

Polychlorinated dibenzothiophenes in Japanese environmental samples and their photodegradability and dioxin-like endocrine-disruption potential

Satoshi NAKAI*, Saeko KISHITA, Yugo NOMURA and Masaaki HOSOMI

Department of Chemical Engineering, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan

*Corresponding author. Tel.: +81-42-388-7069; fax.: +81-42-388-7693.

E-mail address: nakai@cc.tuat.ac.jp (S. Nakai)

Abstract

Polychlorinated dibenzothiophenes (PCDTs) in sediment, soil, and fly ash samples collected in Japan were analyzed and their dioxin-like endocrine-disruption potential and photodegradability investigated. PCDTs were detected in all three types of sample, although the concentrations were lower than those of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). An Ah-immunoassay confirmed that 2,3,7,8-TeCDT, 1,2,3,7,8-PeCDT, 1,2,3,7,8,9-HxCdT, and 1,2,3,4,7,8,9-HpCDT had Ah receptor-binding activities, which means that these PCDTs have the potential to cause dioxin-like endocrine disruption. Photodegradation of PCDTs by UV irradiation was accompanied by

dechlorination. The photodegradation rates of 2,3,7,8-TeCDT and OCDT were lower than the rates for the corresponding PCDF isomers (2,3,7,8-TeCDF and OCDF), which indicates that PCDTs are more stable than PCDFs to photodegradation.

Key words: Ah receptor; fly ash; photodegradation; polychlorinated dibenzothiophenes; sediment, soil

1. Introduction

Polychlorinated dibenzothiophenes (PCDTs) are sulfur analogues of polychlorinated dibenzofurans (PCDFs). PCDTs have been detected in environmental samples such as soil and sediment (Peterman et al., 1986, 1988; Buser and Rappe, 1991; Pruell et al., 1993; Claus et al., 1998; Sinkkonen et al., 1994a, 1995, 2001), and in some samples PCDT homologues are present at higher levels than the corresponding PCDD/DFs. Pruell et al. (1993) found that the concentration of 2,4,6,8-TeCDT (3680 pg g^{-1}) was five times that of 2,3,7,8-TeCDD (656 pg g^{-1}) in sediment that was collected from the Passaic River (NJ, USA) and had been contaminated with 2,4,5-T containing 2,3,7,8-TeCDD as an impurity. Cai et al. (1994) reported that blue crabs (*Callinectes sapidus*) collected from Newark Bay, at the mouth of the Passaic River, contained more 2,4,6,8-TeCDT than 2,3,7,8-TeCDD in their muscle and hepatopancreatic tissues. These results indicate the necessity for monitoring PCDTs as a class of environmental pollutants.

Unfortunately, information on the environmental levels and emissions of PCDTs is limited. Although incineration of municipal waste and polychlorinated biphenyls (Peterman et al., 1986; Buser et al., 1991; Sinkkonen, 1991), pulp bleaching (Sinkkonen, 1994a), metal reclamation (Sinkkonen, 1994b, 2003), and production of chlorinated thiophenols (Benz et al., 1992) have been reported as emission sources of PCDTs, the high level of 2,4,6,8-TeCDT in

the Passaic River has not been explained. In addition, there have been no reports of PCDTs in environmental samples collected in Japan, because Japanese environmental samples have not been tested for PCDTs.

Owing to their structural similarity to PCDFs, PCDTs are suspected of causing dioxin-like endocrine disruption. Kopponen et al. (1994) measured aryl hydrocarbon hydroxylase and ethoxyresorufin-O-deethylase (AHH/EROD) activities induced by 2,3,7,8-TeCDT in mouse hepatoma cell cultures and calculated its toxic equivalency factor (TEF) with respect to 2,3,7,8-TeCDD. The experimental TEF was 0.001, indicating that 2,3,7,8-TeCDT has the potential to cause dioxin-like endocrine disruption. However, no data are available for more highly chlorinated dibenzothiophenes.

To determine whether or not PCDTs are environmental pollutants that require high priority for monitoring, the toxicity, environmental levels, and persistence of PCDTs must be evaluated. However, information on the environmental levels, sources, and endocrine-disruption potency of PCDTs is limited, and the degradability of PCDTs in the environment has not yet been investigated. Therefore, we analyzed PCDTs in sediment, soil, and fly ash samples collected in Japan and estimated the dioxin-like endocrine-disruption potential and photodegradability of these compounds.

