

**THE IDENTIFICATION AND QUANTITATIVE ANALYSIS OF THE
VERY TOXIC 2,3,7,8-TETRACHLORDIBENZO-P-DIOXIN IN THE
PRESENCE OF POLYCHLORINATED DIBENZODIOXINS (PCDDs)
AND POLYCYCLOPORINATED DIBENZOFURANS (PCDFs)
CONTAMINATED SOIL SAMPLES**

Key Words: Polychlorodibenzodioxins, Toxic Materials, GC-MS, TCDD,
PCDD, PCDF

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ABSTRACT

This research describes the nature and extent of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) contamination in the soil samples from a waste cable incineration plant. The samples are collected at different places in the nearest and far away points of the soil around the incineration plant. A procedure which is described for the selective separation of 2,3,7,8-TCDD from all other PCDDs and PCDFs fractionated on Alumina Woelm B Super I in such a manner that all PCDDs and PCDFs are eluted prior to 2,3,7,8-TCDD. This procedure allows more sensitive quantitative determination of 2,3,7,8-TCDD in the soil samples.

INTRODUCTION

We have recently published a procedure¹ which allows the selective determination of PCDDs and PCDFs on the roadside of motorways. Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) have been identified as by products in industrial compounds, formed during the combustion processes in the municipal incinerators, in the waste cable burning processes and in the production of some chlorinated aromatic compounds^{2,7}

Problems concerning the correct determination and interpretation of soil pollution have been considered to be important due to toxic effects. Some of the PCDDs and PCDFs have extremely toxic properties and they are a risk to human health. The most toxic compound appears to be the 2,3,7,8-substituted tetrachlorodibenzo-dioxin.⁸⁻¹⁰ Due to its potential bioaccumulation and resistance to metabolism, this halogenated compound has received much scientific attention. One of the sensitive signs of 2,3,7,8-TCDD toxicity in animals is the loss of body weight. Although doses are low, it leads to embryotoxicity^{11,12}. If a low detection

limit is desired, the quantitative determination of 2,3,7,8-TCDD in soil samples containing a large amount of TCDDs and TCDFs is difficult. In this study, 2,3,7,8-TCDD determination in soil samples coming from the chlorine source of a waste cable incineration plant is described.

The most toxic isomer of PCDDs is 2,3,7,8-TCDD and it has a structural formula shown in Fig. 1³

EXPERIMENTAL

Equipment

1. Gas Chromatography (GC;HP): Hewlett-Packard GmbH, Germany.
2. Mass Spectrometer (MS 5970 B): Hewlett-Packard GmbH.
3. Bio-Beads S-X3 G I chromatography: Bio Rad, Germany.
4. Capillary Column (Chrompack, CP-Sil 88): Chrompack International, Holland.

Chemicals

1. ¹³C-PCDD/PCDF standards: Promochemie GmbH, Germany.
2. Nitrogen Gas, Helium Gas: Fa. Messer, Germany.
3. Organic Solvents: Dichloromethane, Cyclohexane, Hexane, Acetone, Benzene, Toluene (all of analytical purity): Promochemie.
4. Kieselgel-Silica Woelm 63-Active, Na₂SO₄, Conc. H₂SO₄, AgNO₃, Alumina B Super 1, Woelm Pharma Eschwege: Promochemie.

Experimental Procedure

The analytical methods developed for the determination of PCDDs and PCDFs and 2,3,7,8-TCDD in the various soil samples entail a sequence of five operations:

1. Extraction of PCDD and PCDF from the soil samples
2. Preliminary separation of PCDDs and PCDFs from the major matrix constituents



FIG.1. The structural formula of 2,3,7,8-TCDD

3. Detection of PCDDs and PCDFs in the cleaned-up sample extract
4. Preliminary separation of 2,3,7,8-TCDD from the PCDDs and PCDFs
5. Detection of 2,3,7,8-TCDD

