

# Analysis of Polychlorinated Dibenzofurans (PCDFs) Isomers in Soil Samples\*

Mustafa PEHLİVAN

*Dept. of Science E., Fac. of Edu., Selcuk University,  
42090 Konya-TURKEY*

Erol PEHLİVAN

*Dept. of Chemistry, Selcuk University,  
42031 Konya-TURKEY*

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This study presents an analytical method for the separation of a series of polychlorinated dibenzofurans (PCDFs) emitted to the soil. PCDF contaminants were concentrated from soil samples and isolated from other materials by chromatographic methods and their quantitative determinations were performed by GC/MS (gas chromatography/mass spectroscopy).

**Key Words:** Polychlorodibenzofurans, Toxic Materials, GC/MS, hyphenated technique

## Introduction

Dioxin is one of the world's most toxic chemicals. It is formed as an accidental and unavoidable by-product of some industrial uses of chlorine. The presence of PCDFs has been reported in the fly ash and flue gas of hazardous waste incinerators of municipalities and hospitals. The majority of these compounds are probably produced in the incinerator itself, by formation from precursors at high temperatures in the flame or by de novo synthesis at low temperatures in the post-combustion zone of the incinerator. The formation of PCDFs is explained by the extensive chlorine exchange reactions. A large number of mixed halogenated congeners are theoretically possible: 1550 brominated/chlorinated dibenzo-p-dioxins (PXDDs) and 3050 brominated/chlorinated dibenzofurans (PXDFs). Because of the complexity of the analytical procedures, it has been possible to characterize and determine only a small number of these compounds. The most toxic congeners are those substituted at positions 2, 3, 7 and 8, and they are considered to be among the most dangerous environmental pollutants the "super-poisons"<sup>1-6</sup>.

PCDFs are not known to occur naturally. They are not intentionally produced but are generated as undesired by-products in various processes. They can be formed by chemical, photochemical, or thermal reactions from precursors. Fires and incinerators cause the release of PVC's toxic chemicals such as dioxin. Halogenated aromatic hydrocarbons (HAHs), such as polychlorinated dibenzo-p-dioxins, biphenyls and

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dibenzofurans, are widespread environmental contaminants. During waste incineration, traces of dioxins have also been found. If dioxins or waste containing dioxins are introduced in high concentrations into the environment, entire tracts of land can be poisoned. 2,3,7,8- TCDF, the most biologically active and toxic member of this class of compounds, produces a wide variety of species- and tissue-specific effects<sup>7-10</sup>. Dioxin is one of a family of compounds that has the ability to disrupt the body's endocrine system. Because of the toxic nature of these compounds, care must be taken during sampling and analysis. Highly sensitive, selective and specific analytical methods (gas chromatography/mass spectrometry, or GC/MS) are required because of the large number of PCDF congeners. Sampling procedures are identical for all PCDFs, but the separation and determination of them differ slightly from those of their chlorinated analogues.

## Experimental

### Equipment

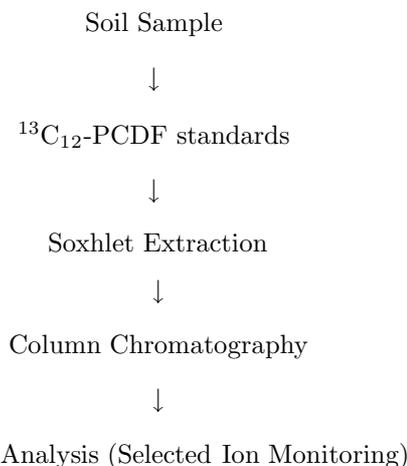
A gas chromatograph (HP 5890) and a mass spectrometer (HP 5970B) from Hewlett-Packard (Böblingen, Germany) were used along with a Bio-Beads S-X3 gel chromatographic column (Bio-Rad, München, Germany), a CP-Sil 88 capillary column (Chrompack International, Middelburg, The Netherlands), and a DB-5 column from J&W Scientific (Frankfurt, Germany).

