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Biotransformation of tetrabromobisphenol A dimethyl ether back to tetrabromobisphenol A in whole pumpkin plants★

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Abstract

As the metabolites of tetrabromobisphenol A (TBBPA), tetrabromobisphenol A mono- and dimethyl ethers (TBBPA MME and TBBPA DME) have been detected in various environmental media. However, knowledge of the contribution of plants to their environmental fates, especially to the interactions between TBBPA DME and TBBPA, is quite limited. In this study, the metabolism and behaviors of TBBPA DME was studied with pumpkin plants through 15-day hydroponic exposure. The TBBPA were also studied separately using in-lab hydroponic exposure for comparison. The results showed that more TBBPA DME accumulated in pumpkin roots and translocated up to stems and leaves compared with TBBPA. Transformation of TBBPA DME occurred later and more slowly than that of TBBPA. Interconversion between TBBPA DME and TBBPA was verified in intact plants for the first time. Namely, TBBPA DME can be biotransformed to TBBPA MME (transformation ratio in mole mass, TRMM 0.50%) and to TBBPA (TRMM 0.53%) within pumpkin; and TBBPA can be biotransformed to TBBPA MME (TRMM 0.58%) and to TBBPA DME (TRMM 0.62%). In addition, two single benzene-ring metabolites, 2,6-dibromo-4-(2-(2-hydroxyl)-propyl)-anisole (DBHPA, TRMM 3.4%) with an *O*-methyl group and 2,6-dibromo-4-(2-(2-hydroxyl)-propyl)-phenetole (DBHPP, TRMM 0.57%) with an *O*-ethyl group, were identified as the transformation products in the TBBPA exposure experiments. The transformation and interconversion from TBBPA DME back to TBBPA is reported as a new pathway and potential source for TBBPA in the environment.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.envpol.2018.05.075>.

Keywords

Tetrabromobisphenol A; Tetrabromobisphenol A monomethyl ether; Tetrabromobisphenol A dimethyl ether; Interconversion; Single benzene-ring compounds

1. Introduction

As one of the most predominant brominated flame retardants (BFRs), tetrabromobisphenol A (TBBPA) has been applied widely in various plastic products, electronics and textiles (de Wit, 2002; Voordeckers et al., 2002; Morf et al., 2005). It was frequently found in air, house dust, water, soil and sediments, human and animal tissues (Sellström and Jansson, 1995; Johnson-Restrepo et al., 2008; Fan et al., 2013; Lee et al., 2015; Sühling et al., 2015; Li et al., 2016). TBBPA and its derivatives are regarded as potential endocrine disruptors due to the similar molecular structures to thyroid hormones (Ren and Guo, 2013). Though TBBPA shows low toxicity to mammals, it has acute negative physiological effects on fish and algae (Lee et al., 1993; Darnerud, 2003; McCormick et al., 2010; Liu et al., 2013; Sun et al., 2014a; Linhartova et al., 2015). For example, TBBPA exposure to zebrafish (*Danio rerio*) embryos at a concentration of 0.80 mg L⁻¹ can lead to total mortality; and it can cause edema, hemorrhaging, decreased heart rate and tail malformation at lower concentrations (McCormick et al., 2010).

Tetrabromobisphenol A dimethyl ether (TBBPA DME) as well as tetrabromobisphenol A monomethyl ether (TBBPA MME) has no either industrial production or application, and also no reported natural origins. They are generally considered as transformation products of TBBPA. Biotransformation from TBBPA to TBBPA DME and TBBPA MME has been reported in various studies (George and Hägglom, 2008; Sun et al., 2014a; Chen et al., 2017; Gu et al., 2017). Transformation ratio in mass or mole mass (TRM or TRMM, respectively) was used to describe the transformation efficiency from a parent compound to its metabolite. TBBPA was transformed to *O*-methylation metabolites including TBBPA DME in submerged soil and soil-plant systems with a total TRM of 11% (Sun et al., 2014a). TBBPA was transformed to TBBPA MME and TBBPA DME in the Kearny Marsh and Kymijoki sediments with reported TRMs of 10–50% (George and Hägglom, 2008). In soil, the earthworm *Metaphire guillelmi* enhanced the *O*-methylation transformation of TBBPA with a total TRM of 59.5 ± 5% (Gu et al., 2017).

