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Review

Gas chromatographic analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans

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Abstract

Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans are toxic compounds formed during natural processes and human activities. The extraction and clean-up for these compounds from various environmental and biological sources has been described. The gas chromatographic analysis of polychlorinated dibenzo dioxins/furans with different columns and detectors has been reviewed. The advantages of using a mass detector in the analysis are discussed. © 1997 Elsevier Science B.V.

Keywords: Sample handling; Environmental analysis; Polychlorinated dibenzo-*p*-dioxins; Polychlorinated dibenzofurans

Contents

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) belong to the class of most hazardous environmental pollutants that have received prolonged attention by the scientific community and by environmental regulators. According to the Environmental Protection Agency (EPA) Assistant Administrator, Lynn Goldman, dioxin, one of the most toxic chemicals regulated by EPA has been

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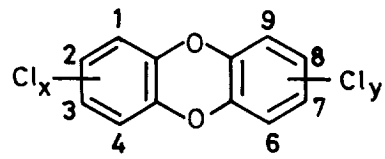
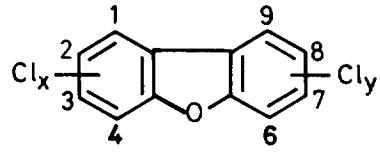
the subject of a series of agency-assessment dating back to the early 1980s [1]. The most recent evaluation began in 1991 because of several new studies as well as controversy about the health threat from dioxin-like compounds. One key piece of new information emerged from a 1990 meeting of scientists at the Banbury Centre in New York, where a consensus was reached that dioxin-like compounds gain entry to cells by binding to a particular protein, the Ah receptor.

Four primary sources responsible for the generation of these compounds are: combustion and incineration, chemical manufacturing, industrial processes like chlorine bleaching of pulp, paper and smelting etc. and reservoir source in which dioxin may be recirculated throughout the environment once it is generated. Dioxins and furans are produced when chlorine based compounds like polyvinylchloride (PVC) and chlorophenols are exposed to high temperatures in the presence of organic materials. These compounds are semivolatile and hydrophobic, hence they accumulate in organic rich media such as soils, sediments and biota. EPA's draft reassessment presents the first compilation of nationwide PCDD/PCDF emission estimates. It shows that emission from municipal and medical waste incinerations to the atmosphere is the dominant dioxin source in the United States [2].

The class of chlorinated dibenzodioxins and furans contain 75 possible PCDDs and 135 possible PCDFs. The basic structure of these compounds is presented in Fig. 1. Out of 75 isomers of PCDDs, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic [3]. TCDD is a by product of the manufacturing of 2,4,5-trichlorophenol (TCP) from tetrachlorobenzene. TCDD is not known to be produced biologically. Because TCP was used as a feedstock for the production of chlorinated phenoxy acetic acid pesticides such as 2,4,5-T and silvex [2-(2,4,5-trichlorophenoxy)propionic acid] and is currently used in the production of hexachlorophene, TCDD has become a generally widespread trace environmental pollutant. Levels at which health effects from dioxin-like compounds are observed vary widely among species [4]. A sample of the studies presented in the EPA dioxin health assessment indicates that some health effects are observed at estimated body burden levels close to the average human 'background' body burden level.



Polychlorinated dibenzo-p-dioxins



Polychlorinated dibenzofurans

Fig. 1. Chemical structure of PCDD and PCDF.

The problem becomes more serious when bioaccumulation of these compounds is taken into consideration. The bioaccumulation of such hydrophobic organic chemicals in aquatic ecosystems has been the subject of intense research for almost the last 20 years. Bioaccumulation in terrestrial ecosystems has also been examined by McLachlan [5]. Air–plant–soil–cow–human food chain was examined using field data collected in southern Germany when the chemical concentrations given in Table 1 were found in the agricultural food chain.

