

ANALYSIS OF POLYCHLORINATED DIBENZOFURANS AND
DIOXINS IN ECOLOGICAL SAMPLES

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INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are two series of tricyclic almost planar aromatic compounds which exhibit similar physical, chemical and biological properties and have been the subject of much concern in recent years. They have been involved in accident like that in Seveso, Italy in 1976, the fire in the Binghamton State Office Building in 1981, the Love Canal incident in Niagara Falls in 1979, the intoxications at horse arenas in Missouri in 1971 and Times Beach, Missouri in 1982-83, the Yusho accidents in Japan in 1968 and in Taiwan in 1979 and also in the herbicide spraying program in Vietnam in the late 1960s.

The chemical structures and numbering of these hazardous compounds are given in Figure 1. The number of chlorine atoms in these compounds can vary between one and eight producing up to 75 PCDD and 135 PCDF positional isomers as shown in Table 1.

Animal studies and in vitro experiments have indicated that there is a pronounced difference in toxic and biologic effects among different PCDD and PCDF isomers. A factor of 1 000 - 10 000 in difference can be found for so closely related isomers as 2,3,7,8- and 1,2,3,8-tetra-CDD and 1,2,3,7,8- and 1,2,4,7,8-penta-CDD (1,2). The isomers with the highest activity and the highest acute toxicity are those having 4-6 chlorine atoms and all lateral (2,3,7 and 8) positions substituted for chlorine, see Table 2. The LD₅₀-values for guinea pigs for 2,3,7,8-tetra-CDD, 1,2,3,7,8-penta-CDD, 2,3,7,8-tetra-CDF and 2,3,4,7,8-penta-CDF are all below 10 µg/kg (3,4,5).

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Because of the extreme toxicity of some of the isomers of PCDDs and PCDFs, highly sensitive and specific analytical techniques are required for the measurements. Detection levels in human and ecological samples should be orders of magnitude below the usual detection levels obtained in pesticide analysis.

Another complication is the large number of isomers (Table 1) and the large variation in toxicity and biological potency between closely related isomers. A risk evaluation should be based on the levels of the highly toxic isomers found in isomer specific analyses (Table 2).

In recent years a great number of analytical methods have been developed for the analysis of trace amounts of PCDDs and PCDFs and especially for the most toxic 2,3,7,8-tetra-CDD in technical and environmental samples.

A general procedure for isomer specific analyses of Cl₄-Cl₈ congeners of PCDDs and PCDFs in environmental samples has been described by Stalling et al., (6,7) and Buser et al., (8).

There are several requirements to be met by such an analytical method:

- 1) Representative sampling and good storage of the samples.
- 2) Efficient extraction and sample purification (clean-up), which can be tested by good recovery of labelled spikes.
- 3) High selectivity in the clean-up. The PCDDs and PCDFs present in the sample should be separated from a multitude of other co-extracted and possibly interfering compounds (6,7,8).

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- 4) High specificity. The highly toxic 2,3,7,8-substituted isomers should be separated from less toxic PCDD and PCDF isomers. Very few isomer specific analytical methods have been described, but recently the use of a 60 m SP 2330 fused silica column has been reported for such analyses (9, 10, 11).
- 5) High sensitivity. The detection levels should be in the lower program (10^{-12} g)/g (ppt) range. A mass spectrometer working in the EI or NCI mode fulfills this requirement.
- 6) Safe quantification. This can be done by a mass spectrometer using internal ^{13}C or ^{37}Cl labelled standards (8) or calibration curves of pure standards. A remarkable variation in response has been found for closely related isomers (12), consequently each isomer has to be calibrated.
- 7) Good reproducibility for the complete analytical method.

PCDDs and PCDFs are known contaminants in a series of industrial chemicals such as chlorophenols, chlorophenoxy acetic acid derivatives and polychlorinated biphenyls (PCBs). Together with other polyhalogenated aromatics they have also been detected in emissions from both municipal and industrial incinerators (10).

Fish and shellfish taken from areas in South Vietnam, that were heavily exposed to "Agent Orange" during military defoliation operations in the 1960s have been reported to contain 18-810 pg/g of TCDD (13). The analytical technique of direct inlet-high resolution MS used in this study is not considered isomer specific. It did not include any GC separation at all.

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Rappe et al., (14) identified a series of tetra to octa-CDFs in fat samples of a snapping turtle from Hudson River and gray seal from Baltic Sea. The total levels of PCDFs in these samples were 3 ppb and 40 ppt, respectively. In both samples the major PCDFs consisted of the most toxic isomers (2,3,7,8-tetra-, 2,3,4,7,8-penta- and 1,2,3,4,7,8- and 1,2,3,6,7,8-hexa-CDF).