### Chemicals

<sup>13</sup>C<sub>12</sub>-PCDF standards were purchased from Promochemie (Wesel, Germany). Nitrogen and helium gas were both purchased from Fa. Messer Griesheim (Germany). The organic solvents used were hexane, acetone, dichloromethane, benzene, toluene, cyclohexane, and ethyl acetates. All solvents were of nanograde purity and were obtained from Promochemie. Kieselgel-Silica Woelm 63 (active), Na<sub>2</sub>SO<sub>4</sub>, concentrated H<sub>2</sub>SO<sub>4</sub>, AgNO<sub>3</sub>, Alumina B Super 1, and Woelm Pharma Eschwege were also from Promochemie.

### Procedure

Soil samples were collected, freeze-dried and ground to a particle size of about 0.1 mm. The extraction and clean-up procedure for the PCDFs analysis were completed as shown in the Figure.



**Figure.** Clean-up for PCDF analysis of soil sample

In the separation technique, known amounts of  $^{13}\text{C}_{12}$ -PCDF standards were injected into the soil samples, and these samples were then extracted in a Soxhlet type extractor. The total PCDF isomers of the samples were first isolated and then concentrated by passing the extract through columns packed with Kieselgel/44 vol. %  $\text{H}_2\text{SO}_4$ , Macro Alumina B Super 1, Mix Column, Bio Beads S-X3 Gel Chromatography and Mini Alumina B Super 1 columns, all of which are basically chromatographic separation procedures. Isomer-specific PCDF analysis was carried out by GC/MS in multiple ion selection mode. Quantitative determinations were performed by recording the spectra of all PCDF extracts on a GC/MS instrument, and the results are given in the Table.

## Experimental Procedure

The soil sample was vacuum dried at  $-3^\circ\text{C}$  and ground to a fine powder. To 100-g aliquots of the dry sample; 5 ng each of 2,3,7,8-tetraCDF, 1,2,3,7,8-pentaCDF, and 1,2,3,6,7,8-hexaCDF and 10 ng each of 1,2,3,4,6,7,8-heptaCDF and 1,2,3,4,6,7,8,9-octa CDF, were added from PCDF standards containing the  $^{13}\text{C}_{12}$ -isotope. The samples were extracted in a Soxhlet apparatus with toluene for 18 hours. The extract was concentrated in vacuo to about 5 mL

In the first chromatographic step, the concentrate was transferred to a chromatography column, which fully packed with a mixture of 40 g (kieselgel/44 vol. % of concentrated  $\text{H}_2\text{SO}_4$ ) and 15 g  $\text{Na}_2\text{SO}_4$ . The concentrate was eluted with a 250 mL (80 vol. % n-hexane/20 vol. % dichloromethane) mixture. The solution was evaporated to a volume of 5 mL in vacuo. This concentrate was transferred to a chromatographic column packed with a mixture of 30 g Alumina B Super 1 and 15 g  $\text{Na}_2\text{SO}_4$ , and then washed with 120 mL benzene and a 250 mL (98 vol. % n-hexane/2 vol. % dichloromethane) mixture respectively. The mixture was discarded, and the column was eluted with a 200 mL (50 vol. % n-hexane/50 vol. % dichloromethane) mixture. The obtained eluent was evaporated to 5 mL. This concentrate was transferred into a chromatographic column which was packed with a mixture of 1 g kieselgel + (2.5 g kieselgel/33 vol. % 1 N NaOH) + 1 g kieselgel + (10 g kieselgel/44 vol. % of concentrated  $\text{H}_2\text{SO}_4$ ) + 5 g  $\text{Na}_2\text{SO}_4$ . The column was then eluted with 250 mL n-heptane. The eluent was collected, and evaporated to a volume of 5 mL. This concentrate was injected into a Bio-Beads S-X3 gel chromatographic column, which was equilibrated with a mixture of 50 vol. % cyclohexane - 50 vol. % ethyl acetate prior to injection. The column was eluted with 200 mL 50 vol. % cyclohexane - 50 vol. % ethyl acetate mixture. Thus, the eluent contained PCDF in a total volume of 100-180 mL. This eluent was evaporated to 1 mL, dried in a nitrogen atmosphere, and mixed with 2 mL benzene. This solution was then injected into a chromatographic column, which was pre-washed with n-hexane and packed with a mixture of 2.5 g Alumina B Super 1 + 0.5 g kieselgel/ $\text{AgNO}_3$  solution (obtained from 270 g kieselgel - 70 g 40 vol. %  $\text{AgNO}_3$  (aq)) + 1.5 g  $\text{Na}_2\text{SO}_4$ . This column was first washed with a 30 mL (98 vol. % n-hexane - 2 vol. % dichloromethane) mixture. The washing mixture was discarded. Then the column was eluted with a 30 mL (50 vol. % n-hexane - 50 vol. % dichloromethane) mixture. Eluent was collected and evaporated to 5 mL, and dried in a nitrogen atmosphere and 20 mL benzene was added.

