

Analysis of Polychlorinated Dibenzofurans, Dioxins and Related Compounds in Environmental Samples

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The analysis of polychlorinated dibenzofurans (PCDFs), dibenzo-*p*-dioxins (PCDDs) and to some extent biphenylenes (PCBPs) by high-resolution gas chromatography (HRGC) and mass spectrometry is described. Electron-impact (EI) and negative chemical ionization (NCI) mass spectrometry were used, and their application in environmental analyses documented. NCI shows increased sensitivity to all PCDFs and PCDDs except for 2,3,7,8- and other tetra-CDD isomers. The identification of the various PCDFs and PCDDs, specifically the identification of the toxic and hazardous 2,3,7,8-substituted isomers is emphasized; the unambiguous identification of all 2,3,7,8-substituted PCDDs is documented. The application of HRGC and mass spectrometry to isomer-specific analyses of samples of aquatic species from the Baltic and the Great Lakes is shown. Results from this and previous studies indicate PCDF and PCDD residues in these organisms including significant levels of 2,3,7,8-substituted isomers. These aquatic species apparently show preferential retention or reduced metabolization rates for these toxic isomers. The results of samples from the Baltic indicate a contamination primarily due to PCDFs. The origin of this contamination seems complex and related to PCBs as well as chlorophenols. PCDF and PCDD isomer distribution patterns from analyses using HRGC and mass spectrometry aid in the identification of origin, sources and elucidation of routes of formation of these compounds. These methods are indispensable in taking measures to reduce future contaminations and to protect our environment.

Introduction

Polychlorinated dibenzofurans (PCDFs), dibenzo-*p*-dioxins (PCDDs) and biphenylenes (PCBPs) are three series of related tricyclic, planar, aromatic chlorinated compounds. Each series consists of a number of chloro homologs (mono- to octachlorinated) and isomers (total numbers: 135 PCDFs, 75 PCDDs and 75 PCBPs), (1). Some of these compounds possess extraordinary toxic properties, especially isomers fully substituted with chlorine at the lateral positions (2-, 3-, 7- and 8- in case of PCDFs and PCDDs; 2-, 3-, 6- and 7- in case of the PCBPs), the so-called 2,3,7,8- (or 2,3,6,7-) substituted isomers (2-6). The chemistry, the formation and occurrence of these hazardous compounds have been previously reviewed (1).

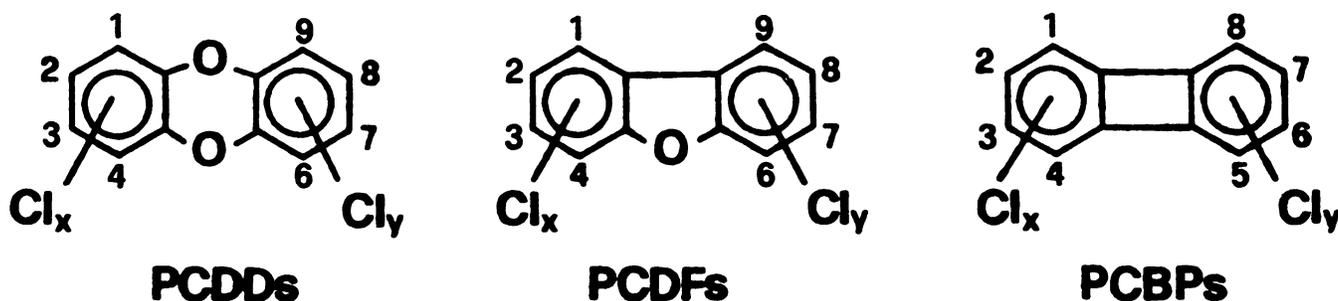
PCDFs and PCDDs are known contaminants of industrial chemicals such as the chlorophenols and their derivatives, and also of polychlorinated biphenyls

(PCBs). The best studied compound is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-tetra-CDD, TCDD); it has received wide public attention as an environmental contaminant in cases like Vietnam, Seveso, Love Canal and Times Beach. PCDFs received unfavorable attention in the Yusho poisonings in Japan and Taiwan. PCBPs are much less well known. They were identified in soot from accidents (fire, explosions) with electrical systems; however, the relevance of this finding is not completely clear. From structural considerations and from the biological activity of one of the isomers studied (2,3,6,7-tetra-CBP) they may be of similar importance as the PCDFs and PCDDs (5,6).