TBBPA DME and TBBPA MME have been detected together with TBBPA in aquatic systems, sediments and animals (Watanabe et al., 1983; Sellström and Jansson, 1995; Vorkamp et al., 2005; Kotthoff et al., 2017). TBBPA generally exhibits higher concentrations in the environment than TBBPA DME and TBBPA MME. Concentrations of TBBPA and TBBPA DME in sewage sludge were reported in the range of 31–56 ng g⁻¹ dry weight (dw) and <1.9 ng g⁻¹ dw, respectively (Sellström and Jansson, 1995). However, the concentrations of TBBPA DME and TBBPA MME were sometimes comparable or even higher than that of TBBPA. For example, the concentrations of TBBPA and TBBPA DME in surficial sediments were 34 and 24 ng g⁻¹ dry weight (dw) upstream and 270 and 1500 ng g⁻¹ dw downstream of a plastic factory, respectively (Sellström and Jansson, 1995). It was also

reported that TBBPA MME showed higher concentrations than TBBPA in some fish (Kotthoff et al., 2017). Even in some samples, such as those in mussels and peregrine falcon (*Falco peregrinus*) eggs, only TBBPA DME was detectable without measurable TBBPA (Watanabe et al., 1983; Vorkamp et al., 2005). Such observations are surprising considering the continual input of TBBPA into the environment. Thus, to better understand the fate and occurrence of TBBPA and TBBPA DME in the environment, greater knowledge of their transformation processes is needed.

Plants exhibit essential roles in the translocation and transformation of organic compounds. Understanding the behaviors of TBBPA and its methylated derivatives in intact plants will help to elucidate biogeochemical cycling in the environment. Pumpkin, as a member of the genus Cucurbita, is known to have a special ability to uptake and accumulate a large mass of various POPs, such as DDT and PCBs (Hülster et al., 1994; Lunney et al., 2004). And it also has demonstrated metabolic transformation capabilities for PBDEs, OH-PBDEs and MeO-PBDEs (Sun et al., 2013; Yu et al., 2013). Therefore, pumpkin is regarded as a model plant to investigate the fates of persist organic compounds (Hülster et al., 1994; Lunney et al., 2004; Sun et al., 2013; Yu et al., 2013; Hou et al., 2017; Li et al., 2017). Hydroponic exposures were chosen to focus on the plant metabolism of these compounds without parallel soil microorganism interactions.

In this study, TBBPA DME and TBBPA were separately exposed to pumpkin seedlings through a hydroponic cultivation system. The uptake, translocation and biotransformation, especially the interconversion between TBBPA DME and TBBPA, were determined within intact plants. Because transformation from TBBPA DME to TBBPA was found in plant tissues, interconversion of the transformation product (TBBPA DME) back to the more toxic TBBPA is proposed as a novel environmental pathway. The process can be considered as a potential new source for TBBPA in the environment. TBBPA can be regenerated by pumpkin from its transformation product TBBPA DME, leading to the hypothesis that TBBPA might be more persistent in the environment than previously considered. These findings provide new perspectives for our understanding and evaluation on the environmental occurrences and risks of TBBPA and its methylated derivatives.

2. Material and methods

2.1. Chemicals and reagents

TBBPA DME (50 $\mu\text{g mL}^{-1}$ in nonane, 98%), surrogate standards of $^{13}\text{C}_{12}$ -TBBPA (50 $\mu\text{g mL}^{-1}$ in methanol, 99%) and $^{13}\text{C}_{12}$ -TBBPA DME (100 $\mu\text{g mL}^{-1}$ in toluene, 99%) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). TBBPA (solid, 97%) was purchased from Alfa Aesar (Ward Hill, MA, USA). D_{10} -TBBPA (100 $\mu\text{g mL}^{-1}$ in acetonitrile, 98.5%), as the injection internal standard, was obtained from Dr. Ehrenstorfer GmbH (Augsburg, GER). Working solutions of TBBPA DME and TBBPA for hydroponic exposure were prepared at a concentration of 100 $\mu\text{g mL}^{-1}$ in methanol.

Chromatographic grade hexane, methylene chloride and methanol were obtained from J.T. Baker (Philipsburg, USA). Anhydrous sodium sulfate (Sinopharm Chemical Reagent,

Beijing, China) was heated at 600 °C for 6 h before use. Ultra-pure water (18.2 Ω) used in experiments was produced by a Milli-Q advantage A10 system, Billerica, USA.

