrier gas was helium at a flow rate of 1.5 mL min^{-1} . More detailed information is available in the Supplementary material.

The extraction and cleanup procedures for OH-PBDEs in plants have been reported in our previous study (Wang et al., 2011a), and the detailed method is provided in the [Supplementary material](#). A UPLC-MS/MS system was used to detect and quantify OH-PBDEs. Chromatographic analyses were performed using ACQUITY UPLC system (Waters, Milford, MA, USA). A reversed-phase chromatography was performed by Waters ACQUITY UPLC BEH C₁₈ column (2.1 mm × 100 mm i.d., 1.7 μm particle size, Waters, Milford, MA, USA). A binary mixture with acetonitrile (A) and water (B) was modified and used as the mobile phase. A linear gradient with a flow rate of 0.4 mL min⁻¹ was applied in the following manner: 70% B (0–0.8 min), 70–30% B (0.8–5 min), 30–5% B (5–7 min), 5% B (7–9 min), 5–70% B (9–10 min), 70% B (10–13 min). Mass spectrometry analysis was carried out using a Waters Xevo TQ MS triple quadrupole mass spectrometer equipped with an ESI source (Waters, Milford, MA, USA). The details of the UPLC-MS/MS method are provided in the [Supplementary material](#).

2.5. Quality assurance and quality control

Quality assurance protocol included the addition of surrogate standards and random injection of solvent blanks and standards. The limits of detection (LOD) were in the range of 25–52, 32–58 and 30–210 pg g⁻¹ in plant tissues for all the PBDE, MeO-PBDE and OH-PBDE congeners (*n* = 6), respectively. Recoveries of the surrogate standards of ¹³C-PCB-141 were 70.6–95.8%. Recoveries of PBDEs, MeO-PBDEs and OH-PBDEs were within the ranges 73.7–108.4%, 67.8–102.3% and 68.2–94.6% in the matrix spiked samples, respectively.

2.6. Data analysis

The data were subjected to statistical analysis by using the SPSS version 12.0 software package. All of the data represent the mean and standard deviation of triplicates.

3. Results and discussion

3.1. Debrominated metabolites of PBDEs in maize

Debrominated metabolites of PBDEs were detected in maize root, stem and leaf samples. A total of two, six and five de-PBDEs were determined in maize exposed to BDE-15, -28 and -47, respectively, after exposure for 12 h (Fig. 1), indicative of a very rapid debrominated metabolism. Among these debrominated metabolites, BDE-2 and -3 had relatively high concentrations, suggesting that PBDEs had a high debrominated metabolic ability in maize. This phenomenon was also found in numerous previous studies. For example, studies showed that BDE-209 was debrominated to the lower PBDEs such as di- and tetra-PBDEs in sediments, soils and plants (La Guardia et al., 2007; Tokarz et al., 2008; Huang et al., 2010). No de-PBDE was detected in the hydroponic solutions with the exception of the BDE-28 exposure experiment in which BDE-2, -12, -13, -15, -32 and -37 were detected in the solutions before plant exposure. However, altogether they accounted for less than 0.8% of the amount of BDE-28 added, and this was much lower than the concentrations in the exposed maize (Table S3, [Supplementary material](#)). These results suggest the occurrence of debromination of PBDEs in maize.

BDE-15, -28 and -47 investigated in this study were congeners with bromine at the *ortho*- or *para*-positions. Based on the de-PBDEs detected, BDE-3, BDE-3, -15 and BDE-3, -15, -28 were the debrominated metabolites of BDE-15, -28 and -47, BDE-2, BDE-2, -12, -13, -32, -37 and BDE-2, -12, -13 were the products of bromine atom rearrangement of BDE-15, -28 and -47, respec-

Fig. 1. Time-dependent concentrations of the debrominated PBDEs (de-PBDEs) in maize.

tively. In a previous study, chlorine dechlorination and rearrangement on the biphenyl rings were observed in poplars and switchgrass exposed to PCB-77 (Liu et al., 2009). However, no metabolite of the debromination at *para*-positions (BDE-8 and -17) was detected in the BDE-28 and -47 exposed maize, suggesting that bromine atoms at the *ortho*-positions departed more easily to form BDE-15 and -28. A previous study on the debromination metabolism of PBDEs in hexane under ultraviolet irradiation has shown that the bromine atoms at *ortho*-positions have much high-

er elimination efficiency than those at *meta*- and *para*-positions (Fang et al., 2008). However, another study has demonstrated that the bromine atoms are preferentially removed from the *meta*- and *para*-positions on the biphenyl structure by microbial degradation (Gerecke et al., 2005).

