SELECTIVE ACCUMULATION OF COPLANAR PCB CONGENERS BY *ARABIDOPSIS THALIANA*

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Introduction

Polychlorinated biphenyls (PCBs), one of the halogenated aromatic hydrocarbons, are ubiquitous pollutants of great concern due to their characteristics of recalcitrance towards environmental breakdown and human toxicity such as endocrine disruption and carcinogenic effects. The high chemical stability and hydrophobic nature of PCBs make them a persistent environmental hazard[1-4]. Among the possible 209 PCB congeners, the environmental toxicity of coplanar PCBs (Co-PCBs) is becoming more severe. To clean up the polluted environment, biological remediation system using plants, so-called phytoremediation, is expected to solve the environmental pollution problem. There are some reports on the remediation of PCBs by plants; however, few reports referred to the precise analysis of Co-PCBs congeners accumulated in plants[5-8]. In this study, we investigated the accumulation of Co-PCB congeners into the model plant, *Arabidopsis thaliana*, by high-resolution GC-MS. As a result, it was found that the accumulation rate greatly depended on the chlorination rate of Co-PCB congeners. Lower chlorinated congeners were accumulated to a much greater level than higher chlorinated congeners, and non-ortho congeners were accumulated to a lower level than ortho congeners.

Materials and Methods

Chemicals

Co-PCB congeners of tetrachlorobiphenyl (TCB), pentachlorobiphenyl (PeCB), hexachlorobiphenyl (HxCB) and heptachlorobiphenyl (HpCB) were purchased from Wellington Labs (Ontario, Canada). Each congener in toluene was mixed and diluted to a concentration of 0.5mg/l in dimethylsulfoxide, and then this standard solution was diluted to 0.5 ng/ml in distilled water for the exposure experiment to plants. All solvents were pesticide-free, reagent grade. All other chemicals used were of analytical grade.

Plant material and exposure to Co-PCB congeners

Arabidopsis thaliana ecotype Columbia (The Sendai Arabidopsis Seed Stock Center, Japan) were grown on the rock wool at 26°C under a 16hr light / 8hr dark cycle. One and half month old seedlings were exposed to Co-PCBs congeners in the closed chamber for one week and two weeks, respectively.

Preparation of Co-PCBs from harvested plants for GC-MS

The above-ground parts of 3 seedlings were carefully cut off and used for one experiment. As internal standards for the precise measuring of Co-PCBs, ^{13}C-3,3',4,4'-tetrachlorobiphenyl for tetrachloro-congeners analysis and ^{13}C-3,3',4,4',5'-pentachlorobiphenyl for pentachloro-, hexachloro-, and heptachloro-congeners analysis were added to the harvested seedlings, and then the mixture was ground immediately under liquid nitrogen and hydrolyzed with 1 mol/L KOH in ethanol overnight. Co-PCBs were extracted with hexane 3 times, and then sulfuric acid was added to the hexane extract at a concentration of 18 mol/L for acid hydrolysis. After rinsing the hexane extract with distilled water, dehydration and concentration, column chromatography was carried out on a two-layer column packed with 3g of silica gel for the upper layer and 10g of alumina for the lower layer. The elution of Co-PCBs was performed with 2%(v/v) dichloromethane in hexane, and then ^{13}C-3,3',4,4',5,5'-hexachlorobiphenyl was added to the concentrated solution of Co-PCBs as an internal standard with the syringe spike for a correction of injection volume in GC-MS.

GC-MS condition

Gas chromatograph (HP6890/HEWLETT PACKARD, USA) having SPB-5 capillary column (30m/SUPELCO, USA) equipped with high-resolution mass spectrometry detector (MStation JMS-700/JEOL, Japan) was used for the analysis of Co-PCB congeners. The mass values used for single ion monitoring were 291.9194, 325.8804, 359.8415 and 393.8025 for TCBs, PeCBs, HxCBs and HpCB, respectively.

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Results and Discussion

Selective accumulation of Co-PCB congeners

Co-PCB congeners were sharply separated except PCB156 and PCB157. Therefore, 11 of 12 congeners, with the exception of PCB157, were investigated in this study. As shown in Fig.1, the lower chlorinated Co-PCBs such as the two TCBs, PCB77 and PCB81, and four of the five PeCBs, PCB105, PCB114, PCB118 and PCB123, were accumulated to a much greater level than the higher chlorinated congeners, the PeCB, PCB126, the HxCBs and HpCB. It seems that this segregated result is due to the difference in water solubility among the Co-PCB congeners rather than to the selective uptake of congeners by plant. The highly accumulated TCBs and PeCBs have higher water solubility, compared to the HxCBs and HpCB. The solubility value of each TCB, PeCB, HxCB and of HpCB at 25°C is 2.70-3.13, 0.899-2.63, 0.130-1.10 and 0.063 ng/ml,

respectively[9].

Interestingly, non-ortho congeners in the same chlorinated congeners, such as PCB126 of the PeCBs and PCB169 of the HxCBs, were accumulated to a lower level than the ortho congeners. This segregated result may also be due to the difference in water solubility between non-ortho and ortho congeners. The accumulation levels of lower chlorinated congeners were proportionally increased in proportion to the exposure levels of the congeners, whereas the accumulation levels of higher chlorinated congeners were not. Fig.2. shows that for all congeners the accumulation level increased with the doubling of the time of exposure from 7 to 14 days. Especially, the accumulation rate of the higher chlorinated congeners, HxCBs and HpCB, was remarkably increased with the doubling of exposure time.