2.2. Hydroponic exposure

Seeds of pumpkin (*Cucurbita maxima* × *C. moschata*, Taigu Yinong Seed Co., Ltd., Shanxi Province, China) were germinated on wet sterile gauze at approximately temperature of 28 °C. Then, the seedlings were cultivated on sterile perlite beds in an illumination growth chamber at 25 °C for a 16 h light period, and at 22 °C for an 8 h dark period. After the seedling shoots grew to 4–5 cm height, the healthy seedlings with similar strength were transferred into 50 mL glass reactors and cultivated in sterile water for another 2 d. Then hydroponic exposures were conducted.

Pumpkin plants were exposed to individual standard of TBBPA and TBBPA DME. Each exposure reactor contained 45 mL sterile DI-water, 100 ng mL⁻¹ of single exposure chemical and three pumpkin seedlings. The pH of these solutions was detected with value of 5.51 ± 0.05 (Thermo Scientific Orion 3-Star pH Benchtop). Both blank controls (with plants but without exposure chemical) and unplanted controls (without plants but with exposure chemical) were set-up simultaneously for each exposure chemical. All the reactors were wrapped with aluminum foil to support root growth in darkness and to avoid the possible photolysis of parent chemicals. Transpired water lost each day from both treatment groups and blank controls was determined gravimetrically and replenished by sterile syringes.

2.3. Sampling and sample pretreatment

The hydroponic exposure lasted for 15 d. Exposure groups were sampled at day 2, 4, 6, 9, 12, and 15. Blank and unplanted controls were only sampled at the end of experiment (day 15). Treatments and controls were all conducted in triplicates for each sampling time point. The leaf, stem and root samples of the pumpkin seedlings were sampled, freeze-dried and stored at -20 °C before analysis. The dry weights of plant tissue samples for each reactor were 0.15–0.25 g for leaves, 0.04–0.06 g for stems and roots. All hydroponic solution in one reactor was collected and extracted immediately after sampling. Before extraction, 10 ng ¹³C₁₂-TBBPA DME and 10 ng ¹³C₁₂-TBBPA were added to all solutions and plant samples as surrogate standards.

Exposure solutions were shaken and extracted by 10 mL methylene chloride three times, and each extraction lasted for 5 min. After dehydration with 5 g anhydrous sodium sulfate, the combined extracts were concentrated to 1 mL for further purification.

The plant tissue was placed into a 2 mL centrifuge tube with small steel balls and 0.8 mL methylene chloride, vigorously shaken, homogenized and extracted at 30 Hz for 2 min on a TissueLyser II (QIAGEN, Hilden, Germany). After centrifugation at 8000 rpm for 3 min, the supernatant was transferred into a clean 5 mL tube. Extraction was conducted three times and the extracts were combined for purification.

The ENVI™-carb SPE columns (Supelclean™ 0.5 g, 6 mL, SUPELCO, Bellefonte, USA) were used for further purification. The SPE column was preconditioned with 6 mL methylene chloride and 6 mL hexane in sequence. After loading the extract, the analytes

were eluted by a 15 mL mixture of hexane and methylene chloride (1:1 v/v). The organic phase was divided into two parts. One half of the sample in hexane was used for neutral compound (TBBPA DME) analysis on GC/MS, and the other half in methanol was used for phenolic compound (TBBPA) analysis on LC/MS/MS. Before LC/MS/MS analysis, 10 ng D₁₀-TBBPA (as an injection internal standard) was added to all samples.

2.4. Instrumental analysis

TBBPA DME and TBBPA MME were detected by an Agilent 6890 gas chromatograph (GC) coupled with a 5975C mass spectrometer (MS) detector (Agilent Technologies, Palo Alto, CA). Samples were injected (2 μ L) by a 7683B Series Injector into a DB-5MS column (15 m $\times$ 0.25 mm $\times$ 0.1 μ m J&W Scientific, Folsom, CA) with a splitless mode (300 °C). Helium was used as carrier gas at a constant flow of 1.0 mL min⁻¹. The oven program commenced at 100 °C (held for 1 min), increased to 180 °C at 20 °C min⁻¹, then to 200 °C at 10 °C min⁻¹ and to 210 °C at 1 °C min⁻¹, and finally to 230 °C at 5 °C min⁻¹. The post run was set at 320 °C and held for 3 min. Quantitative determination was carried out under an optimized selected ion monitoring (SIM) mode on MS detector with Electron Impact Ionization (EI) source. The qualitative and quantitative ions for TBBPA DME and TBBPA MME are shown in Supporting Information (Tables SI-1). Full scan (m/z 200–600) by GC/MS was performed to confirm TBBPA DME and TBBPA MME.