Fig. 1 describes the time-dependent accumulation of de-PBDEs in maize. Accumulation of the de-PBDEs in each part of the maize plant generally increased firstly and then decreased to different extents for BDE-15, -28 and -47. The decrease in accumulation may be caused by further metabolism to other lower brominated congeners or hydroxylated and methoxylated analogs. In comparison, the total concentration of the de-PBDEs was relatively low in leaves compared with roots and stems with the exception of a higher concentration in leaves than in stems of BDE-28 exposed maize. It was speculated that the lowest concentration of the parent PBDEs in leaves (Wang et al., 2011c) and the limited acropetal translocation of PBDEs inside plants with higher log k_{ow} contributed to the lower concentrations of de-PBDEs in leaves than in roots or stems.

3.2. Hydroxylated metabolites of PBDEs in maize

Hydroxylated metabolites of BDE-15, -28 and -47 were analyzed in maize root, stem and leaf samples. A total of four OH-PBDEs of the seven OH-PBDE standards, two unidentified OH-di-PBDEs (OH-di-BDE(1) and OH-di-BDE(2)) and one unidentified OH-tri-PBDE were detected in maize (Fig. 2). All these metabolites were detected in maize only after exposure for 12 h, indicating that hydroxylated metabolism in maize also occurred rapidly. Except that no OH-PBDE detected in the BDE-47 exposed maize leaf samples, two unidentified OH-di-PBDEs (OH-di-BDE(1) and OH-di-BDE(2)), five OH-PBDEs (3'-OH-BDE28, 4'-OH-BDE17, OH-di-BDE(1), OH-di-BDE(2) and one unidentified OH-tri-PBDE), and four OH-PBDEs (5-OH-BDE47, 6-OH-BDE47, OH-di-BDE(1) and OH-di-BDE(2)) were detected in maize exposed to BDE-15, -28 and -47, respectively. There were more types of OH-PBDE congeners in roots than in stems or leaves. No OH-PBDE was measured in the blank maize controls (without PBDE exposure), suggesting that the OH-PBDEs detected were derived from metabolism of PBDEs in maize.

Among all the detected OH-PBDEs, the concentrations of the two unidentified OH-di-PBDEs (OH-di-BDE(1) and OH-di-BDE(2)) were higher than the other OH-PBDE congeners in the exposed maize, with the concentrations following the order: BDE-15 >> BDE-28 > BDE-47. Furthermore, for di-PBDEs metabolites, the BDE-15 concentration was much higher than BDE-12 or -13, and it is speculated that these two OH-di-PBDEs were OH-BDE15. In the BDE-47 exposed maize roots, the concentration of 6-OH-BDE47 was higher than that of 5-OH-BDE47 and no 3-OH-BDE47 was detected, which suggests that the hydroxyl group was preferentially substituted at the *ortho*-positions to form 6-OH-BDE47 in comparison with 5-OH-BDE47 and 3-OH-BDE47. A similar result was obtained with CB77 being primarily metabolized to 6-OH-CB77 and 5-OH-CB77 (in a ratio of 10:1) in poplar tissues (Zhai et al., 2010a). The study of OH-PBDE metabolites in human serum samples from an electronic waste dismantling site showed that all three OH-PBDE metabolites identified were of substitution of hydroxyl groups at the *ortho*-positions (Yu et al., 2010).

The total concentration of OH-PBDEs in the BDE-28, -47 exposed maize roots and stems increased with increasing exposure time until 48 and 24 h, respectively, and then decreased, whereas the total concentration of OH-PBDEs in roots and stems increased with increasing exposure time for the BDE-15 exposure, which were similar to the trend of their parent PBDE congeners in our previous study (Wang et al., 2011c). No significant difference in the total concentration of OH-PBDEs in leaves over time was

Fig. 2. Time-dependent concentrations of the hydroxylated PBDEs (OH-PBDEs) in maize.

observed between the BDE-15 and -28 exposure. Some studies have shown that the elimination half-life of 6-OH-BDE47 in rat plasma is 71.7–85.6 min (Lai et al., 2011) and much shorter than the half-life of the parent compound BDE-47 (664, 30–40, and 31 d in humans, mice and rats, respectively) (Staskal et al., 2005), and this is likely due to the insignificant accumulation 6-OH-BDE47 in rats. In the present study the total concentration of OH-PBDEs in each part of maize was unlikely to increase significantly with increasing exposure time, and decreased in the BDE-28 and -47 exposed maize roots and stems at the later exposure time. This result may be attributed to a combination of the short

elimination half-life of OH-PBDEs and further metabolism of the OH-PBDEs inside maize.