Tetrachlorodioxins, the most toxic of the class, are 22 isomers [6] that have the same molecular mass, thus showing the difficulty of analyzing one individual compound. Each of these substances and isomers has different, toxicological relevance. Analysis of dioxins therefore, requires an extremely selective and sensitive method for separation and detection. Since different substrates can accumulate dioxins, the method of extraction and clean-up normally will be different for different matrices. Collection of these toxic chemicals from environmental samples, enough for analysis, itself also requires skill which basically depends upon the methods of extraction and clean-up. Hence while dealing with a technique of analysis or estimation, extraction and clean-up of dioxins and furans also requires selection for quantitative assessment. The

Table 1
Chemical concentrations in the agricultural food chain

Chemical	Gaseous (pg/m^3)	Particle (pg/m^3)	Soil (pg/g dw)[a]	Grass (pg/g fw)[a]	Corn (pg/g fw)[a]	Cows' milk (pg/g of fat)	Human milk (pg/g of fat)
2,3,7,8-Cl_4DD	0.0027	0.0009	0.05	0.0019	0.027	0.19	3.6
1,2,3,7,8-Cl_5DD	0.0017	0.0024	0.18	0.0036	0.044	0.38	10.2
1,2,3,4,7,8-Cl_6DD	0.0016	0.0030	0.19	0.0029	0.033	0.23	8.7
1,2,3,6,7,8-Cl_6DD	0.0020	0.0079	0.37	0.0068	0.057	0.81	45.8
1,2,3,7,8,9-Cl_6DD	0.0011	0.0055		0.0050	0.045	0.25	6.7
1,2,3,4,6,7,8-Cl_7DD	<0.008	0.088	5	0.07	0.72	1.04	58.1
Cl_8DD	<0.050	0.28	16	0.19	3.3	2.28	670
2,3,7,8-Cl_4DF	0.014	0.0062	0.48	0.023	0.15	0.21	0.7
1,2,3,7,8-Cl_5DF	0.0071	0.0069	0.76	0.016	0.17	0.15	0.1
2,3,4,7,8-Cl_5DF	0.0080	0.0099	0.59	0.013	0.11	1.00	21.6
1,2,3,4,7,8-Cl_6DF	0.0050	0.0079	0.92	0.017	0.11	0.53	6.4
1,2,3,6,7,8-Cl_6DF	0.0051	0.0086	0.74	0.011	0.10	0.56	5.5
1,2,3,7,8,9-Cl_6DF	0.0008	0.0014					0.1
2,3,4,6,7,8-Cl_6DF	0.0047	0.0087	0.65	0.0091	0.08	0.38	3.0
1,2,3,4,6,7,8-Cl_7DF	<0.006	0.057	7	0.09	0.53	0.23	6.3
1,2,3,4,7,8,9-Cl_7DF	<0.001	0.011	0.4	0.01	0.04	0.12	0.2
Cl_8DF	<0.003	0.035	3.7	0.029	0.22	0.37	0.4

Concentration values are the mean of 12–27 samples except for human milk which are the mean of 121 samples which were collected continuously around the south of Bayreuth between 1989–1990 and are not influenced by PCDD/PCDF sources [5].
[a] dw refers to dry mass, fw refers to fresh mass.

method of extraction from different substrates are described below.

2. Extraction

2.1. From soil, sediment, ash, fly ash

Soxhlet extraction is a well recognized procedure for extraction of PCDDs/PCDFs from soil, sediment, ash and fly ash from open burn sites or metal recovery facilities. The solvents used for Soxhlet extraction are toluene [7–12], hexane [13], dichloromethane [14,15], dichloromethane–acetone (1:1, v/v), 2-propanol-dichloromethane (1:1, v/v) [16] and benzene [17,18]. The extraction time varies from 12 to 60 h.

Hengstmann studied five different extraction methods for chlorodioxins in soil and reported almost quantitative extraction in 2 h using 'supersonic' Soxhlet extraction [19].

Supercritical fluid extraction (SFE) one of the advanced techniques, is also used for extraction of soil, sediments, ash and fly ash samples. Extraction of PCDDs/PCDFs from municipal incinerator fly ash with supercritical CO_2 has been quantitatively achieved [20]. Also reported are 2,3,7,8-TCDD from sediments [21] and coal tar contaminated soil [22]. On-line SFE–GC was also employed for the analysis of soil [23]. A review on supercritical fluid extraction of such anylates from environmental samples has been compiled by Barnabas et al. [24]. The extraction of PCDDs/PCDFs has also been reported from municipal sewage sludge [25], soil from a former 2,4,5-T plant [26], chlorine production plant [27], soil contaminated with graphite sludge [28], soil beneath waste electrical equipment incineration [29], soil from British semirural [30] and urban [31] areas, municipal water treatment plant sludge [28], soil from scrap wire, and car incinerator sites [32] and compost from municipal yard waste facility [33].