TBBPA and the phenolic metabolites were quantified by an Agilent 1290 Series LC system coupled with an Agilent 6460 Triple Quadrupole LC/MS/MS system (electron spray ionization source, ESI) using a ZORBAX ODS column (150 mm $\times$ 3 mm $\times$ 5 μ m, Agilent Technologies, Santa Clara, California, USA). The mobile phase consisting of water (Solvent A) and methanol (Solvent B) was in a gradient elution mode. The ratio of A:B started at 70:30 and was held for 1 min, changed to 25:75 within 4 min then to 0:100 within 6 min and finally back to 70:30 within 1 min, and held for 4 min. The flow rate was set at 0.5 mL min⁻¹. Column temperature was 40 °C and the injection volume was 10 μ L. The MRM and ESI parameters are provided in Supporting Information (Tables SI-2 and SI-3).

Since the low levels of low-molecular weight metabolites generally have very low responses using MS with an EI source for detection, high resolution mass spectrometry with negative chemical ionization (NCI) was used to identify unknown metabolites. The Agilent 7200 Accurate Mass Q-TOF GC/MS (NCI) (Agilent Technologies, Palo Alto, CA) was employed with full scan mode. To confirm the molecular weights of the metabolites obtained by Q-TOF GC/MS, UltiMate 3000 LC-Orbitrap Tribrid HRMS (Thermo Scientific, Waltham, MA) coupled with negative heated electrospray-ionization (H-ESI) was utilized. Detailed parameters for the two instruments are shown in Tables SI-4 and SI-5.

2.5. Quality assurance and quality control

Laboratory procedural blanks were conducted accompanying sample pretreatment. No target parent chemicals or metabolites were detected in laboratory procedural blanks, illustrating that there was no contamination from laboratory background concentrations. Blank solvents were injected for every three samples and no memory effects were found. Recoveries of the surrogate standards in leaf, stem, root and solution samples were in the range of 47–90%,

75–107%, 87–110% and 49–81% for $^{13}\text{C}_{12}$ -TBBPA DME and 43–52%, 47–79%, 51–87% and 42–57% for $^{13}\text{C}_{12}$ -TBBPA, respectively. The method detection limits (MDLs) for TBBPA DME were 5.7 pg mL^{-1} for solution samples, 1.9 ng g^{-1} for leaf samples, and 5.0 ng g^{-1} for stem and root samples. The MDLs for TBBPA were 0.7 pg mL^{-1} for solution samples, 230 pg g^{-1} for leaf samples, and 580 pg g^{-1} for stem and root samples. All data were corrected by the surrogate recoveries.

2.6. Transformation ratio in mole mass

The transformation ratio in mole mass (TRMM) was the mole ratio between a metabolite and its parent compound. It was calculated based on the following equation:

$$\text{TRMM} = \frac{m_{\text{D}} \cdot M_{\text{P}}}{m_{\text{P0}} \cdot M_{\text{D}}} \times 100\%$$

in which m_{D} is the amount of a metabolite, m_{P0} is the initial mass of parent compound, M_{P} and M_{D} are the molecular weights of the parent compound and the metabolite. Molecular weights (MWs) of TBBPA DME, TBBPA and their metabolites found in this experiment were shown in Tables SI-6.

2.7. Statistical analysis

The software OriginPro 8.5.1 was used to analysis the data and plotting the distribution tendency over time. The nonlinear regressions were used to fit the mass changes of parent and daughter compounds over time. The confidence level was 95%. Analysis of variance (ANOVA) was performed for all data. Significant differences were considered for $p < 0.05$. Adjusted coefficients of determination (R^2) were used to evaluate the optimization indexes.

3. Results and discussion

3.1. Distribution of the parent chemicals

After the pumpkin seedlings were separately exposed to individual parent compound of TBBPA DME and TBBPA, the uptake, translocation and distribution of the parent chemicals in the seedlings were determined. Temporal variations of two parent compounds, TBBPA DME and TBBPA in different compartments of the exposure and control reactors are shown in Figs. 1 and 2. Total recoveries of TBBPA DME and TBBPA in unplanted controls were $97 \pm 2\%$ and $101 \pm 11\%$ at the end of the experiment, indicating the stability of the parent chemicals in the reactors. Parent compounds were not detected in their blank controls, suggesting that there was no cross contamination between reactors, and that parent TBBPA DME and TBBPA were not volatilized or phytovolatilized.