Similar to the de-PBDEs, the total concentration of the OH-PBDEs was relatively low in leaves compared with roots and stems. Although the concentrations of the parent PBDEs (BDE-15, -28 and -47) were much higher in roots than in stems (Wang et al., 2011c), the total concentration of the OH-PBDEs between roots and stems showed no discernible difference. It might be speculated that different activities of metabolic enzymes in different plant parts contribute to the different metabolic ability (Chhikara et al., 2010; Zhai et al., 2010b).

3.3. Methoxylated metabolites of PBDEs in maize

Methoxylated metabolites were analyzed in maize root, stem and leaf samples after exposure to BDE-15, -28 and -47. Four MeO-PBDEs (2'-MeO-BDE3, 3'-MeO-BDE28, 5-MeO-BDE47 and 6-MeO-BDE47) of the seven MeO-PBDE standards were detected, and no 3'-MeO-BDE7, 4'-MeO-BDE17 or 3-MeO-BDE47 was detected in the plant samples (Fig. 3). Consistent with the debromination and hydroxylation results, MeO-PBDEs were also detected in maize only after exposure for 12 h. One MeO-PBDE (2'-MeO-BDE3), two MeO-PBDEs (2'-MeO-BDE3 and 3'-MeO-BDE28), and four MeO-PBDEs (2'-MeO-BDE3, 3'-MeO-BDE28, 5-MeO-BDE47 and 6-MeO-BDE47) were detected in maize exposed to BDE-15, -28 and -47, respectively. No MeO-PBDE was measured in the blank maize controls (without PBDE exposure) or exposure solutions, suggesting that MeO-PBDEs resulted from metabolism inside the plants. However, it is difficult to determine whether the MeO-PBDEs are formed by methylation of the OH-PBDEs or directly by the methoxylation of PBDEs. The concentration of 6-MeO-BDE47 was much higher than those of 3'-MeO-BDE28 and 5-MeO-BDE47, but no 3-MeO-BDE47 was detected in maize after BDE-47 exposure. This result indicates that the methoxy group might also be preferentially substituted at the *ortho*-positions to form 6-MeO-BDE47. Similar observations of the most frequently detected MeO-PBDE of 6-MeO-BDE47 in many environmental matrices and biotic samples have also been reported previously (Malmvärn et al., 2008; Lacorte and Ionomou, 2009).

Fig. 3 shows the time-dependent accumulation of MeO-PBDEs in maize exposed to BDE-15, -28 and -47. The total MeO-PBDE concentration was also in the order: roots > stems > leaves. With increasing exposure time, the total MeO-PBDE concentration in maize exposed to BDE-28 and -47 increased until 96 h and then decreased, whereas the total concentration of MeO-PBDEs in maize exposed to BDE-15 continued to increase. It is suggested that MeO-PBDEs would be further degraded to other metabolites in maize.

3.4. Transformation of PBDEs, OH-PBDEs and MeO-PBDEs in maize

Debromination, hydroxylation, and methoxylation of PBDEs in maize occurred rapidly, and all of the metabolites were detected in each plant part only after 12 h exposure to PBDEs. A significantly positive linear relationship was found between each parent PBDE and its hydroxylated and methoxylated metabolites over the exposure time (BDE-28 and 3'-OH-BDE28: $R = 0.84$, $P < 0.0001$; BDE-28 and 3'-MeO-BDE28: $R = 0.51$, $P < 0.05$; 3'-OH-BDE28 and 3'-MeO-BDE28: $R = 0.56$, $P < 0.05$; BDE-47 and OH-BDE47: $R = 0.85$, $P < 0.001$; BDE-47 and MeO-BDE47: $R = 0.93$, $P < 0.0001$; OH-BDE47 and MeO-BDE47: $R = 0.72$, $P < 0.001$), suggesting the existence of the transformation of PBDEs, OH-PBDEs and MeO-PBDEs inside maize and a similar metabolic pathway between OH-PBDEs and MeO-PBDEs. Furthermore, it was found that the bromine atoms at the *ortho*-positions of BDE-28 and -47 departed more

Fig. 3. Time-dependent concentrations of the methoxylated PBDEs (MeO-PBDEs) in maize.

easily than those at the *para*-positions, and the hydroxyl/methoxy group was also preferentially substituted at the *ortho*-positions.

