Detection of polychlorinated dibenzothiophenes in Japan and investigation of their dioxin-like endocrine disrupting potency

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Introduction

Polychlorinated dibenzothiophenes (PCDTs) are sulfur analogues of polychlorinated dibenzofurans (PCDFs). Several papers have reported the existence of PCDTs in the environment[1,2], and sometimes, some PCDT homologues exist in the environment at higher levels than the corresponding PCDD/DFs. For example, Buser & Rappe found that the concentration of 2,4,6,8-T4CDT was more than five times higher than that of 2,3,7,8-T4CDD in the sediment collected from Passaic river that had been contaminated with 2,4,5-T containing 2,3,7,8-T4CDD as an impurity[3], while their bioaccumulation was experimentally confirmed[4]. In addition, PCDTs have been reported to demonstrate dioxin-like endocrine disrupting activities[5,6]. Although PCDTs are environmental contaminants of a great concern, limited information is available for the environmental levels, source, and endocrine disrupting potency of PCDTs. As for the endocrine potency, only T3 and T4CDTs have been investigated[5,6], while, unfortunately, no data is available for the environmental levels in Japan. Therefore, this research was carried out to analyze PCDTs in soil and fly ash samples collected in Japan and to estimate dioxin-like endocrine disrupting potency of PCDTs.

Materials and Methods

Soil samples A and B were collected from municipal waste incineration fly ash-origin dioxin contaminated sites in Osaka and Tokyo, while a fly ash sample was obtained from a municipal waste incineration plant in Tokyo. All samples were extracted and purified according to the methods designed for the measurement of dioxins[7]. The purified samples were then analyzed for PCDTs by capillary gas chromatography/high resolution mass spectrometry of which the operating

conditions are shown in Table 1. As the standard compounds of PCDTs, commercially obtained 2,3,7,8-T4CDT, 1,2,3,7,8-P5CDT, 1,2,3,7,8,9-H6CDT, 1,2,3,4,7,8,9-H7CDT, and O8CDT were used. Since ^{13}C-labeled PCDTs were not available, corresponding ^{13}C-labelled PCDFs were used as the internal standards for quantification of PCDTs in the samples. The quantification was made by assuming that the mass spectrometry responses for PCDTs were equal to those for the corresponding ^{13}C-labeled PCDFs.

In order to estimate dioxin-like endocrine disrupting potency of PCDTs, we measured the aryl hydrocarbon (Ah) receptor binding activities of PCDTs. Briefly, the 5 isomers were respectively dissolved in dimethyl sulfoxide and then subjected to Ah-immnoassay® (Paracelsian, USA). As a reference compound, 2,3,7,8-T4CDD was used in the assay.

Table 1 Operating condition of GC/HR-MS

Homologue	T4 H6CDTs	T4 H6CDTs
Resolution	22000	24000
Column	DB-5MS J&W Scientific 0.25 mm I.D.×30 m, 0.25μm	
Oven temp.	80 2 min 8 /min 320 5 min	
Ion source temp.	275	
Ionization	Electron Impact	
Ionization current	500 μA 42 keV	
Injection	1 μL (splitless)	
Masses for selected ion monitoring	T4CDTs: 319.8788, 321.8758 P5CDTs: 355.8369, 357.8339 H6CDTs: 389.7979, 391.7949 H7CDTs: 423.7589, 425.7560 O8CDT: 457.7199, 459.7170	

Results and Discussion

Ah receptor binding activities

Table 2 summarizes the relative responses of the 5 isomers to 2,3,7,8-T4CDD determined by the Ah-immnoassay®. Except for O8CDT, the tested isomers demonstrated Ah receptor binding activities, indicating that these isomers have potency to cause dioxin-like endocrine disruption. Based on the dioxin equivalency quantity (DEQ), dioxin equivalency factors (DEFs) were calculated. Note that DEFs have similar meaning to that of Toxicity Equivalency Factors (TEFs).

Table 2 Relative response of PCDTs to 2,3,7,8-T4CDD

Isomer	2,3,7,8-T4CDD [pg-DEQ/ng]	DEF [-]
2,3,7,8-T4CDT	111	0.1
1,2,3,7,8-P5CDT	117	0.1
1,2,3,7,8,9-H6CDT	44	0.04
1,2,3,4,7,8,9-H7CDT	50	0.05*
OCDT	N.D.	-

*: Value could not be calculated.