Norstrom et al., (15) have analyzed pooled samples of herring gull eggs collected in 1982 from various parts of the Great Lakes. In all samples 2,3,7,8-tetra-CDD was found in levels ranging from 9 to 90 pg/g. The identity of the 2,3,7,8-isomer was confirmed by retention times on three capillary columns. In another study Stalling et al., (7) were not able to identify measurable levels of tetra-CDDs and other PCDDs in fish samples from Lake Superior (the detection level was 2-5 ppt). The difference could be explained by the migration of the herring gulls during the winter. On the contrary a series of PCDFs could be identified in the Lake Superior fish samples, indicating a more widespread background levels for the PCDFs than for the PCDDs. Stalling et al., (7) also reported on the analysis of fish samples from Lakes Michigan, Huron and Ontario. The toxic 2,3,7,8-substituted PCDDs and PCDFs (Table 2) were present in all samples, the highest levels being found in samples from Lake Huron, Lake Ontario and Tittabawasee River. The residue pattern found in the fish and locally high levels suggest a strong influence by point source discharges.

Similar conclusions could be drawn from a study by Ryan et al., (16). This study showed that 2,3,7,8-tetra-CDD is a relatively common contaminant of Lake Ontario commercial fish collected in 1980. About 25 % of the samples contained levels higher than 10 ppt. They also report on the presence of

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2,3,7,8-tetra-CDF at about the same magnitude as 2,3,7,8-tetra-CDD.

In the present study we report on a recovery study of the clean-up system analyzing fish samples fortified at three different spiking levels using the synthetic PCDD and PCDF standards available in our laboratory. We have also studied the elution order of these standards on a acidic alumina column. Moreover we report on the levels of PCDDs and PCDFs in samples from the Baltic Sea.

EXPERIMENTAL

The experimental conditions are given in Refs. 7,8,10,12 and 14.

RESULTS

Recovery tests

Fish samples (300 g Na_2SO_4 -fish homogenate corresponding to 50 g fish) were spiked at three different levels with a mixture of 14 tetra-CDFs, 5 penta-CDFs, 5 hexa-CDFs, 3 hepta-CDF and octa-CDF, one penta-CDD, one hexa-CDD, one hepta-CDD and one tetrachloro biphenylene, see Table 3. The spiking levels were 0 ppt, 20 ppt and 100 ppt for each isomer, and the experiments were run in duplicate.

The PCDFs and PCDDs were extracted and separated from interfering substances in two series of sequential chromatographic processes (1-3). The first process involved passage through a column containing a segment of potassium silicate prior to another segment of silica gel, the eluate then passes directly

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through a column containing carbon dispersed on glass fibers. The carbon adsorbs most planar polynuclear aromatics from the eluate of the first column whereas most biological co-extractives are not retained on this column. The PCDFs and PCDDs together with related compounds are subsequently recovered from the carbon by reverse elution with toluene.

In the second chromatographic process the sample is redissolved in hexane and applied to two columns in tandem, the first column contains sulfuric acid dispersed on silica gel and cesium silicate and the second contains acid alumina.

Detection of the PCDDs and PCDFs was accomplished by HRGC/MS, a Finnigan Model 4021 system updated with the new ion source to the 4500 model. A 30 m bonded SE 54 column was used and the instrument was operating in the NCI mode. The results are collected in Table 3.

Studies of elution order on alumina column

The same standard mixture was applied to an alumina column prepared from 3.25 g of Acid Alumina (Woelm Pharma, Akt. 1, stored at 190⁰ C for several days) in a column ϕ 6.0 mm. For the elution we collected 17 fractions consisting of n-hexane and methylene chloride in varying proportions, see Table 4. Each fraction was analyzed by GC/MS (NCI, methane) and the results are given in Figure 2.

It is interesting to notice that the highly toxic 2,3,7,8-tetra-CDF and 2,3,6,7-tetra-CBP are both eluting very late. Using a too narrow window

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in this step these two interesting compound can easily be lost during the clean-up.

Analyses of samples from the Baltic Sea

We have also analyzed two samples of Baltic Herring collected 1981 from Utlängen (S. Karlskrona, Sweden) and W. Island of Gotland (Sweden) collected 1979 and Guillemot (Stora Karlsö, Sweden) collected 1971. The clean-up technique used is described above and in Refs. 6 and 7. The fragmentograms are given in Figures 3-8 and the quantitative results in Table 5.

A series of PCDDs and PCDFs has been identified in these samples at the ppt level. Of special importance is the observation of 1,2,3,7,8-penta-CDD. This particular toxic isomer has never been reported as a contaminant in any commercial product, but it is normally found as a middle component in fly ash and other samples from municipal and hazardous waste incinerators (10).

Consequently it cannot be excluded that incinerators are the ultimate sources of the background levels of PCDFs and PCDDs found in the Baltic Sea and Great Lakes (6,7).

2,3,4,7,8-Penta-CDF is the dominating isomer among the PCDFs. This isomer is also dominating isomer in fly ash and other samples from municipal and hazardous waste incinerators (10).

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Table 1. Number of positional isomers for PCDDs and PCDFs.

Number of Cl	PCDDs	PCDFs
Mono-	2	4
Di-	10	16
Tri-	14	28
Tetra-	22	38
Penta-	14	28
Hexa-	10	16
Hepta-	2	4
Octa-	1	1

Total	75	135

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Table 2. List of highly toxic PCDD and PCDF isomers.

