

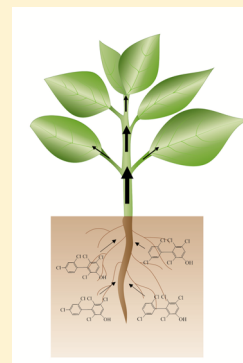
Enantioselective Transport and Biotransformation of Chiral Hydroxylated Metabolites of Polychlorinated Biphenyls in Whole Poplar Plants

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ABSTRACT: Hydroxylated metabolites of polychlorinated biphenyls (OH-PCBs) have been found to be ubiquitous in the environment due to the oxidative metabolism of their parent PCBs. With more polarity, OH-PCBs may be more toxic and mobile than their parent compounds. However, the behavior and fate of OH-PCBs have been neglected in the environment because they are not the original contaminants. Some of these hydroxylated metabolites are chiral, and chiral compounds can be used to probe biological metabolic processes. Therefore, chiral OH-PCBs were selected to study their uptake, translocation, transformation, and enantioselectivity in plants in this work. Poplars (*Populus deltoides* × *nigra*, DN34), a model plant with complete genomic sequence, were hydroponically exposed to 5-hydroxy-2,2',3,4',6-pentachlorobiphenyl (5-OH-PCB91) and 5-hydroxy-2,2',3,5',6-pentachlorobiphenyl (5-OH-PCB95) for 10 days. Chiral 5-OH-PCB91 and 5-OH-PCB95 were clearly shown to be sorbed, taken up, and translocated in whole poplars, and they were detected in various tissues of whole poplars. However, the enantioselectivity of poplar for 5-OH-PCB91 and 5-OH-PCB95 proved to be quite different. The second-eluting enantiomer of OH-PCB95, separated on a chiral column (Phenomenex Lux Cellulose-1), was enantioselectively removed in whole poplar. Enantiomeric fractions in the middle xylem, top bark, top xylem, and stem, reached 0.803 ± 0.022 , 0.643 ± 0.110 , 0.835 ± 0.087 , and 0.830 ± 0.029 , respectively. Therefore, 5-OH-PCB95 was significantly enantioselectively biotransformed inside poplar tissues, in contrast to nearly racemic mixtures of 5-OH-PCB95 remaining in hydroponic solutions. Unlike 5-OH-PCB95, 5-OH-PCB91 remained nearly racemic in most tissues of whole poplars during 10 day exposure, suggesting the enantiomers of 5-OH-PCB91 were equally transported and metabolized in whole poplars. This is the first evidence of enantioselectivity of chiral OH-PCBs and suggests that poplars can enantioselectively biotransform at least one chiral OH-PCB: namely, 5-OH-PCB95.



INTRODUCTION

Wide application and release of polychlorinated biphenyls (PCBs) in past decades have led to their ubiquity in the environment.¹ The production of PCBs was banned in the 1970s,^{2,3} but their persistence, high toxicity, and bioaccumulation continue to raise concerns regarding their long-term damage to living organisms. Once released into the environment, PCBs can be transformed by living organisms to a variety of metabolites, such as hydroxy-PCBs (OH-PCBs),⁴ methoxy-PCBs,⁵ and methylsulfony-PCBs.⁶ OH-PCBs are especially prevalent in various environmental media, including water,⁷ sediment,⁸ fish,⁹ mammals, birds,^{10,11} and human beings.¹² Furthermore, OH-PCBs were recently found in the original commercial Aroclors.⁸

OH-PCBs are mainly formed via oxidative mechanisms from their parent PCBs, such as the epoxide intermediates^{13,14} and direct insertion of the hydroxyl group to a biphenyl¹⁵ by living organisms. The produced OH-PCBs could be released into the environment and move through the food chain by trophic transfer. Thus, once formed, they may be considered a new class of environmental contaminants. In addition, OH-PCBs are gaining more attention because they are increasingly found in environmental samples due to new analytical techniques, and

they can exhibit greater toxic effects than their parent PCBs. For example, OH-PCBs were found to inhibit human hydroxysteroid sulfotransferase 2A1 (hSULT2A1) and act as substrates for sulfation reactions catalyzed by the enzyme.¹⁶ OH-PCB 3s inhibited the sulfonation of 4-nitrophenol in human liver cytosol¹⁷ and were mutagenic in the rat lung.¹⁸ Also, OH-PCB 3s can induce a surge in estradiol secretion in ovarian follicle cells, which would be expected to disrupt reproductive processes.¹⁹ Furthermore, OH-PCBs can have a great influence on brain development and function^{20–22} and on the endocrine system.^{23–26} Several OH-PCBs, such as 4-OH-PCB52 and 5-OH-PCB66, are more toxic than their parent PCBs in cell toxicity assays in mice.^{27,28}

