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Inhibition of Cytochromes P450 and the Hydroxylation of 4-Monochlorobiphenyl in Whole Poplar

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Abstract

Cytochromes P450 (CYPs) are potential enzymes responsible for hydroxylation of many xenobiotics and endogenous chemicals in living organisms. It has been found that 4-monochlorobiphenyl (PCB3), mainly an airborne pollutant, can be metabolized to hydroxylated transformation products (OH-PCB3s) in whole poplars. However, the enzymes involved in the hydroxylation of PCB3 in whole poplars have not been identified. Therefore, two CYP suicide inhibitors, 1-aminobenzotriazole (ABT) and 17-octadecynoic acid (ODYA), were selected to probe the hydroxylation reaction of PCB3 in whole poplars in this work. Poplars (*Populus deltoides* × *nigra*, DN34) were exposed to PCB3 with or without inhibitor for 11 days. Results showed both ABT and ODYA can decrease the concentrations and yields of five OH-PCB3s in different poplar parts via the inhibition of CYPs. Furthermore, both ABT and ODYA demonstrated a dose-dependent relationship to the formation of OH-PCB3s in whole poplars. The higher the inhibitor concentrations, the lower the total yields of OH-PCB3s. For ABT spiked-additions, the total mass yield of five OH-PCB3s was inhibited by a factor of 1.6 times at an ABT concentration of 2.5 mg L⁻¹, 4.0 times at 12.5 mg L⁻¹, and 7.0 times at 25 mg L⁻¹. For the inhibitor ODYA, the total mass of five OH-PCB3s was reduced by 2.1 times compared to the control at an ODYA concentration of 2.5 mg L⁻¹. All results pointed to the conclusion that CYP enzymes were the agents which metabolized PCB3 to OH-PCB3s in whole poplars because suicide CYP inhibitors ABT and ODYA both led to sharp decreases of OH-PCB3s formation in whole poplars. A dose-response curve for each of the suicide inhibitors was developed.

Introduction

The ubiquity of polychlorinated biphenyls (PCBs) in the environment and their multiple reactions and effects have attracted significant attention during past decades (1). In recent years, hydroxylated metabolites of PCBs (OH-PCBs) have been detected in many species and habitats (2-6). Furthermore, the total concentrations of OH-PCBs detected in the human body have occasionally reached the same order of magnitude of concentration as the parent PCB congeners (6-8). More seriously, some metabolites of PCBs, including OH-PCBs, showed higher toxicity than their parent PCBs (9). In our previous work, hydroxylated metabolites of PCB3 and PCB77 were measured in exposed whole poplars in hydroponic solution (10-12). It was evident that plants produced the hydroxyl-metabolites in these experiments because dead plant controls, autoclaved plants, and abiotic systems produced very low concentrations of metabolites, indicating that bacteria, fungi, and abiotic processes, were of less importance. Moreover, OH-PCB3s further underwent phase II metabolism to

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produce PCB3 sulfates in whole poplar plants and rats in the recent publications (13-14). However, up to now, the enzymes responsible for hydroxylation of PCBs in living organisms were not identified, although cytochromes P450 (CYPs) were implicated in some *in vitro* experiments (15-16).

The CYP superfamily includes a large group of enzymes ranging from bacteria to plants and animals. The main function of CYPs is to catalyze the oxidation of organic compounds, including many xenobiotic substances (17), by activating molecular oxygen. Plants, like other organisms in the environment, can take-up and accumulate potentially toxic xenobiotics. Therefore, detoxification systems to remove xenobiotic chemicals are very important for plants. One of major detoxification processes in plants is chemical transformation, which includes phase I activation reactions, involving oxidation; and phase II reactions, involving conjugation or synthesis. In phase I, CYPs are thought to catalyze the major oxidation reactions of many xenobiotics. For example, plant CYPs have been reported to mediate hydroxylation and epoxidation in the biotransformation of xenobiotics *in vitro* (18-19). In addition, animal and recombinant human CYPs (15-16) were proposed as the enzymes metabolizing PCBs into OH-PCBs in *in vitro* experiments. However, little information existed on formation of OH-PCBs catalyzed by CYPs in whole plants. In this research, CYP inhibitors, 1-aminobenzotriazole (ABT) and 17-octadecynoic acid (ODYA) were employed as probes to shut-off the CYP-metabolic processes of hydroxylation of PCBs in plants.

