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Hydroxylated Metabolites of 4-Monochlorobiphenyl and Its Metabolic Pathway in Whole Poplar Plants

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Abstract

4-Monochlorobiphenyl (CB3), mainly an airborne pollutant, undergoes rapid biotransformation to produce hydroxylated metabolites (OH-CB3s). However, up to now, hydroxylation of CB3 has not been studied in living organisms. In order to explore the formation of hydroxylated metabolites of CB3 in whole plants, poplars (*Populus deltoides* × *nigra*, DN34) were exposed to CB3 for 10 days. Poplars are a model plant with complete genomic sequence, and they are widely used in phytoremediation. Results showed poplar plants can metabolize CB3 into OH-CB3s. Three monohydroxy metabolites, including 2'-hydroxy-4-chlorobiphenyl (2'OH-CB3), 3'-hydroxy-4-chlorobiphenyl (3'OH-CB3) and 4'-hydroxy-4-chlorobiphenyl (4'OH-CB3), were identified in hydroponic solution and in different parts of poplar plant. The metabolite 4'OH-CB3 was the major product. In addition, there were two other unknown monohydroxy metabolites of CB3 found in whole poplar plants. Based on their physical and chemical properties, they are likely to be 2-hydroxy-4-chlorobiphenyl (2OH-CB3) and 3-hydroxy-4-chlorobiphenyl (3OH-CB3). Compared to the roots and leaves, the middle portion of the plant (the middle wood and bark) had higher concentrations of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3, which suggests that these hydroxylated metabolites of CB3 are easily translocated in poplars from roots to shoots. The total masses of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 in whole poplar plants were much higher than those in solution, strongly suggesting that it is mainly the poplar plant itself which metabolizes CB3 to OH-CB3s. Finally, the data suggest that the metabolic pathway be via epoxide intermediates.

Introduction

Polychlorinated biphenyls (PCBs) were extensively used in past decades as flame retardants, heat transfer fluids and dielectric fluids for transformers and capacitors and released worldwide into different environmental matrices (1). Once introduced into the environment, PCBs persist because of their high chemical stability. Furthermore, PCBs may cause harm to biota because such lipophilic compounds preferentially bioaccumulate and biomagnify in higher trophic levels of the food chain (2). As environmental pollutants, more than ten thousand papers about PCBs have been published in past decades. However, hydroxylated PCBs, converted from parent compounds through various chemical and biological transformation processes (e.g., metabolism and biodegradation), have been relatively neglected (3,4). The hydroxylated

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Supporting Information Available

The structures of OH-CB3s, the analytical method and recoveries of OH-CB3s in the solution and poplar are shown in figure and table format. This material is available free of charge via the Internet at <http://pubs.acs.org/>.

metabolites of PCBs (OH-PCBs) have no known anthropogenic source but have been reported in many species and habitats (4-8).

4-Monochlorobiphenyl (CB3) is a component of commercial PCB products (9) and is mainly an airborne environmental pollutant (10,11). In contrast to highly chlorinated congeners that are more resistant to metabolic attack, CB3 is readily converted by oxidative enzymes to monohydroxy-CB3s and further to dihydroxy metabolites, which can be subsequently oxidized to quinones (12,13). Furthermore, potential hydroxylated metabolites of CB3 (OH-CB3s), including 2'-hydroxy-4-chlorobiphenyl (2'OH-CB3), 3'-hydroxy-4-chlorobiphenyl (3'OH-CB3) and 4'-hydroxy-4-chlorobiphenyl (4'OH-CB3), pose a risk for higher toxicity to organisms than the parent compound. Wang et al. (14) found that these three OH-CB3s inhibited the sulfonation of 4-nitrophenol in human liver cytosol with the sequence of 3'OH-CB3, 2'OH-CB3 and 4'OH-CB3. Moreover, 3'OH-CB3 was the most potent inhibitor with the lowest IC₅₀ concentration of 0.73±0.15 µM among the eighteen OH-PCBs studied. In addition, 3'OH-CB3 and 4'OH-CB3 also proved to be potent inhibitors of SULT1A1 (Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1) and SULT1B1 (Sulfotransferase family, cytosolic, 1B, member 1). The metabolite 4'OH-CB3 was shown to be mutagenic in the rat lung (15). Also, CB3 and its hydroxylated metabolites were reported to induce a surge in estradiol secretion in ovarian follicle cells. Such a response *in vivo* would be expected to disrupt reproductive processes (16). Low molecular weight OH-PCBs, such as 3'OH-CB3 and 4'OH-CB3, also elicited significant estrogenic activity and potentiated the effect of 17β-estradiol (17). Therefore, it is necessary to study the metabolism of CB3 in living organisms including plants, which can serve also as a food source for higher order organisms.