PCDFs and PCDDs as well as other polychlorinated aromatics have been detected in emissions from municipal and industrial incinerators. This was the first finding of PCDFs and PCDDs in samples with no apparent relation to the industrial chemicals listed above. The origin of these compounds in these emissions is still not completely known and a matter of debate. Some indications are that these compounds form through more general reactions from a variety as well as from specific chlorinated precursors. In addition, PCDFs and PCDDs

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are toxic thermolysis products from PCBs and chlorobenzenes (PCBZs) (1). The formation of PCDFs from accidental burning or incineration of PCBs and PCBZs is expected to be an important potential source of these hazardous compounds, and all decomposition and disposal of PCBs should be carefully controlled in order to prevent accidental formation and environmental contamination with these compounds (7).

Very recently, these tricyclic aromatic compounds have also been implicated in several accidents involving transformers and capacitors with PCBs and PCBZs (8-10). In addition to PCDFs and PCDDs, these accidents sometimes also produced PCBPs.

PCDFs and PCDDs have a potential for accumulation in the food chain and are resistant to metabolism or chemical degradation. Only recently, metabolites of 2,3,7,8-tetra-CDD and 2,3,7,8-tetra-CDF in mammals (dog and rat) have been identified; hydroxylated and ring-cleaved products were isolated from the bile of these animal species (11). The heat stability of these compounds (700°C or higher) is the reason that they survive high temperatures in some of these accidents involving electrical systems. These properties and the high toxicity of some of the isomers are the basis of the threat these compounds present to man and the environment. The release of these compounds from electrical system fires, explosions and overheating with involvement of PCBs and PCBZs constitutes a previously unrecognized health hazard, and environmental contamination with PCDFs, PCDDs or PCBPs through such accidents must be prevented.

In this report, we discuss analytical techniques to detect and quantify these compounds in environmental and biological samples. There has been a major international effort for more than a decade in a large number of studies to develop methodology for the analysis of these compounds, notably the 2,3,7,8-tetra-CDD (12). The most sensitive and specific analytical techniques use mass spectrometry for detection, quantification and confirmation of these compounds after extensive and sophisticated purification of sample extracts. However, often these methods are designed for the recovery of specific compounds, e.g., 2,3,7,8-tetra-CDD, with no provisions made to recover other or all PCDFs or PCDDs. Nevertheless these procedures prove generally applicable or may be easily modified for complete PCDF and PCDD analyses (13).

The technique described here uses high-resolution gas chromatography (HRGC) and mass spectrometry. Emphasis is on isomer identifications. Isomer distribution patterns obtainable from such analysis aid in the identification of contamination sources. Sample preparation procedures for such analyses have to be designed to recover all PCDFs and PCDDs as a group. Isomer-specific analyses using HRGC and mass spectrometry are then followed. Extremely useful would be methods that allow an unambiguous identification of all the toxic congeners, such as the 2,3,7,8-substituted isomers. This has been achieved for the PCDDs (14) and also for the PCDFs (15). The main discussion here will be on the analysis of PCDFs and PCDDs, and less on PCBPs and other polychlorinated polynuclear aromatics (PCPNAs). The latter two series of compounds have also been formed in some accidents involving electrical systems, but little is known of their toxicity and the routes and conditions required for their formation; reference materials are only sparingly available. Results of some environmental analyses will be discussed. Although the samples studied have no direct relation to the incidents in discussion here, they should document the use of the described analytical methodology for identifying sources and origin of contaminations with these compounds. These methods are indispensable in taking measures to reduce contamination and to protect our environment.

Analytical Requirements

Prior to the analysis by HRGC and mass spectrometry, samples must be extracted and the extracts purified. Extensive methodology is available for the analysis of 2,3,7,8-tetra-CDD by multistep cleanup procedures. Fairly simple and fast procedures involving multiple chromatography on silica, modified (HSO) silica, and alumina usually recover all the PCDFs and PCDDs and are expected to recover most of the PCBPs (13,16). These methods are applicable to levels of 1 to 10 ppt (part per trillion, $1:10^{12}$) 2,3,7,8-tetra-CDD in environmental samples, but may result in interference for some other PCDFs and PCDDs at levels below 100 ppt.