Both ABT and ODYA are CYP suicide inhibitors (20). ABT is a well-known nonselective substrate inhibitor of both human and nonhuman CYPs *in vitro* and *in vivo*. It has been used extensively to distinguish CYP-mediated metabolism from non-CYP-mediated metabolism *in vitro* (21-24). Furthermore, ABT has also been proven to be safe in rats after an acute high dose and upon multiple dosing, making it an attractive agent for differentiating parent- or metabolite-based toxicities in safety assessment studies (25-26). Mico et al. (25) reported an examination of the time-course of inhibition of phenacetin elimination by ABT, a demonstration of dose-dependent inhibition of phenacetin and antipyrine clearances by ABT, and an examination of the acute toxicity of ABT in rats, as well as the effect of ABT on phenacetin metabolism in beagles. Their results demonstrated that ABT pretreatment caused long-lasting inhibition of oxidative metabolism of two drug species, phenacetin and antipyrine, without disruption of normal physiological processes. Meschter et al. (26) found ABT had only slight influence on the pharmacologic, toxicological, and microscopic effects of in male Sprague-Dawley rats over a 13-week period, which were considered to be well-tolerated under controlled laboratory conditions.

ODYA is a fatty acid analog with an ω -terminal acetylene and a suicide inhibitor of CYP fatty acid ω -hydroxylase (27-28). For example, Zou et al. (27) found that ODYA inhibited the metabolism of arachidonic acid by CYPs in renal cortical microsomes of rats. Furthermore, ODYA inhibited both ω -hydroxylation and epoxidation of arachidonic acid with IC₅₀ values of 7 and 5 μ M, respectively, using rat renal cortical microsomes and recombinant CYP proteins *in vitro*.

Even though five OH-PCB3s and two OH-PCB77s have been detected in PCB3 and PCB77 exposed whole poplars in our previous work (10-12), the active enzymes at play in whole poplars were still unclear. Therefore, two CYP inhibitors, ABT and ODYA, at various concentrations were applied in poplars exposed to PCB3 to confirm the functionality of CYPs in the oxidation of PCBs and to elucidate the mechanisms of transformation. The objective of this research was to prove the functionality of CYP on hydroxylation of PCB3 using CYP inhibitors.

Experimental Section

Chemicals

Five OH-PCB3s (2OH-PCB3, 3OH-PCB3, 2'OH-PCB3, 3'OH-PCB3 and 4'OH-PCB3; 98% purity or better) were synthesized and characterized using established procedures (29-30). Stock solutions of 2OH-PCB3, 3OH-PCB3, 2'OH-PCB3, 3'OH-PCB3 and 4'OH-PCB3 were prepared in acetonitrile at 1.0 mg mL⁻¹. Working solutions of 2OH-PCB3, 3OH-PCB3, 2'OH-PCB3, 3'OH-PCB3 and 4'OH-PCB3 were prepared by gradual dilution of the stock solution with acetonitrile. All standards and solutions of 2OH-PCB3, 3OH-PCB3, 2'OH-PCB3, 3'OH-PCB3 and 4'OH-PCB3 were stored in amber glass vials at 4 °C.

Two CYP inhibitors, ABT (98% purity) and ODTA (minimum 97% purity), were purchased from Sigma-Aldrich. Florisil (60-100 mesh, Acros Organics) was activated at 450 °C for 12 h, followed by deactivation with 1% water (w/w). Anhydrous sodium sulfate was purchased from Fisher Scientific. Methyl-*tert* butyl ether (MTBE) (HPLC grade), dichloromethane (HPLC grade), hexane (pesticide grade) and sodium hydroxide (98.6%) were 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 were of analytical reagent grade or better in this experiment.