Plants, such as poplar, have been widely applied to remediate the pollution of organic compounds. Metabolic pathways in plants have been characterized as the “green-liver” model of metabolism, which transforms and degrades xenobiotic contaminants (18,19). Studies of the metabolism of PCBs by plant cell tissue cultures have found some hydroxylated metabolites of PCBs (20,21). Furthermore, poplars have been shown to uptake and translocate some lower chlorinated PCBs (22) and one hydroxylated metabolite of 3,3',4,4'-tetrachlorobiphenyl (CB77) was detected after its exposure to whole poplar plants (23). However, there is a lack of metabolic studies on CB3 *in vivo* in poplar plants with the potential to form toxic OH-PCB metabolites.

In this paper, the metabolism of CB3 and its hydroxylated metabolites (OH-CB3s) were studied in detail in order to elucidate the pathways of transformation in whole poplar plants. The possible pathways of CB3 metabolism in poplar plants were studied using a newly developed, highly sensitive and selective HPLC-MS method (supporting information), which can directly determine OH-CB3s and avoid the derivatization step frequently used in GC-based methods. In this research, we propose a metabolic pathway based on results of the pattern and concentration of OH-CB3s detected.

Experimental Section

Chemicals

The chemical structures of three OH-CB3s (2'OH-CB3, 3'OH-CB3 and 4'OH-CB3; 98% purity or better) are shown in Figure S1. They were synthesized using established procedures (24, 25). Stock solutions of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 were prepared in acetonitrile at 1.0 mg mL⁻¹. Working solutions of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 were prepared by gradual dilution of the stock solution with acetonitrile. All standards and solutions of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 were stored in amber glass vials at 4 °C.

Florisil (60-100 mesh, Acros Organics) was activated at 450 °C for 12 h (deactivated 1% (w/w) water). Anhydrous sodium sulfate was obtained from Fisher Scientific. Methyl-*tert* butyl ether (MTBE) (HPLC grade), dichloromethane (HPLC grade), hexane (pesticide grade) and sodium hydroxide (98.6%) were purchased from Fisher Scientific. Methanol was HPLC grade solvent (Acros Organics, NJ, USA). The deionized water (18.3MΩ) was from an ultrapure water system (Barnstead International, Dubuque, IA, USA). All other chemicals and reagents used in this experiment were of analytical reagent grade or better.

Exposure Method

Cuttings from male clones of the adult Imperial Carolina hybrid poplar tree (*Populus deltoides* × *nigra*, DN34) were used as the model plant in this work. Each cutting was fit snugly into a pre-drilled screw cap with PTFE-faced septum. The interface of the cutting and the cap was sealed using 100% silicone sealant (DAP inc. USA). All the poplar cuttings hydroponically grow in half strength Hoagland nutrient solution (26).