2.2. From biota samples

Biota samples are generally frozen prior to extraction to disintegrate the tissues. The water in the tissues freezes, thus rupturing the cell walls and destroying the tissues. The tissues are then homogenized with sodium sulphate which absorbs water from tissues giving better extraction. The tissue samples with high water content require overnight equilibration with sodium sulphate before blending [34]. In

that case tissue samples are first cut into small pieces, ground in a meat grinder and mixed thoroughly with anhydrous sodium sulphate. The mixture is then spread out to a depth of less than 3 cm so that the mass, which solidifies after 3–6 h, can be readily broken up after drying overnight. The mixture is then dry blended to a fine powder. It is then extracted with organic water-immiscible solvents for the isolation of PCDDs/PCDFs. Dichloromethane is often used for extraction of fish [10,35], mink [36], other animal tissues [14] and human adipose tissues [37]. Sea animals like pike and seal are extracted with light petroleum–acetone–hexane–diethyl ether (18:10:5:2, v/v) and acetone–hexane (1:1 v/v) respectively [12]. Acetone–pentane (3:7, v/v) mixture has also been used [38] for the extraction of three marine species namely sand worm, clams and grass shrimp while investigating accumulation of these contaminants from sediments by these species. Dichloromethane–hexane (1:1, v/v) mixture has also been used for human adipose tissues [39]. Human blood samples are extracted with hexane after adding ethanol and aqueous saturated ammonium sulphate solution [40].

Human milk samples are extracted with either acetonitrile or a mixture of chloroform–methanol–hexane (1:1:1, v/v) after adding formic acid and lipidex 5000 [41,42]. An automated clean-up procedure for milk samples, used by Van Rhijn, also includes the use of gel permeation chromatography (GPC) [43].

All the biota samples are generally Soxhlet extracted with Dean Stark's apparatus except blood and milk samples which are shaken at room temperature.

2.3. From water

Drinking waters are also the subject for the development of specialized methods for the detection of PCDDs and PCDFs. A GC method for 2,3,7,8-TCDD was described by Tausch et al. [44]. Dichloromethane was used for extracting rain water samples for investigating wet deposition contribution [45]. In water, these hydrophobic compounds settle down primarily with large rapidly settling particles [46]. This settling particulate matter (SPM) often consists of aggregates of smaller particles which are formed by biotic processes, such as fecal pellet formation, and by abiotic processes, such as flocculation and agglomeration. Flocules are inorganic particle aggregates that are held together by electrostatic forces and which often get colonized by bacteria and protozoans. Agglomerations are comprised of organic and inorganic matter weakly held together by surface tension and organic cohesion due to various biological activities. These mechanism probably also facilitates relatively fast settling of the combustion derived particles like PCDDs/PCDFs. Further, the comparatively high organic content of SPM results in a high absorbing capacity for the dissolved PCDDs/PCDFs due to high organic carbon–water partition coefficients displayed by these compounds. SPM collected in sediment traps during a longer period of time, therefore, reflects an integrated picture of polycyclic aromatic hydrocarbons (PAHs) and PCDDs/PCDFs found in water mass due to the efficient scavenging capacity of SPM for both dissolved and suspended particulate forms of these compounds.

Using this technique the extraction of PCDDs/PCDFs has been carried out by Broman et al, [47] and Naf et al. [48] from waters outside various emission sources. The SPM samples are extracted with toluene in a Soxhlet apparatus for 24 h equipped with a Dean–Stark trap for the collection of water.

2.4. From air

Air samples are collected in accordance with EPA reference method TO9 A [49]. Samples are collected using a General Metal Works Model PS-1 polyurethane foam (PUF) sampler with a flow-rate of approximately 0.27 m^3/min. Total elapsed sample time varied in different studies but ranged between 24 h to 170 h. The glass fiber filter and PUF plugs are combined, extracted and the extract is analysed for PCDDs and PCDFs. These PUF plugs along with glass fiber filters are initially spiked with labeled PCDDs/PCDFs to serve as internal standard. These are Soxhlet extracted with benzene for 16 h in a monitoring study of PCDDs/PCDFs in ambient air [50,51]. Volatile organics sampling train (VOST) is also used for monitoring PCDDs and PCDFs in incinerator flue gas [52]. Extraction of PUFs has also been carried out in a sequence of acetone, toluene

and benzene for the work area air monitoring for PCDDs and PCDFs at a municipal waste power boiler facility [53,54]. Similar extraction for PCDDs/PCDFs with toluene and dichloromethane has also been carried out for air samples from different sources like air from automobile traffic tunnel [55], ambient air near a municipal sewage waste (MSW) incinerator [56] and from urban air [57,58] from Hamburg, Germany and Bridgeport, CT USA respectively. Although the concentration of these compounds in urban air varied from 0.001 to 1.00 pg/m^3, in ambient air near a MSW incinerator, it was up to 2100 pg/m^3.