Hydroponic Exposure

The exposure method was the same as our previous work (10). In brief, cuttings from male clones of the adult Imperial Carolina hybrid poplar tree (*Populus deltoides* × *nigra*, DN34) were fitted snugly into a pre-drilled screw cap and sealed with 100% silicone sealant. After 25 days of growth in half strength Hoagland nutrient solution, vigorously-growing whole poplar plants were selected for the following experiments. The exposure reactors were conical glass flasks (500 ml) with a sampling port. Hoagland solution (400 mL) prepared from autoclaved de-ionized and oxygen-saturated water was added to the autoclaved reactors. Except for the blank control trees and inhibitor control trees without PCB3, the starting concentration of PCB3 in each reactor was 1.0 mg L⁻¹. In addition, the different concentrations of ABT and ODTA were conducted in hydroponic solution of each reactor with PCBs. According to their solubility, the concentrations of ABT were set 0, 2.5, 12.5 and 25 mg L⁻¹ and the concentrations of ODTA were set 0, 0.25, 1.25 and 2.5 mg L⁻¹. Triple whole healthy poplar plants were used at each condition. All the procedures were conducted in a laminar flow hood and all reactors were wrapped with aluminum foil to eliminate photolysis of PCB3. 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 deionized water saturated with oxygen was injected into reactors to compensate for the transpiration losses. Approximately 60 mL d⁻¹ of water was transpired due to the growth of poplar plants during the exposure period.

In order to investigate the translocation and distribution of OH-PCB3s and the influence of CYP inhibitors on the formation of OH-PCB3s in different parts of poplar plants after 11 days of PCB3 exposure, each reactor specimen was divided into hydroponic solution, root, bottom bark, bottom wood, middle bark, middle wood, top bark, top wood, stem and leaf as described previously (10). Roots and leaves were ground in liquid nitrogen with a ceramic mortar and pestle. Other parts of the poplar plant were cut into very small pieces for efficient extraction of OH-PCB3s. All equipment was rinsed 3 times with reagent-grade acetone between samples.

Pretreatment and analysis

The extraction, separation and cleanup procedure for OH-PCB3s was the same as that in our previous work (10). The analysis of five OH-PCB3s was performed by Agilent Zorbax Bonus RP columns (2.1×150 mm, 5 μ m) on HPLC-MS (Agilent 1100 Series LC/MSD) with an autosampler. The operation parameters were the same as before (10).

Results and discussion

Lack of toxicity of ABT and ODYA to whole poplars

The toxicity of ABT and ODYA in whole poplars was investigated at different inhibitor concentrations (ABT: 2.5, 12.5 and 25.0 mg L⁻¹; ODYA: 0.25, 1.25 and 2.50 mg L⁻¹). Results suggested that both CYP inhibitors were not phytotoxic to whole poplars at all the applied concentrations. Furthermore, both CYP inhibitors in combination with PCB3 showed no phytotoxicity in whole poplars during 11 days of exposure. No particular symptom or phytotoxic effect was observed in either treatment group of plants with or without inhibitor, and all the poplars had similar water uptake due to transpiration and growth. Therefore, we concluded that ABT and ODYA did not produce any noticeable phytotoxic effect at the concentrations employed. Furthermore, no OH-PCB3s were detected in blank controls, which consisted of poplar plants without PCB3 exposure – this indicated that poplar plants were not contaminated by PCB3 from the laboratory during the experimental process.

ABT inhibits the formation of OH-PCB3s

ABT, a suicide inhibitor of CYPs, caused less formation of OH-PCB3 metabolites in poplar tissues compared to controls without ABT. Figure 1 showed that the concentrations of 2OH-PCB3, 3OH-PCB3, 2'OH-PCB3, 3'OH-PCB3 and 4'OH-PCB3 in whole poplars decreased sharply from 0, 2.5, 12.5 to 25.0 mg L⁻¹ of ABT, suggesting that ABT strongly inhibited the formation of OH-PCB3s by its effect on CYPs in whole poplars with a classic dose-response.