2. Materials and Methods

2.1. Environmental samples and PCDT standard compounds

Soil samples were collected from Japanese sites (in Nose, Osaka, and Hachiouji, Tokyo) contaminated with dioxins from municipal waste incineration. A fly ash sample was obtained from a municipal waste incineration plant located on the Tamagawa River in Tokyo, Japan, and a sediment sample was taken from the mouth of the same river, about 35 km downstream from the incineration plant. 2,3,7,8-TeCDT (Wellington Laboratories, Canada) and 1,2,3,7,8-PeCDT, 1,2,3,7,8,9-HxCdT, 1,2,3,4,7,8,9-HpCDT, and OCdT (EnBio Tec Laboratories, Japan) were used as standard PCDTs. 2,3,7,8-TeCDF and OCDF were purchased from Cambridge Isotope Laboratories (USA). Because ^{13}C -labeled PCDTs were not available, the corresponding ^{13}C -labeled PCDFs (NK-LCS-I, Wellington Laboratories, Canada) and a mixture of ^{13}C -1,2,3,4-TeCDD and ^{13}C -1,2,3,7,8,9-HxCDD (NK-IS-A, Wellington Laboratories, Canada) were used as internal standards for quantification of PCDTs in the samples.

2.2. Analysis of PCDTs in environmental samples

Prior to qualification and quantification of PCDTs, the environmental samples were extracted and purified. Because previous research has shown that PCDTs behave similarly to dioxins during conventional extraction and purification processes designed for dioxins (Sinkkonen et al. 2001; Sielex and Andersson, 1997), we used the Japanese standard analytical methods for dioxins in soil and sediment (Water Quality Bureau, 2000a, 2000b),

and waste (Environmental Health Bureau, 1997) to analyze PCDTs in the environmental samples. The purified samples were then analyzed for PCDTs by high resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS; JMS700, Jeol, Japan; see Table 1 for operating conditions). To quantify the PCDTs, we assumed that their mass spectrometry responses were equal to those of the corresponding ¹³C-labeled PCDFs.

2.3. Photodegradation of PCDTs

To investigate the persistence of PCDTs, we compared the photodegradabilities of 2,3,7,8-TeCDT and OCDT with those of 2,3,7,8-TeCDF and OCDF. Briefly, an *n*-hexane solution of each compound (1000 pg μl⁻¹) was poured into a 4-cm³ quartz cuvette (S11-UV-10, GL Science, Japan) and subjected to UV irradiation for 1 h with a 5.3 W low-pressure mercury lamp that emits mainly 254 nm (Germipak GCL212/11.4/Cell/C, Light Sources, USA) (Fig. 1). During irradiation, a portion of the *n*-hexane solution was sampled and analyzed by high resolution gas chromatography/low-resolution mass spectrometry (HRGC/LRMS (HP6890/HP5973, Agilent, USA; see Table 2 for operating conditions).

2.4. Estimation of Ah receptor-binding activities of PCDTs

Dioxins are known to cause endocrine disruption by binding to Ah receptors in cells. Therefore, we decided to estimate the endocrine-disruption potential of PCDTs by comparing the Ah receptor-binding activities of PCDTs with the activity of 2,3,7,8-TeCDD. 2,3,7,8-

TeCDT, 1,2,3,7,8-PeCDT, 1,2,3,7,8,9-HxCdT, 1,2,3,4,7,8,9-HpCDT, and OCdT were separately dissolved in dimethyl sulfoxide and then analyzed with an Ah-immunoassay kit (Paracelsian, USA) according to the instructions supplied with the kit. 2,3,7,8-TeCDD was used as a reference compound in the assay to determine the dioxin equivalency quantity (DEQ) of each isomer. Finally, an experimental toxicity equivalency factor (e-TEF) was calculated on the basis of the DEQ.

3. Results and discussion

3.1. Environmental sample analysis

2,3,7,8-TeCDT, 1,2,3,7,8-PeCDT, and other TeCDT and PeCDT isomers were detected in the selected-ion monitoring (SIM) chromatograms of the soil sample collected from Nose (Fig. 2). As in previous studies, in which ionization of PCDTs using the electron impact mode was confirmed to produce $[M-Cl]^+$ and $[M-2Cl]^+$ ions (Buser and Rappe, 1991; Buser et al., 1991; Sinkkonen et al., 1993), more highly chlorinated dibenzothiophenes than the target homologues might appear at each channel (Fig. 2). Each homologue was identified on the basis of the retention time and the isotope ratios. The results confirmed the presence of PCDTs in the environmental samples collected in Japan.