An efficient, sophisticated procedure for the cleanup of environmental and biological samples was described by Stalling and collaborators (17,18). This procedure makes use of carbon adsorption chromatography which

preferentially retains all the planar, polynuclear aromatic compounds which are thus specifically recovered and analyzed. This cleanup was judged among the best available in a comparative study for the analysis of 2,3,7,8-tetra-CDD in fish (19). It has also been tested extensively and found to be suitable for the recovery of other PCDDs and PCDFs (13). Often this has not been shown for other cleanup procedures. For the analysis of samples from the sites of accidents, simpler procedures may be applicable (10,20). In this case, special attention must be given to the difficult recovery of these planar aromatic compounds from soot (Soxhlet extraction with boiling toluene may be required). Good recovery and reproducibility are prerequisites for extraction and clean-up.

There are three requirements to be met by the actual analytical procedure for these compounds: (1) high sensitivity: detectable quantities have to be in the picogram (10^{-12} g) range and detectable concentrations in the part per trillion range because of the extreme toxicity of some of these compounds; (2) high selectivity: a distinction is required between PCDFs and PCDDs and a multitude of other, co-extracted and possibly interfering compounds present at concentrations up to several magnitudes above those of the compounds of interest; (3) high specificity: a differentiation among various isomers is desired, as between toxic (2,3,7,8-substituted) and other isomers.

Mass spectrometry is ideally suited for the first two requirements. Sensitivity of this method with single or multiple ion detection (SID or MID) is of the order of picograms and even below when special ionization techniques (negative chemical ionization, NCI) are used. However, mass spectrometry alone cannot differentiate among most isomers; most isomers of a PCDF or PCDD yield very similar spectra. Mass spectrometry alone thus cannot give the required isomer specificity in these analyses. For this differentiation, highly efficient separation techniques are required. Most suitable is HRGC, because it can be combined easily and directly with mass spectrometry. High-performance liquid chromatography (HPLC) has also been suggested and is sometimes used (21); however, the necessity of analyzing several fractions for complete analysis makes the method impractical. Its applicability to PCDF and penta-CDD analyses has never been documented.

Mass Spectrometry of Polychlorinated Tricyclic Aromatic Compounds

In our analyses we used a low-resolution, quadrupole mass spectrometer with two ionization techniques. Electron-impact (EI) ionization (50–70 eV, 250°C) is preferred for tetrachlorinated compounds (sensitivity 1–10 pg, SID) but shows decreasing sensitivity for the higher chlorinated species. NCI with methane as reagent gas (0.35 Torr, 180°C) shows extremely good sen-

sitivity for all PCDFs (tetra- to octachlorinated), and for the higher chlorinated PCDDs (penta- to octa-CDD) sensitivities attainable are in the 10 to 100 fg (10^{-15} g) range using SID, 1 to 2 orders of magnitude better than EI. However, NCI has very poor sensitivity for 2,3,7,8-tetra-CDD under these conditions. NCI also has the disadvantage of requiring more frequent cleaning of the ion source, although this may be circumvented by using newer equipment. NCI using O was suggested and used but requires a specially designed ion source (Townsend discharge); oxygen inclusion (M^-Cl+O , M-19) and cleavage ions from PCDDs are formed (22).

The EI mass spectral properties of PCDFs and PCDDs have been previously described (23,24) and are (with PCBPs) summarized here and in Table 1. Low mass ions (m/z 50–150) from cleavage of ether bridges allow identification of substitution types of PCDD isomers (e.g., distinction of 2–2, 3–1 and 4–0 tetra-CDDs) (23). These low mass ions, however, may be obscured in spectra from environmental or biological samples. Most isomers of a PCDF, however, yield identical EI mass spectra and a distinction of different substitution types is not possible. The molecular (M) and fragment ions of PCDFs and PCDDs show the typical, expected clustering due to the chlorine isotopes. PCDFs and PCDDs are easily identifiable from these EI mass spectra by M^+ , chlorine number and their typical fragmentations (M^+-COCl , etc.). Detection limits are 1 to 10 pg for the tetrachloro compounds up to 10 to 50 pg for the octachloro compounds using SID or MID (M^+ or M^++2 ions, respectively). Full mass spectra require 0.1 to 1 ng (10^{-9} g). Quantification by use of SID or MID has an acceptable accuracy and precision. The ions used to monitor these compounds are listed in Table 2 (M^+ for tetra-, penta- and hexachloro compounds; M^++2 for hepta- and octachloro compounds).