After 25 days of growth, healthy whole poplar plants were selected to carry out the exposure experiments with the conical flasks as reactors. The exposure method was similar to previous papers (22,23). In brief, 500 ml glass conical flasks with a PTFE-faced septum sampling port and a predrilled screw cap were used as the exposure reactors. Autoclaved de-ionized water was used to prepare Hoagland nutrient solution, which was saturated by oxygen in order to reduce the metabolism of anaerobic microorganisms. Hoagland solution (400 mL) and a suitable amount of CB3 were added to the autoclaved reactors. Except for the blank tree control without CB3, the starting concentration of CB3 in each reactor was 1.0 mg L⁻¹. All these procedures were conducted in a laminar flow hood. A variety of relative reference “controls” were tested to elucidate the metabolism of poplar plants with the following rationale: Blank plant control- three whole poplar plants without CB3 (contamination control); Water control- three autoclaved de-ionized water solutions with CB3 but without poplar plants (abiotic reaction control); Solution control- three growth media Hoagland solutions with CB3 but without poplar plants (another abiotic reaction control); Autoclaved plant control- three autoclaved whole poplar plants with CB3 (microorganism control); Dead plant control- three wilted, dead whole poplar plants for 4 days with CB3 (inactive plant control); Washed plant control- three whole poplar plants whose roots were washed with 70% ethanol solution and then rinsed with de-ionized water prior to exposure with CB3 (microorganism control); Whole poplar plant- three whole, growing, intact poplar plants with CB3. All reactors were wrapped with aluminum foil to eliminate photolysis of CB3. The exposure was conducted at 23±1 °C. The photoperiod was set 16 h per day under fluorescent lighting with a light intensity between 120 and 180 μmol m⁻² s⁻¹. Autoclaved de-ionized water saturated with oxygen was injected into reactors to compensate for the transpiration losses according to the weight of the reactors. Approximately 40 mL d⁻¹ of water was transpired due to the growth of poplar plants.

In order to investigate the translocation and distribution of OH-CB3s in different parts of poplar plants after 10 days exposure, each reactor specimen was divided into hydroponic solution, root, bottom bark, bottom wood, middle bark, middle wood, top bark, top wood and leaf as described previously (22). A ceramic mortar and pestle was used to grind the root and leaf samples in liquid nitrogen for further extraction. Other parts of the poplar plant were cut into very small pieces for efficient extraction of OH-CB3s. All equipment was rinsed 3 times with reagent-grade acetone between samples.

Extraction and cleanup

The extraction and cleanup procedure for OH-CB3s was modified from previous literature for poplar plants (23). In brief, 0.5 ml of 37% HCl and 5 mL of 2-propanol were added to de-ionized water and hydroponic solutions prior to extraction. Then these de-ionized water and

hydroponic solutions were extracted twice with 50 mL of hexane/MTBE (1:1, v/v) for 30 minutes. The poplar tissue samples were firstly mixed thoroughly with 2 mL of 37% HCl and 5 mL of 2-propanol, and then shaken vigorously overnight to extract OH-CB3s using 3 mL of hexane/MTBE (1:1, v/v) g⁻¹ of sample. The organic extract containing OH-CB3s was transferred after centrifugation at 3000 rpm for 5 min. A second extraction with 3 mL of hexane/MTBE (1:1, v/v) g⁻¹ of sample was performed for half an hour and then the organic extract was combined with the first organic extract after centrifugation. The combined extracts were evaporated to dryness in rotary evaporator. The extracts were re-dissolved in 2 mL of hexane. The hexane phase was partitioned with 500 µL of NaOH solution (0.5 M in 50% ethanol) and OH-CB3s were changed into ionic form and remained in the alkaline solution. The hexane phase was removed after phase separation. The ionic OH-CB3s in alkaline solution were changed into neutral form using 125 µL HCl (2 M). The neutral OH-CB3s were extracted twice using 1 mL of hexane/MTBE (9:1, v/v) and the extracts were combined. The combined OH-CB3 fraction was cleaned up by a florisil column (2.0 g anhydrous Na₂SO₄ on top, 5.0 g of florisil at the bottom). The column was activated with 3 mL of dichloromethane (DCM)/hexane/methanol (50:45:5) and 7 mL of DCM/hexane/methanol was used to elute the OH-CB3s. The eluate was evaporated to dryness under the nitrogen flow and dissolved in 1 mL of acetonitrile for HPLC-MS analysis after filtration using a 0.4 µm membrane.

Instrument

Qualitative and quantitative analysis of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 was performed on HPLC-MS (Agilent 1100 Series LC/MSD) with an autosampler. These three OH-CB3s were separated by Agilent Zorbax Bonus RP columns (2.1×150 mm, 5 µm) with a mobile phase flow rate of 0.15 mL min⁻¹ at room temperature. The ratio of acetonitrile and water (pH 9.9) in mobile phase was 65:35. The injection volume was 20 µL. The electrospray in negative ionization mode of MS (LC-ESI (—)-MS) was utilized and the ion mass detected in SIM was 203. Other analysis parameters of the LC-MS were: fragmentor: 115 V; capillary voltage: 3500 V; gain: 7.00; drying gas flow: 10 L min⁻¹; nebulizer pressure: 35 psi; drying gas temperature: 250 °C. With the parameters above, these three OH-CB3s are separated perfectly (Figure S2) with the elution sequence of 4'OH-CB3, 3'OH-CB3 and 2'OH-CB3. Furthermore, this method also has very good analytical performance (Table S1). The recoveries of 4'OH-CB3, 3'OH-CB3 and 2'OH-CB3 in the samples, including solution, root, wood, bark and leaf, were shown in the table S2.