Neither OCdT nor HpCDTs were detected in the fly ash sample ($<20 \text{ pg g}^{-1}$ of detection limit), and OCdT was not detected in the Hachiouji soil sample; but all samples contained Te-HxCdT (Table 3). These results indicate that the incineration of municipal waste may produce Te-HxCdT rather than the more highly chlorinated PCDTs. As was the case for the fly ash sample, only Te-HxCdT were detected in the sediment sample, and at trace levels

(13 pg g⁻¹ for TeCDTs and HxCDDs and 5 pg g⁻¹ for PeCDTs), whereas the Bureau of Environment of the Tokyo Metropolitan Government reported that the concentration of PCDD/DFs at the mouth of the Tamagawa River was about 2600 pg g⁻¹ (2000).

Buser et al. (1991) analyzed TeCDTs and PeCDTs in fly ash samples collected from three municipal waste incineration plants and showed that the sum of their concentrations was lower than the sum of Te/PeCDD and Te/PeCDF concentrations. We observed a similar trend: the concentrations of PCDTs were lower than the concentrations of PCDD/DFs in each sample. On the basis of these results, we surmise that the production of PCDTs from municipal waste incineration is low compared with the production of PCDD/DFs.

3.2. Photodegradation of PCDTs

Upon photodegradation of OCDT/DF in *n*-hexane (Fig. 3), the OCDT concentration fell below the detection limit (<10 pg μl⁻¹) after 0.5 h, whereas OCDF disappeared after only 0.1 h. This result indicates OCDT underwent photodegradation more slowly than did OCDF.

Photodegradation of 2,3,7,8-TeCDF/DF and OCDF/DF followed pseudo-first-order kinetics (Fig. 4). The photodegradation rate of OCDF was about seven times that of 2,3,7,8-TeCDF, a result that agrees with the results of previous research on the photodegradability of 2,3,7,8-TeCDF and OCDF in *n*-hexane (Lee et al., 1999). Photodegradation rates for 2,3,7,8-TeCDF and OCDF were lower than the rates for the corresponding PCDF isomers, and the difference between the octachlorinated isomers was bigger than that between the

tetrachlorinated isomers, which indicates that PCDTs are more stable to photodegradation than PCDFs.

Figure 5 shows the total-ion chromatogram of the OCDT solution after 15 min of UV irradiation, at which point Tr-HpCDTs appeared. The presence of lower-chlorinated compounds confirmed that dechlorination of OCDT had occurred. Among the HpCDTs, 1,2,3,4,7,8,9-HpCDT showed the largest peak area, which reached a maximum at 15 min and then decreased (data not shown). 1,2,3,7,8,9-HxCDF was one of the two dominant HxCDFs isomers; the other dominant isomer detected at the same magnitude has not yet been identified.

In OCDF molecules, the C–Cl bonds at the ortho positions are the weakest bonds, and therefore the C–Cl bonds at the 1- and 9-positions are preferably dissociated during the photodegradation of OCDF (Wagenaar et al., 1995; Ballschmiter and Bacher, 1996). In contrast, photodegradation of OCDT produces 1,2,3,4,7,8,9-HpCDT preferentially, which allows us to hypothesize that the C–Cl bonds at the 4- and 6-positions may be the weakest of the eight C–Cl bonds of OCDT. The predominance of 1,2,3,7,8,9-HxCDF supports this hypothesis. To verify this hypothesis, further research is necessary to identify the unknown isomers.

3.3. Ah receptor-binding activity

Table 4 summarizes the responses of the five isomers relative to the response of 2,3,7,8-TeCDD in the Ah-immunoassay. All the tested isomers, except for OCDD, demonstrated Ah receptor-binding activities, which indicates that PCDDs have the potential to cause dioxin-like endocrine disruption. Of the five isomers, 2,3,7,8-TeCDD and 1,2,3,7,8-PeCDD showed the highest e-TEF values, at 0.1. This value is substantially different from the value of 0.001 that Kopponen et al. (1994) obtained for 2,3,7,8-TeCDD by measuring the induced AHH/EROD activities in Hep1c1c 7 cell. The large difference may be due to the difference in the assay systems used.

Kobayashi (2001) investigated the Ah receptor-binding activities of PCDF isomers and reported the following e-TEF values: 0.06 for 2,3,7,8-TeCDF, 0.04 for 1,2,3,7,8-PeCDF, 0.6 for 1,2,3,7,8,9-HxCDF, 0.07 for 1,2,3,4,7,8,9-HpCDF, and 0.008 for OCDF. Note that the e-TEF values for 2,3,7,8-TeCDD and 1,2,3,7,8-PeCDD were higher than the values for the corresponding PCDF isomers, whereas the opposite relationship was observed for the hexa- and octachlorinated isomers. In addition, the e-TEF value of 1,2,3,4,7,8,9-HpCDD was nearly identical to that of 1,2,3,4,7,8,9-HpCDF. These results indicate that PCDDs have dioxin-like endocrine-disruption potential comparable to that of PCDFs.