EI mass spectra of PCBPs show intense molecular ions (with typical clustering) and fragmentation is ac-

Table 1. EI mass spectral data of tricyclic aromatic compounds.^a

	Ion	Intensity ^b
PCDFs	M^+	+++++
	M^+-Cl	+
	M^+-COCl	+++
	M^+-Cl_2	+
	$M^+-COCl-Cl_2$	++
PCDDs	M^{2+}	+
	M^+	+++++
	M^+-Cl	+
	M^+-COCl	+++
	M^+-Cl_2	+
	$M^+-2COCl$	++
PCBPs	M^{2+}	+
	M^+	+++++
	M^+-Cl_2	+
	M^+-Cl_2	++
	M^+-Cl_3	+
	M^+-Cl_4	+
	M^{2+}	+

^a70 eV, 240°C source temperature.

^bIntensity of ions semiquantitatively given by number of marks (+).

complicated by losses of Cl \cdot and Cl $_2$. Very few authentic standards, however, have been studied. It should be pointed out that most PCBs yield intense fragment ions (M $^+$ -Cl $_2$) with the same exact mass as a PCBP. The identification of PCBPs therefore is not as simple and may easily be obscured by the presence of PCBs; the presence of PCBPs therefore has to be checked carefully by confirming the absence of interfering PCBs. The new technique of mass spectrometry-mass spectrometry (MS-MS) may be especially useful for this confirmation (10).

NCI mass spectrometry is extremely useful for PCDFs and the higher chlorinated PCDDs, and its sensitivity is orders of magnitude better than EI (2,3,7,8-tetra-CDF, 22 \times ; 1,2,3,6,8,9-hexa-CDD, 75 \times ; octa-CDD, 170 \times ; octa-CDD, 440 \times). This high sensitivity allows acquisition of complete or partial mass spectra from picogram amounts of a PCDF or PCDD. The NCI sensitivity and mass spectra (ratio fragment ions to molecular ions) however, are much more dependent on the actual ion source conditions (temperature, pressure, oxygen content, residence time of ions), especially for PCDDs.

Although not all PCDF and PCDD isomers have been systematically studied under NCI conditions, the following seems valid, as summarized in Table 3. In case of the PCDFs, the base peak is usually due to M $^-$ and fragmentation is by an unexpected addition of H \cdot and loss of Cl \cdot yielding unusual M $^-$ -34 ions. Fragmentation of PCDDs is more conventional, with loss of Cl \cdot yielding M $^-$ -35 ions; very little inclusion of H is observed. At-

tention should be given to the fact that in SID analysis these M $^-$ -34 ions of higher chlorinated PCDFs give rise to signals at the m/z values for lower chlorinated congeners. Among the compounds studied (four-hepta-, seven hexa- and four tetra- CDF isomers), all isomers of the PCDF showed practically identical NCI mass spectra. However, among PCDD isomers, some marked differences were observed. Whereas molecular ions (M $^-$) are observable for all PCDDs they are intense (base peak) only in case of the penta-CDDs and some of the hexa-CDD isomers. Other hexa-CDD isomers and hepta- and octa-CDD show more intense M $^-$ -Cl fragment ions (tetra-CDD isomers not yet systematically investigated). The ions used for monitoring PCDFs are M $^-$ or M $^-$ + 2; for PCDDs they are M $^-$ (lower chlorinated congeners) and M $^-$ -Cl + 2 (higher chlorinated congeners) (see Table 2). The main disadvantage of NCI mass spectrometry is the poor sensitivity of 2,3,7,8-tetra-CDD (sensitivity NCI/sensitivity EI / 0.2).