Extraction, Separation and Detection of the PCDDs and PCDFs

To obtain pure PCDDs and PCDFs fraction free of other compounds, the extract is subjected to a clean-up procedure using the method below:

Method

The PCDD/PCDF isomers of the soil samples, were isolated and concentrated by chromatographic techniques in the following sequence:

Soil Sample

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¹³C-PCDD/PCDF standards

Soxhlet Extraction

Column Chromatography

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GC/MS Analysis (Selected Ion Monitoring)

The following adsorbents were utilised for the column chromatographic separation:

- 1- Kieselgel % 44 H₂SO₄ (2.5 X 60 cm)
- 2- Macro Alumina B Super 1 (2.5 X 60 cm)
- 3- Mix Column (2 X 30 cm)
- 4- Bio-Beads S-X3 Gel Chromatography (3 X 60 cm)
- 5- Mini Alumina 8 Super 1 (0.7 X 11 cm)

in the separation technique, known amounts of ¹³C-PCDD/PCDF standards were injected to the soil samples, and these samples were then extracted in a Soxhlet type extractor. The total PCDD/PCDF isomers of the sample were first isolated and then concentrated by passing the extract through columns packed with Kieselgel(% 44 H₂SO₄, Macro Alumina 8 Super 1, Mix. Column, Bio Beads S-X3 Gel Chromatography, Mini Alumina B Super 1 columns, all of which are "basically chromatographic separation procedures. Quantitative determinations were then performed by recording the spectra of ali PCDD/PCDF extracts on a GC/MS instrument.

Separation of 2,3,7,8-TCDD

The GC/MS analyses of the clean-up fraction containing the PCDDs and PCDFs don't allow a correct quantitative determination of 2,3,7,8-TCDD. For that reason, the eluant is evaporated and the residue is dissolved in 5 ml of benzene. This solution is injected onto a column filled with 3 g Alumina Woelm 8 Super 1 and 2.5 g Na₂SO₄, prewashed with 50 ml of hexane. This column is eluted with 35 ml of 20 % dichloromethane in hexane mixture. This fraction contains all PCDDs and PCDFs except 2,3,7,8-TCDD. Then the column is eluted with 30 ml 50 % dichloromethane in hexane mixture and 2,3,7,8-TCDD is obtained. This eluant is evaporated to 4 ml and dried in a nitrogen atmosphere and 10 µl benzene is added.

Detection of 2,3,7,8-TCDD

The final solution was analysed on a GC/MS instrument under the following experimental conditions: A CP Sil 88 capillary column was employed,

and helium was used as a carrier gas, and the temperature of the chromatographic separation was programmed as follows: 130 °C for 1 min. and raised from 130 °C to 240 °C at a rate of 15 °C/min. 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 analyses in the following manner:

The "response factor" of the 2,3,7,8-TCDD isomer was calculated from the mass fragmentogram peak of the ^{13}C -2,3,7,8-TCDD standard obtained experimentally. Then, quantitative calculation was carried out utilizing the mass fragmentogram of the isomer and its response factor.

The m/z ($M+2$) ions of all the TCDD isomers, and 2,3,7,8-TCDD isomer and ^{13}C -2,3,7,8-TCDD obtained from the analyses of the soil samples are illustrated in Fig. 2, Fig. 3 and Fig. 4.

The Suitability of The Method

In the beginning of the analysis, ^{13}C -2,3,7,8-TCDD standard was injected to the soil sample to determine how much ^{13}C -2,3,7,8-TCDD standard will be lost during the chromatographic separation. If there is some decrease in the amount of ^{13}C -2,3,7,8-TCDD standard, the same ratio will be considered in our sample. For this, a definite amount of ^{13}C -1,2,3,4-TCDD comparison sample was injected to the concentrated sample after chromatographic steps. Then GC-MS analysis of ^{13}C -2,3,7,8-TCDD standard and ^{13}C -1,2,3,4-TCDD comparison sample was done together. The obtained mass fragmentogram was given in Fig. 5. Then, the area and peak height of the mass fragmentogram of ^{13}C -2,3,7,8-TCDD standard and ^{13}C -1,2,3,4-TCDD comparison sample were compared with each other. As seen in Fig. 5, there wasn't any loss. This result proves the reliability of this method for the quantitative determination of ^{12}C -2,3,7,8-TCDD in soil samples. The difference between peak heights is smaller than the