4. Conclusions

In this research, environmental samples collected in Japan were analyzed for PCDTs, and the photodegradability and dioxin-like endocrine-disruption potential of the PCDTs were estimated and compared with the potencies of PCDFs. PCDTs were detected in the soil, sediment, and fly ash samples, although the concentrations of PCDTs were lower than those of PCDD/DFs. Photodegradation of PCDTs by UV irradiation was accompanied with dechlorination. The photodegradation rates of 2,3,7,8-TeCDT and OCdT were lower than the photodegradation rates of the corresponding PCDF isomers (2,3,7,8-TeCDF and OCDF), which indicates that PCDTs were more stable than PCDFs to photodegradation. An Ah-immunoassay demonstrated that 2,3,7,8-TeCDT, 1,2,3,7,8-PeCDT, 1,2,3,7,8,9-HxCdT and 1,2,3,4,7,8,9-HpCDT bind to the Ah receptor, which indicates that PCDTs have the potential to cause dioxin-like endocrine disruption.

Acknowledgements

This research was supported by the Tokyu Foundation for Better Environment in Japan. The authors wish to thank Prof. J. T. Andersson of the Universität Münster for helpful suggestions regarding the analysis of PCDTs and for supplying PCDT standards.

References

- Ballschmiter, K. und Bacher, R., 1996. Dioxine Chemie, Analytik, Vorkommen, Umweltverhalten und Toxikologie der halogenierten Dibenzo-p-dioxine und Dibenzofurane. VCH, Weinheim. (in German)
- Benz, T., Hagenmaier, H., Lindig, C., and She, J., 1992. Occurrence of the sulphur analogue of octachlorodibenzo-p-dioxin in the environment and investigations on its potential source. *Fresen. J. Anal. Chem.* 344, 286-291.
- Bureau of Environment, Tokyo Metropolitan Government, 2000. Results of Dioxins Measurement in Rivers and Bays in Tokyo in Heisei 11th year. (in Japanese)
- Buser, H.-R. and Rappe, C., 1991. Determination of polychlorodibenzothiophenes, the sulfur analogues of polychlorodibenzofurans, using various gas chromatographic/mass spectrometric techniques. *Anal. Chem.* 63, 1210-1217.
- Buser, H.-R., Dolezal, I. S., and Wolfensberger, M., 1991. Polychlorodibenzothiophenes, the sulfur analogues of the polychlorodibenzofurans identified in incineration samples. *Environ. Sci. Technol.* 25, 1637-1643.
- Cai, Z., Giblin, D. E., Ramanujam, V. M. S., Gross, M. L., and Cristini, A., 1994. Mass-profile monitoring in trace analysis: identification of polychlorodibenzothiophenes

- in crab tissues collected from the Newark/Raritan Bay system. *Environ. Sci. Technol.* 28, 1535-1538.
- Claus, E., Heininger, P., Bade, M., Jurling, H., and Raab, M., 1998. Mass spectrometric identification of polychlorinated dibenzothiophenes (PCDBTs) in surface sediments of the river Elbe. *Fresen. J. Anal. Chem.* 361, 54-58.
- Environmental Health Bureau, Ministry of Health and Welfare, Japan, 1997. Standard Manual for Determination and Analysis of Dioxins in Waste Treatment Process. Japan Waste Research Foundation, Tokyo, Japan (in Japanese)
- Kobayashi, Y., 2001. Quick measurement of dioxins using Ah receptor. *J. Resour. Environ.* 9, 65-70. (in Japanese)
- Lee, B. D., Yamane, A., and Hosomi, M., 1999. Degradability of persistent organic pollutants (POPs). III. photolysis (1). *J. Water Waste* 41, 483-491. (in Japanese)
- Kopponen, P., Sinnkonen, S., Poso, A., Gynther, J., and Kärenlamp, S., 1994. Sulfur analogues of polychlorinated dibenzo-p-dioxins, dibenzofurans and diphenyl ethers as inducers of CYP1A1 in mouse hepatoma cell culture and structure-activity relationships. *Environ. Toxicol. Chem.* 13, 1543-1548.
- Peterman, P. H., Smith, L. M., Stalling, D. L., and Petty, J. D., 1986. Identification of chlorinated biphenylenes and other polycyclic aromatic compounds formed from the incineration of PCB-dielectric fluids at a capacitor plant's disposal site.

