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Evidence of PCDD/Fs and PCBs Contamination in Trees Grown in Forests Far from Their Production and Contamination-Free Areas

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Abstract Polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) in trees grown in pathless forests were analyzed to investigate their dispersal patterns in the atmosphere. The pg/g levels of 23 PCCD, 22 PCDF, and 54 PCB congeners were identified. The total amount of PCDDs in the red pine tree sample (95.8 pg/g) was approximately 7 times that in the beech tree sample (13.2 pg/g). The total amount of PCDFs in the red pine tree sample (71.1 pg/g) was also approximately 7 times that in the beech tree sample (11.1 pg/g). The total amounts of PCBs in the red pine tree and beech tree samples were 1,380 and 1,150 pg/g, respectively. The (Cl₁–Cl₃)-PCBs comprised 63.1 % and 67.6 % of total PCBs in the red pine and in the beech, respectively. The results of the present study indicate that PCDD/Fs and PCBs are transferred over long distances in the atmosphere and accumulated in the contamination-free areas.

Keywords Dioxins · PCBs · Long range transport · Tree samples

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The presence of polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (PCDD/Fs) in the atmosphere has long been known because they form during the incineration of the chlorine atom, which contains materials such as polyvinyl chloride plastics, and escape into ambient air (Yasuhara et al. 2001). Also, 2,3,7,8-tetrachloro dibenzo-*p*-dioxin (2,3,7,8-TCDD), which is one of the most potent carcinogens, is formed during the synthesis of herbicide 2,4,5-trichlorophenoxy acetic acid (2,4,5-T) as a byproduct and stays in the final product of 2,4,5-T. Additionally, 2,4,5-T has been used widely for many years, and 2,3,7,8-TCDD is expected to be deposited into the environment and eventually entered into the food chain. Polychlorinated biphenyls (PCBs) had been used widely in various electric appliances, such as transformers, vacuum pumps, and air conditioners, as well as for cutting oils, flame retardants, and pesticide formulation (Rudel et al. 2008). Therefore, PCBs were deposited into the environment continuously until they were banned by the United States Congress in 1979 (Porta and Zumeta 2002).

PCDD/Fs and PDBs are some of the most persistent organic pollutants (POPs), and they are transferred into the atmosphere as particulates (Diamond et al. 2001). Background levels of PCDD/Fs have been found in soil and sediment samples from uncontaminated areas, which are located far from their point of origin (Hashimoto et al. 1995; Porta and Zumeta 2002). For example, background levels of total PCDD/Fs in plants are reportedly around 50 ng/kg in England (Startin et al. 1989). In the US, however, the concentrations of individual PCB congeners in the bark of trees at a landfill site averaged 18 µg/kg, whereas level at the site far from the landfill was 0.5 µg/kg (Meredith and Hites 1987).

The accumulation of POPs, including PCDDs/Fs, in vegetation has been reviewed from several perspectives,

including research on the uptake mechanism, the use of vegetation as a qualitative indicator of contaminant levels, and the importance of vegetation as a pollutant sink (Simonich and Hites 1995). Several investigations have revealed that the uptake of PCDD/Fs in contaminated soil via vegetation roots was not significant compared to their uptake from the atmosphere (Jones and Duarte-Davidson 1997; Wagrowski and Hites 1998). PCDD/Fs and PCBs are proposed to be deposited into atmospheric particles, which are absorbed into leaves. Once they are absorbed into leaves, they move to other part of trees, such as trunks.

In the present study, PCDD/Fs and PCBs in the center sections of evergreen needle-leaved trees and broad-leaved deciduous trees, which grew naturally in pathless forests far from their occurrence, were analyzed to investigate their possible long-range spreading in the atmosphere.