Results and discussion

OH-CB3s in different solutions

Abiotic controls were established in de-ionized water and hydroponic solution to study the oxidation of CB3 because lower chlorinated congeners of PCBs are known to be less stable and more easily attacked in aqueous solution (12). Three OH-CB3 degradates were detected, including 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 during the 10 d experiment, when 1.0 mg L⁻¹ of CB3 was spiked into the reactors. These three OH-CB3s were detected in de-ionized water controls and hydroponic solution controls (pH 6.8), even though all the materials, such as water and reactors, were autoclaved and the photodegradation of CB3 was also eliminated by wrapping the exposure reactors with aluminum foil. It can be seen from figure 1 that the concentrations of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 continuously increased during the course of 10 days. These OH-CB3s could not be detected at the beginning of experiment (0 day), which confirmed that the CB3 standard and exposure reactors were not contaminated by OH-CB3s. Furthermore, the concentrations of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 in hydroponic solution controls were significantly higher than those in de-ionized water controls likely due to the catalytic effect of higher cation and anion concentrations in the nutrient solution. The concentration of 4'OH-CB3 in hydroponic solution controls was about 10 times

higher than that in de-ionized water controls; and the concentrations of 2'OH-CB3 and 3'OH-CB3 in hydroponic solution controls were about 5 times higher than those in de-ionized water controls. This indicates that 4'OH-CB3 was more easily formed than 2'OH-CB3 and 3'OH-CB3 in de-ionized water and hydroponic solutions. Overall the abiotic transformation of CB3 represents a small yield of the parent compound; approximately 0.001-0.01% of CB3 reacted to form total OH-CB3s.

The formation of OH-CB3s in hydroponic solutions due to microorganisms and poplar roots were monitored after 10 days of exposure of poplar plants to CB3 (figure 2). CB3 was not added to the "blank poplar controls", and formation of OH-CB3s was not detected in those controls, indicating that the reactors were not contaminated during the course of the experiment. However, it can be seen from figure 2 that 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 were detected in all other hydroponic solutions spiked with CB3. The concentrations of OH-CB3s in solutions with poplar plants were much higher than those produced by oxidation in de-ionized water controls and hydroponic solution controls. The metabolites of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 detected in solutions of autoclaved poplar plant controls and dead poplar plant controls were considerably higher, suggesting that these OH-CB3s came from the metabolism of CB3 by microorganisms, even though autoclaving poplar plants should reduce greatly the activity of microorganisms. Among these treatments in figure 2, 4'OH-CB3 had the highest concentration in solutions of autoclaved poplar plant controls and dead poplar plant controls, reaching $171.5 \pm 15.5 \text{ ng L}^{-1}$ and $395.2 \pm 176.7 \text{ ng L}^{-1}$, respectively. There was a similar hierarchy of OH-CB3s in de-ionized water controls and hydroponic solution controls, with 4'OH-CB3 consistently showing the highest concentration, except in the solution of washed poplar plant controls whose roots were washed with 70% ethanol and in the solution of whole poplars. The concentration sequence of OH-CB3s in solutions of washed poplars and whole poplars was 2'OH-CB3, 4'OH-CB3 and 3'OH-CB3, in descending order. The reason for this difference in ratios can be seen in Figure 3. In the root sample of Figure 3, the three OH-CB3s showed the same concentration sequence, suggesting that roots are the primary source of OH-CB3 production in solution, but 4'OH-CB3 is the dominant metabolites in bark, wood and leaf tissues.