- Proceedings of the 34th Annual Conference on Mass Spectrometry and Allied Topics. Cincinnati, OH, 486-487.
- Peterman, P. H., Lebo, Jon. A., M., and Hilary J., 1988. Accurate Mass Determinations of Polychlorinated Dibenzothiophenes in Soil from a Capacitor Plant's Incineration Site. Proceedings of the 36th ASMS Conference on Mass Spectrometry and Allied Topics. San Francisco, CA, 240-241.
- Pruell, R. J., Rubinstein, N. I., Taplin, B. K., LiVolsi, J. A. A., and Bowen, R. D., 1993. Accumulation of polychlorinated organic contaminants from sediment by three benthic marine species. *Arch. Environ. Con. Tox.* 24, 290-297.
- Sielex, K. and Andersson, J. T., 1997. Separation of polychlorinated dibenzothiophenes from polychlorinated dibenzodioxins and -furans. *Fresen. J. Anal. Chem.* 359, 261-266.
- Sinkkonen, S., Paasivirta, J., Koistinen, J., and Tarhanen, J., 1991. Tetra- and pentachlorodibenzothiophenes are formed in waste combustion. *Chemosphere* 23, 583-587.
- Sinkkonen, S., Kolehmainen, E., Koistinen, J. and Lahtiperä, M., 1993. High-resolution gas chromatographic-mass spectrometric determination of neutral chlorinated aromatic sulphur compounds in stack gas. *J. Chromatogr. A* 641, 309-317.
- Sinkkonen, S., Kolehmainen, E., Paasivirta, J., Koistinen, J., Lahtiperä, M. and Lammi, R., 1994a. Identification and level estimation of chlorinated neutral aromatic sulfur

- compounds and their alkylated derivatives in pulp mill effluents and sediments. *Chemosphere* 28, 2049-2066.
- Sinkkonen, S., Vattulainen, A., Aliotta, J.-P., Paasivirta, J., Tarhanen, J. and Lahtiperä, M., 1994b. Metal reclamation produces sulphur analogues of toxic dioxins and furans. *Chemosphere* 28, 1279-1288.
- Sinkkonen, S., Rantio, T., Vattulainen, A., Aliotta, J.-P., Paasivirta, J., and Lahtiperä, M., 1995. Chlorohydrocarbons, PCB congeners, polychlorodioxins, furans, and dibenzothiophenes in pine needles in the vicinity of a metal reclamation plant. *Chemosphere* 30, 2227-2239.
- Sinkkonen, S., Paasivirta, J., and Lahtiperä, M., 2001. Chlorinated and methylated dibenzothiophenes in sediment samples from a river contaminated by organochlorine wastes. *J. Soils Sediments* 1, 1-6.
- Sinkkonen, S., Lahtiperä, M., Vattulainen, A., Takhiskov, V. V., Viktorovskii, I. V., Utsal, V. A., and Paasivirta, J., 2003. Analyzes of known and new types of polyhalogenated aromatic substances in oven ash from recycled aluminium production. *Chemosphere* 52, 761-775.
- Wagenaar, W. J., Boelhouwers, E. J., Kokde, H. A. M., and Groen, C. P., 1995. A comparative study of the photolytic degradation of octachlorodibenzofuran (OCDF) and octachlorodibenzo-p-dioxin (OCDD). *Chemosphere* 31, 2983-2992.

Water Quality Bureau, Environment Agency, Japan, 2000a. Manual on Determination of
Dioxins in Sediment. <http://www.env.go.jp/water/dioxin/teishitsu-m.pdf> (in
Japanese)

Water Quality Bureau, Environment Agency, Japan, 2000b. Manual on Determination of
Dioxins in Soil. <http://www.env.go.jp/chemi/dioxin/manual/dojo-manual.pdf> (in
Japanese)

Table 1
HRGC/HRMS operating conditions

Homologue	Te-HxCDTs	Hp-OCDTs
Resolution	22000	24000
Column ^a	DB-5MS 0.25 mm I.D. × 30 m, 0.25 μm (J&W Scientific, USA)	
Oven temp. ^a	80°C (2 min), 8°C min ⁻¹ to 320°C (5 min)	
Ion source temp.	275°C	
Ionization	Electron impact	
Ionization current	500 μA (42 keV)	
Injection	1 μL (splitless)	
Masses for SIM	TeCDTs: 319.8788, 321.8758 HxCDTs: 389.7979, 391.7949 OCDT: 457.7199, 459.7170	PeCDTs: 355.8369, 357.8339 HpCDTs: 423.7589, 425.7560

^a The column and temperature program were used by Sielex and Andersson (1997).