Materials and Methods

A Japanese beech tree (40 years old) and a Japanese red pine tree (30 years old) were obtained from the northern part of Takayama City, Gifu Prefecture, Japan, and the central part of Mino City, Gifu Prefecture, Japan, respectively. Both collection areas are located in pathless forests. A tree (trunk diameter is apx. 50 cm at the bottom) was cut down and a trunk sample was prepared using a chain saw in the forest. The trunk sample was carried out of the forest to the area with an auto-path by a hand-cart and then taken to the research laboratory by a van.

After removal of the bark, the heartwood was ground into powder (sawdust) by a model HE-100 saw (Hirota Co., Shimada, Shizuoka, Japan).

A 500 μL nonane solution containing 0.0005 $\text{ng}/\mu\text{L}$ each of $^{13}\text{C}_{12}$ -1,2,3,4-TeCDD, $^{13}\text{C}_{12}$ -1,2,3,4,7,8-HxCDD and $^{13}\text{C}_{12}$ -1,2,3,4,7,8,9-HpCDF was added to sawdust (50 g) as the standard for a sampling spike, and then the sawdust dust (50 g) was mixed with a 1,000 mL HCl solution (over 0.5 mol/L) and allowed to stand for 1 h. After the sample solution was vacuum-filtered, the residual sample was extracted with toluene (500 mL) using a Soxhlet extractor. The aqueous filtrate was extracted three times with a 50 mL dichloromethane. The dichloromethane extract and the toluene extract were combined, and a clean-up spike solution (a 100 μL nonane solution containing 0.005 $\text{ng}/\mu\text{L}$ of each $^{13}\text{C}_{12}$ -2,3,7,8-TeCDD, $^{13}\text{C}_{12}$ -1,2,3,7,8-PeCDD, $^{13}\text{C}_{12}$ -1,2,3,6,7,8-HxCDD, $^{13}\text{C}_{12}$ -1,2,3,4,6,7,8-HpCDD, $^{13}\text{C}_{12}$ -1,2,3,4,6,7,8,9-OCDD, $^{13}\text{C}_{12}$ -2,3,7,8-TeCDF, $^{13}\text{C}_{12}$ -1,2,3,7,8-PeCDF, $^{13}\text{C}_{12}$ -1,2,3,4,7,8-HxCDF, $^{13}\text{C}_{12}$ -1,2,3,4,6,7,8-HpCDF, $^{13}\text{C}_{12}$ -1,2,3,4,6,7,8,9-OCDF, $^{13}\text{C}_{12}$ -2,3,3',4,4'-TeCB, $^{13}\text{C}_{12}$ -3,4,4',5'-TeCB, $^{13}\text{C}_{12}$ -3,3',4,4',5'-PeCB, $^{13}\text{C}_{12}$ -2',3,4,4',5'-PeCB, $^{13}\text{C}_{12}$ -3,3',4,4',5,5'-HxCB, $^{13}\text{C}_{12}$ -2,3',4,4',5,5'-HxCB, $^{13}\text{C}_{12}$ -2,3,3',4,4',5,5'-HpCB) was added at

this point, according to the previously reported method (Katami et al. 2002), and kept as a stock solution.

A 400 mL portion of stock solution was condensed using a rotary evaporator under reduced pressure (around 10^{-4} Pa) to approximately 5 mL in volume and then further condensed by purging with a purified nitrogen stream to 500 μL in volume. The sample was cleaned for PCDD/F analysis by multilayer silica-gel column chromatography and alumina column chromatography. For multilayer silica-gel column chromatography, a glass column (300 mm \times 15 mm i.d.) was packed with, in order of insertion, 0.9 g silica-gel, 3 g silica-gel containing potassium hydroxide (2 %, w/w), 0.9 g silica-gel, 4.5 g silica-gel containing sulfuric acid (44 %, w/w), 6 g silica-gel containing sulfuric acid (22 %, w/w), 0.9 g silica-gel, 3 g silica-gel containing silver nitrate (10 %, w/w), and 6 g anhydrous sodium sulfate. After the column was eluted with 50 mL hexane, the condensed sample (500 μL) was placed at the top of the column. The column was eluted with 120 mL hexane at 2.4 mL/min. The eluate was condensed with a rotary evaporator to approximately 2 mL and then further condensed with a purified nitrogen stream to 500 μL in volume.