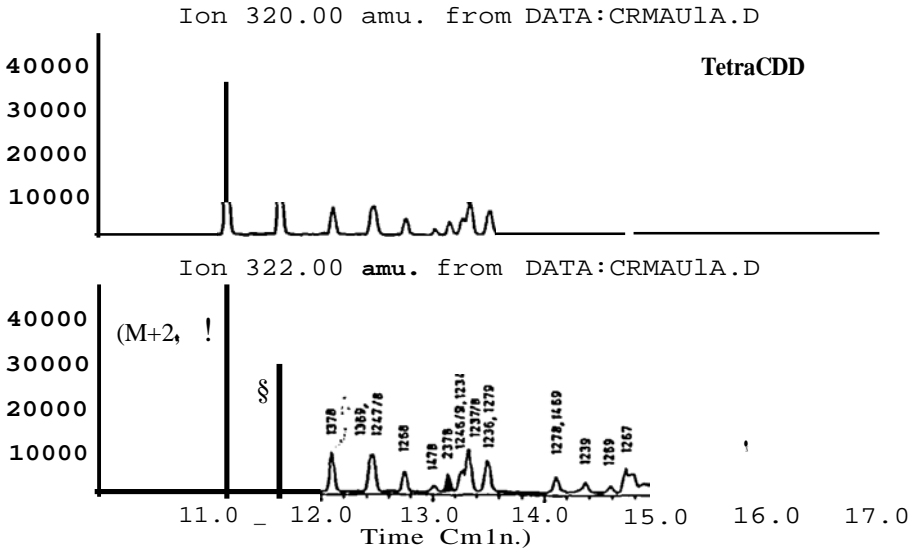


FIG. 2. The massfragmentogram of M⁺ and (M+2t) ions of tetraCDDs

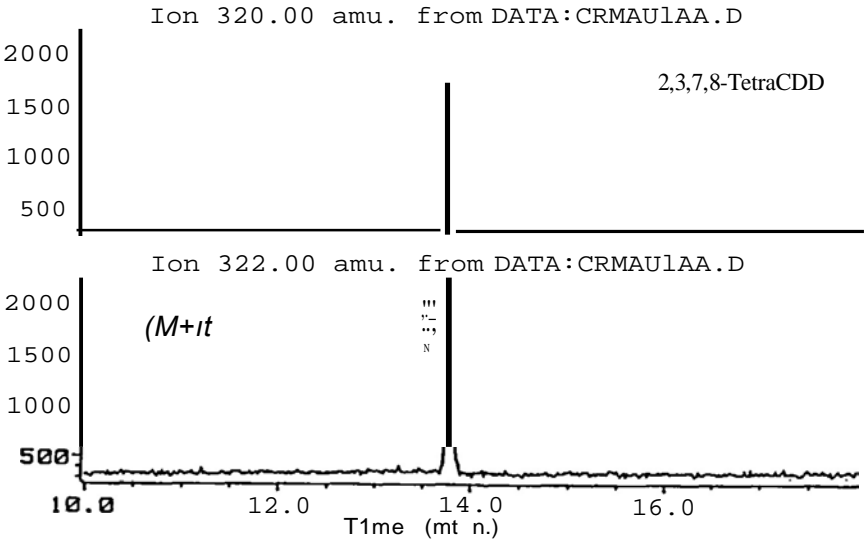


FIG. 3. The massfragmentograms of M⁺ and (M+2t) ion., of 2,3,7,8-tetraCDD

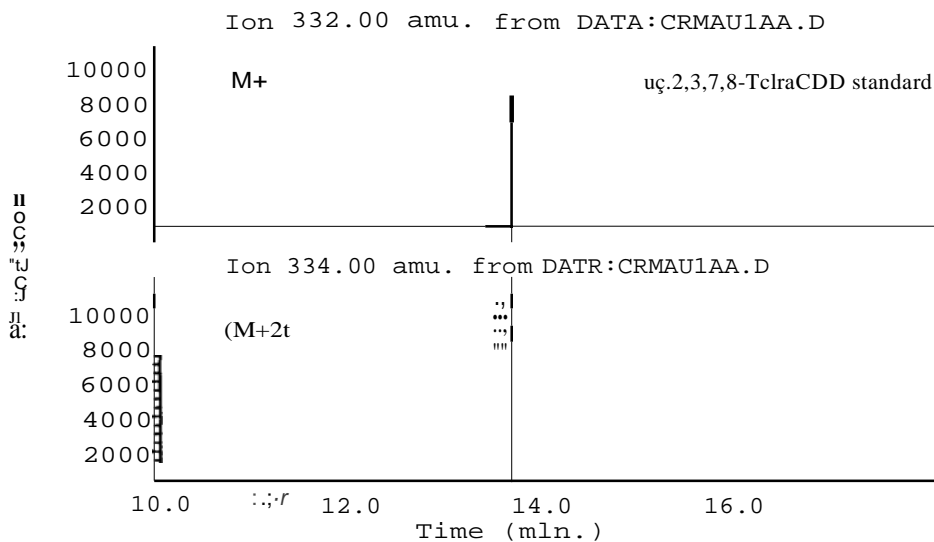


FIG. 4. The mass fragmentograms of M^+ and $(M+2t)$ ions of ^{13}C -2,3,7,8-tetraCDD standard

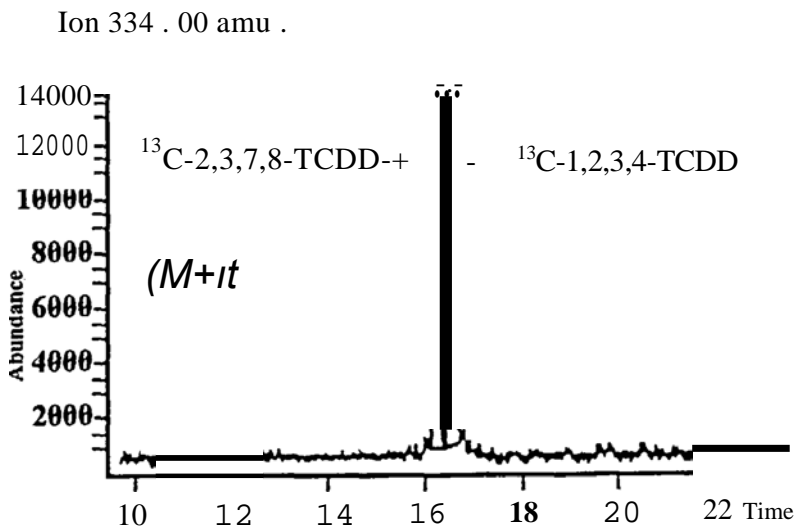


FIG. 5. The mass fragmentograms of $(M+1t)$ ions of ^{13}C -2,3,7,8-tetraCDD standard and ^{13}C -1,2,3,4-tetraCDD comparison sample

difference between peak areas. For that reason, quantitative calculations were done by using the peak heights.

RESULTS AND DISCUSSION

The aim of the present study is to find the specific concentration of 2,3,7,8-TCDD in the PCDDs and PCDFs contaminated soil samples. We report on the GC/MS properties of chlorinated PCDDs and PCDFs and we also present an analytical method for 2,3,7,8-TCDD.