To compare the metabolites in different plant parts expected for higher concentration in leaves than in stems after BDE-28 exposure, the total concentration of each type of the metabolites (de-PBDEs, OH-PBDEs and MeO-PBDEs) was lower in leaves than in roots and stems. The concentration ratio of the total debrominated, hydroxylated or methoxylated metabolites to their parent

Table 1

The mean ratios of the total metabolites to their parent congeners in maize roots, stems and leaves.^a

	Roots	Stems	Leaves
∑de-PBDEs/BDE-15	0.015 (0.0050)	10 (4.4)	17 (5.3)
∑de-PBDEs/BDE-28	0.021 (0.0042)	2.0 (0.91)	15 (6.3)
∑de-PBDEs/BDE-47	0.0081 (0.0016)	27 (6.1)	12 (5.2)
∑MeO-PBDEs/BDE-15	0.0010 (0.00037)	0.31 (0.16)	1.1 (0.58)
∑MeO-PBDEs/BDE-28	0.019 (0.011)	3.1 (2.0)	5.9 (4.5)
∑MeO-PBDEs/BDE-47	0.053 (0.014)	28 (5.7)	34 (18)
∑OH-PBDEs/BDE-15	0.0010 (0.00030)	0.55 (0.25)	0.80 (0.35)
∑OH-PBDEs/BDE-28	0.0014 (0.00050)	0.49 (0.23)	1.8 (1.0)
∑OH-PBDEs/BDE-47	0.0011 (0.00013)	2.8 (1.4)	–

^a Data within parenthesis are standard errors.

congener in each part of maize was further calculated (Table 1). The ratios were in the increasing order of roots < stems < leaves for all the three types of metabolites with the only exception of the lower ratio of the debrominated metabolites in leaves than in stems after BDE-47 exposure. It is difficult to make a detailed interpretation of the metabolites in plants since accumulation of the metabolites in maize may result from a combination of metabolism and translocation of the metabolites inside the plants. Nevertheless, these findings suggest that PBDEs in maize leaves and stems are more susceptible to metabolism compared with those in the roots. The concentration ratios of the total debrominated, hydroxylated or methoxylated metabolites to the parent congener (BDE-28 or -47) in each part of maize were in the decreasing order MeO-PBDEs > de-PBDEs > OH-PBDEs with the exception of the metabolites in leaves of the BDE-28 exposed maize, for which an order of OH-PBDEs < MeO-PBDEs < de-PBDEs was obtained. However, after BDE-15 exposure, the order of de-PBDEs > MeO-PBDEs > OH-PBDEs was obtained, possibly due to the fact that only limited MeO-PBDE standards are available to date, particularly without MeO-BDE15. The evidence that much lower concentrations of OH-PBDEs than of de-PBDEs or MeO-PBDEs in maize might be attributed to the shorter elimination half-life (Staskal et al., 2005; Kelly et al., 2008; Lai et al., 2011) and as well as less transformation of OH-PBDEs due to their greater toxicity compared with PBDEs and MeO-PBDEs.

4. Conclusions

The results of this study suggest that debromination, hydroxylation and methoxylation of BDE-15, -28 and -47 in maize occurred rapidly, and the metabolites might be further metabolized. Compared to *para*-positions, bromine atoms at the *ortho*-positions on the biphenyl structure departed more easily, and the introduction of a hydroxyl/methoxy group at the *ortho*-positions was also easier. Furthermore, it is suggested that PBDEs are more susceptible to metabolism in leaves and stems than in roots, and the total concentration of MeO-PBDEs was significantly higher than the concentration of OH-PBDEs. These results thus provide important information about the metabolic behavior of PBDEs in plants.

Acknowledgments

This work was funded by the National Basic Research Program of China (Project 2009CB421603) and National Natural Science Foundation of China (Projects 21177139 and 20921063).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.chemosphere.2012.05.026>.

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