Nineteen of 209 PCB congeners are chiral, and they can form stable rotational isomers.²⁹ Furthermore, at least 12 chiral PCBs, including PCBs 91 and 95, have been detected in commercial PCB mixtures.³⁰ As might be expected, chiral PCBs have been detected in a variety of environmental media, such as

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soil,^{31,32} sediment,³³ aquatic and riparian biota,³⁴ birds,³⁵ sharks and groupers,³⁶ dolphins,³⁷ whales,³⁸ and humans.^{39,40} Chiral PCBs were produced and released as racemic mixtures (two enantiomers of the chiral PCBs having the same ratio), such as Aroclors, but are frequently detected as nonracemic mixtures in biota⁴¹ because of the enantioselective formation of PCB metabolites in living organisms. The metabolites of chiral PCBs, such as OH-PCBs, are still chiral compounds.⁴² Chiral OH-PCBs have identical physical and chemical properties in the environment, and only biochemical processes can distinguish between the enantiomers of chiral OH-PCBs. Chiral OH-PCBs have been found to be enantioselectively formed from chiral PCBs by rats and rat liver microsomes.^{43–45} However, little is known about the behavior of chiral OH-PCBs after they are produced in living organisms. A nonracemic signature of chiral OH-PCBs suggests that the enantiomers of the chiral OH-PCBs behave differently in biochemical processes in living organisms. Therefore, the change in enantiomeric fractions (EFs) of chiral OH-PCBs from the racemic signature is a useful indicator of biological selectivity and can probe the metabolic processes and toxicological differences of chiral OH-PCBs and further predict their environmental fate.

Plants are the base of food chains and well-known for the “green-liver” metabolic model to transform and degrade of xenobiotic contaminants.^{46,47} Poplars are a widely used genetic model and widely used plant in the field of phytoremediation. They have been shown to accumulate and translocate some lower chlorinated PCBs⁴⁸ and to metabolize PCB3 and PCB77 to their OH-PCBs^{49,50} in previous work. However, whether whole poplars can take up and translocate chiral OH-PCBs enantioselectively was still unknown. To answer that question, chiral 5-hydroxy-2,2',3,4',6-pentachlorobiphenyl (5-OH-PCB91) and 5-hydroxy-2,2',3,5',6-pentachlorobiphenyl (5-OH-PCB95) were selected as model chiral OH-PCBs in this research because chiral PCB91 and PCB95 exist widely in the environment. The results will serve to elucidate the enantioselective behavior of chiral OH-PCBs in poplar.

■ EXPERIMENTAL SECTION

Chemicals. Three chiral OH-PCBs (5-OH-PCB91, 5-OH-PCB95, and 4-OH-PCB95; 98% purity or better) were synthesized by the Synthesis Core of the Iowa Superfund Research Center at the University of Iowa as described previously.^{51,52} The concentrations of these chiral OH-PCBs (1.0 mg mL⁻¹) were prepared in methanol as stock solutions. Working solutions of the OH-PCBs were prepared by gradual dilution of the stock solution with methanol. All solutions of these chiral OH-PCBs were stored hermetically in amber glass vials at 4 °C.

Methanol (HPLC grade), hexane (pesticide grade), 2-propanol (pesticide grade), and methyl *tert*-butyl ether (MTBE; pesticide grade) were purchased from Fisher Scientific (Pittsburgh, PA). The deionized water (18.2 MΩ) was collected from an ultrapure water system (Barnstead International, Dubuque, IA, USA). All other chemicals and reagents were of analytical reagent grade or better.