OH-CB3s in poplar plants

Previous study in our laboratory found that CB3 can be taken up and translocated to the upper stem of poplar plants (22), but metabolites were not investigated. In the research reported here, the metabolites of OH-CB3s were also measured in hydroponic solution and in the exposed whole poplars. It can be seen from figure 3 that 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 were detected in all the parts of poplar plants. However, there were different concentration rank orders in different parts of the whole poplars: the concentration sequence was 4'OH-CB3, 2'OH-CB3 and 3'OH-CB3 in the middle wood, middle bark, bottom wood and bottom bark; in top wood, top bark and leaf, the sequence was 4'OH-CB3, 3'OH-CB3 and 2'OH-CB3; and the sequence was 2'OH-CB3, 4'OH-CB3 and 3'OH-CB3 in the root portion of the plant. Peak metabolite concentrations were formed in the middle and bottom woody portion of the plant (wood and bark), and the metabolites were translocated from there to the top wood and bark. The concentrations of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 in the bark were higher than those in the wood, which might have two reasons. One is the bark is located near the transport path for water and nutrients, which is also the transport path for CB3 and OH-CB3s. Second, the bottom and middle bark was surrounded (in direct contact) with the gas phase of volatilized CB3 and OH-CB3s inside the sealed reactor. The lower and middle bark can absorb OH-CB3s from the hydroponic solution and diffuse them up through the bark tissue, and it can also absorb them from the gas phase in the head space above the solution.

Except for the roots, the concentration of 4'OH-CB3 was the greatest among the OH-CB3s in all portions of the plant. The highest concentration of 4'OH-CB3 reached $622.5 \pm 134.8 \text{ ng g}^{-1}$ in the middle bark. However, the highest concentration of OH-CB3s in the root was for 2'OH-CB3, and the concentration sequence was 2'OH-CB3, 4'OH-CB3 and 3'OH-CB3, in rank order, which is the same sequence as in hydroponic solutions of whole poplars. This suggests that OH-CB3s in the solution (figure 2) are mainly derived from CB3 transformation in the roots. Figure 3 also shows that 4'OH-CB3 is produced and translocated more easily than 2'OH-CB3 and 3'OH-CB3 in different parts of poplar plants. Even in the leaves, the three OH-CB3s were detected (under 2.0 ng g^{-1}), further indicating that poplar plants can metabolize CB3 into hydroxylated products.

Total masses of OH-CB3s in the solutions and poplar plants

The total masses of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 were compared in both solution and poplars in order to quantify the metabolism of CB3 (table 1). Masses of OH-CB3s in the solutions of the autoclaved poplars and dead poplars were 142.4 ng and 190.5 ng which are much smaller than those in the washed poplars and whole poplars with masses of 239.0 and 239.3 ng, respectively. All in all, the total masses of OH-CB3s followed the same rank order as their concentrations in the solutions. However, the total masses of OH-CB3s in poplars were much higher than in solution indicating that metabolism is primarily occurring in the plant tissue itself. The total masses varied greatly in the poplars for each of the different treatments (figure 4 and table 1). The existence of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 were not found in blank control poplars (no CB3 was added to the solution) which excludes the possibility of background contamination of CB3 and OH-CB3s from the environment. The total masses of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 in autoclaved poplars and dead poplars were much lower than those in washed poplars and whole poplars because the activities of tissues in autoclaved and dead poplars were damaged and there would be no translocation in them. However, the total masses of metabolites in the autoclaved poplars were greater than the total masses analyzed in the dead poplars. The reasons might be that autoclaved poplars (detritus organic materials) were susceptible to the growth of microorganisms during the 10 day experiment, and at the same time they could absorb more OH-CB3s from the solutions. There were higher total masses of OH-CB3s in washed poplars and whole poplars. Moreover, the total masses of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 in whole poplars (555.8, 249.1 and 3422 ng, respectively) were greater than those in washed poplars (229.6, 78.3 and 574.4 ng, respectively). For 4'OH-CB3, the total mass in whole poplars was 6 times higher than that in washed poplars, suggesting that 70% ethanol kills a significant fraction of the microorganisms in roots, while at the same time partly hurting the root tissues and decreasing the enzymatic activity to metabolize CB3. More importantly, the total masses of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 in the whole poplars were 14.5, 5.5 and 37.4 times as high as those in the solutions of whole poplars, which clearly indicates that whole poplars can metabolize CB3 into OH-CB3s, although microorganisms make a small metabolic contribution of OH-CB3s in these experiments. Whole intact poplars exposed to CB3 produced the greatest mass of metabolites compared to all other treatments, and the yield of these three OH-CB3s is about 1% of total CB3 spiked in the solution.