The total concentrations of five OH-PCB3s in different poplar tissues were increasingly inhibited at 2.5, 12.5 and 25.0 mg L⁻¹ of ABT. It can be seen from Figure 1 that the yield of five OH-PCB3s decreased sharply following increases in ABT concentration. First, the total concentrations of OH-PCB3s were slightly decreased at ABT concentrations from 0 to 2.5 mg L⁻¹, which suggested that an ABT concentration of 2.5 mg L⁻¹ inhibited the oxidation activities of CYPs in whole poplars. Second, the formation of OH-PCB3s was strongly inhibited by ABT at concentrations of 12.5 and 25.0 mg L⁻¹. None of the five OH-PCB3s were detected in stem, top wood and top bark samples at 12.5 and 25.0 mg L⁻¹ of ABT. Furthermore, compared with the total concentration of OH-PCB3s in poplar tissues without ABT, the corresponding total concentration of OH-PCB3s in poplar tissues at 25.0 mg L⁻¹ of ABT decreased by 17 times in middle wood, 4.7 times in middle bark, 66 times in bottom wood, 6.7 times in bottom bark, and 33 times in root. These results showed that ABT at a concentration of 25.0 mg L⁻¹ can almost completely inhibit the CYP oxidation activity to metabolize PCB3 in whole poplars. Therefore, the metabolism and translocation of OH-metabolites to the top stem and top wood was decreased markedly by the exposure of plants to suicide inhibitors. Also, the total concentrations of OH-PCB3s decreased from bottom wood to top wood and in bark samples at ABT concentrations of 0 and 2.5 mg L⁻¹. However, this effect was not observed at ABT concentrations of 12.5 and 25 mg L⁻¹. All in all, it can be concluded that ABT, a suicide CYP inhibitor, indeed inhibited CYP activities, leading to the reduction of OH-PCB3s in whole poplars.

More evidence of the tendency for ABT to inhibit the formation of OH-PCB3s in whole poplars can be found from their concentrations in different tissues shown in Figure 1. All five OH-PCB3s were detected in most of the plant tissues with or without ABT. Among these five OH-PCB3s, the 4'OH-PCB3 was the metabolite of greatest yield in all plant tissues except for the roots. The presence of ABT did not affect the outcome that 4'OH-PCB3 was the maximum metabolite formed. The concentrations of 4'OH-PCB3 in bark tissues were higher than those in the corresponding woods. However, the influence of ABT on the concentration of 4'OH-PCB3 was not the same at every concentration level. The concentration of 4'OH-PCB3 decreased from top bark, top wood and stem (the plant parts external to the aqueous exposure-reactor) at ABT concentrations of 0 and 2.5 mg L⁻¹. In addition, the concentrations of 4'OH-PCB3 in the stem, top wood and top bark samples at 2.5 mg L⁻¹ of ABT increased from 9.82±3.67, 10.71±6.69 to 17.66±2.01 ng g⁻¹. But all five OH-PCB3 metabolites were undetected in the stem, top wood and top bark samples at higher ABT concentrations of 12.5 and 25 mg L⁻¹, which suggested that the formation and translocation of OH-PCB3s was completely inhibited by higher ABT concentrations. Furthermore, the concentrations of five OH-PCB3s decreased in the bark and wood inside the aqueous exposure-reactor, including the middle and bottom parts, at ABT concentrations from 0, 2.5, 12.5 to 25 mg L⁻¹.

In the roots, the metabolite concentration of 2OH-PCB3 was the greatest of the five OH-PCB3s at the various ABT concentrations, except for ABT of 12.5 mg L⁻¹. Moreover, the concentrations of the five OH-PCB3s in the root samples clearly showed a decreasing tendency with the increase of ABT concentrations. For example, the concentrations of 2OH-PCB3 in root samples at ABT concentrations from 0, 2.5, 12.5 to 25 mg L⁻¹ decreased from 67.06±39.12, 29.96±8.16, 4.63±0.33 to 1.39±0.96 ng g⁻¹, respectively.