Some of the PCDDs and PCDFs have extraordinary toxic properties, and have been the subject of much interest. They are now generally associated with the combustion processes when organic materials and a chlorine source are present. The isomer analyses of PCDDs and PCDFs contaminated soil samples exhibited the known broad spectrum of toxic dioxin and furan isomers. Because of the extreme toxicity of the 2,3,7,8-TCDD isomer, very sensitive analytical techniques are required. The separation, identification and quantification of 2,3,7,8-TCDD has become very important.

An extract from the soil sample was cleaned up for PCDDs and PCDFs analyses. Total concentration of PCDDs and PCDFs obtained for the different soil samples after GC/MS analyses are given in Tables 1 and 2. The PCDDs and PCDFs results reported in these Tables are the emission from the waste cable incineration plant to the soil for 12 months accumulation.

The PCDDs and PCDFs samples extracted from the soil sample were fractionated as described in the reference 1. The separation of 22 mass fragmentograms of (M+) and (M+2) isomers of TCDDs homologous group from PCDDs and PCDFs are shown in Fig. 2. In this figure, the most toxic TCDD congener substituted 2,3,7,8-TCDD is shown as the dark black peak. This fraction containing all PCDDs and PCDFs was fractionated on Alumina (Voelm

TABLE 1
The Contribution of Individual Congeners of Ali PCDD/PCDF (ng/g) in The
Different Soil Samples

	Sam le 1	Sam le2	Sam le3
Total TetraCDD	20.50	13.00	9.00
-2,3,7,8-TetraCDD	0.63	0.55	0.27
Total PentaCDD	37.80	21.00	10.40
-1,2,3,7,8-PentaCDD	2.92	2.04	1.29
Total HexaCDD	41.60	24.40	21.30
-1,2,3,4,7,8-HexaCDD	2.39	1.98	1.62
-1,2,3,6,7,8-HexaCDD	4.79	2.83	2.59
-1,2,3,-7,8,9-HexaCDD	3.59	2.26	1.83
Total HeptaCDD	66.80	43.30	42.50
-1,2,3,4,6,7,8-HeptaCDD	34.01	23.30	23.00
Total OctaCDD	63.80	16.20	50.00
Total PCDD	230.50	118.00	141.20
Total TetraCDF	154.00	52.40	45.10
-2,3,7,8-TetraCDF	24.73	6.85	6.41
Total PentaCDF	218.70	92.20	66.70
-1,2,3,7,8-PentaCDF	50.73	13.65	10.42
-2,3,4,7,8-PentaCDF	16.62	7.03	4.62
Total HexaCDF	201.70	114.00	101.40
-1,2,3,4,7,8-HexaCDF	46.26	16.29	20.20
-1,2,3,6,7,8-HexaCDF	27.91	13.33	13.76
-1,2,3,7,8,9-HexaCDF	3.99	3.70	2.90
-2,3,4,6,7,8-HexaCDF	5.88	6.67	6.52
Total HeptaCDF	357.60	102.10	110.50
-1,2,3,4,6,7,8-HeptaCDF	201.39	65.40	74.40
-1,2,3,4,7,8,9-HeptaCDF	59.03	18.20	21.90
Total OctaCDF	179.00	40.20	45.00
Total PCDF	1111.00	409.70	376.70
Total Toxic Equivalent (TE)	27.77	11.05	10.61

TABLE2

The Contribution of Individual Congeners of Ali PCDD/PCDF (ng/g) in The Different Soil Samples