Other unknown hydroxylated metabolites of CB3 in poplar plants

There were two other mono-OH-CB3s detected in different parts of poplars besides 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3. They had the same mass weights as 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 using SIM mode of LC-MS (figure 5). Due to the unavailability of their standards, the structures could not be unambiguously determined. However, a reasonable hypothesis can be advanced based on their physical and chemical properties. In theory, CB3 should produce five monohydroxy metabolites of CB3 without a chlorine shift, namely the three compounds in the adjacent biphenyl ring (2'OH-CB3, 3'OH-CB3 and 4'OH-CB3) and two compounds with

hydroxyl and chlorine moieties in the same ring (2OH-CB3 and 3OH-CB3). Therefore, the other two unknown OH-CB3s should be 2OH-CB3 and 3OH-CB3. It can be seen from the chromatogram of the root sample (figure 5) that these two unknown OH-CB3s eluted earlier than 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3, which suggests they are more polar than 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 because more polar compounds elute first in reversed phase liquid chromatography. According to the steric positions of hydroxyl and chlorine in their molecular structures, monohydroxy CB3s with hydroxyl and chlorine in the same ring should have more polarity than those with hydroxyl and chlorine in the different rings. Therefore, the elution sequence from column also suggests the unknown monohydroxy CB3s should be 2OH-CB3 and 3OH-CB3. For reversed HPLC, the more polar compounds elute earlier from the column. Therefore, the sequence of 2OH-CB3 and 3OH-CB3 can be estimated according to the Kow values of 2OH-CB3 and 3OH-CB3. The lower Kow value should elute earlier from the column. However, the Kow values of 2OH-CB3 and 3OH-CB3 could not be found in the literature, so the Kow values of 2-chlorophenol and 3-chlorophenol were used as surrogates for the elution sequence because they have only the difference of one benzene ring with 2OH-CB3 and 3OH-CB3. According to the Kow values of 2-chlorophenol (2.17) and 3-chlorophenol (2.50) (27), the Kow value of 2OH-CB3 should be larger than that of 3OH-CB3. As a result, 3OH-CB3 should elute earlier than 2OH-CB3 according to the chromatographic properties (figure 5). Actually, McLean et al. (12) also found 5 OH-CB3s in rat liver microsomes *in vitro*. They identified peaks of 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3 using GC-MS. For the two peaks of unknown OH-CB3s without standards, they also proposed them to be 2OH-CB3 and 3OH-CB3. In summary, these chromatograms provide preliminary evidence for the metabolism of CB3 to 2OH-CB3 and 3OH-CB3 *in vivo* in whole plants, but confirmation with standards is needed.

Metabolic pathway of CB3 in poplar plants

There are two possible mechanisms explaining the formation of OH-PCBs from PCBs. One involves the formation of the epoxide intermediates under the functions of enzymes and then shifted to different OH-PCBs (28-30), which is a widely accepted theory with substantial supporting experimental evidence (31). The other is the formation of OH-PCBs from PCBs via direct insertion of the hydroxyl group to a biphenyl, which mainly applies to the formation of a single OH-PCB (32). In this work, three monohydroxy metabolites of CB were identified in whole poplar plants: 2'OH-CB3, 3'OH-CB3 and 4'OH-CB3. Assuming no chlorine shift, there are five possible monohydroxy metabolites of CB3. Though the other two monohydroxy metabolites of CB3 were not confirmed due to the unavailability of their standards, they were proposed to be 2OH-CB3 or 3OH-CB3 according to their physical and chemical properties. These data suggest that the metabolic pathway from CB3 to various OH-CB3s can be easily explained via epoxide intermediates. The proposed metabolic pathway from CB3 to OH-CB3s is shown in figure 6. The parent compound, 4-monochlorobiphenyl, was likely first catalyzed by cytochrome P450 (32) to yield 2',3'-epoxide, 3',4'-epoxide and 2,3-epoxide. These three epoxide intermediates then were isomerized to five possible monohydroxy CB3s. The major product in these five monohydroxy metabolites of CB3 was 4'OH-CB3, which is probably due to the lower steric hindrance of the 4'-position of CB3.