ODYA inhibits the formation of OH-PCB3s

The influence of ODTA, another suicide CYP inhibitor of OH-PCB3s production, was also investigated in whole poplars at ODTA concentrations of 0, 0.25, 1.25 and 2.5 mg L⁻¹. These concentrations were selected to be 10 times lower than ABT concentrations because of the low water solubility of ODTA. The yield of OH-PCB3s was comparable between ODTA and ABT when the same (low) inhibitor concentrations were employed. The inhibition of ODTA on the production of OH-PCB3s in whole poplars was shown in Figure 2 for 0.25, 1.25 and 2.5 mg L⁻¹ of ODTA.

The inhibition of ODTA on the total formation of OH-PCB3s in different poplar parts was presented in Figure 2. It was likely that PCB3 was translocated from the roots to the wood and eventually to the stem in gradually decreasing concentrations, but it was not translocated all the way to the leaves. Thus, the yield of OH-PCB3s metabolites decreased from the roots to the top wood and stem. Generally, the yield of OH-PCB3s decreased with increasing concentration of ODTA in the solution, and in plant tissues such as roots, bottom wood and top wood (those plant tissues most affected by PCB3 exposure and translocation). Results suggested that metabolism to OH-PCB3s occurred in those tissues and was inhibited by the presence of ODTA. Other tissues such as bottom bark, middle and top woods, did not show the characteristic inhibition pattern of lower OH-PCBs yields at higher ODTA inhibitor concentration. This was likely due to the fact that PCB3 (Log Kow=4.80) was translocated to higher portions of the plant than ODTA (Log Kow=7.17). Thus, OH-metabolites were formed in those tissues but were relatively less affected by ODTA inhibitor concentration (which was not as readily translocated).

Similar to the ABT experiment, 4'OH-PCB3 was the major product among the five OH-PCB3s in poplar at all ODTA concentrations, except for the root samples. Secondly, ODTA did not show a strong inhibition on formation of OH-PCB3s in poplar at lower

concentrations, such as 0.25 and 1.25 mg L⁻¹. These results suggested that ODYA concentrations at 0.25 and 1.25 mg L⁻¹ were only slightly inhibitory of the CYP activities. But compared to the samples without ODYA, there was a big influence on the yield of OH-PCB3s at an ODYA concentration of 2.5 mg L⁻¹, the highest ODYA concentration in this work. In addition, the inhibitor ODYA affected the relative proportion of the various OH-PCB3s isomers in whole poplars. For example, the concentrations of 2OH-PCB3, 2'OH-PCB3 and 3'OH-PCB3, in roots at the ODYA concentration of 2.5 mg L⁻¹ were much lower than those in roots without ODYA; and 3OH-PCB3 and 4'OH-PCB3 in roots at the ODYA concentration of 2.5 mg L⁻¹ had similar concentrations to those in roots without ODYA. This was because the roots directly contacted the ODYA in the solution such that the inhibitory function of ODYA on the CYP activity was very apparent in the roots, leading to lower concentrations of OH-PCB3s.

Inhibition of total masses of OH-PCB3s by two inhibitors

Suicide inhibitors exhibited a dose-response curve in their inhibitory effect on the transformation of PCB3 in poplar. The yield of OH-PCB3s was greatly decreased as the concentrations of suicide inhibitors were increased. The total masses of each OH-PCB3 at different inhibitor conditions were shown in Figure 3. First, the profiles of five OH-PCB3s were somewhat similar for different inhibitors and at various different concentrations. 4'OH-PCB3 was the major product in whole poplars at ODYA concentrations of 0, 0.25, 1.25 and 2.5 mg L⁻¹ and ABT concentration of 2.5 mg L⁻¹ and without inhibitor. However, 3'OH-PCB3 was the major product in whole poplar at ABT concentrations of 12.5 and 25 mg L⁻¹. These results suggested that the inhibitor species and concentrations together affected the profile of OH-PCB3 in whole poplars.