Exposure of Chiral OH-PCBs. Cuttings (8 in., 29.6 ± 1.8 g) of hybrid poplar tree (*Populus deltoides* × *nigra*, DN34, Segal Ranch, WA, USA) were grown hydroponically for 30 days. Only healthy, actively growing poplar plants were selected for chiral OH-PCBs exposure experiments. The exposure setup was the same as that described in previous papers.^{48–50} In brief, 5-OH-PCB91 and 5-OH-PCB95 with 0.10 mg L⁻¹ as the final

concentrations were added into autoclaved reactors containing 400 mL of half-strength of Hoagland solution (pH 6.8) except for the blank poplar control without chiral OH-PCBs. Specifically, blank plant controls were run in triplicate with whole poplar plants but without chiral OH-PCBs to control the background contamination during the experiment. Solution controls were run with half-strength Hoagland solution with chiral OH-PCBs. Dead plant controls were run in triplicate consisting of wilted, dead whole poplar plants with chiral OH-PCBs as inactive plant controls. Whole poplar plants were triplicate treatments of whole, growing, intact poplar plants with chiral OH-PCBs. The exposure experiment was conducted for 10 days. The deionized water saturated with oxygen was added to each reactor by weighing the reactor to compensate for the water loss due to evapotranspiration every day.

Each reactor specimen was divided into hydroponic solution, root, bottom bark, bottom xylem, middle bark, middle xylem, top bark, top xylem, stem, and leaf to study the dynamic processes of uptake and translocation of two chiral OH-PCBs in whole poplar plants. Roots and leaves were ground in liquid nitrogen using mortar and pestle. Other parts of the poplar plants were cut into small pieces to effectively extract chiral OH-PCBs.

Extraction and Cleanup. The extraction and cleanup procedure for PCBs was modified from the previous literature for poplar plants.⁴⁹ Briefly, 0.5 mL each of hydroponic solution samples was directly taken from the reactors and diluted with 0.5 mL of methanol, and then the mixed samples were filtered with 0.45 μm membrane into the vials for HPLC-MS/MS analysis.

The poplar tissue samples (1.0 g, wet weight) were put in centrifuge tubes. Then 100 ng of 4-OH-PCB95 was added as an internal standard. The poplar samples were mixed with 0.5 mL of 37% HCl and 2 mL of 2-propanol, homogenized, extracted with 10 mL of hexane/MTBE (1:1, v/v) and shaken vigorously overnight. The organic extract with OH-PCBs was transferred into another vial after the samples were centrifuged at 3000 rpm for 5 min. A second extraction of the samples using 5 mL of hexane/MTBE (1:1, v/v) was conducted for a half-hour, and then the organic extract was combined with the first organic extract after centrifugation. The combined organic extracts were evaporated to dryness under gentle nitrogen flow. The dry extracts were re-dissolved in 2 mL of hexane. Then 500 μL of NaOH solution (0.5 M in 50% ethanol) was added to partition the hexane phase and change the OH-PCBs into an ionic form to remain in the alkaline solution. After phase separation, the hexane phase was removed. The samples were extracted one more time using 2 mL of hexane, and the hexane phase was removed again. Next, the alkaline solution was acidified with 125 μL of HCl (2 M) and extracted twice with 2 mL of hexane. The organic extracts containing chiral OH-PCBs were combined, evaporated to dryness under a gentle nitrogen flow, and dissolved in 1 mL of methanol for HPLC-MS/MS analysis after filtration using a 0.45 μm membrane.

Instrumentation. Qualitative and quantitative analysis of chiral OH-PCBs was performed on HPLC-MS/MS (Agilent 1260-6460) with an autosampler. Sample separation was optimized using Phenomenex Lux Cellulose-1 (250 mm × 4.6 mm, 3 μm). The injection volume of each column was 5 μL with a column temperature of 6 °C. The mobile phase was methanol and water. For the gradient elution, a binary mobile phase of an aqueous solution of water (A) and methanol (B) at a flow rate of 0.5 mL/min was utilized. The gradient was set as

follows: 0 min (80% B), 55 min (80% B), 56 min (100% B), 90 min (100% B), and 91 min (80% B). The total run time was 100 min.

Mass spectrometry (MS) electrospray in negative ionization mode was utilized. Other analysis parameters were as follows: selected ion monitoring (SIM) ion mass, m/z 341; fragmentor, 100 V; capillary voltage, 3500 V; gas temperature, 350 °C; gas flow, 10 L min⁻¹; nebulizer pressure, 20 psi; sheath gas temperature, 400 °C; sheath gas flow, 12 L min⁻¹.