In Figs. 1(a) and 2(a), the mass of TBBPA DME and TBBPA in the hydroponic solution both displayed nonlinearly decreasing trends, as the fitted dashed lines (R^2 0.960, $p < 0.05$ and R^2 0.975, $p < 0.05$, respectively) showed, and finally reached a plateau (steady state). Between the two parent compounds, TBBPA is an ionic compound with dissociate constants pK_{a1} (7.5) and pK_{a2} (8.5). At the pH value (5.51 ± 0.05) for exposure, only 1% of the parent

TBBPA was dissociated to ions in the hydroponic solution. Majority of the TBBPA was in molecular form. Thus, the decreasing trends of the two compounds showed similar patterns.

Parent TBBPA DME and TBBPA were detected in various pumpkin tissue samples. The mass distributions of parent compounds in the pumpkin displayed the following order: roots >> stems >> leaves. It was shown that TBBPA DME and TBBPA can be taken up and translocated within intact pumpkin seedlings. As shown in Figs. 1(b) and 2(b), roots showed the ability to accumulate both TBBPA DME and TBBPA over the time. The mass of TBBPA DME and TBBPA in pumpkin roots reached a balance within 2 d as the regression model lines show ($R^2 = 0.992$, $p < 0.05$ and $R^2 = 0.967$, $p < 0.05$, respectively). The trends were consistent with previous hydroponic experiments that the sorption of roots can come to a steady state within 48 h (Briggs et al., 1982; Trapp, 2004; Liu and Schnoor, 2008).

Comparing the mass of parent TBBPA accumulated in roots, parent TBBPA DME was a little higher. In Figs. 1(c) and 2(c), the mass of TBBPA DME and TBBPA in stems displayed different patterns. The mass of TBBPA DME in stems was in the range from 21 ± 6 to 55 ± 14 ng; it increased within the first 6 d and then decreased, and the trend fitted well with a nonlinear regression ($R^2 = 0.899$, $p < 0.05$). The mass of TBBPA in stems was in the range from 0.79 ± 0.24 to 2.1 ± 1.0 ng and increased at the beginning and then came to a plateau as the regression model indicated ($R^2 = 0.854$, $p < 0.05$). Compared their stem contents at the same sampling time, the mass of TBBPA DME was 17–51 times greater than that of TBBPA. During the 15-day exposure, parent TBBPA was not detectable in the leaves of the pumpkin seedlings. However, parent TBBPA DME was detected in the leaves with an increasing tendency ($R^2 = 0.849$, $p < 0.05$) as shown in Fig. 1(d).

The K_{ow} value of TBBPA DME (7.36) is higher than TBBPA (6.70) (Jonsson and Hörsing, 2009). Previous studies found that the translocation of organic compounds within plants related to their hydrophilicity: compounds with higher K_{ow} would be easier to be accumulated in roots and more difficult to translocate in plants (Briggs et al., 1982; Trapp, 2004; Liu and Schnoor, 2008). However, in this case, the more hydrophobic TBBPA DME translocated faster, easier and farther than TBBPA. Compared with TBBPA DME (a nonionic compound), the different ionization behavior of TBBPA (an ionic compound with pK_{a1} of 7.5 and pK_{a2} of 8.5) might influence its translocation in pumpkin seedlings (Briggs et al., 1987; Bromilow and Chamberlain, 1995; Sicbaldi et al., 1997).

3.2. Mass balance of the parent TBBPA DME and TBBPA

Temporal variations in the mass balance for parent compounds TBBPA DME and TBBPA are shown in Fig. 3. Volatilization, phytovolatilization and chemical transformation of parent TBBPA DME and TBBPA did not occur during the experiments based on results from unplanted and blank controls. Thus, the decrease in recoveries from 100% can be assumed to be due to plant biotransformation. Total recoveries of TBBPA DME and TBBPA gradually decreased to $69 \pm 5\%$ and $63 \pm 11\%$, respectively. According to the regressions (TBBPA DME, $R^2 = 0.652$, $p < 0.05$ and TBBPA, $R^2 = 0.865$, $p < 0.05$), the total recoveries of TBBPA DME were generally higher than that of TBBPA, and decreased much more slowly than that of TBBPA, indicating that the biotransformation of TBBPA was to a greater extent and faster.

