

6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring CDFs, its metabolites, and other biomarkers of exposure and effect to CDFs. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

Some of the methods used to analyze CDFs in biological samples are shown in Table 6-1. These methods are sufficiently sensitive to determine CDF levels in important biological tissues and body fluids. Besides these methods, the International Agency for Research on Cancer (IARC) has published several methods for the determination of CDFs in a variety of biological matrices (Norstrom and Simon 1991; Patterson et al. 1991; Ryan 1991a; Turner et al. 1991). The biological samples used for the determination of CDFs usually contain trace quantities of these compounds in a large matrix of the tissue or fluids. Other contaminants are usually present in biological matrices at much higher concentrations than CDFs, and some of the chlorinated aromatic contaminants are difficult to separate from CDFs. For these reasons, biological samples are subjected to extensive clean up procedures before quantitation. Since the use of high resolution gas chromatography (HRGC) provides an additional useful separation and mass spectrometry (MS) provides the most unambiguous identification, HRGC-MS is the preferred, or even exclusive method for the quantitation of CDFs (see Table 6-1). Sometimes, HRGC with electron capture detection is used for screening CDFs in samples, but quantitation is usually performed by MS. The use of high resolution is preferred over low resolution MS, because the high resolution provides more definitive identification and a lower limit of detection, Negative chemical ionization (NCI)-MS is preferable over electron impact mass

TABLE 6-1. Analytical Methods for Determining CDFs in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human serum	Sample labeled with ^{13}C -CDF containing 2,3,7,8-substituted congeners fractionated into lipo-protein, chylomicrons and red blood cells by centrifugation/ultracentrifugation, extract with $(\text{NH}_4)_2\text{SO}_4$, ethanol, and hexane; clean hexane layer with concentrated H_2SO_4 , concentrate, and cleanup by column chromatography	HRGC/HRMS	5 ppq (pg/kg)	89–103.5	Patterson et al. 1989, 1987 (CDC method)
Human plasma	Spike plasma with ^{13}C -CDF and mix with formic acid and degas under vacuum, cleanup by reversed phase C-18 column, H_2SO_4 and multiple adsorbent column chromatography	HRGC/NCI-MS	No data	66–87	Chang et al. 1990
Human plasma/adipose tissue	Extract sample with added ^{13}C -surrogate and internal standards with methanol and chloroform, separate chloroform layer by adding more chloroform, concentrate, and cleanup by multiple column chromatographic steps	HRGC/HRMS	0.003–0.02 ppt	64–135	Nygren et al. 1988
Human adipose tissue	Spike sample with ^{13}C -CDF, extract with methylene chloride, concentrate, cleanup by multiple column chromatography, add internal quantitation standard	HRGC/MS	2–10 ppt	No data	Stanley et al. 1986

TABLE 6-1. Analytical Methods for Determining CDFs in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human adipose tissue	Spike tissue with isotopically labeled compounds, digest in concentrated HCl, extract with hexane, cleanup and fractionate by multiple column chromatography	HRGC/MS	1–10 ppt	No data	Graham et al. 1986
Human adipose tissue	Spike tissue with ¹³ C-CDF congeners and internal standard, extract with acetone/hexane, redissolve in dichloromethane/cyclohexane, cleanup by gel permeation chromatography, further cleanup by multiple column chromatography	HRGC/HRGC	1 ppt	65.5–180.3 at 20 ppt	Le Bel et al. 1990
Human adipose and other tissues (adrenal, bone marrow, liver, muscle, spleen, kidney, and lung)	Homogenize tissue, extract with acetone/hexane, spike with ³⁷ Cl-CDF, cleanup with H ₂ SO ₄ and multiple column chromatography	HRGC/MS	2 ppt	No data	Ryan et al. 1986, 1987b
Tissues (adipose, whole blood, serum, or organ section)	Spike tissue with ¹³ C-CDF internal standard, digest/extract with (1) HCl/hexane in ultrasonic bath, or (2) potassium hydroxide/ethanol and extract with hexane, or (3) hexane/acetone in shaker; cleanup with concentrated H ₂ SO ₄ and multiple column chromatography	HRGC/MS or HRGC/HRMS	2–25 ppt (for 2,3,7,8-tetraCDD)	50–90 (for 2,3,7,8-tetraCDD)	Tiernan et al. 1984