Table 2
HRGC/LRMS operating conditions

Column	HP-5MS 0.32 mm I.D. × 30 m, 0.25 μm (Agilent, USA)
Oven temp.	50°C (1 min), 3°C min ⁻¹ to 310°C
Ion source temp.	230°C
Ionization	Electron impact
Mode	Scan
Injection	1 μL (splitless)

Table 3
 Concentrations (pg g^{-1}) of PCDTs, PCDDs, and PCDFs in (a) Nose soil, (b) Hachiouji soil, and (c) municipal waste fly ash

	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

(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	13300	55100

(c)

	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

Table 4
Response of PCDTs relative to 2,3,7,8-TeCDD

Isomer	2,3,7,8-TeCDD [pg-DEQ g ⁻¹]	e-TEF [-]
2,3,7,8-TeCDT	111	0.1
1,2,3,7,8-PeCDT	117	0.1
1,2,3,7,8,9-HxCdT	44	0.04
1,2,3,4,7,8,9-HpCDT	50	0.05
OCdT	N.D.	- ^a

^a Value could not be calculated.

Fig. 1. Schematic of the photodegradation reactor (1, quartz cuvette; 2, UV lamp).

Fig. 2. Selected ion chromatograms of Nose soil sample at the channels for (a) M^+ ion of TeCDTs (m/z 319.8788) and (b) $(M+2)^+$ ion of PeCDTs (m/z 355.8369).

Fig. 3. Time course of OCDT and OCDF concentrations during UV irradiation.

Fig. 4. Comparison of the photodegradation rates of 2,3,7,8-TeCDF/DT and OCDF/DT in *n*-hexane.

Fig. 5. Total ion chromatogram of the OCDT solution after 15 min of UV irradiation.

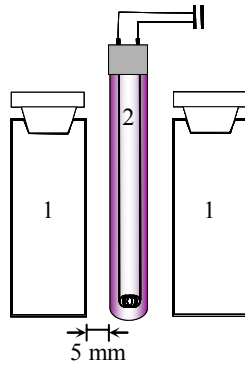


Fig. 1. Nakai et al.

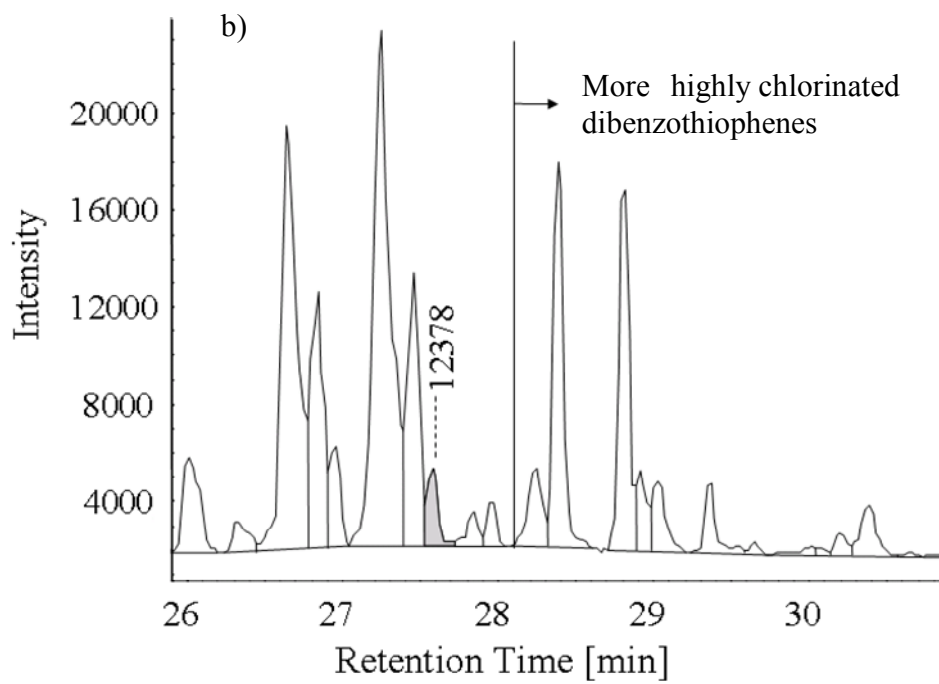
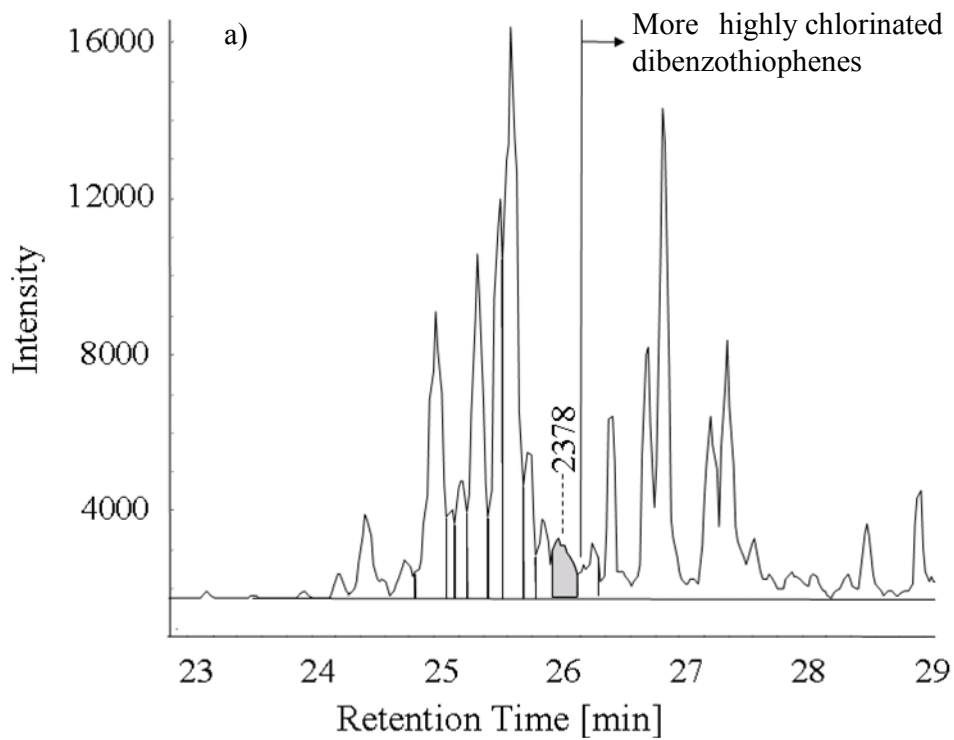