For alumina column chromatography, a glass column (300 mm \times 10 mm i.d.) was packed with 10 g alumina. After the column was eluted with hexane (10 mL), anhydrous sodium sulfate was added to the top of the alumina (10 mm thickness). The eluate prepared (500 μL) with a multilayer silica-gel column chromatography was added to this column and then eluted with a 100 mL dichloromethane/hexane (2/98) solution at 2.5 mL/min and a 150 mL dichloromethane/hexane (50/50) solution at 2.5 mL/min in series. The eluate of 50/50 dichloromethane solution was condensed to 2 mL, and the syringe-spike internal standard (a 2 μL nonane solution containing 0.25 $\text{ng}/\mu\text{L}$ of each $^{13}\text{C}_{12}$ -1,3,6,8-TeCDD, $^{13}\text{C}_{12}$ -1,2,3,7,8,9-HxCDD, and $^{13}\text{C}_{12}$ -2,2',3,4,4',5,5'-HpCB) was added for GC/MS analysis.

After a 200 mL portion of the stock solution was condensed to 5 mL, it was dissolved in 25 mL hexane. The hexane solution was shaken with a 25 mL portion of hexane-saturated DMSO four times in a separatory funnel. The DMSO layer combined (100 mL) was washed with 40 mL hexane. The DMSO layer was mixed with a hexane (75 mL) and hexane-washed water (100 mL), shaken and the hexane layer was separated using a separatory funnel. Extraction with hexane (75 mL) was repeated two times. The hexane extract combined (approx. 225 mL) was washed with a NaOH solution and water and then condensed to 1 mL, which was subsequently cleaned utilizing the multilayer silica-gel column chromatography method used for PCDD/F analysis. After the column was eluted with 50 mL hexane, the condensed sample (1 mL) was placed at the top of the column. The column was eluted with 120 mL hexane at 2.5 mL/min. The eluate was condensed with a rotary evaporator to approximately 2 mL, and the syringe-spike

Table 1 LOQ and LOD in pg/g for each chemical identified in the present study

Congener	LOQ	LOD	Congener	LOQ	LOD
2,3,7,8-TeCDD	0.25	0.08	Non-ortho co-planar chlorobiphenyls		
1,2,3,7,8-PeCDD	0.31	0.09			
1,2,3,4,7,8-HxCDD	0.7	0.2	3,4,4',5-TeCB	0.3	0.1
1,2,3,6,7,8-HxCDD	0.8	0.2	3,3',4,4'-TeCB	0.4	0.1
1,2,3,7,8,9-HxCDD	0.5	0.2	3,3',4,4',5-PeCB	0.4	0.1
1,2,3,4,6,7,8-HpCDD	0.3	0.1	3,3',4,4',5,5'-HxCB	0.5	0.2
OCDD	0.5	0.1			
2,3,7,8-TeCDF	0.20	0.06	Mono-ortho co-planar chlorobiphenyls		
1,2,3,7,8-PeCDF	0.24	0.07			
2,3,4,7,8-PeCDF	0.30	0.09	2',3,4,4',5-PeCB	0.5	0.1
1,2,3,4,7,8-HxCDF	0.7	0.2	2,3',4,4',5-PeCB	0.3	0.1
1,2,3,6,7,8-HxCDF	0.6	0.2	2,3,3',4,4'-PeCB	0.4	0.1
1,2,3,7,8,9-HxCDF	0.6	0.2	2,3,4,4',5-PeCB	0.4	0.1
2,3,4,6,7,8-HxCDF	0.7	0.2	2,3',4,4',5,5'-HxCB	0.3	0.1
1,2,3,4,6,7,8-HpCDF	0.31	0.09	2,3,3',4,4',5-HxCB	0.3	0.1
1,2,3,4,7,8,9-HpCDF	0.4	0.1	2,3,3',4,4',5'-HxCB	0.5	0.1
OCDF	0.5	0.2	2,3,3',4,4',5,5'-HpCB	0.4	0.1

internal standard (a 2 μ L nonane solution containing 0.25 ng/ μ L of each $^{13}\text{C}_{12}$ -2,2'-DiCB, $^{13}\text{C}_{12}$ -2,3',4',5-TeCB, and $^{13}\text{C}_{12}$ -2,3,3',4,4',5,5',6'-OCB) was added for GC/MS analysis of PCBs.