TABLE 6-1. Analytical Methods for Determining CDFs in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Liver (rat, guinea pig, hamster, mouse)	Homogenize sample with sodium sulfate and ^{13}C -CDF internal standard, extra/cleanup in a column of multiple adsorbents with cyclohexane-methylene chloride; further cleanup by column chromatography	HRGC/HRMS	0.1–1.0 ppt	55–110 ppt	Lindstroem and Rappe 1990
Egg yolk (OCDF only)	Homogenize with acetone-hexane, extract hexane, concentrate and cleanup by Biobeads S-X-3 column	Megabore GC/EDC (ASTM STP1075)	0.5–1.0 ppt lipid	53–120	Draper et al. 1991
Chicken egg and chicken liver	Homogenize sample with acetonitrile, add ^{13}C -CDF, separate acetonitrile layer, concentrate, and cleanup by reverse phase C-18 column; cleanup further with H_2SO_4 and multiple adsorbent column chromatography	HRGC/NCI-MS	No data	64–80	Chang et al. 1990
Cow milk	Spike sample with ^{13}C -tetraCDF, add acetone, extract with hexane, cleanup by multi-column chromatography and concentrated H_2SO_4 and further column and HPLC	HRGC/HRMS	0.01–0.7 ppt	47.3–57.9	Glidden et al. 1990

TABLE 6-1. Analytical Methods for Determining CDFs in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human milk	Spike sample with internal standard, mix with formic acid and Lipidex 5000; wash mixture with methanol and elute into acetonitrile, cleanup by multiphase column chromatography	HRGC/MS	No data	64–100	Noren and Sjoval 1987

CDF = chlorinated dibenzofuran; H₂SO₄ = sulfuric acid; HCl = hydrochloric acid; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; HRMS = high resolution mass spectrometry; MS = mass spectrometry; (NH₄)₂SO₄ = ammonium sulfate; NCI-MS = negative chemical ionization mass spectrometry; tetraCDD = tetrachlorodibenzo-*p*-dioxin; tetraCDF = tetrachlorodibenzofuran

6. ANALYTICAL METHODS

spectrometry (EI-MS) because the sensitivity of the negative chemical ionization is orders of magnitude better than EI-MS (Buser et al. 1985).

Since the concentrations of CDFs in most baseline biological samples are very low, extreme care must be used to ensure that all the reagents and equipments used during the analysis are scrupulously free of contamination. Glass bottles sealed with screw caps can be a source of contamination (Fürst et al. 1989). Owing to their lipophilic nature, CDFs in biological samples are largely associated with the lipid fraction. Procedures commonly used to eliminate lipid interference are saponification, concentrated sulfuric acid treatment, gel permeation chromatography, and column chromatography with suitable adsorbents (Chang et al. 1990). Saponification with hot ethanolic alkali has been shown to degrade higher chlorinated CDFs into lower chlorinated CDFs and ethoxy-CDFs as artifacts (Ryan et al. 1989). Because of the variability in the per cent lipid determination by different laboratories, it is advisable to take this into account when comparing CDF levels in blood and breast milk from different laboratories (Patterson et al. 1989b).

Because CDFs are usually present in biological samples in trace quantities, the more acceptable methods of analysis use internal standards to monitor method performance and quantitation purpose. Normally, ^{13}C - or ^{37}Cl -labeled CDFs are used as internal and recovery standard. In the absence of standard reference materials, the best method to ensure the reliability of quantitation is interlaboratory study (Albro et al. 1985). The quality assurance/quality control procedures used for the determination of CDFs in biological and environmental samples have been discussed (Mitchum and Donnelly 1991). A good review of different methods to analyze biological samples is available (Firestone 1991). An automated method has been proposed to reduce the labor intensive aspects of CDF analysis (Bicking and Wilson 1991).