3.3. Identification of the metabolites

In the exposure group with TBBPA DME as parent compound, TBBPA and an unknown methoxyl substituted compound were identified as the metabolites using the Agilent GC/MS (EI source) in the pumpkin roots and hydroponic solutions. The methoxyl metabolite was found to flow out after TBBPA DME and before TBBPA on the GC chromatogram (Retention Time (RT), 39.300 min). Its typical full scan mass spectrum in a root sample is shown in Fig. SI-1. The spectrum showed the isotope peaks ratio of 1:4:6:4:1 for molecular ions, suggesting that this metabolite contained four bromine atoms. The highest molecular ion $[M]^+$ is m/z 557.6, giving information on the molecular weight of the metabolite and the peak of m/z 542.8 is a fragment ion of $[M-CH_3]^+$. Similar MS spectrum behaviors were observed for TBBPA and TBBPA DME, both of which have the molecular ions $[M]^+$ at m/z 543.7 and 571.8, and fragment ions of $[M-CH_3]^+$ at m/z 528.7 and 556.8, respectively. The similarity in spectrum behaviors suggested that this metabolite has a similar structure to that of TBBPA and TBBPA DME. All of these observations suggest that the compound was TBBPA MME.

Compared with results of the unplanted controls and the impurity detection of parent TBBPA DME standard in which TBBPA and TBBPA MME were detected at the same negligible levels (accounting for only 0.081 and 0.022% in mole mass, respectively), the metabolites, TBBPA MME and TBBPA, detected in the exposure systems were much higher, with highest TRMMs accounting for 0.50% and 0.53%. In addition, TBBPA or TBBPA MME was not detected in blank controls set for parent TBBPA DME exposure. All these results confirmed the surprising result that TBBPA DME can be interconverted back to TBBPA, and the biotransformation process is solely due to the presence of pumpkin seedlings in the exposure experiments.

In the exposure group with TBBPA as parent compound, TBBPA MME, TBBPA DME, and another two unknown metabolites were identified. The two unknown metabolites found in the pumpkin tissue samples were confirmed using high sensitive accurate mass Q-TOF GC/MS with NCI source. As the qualitative analysis shown in Section SI-1 and data displayed from Figs. SI-2 to SI-5, these two compounds were both identified as metabolites containing a single benzene-ring. One of them was proposed to be 2,6-dibromo-4-(2-(2-hydroxyl)-propyl)-anisole (DBHPA) which was previously reported in soil-organism systems and rice cells exposed to TBBPA (Sun et al., 2014a; Wang et al., 2016). The other one was considered to be 2,6-dibromo-4-(2-(2-hydroxyl)-propyl)-phenetole (DBHPP) which contained an ethyl group. This is the first report of a TBBPA metabolite containing an ethyl group, which suggests a new transformation product and a new potential metabolism pathway of TBBPA.

The detection of the standard purity showed that TBBPA MME, TBBPA DME and the two single-ring metabolites were not found in the standard of parent TBBPA. The four metabolites were also not found in blank and unplanted controls. Therefore, the metabolites detected in the exposure group of TBBPA were all biotransformed by pumpkin seedlings.

Because of the lack of authentic standards, metabolites TBBPA MME, DBHPA and DBHPP were all semi-quantified by the standard of TBBPA DME. The quantitative ion for TBBPA

MME was m/z 543, and for DBHPA and DBHPP, it was m/z 79. According to the quantitative analysis, the accumulation and distribution of metabolites were further studied.

3.4. Distribution of metabolites of parent TBBPA DME

The metabolites, TBBPA MME and TBBPA, were only detected in roots and solutions, and not detectable in stems and leaves after pumpkin exposed to TBBPA DME, suggesting that the transformation from TBBPA DME to TBBPA MME and TBBPA happened at the root/water interface of pumpkin seedlings. The occurrence of TBBPA MME suggested that the transformation from TBBPA DME to TBBPA MME and then to TBBPA was a sequential process.

As the regressions show ($R^2 = 0.929$, $p < 0.05$ and $R^2 = 0.864$, $p < 0.05$, respectively), TBBPA MME and TBBPA in roots (Fig. 4(a)) remained at relatively low mass and increased only slowly to 3.3 ± 1.8 ng and 1.4 ± 0.4 ng on day 9, but then rose rapidly to 9.0 ± 1.2 and 19 ± 9 ng at the end of exposure. In Fig. 4(b), the mass of TBBPA MME and TBBPA in solutions continued to rise during the experiment as shown by the regressions ($R^2 = 0.793$, $p < 0.05$ and $R^2 = 0.800$, $p < 0.05$, respectively). The highest masses of TBBPA MME and TBBPA in solution were 13 ± 5 and 3.5 ± 1.5 ng, respectively. For the whole exposure system, the highest TRMMs from parent TBBPA DME to TBBPA MME and TBBPA occurred on day 15 with the close values of $0.50 \pm 0.14\%$ and $0.53 \pm 0.23\%$.