Furthermore, the ratios of the total mass of OH-PCB3s produced were also influenced by inhibitors and their concentrations. It can be seen from Figure 4 that there were different tendencies for the various ratios with different inhibitor conditions. First, the total mass of isomers on the opposite ring divided by the isomers on the chloro-ring (inverted triangles in Figure 4) showed a monotonic increase with ABT concentrations. But ODYA was relatively constant in that ratio. This means that ABT preferentially inhibits the formation of OH-PCBs on the same ring as the chlorine moiety. Furthermore, the ratio comparison of hydroxyl group in the same ring could elucidate how the inhibitors influence the isomerization process of intermediate epoxides by influencing the activity of CYPs in whole poplar plants (see Figure 4 results for 2OH/3OH, 4'OH/3'OH, and 2'OH/3'OH). For example, the total mass ratios of 2OH-PCB3 and 3OH-PCB3 were 3.02 without inhibitor, 2.05 at an ODYA concentration of 0.25 mg L⁻¹, 1.80 at an ODYA concentration of 1.25 mg L⁻¹, 1.77 at an ODYA concentration of 2.5 mg L⁻¹, 1.81 at an ABT concentration of 2.5 mg L⁻¹, 1.16 at an ABT concentration of 12.5 mg L⁻¹ and 1.25 at an ABT concentration of 25 mg L⁻¹, which suggested that the decrease of mass formation of 2OH-PCB3 was more than that of 3OH-PCB3 due to the formation of epoxide and its isomerization process following the concentration increase of ODYA and ABT.

Moreover, the inhibition of CYPs on the metabolism of PCB3 to OH-PCB3s can be explained via the total masses of the five OH-PCB3s in whole poplar at different inhibitor conditions shown in Figure 5. There was a clear dose-response relationship between the concentrations of ABT and the total mass of OH-PCB3s yielded. The general decreasing tendency of the curve for the dose-response relationships in Figure 5 for ODYA was similar to that of ABT, although the total mass of OH-PCB3s at 1.25 mg L⁻¹ of ODYA was a little higher than that at 0.25 mg L⁻¹ of ODYA. ABT strongly inhibited the formation of the five OH-PCB3s, and the dose-response curve showed a monotonic decrease in OH-PCB yield with increasing ABT concentration. All the total mass yield data showed that these two inhibitors inhibited formation of the OH-PCB metabolites, corresponding with the inferred

decrease in CYP activity from the suicide inhibitors. Therefore, it can be inferred that CYP enzymes were the catalysts necessary to metabolize PCB3 into OH-PCB3 in whole poplars.

CYP enzymes on hydroxylation of PCB3 in whole poplars

CYP enzymes are important enzymes that metabolize xenobiotics and detoxify pollutants in living organisms. Actually, both of the possible mechanisms explaining the formation of OH-PCBs from PCBs include the involvement of CYP enzymes. One mechanism was the formation of epoxide intermediates under the influence of CYP enzymes and subsequent rearrangement to form OH-PCBs (31-33). The other mechanism was a direct insertion of the hydroxyl group on the PCB phenyl rings to form OH-PCBs in the presence of CYP enzymes (34). However, up to now, little work has been done to prove that CYP enzymes were responsible for ring, or ring-alkyl hydroxylation of PCBs *in vivo*. In this research, the use of CYP inhibitors to study the formation of total OH-PCB3s was a good method to probe the role of CYP enzymes in the oxidation of PCBs in whole poplars.