Internal Standards in Mass Spectral Analyses

Stable isotope-labeled (^{37}Cl and ^{13}C) congeners are available for use in mass spectral analyses of PCDDs and PCDFs (KOR Isotopes, Cambridge, MA; Cambridge Isotope Laboratory, Cambridge, MA). Known quantities of these internal standards are usually added to samples prior to extraction and purification; they help to account for losses during cleanup and for identification and quantification. In Figure 1 we present the spectra of native 2,3,7,8-tetra-CDD, and for comparison those of $^{37}\text{Cl}_4$ -2,3,7,8- and $^{13}\text{C}_{12}$ -2,3,7,8-tetra-CDD. From these spectra the purity of the isotope labels of our internal standards are calculable as 95% ^{37}Cl (25% presence of m/z 326, $^{37}\text{Cl}_3$ ^{35}Cl -tetra-CDD) and 98.5% ^{13}C (21.8% presence of m/z 331, $^{13}\text{C}_{11}$ ^{12}C -tetra-CDD). Interesting, but not unexpected, is the finding of the main fragment ions at M $^+$ -63 (loss of $^{12}\text{C}^{35}\text{Cl}$) for the native, at M $^+$ -64 (loss of $^{13}\text{C}^{35}\text{Cl}$) for the ^{13}C -labeled and M $^+$ -65 (loss of $^{12}\text{C}^{37}\text{Cl}$) for the ^{37}Cl -labeled material. Although the molecular weights (ions) of these internal standards are significantly increased (+ 8 and 12 daltons, respectively), they practically coelute with the native compounds even on HRGC columns (55 m Silar 10 c glass capillary column; elution of $^{13}\text{C}_{12}$ -2,3,7,8-tetra-CDD 0.5 sec earlier, that of $^{37}\text{Cl}_4$ -2,3,7,8-tetra-CDD 0.4 sec earlier than native compound). Possible interferences from other compounds at the m/z values used to monitor these internal standards may occur such as at m/z 328 (^{37}Cl -tetra-CDD) by PCBs (pentachlorobiphenyls, M $^+$ + 4) and at m/z 332 (^{13}C -tetra-CDD) by PCNs (hexachloronaphthalenes, M $^+$). In addition, native tetra-CDDs (M $^+$ = 320) give a response at m/z 328 (M $^+$ + 8) but not at m/z 332. Interference from the labeled compound at the m/z values used for the native compounds are expected to be negligible. Nevertheless, it is good practice that the amounts of internal stan-

Table 2. Ions (m/z) used for monitoring PCDFs and PCDDs in environmental samples

	Tetra-	Penta-	Hexa-	Hepta-	Octa-
PCDFs					
EI (M $^+$)	304	338	372	—	—
(M $^+$ + 2) ^a	306	340	374	408	442
NCI (M $^-$)	304	338	372	—	—
(M $^-$ + 2) ^a	306	340	374	408	442
PCDDs					
EI (M $^+$)	320	354	388	—	—
(M $^+$ + 2)	—	—	—	424	458
NCI (M $^-$)	(320)	354	388	—	—
(M $^-$ - 35)	—	319	353	—	—
(M $^-$ - 35 + 2)	—	—	—	389	423

^aLess interference from PCNs and PCTs but possible interference from PCDFEs.

Table 3. NCI mass spectral data for PCDFs and PCDDs.^a

	Ion	Intensity ^b
PCDFs		
	M $^-$,	+++++
	M $^-$ + H - Cl (M $^-$ -34),	++
	M $^-$ + 2H - 2Cl (M $^-$ -68),	+
	M $^-$ + 3H - 3Cl (M $^-$ -102),	+
PCDDs		
	M $^-$,	++++ (+++++)
	M $^-$ -Cl (M $^-$ -35),	+++++ (++++)
	M $^-$ + H - 2Cl (M $^-$ -69),	+
	M $^-$ -2Cl (M $^-$ -70),	++
	M $^-$ + H - 3Cl (M $^-$ -104),	+

^aCH $_4$ reagent gas, 0.35 Torr; 180°C source temperature.

^bIntensity of ions semiquantitatively given by number of marks (+).

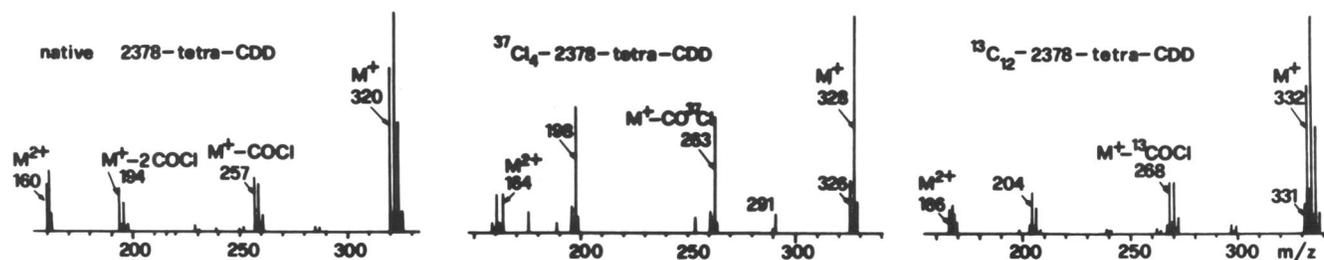


FIGURE 1. EI mass spectra of native and stable isotope (^{37}Cl - and ^{13}C -) labeled 2,3,7,8-tetra-CDD.