3.5. Distribution of metabolites in TBBPA exposure group

TBBPA MME, TBBPA DME, DBHPA and DBHPP were all detected in the pumpkin roots, while TBBPA MME and TBBPA DME were detected in hydroponic solutions, and only DBHPA was detected in stems. As shown in Fig. 5(a), the production of DBHPA in roots increased very slowly before day 9 (9.0 ± 7.8 ng), but increased faster after that day and continued to increase to 89 ± 39 ng on day 15 as the regression curve shows ($R^2 = 0.977$, $p < 0.05$). The mass of DBHPP, TBBPA MME and TBBPA DME in roots reached the highest, 16 ± 5 ng on day 6, 27 ± 4 ng and 29 ± 2 ng on day 2, respectively, and then rapidly decreased to 1.9 ± 0.8 ng on day 9, 4.1 ± 4.2 ng and 5.4 ± 0.5 ng on day 6, and continued at low levels. DBHPP, TBBPA MME and TBBPA DME displayed similar trends that were to increase in early time periods and then decline in pumpkin roots at later times. The fitted curves ($R^2 = 0.760$, $p < 0.05$; $R^2 = 0.775$, $p < 0.05$ and $R^2 = 0.822$, $p < 0.05$, respectively) were consistent with the typical kinetics of intermediates, showing that these three compounds may be intermediate products in consecutive transformation reactions.

The results in Fig. 5(b) show that the temporal variations of TBBPA MME and TBBPA DME in solutions have similar tendencies ($R^2 = 0.711$, $p < 0.05$ and $R^2 = 0.798$, $p < 0.05$, respectively) and their mass was 50 and 25 times lower in average than those in roots, respectively. The temporal pattern of DBHPA in stems was similar to that in roots (Fig. 5(c), $R^2 = 0.815$, $p < 0.05$), indicating that DBHPA was translocated from roots to stems. The average mass ratio of DBHPA between stems and roots was 6.3%, while the translocation of TBBPA MME, TBBPA DME and DBHPP from roots to stem was too small to detect. The highest TRMMs from parent TBBPA to DBHPA, DBHPP, TBBPA MME and TBBPA DME were $3.4 \pm 1.5\%$ (on day 15), $0.57 \pm 0.18\%$ (on day 6), $0.58 \pm 0.08\%$ and $0.62 \pm 0.05\%$ (on

day 2). Compared with the transformation from TBBPA DME to TBBPA, the transformation from TBBPA to TBBPA MME and TBBPA DME was significantly earlier in time.

3.6. Environmental impact of the interconversion

Using separate exposures of TBBPA DME and TBBPA to pumpkin clearly demonstrated the interconversion between these two compounds. Similar reciprocal transformations between OH-PBDE and MeO-PBDE, between 4'-OH-PCB-61 and 4'-MeO-PCB-61 and between triclosan and methyl triclosan were also reported in Japanese medaka and plants (Wan et al., 2010; Sun et al., 2014b; Sun et al., 2016; Fu et al., 2018). It indicates that the interconversion between phenolic compounds and their methoxyl forms might be a common biotransformation reaction. TBBPA MME and TBBPA DME found in the environment are generally considered as the transformation products of TBBPA (George and Häggblom, 2008). However, our study suggests that TBBPA DME and TBBPA MME occurring in the environment are new potential sources of TBBPA by interconversion.