2.5. From plant sample and pulp mill sludges

Plant samples are extracted after freezing. The purpose here is also to destroy the cells as in biota samples. Extraction from pine needles [59] used for biomonitoring near a wood preserving chemical site was carried out by packing the branches of pine in aluminium foil and then submerging them in liquid nitrogen for 15 min. The package was removed from liquid nitrogen and thumped repeatedly to detach the frozen needles from branches. After opening the package the branch part were removed using tweezers leaving the still intact needles. These were transferred into a beaker with dichloromethane and placed in a ultrasonic bath for 15 min. The solvent was decanted through glasswool. Dichloromethane has also been used for extracting water–pulp mixture [60]. Toluene–acetone (1:1) was used for Soxhlet extraction of park grass and herbage samples to report the trends of PCDDs/PCDFs in vegetation samples from the control plot of the park grass experiment started in 1856 at Rothamsted Experimentation Station [30]. Toluene and toluene–ethanol (3:1) have also been used for the extraction of pulp mill sludge [10] and dried pulp sheet [61] respectively.

2.6. From other sources

Particulate samples from diesel vehicles exhaust are extracted with dichloromethane [62]. Particles from chimney deposits from wood burning are also Soxhlet extracted with toluene [63]. Chimney soot from coal fire places has also been extracted as reported by Harrad et al. [64]. Leaded and unleaded gasoline engine lube oils have also been extracted for PCDDs and PCDFs [65,66].

3. Clean-up and fractionation

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans are such a group of environmental contaminants that are always associated with high-molecular molecular-mass pollutants like polychlorobiphenyls (PCBs) and PAHs. During extraction these type of compounds are co-extracted with dioxins and furans. Similarly other compounds from soil organic matter, lipids from biological materials and chlorophyll from plant samples are also extracted and can interfere with gas chromatographic analysis by electron capture detection (GC–ECD) or by mass spectrometry (GC–MS). The interferants are typically present in greater amount than these toxic compounds to be estimated [67]. Different types of clean-up procedures are used for the purification of PCDDs and PCDFs which depends upon the substrate from which it has been extracted. The concentrated extracts are sometimes partitioned with different non-polar solvents for a clean-up to some extent. But the major clean-up is achieved by passing the extract through different adsorbent columns. The elution solvent for these columns are hexane, dichloromethane, cyclohexane, pentane and nonane. Biological materials contain a large amount of lipid which is removed by shaking with sulfuric acid-impregnated silica, followed by column chromatography using a multilayer column [12,38–40].

In general, the clean-up procedure consists of two parts. In the first part, the extract of tissues and sediment or soil samples (spiked with isotopic marker compounds) passes through the adsorbents in the following order: potassium silicate, silica gel, cesium or potassium silicate, silica gel and finally on activated carbon adsorbent. The residues of interest are retained on the carbon adsorbent and subsequently recovered by reverse elution. In the second part, sample is applied to a second series of adsorbents containing two tandeum columns of cesium or potassium silicate and sulphuric acid impregnated silica gel and activated alumina. Thus seven processes are integrated into a two step procedure,

significantly reducing the time [34]. Similarly an improved automated sample cleanup apparatus used in the Centres for Disease Control (CDC) procedure is developed for serum and adipose tissues [68]. Compared to the earlier prototype model, this device is more versatile, easier to use and has reduced the time from 20 to 4 h.

Florisil microcolumns (activated at 130°C) are used to separate the non-*o*-substituted PCBs (planar PCBs) and PCDDs/PCDFs from *o*-substituted PCBs which are nonplanar [39,40,62]. The Florisil column is eluted first with hexane and then with dichloromethane in order to obtain two fractions, the former containing the *o*-substituted PCBs and the latter containing non ortho-PCB congeners and PCDDs/PCDFs. The second fraction is then analysed by GC.

Use of activated carbon [68] and carbon dispersed on silica gel [69–71] or glass fibres [72,73] has also been reported in the experiments of carbon chromatography for isolation of these compounds. Three recent developments for fractionating planar PCBs, PCDDs and PCDFs are (A) porous graphitic carbon (PGC) [74]; (B) 2-(1-pyrenyl)-ethyl dimethyl silylated silica gel (PYE) [75]; and (C) C_{60}/V_{70} fullerenes bonded to polystyrene–divinylbenzene ($C_{60/70}$-PS-DVB) [76]. The three adsorbents, PGC, PYE and $C_{60/70}$–PS–DVB have similar elution characteristics for PCBs and PCDDs/PCDFs to activated carbon and require less solvent volume and strength, but they are costly, have lower sample capacity or require sample to be almost completely devoid of lipids and co-extractants [75,77,78].