Enantiomer fraction or enantiomeric fraction (EF)⁵³ was used to calculate the enantiomer composition in this work:

$$EF = E1/(E1 + E2)$$

where E1 and E2 are the concentrations of the first-eluting enantiomer and the second-eluting enantiomer of 5-OH-PCB91 and 5-OH-PCB95 on the enantioselective chromatographic column. The enantiomers of internal standard 4OH-PCB95 cannot be separated by the chiral column used in this work. The standards of 5-OH-PCB91 and 5-OH-PCB95 were racemic with EF values of 0.500 ± 0.006 ($n = 6$) and 0.500 ± 0.004 ($n = 6$), respectively. The retention times of chiral OH-PCBs are 64.353 min (E1) and 64.860 min (E2) for 5-OH-PCB91, 67.164 min (E1) and 67.619 min (E2) for 5-OH-PCB95, and 71.483 min for 4OH-PCB95. Calculated detection limits ($S/N = 3$ according to their height) of two chiral OH-PCBs were 0.069 ng mL⁻¹ (E1(5-OH-PCB91)), 0.068 ng mL⁻¹ (E2(5-OH-PCB91)), 0.129 ng mL⁻¹ (E1(5-OH-PCB95)), and 0.127 ng mL⁻¹ (E2(5-OH-PCB95)), respectively. The recoveries of internal standard (4OH-PCB95) were $90.1 \pm 4.8\%$ for root, $80.6 \pm 2.5\%$ for bark, $80.4 \pm 3.9\%$ for wood, $83.0 \pm 2.7\%$ for stem, and $73.8 \pm 3.3\%$ for leaf samples.

The data of statistical analysis of 5-OH-PCB91 and 5-OH-PCB95 are presented for significant differences by one way ANOVA with Tukey test at $p = 0.05$.

RESULTS AND DISCUSSION

Effect of 5-OH-PCB91 and 5-OH-PCB95 on Evapotranspiration of Poplars. Toxicity of 5-OH-PCB91 and 5-OH-PCB95 to the poplars was inferred by measuring the rate of evapotranspiration. Decreased rates of evapotranspiration are a reliable indicator of decreased biomass growth and toxicity to the plant.⁵⁴ Poplars exposed to 0.1 mg L⁻¹ 5-OH-PCB91 and 5-OH-PCB95 had evapotranspiration rates comparable to those of blank poplar controls, suggesting that 5-OH-PCB91 and 5-OH-PCB95 did not have any adverse effects on the poplars at the experimental concentrations (0.1 mg L⁻¹) in 10 days (Figure 1).

Dynamic Concentrations and EFs of 5-OH-PCB91 and 5-OH-PCB95 in Solutions. The concentration decrease of 5-OH-PCB91 and 5-OH-PCB95 in the solutions over time suggests that 5-OH-PCB91 and 5-OH-PCB95 were continuously taken up and/or sorbed by poplar plants from the solutions. It can be seen from Figure 2 that 5-OH-PCB91 and 5-OH-PCB95 were rapidly removed from the solutions within 18 h, but the difference between 5-OH-PCB91 and 5-OH-PCB95 is not statistically significant ($p < 0.05$). Approximately 95.3% of 5-OH-PCB91 and 94.6% of 5-OH-PCB95 were taken up and sorbed in the whole poplars within 18 h, in contrast to 12.8% of 5-OH-PCB91 and 17.6% of 5-OH-PCB95 sorbed to the glass containers and 65.9% of 5-OH-PCB91 and 72.8% of 5-OH-PCB95 sorbed to the dead poplar and glass containers within 18 h. Furthermore, the concentrations of 5-OH-PCB91 and 5-OH-PCB95 slowly decreased in the solutions of dead

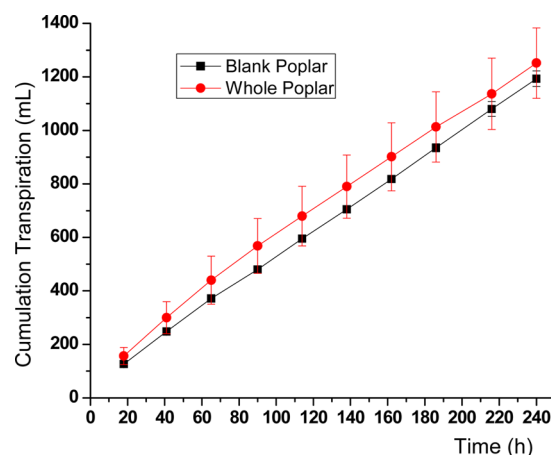


Figure 1. Cumulative evapotranspiration of water by blank poplar (unexposed to chiral OH-PCBs) and exposed whole poplar ($n = 3$) in 10 day experiments.