Fig. 2. Nakai et al.

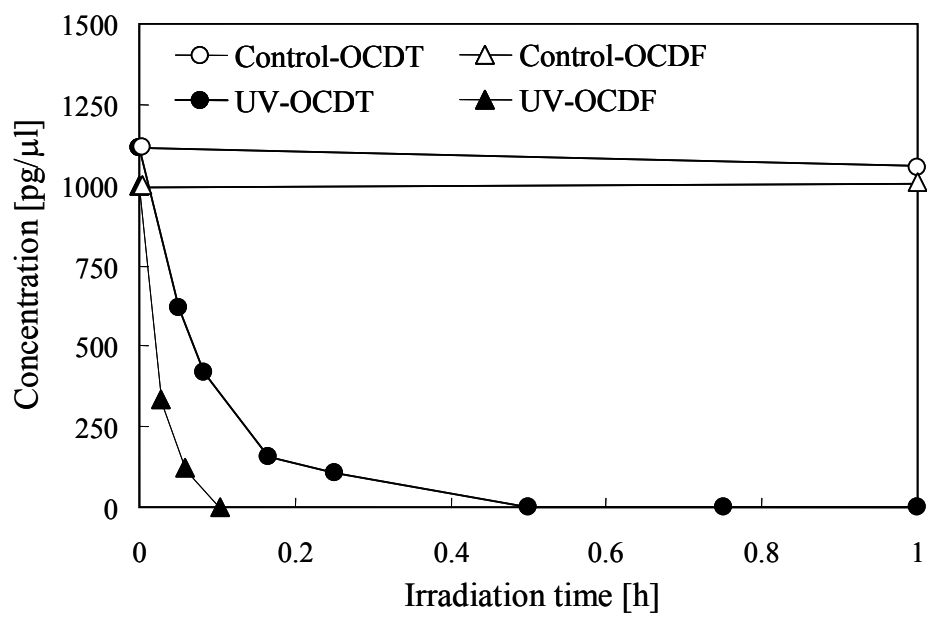


Fig. 3. Nakai et al.