In a very recent method, the extracted material is fractionated by HPLC and each fraction is then cleaned up before the analysis. This technique is used for the purification of dioxins and furans in the waters outside various emission sources on the Swedish Baltic Coast [48]. The samples after extraction were fractionated on HPLC according to the method described by Zebuhr et al. [78] and Broman et al. [47] which efficiently separates PCD dioxins and furans from PAHs. Similarly a method of separation of PCDDs/PCDFs and a number of specific di-, mono-, and non-*ortho*-, *ortho*-chlorine (*o,o'*-Cl) substituted chlorobiphenyl congeners of toxicological significance has been developed that uses HPLC columns containing PX-21 activated carbon dispersed by C_{18} (octadecysilane) sorbent in combination with an automated, quaternary HPLC auto injector and fraction collector apparatus [14]. The system produces four discrete fraction containing bulk PCBs (di to tetra) mono-*o,o'*-Cl PCBs, non *o,o'*-Cl PCBs and PCDDs/PCDFs. The fractionation is achieved with reduced manpower and easy to meet specific analytical needs.

Soil sediments and fly ash sample extracts are generally cleaned up on silica, alumina and carbon adsorbents. Extracts from biological materials are purified by sulfuric acid and finally on GPC columns with biobeads. Plant samples are cleaned on multilayer silica columns. Air samples need much less clean-up in comparison to other samples. A list of the different absorbents used for different substrates is given in Table 2.

3.1. GC analysis

PCDDs/PCDFs form a large group of toxic materials representing 75 dioxins and 135 dibenzofurans. Such a big group is equally difficult to analyse with a single method of analysis. A good separation and a sensitive method of detection can only be of help. Capillary columns with high resolution GC are quite helpful in solving the problem of separation of such a large group of pollutants.

Table 3 gives a list of some of the GC columns, stationary phases, column temperature programming, carrier gas with linear velocity, detector used and limit of detection used in various recent studies. A general glance at Table 3 shows that mostly DB-5, SP-2331 stationary phases are used for the separation of PCDDs/PCDFs. The column used is always a capillary column which advocates the better resolution capacity of these columns. Although the length of the column varies between 25 to 60 m but the internal diameter and film thickness is mostly in the range of 0.20 to 0.32 mm and 0.15 to 0.33 μm respectively. A comparison of estimation of 2,3,7,8-class congeners of tetra to hexa chlorinated DDs/DFs in a fly ash extract determined by HRGC–MSD using three columns of different polarity is also given [79]. This comparison also verifies the efficiency of DB-5 and SP-2331 stationary phases, while SE-54 is suitable only for few congeners.

GC separation of all 136 tetra to octa PCDDs/PCDFs on nine different stationary phases of diverse

Table 2
Various adsorbents used for clean-up

Compounds	Substrate	Adsorbent	Ref.
TCDD	Soil	Alumina	[13]
PCDDs/PCDFs tetra to octa	Sediment	Silica, alumina, carbon	[7]
	Soil, ash Fly ash	Potassium silicate–silica gel, basic alumina, AX-21/ celite 545-carbon	[8]
PCDDs/PCDFs	Soil	Deactivated silica, activated alumina	[16]
	Soil sediment	Acid/basic silica gel	[9]
	Fly ash, soil	Partition into nonane Silica, NaOH/silica silica, H_2SO_4/silica silica/Na_2SO_4	[10]
	Fly ash	Deactivated alumina	[17]
	Sediment	activated copper, deactivated silica gel, alumina	[6]
TCDD	2,4,5-T acid	Silica microcolumn, alumina	[6]
	2,4,5-T ester	Alumina	[6]
PCDDs/PCDFs	Fish	GPC col. with Biobeads S-X3	[10]
	Mink	GPC	[36]
	Biogenic material	Na_2SO_4, Potassium silicate, H_2SO_4/silica gel Na_2SO_4 and coarse H_2SO_4/silica gel Na_2SO_4, potassium silicate, H_2SO_4/silica gel with size exclusion, chromatography (SEC)	[14]
	Human blood	H_2SO_4/silica, Cesium hydroxide/silica, Florisil, Carbopack-C dispersed on silica	[62]
	Milk	Lipophilic gels lipidex 1000,	[42]
		lipidex 5000, magnesia–celite, alumina, Florisil	[41]
	Sediment	Potassium silicate, sulfuric	[17]
	Animal tissue, Eggs	acid, silica gel, coarse sulfuric acid/silica gel Silica gel, Potassium silicate, sulfuric acid/silica gel Size exclusion chromatography (SEC) with phenol gel and phenomenex. PX-21 dispersed with C_{18} H_2SO_4/ silica, multilayer col.	
	Human adipose tissue	Sulfuric acid/silica, multilayer col.	[39]
	Pine needles	Acid–base column 1 *M* NaOH–silica, activated silica and 44% H_2SO_4–silica Florisil, Al_2O_3 and Na_2SO_4	[59]