6.2 ENVIRONMENTAL SAMPLES

Some of the methods used to determine CDF levels in environmental samples are shown in Table 6-2. Besides these methods, IARC has published several methods for the determination of CDFs in a variety of environmental samples (Luksemburg 1991; Ryan 1991b; Smith et al. 1991; Tondeur and Becker-t 1991; Tondeur et al. 1991). Other methods, including monoclonal antibodies for the immunoassay of CDFs (Stanker et al. 1987; Vanderlaan et al. 1988) and radioimmuno assay for 2,3,7,8-tetraCDF, are also available (Luster et al. 1980). Generally, the sensitivity of immunoassay

TABLE 6-2. Analytical Methods for Determining CDFs in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Ambient air	Spike sample collected on polyurethane foam/XAD-2 cartridge with a ³⁷ Cl-CDF internal standard, extract with toluene, wash with acid and base, cleanup by multisection and multicolumn chromatography	HRGC/MS	0.01 pg/m ³	38-109 (for field samples)	Wagel et al. 1989
Ambient air	Spike sample collected on quartz fiber filter and polyurethane foam with ¹³ C-CDFs internal standard; Soxhlet extract with benzene; cleanup by acid-base separation and multicolumn chromatography on silica gel, alumina, and carbon	HRGC/HRMS	0.02–0.2 pg/m ³	74–98% at 0.2 ng spike	Harless et al. 1992
Ambient air	Spike sample collected on polyurethane foam/glass fiber filter with ¹³ C-CDFs internal standard, extract with acetone/toluene, cleanup by multicolumn chromatography and HPLC	HRGC/NCI-MS	0.1–0.5 pg/m ³	88.3–113	Oehme et al. 1986
Stack gas effluent	Collect sample isokinetically with a modified EPA method 5 collection train, extract with benzene, spike with ¹³ C-tetraCDF internal standard, cleanup by 2-dimensional HPLC	HRGC-MS	No data	68–94	Nestrick and Lamparski 1989; Lamparski and Nestrick 1989

TABLE 6-2. Analytical Methods for Determining CDFs in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air, water, soil, sediment, ash, and fish	Spike sample with ^{13}C -CDF internal standard, extract (1) air filters, soil, sediment, and ash with toluene; (2) water with methylene chloride; and (3) fish with methylene chloride/cyclohexane; cleanup extract in multiphase column chromatography	HRGC/HRMS	No data	No data	Kleopfer et al. 1989
Precipitation (rain, snow)	Collect sample in a jug, pass collected water through XAD-2 cartridge with a glass fiber prefilter, spike cartridge with ^{13}C -CDF; extract cartridge with acetone-hexane and filter with toluene; cleanup by multilayer column chromatography and HPLC	HRGC/MS	1–7 ppq (for CDD)	42–86 (for CDD)	Tashiro et al. 1989
Water, soil, sludge, chemical wastes, fly ash	Spike sample with ^{13}C -CDFs, perform matrix-specific extraction, perform multicolumn cleanup, add recovery standard	HRGC/MS	0.63–2.53 ppt (reagent water) 0.11–0.83 ppb (soil) 0.06–0.30 ppb (fly ash) 0.46–2.17 ppb (sludge)	54.2–105.8	EPA 1986b (SW 846 [method 8280])