## GC/MS analyses

Analyses for PCDF was carried out with the gas chromatography and the mass-selective detector, coupled with a direct interface. Sample aliquots of 1  $\mu\text{L}$  in benzene solution were injected splitless (injector

temperature 300°C). A CP Sil 88 capillary column was employed, and helium was used as a carrier gas, and the temperature of the chromatography was programmed as follows: 130°C in one minute from 130°C to 240°C at a rate of 15°C/min. For the HeptaCDF/OctaCDF congeners, a DB-5 capillary column was employed and the temperature programme was adjusted at 20°C/min. from 130°C to 220°C and at 5°C/min. from 220°C to 300°C. The capillary column was directly connected to a mass-selective detector in order to achieve efficient chromatographic separation.

The quantitative evaluation was accomplished by the use of mass-fragmentograms obtained from GC/MS analysis in the following manner: The “m/z (M+) and (M+2)+” ions of all the PCDF isomers were obtained from the analysis of the soil sample and standards. The minimum, average and maximum values (ng/kg) in specific isomer determination of PCDF found in the soil sample are given in the Table.

**Table.** The Contribution of Individual Congeners of All PCDF (ng/kg)

		Minimum	Average	Maximum
Total TetraCDF		0.045	11.20	19.90
	-2,3,7,8-TetraCDF	0.008	1.34	2.75
Total PentaCDF		0.048	16.30	28.6
	-1,2,3,7,8-PentaCDF	0.014	3.30	6.33
	-2,3,4,7,8-PentaCDF	0.005	0.94	1.78
Total HexaCDF		0.077	19.70	32.00
	-1,2,3,4,7,8-HexaCDF	0.017	4.43	7.92
	-1,2,3,6,7,8-HexaCDF	0.009	2.41	4.10
	-1,2,3,7,8,9-HexaCDF	0.001	0.38	0.57
	-2,3,4,6,7,8-HexaCDF	0.005	0.66	1.30
Total HeptaCDF		0.106	26.60	47.20
	-1,2,3,4,6,7,8-HeptaCDF	0.070	13.56	27.73
	-1,2,3,4,7,8,9-HeptaCDF	0.010	2.91	6.62
Total OctaCDF		0.089	19.00	38.60
Total PCDF		0.365	92.90	166.30
Total Toxic Equivalent (TE BGA)		0.008	2.028	3.709

## Results and Discussion

The objective of this work is to develop an analytical method for determining PCDF congeners in soil samples. The analytical data for samples taken at soil around industrial regions and the toxicity equivalents calculated according to the Federal Health Office of the Federal Republic of Germany (TE, BGA)<sup>11</sup> are illustrated in the Table. We reported on the PCDF extraction procedures from soil sample and isolation techniques of PCDF from materials having the same specific characteristics.