(*continued on page 104*)

Table 2 (*continued*)

Compounds	Substrate	Adsorbent	Ref.
	Herbage samples	Silica, NaOH–silica silica, H_2SO_4–silica, alumina	[11,30]
	Water	HPLC fractionation	[48]
	Rain water	silica, alumina	[45]
	Sediment	GPC, carbon, acidic biosil/alumina	[45]
	Chimney deposit or soot	Basic Al_2O_3, H_2SO_4, silica, basic Al_2O_3	[63]
	Air	multistep clean-up	[53,54]
	Air	Acid–base, silica gel Al_2O_3, carbon	[50]
	Pulp-mill sludge, pulp sheet	Acid extraction, silica, NaOH/silica, H_2SO_4/silica	[61]
	Oil sample	Silica, acidic alumina, AX-21	[66]

polarity (100% Me, 55% PhMe, 50% PhMe, 50 Me trifluoropropyl, 50%, 75%, 90% and 100% cyanopropyl and liquid crystal smectic) is described by Ryan et al. [80]. The data are expressed in a series of GC chromatograms and in a table of relative retention time. They also reported that all 136 compounds including the biologically important 2,3,7,8-substituted congeners can be separated from each other mostly with two stationary phases. However possible variation in GC conditions and stationary phases necessitates assessment of the resolution of near eluting isomers.

Temperature programming of the oven is generally a two step linear temperature programming. In the first phase, rate of increase in temperature is always higher i.e. 10 C°/min to 25 C°/min, while in the second phase, it is a slow-rate i.e. 1 C°/min to 5 C°/min. Initial temperature is different in different studies depending upon the number of congeners to be analysed and ranges between 70°C to 150°C. The final temperature generally touches to 280°C and above except for a SP-2331 column where the maximum temperature used is 260°C.

Various detection methods can be used for the detection of this group of compounds but mass spectrometry (MS) is the best suited and most widely used detection method. Being a very large group of various isomeric congeners, without mass spectral fragmentation it becomes almost impossible to identify the chemical structure of each component. Moreover, in the detectors other than mass spectrometric detector, a standard reference is always required as authentic compound for comparison. Some of these chemicals are too toxic to handle, thus making the analysis more problematic. Although internal standards are used in most of the studies, MS is still the most popular detector for the analysis of PCDDs/PCDFs.

Although flame ionization detection (FID) [79,81], ECD [9,36,79] and atomic emission detection (AED) [79] are also used, a large number of studies are with MSD [7–18,38–54,59–79,82–89]. MS with electron ionization gives a molar response for Cl_xDD/Cl_xDF ($x=1-8$). This response does not depend on the position or the number of chloride atoms but on the molecular selection of Cl_xDD/Cl_xDF alone. The carbon skeleton based molar response of MS can simplify the quantitation of PCDDs/PCDFs in GC–MS substantially. Used in an appropriate way it may supplement the dilution technique. FID and AED, due to their molar response characterisation for defined elements, can be used for the quantitation of standard solutions in the microgram per millilitre range of PCDDs/PCDFs. Specifically, the highly toxic compounds of group 2,3,7,8 (tetra) do not have to be handled in bulk and can be quantified after capillary GC with non-toxic congeners references. The molar response of ECD is structure-dependent and is higher for PCDFs than PCCDs [79].