Very highly toxic LD ₅₀ = 1-10 µg/kg	Highly toxic LD ₅₀ = 50-500 µg/kg
2,3,7,8-Tetra-CDD	1,2,3,6,7,8-Hexa-CDD
1,2,3,7,8-Penta-CDD	1,2,3,7,8,9-Hexa-CDD
	1,2,3,4,7,8-Hexa-CDD
2,3,7,8-Tetra-CDF	
1,2,3,7,8-Penta-CDF	1,2,3,6,7,8-Hexa-CDF
2,3,4,7,8-Penta-CDF	1,2,3,7,8,9-Hexa-CDF
	1,2,3,4,7,8-Hexa-CDF
	2,3,4,6,7,8-Hexa-CDF

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Table 3. Recoveries from spiking experiments.

	Spiking		levels			
	0 ppt		20 ppt		100 ppt	
	D ^a	C	A	B	E	F
	%	%	%	%	%	%
1,3,6,8-tetra-CDF	0	-	58	46	47	55
2,4,6,8- "-	0	-	62	52	52	58
1,3,6,7-/1,3,7,9--"-	0	-	68	52	47	54
1,4,6,7-tetra-CDF ^b)	-	-	55	36	33	43
1,3,6,9-/2,3,6,8 -"-	-	-	67	52	53	62
2,4,6,7-/1,4,6,9--"-	-	-	68	52	52	58
1,2,7,8-tetra-CDF	-	-	72	52	49	57
2,3,7,8-/1,2,7,9- "-	1	-	71	56	54	58
2,3,6,7-tetra-CDF	-	-	69	54	48	59
3,4,6,7-tetra-CDF ^b)	-	-	69	50	39	52
1,2,4,6,8-penta-CDF	1	0	71	56	53	57
1,2,4,7,8- "-	2	1	71	60	50	53
1,2,3,4,8- "-	0	-	68	58	52	59
2,3,4,6,8- "-	1	-	63	58	51	59
2,3,4,7,8- "-	2	1	70	62	59	58
1,2,3,4,6,8-hexa-CDF	1	-	76	66	43	46
1,2,4,6,7,8- "-	1	-	74	62	51	55
1,2,4,6,8,9- "-	1	-	68	58	47	52
1,2,3,4,7,8- "-	2	1	66	56	53	56
2,3,4,6,7,8- "-	2	-	57	56	60	64
1,2,3,4,6,7,8-hepta-CDF	3	-	67	70	62	59
1,2,3,4,6,8,9- "-	2	-	66	70	54	52
1,2,3,4,7,8,9- "-	-	-	63	42	56	53
octa-CDF	3	-	66	52	54	50
1,2,3,7,8-penta-CDD ^b)	-	-	44	38	85	83
1,2,3,4,7,8-hexa-CDD	-	-	44	54	59	60
1,2,3,4,6,7,8-hepta-CDD	4	-	56	60	52	49
2,3,6,7-tetra-CBP	-	-	58	46	57	60

a) possibly due to carry over from the previous injection on the GC/MS system

b) low MS-response factor for this isomer

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Table 4. Composition of the eluent used in the study of the alumina column. Each fraction consisted of 4 ml. The main solvent was n-hexane with minor amounts of methylene chloride (MC) added.

Fraction number	Composition
1 + 2	0 % MC
3	2 ml 0 % MC followed by 2 ml 2 % MC
4 - 6	2 % MC
7	1 ml 2 % MC followed by 3 ml 5 % MC
8 - 10	5 % MC
11 - 15	8 % MC
16 + 17	50 % MC

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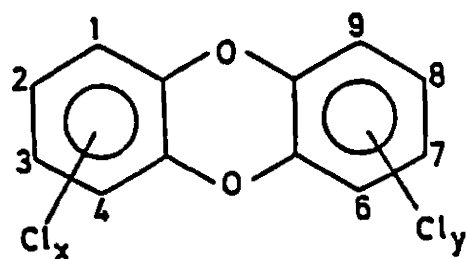
Table 5. Levels of PCDDs and PCDFs in samples from the Baltic Sea (ppt).

Isomer	Herring Utlängen	Herring W. Gotland	Guillemot Karlsö
2,3,7,8-Tetra-CDF	4	3	2
2,3,6,7- "-	1	1	T
1,3,4,8,9-Penta-CDF	-	-	T
1,2,4,6,8- "-	T	T	-
1,3,4,7,9-/1,2,3,6,8- "-	T	T	T
1,2,4,7,8- "-	1	1	-
1,2,3,7,8-/1,2,3,4,8- "-	1	1	4
2,3,4,7,8- "-	6	6	180
1,2,3,4,6,8-Hexa-CDF	T	T	-
1,3,4,6,7,8- "-	T	T	T
1,2,3,4,7,8-/1,2,3,4,7,9- "-	1	1	9
1,2,3,6,7,8- "-	T	T	25
1,2,4,6,8,9- "-	T	T	-
2,3,4,6,7,8- "-	1	1	7
2,3,7,8-Tetra-CDD	-	-	7
1,2,3,7,8-Penta-CDD	-	T	22
1,2,3,4,7,8-Hexa-CDD	-	-	15
¹³ C ₁₂ -Tetra-CDF (recovery)	88 %	78 %	79 %
¹³ C ₁₂ -Tetra-CDD ("-)	71 %	78 %	67 %
¹³ C ₁₂ -Octa-CDD ("-)	44 %	33 %	49 %

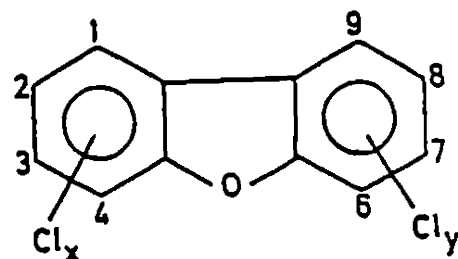
T = Traces; identified but normally less than 1 ppt.