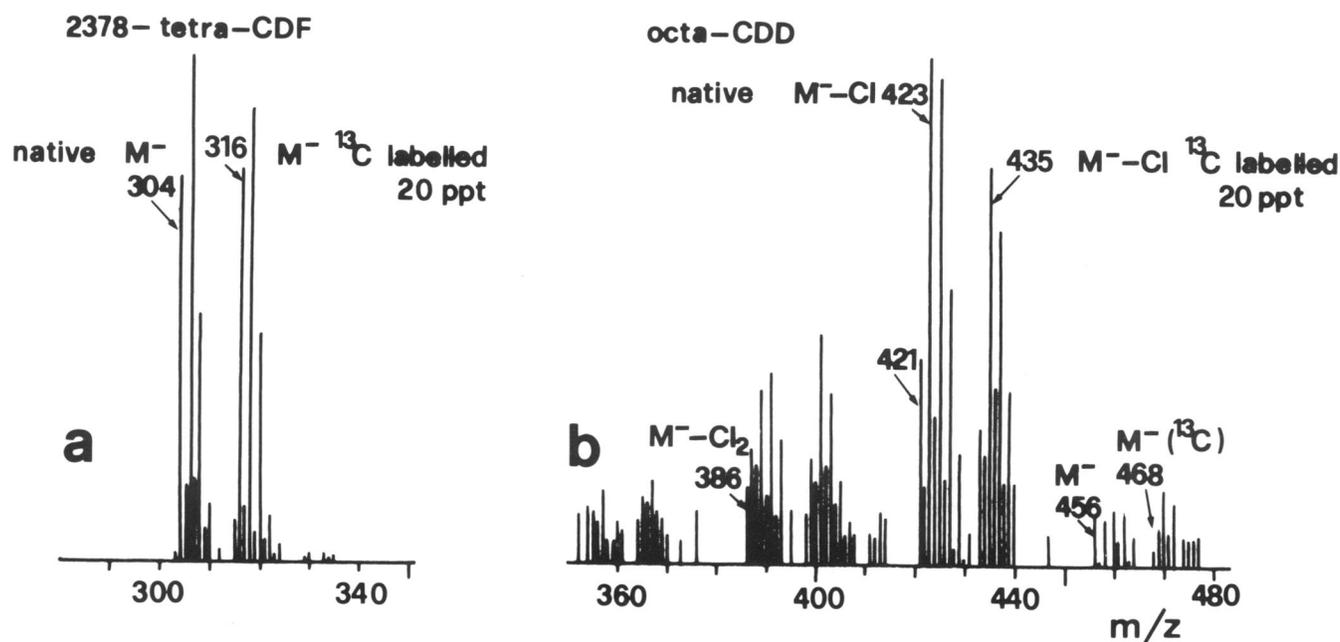


FIGURE 2. Partial mass spectra (NCI) of a fish extract showing presence of native in addition to ^{13}C -labeled 2,3,7,8-tetra-CDF and octa-CDD. The ^{13}C -labeled internal standards were added at concentrations of 20 ppt; the native compounds are calculated to be present at concentrations of about 25 ppt.

dards added are in the range (1-100 \times) of the native compounds present.

As illustrated in Figure 1, the ^{13}C -labeled compounds retain the typical chlorine clustering of the molecular and fragment ions, which greatly aids in their identification and simplifies quantification of the native compounds. This is clearly shown in Figure 2 with the analysis of a fish extract fortified with 20 ppt each of $^{13}\text{C}_{12}$ -2,3,7,8-tetra-CDF and $^{13}\text{C}_{12}$ -octa-CDD (NCI analysis, partial mass spectra recorded). The levels of the corresponding native compounds in this sample were determined at 25 ppt each.

Interference from Other Coextracted Compounds

Although very efficient cleanup and purification procedures are used, very often additional chlorinated compounds are encountered in the extracts and may inter-

fere in the analysis. The presence of PCDFs, PCDDs and PCBs should be confirmed by determining proper signal ratios from molecular or fragment ion clusters or possibly by obtaining partial or complete mass spectra. Some interfering chlorinated compounds occasionally observed in purified extracts of environmental samples are listed below and in Table 4.