A comparison of molar response of PCDDs/PCDFs by MS with AED/FID has also been made [79]. The results demonstrate the linear relationship

Table 3
Gas chromatographic conditions and limit of detection of PCDDs/PCDFs

Compound	Column (capillary)	Temperature (°C)	Carrier gas with linear flow ($cm\ s^{-1}$)	Detector	Limit of detection	Ref.
PCDDs/Fs	DB-5 or SP-2330 (60 m×0.25 mm×0.2 μm)	60 (2 min)..30/min ..210..2/min.. 300 (10 min)	He 30	MS ECD	60 fg/g	[7,26,50]
PCDDs/Fs	DB-5 (60 m×0.25 mm, 0.25 μm)	220 (2 min)..5/min ..260..1/min ..300 (10 min)	He 25	MS	0.003 pg	[8]
PCDDs/Fs	DB-5 (30 m×0.25 mm, 0.25 μm)	40 (2 min)..30/min ..210..2/min ..285 (10 min)	He 22	MS	1 pg/g	[16]
PCDDs/Fs	DB-5 (50 m×0.32 mm, 0.25 μm)	250 (5 min)..1.2/min ..300 (5 min)	He 25	ECD	1 pg/g	[9]
PCDDs/Fs	DB-5 (60 m×0.25 mm, 0.15 μm)	90 (5 min)..25/min ..200 (15 min)..4/min ..250 (15 min)	He 25	MS	14 fg/g	[9,11,1]
PCDDs/Fs	HP-5 (25 m×0.2 mm, 0.33 μm)	100 (1 min)..20/min ..200..5/min..280	He 30	MS	1 pg/g	[26]
PCDDs/Fs	DB-5 (60 m or 40 m×0.25 mm, 0.25 μm)	120 (1 min)..20/min ..210..3/min ..300 (15 min)	He 25–35	MS	19 pg/g	[10,14]
2,3,7,8-Cl_4–Cl_6DD/DF	HP Ultra-2 (25 m×0.2 mm, 0.33 μm)	120 (3 min)..10/min ..180..2/min ..280 (15 min)	He 30	AED	6 ng	[79]
PCDDs/Fs	DB-5 (40 m×0.32 mm, 0.25 μm)	90 (3 min)..10/min ..180..2/min ..280 (5 min)	H_2 50	FID	6 ng	[79]
Cl_4–Cl_7DD/DF	DB-5 (40 m×0.32 mm, 0.25 μm)	120 (3 min)..20/min ..170..1.7/min ..285 (1 min)	H_2 50	ECD	1 pg	[79]
Cl_7–Cl_8DD/DF	DB-5 (40 m×0.32 mm, 0.25 μm)	120 (3 min)..20/min ..170..4/min ..285 (1 min)	H_2 50	ECD	9 ng	[79]
17-Congeners of 2,3,7,8-Cl_4–Cl_8DD/DF	HP-Ultra-2 (25 m×0.32 mm, 0.17 μm)	120 (5 min)..20/min ..180..2/min ..260 (5 min) and 70 (3 min)..20/min ..180..2/min...260 (5 min)	He 30	MS	<1 pg	[79]
2,3,7,8-Cl_4–DD/DF	SP-2331 (60 m×0.25 mm, 0.2 μm)	100 (1 min)..20/min ..180..5/min ..250 (120 min)	He 22	MS	0.1 pg/g	[59]
PCDDs/Fs (All congeners Cl_1–Cl_8)	SP-2331 (60 m×0.32 mm, 0.2 μm)	120 (3 min)..20/min ..180..2/min ..250 (20 min)	He 50	MS	50 fg/g	[63]
PCDDs/Fs (All congeners Cl_1–Cl_8)	DB-5 (60 m×0.25 mm, 0.25 μm)	150 (1 min)..20/min ..190..3/min ..300 (10 min)	He 25	MS MS/MS	<1 pg/g	[81]
Cl_4–Cl_8DD/DF	HP Ultra-2 (50 m×0.2 mm, 0.11 μm)	120 (5 min)..20/min ..180..2/min ..260 (5 min)	He 30	MS	8 pg/g	[39]

A few examples are taken in Table as most of the studies used same columns with similar temperature programming and detectors.

of the signals of AED (carbon mode) of FID and of MS. The response of ECD depends on the number of chlorines per molecules. The response increases with increasing number of chlorine substituent especially between the groups of chloro homologues with four to six chlorines. The reason for the slight decrease of the molar ECD response of the octachloro homologues could be a chromatographic phenomenon caused by discrimination of the strongly adsorbing octachlorohomologues.