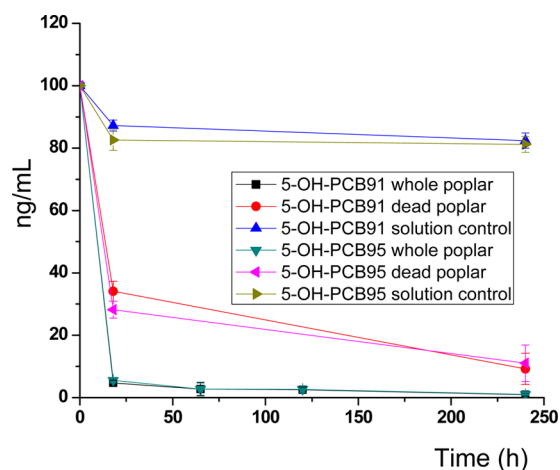


Figure 2. Dynamic concentrations of 5-OH-PCB91 and 5-OH-PCB95 ($n = 3$) in the solutions during 10 day exposures to whole poplar plants, dead poplars, and solution controls.

poplars and whole poplars after the first 18 h, while those in the solution controls almost remained the same. Therefore, these concentration changes in the solutions clearly showed whole poplars can take up and remove chiral OH-PCBs rapidly from the solutions.

The EF change is a powerful metric to indicate selective biotransformation of chiral compounds in biota; thus it was used in this work to show the enantioselectivity of 5-OH-PCB91 and 5-OH-PCB95 in poplar plants. Figure 3 shows that all of the EFs of 5-OH-PCB91 and 5-OH-PCB95 in the solutions, including the solution control, dead poplar, and whole poplar, were nearly racemic, which suggested that enantiomers of 5-OH-PCB91 and 5-OH-PCB95 were nearly equally taken up, transformed and sorbed to roots. The EF ratios were not significantly affected during the 10 day experiment in the hydroponic solution.

Distribution and EFs of 5-OH-PCB91 and 5-OH-PCB95 in Poplar Plants. Two chiral OH-PCBs (5-OH-PCB91 and 5-OH-PCB95) were not detected in any of the tissues of the "blank poplar controls", indicating that the reactors and poplars had no background contamination during the course of the experiment.

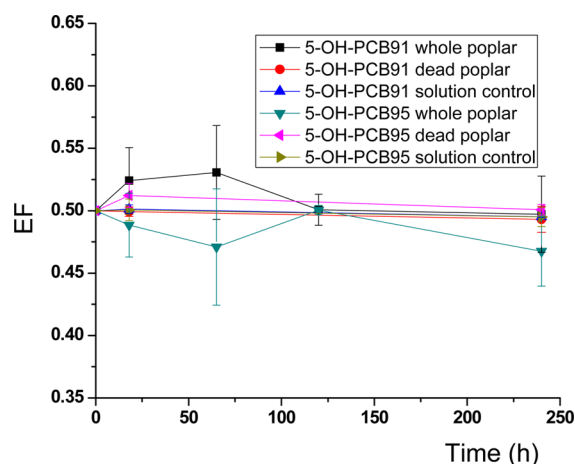


Figure 3. EFs of 5-OH-PCB91 and 5-OH-PCB95 ($n = 3$) in the various solutions over 10 days.

The chiral OH-PCBs were efficiently taken up and translocated by the whole poplars. Table 1 includes the results from a negative control (dead poplar plants) where living plant tissues were not present, but physical sorption (removal from solution) and microbial enantioselective transformation by microorganisms were possible. Transformation products (metabolites of OH-PCBs) were not measured directly in this research; rather we were interested in the selectivity and concentration of one enantiomer over another in plant tissues, which would indicate an enzymatic selectivity for one enantiomer over another (enantioselective transformation).

Table 2 gives the results of recoveries of 5-OH-PCB91 and 5-OH-PCB95 in the various reactor systems, including solution control, dead poplar control, and whole poplar plants exposed to 5-OH-PCB91 and 5-OH-PCB95. Approximately $84.3 \pm 6.3\%$ of 5-OH-PCB91 and $82.4 \pm 2.9\%$ of 5-OH-PCB95 masses added on day zero were recovered from the dead poplar controls (Table 2), while $58.6 \pm 3.7\%$ of 5-OH-PCB91 and $64.6 \pm 3.5\%$ of 5-OH-PCB95 masses added were recovered from whole poplars. It is likely that the remainder (the unrecovered mass) was due to volatilization through the reactor seal, unextractable or irreversible binding of chiral OH-PCBs and their metabolites to plant tissues and microbes, and/or experimental error during the course of the 10 day experiment. In particular, the lower recoveries of 5-OH-PCB91 and 5-OH-PCB95 in the whole poplar plants compared to those in dead poplar controls suggests that whole poplar plants can metabolize 5-OH-PCB91 and 5-OH-PCB95, though the possible metabolites of 5-OH-PCB91 and 5-OH-PCB95, for example sulfated metabolites, were not examined in this work.