Among the 5 isomers, 2,3,7,8-T4CDT and 1,2,3,7,8-P5CDT showed the highest DEFs at 0.1, whereas Kopponen et al. obtained 0.001 as TEF of 2,3,7,8-T4CDT by measuring the induced AHH/EROD activities in Hep1c1c 7 cell[5]. Such big difference might be due to the difference in the assay systems used. These results indicate the possibility that PCDTs may cause dioxin-like endocrine disruption at the same or less magnitude on their corresponding PCDFs.

PCDT concentrations

Figure 1 shows the SIM chromatogram of P5CDTs in soil A at m/z = 355.8369, where 1,2,3,7,8-P5CDT appears. As far as we know, this is the first data indicating the existence of 1,2,3,7,8-P5CDTs in an environmental sample, while the other isomers are still unknown. As previously reported[3], H6CDTs appeared as $[M\text{-}Cl]^+$ ion at the channels used for the detection of P5CDTs. The data confirmed the existence of PCDTs in the environment of Japan.

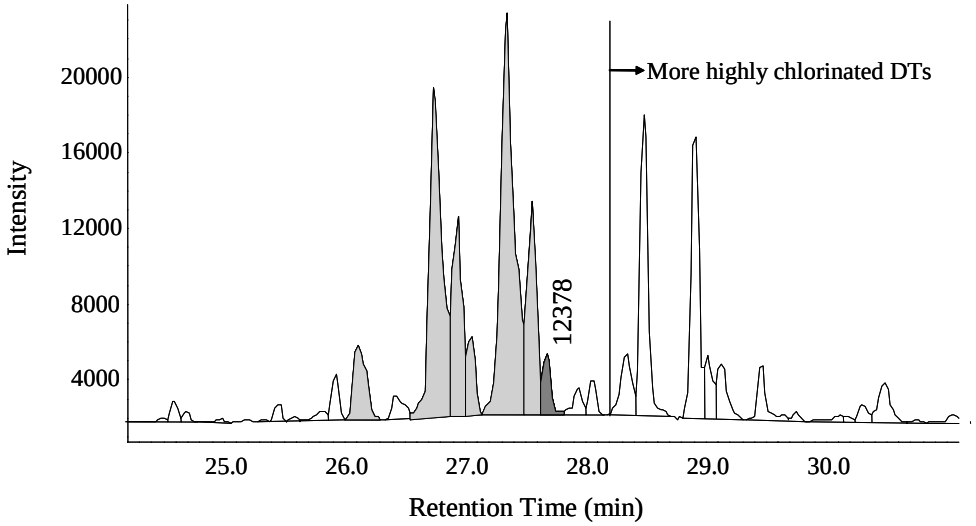

Fig. 1 SIM chromatogram (m/z = 355.8369) of P5CDTs in soil A.

The concentrations of PCDTs in the soil and the fly ash samples are shown in Table 3. Although H7 and/or O8CDTs were not detected in the soil B and fly ash samples, all samples contained T4 H6CDTs, thereby indicating that the incineration of municipal waste may produce T4 H6CDTs rather than the more highly chlorinated PCDTs.

Buser et al. analyzed T4 and T5CDTs in the fly ash samples collected from 3 municipal waste incineration plants and showed that the sum of their concentrations was lower than that of T4 and P5CDDs and T4 and P5CDFs[8]. A similar tend was observed in our results. In each sample, the total concentration of PCDTs was lower than those of PCDDs and PCDFs.

Table 3 Concentrations of PCDTs in the environmental sample analyses [pg/g].

SOIL A	PCDTs	PCDDs	PCDFs
Tetra	990	630	12000
Penta	610	4800	49000
Hexa	2900	23000	130000
Hepta	750	48000	160000
Octa	1300	44000	88000
Total	6550	120430	439000

SOIL B	PCDTs	PCDDs	PCDFs
Tetra	1700	3600	24000
Penta	1700	3600	15000
Hexa	720	3100	11000
Hepta	64	1800	4600
Octa	N.D.	1200	500
Total	4184	12700	55100

FLY ASH	PCDTs	PCDDs	PCDFs
Tetra	440	2800	12000
Penta	480	4100	8900
Hexa	460	6100	5900
Hepta	N.D.	5600	2900
Octa	N.D.	6000	850
Total	1380	24600	30550

Finally, it is surmised that the production of PCDTs from municipal waste incineration occurs at comparatively low levels, as compared to PCDD/DFs.

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