4. Conclusions

TBBPA DME and TBBPA were taken-up, translocated and bio-transformed by pumpkin plants. The uptake and translocation to leaves and the bioaccumulation of TBBPA DME was greater than TBBPA within the pumpkin seedlings. Two single-ring metabolites, one with an *O*-ethyl group and the other with an *O*-methyl group, were reported in TBBPA exposed plant systems. The *O*-ethylation of TBBPA, detected for the first time, revealed a new metabolic pathway of organic compounds in plants. To our knowledge, this is also the first paper to report the interconversion between TBBPA and TBBPA DME mediated by plants. The transformation from TBBPA to TBBPA MME and TBBPA DME was faster than the opposite biotransformation of TBBPA DME to TBBPA MME and TBBPA. The transformation of TBBPA DME and TBBPA MME to TBBPA was reported as new potential sources for TBBPA in the environment. These findings provide new understanding of the fate of TBBPA, TBBPA MME and TBBPA DME in the environment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Time series of parent TBBPA DME mass in (a) solutions, (b) roots, (c) stems and (d) leaves of pumpkin seedlings in TBBPA DME exposure reactors and its controls. The UCs stands for “unplanted controls”. The dashed lines in (a), (b) (c) and (d) were nonlinear regressions (a. $Y = 3583 \cdot \exp(-X/1.1) + 894$ $R^2 = 0.960$; b. $Y = 2570 \cdot (1 - \exp(-1.1X))$ $R^2 = 0.992$; c. $Y = 0.093X^3 - 2.7X^2 + 22X + 0.0037$ $R^2 = 0.899$; d. $Y = 5.0 \cdot (1 - \exp(-0.18X))$ $R^2 = 0.849$. X stands for the time (d) and Y stands for the mass (ng), R^2 is the determinant coefficient).

Fig. 2.

Time series of parent TBBPA mass in (a) solutions, (b) roots and (c) stems of pumpkin seedlings in TBBPA exposure reactors and its controls (no TBBPA was found in leaves). The UCs stands for “unplanted controls”. The dashed lines were nonlinear regressions (a. $Y = 3597 \cdot \exp(-X/0.72) + 900$ $R^2 = 0.975$; b. $Y = 2508 \cdot (1 - \exp(-0.95X))$ $R^2 = 0.967$; c. $Y = 0.99 \cdot (1 - \exp(-1.8X))$ $R^2 = 0.854$. X stands for the time (d) and Y stands for the mass (ng), R^2 is the determinant coefficient).

Fig. 3.

Variations of the total recoveries (mass balance) of parent TBBPA DME and parent TBBPA in separate hydroponic exposure systems over time. The dashed lines were nonlinear regressions (For TBBPA DME: $Y = 24 \cdot \exp(-X/2.3) + 76$ $R^2 = 0.652$; for TBBPA: $Y = 11 \cdot \exp(-X/0.073) + 207 \cdot \exp(-X/102) - 118$ $R^2 = 0.865$. X stands for the time (d) and Y stands for the mass (ng), R^2 is the determinant coefficient).

Fig. 4.

Mass variations of metabolites TBBPA and TBBPA MME in (a) roots and (b) hydroponic solutions in the exposure experiments of TBBPA DME over time. The colored dashed lines were nonlinear regressions (For TBBPA: a. $Y = 0.13 \cdot \exp(X/3.0) - 0.13$ $R^2 = 0.864$; b. $Y = 0.0070 \cdot \exp(-X/2) - 0.0068$ $R^2 = 0.800$. For TBBPA MME: a. $Y = 83 \cdot \exp(-X/155) + 83$ $R^2 = 0.929$; b. $Y = 0.010 \cdot \exp(X/2.5) - 0.012$ $R^2 = 0.793$. X stands for the time (d) and Y stands for the mass (ng), R^2 is the determinant coefficient).

Fig. 5.

Mass variations of the metabolites DBHPA, DBHPP, TBBPA MME and TBBPA DME in (a) roots, (b) hydroponic solutions and (c) stems in the exposure experiments of TBBPA over time. The colored dashed lines were nonlinear regressions for DBHPA and DBHPP, TBBPA MME and TBBPA DME (For DBHPA: a. $Y^2 = 0.12X^3 - 2.1X^2 + 10X + 0.0044$ $R^2 = 0.977$; c. $Y = 0.0027X^3 - 0.06X^2 + 0.43X + 0.00053$ $R^2 = 0.815$. For DBHPP: a. $Y = 211 * [\exp(-0.30) - \exp(-0.33)]$ $R^2 = 0.760$. For TBBPA MME: a. $Y = 125 * [\exp(-0.46X) - \exp(-0.83X)]$ $R^2 = 0.775$; b. $Y = 0.36 * [\exp(-0.14X) - \exp(-7.9X)]$ $R^2 = 0.711$. For TBBPA

DME: a. $Y = 147 * [\exp(-0.43X) - \exp(-0.73X)]$ $R^2 = 0.822$; b. $Y = 0.38 * [\exp(-0.05X) - \exp(-2.7X)]$ $R^2 = 0.798$. X stands for the time (d) and Y stands for the mass (ng), R^2 is the determinant coefficient).