Analysis was performed according to “The Test Method for Disposal Standard of Domestic and Industrial Waste Subject to Special Control,” described on July 3, 1995 in Notification #192 of the Ministry of Health, Labor, and Welfare in Japan. PCDD/Fs and PCBs in the samples were analyzed using a gas chromatograph (Agilent Model 6890, Palo Alto, CA, USA) interfaced to a JMS-700 double-focus, high-resolution mass spectrometer (JEOL, Akishima, Tokyo, Japan). Isotope-labeled PCDDs/PCDFs and PCBs (10 ng/mL nonane solution) were used as internal standards for quantitative analysis. Details of the procedures for PCDD/F analysis were described in a previous publication (Katami et al. 2002). Table 1 shows limit of quantitation (LOQ) and limit of detection (LOD) in pg/g for each chemical identified in the present study.

Table 2 Concentrations (pg/g) of predominant PCDD/Fs congeners found in Japanese red pine and beech trees

Congener	Red Pine	Beech
1,2,3,4- + 1,2,3,8- + 1,2,4,6- + 1,2,4,9-TeCDD	2.3	0.25
1,2,3,6- + 1,2,7,9-TeCDD	1.4	0.18
1,2,4,7- + 1,2,4,8- + 1,3,6,9-TeCDD	1.7	0.16
1,3,6,8-TeCDD	5.4	0.94
1,3,7,9-TeCDD	3.1	0.44
2,3,7,8-TeCDD	0.39	ND
1,2,3,6,8-PeCDD	5.8	0.38
1,2,3,7,8-PeCDD	1.2	0.12
1,2,3,7,9-PeCDD	3.9	0.30
1,2,4,6,8- + 1,2,4,7,9-PeCDD	7.4	0.50
1,2,4,6,9- + 1,2,3,4,7-PeCDD	1.7	0.16
1,2,3,4,6,8- + 1,2,4,6,7,9- + 1,2,4,6,8,9-HxCDD	24	1.2
1,2,3,4,7,8-HxCDD	0.7	ND
1,2,3,6,7,8-HxCDD	1.5	ND
1,2,3,4,8,9-HxCDD	1.2	ND
1,2,3,6,7,9- + 1,2,3,6,8,9-HxCDD	8.6	0.70
1,2,3,4,6,7,9-HpCDD	7.8	1.9
1,2,3,4,6,7,8-HpCDD	3.9	1.9
OCDD	3.2	3.4
Total	85	13
1,2,4,6- + 1,2,6,8-TeCDF	1.1	0.22
1,2,3,7- + 1,3,6,9- + 1,4,7,8-TeCDF	1.1	0.20
1,6,7,8- + 1,2,3,4-TeCDF	1.1	0.22
2,3,7,8-TeCDF	0.7	0.1
2,4,6,7-TeCDF	1.2	0.23
1,2,3,6,8- + 1,3,4,7,8-PeCDF	1.9	0.24
1,2,3,4,8- + 1,2,3,7,8-PeCDF	1.9	0.26
2,3,4,7,8-PeCDF	1.6	0.17
2,3,4,6,7-PeCDF	1.5	0.31
1,2,4,6,7,8-HxCDF	1.5	0.40
1,2,3,4,7,8- + 1,2,3,4,7,9-HxCDF	3.5	0.40
1,3,4,6,7,8- + 1,3,4,6,7,9-HxCDF	1.6	0.30
1,2,3,6,7,8-HxCDF	1.6	0.30
1,2,3,4,6,7-HxCDF	1.5	0.30
2,3,4,6,7,8-HxCDF	0.8	ND
1,2,3,4,6,7,8-HpCDF	4.8	0.98
1,2,3,4,6,8,9-HpCDF	1.6	0.30
1,2,3,4,7,8,9-HpCDF	3.0	0.40
OCDF	13.0	1.7
Total	45	7.0
Grand total	130	20