PCBs. Most PCBs are efficiently removed, except for some specific isomers (PCB isomers which can attain a planar conformation, such as those with no *ortho*-Cl substituents M^+-Cl_2 and M^+-Cl_4 ions (EI) sometimes interfere at m/z values (M^++2) for PCDDs and at m/z 328 (pentachlorobiphenyls, M^++4) for the ^{37}Cl -tetra-CDD internal standard. PCBs are easily recognized from mass spectra by their intense molecular ions (M^+ or M^-) and by the number of chlorine substituents deducible from these ion clusters. Main fragment ions are: EI, M^+-Cl , M^+-Cl_2 ; NCI, $\text{M}^- -\text{Cl}$ for some, $\text{M}^- +\text{H}-\text{Cl}$ for other isomers. The interference of PCBs is signifi-

Table 4. List of molecular ions of polychlorinated compounds present in some environmental samples and possibly interfering in the mass spectral analysis of PCDFs, PCDDs and PCBPs.

Compounds	Molecular ions (m/z , M^+ , M^-) (chlorination)									
	Mono-	Di-	Tri-	Tetra-	Penta-	Hexa-	Hepta-	Octa-	Nona-	Deca-
PCDFs				304	338	372	406	440	—	—
PCDDs				320	354	388	422	456	—	—
PCBPs				288	322	356	390	424	—	—
PCBs				290	324	358	392	426	460	494
PCNs				264	298	332	366	400	—	—
PCTs		298	332	366	400	434	468	502	536	570
PCDPEs		238	272	306	340	374	408	442	476	510
PCPNAs (PCPYs)	236	270	304	338	372	406	440	474	508	542

cant in the analysis of PCBPs due to the formation of intense $M^+ - Cl_2$ ions with the same exact mass as PCBPs.

PCNs (Polychlorinated Naphthalenes). These planar aromatic compounds are retained to some extent in the purified extracts. They may give rise to signals in EI and NCI at the m/z values for PCDFs due to $M^+ + 6$ ($M^- + 6$) ions. However, no confusion usually occurs because PCNs elute prior to corresponding PCDFs and they are easily identifiable from mass spectra (M^+ or M^- , respectively and number of chlorine substituents deducible from ion clusters). Analysis of PCDFs using $M^+ + 2$ ($M^- + 2$) ions minimizes the influence of PCNs. Hexachloronaphthalenes ($M^+ = 332$) may interfere with the $^{13}C_{12}$ -tetra-CDD internal standard. Fragmentation of PCNs is by loss of $Cl\cdot$ and Cl_2 (EI) or inclusion of H \cdot and loss of $Cl\cdot$ and Cl_2 ($M^- - 34$ and $M^- - 68$, NCI).

PCTs (Polychlorinated Terphenyls). These compounds are usually present at much lower levels than PCBs and they are removed in the cleanup procedure. $M^+ + 6$ ($M^- + 6$) ions may interfere in PCDF analyses but monitoring $M^+ + 2$ ($M^- + 2$) ions of PCDFs again minimizes their influence. Identification from complete mass spectra is easily achieved.

PCDPEs (Polychlorinated Diphenyl Ethers). These are common impurities in chlorophenols and other industrial chemicals, but they are less polar and usually removed in the cleanup scheme (25). In EI, they yield M^+ and intense $M^+ - Cl_2$ ions (NCI: $M^- - HCl$ ions) with the same exact mass as corresponding PCDFs. They can be identified by observation of their M^+ (M^-) ions.

PCPNAs. Additional chlorinated compounds were observed in some environmental samples. They were tentatively identified as chlorinated anthracenes/phenanthrenes, fluorenones and pyrenes/fluoranthenes (PCPYs, parent molecular weight 202). These latter compounds were also found in soot from capacitor accidents in Sweden (8–10). The PCPYs have the same nominal M^+ (M^-) values as corresponding PCDFs but different mass spectra (lower number of chlorine substituents for same M^+ , fragmentation in EI via loss of Cl , HCl and Cl_2). They elute significantly later from GC columns than PCDFs, and usually no confusion occurs. Several isomers were also detected in emissions from municipal incinerators, in contaminated soil and in marine sediments (26). Confirmation and quantification

is presently not possible because few authentic standards are available.

PCBZs, Chlorinated Benzofurans and Styrenes. PCBZs, chlorinated benzofurans and styrenes were sometimes observed but do not interfere in these analyses.