Results from the negative control (dead poplar) in Figure 2 indicated that most of 5-OH-PCB91 and 5-OH-PCB95 were

Table 2. Total Mass Recovery of 5-OH-PCB91 and 5-OH-PCB95 in the Exposure System ($n = 3$)

recovery (%)	5-OH-PCB91	5-OH-PCB95
whole poplar	58.6 ± 3.7	64.6 ± 3.5
dead poplar	84.3 ± 6.3	82.4 ± 2.9
solution control	82.4 ± 2.5	81.2 ± 2.2

removed from solution, and it was sorbed by the plant along the bottom bark, bottom wood and middle bark which were directly exposed to 5-OH-PCB91 and 5-OH-PCB95 in the hydroponic solution or headspace (Table 1). This mass movement is consistent with physical sorption to bark tissues. Some uptake and transformation could have occurred in the dead poplar controls by regrowth of microbes, but it was small based on the solution control. EFs were not significantly different between hydroponic solution and various tissues from the negative controls, with a range from 0.484 ± 0.015 to 0.493 ± 0.007 for 5-OH-PCB91 and from 0.496 ± 0.006 to 0.521 ± 0.004 for 5-OH-PCB95 in dead poplars during the 10 day exposure. Therefore, microorganisms had no significant enantioselective influence on 5-OH-PCB91 and 5-OH-PCB95 in dead poplar plants.

In whole poplar plants, 5-OH-PCB91 and 5-OH-PCB95 were detected in a variety of plant tissues inside and outside the aqueous exposure of the reactors (Figure 4), suggesting that

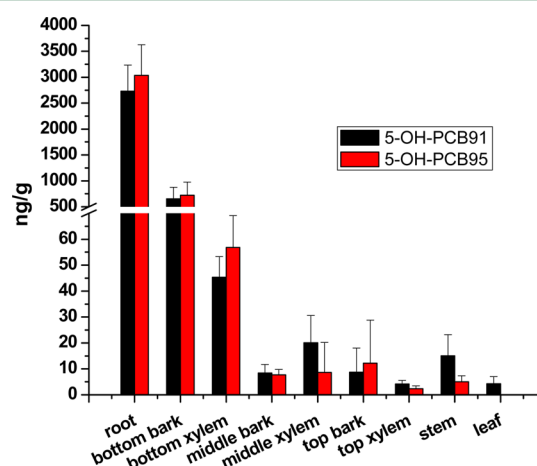


Figure 4. Concentrations of 5-OH-PCB91 and 5-OH-PCB95 ($n = 3$) in the various tissues of exposed whole poplar plants.

chiral 5-OH-PCB91 and 5-OH-PCB95 were readily translocated in whole poplars compared to PCB95, which is less polar and did not readily translocate to tissues outside the aqueous exposure of the reactors.⁵⁵

Table 1. Concentrations (ng g^{-1}) and EFs of 5-OH-PCB91 and 5-OH-PCB95 in the Dead Poplar Controls ($n = 3$)^a

	5-OH-PCB91		5-OH-PCB95	
	ng g^{-1}	EFs	ng g^{-1}	EFs
middle wood	ND		ND	
middle bark	75.4 ± 12.3	0.484 ± 0.015	5.86 ± 1.09	$0.521 \pm 0.004^*$
bottom wood	437 ± 38.4	0.491 ± 0.007	40.2 ± 5.86	0.496 ± 0.006
bottom bark	$(8.64 \pm 2.21) \times 10^3$	0.493 ± 0.007	$(8.58 \pm 1.83) \times 10^3$	0.502 ± 0.014
solution	9.23 ± 5.03	0.493 ± 0.010	11.0 ± 5.85	0.501 ± 0.004

^aThe asterisks (*) denotes the statistically significant ($p < 0.05$) change of EF compared to standard. ND = not detectable.