TABLE 6-2. Analytical Methods for Determining CDFs in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water, soil, sludge, chemical wastes, fly ash	Spike sample with ^{13}C -CDFs, perform matrix-specific extraction, cleanup by acid-base and multicolumn chromatography, add recovery standard	HRGC/HRMS	0.025–0.1 ppt (water) 2.5–12.5 ppt (soil and sediment) 2.5–12.5 ppt (fly ash) 12.5–62.5 ppt (sludge)	No data	Tondeur et al. 1989 (EPA method 8290)
Water, sludge	Pass river water and drinking water through a series of polyurethane foam; filter waste water through a spiked glass-fiber filter, and extract filtrate with methylene chloride; extract polyurethane foam, glass-fiber filter, and sludge with acetone/methylene chloride; concentrate all extracts, dissolve in hexane, cleanup by three column system	HRGC/HRMS	0.02 ppq (water) 0.3–18 ppt	No data	Rappe et al. 1989c
Soil	Spike sample with ^{13}C -CDF internal standard, extract with hexane/acetone, cleanup by multilayer and multicolumn, add recovery standard	HRGC/HRMS	No data	53–86	Creaser and Al-Haddad 1989
Soil (OCDF only)	Soxhlet extract sieved sample with hexane, concentrate and cleanup by Florisil [®]	Megabore GC/ECD (ASTM STP1075)	0.4–0.8 ng/g	101	Draper et al. 1991

TABLE 6-2. Analytical Methods for Determining CDFs in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Fish, fish oil, soil, sediment	Spike sample with isotopic markers, homogenize with sodium sulfate, extract with selective solvent, cleanup by multilayer and multicolumn chromatography	HRGC/NCI-MS	<5 ppt	52–98 (for fish) 62–117 (for fish oil)	Smith et al. 1984
Canned meat	Spike sample with ¹³ C-tetraCDF, mix with sodium sulfate, extract with methylene chloride, cleanup by multi-layer and multicolumn chromatography	HRGC/HRMS	16–39 ppq (pg/kg) (lipid wet basis)	70–120	LeFleur et al. 1990
Fish	Homogenize spiked sample, add concentrated HCl, extract with pentane, concentrate, cleanup by concentrated H ₂ SO ₄ and multilayered, multicolumn chromatography	HRGC/MS	0.03–20 ppt	No data	Zacharewski et al. 1989
Fly ash	Extract sample with benzene, cleanup by HPLC fractionation	HRGC/MS (separation not isomer specific)	No data	No data	Tong et al. 1984
PCB, fly ash, and hexachlorophene	Dissolve or extract spiked (¹³ C-tetraCDF) with suitable solvent, separate by 2-dimensional gas chromatography	GC/GC/MS	No data	No data	Lignon and May 1986
Soot from PCB fire	Spike sample with ³⁷ Cl-CDFs, saponify with alkali, extract with hexane, clean with H ₂ SO ₄ and column chromatography	HRGC/HRMS	1–10 ppt	No data	Harless et al. 1983

TABLE 6-2. Analytical Methods for Determining CDFs in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Paper mill effluent, sludge	Spike sample with ¹³ C-tetraCDF, filter sample, extract filtrate with methylene chloride and residue or sludge with benzene/acetone; concentrate extract, cleanup with acid-base extraction, multicolumn chromatography	HRGC/HRMS	3.8 ppq (water) 0.34 ppq (solid sample)	40–65	Tiernan et al. 1989c
Polyethylene	Extract with decahydronaphthalene at 160°C, dilute with isooctane, filter; pass filtrate through activated basic alumina column and further cleanup by two stage HPLC fractionation	HRGC/MS	20–40 ppq (2,3,7,8-TCDF)	106% at 500 ppq	Nestrick et al. 1991
Paper products	Homogenize slurried sample, spike with ¹³ C-CDFs, extract with ethanol, cleanup by gel permeation chromatography, H ₂ SO ₄ treatment and multicolumn chromatography	HRGC/HRMS	0.2–0.4 ppt	No data	Le Bel et al. 1991

CDD = chlorodibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; H₂SO₄ = sulfuric acid; HCl = hydrochloric acid; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; HRMS = high resolution mass spectrometry; MS = mass spectrometry; NCI-MS = negative chemical ionization mass spectrometry; tetraCDF = tetrachlorodibenzofuran

6. ANALYTICAL METHODS

methods is lower than that attained by high resolution gas chromatography-high resolution mass spectrometry (see Table 6-2), and they require extensive cleanup. Induction bioassay analysis *is* also used for analysis of toxic CDFs in environmental samples. The 2,3,7,8-TCDD equivalents in chemically cleaned fish extracts were determined by their activities as inducers of AHH and EROD in rat hepatoma H-4-11 E cells in culture (Zacharewski et al. 1989). Analytical methods sensitive enough to determine the very low concentrations of CDFs present in most drinking waters are not yet available.