The procedures followed in this study differ in various details, such as the choice of chromatography columns and extraction reagents. Based on an effective extraction procedure, the samples were extracted in a Soxhlet apparatus with toluene rather than with benzene and ethoxy-ethanol as described in literature<sup>12</sup>. Since benzene is carcinogenic, toluene is preferred as a solvent. In GC/MS analysis, a CP-Sil 88 capillary column was used for all PCDF isomers. The clean-up procedures employed in this work were based on solid-liquid adsorption chromatography in a glass column, in contrast to the other methods. Five types of column were used for clean-up. This sequential chromatographic method allows a more sensitive separation than the method given in the literature<sup>13-14</sup>. Mass fragmentograms of “M+” and “(M+2)+” ions, and

mass fragmentograms of  $^{13}\text{C}$ -standard ions, which are necessary for the quantitative determination of these ions, have been obtained. Furthermore, mass fragmentograms of HeptaCDF and OctaCDF isomers and the  $^{13}\text{C}$ -standard ion of these obtained with a DB-5 capillary column have been also obtained. All PCDF isomers were found in the fly ash sample and well separated from each other.

We analyzed a series of soil samples which contaminated with PCDF in the surroundings of industrial plants in Konya. Samples were taken during a period of 1-3 months. Samples near the industrial region and around an area 1000 m from the industrial region were collected. The total concentrations of some PCDFs for soil layers of 0-30 cm depth are presented in the Table. There is a good agreement in the concentration of homologue groups and individual super toxic 2,3,7,8-substituted congeners in soil samples taken from the contaminated site.

The toxicology of PCDF is discussed much more extensively elsewhere<sup>15-18</sup>, but it is clear from the foregoing that there is a need to accomplish isomer-specific analysis of PCDF in many types of sample media. It is likely that the toxic hazard posed by PCDF depends on several factors, including the nature of the major components of the mixture, the concentration of specific PCDF, the route of exposure, and various other factors. In any case, the distribution of PCDF in environments where human exposure is likely to occur is a matter of considerable concern.

The procedures followed differ in various details, such as the choice of liquid chromatography columns and extraction reagents. In GC/MS analysis, a CP-Sil 88 capillary column was used for all PCDF isomers. The obtained mass fragmentograms of "M+" and "(M+2)+" ions and mass fragmentograms of  $^{13}\text{C}$ -standard ions were necessary for quantitative determination. In the analysis done with a CP-Sil 88 capillary column, some isomers of HeptaCDF and OctaCDF homologue groups were irreversibly adsorbed by capillary columns, reducing the errors in the quantitative determination of HeptaCDF and OctaCDF homologue groups. A DB-5 capillary column was also used. This column cannot adsorb HeptaCDF, and OctaCDF and it is highly temperature resistant. Because of this characteristic, this column is ideal for the HeptaCDF/OctaCDF homologue groups.

All PCDF isomers were found in soil sample and well separated from each other. These results show that the chromatographic separation methods applied in PCDF analysis identification techniques of PCDF specific isomers with GC/MS are quite satisfactory.

The results of these studies compiled in the Table show that the maximum and minimum levels of PCDF were 166.30 and 0.365 ng/kg respectively in the soil sample, and the corresponding total TEQs were 3.709 and 0.008. The toxicity of these compounds depends on both the number and position of the chlorine substituents. 2,3,7,8-TetraCDF is clearly the most toxic among them. 2,3,7,8-TetraCDF, 1,2,3,7,8/2,3,4,7,8-PentaCDF and 1,2,3,4,7,8/1,2,3,6,7,8/1,2,3,7,8,9/2,3,4,6,7,8-HexaCDF were identified as the major constituents in the samples.

Based on these results, PCDF emissions from the industrial region to the soil present a significant health risk for people living around these locations. The results of these investigations show that the procedure given in this particular study for the identification of PCDFs formed in soil around industrial regions can be used as an alternative method.

## Conclusion

Procedures for the clean-up of samples and quantitation of GC/MS results were further developed, and it is concluded that the analysis for PCDF in the soil samples from the industrial region can be easily completed.

This study shows that PCDF emissions from industrial sources present a significant health risk for people living around these locations. The procedures described can be applied to other waste samples for PCDF determination.

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