Previous research (35) demonstrated that ABT strongly inhibited the metabolism of both chlortoluron and isoproturon in wheat plants, a higher plant species; and the wheat enzymes responsible for the ring-alkyl hydroxylation of these two herbicides were CYP enzymes, inferred via an ABT inhibition experiment. Similar to our results, ABT showed strong inhibition on the formation of OH-PCB3s at all the studied concentrations via the inhibition of CYP enzymes. Furthermore, the influence of CYP on the hydroxylated metabolism of 3-chlorobiphenyl (PCB2) was also studied in marine macroalgae, a lower plant species (36). ABT as an inhibitor of CYPs and phenobarbital as an inducer of CYPs were used to test for involvement of a P450 monooxygenase system in metabolism of PCB2 in marine macroalgae. Results demonstrated that ABT (80 mg L⁻¹) led to complete inhibition of the *in vivo* metabolism of PCB2, and phenobarbital (80 mg L⁻¹) led to other metabolites of PCB2 with total metabolism and yields increasing 5-fold, suggesting that CYP enzymes are the enzymes which metabolize PCB2 to OH-PCB2s in macroalgae. The maximum ABT concentration employed was 25 mg L⁻¹ in our research reported here, which was much lower than 80 mg L⁻¹ employed in the marine macroalgae work.

Plants and animals may use similar enzyme systems and gene families to metabolize a wide range of xenobiotics. The CYP super-family of enzymes has been detected universally in animal and higher plant species. But there have not been many studies of the influence of CYP inhibitors on the metabolism of xenobiotic chemicals in plants. Dose-dependent responses for ABT inhibition of CYP activities was concluded for some animal experiments (37-38). Balani et al. (37) found that antipyrine clearance by CYP enzymes was inhibited by ABT in animals, including rats, dogs, and monkeys and ABT also showed different degrees of inhibition on CYP activities for different animal species and a dose-dependent response. Moreover, their results showed *in vitro* inhibition of various expressed CYP enzymes upon 30 min preincubation with ABT (0-500 μM). Individual human enzymes CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A4 were inhibited in a dose-dependent manner by ABT, and the higher the ABT concentrations, the lower the CYP activities observed. Thus, the previously reported results with human enzymes were in relative agreement with our research reported here.

Compared with ABT, little work has been done regarding the inhibition of CYP enzymes by ODYA. Both ω-hydroxylation and epoxidation of arachidonic acid were inhibited by ODYA using rat renal cortical microsomes and recombinant CYP proteins *in vitro* (27-28). Our finding that ODYA inhibited the total yield of OH-PCB3s was the first demonstration in whole plants which inferred an active role for CYP enzymes in metabolism of PCBs by poplar.

Plants can take up volatile PCBs from water, soil and air. Therefore, it was important to understand the mechanism of metabolism of PCBs in plants. As a model plant widely used in phytoremediation, poplar was known to take up and metabolize PCB3 to corresponding OH-PCBs products. These products might be more toxic than the parent congener. In this research, two CYP suicide inhibitors, ABT and ODTA, clearly showed their abilities to inhibit the formation of hydroxylated metabolites of PCB3 in whole poplar. These results strongly suggested that CYP enzymes were the enzymes responsible for hydroxylation of PCB3 in whole poplars.

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Figure 1.
Influence of different ABT concentrations on total concentrations of OH-PCB3s in different poplar tissues.

Figure 2.
Influence of different ODYA concentrations on total concentrations of OH-PCB3s in different poplar tissues.

Figure 3.

Comparison of total mass of five OH-PCB3s in whole poplars at different inhibitor conditions: PCB3, only PCB3 without inhibitors, ODYA1, 0.25 mg L⁻¹, ODYA2, 1.25 mg L⁻¹, ODYA3, 2.5 mg L⁻¹, ABT1, 2.5 mg L⁻¹, ABT2, 12.5 mg L⁻¹, ABT3, ABT 25 mg L⁻¹.

Figure 4.

Comparison of total mass ratios of OH-PCB3s in whole poplars at different inhibitor conditions: PCB3, only PCB3 of 1.0 mg L^{-1} without inhibitor, ODYA1, 0.25 mg L^{-1} , ODYA2, 1.25 mg L^{-1} , ODYA3, 2.5 mg L^{-1} , ABT1, 2.5 mg L^{-1} , ABT2, 12.5 mg L^{-1} , ABT3, ABT 25 mg L^{-1} .

Figure 5. Comparison of total mass of five OH-PCB3s in whole poplars at different ABI and ODYA inhibitor concentrations.