It can be seen from Figure 4 that 5-OH-PCB91 and 5-OH-PCB95 can be sorbed and taken up by the healthy root tissues, and translocated through xylem and bark to the stems and leaves. In particular, 5-OH-PCB91 was found in the leaf, which suggested that 5-OH-PCB91 was easier to be translocated than 5-OH-PCB95 in whole poplars. Once again, large fractions of 5-OH-PCB91 and 5-OH-PCB95 were removed from solution (Figure 2), but much more resided on the bottom xylem (2730 ± 502.9 and 3036 ± 589.9 ng g⁻¹, respectively), bottom bark (654 ± 223 and 725 ± 254 ng g⁻¹, respectively), and the roots (45.3 ± 8.0 and 56.8 ± 12.3 ng g⁻¹, respectively). Although the concentrations of 5-OH-PCB91 and 5-OH-PCB95 in the rest of the tissues, such as the top xylem, top bark, stem, and leaf, are relatively lower, they are of importance because these data definitely showed that these two chiral OH-PCBs can be translocated inside the whole poplar to the aerial portion of the plant which makes it more available for food chain transfer. For 5-OH-PCB91 and 5-OH-PCB95 in xylem and bark samples, their concentrations decreased following the same pattern from bottom → middle → top. In addition, the total masses of 5-OH-PCB91 and 5-OH-PCB95 in the poplar plants are not significantly different ($p < 0.05$) (Figure 4).

The changes of EF ratios elucidate the enantioselective biological processes of poplar for these chiral compounds. Enantioselective translocation (e.g., rejection at a membrane by one enantiomer and not the other) would have yielded EFs of nearly 0.5 (the racemic mixture) because both enantiomers would have been extracted from the tissue sample and analyzed. On the other hand, enantioselective biotransformation results in one enantiomer complexing with an enzyme to form an enzyme–substrate complex and being transformed. Upon extraction, this case would result in EFs greater or less than 0.5. Figure 5 showed that 5-OH-PCB95 underwent enantiose-

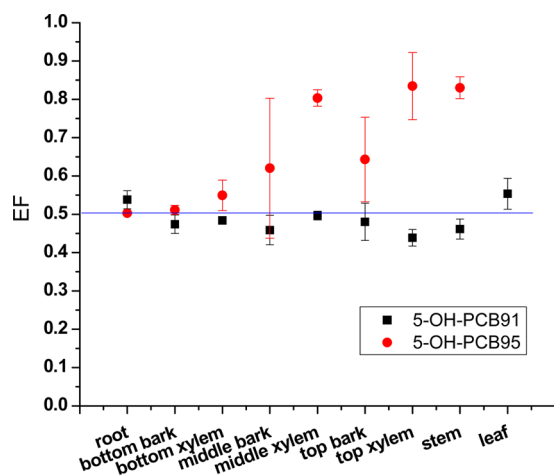


Figure 5. EFs of 5-OH-PCB91 and 5-OH-PCB95 ($n = 3$) in the various tissues of exposed whole poplar plants.

lective biotransformation, not enantioselective translocation, in the whole poplars because all of the EFs in the various tissues in 10 days are not less than the original EF (0.5). EFs of 5-OH-PCB95 are significantly different compared to the initial EF of 5-OH-PCB95 in various tissues of whole poplars, except for the root and bottom bark, where high concentrations of 5-OH-PCB95 may have overshadowed the EF ratio changes. Due to the different properties and functions of xylem and bark, it is likely 5-OH-PCB95 can be transported by two routes: by bark

and xylem routes. The bark route is root → bottom bark → middle bark → top bark → stem and the xylem route is root → bottom xylem → middle xylem → top xylem → stem. The EF value in the stem is between that of the top xylem and top bark because the tender stem was not separated into bark and xylem. Significantly, EFs increased following either the bark route or xylem route. Especially in middle xylem, top bark, top xylem and stem, EF reached 0.803 ± 0.022 , 0.643 ± 0.110 , 0.835 ± 0.087 , and 0.830 ± 0.029 , respectively, which indicated a preferential loss (binding) of E2(5-OH-PCB95) as it was translocated from the root to the top tissues for 5-OH-PCB95. However, these two routes are not independent. There is likely some interaction and exchange of 5-OH-PCB95 between bark and xylem during the translocation. These EF data strongly suggest that 5-OH-PCB95 was enantioselectively biotransformed, either by enzymatic reaction (e.g., formation of PCB95 sulfate compound/s) or by enzyme–complex formation and binding to cellular tissues (e.g., complexation with reduced glutathione, GSH), or both in the whole poplar plants.