Results and Discussion

The recovery efficiency of the sampling spike was 88.4 % from $^{13}\text{C}_{12}$ -1,2,3,4-TeCDD, 82.3 % from $^{13}\text{C}_{12}$ -1,2,3,4,7,8-HxCDD and 79.1 % $^{13}\text{C}_{12}$ -1,2,3,4,7,8,9-HpCDF. The

Table 3 Concentrations (pg/g) of PCBs congeners found in trees

Congener	Red Pine	Beech
2-CB	27.4	1.9
3-CB	18.1	5.4
4-CB	48.4	14.5
2,3- + 2,4'-DiCB	37.5	25.2
3,3'-DiCB	116	218
2,2',5'-TrCB	41.7	22.5
2,4',5'-TrCB	74.4	50.7
2,4,4'-TrCB	63.5	48.6
2,3,3'- + 2,3,4- + 2',3,4-TrCB	42.1	32.4
2,3,4'-TrCB	20.6	17.5
3,3',4-TrCB	22.3	10.2
3,4,4'-TrCB	13.1	11.7
2,2',3,6'- + 2,2',5,5'- + 2,3',4,6- + 2,3',5',6'-TeCB	53.4	29.9
2,2',3,5- + 2,2',4,5'-TeCB	29.5	16.7
2,2',4,4'- + 2,2',4,5- + 2,3,5,6- + 2,4,4',6-TeCB	19.1	12.2
2,2',3,5'- + 2,3,3',6-TeCB	32.6	19.1
2,3',5,5'-TeCB	20.0	14.5
2,3,4',6- + 2,3',4',6-TeCB	12.3	9.4
2,3',4',5-TeCB	24.4	21.0
2',3,4,5- + 3,3',5,5'-TeCB	60.2	53.8
2,3',4,4'-TeCB	36.5	40.9
2,3,3',4'- + 2,3,4,4'-TeCB	27.9	34.7
3,4,4',5-TeCB*	1.37	0.92
3,3',4,4'-TeCB*	20.6	13.8
2,2',3,5,6'-PeCB	30.0	16.6
2,2',3,4,6'- + 2,2',3,4,5'- + 2,2',3,5,5'- + 2,2',4,5,5'-PeCB	47.8	30.0
2,3,3',5',6-PeCB	17.0	10.5
2,2',3,4,5'- + 2,3,3',5,5'- + 2,3,4,4',6- + 2,3,4,5,6-PeCB	17.3	13.7
2,3,3',4',6-PeCB	41.9	35.2
2',3,4,4',5-PeCB*	0.6	1.08
2,3,3',4,5- + 2,3',4,4',5-PeCB*	34.9	39.3
2,3,4,4',5-PeCDF*	2.39	2.39
2,3,3',4,4'-* + 3,3',4,5,5'-PeCB	13.5	25.4
3,3',4,4',5-PeCDF*	3.42	0.77
2,2',3,4,4',6- + 2,2',3,4,4',5,6-HxCB	21.7	13.9
2,2',4,4',5,5'-HxCB	27.8	21.9
2,2',3,4,4',5'-HxCB	15.9	14.4
2,3',4,4',5,5'-HxCB*	0.99	1.27
2,3,3',4,4',5-HxCB*	2.67	2.75
2,3,3',4,4',5'-HxCB*	ND	0.73
3,3',4,4',5,5'-HxCB*	0.57	0.54
Total	1,140	956