Besides low resolution MS (LRMS) and high resolution MS (HRMS) the tandem mass spectrometry (MS–MS) has also been used for the quantification of PCDDs and PCDFs [90–92]. Comparative analysis of fly ash and sediment extracts indicate that in the absence of interferences, HRMS and MS–MS methods provide similar quantitative data. In certain cases where greater selectivity is needed, MS–MS can provide accurate quantitative data [90].

Bacher et al., have reported a full spectrum of chloro homologue profile and chloro isomer patterns of both the Cl_xDD ($x=1-8$) and Cl_x DF ($x=1-8$) of deposits in the upper part of a chimney of an old farm house in Southern Germany [63] using the GC–MS. According to them, the widely used approach of analysing only for tetra through octachloro congeners, or even only for the sum of these congeners, omits valuable information and possibly the most relevant part, hence their occurrence should be discussed on the basis of full spectrum of profiles and patterns. Levels, profiles and pattern of PCDDs and PCDFs in samples related to the production and use of chlorine are described by Rappe et al. [93,94].

PCDDs and PCDFs were also quantified by homologues. In this case, a homologue is the sum of the concentration of each isomer at a particular level of chlorination [16]. Thus there are ten homologues for tetrachloro through octochlorodioxins and benzofurans. The electron impact mode (EI) and multiple ion detection are routinely used for GC–MS [34]. For increased sensitivity, selective ion monitoring (SIM) is used. Quantitation is performed by finding the ratio of appropriate peak area to the internal standard correcting for relative response factor that were obtained from running a standard mixture of PCDDs/PCDFs. Using this method global mass balance for PCDDs/PCDFs has been discussed [85], For maximum sensitivity care must be taken to ensure that the system is leak free to avoid the formation of $(M-Cl+O)^-$ ions.

4. Conclusion

PCDDs and PCDFs belong to one of the most toxic and hazardous environment pollutants listed by EPA which are generated in combustion and incineration of medical and municipal wastes, chemical manufacturing of 2,4,5-T (herbicide) and industrial processes. Other important sources of PCDDs and PCDFs are: industrial and hazardous waste incineration, fires, automobile exhaust and the manufacture of polychlorinated phenols and biphenyls. Since these chemicals have a tendency to bioaccumulate in biological materials, the risk becomes more severe which necessitates a sensitive method of analysis. Extraction methods for these trace pollutants include apart from solvent extraction, the supercritical fluid extraction and supersonic Soxhlet extraction. A new thermospray liquid–liquid extractor that recovers 80–100% of semi volatile compounds in water samples in less than an hour can increase the extraction efficiency of PCDDs and PCDFs [95]. Clean-up of these trace compounds from a large mass of substrates is again a tedious job. Extensive preconcentration and clean-up which generates large amounts of wastes are required to determine these compounds. A very recent technique is based on micelle-mediated extraction in which serum samples spiked with PCDD are extracted by adding Triton X and sodium chloride to avoid the extensive clean-up procedure [96]. Coextractives are removed just by precipitation with acetonitrile.

Gas chromatographic analysis is the best technique for the analysis of PCDDs/PCDFs in environmental samples. ECD, FID and AED are used for the analysis, but MS is the most widely used detection method, since this particular group contains different congeners with same molecular mass. Multidimensional GC with ECD and MS, and MS–MS have also been exploited by various workers [9,36,79]. Fused-silica capillary column with DB-5 and SP-2331 stationary phase and two step linear temperature programming separates the compounds well for detection. The constancy of the molar response for

these groups of congeners by MS can simplify the quantitation of PCDDs/PCDFs by GC–MS quite significantly [79]. The occurrence of these compounds in various substrates has been preferentially discussed on the basis of full spectrum of congeners including their profiles and patterns [5,9,93,94]. With these dimensions, GC–MS particularly the HRGC becomes an efficient and reliable method of analysis for the trace amounts of toxic compounds like PCDDs and PCDFs in the environmental samples.

Nevertheless, potential advantages of supercritical fluid extraction in a logical step can extend the technique in a supercritical fluid chromatography (SFC). Since, the detection methods which have proved extremely useful in GC, such as ECD, nitrogen–phosphorus detection and flame photometric detection are introduced in SFC, powerful identification possibilities for unknown compounds are provided by the compatibility of SFC with MS and Fourier transform infrared spectroscopy (FTIR) [97]. In the case of extremely complex samples, multidimensional SFC with a series of coupled columns of different selectivities provides enhanced separation power [98]. The use of multidimensional chromatographic techniques for separation is expected to increase in future owing to the ever increasing complexity of the samples to be analysed for these compounds.

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