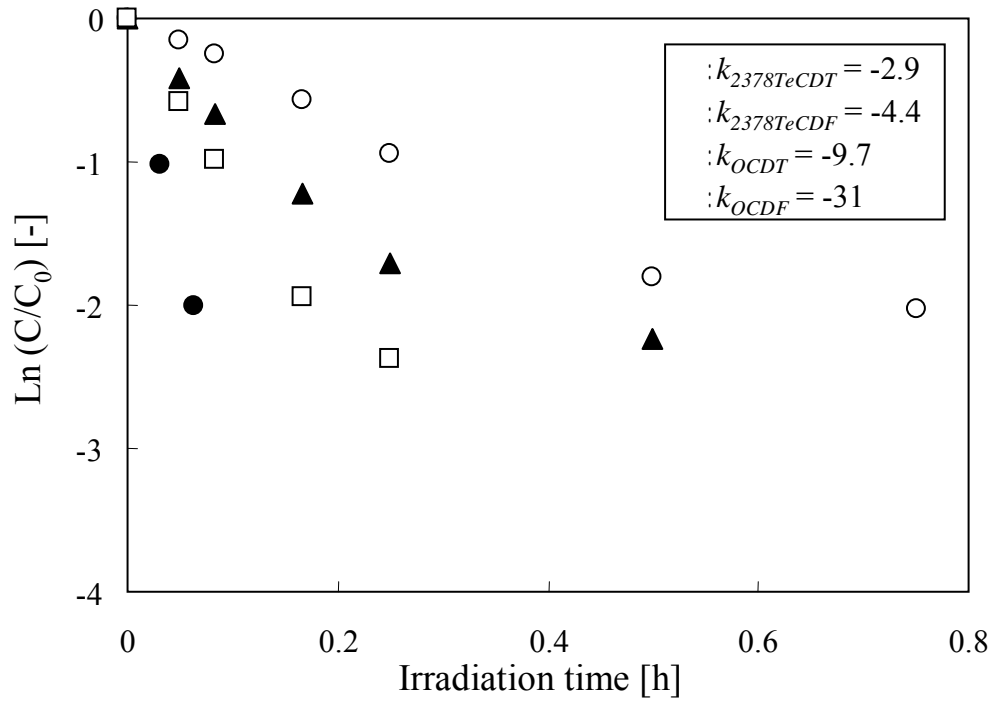


Fig. 4. Nakai et al.

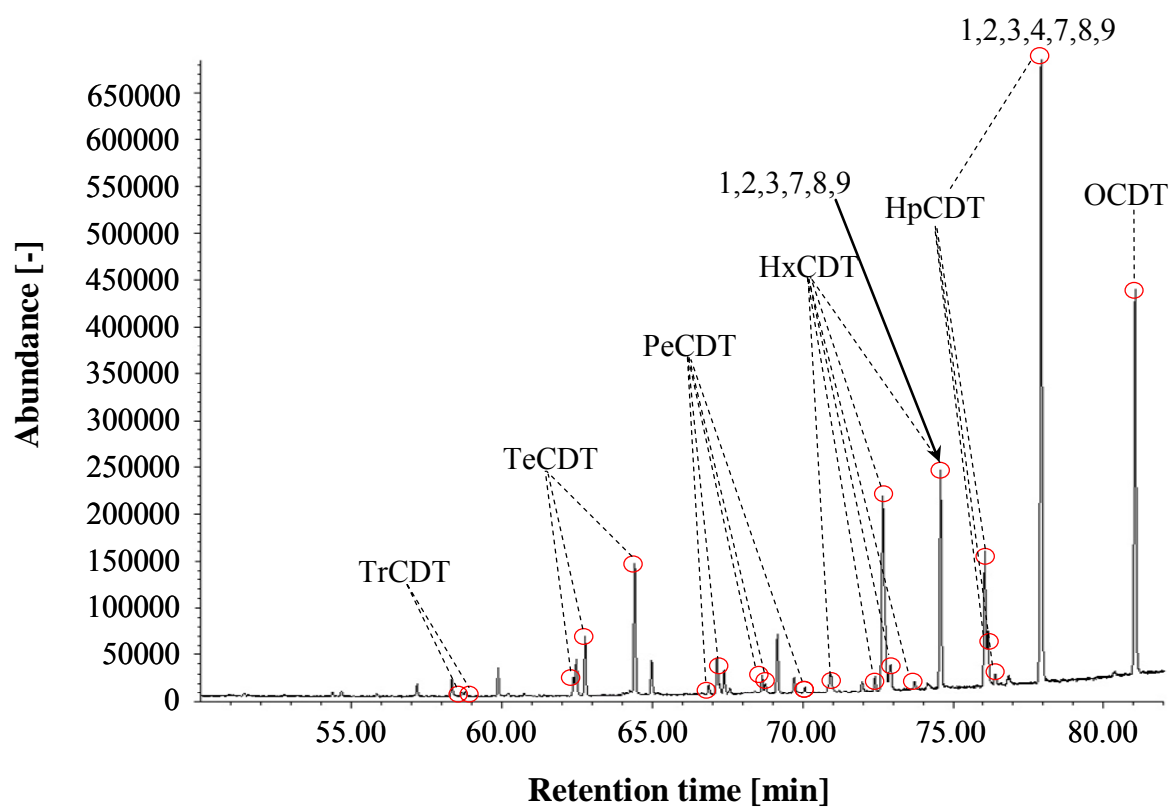


Fig. 5. Nakai et al.