* Co-planar PCB

recovery efficiency of the clean-up spike ranged from 74.2 % ($^{13}\text{C}_{12}$ -2,3,7,8-TeCDF) to 91.8 % ($^{13}\text{C}_{12}$ -1,2,3,7,8-PeCCDD). Table 2 shows the concentrations of the predominant (over 6 % of the total amount in each homologue) PCDD/F congeners found in the tree samples. Virtually no low chloride-numbered PCDD/Fs (Cl_1 – Cl_3) were found in the samples in the present study. The levels of all congeners found in red pine were much higher than those in beech, with the exception of OCDD. In the red pine samples, 1,2,3,4,6,8- + 1,2,4,6,7,9- + 1,2,4,6,8,9-HxCDD (24.0 pg/g) were found in the greatest amount, followed by OCDF (13 pg/g) and 1,2,3,4,6,7,9- + 1,2,3,6,8,9-HxCDD (8.6 pg/g). In the beech samples, OCDD (3.4 pg/g) was found in the greatest amount, followed by 1,2,3,4,6,7,9-/1,2,3,4,6,7,8-HpCDD (1.9 pg/g) and OCDF (1.7 pg/g). The results of the present study are consistent with those reported in vegetables collected from non-contaminated areas (Startin et al. 1989).

Table 3 shows the concentration of the PCBs found in the red pine and beech samples. The highest levels of isomers were 3,3'-DiCB (116 pg/g in red pine and 218 pg/g in beech), followed by 2,4',5'-TrCB (74.4 pg/g in red pine and 50.7 pg/g in beech). A greater total concentration of PCBs was found in red pine (1,140 pg/g) than in beech (956 pg/g). The low chloride-numbered PCBs (Cl_1 – Cl_5) were the major PCBs present in the trees. One previous study reported that the amounts of PCBs in tree samples collected relatively near the sites of their occurrence were much larger (ng/g levels) than those of the present study (Meredith and Hites 1987). The tree samples used in the present study were collected from an area that virtually no people enter and is significantly distant from industrial areas. Therefore, the amounts found in the present study may be informative as background values.

Background levels of PCDD/Fs have been found in soil and sediment samples from uncontaminated areas, which are located far from their source (Hashimoto et al. 1995). For example, background levels of total PCDD/Fs in plants are reportedly around 50 ng/kg in England (Startin et al. 1989). The concentrations of individual PCB congeners in the bark of trees at a landfill site in the US averaged 18 $\mu\text{g}/\text{kg}$, whereas they were 0.5 $\mu\text{g}/\text{kg}$ at sites more remote from a landfill (Meredith and Hites 1987).

There may be two factors in the ability to absorb PCDD/Fs and PCBs by leaves. One is the amount of lipid materials present in leaves (Schuhmacher et al. 1997; Chen et al. 2006). The composition of lipid materials is 4.1 % in red pine and 1.0 % in beech (NFEAJ 2001), suggesting that the ability to absorb organic contaminants by red pine trees is greater than that of beech trees. The other is the size and

life of leaves. Therefore, it can be estimated that the ability of red pine trees to accumulate dioxins is greater than that of beech trees, which is also consistent with the results obtained in the present study. Once chemicals are absorbed into leaves, they would move into other parts of the tree, including trunks. The differences of PCDDs/Fs and PCBs concentration in red pine and beech trees in the present study are reasonable based on factors such as the amount of lipids in the respective species of tree, the size of their leaves, and the duration of the period when leaves are on the tree.

Contamination by PCDD/Fs and PCBs in areas near their origin points has been a matter of concern for the people living near those areas for some time. However, now the possibility has been raised that these toxic contaminants may be transferred over long distances in the ambient air. In the present study, the presence of trace levels (pg/g) of PCDD/Fs and PCBs was detected in trees far from industrial areas. Therefore, we argue that it is necessary to survey the levels of these toxic chemicals not only in the immediate environs of their sources but also in areas far from their source in order to evaluate the air quality in a wide range of environmental ecosystems in order to protect them from toxic contaminants, even though obtaining samples from remote areas such as the pathless and uninhabited forests in this study is extremely difficult.

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