In contrast to 5-OH-PCB95, 5-OH-PCB91 never showed the apparent enantioselectivity in whole poplars (Figure 5). The EFs of 5-OH-PCB91 were nearly racemic, which suggests that chiral compounds of 5-OH-PCB91 were translocated equally, in the whole poplars. Also, they were biotransformed compared to controls (Table 2), but equally so, and not enantioselectively (EF ~ 0.5).

Comparison of 5-OH-PCB91 and 5-OH-PCB95 in Whole Poplars. Two chiral OH-PCBs (5-OH-PCB91 and 5-OH-PCB95) showed completely different enantioselectivity in whole poplars. The compound 5-OH-PCB95 showed significant enantioselective biotransformation as measured by the steadily increasing EF (Figure 5). However, 5-OH-PCB91 remained nearly racemic and showed only very slight enantioselective biotransformation and/or translocation in some tissues of whole poplars, as measured by small deviations from EF = 0.5 (Figure 5). However, it is clear that both of them (5-OH-PCB91 and 5-OH-PCB95) are biotransformed in the whole poplar plants due to their similar total mass recoveries (58.6 ± 3.7 and $64.6 \pm 3.5\%$, respectively), much lower mass recoveries than controls (Table 2).

There were no reports in the literature regarding enantiomeric selectivity of OH-PCB91 and OH-PCB95 in living organisms initially exposed to OH-PCB91 and OH-PCB95. However, we may get clues from enantiomeric differences of their parent compounds—PCB91 and PCB95. It is reported that enantiomeric enrichment of chiral PCB91 and PCB95 was completely different and species-dependent in some living organisms, such as arctic cod (*Boreogadus saida*), black guillemot (*Cepphus grille*), glaucous gull (*Larus hyperboreus*), and ringed seal (*Phoca hispida*).⁵⁶

The different behaviors of 5-OH-PCB91 and 5-OH-PCB95 may be explained by the relationship of their structures, which greatly influenced their biochemical behaviors in living organisms. For example, both PCB91 and PCB95 are pentachlorinated PCBs, but it is reported that PCB91 and PCB95 (and their metabolites 5-OH-PCB91 and 5-OH-PCB95) with more unsubstituted carbons on the meta-position bind better to the CYP enzymes than compounds with more substitutions in the meta-position.⁵⁷ Therefore, the fact that 5-OH-PCB91 has two unsubstituted carbon atoms at the meta-position of the nonphenolic ring, and 5-OH-PCB95 has one unsubstituted carbon atom, may account for the different behaviors of 5-OH-PCB91 and 5-OH-PCB95 in whole poplar.

Similar to our results, E1(5-OH-PCB95) was found to be enriched in mouse liver samples from the high dose of the PCB95 treatment group.⁴³ Furthermore, OH-PCBs were further metabolized to PCB sulfate by sulfotransferase SULT2A1⁵⁸ and PCB3 sulfates were actually detected in the rats and poplars exposed to PCB3.^{59,60} Taking one of the possible metabolic pathways PCBs → OH-PCBs → PCBs sulfates as an example, the enantiomeric formation of OH-PCBs could be due to the transformation of PCBs → OH-PCBs, or OH-PCBs → PCBs sulfates, or both. Liu et al.⁶¹ found that the presence or absence of a chlorine atom at the para-position of the nonphenolic ring of OH-PCBs has a small effect on the magnitude of the 50% inhibition concentration (IC₅₀) value observed. However, a change in chlorine atoms at the meta-positions on the nonphenolic ring of OH-PCBs results in large changes in the interactions with rSULT1A1. That is similar to the structural difference of our OH-PCB91 (with a chlorine atom at the para-position of the nonphenolic ring) and OH-PCB95 (with a chlorine atom at the meta-position of the nonphenolic ring).

It is important to study the behavior and fate of (chiral) OH-PCBs in the environment even though they are the secondary contaminants. The wide distribution and large amount of OH-PCBs in the environment and their potential toxicity make them a major new environmental contaminant class. However, little is known about the behavior and fate of OH-PCBs in living organisms. Plants are the base of the food chain and have a huge biomass on earth. Therefore, plants, such as poplars, might play an underappreciated role in the (enantioselective) biotransformation of (chiral) OH-PCBs. More plant and animal species should be studied for enantioselective transformations of chiral compounds like OH-PCBs because of the potential fate and effects of such compounds in the environment. This work reports the behavior and fate of chiral OH-PCBs in one living organism, poplar. To the best of our knowledge, it is the first report of enantioselective biotransformation of chiral OH-PCBs in plants.

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Notes

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