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PCDDs
Dioxins



PCDFs
Dibenzofurans

$$x + y = 1 - 8$$

Figure 1. Formulaes for PCDDs and PCDFs.

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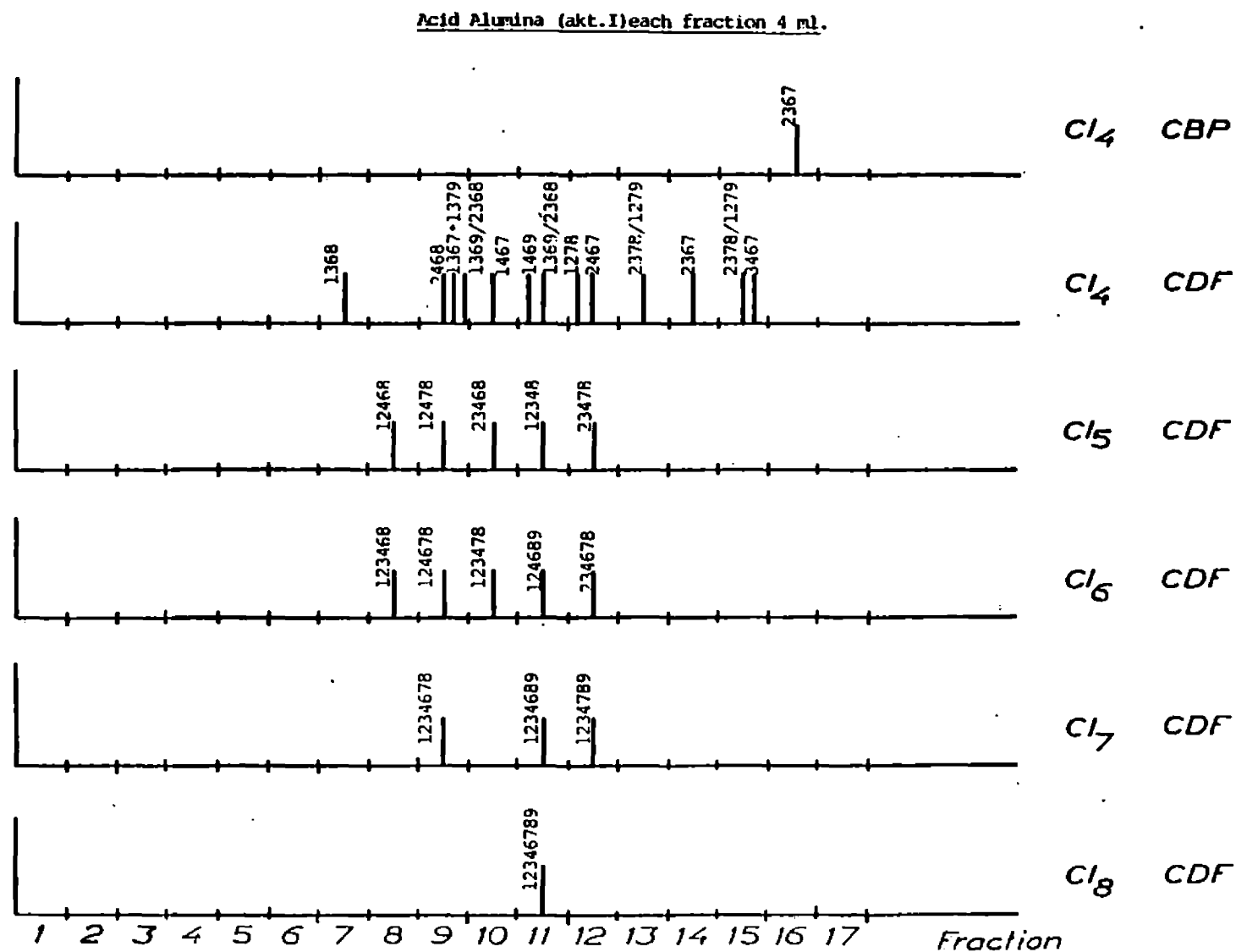


Figure 2. Elution order of the isomers studied.

