UPTAKE, TRANSLOCATION AND TRANSFORMATION OF POLYBROMINATED DIPHENYL ETHERS IN WHOLE PUMPKIN SEEDLINGS

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Introduction

Polybrominated diphenyl ethers (PBDEs) are manufactured as brominated flame retardants and widespread distributed in the environment[1]. The potential metabolites, hydroxylated (OH-) and methoxylated (MeO-) PBDEs, have no known anthropogenic source but have been widely found in marine animals, abiotic samples and humans[2-4]. Several *in vivo* and *in vitro* studies have reported formation of hydroxylated metabolites from various PBDE congeners using animal models [5, 6]. As the beginning of the food chain, plants play an important role in the global cycling of PBDEs. In this study, the metabolism of PBDEs in young whole pumpkin plants was studied by *in vivo* hydroponic exposure.

Materials and methods

Materials. Standards of polybrominated diphenyl ethers included: BDE-1, 3, 7, 8, 15,17, 28, 42, 47, 49, 85, and 99. Standards of hydroxylated polybrominated diphenyl ethers were 2'-OH-BDE-3, 2'-OH-BDE-7, 3'-OH-BDE-7, 4'-OH-BDE-17, 2'-OH-BDE-28, 3'-OH-BDE-28, 5'-OH-BDE-25, 4-OH-BDE-42, 4'-OH-BDE-49, 3-OH-BDE-47, 5-OH-BDE-47, 6-OH-BDE-47, 2'-OH-BDE-68, 6-OH-BDE-85, 5'-OH-BDE-99, and 6'-OH-BDE-99. Methoxylated polybrominated diphenyl ethers were 2'-MeO-BDE-3, 2'-MeO-BDE-7, 3'-MeO-BDE-7, 4'-MeO-BDE-17, 2'-MeO-BDE-28, 3'-MeO-BDE-28, 5'-OH-BDE-25, 4-MeO-BDE-42, 4'-MeO-BDE-49, 3-MeO-BDE-47, 5-MeO-BDE-47, 6-MeO-BDE-47, 2'-MeO-BDE-68, 6-MeO-BDE-85, 5'-MeO-BDE-99, and 6'-MeO-BDE-99. The stock standards were purchased from AccuStandard (New Haven, CT, USA). All solvents were HPLC grade or pesticide grade and were obtained from J. T. Baker (Phillipsburg, NJ, USA).

Hydroponic exposure

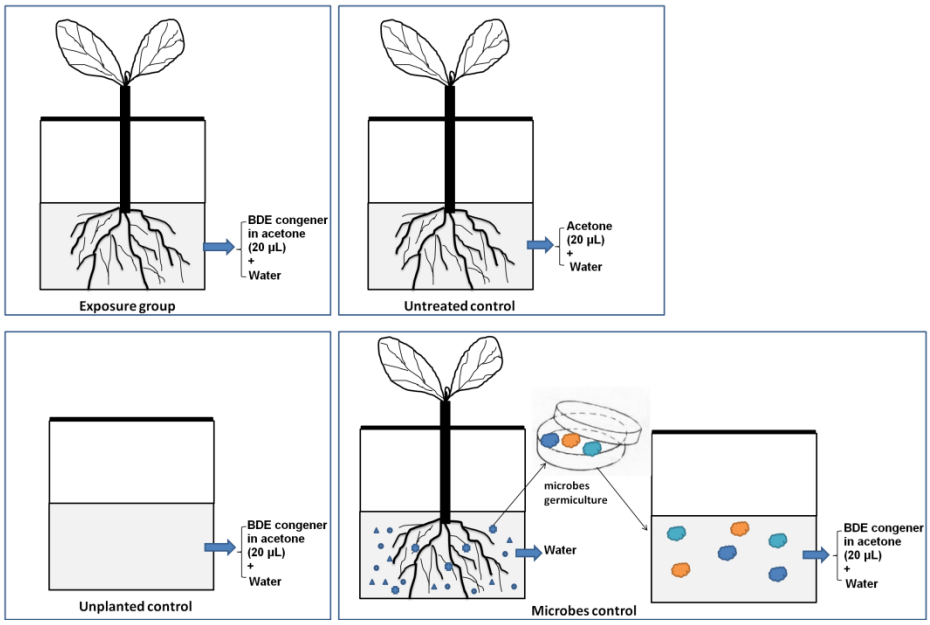

Figure 1. Schematic of the hydroponic exposure and control groups

The seedlings of pumpkins (8-9 cm high) were planted in 60 mL screw top brown glass bottles with 40 mL hydroponic solutions. All the bottles and hydroponic solutions were autoclaved to prevent microbial contamination. BDE-28 and BDE-47 were exposed to pumpkin seedlings individually for ten days with initial concentration of 5 ng/mL, respectively. Reactors were wrapped with aluminium foil and placed under 22 ± 2 °C. The photoperiod for plant growth was 16 h/day. Three control groups were designed and carried out simultaneously as shown in Figure 1. Untreated pumpkins controls were used to control any possible cross contamination of analytes during the experiment. Unplanted controls were designed to control the loss of BDE congeners. Microbes controls were designed as follows: the hydropnic solutions of untreated reactors were applied for germiculture. After growth in solid medium for 48 h, the obtained representative bacterial colonies were transferred to 40 mL of autoclaved hydroponic solution and then exposed to PBDE for ten days. Roots, stem and shoot of whole plants and the hydroponic solutions of exposure group and controls were sampled and anlyzed after exposure.

Method for analysis. The extraction and cleanup procedures for PBDEs, MeO-PBDEs and OH-PBDEs were modified from a previously developed method[7]. In brief, the freeze-dried root, stem, shoot and solution samples were extracted by hexane/MTBE (1:1, *v/v*) twice. Combined extracts were evaporated to dryness and redissolved in 20 mL of DCM. After mixed with acid silica gel, the organic phase were concentrated to dryness, redissolved in 1 mL of hexane, loaded on a column packed with 5 g of silica deactivated with 5% water (*w/w*). Then, 20% DCM in hexane (60 mL) was applied firstly to elute PBDEs and MeO-PBDEs. Secondly, DCM (70 mL) was used to elute OH-PBDEs. Fraction of PBDEs and MeO-PBDEs were concentrated for GC-MS analysis (Agilent 6890 GC coupled with a 5973C mass spectrometer). Solvent of fraction of OH-PBDEs was exchanged with acetonitrile for LC-MS/MS analysis (Agilent 1290 Series LC system coupled with an Agilent 6460 Triple Quadrupole MS/MS system).

Results and discussion

PBDEs can be accumulated by pumpkin roots. The root concentration factors (RCF) were 883 for BDE-47 and 800 for BDE-28. BDE-47 was taken up by the roots and translocated from the roots to the shoots. A debromination product, BDE-28, was identified in the pumpkin plants exposed to BDE-47. And BDE-28 was found much easier to move up and accumulate in shoots than BDE-47 due to the lower hydrophobicity and molecular weight. Four hydroxylated metabolites, 5-OH-BDE47, 6-OH-BDE47, 4'-OH-BDE49 and 4-OH-BDE42 were detected in whole pumpkin plants after exposure to BDE-47. Some hydroxylated tri-brominated diphenyl ethers were found in pumpkin plants exposed to BDE-28 through LC-MS/MS scan. However, none of the peaks consisted with the available commercial standards. A methoxylated metabolite of 4-methoxylated-2,2',3,4'-tetraBDE (4-MeO-BDE42) for BDE-47 exposure was unexpectedly observed in this study. None of these metabolites were found in control groups, indicating that pumpkin plants were responsible for the PBDE metabolism.

Acknowledgements

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