Similar to the results of this work, other authors (12) found five OH-CB3s, including 2'OH-CB3, 3'OH-CB3, 4'OH-CB3 and two unknown OH-CB3s in the *in vitro* experiment of liver microsomes from rats. In previous literature, dihydroxy and methoxy PCBs were also found after exposure to PCBs (12,28,33). For example, the existence of dihydroxylated metabolites of CB3 were detected (12) in the liver microsomes of rats and the dihydroxy and methoxy metabolites of 3,4,3',4'-tetrachlorobiphenyl were found in rats (28). Hydroxylation of PCBs plays a very important function in the total degradation of PCBs in living organisms. It was hypothesized that PCBs are first metabolized to OH-PCBs and then dechlorinated to final

inorganic products because OH-PCBs are dechlorinated much faster than parent-PCBs by microorganisms and animals (34–37). Plants intercept and modify the metabolism of volatile PCBs in the environment. Therefore, it is very important to understand the metabolic processes of OH-CB3s in poplars, a model plant and one used in phytoremediation. To our knowledge, this is the first report of the metabolic pathway of CB3 in whole plants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Abiotic Controls: A) Deionized water control, and B) Hydroponic nutrient-solution control. Concentrations of OH-CB3s ($n = 3$) developed over 10 days following an initial concentration spike of parent compound CB3 at a concentration of 1.0 mg L^{-1} .

Fig. 2.

A comparison of OH-CB3 concentrations ($n = 3$) in the solutions without poplars (deionized water control and hydroponic nutrient-solution control) and with poplars (autoclaved poplar, dead poplar, ethanol-washed poplar roots, and whole poplar) after 10 days exposure to a spiked concentration of 1.0 mg L^{-1} CB3.

Fig. 3.

A comparison of OH-CB3 concentrations ($n = 3$) in the different parts of a whole poplar plant after 10 days exposure (an initial concentration spike of parent compound CB3 was added on day zero at a concentration of 1.0 mg L^{-1}).

Fig. 4.

A comparison of the total mass of OH-CB3s ($n = 3$) in the different solutions and in poplar plants after 10 days. (At day zero, an initial spike of parent compound CB3 of approximately 400,000 ng was added.)

Fig. 5. Chromatogram of five OH-CB3s and their structures in the root sample (Fig.3). (upper chromatogram: root sample; lower: standards of 4'-OH-CB3, 3'-OH-CB3 and 2'-OH-CB3)

Fig. 6. Proposed metabolic pathway from CB3 to OH-CB3s in poplar plant tissues. Confirmed analytes are shown in bold type, and proposed compounds are in regular type.

Table 1

Comparison of the total mass of OH-CB3s (in ng, n = 3) in different solutions and different poplars after 10 days exposure to a spiked concentration of 1.0 mg L⁻¹ CB3 in 400 mL hydroponic solution.

Samples	Mass of 2'OH-CB3, ng	Mass of 3'OH-CB3, ng	Mass of 4'OH-CB3, ng	Total yield of OH-CB3s, %
Deionized water control	1.54±0.08	1.71±0.16	2.09±0.14	0.0013
Hydroponic solution control	11.41±0.84	9.53±0.27	23.82±4.24	0.011
Autoclaved poplar solution	29.27±4.66	44.45±7.52	68.62±6.18	0.036
Dead poplar solution	11.03±1.34	21.39±8.89	158.1±70.68	0.048
Washed poplar solution	134.1±26.56	32.92±6.53	72.03±13.34	0.060
Whole poplar solution	102.1±38.28	45.68±5.87	91.50±12.96	0.060
Blank poplar	ND	ND	ND	ND
Autoclaved poplar	49.09±10.69	34.82±2.77	264.1±140.6	0.087
Dead poplar	20.27±0.95	29.76±0.69	36.73±0.56	0.022
Washed poplar	229.6±36.09	78.28±12.73	574.4±141.6	0.22
Whole poplar	555.8±310.7	249.1±112.3	3422±764.0	1.06

ND=not detectable