SAMPLE: Herring (Baltic Sea)
MPR 53:1

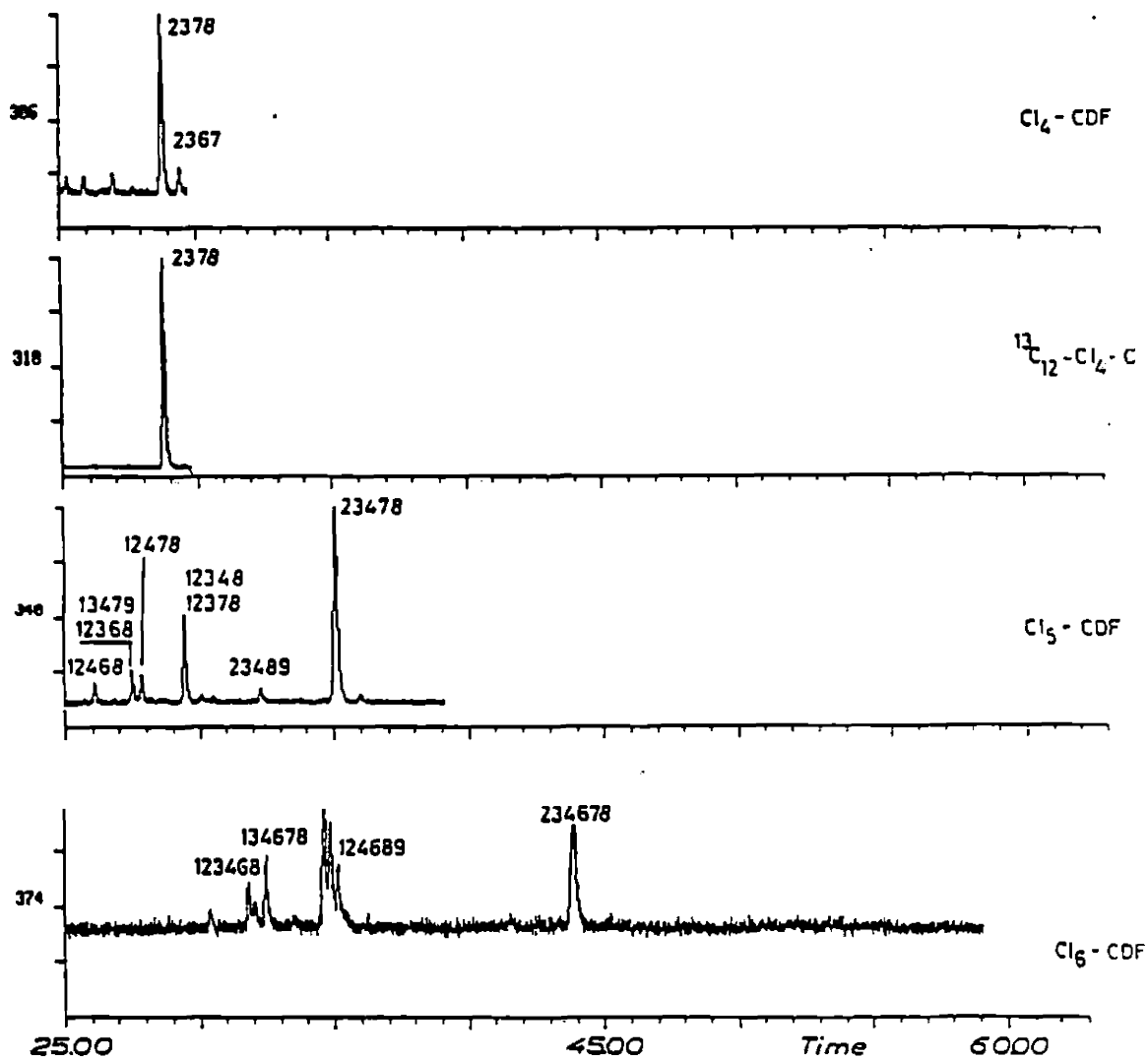


Figure 3. PCDFs in herring sample from Utlängen.

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SAMPLE: Herring (Baltic Sea)
MPR 53:4