Isomer-Specific Analysis of PCDFs and PCDDs by HRGC

High isomer specificity, the additional requirement for accurate PCDF and PCDD analyses, cannot be met by mass spectrometry alone but has to come from highly efficient separation techniques. HRGC is predestined for this purpose because it can be easily combined with mass spectrometry and in fact is routinely used. We have described its application to PCDF and PCDD analyses in a series of papers (7–10,14–16,18).

All PCDD isomers with 4 to 8 chlorine substituents have recently been synthesized and their elution patterns studied by HRGC (14). Conditions were found that allowed a differentiation of all 2,3,7,8-substituted PCDDs (2,3,7,8-tetra-, 1,2,3,7,8-penta-, 1,2,3,4,7,8-, 1,2,3,6,7,8- and 1,2,3,7,8,9-hexa-CDD) from all the other isomers (55 m Silar 10c HRGC column). In Figure 3 we illustrate this separation with a chromatogram of a synthetic composite sample containing all these PCDDs. Although not all the isomers are completely separated from each other, this column allows an isomer-specific analysis for many of the PCDDs including all the 2,3,7,8-substituted congeners. Some coeluting isomers can additionally be separated using other HRGC columns (OV-17, OV-101). The Silar 10c HRGC column was used in the analysis of series of environmental samples for the presence of the 2,3,7,8-substituted PCDDs.

In case of the PCDFs, all the higher chlorinated (tetra- to octa-) compounds have also recently been synthesized and separation of most of these isomers was achieved using HRGC (50 m SP-2330 fused silica capillary column) (15). The toxic 1,2,3,7,8-penta-CDF was found to coelute with 1,2,3,4,8-penta-CDF and the toxic 1,2,3,4,7,8-hexa-CDF with the 1,2,3,4,7,9-substituted isomer, but both isomer pairs were resolved on less polar HRGC columns such as OV-17 and DB-5.

Table 5. PCDF isomers detected in biological samples from the Baltic using HRGC and NCI mass spectrometry.

PCDF isomer	PCDF concn, pg/g		
	Herring	Guillemot	Seal
2,3,7,8-Tetra-CDF	50	10	1
Others	< 2		
1,2,3,7,8-Penta-CDF	80	50	1
2,3,4,7,8-Penta-CDF	250	750	15
1,2,4,7,8-Penta-CDF			
1,2,4,6,8-Penta-CDF	100	< 5	
Others	< 5		
1,2,3,4,7,8-Hexa-CDF	10	50	2
1,2,3,6,7,8-Hexa-CDF	10	100	2
2,3,4,6,7,8-Hexa-CDF	10	50	1
1,2,4,6,8,9-Hexa-CDF	120	500	1
1,2,4,6,7,8-Hexa-CDF	150	150	2
1,2,3,4,6,8-Hexa-CDF	120	150	
1,2,3,4,6,7,8-Hepta-CDF	900	1000	5
1,2,3,4,6,8,9-Hepta-CDF	500	1500	5
Octa-CDF	100	250	3
PCDFs, total	2300	4560	40

were made. Identification of the PCDFs was made from partial mass spectra recorded; isomer identifications were based on comparisons of retention times with those of authentic standards on the 55 m Silar 10c HRGC column. From the results reported in Table 5 and from Figure 5, it is apparent that again 2,3,7,8-substituted PCDFs are present in addition to some other PCDF isomers. Herring and guillemot, with exception of 1,2,4,6,8-penta-CDF, showed the same PCDF isomers present; most of these isomers were also detected in seal fat although surprisingly at a much reduced level. In case of the seal fat, it was previously concluded that the source of the PCDFs was very likely due to a direct contamination by PCBs (27). In case of the herring and guillemot now analyzed, the contamination appears to be more complex. Many of the isomers in these samples were also found in commercial PCBs (6) but some also seem to be related to chlorophenols (1,2,4,6,8-penta-, 1,2,3,4,6,8- and 1,2,4,6,8,9-hexa-CDF).

These environmental samples show contamination with 2,3,7,8-substituted PCDFs and PCDDs. In a previous study we have already reported that aquatic species apparently show a preferential retention of these toxic isomers (18). As discussed, this could result from a decreased excretion of these isomers or a diminished ability of organisms to metabolize components with this pattern of chlorine substitution. The potential adverse effects of these compounds on these organisms remain unknown.

HRGC and MS analyses will detect the presence of these hazardous compounds. Isomer-specific analyses aid in the identification of origin and sources, and in unveiling routes of formation of these toxic contaminants. The methods are indispensable for taking mea-

asures to protect the environment and prevent future contaminations with these compounds.

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