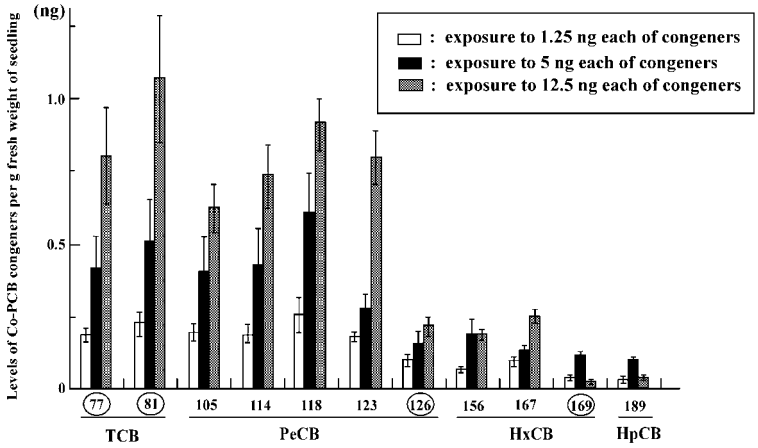

Fig. 1. Effect of exposure levels of Co-PCBs on the accumulation by *Arabidopsis thaliana*

Seedlings were exposed to each levels of Co-PCB congeners for 7 days. Each congener is indicated with the IUPAC number, and open circled IUPAC numbers reveal non-ortho Co-PCBs. Each bar represents the mean and the standard deviation of five determinations.

Accumulation of Co-PCB congeners from air

Co-PCBs have relatively high vapor pressure, therefore, it is expected that some of Co-PCBs accumulated in plants are taken from the air through leaves or some may be just adsorbed on the surface of plants. Levels of congeners taken up from the air or adsorbed on the surface were investigated using plants that were set beside the solution of Co-PCBs in the sealed container. As a result, TCBs, which have a

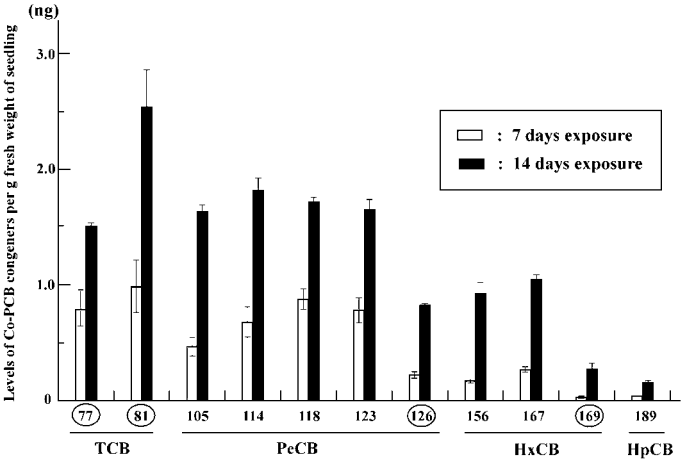

Fig. 2. Time-course of accumulation of Co-PCBs by *Arabidopsis thaliana*

Seedlings were exposed to 12.5 ng each of Co-PCB congeners. Details as in Fig1.1.

higher vapor pressure than the other congeners, were accumulated to a greater level from the air (Fig.3.). Particularly, in the accumulation of PCB 81, 45% of the total level found in plants was from the air.

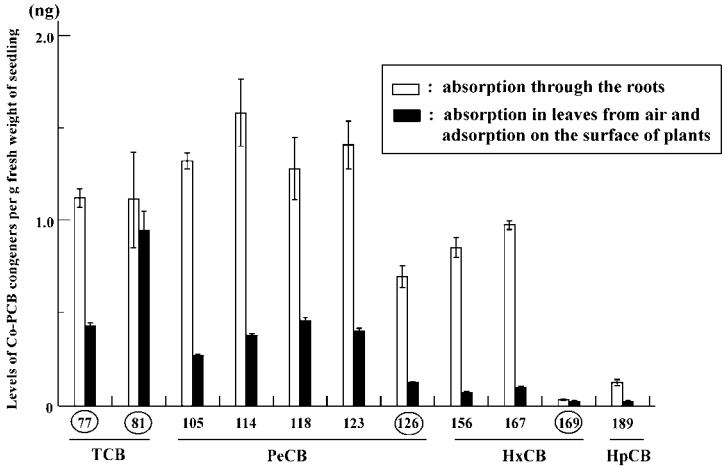

Fig. 3. Accumulation of Co-PCBs by *Arabidopsis thaliana* through the roots or from air
Seedlings were exposed to 12.5 ng each of Co-PCB congeners for 14 days. Details as in Fig.1.

Conclusion

The lower chlorinated congeners, the two TCBs and four of the five PeCBs, except 126, were selectively accumulated, compared to the higher chlorinated congeners, the HxCBs and HpCB. It was determined that the congener-selective accumulation by *Arabidopsis thaliana* highly depended on the chlorine content and on whether the chlorine position isomerism was ortho or non-ortho. The Co-PCBs metabolizing ability of *Arabidopsis thaliana*, such as biodeterioration or biodegradation, is also to be elucidated; however, the finding in this study is expected to be helpful in the future remediation study of contaminated environment using plants.

Acknowledgements

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