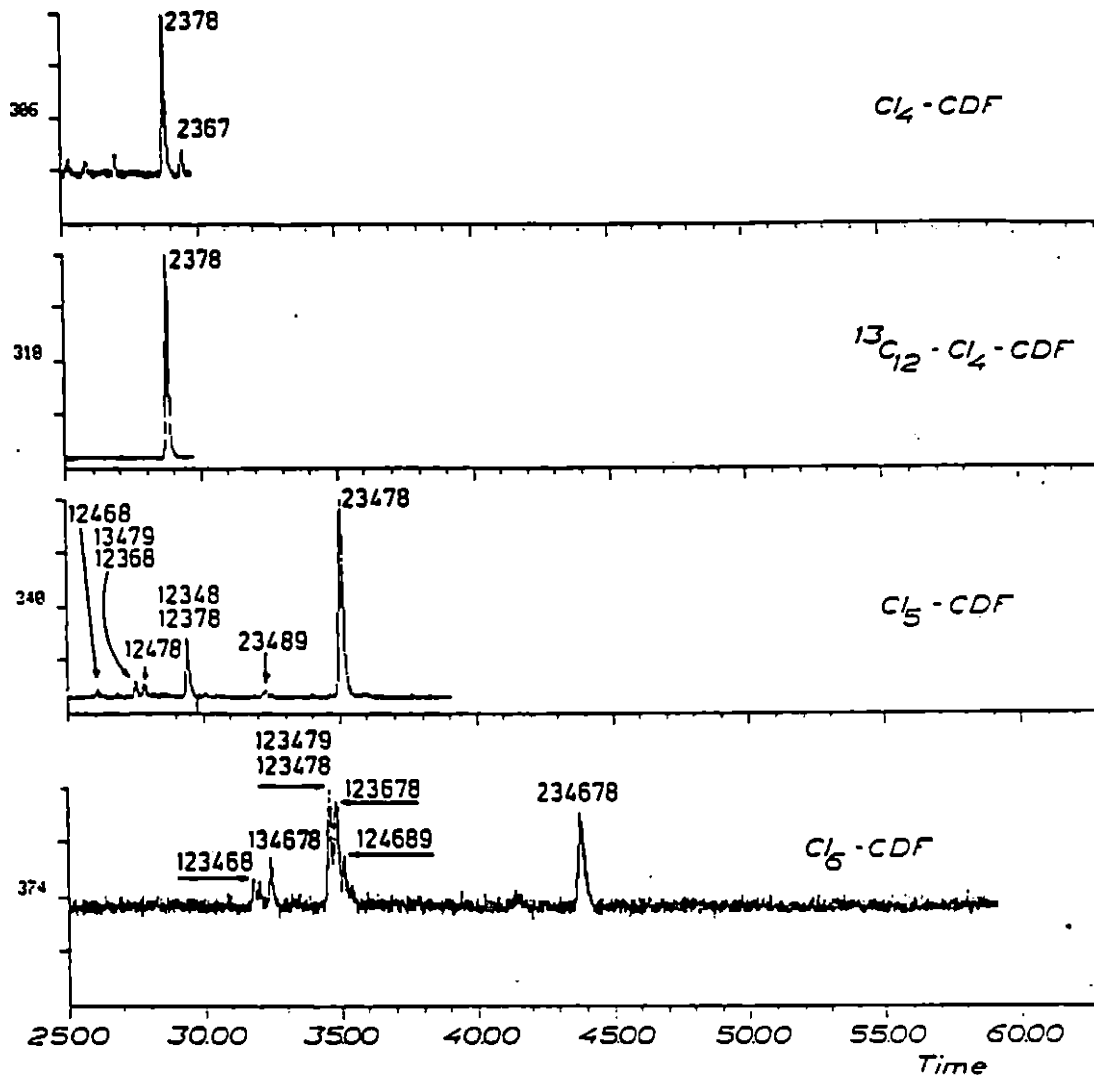


Figure 4. PCDFs in herring sample W. Gotland.

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SAMPLE: Herring (Baltic Sea)
NPR 53:1

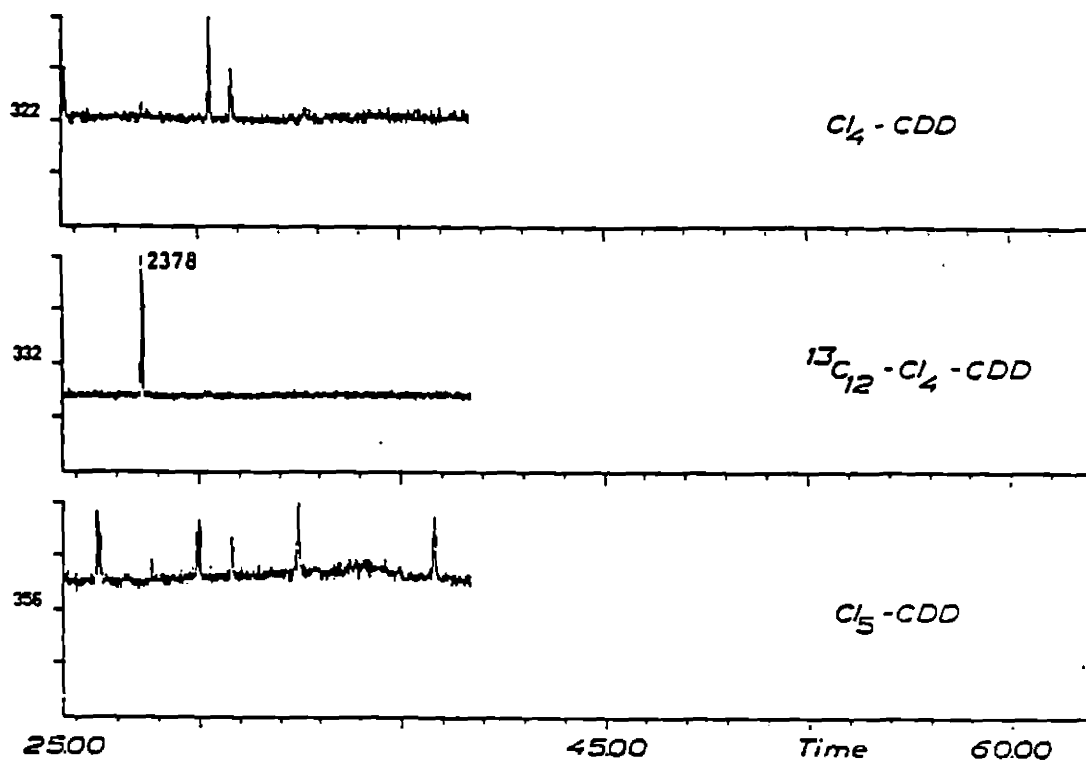


Figure 6. PCDDs in herring sample from Utlängen.