	Sam le 4	Sam le 5	Sam le 6
Total TetraCDD	90.60	1.04	0.65
-2,3,7,8-TetraCDD	0.28	0.03	0.02
Total PentaCDD	13.10	2.57	1.55
-1,2,3,7,8-PentaCDD	0.88	0.17	0.08
Total HexaCDD	15.70	3.16	1.90
-1,2,3,4,7,8-HexaCDD	0.95	0.21	0.10
-1,2,3,6,7,8-HexaCDD	1.77	0.37	0.19
-1,2,3,7,8,9-HexaCDD	1.15	0.24	0.10
Total HeptaCDD	16.80	5.60	3.04
-1,2,3,4,6,7,8-HeptaCDD	10.90	3.29	1.56
Total OctaCDD	17.30	5.26	2.71
Total PCDD	72.50	17.71	9.05
Total TetraCDF	38.30	6.49	3.01
-2,3,7,8-TetraCDF	4.50	0.96	0.43
Total PentaCDF	55.20	9.97	5.30
-1,2,3,7,8-PentaCDF	11.95	1.46	0.76
-2,3,4,7,8-PentaCDF	3.29	0.69	0.35
Total HexaCDF	42.90	10.95	5.84
-1,2,3,4,7,8-HexaCDF	9.21	2.44	1.31
-1,2,3,6,7,8-HexaCDF	5.32	1.40	0.69
-1,2,3,7,8,9-HexaCDF	1.06	0.32	0.13
-2,3,4,6,7,8-HexaCDF	2.48	0.61	0.24
Total HeptaCDF	50.40	15.63	7.75
-1,2,3,4,6,7,8-HeptaCDF	30.46	8.19	4.14
-1,2,3,4,7,8,9-HeptaCDF	7.35	2.68	1.07
Total OctaCDF	28.40	10.51	5.42
Total PCDF	215.20	53.55	27.32
Total Toxic Equivalent (TE)	6.40	1.33	0.68

B Super 1 column and 2,3,7,8-TCDD is separated from the others and the fragmentograms of (M⁺), (M+2f) 2,3,7,8-TCDD are shown in Fig. 3.

The adsorption behaviour of 2,3,7,8-TCDD on the Alumina Woelm B Super 1 shown in Fig. 3, differs significantly from all other PCDDs and has not been obtained by other chromatographic techniques, such as HPLC.

The "response factor" of the Tetra-CDD isomer was calculated from the fragmentogram peaks of the ¹³C-standards obtained experimentally. Then, quantitative calculation was carried out utilising the mass-fragmentogram of the isomer and its response factor. ¹³C₁₂-2,3,7,8-TCDD internal standards are shown in Fig.4. The retention time of M⁺ and (M+2f) mass fragmentograms is seen approximately as 1.7 minutes. Meanwhile a 5 ng ¹³C₁₂-2,3,7,8-TCDD injected sample gives identical results. The quantitative values in Fig. 2 were calculated from the response factors which were obtained from the values of peak height and abundance values as given in Tables 1 and 2.

The determination of 2,3,7,8-TCDD can't be analysed without possibility of its selective loss during clean-up. The (M⁺), (M+2f) mass fragmentograms of ¹³C-2,3,7,8-TCDD as an internal standard is shown in Fig.4. The retention time of the native 2,3,7,8-tetraCDD is the same as that of the labelled standard. The isotope ratio (320/322) agrees with the labelled standard.

The total PCDD and PCDF values were over 1 ng/kg as seen in the Table 1. These toxic amounts are quite big for human health. This pollution affects foods, and the food chain is the primary pathway of human exposure to PCDF. PCDF is easily dissolved in fatty oil and is stored in human fatty tissues³. The human population is exposed to variable mixtures of polychlorinated dibenzo-p-dioxins and dibenzofurans from the environment. The persistence in man of the most toxic substance, 2,3,7,8-TCDD, suggested a half-life of several years. The distribution of various PCDDs and PCDFs was measured in rat tissues. Amounts of the applied dose to rats of over 1 ng/kg can give degeneration of liver tissue, losses of weight, tumors on the liver, lung and nose⁹. The results from this study

should reveal that chlorinated aromatic compounds such as 2,3,7,8-substituted TCDD are a potential risk to the public and the environment.

We have demonstrated that the soil samples can be analysed for 2,3,7,8-TCDD very effectively at low levels of detection with this technique. According to our experience the described procedures can also readily be applied to the other industrial products and corresponding waste samples.

ACKNOWLEDGMENT

We are grateful to Seık University Research Foundation for supporting this research project.

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Received: March 27, 1998

Accepted: November 11, 1998