A review of analytical methods used to determine CDF levels in environmental samples is available (Buser 1991; Buser et al. 1985). A combination of glass fiber filters and polyurethane foam plugs is suitable for collecting airborne CDFs (Tashiro et al. 1989). Ultrasonic extraction has been recommended as the inexpensive, efficient, reliable, and rapid method for the extraction of CDFs from fly ash (Beard et al. 1992). The multiphase silica, acidic alumina, and AX-21 (a porous carbon) are very suitable for cleaning up environmental samples including interference from chlorinated diphenyl ethers (Donnelly et al. 1990; Huestis and Sergeant 1992). The relative retention times of all 87 CDF congeners containing 4 to 8 chlorine atoms on the commonly used capillary chromatographic columns have been determined (Ryan et al. 1991). A minimum of two columns are needed to separate all 87 congener peaks from each other. The capabilities of different mass spectral techniques for determining CDF levels in environmental samples have been compared, and the advantage of the MS/MS system over HRMS and LRMS (low resolution MS) have been discussed (Charles and Tondeur 1990; Marbury et al. 1992; McCurvin et al. 1989; Reiner et al. 1991). The advantages and disadvantages of negative ionization low resolution MS over HRGC have also been discussed (Koester et al. 1992). As in the case of biological samples, the results of CDF analysis from different laboratories should be compared to ensure that the data are reliable (Addis et al. 1989; Bradley et al. 1990; Liem et al. 1989).

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of CDFs is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of CDFs.

6. ANALYTICAL METHODS

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. The levels of CDFs in serum and plasma, human milk, and biological tissues are used as biomarkers of exposure to these compounds (Ryan et al. 1985b; Schecter and Ryan 1985) (see Section 2.5.1). Analytical methods for determining CDF levels in biological tissues and fluids are available that can distinguish the levels of these compounds in control versus exposed populations (see Section 5.4.4 and Table 6-1). Increased sensitivity in the method of determining CDFs in blood would be useful, since blood is the least invasive of the biomedica used as biomarkers of exposure.

No specific biomarkers of effects of CDFs in humans were located (see Section 2.5.2).

Methods for Determining Parent Compounds and Degradation Products in

Environmental Media. Analytical methods of sufficient sensitivity to determine CDF levels in most environmental media are available (see Table 6-2). However, the concentration of CDFs in drinking water is so low that suitable methods for determining the concentration are not available. However, the contribution of drinking water to the total intake of CDFs in humans is so low, there is no compelling need to develop analytical methods for the determination of CDFs in drinking water.

The compounds identified as photodegradation products of higher chlorinated CDFs are lower chlorinated CDFs. In fish, a hydroxylated CDF has been identified as a metabolite. Analytical methods capable of determining the photolytic products and hydroxylated compound in fish are available (Frank and Schrap 1990; Koshioka et al. 1987). Further development of methods from the determination of environmental degradation products of CDFs are not warranted.

6.3.2 On-going Studies

6. ANALYTICAL METHODS

As part of a larger project to determine human health hazards from exposure to PCBs and CDFs for people living near a dumpsite, investigators are developing new analytical methods to monitor congener-specific levels of these compounds in feces and urine. The summary of the proposed analytical method was not provided. This research is being conducted by a group headed by Dr. Carpenter of the State University of New York at Albany, New York (FEDRIP 1992). Dr. Tomer of the National Institute of Health is conducting a research project aimed at elucidating the structures and increasing the sensitivity of CDFs and their conjugates excreted by animals. The investigator is attempting to increase the sensitivity of CDF detection by hybrid MS/MS with a combination of high flux/low level sample introduction systems (FEDRIP 1992).