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SAMPLE: Herring (Baltic Sea)
MPR 53:4

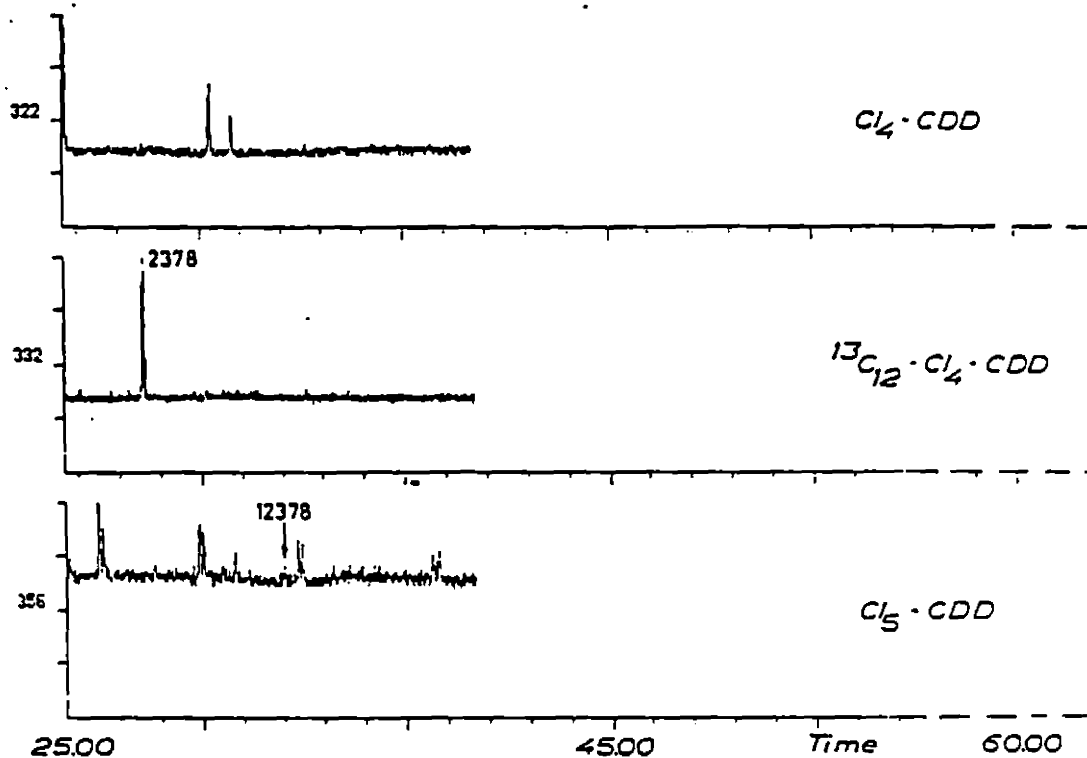


Figure 7. PCDDs in herring sample W. Gotland.

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SAMPLE: Guillemot (Baltic Sea)
MPR 51:5

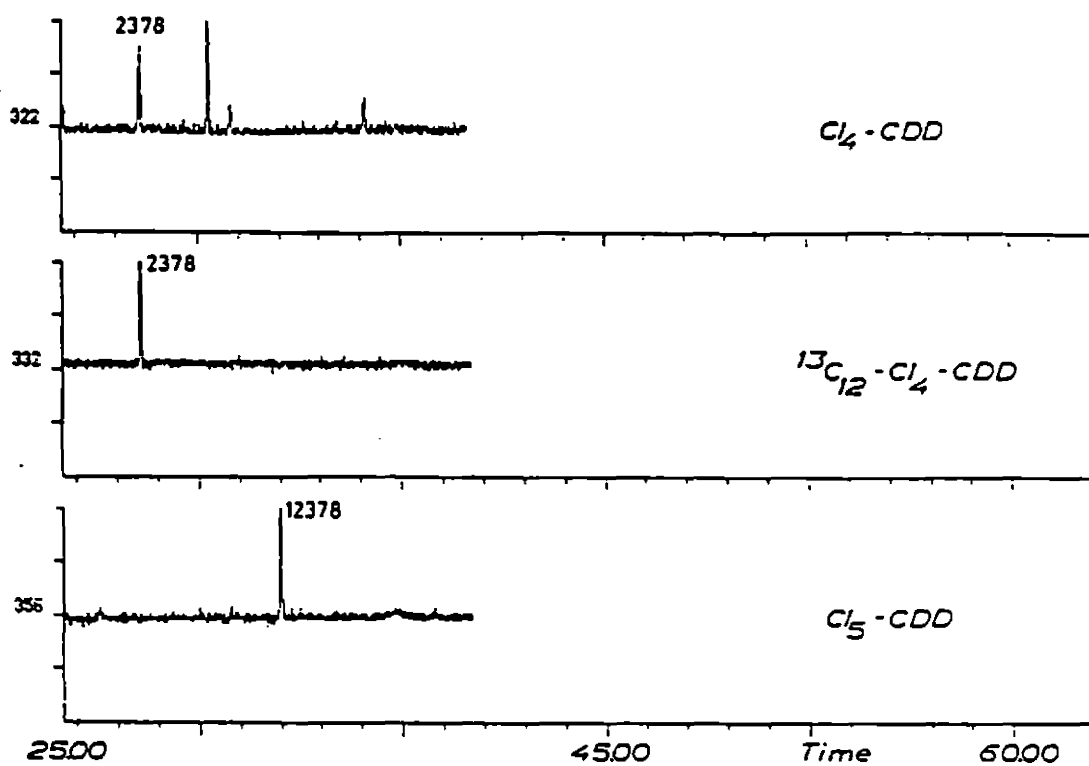


Figure 8. PCDDs in guillemot sample from S. Karlsö.

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SAMPLE: Guillemot (Baltic Sea)
MPR 53:5

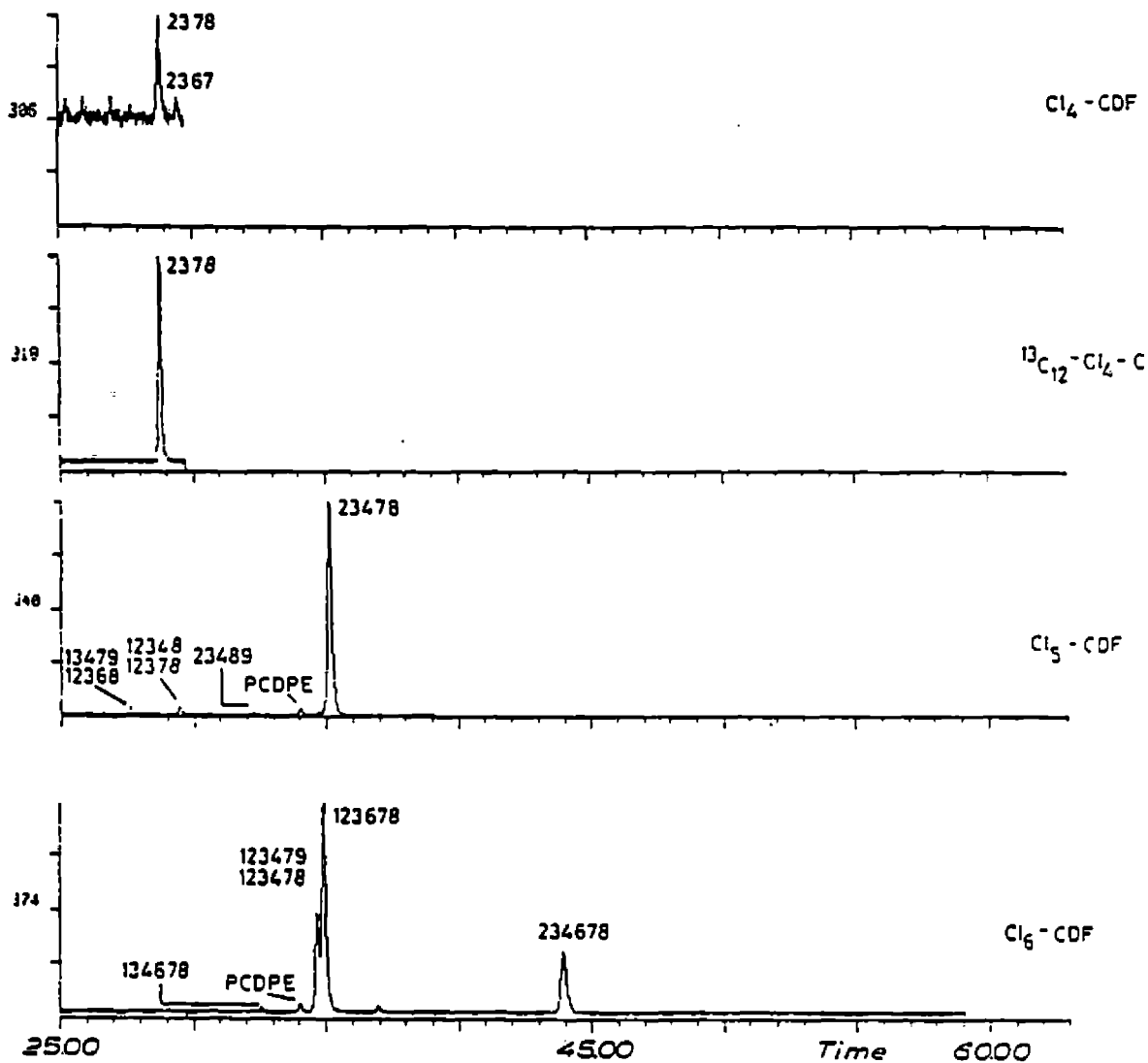


Figure 5. PCDFs in guillemot sample from S. Karlsö.

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