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January 2015

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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 CDDs, its metabolites, and other biomarkers of exposure and effect to CDDs. 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 SAMPLES

The primary method of determining CDDs in biological samples is gas chromatography (GC) with mass spectrometry (MS). Sample preparation is critical, and extensive extraction and sample clean-up are required to separate the CDD homologues/congeners from fatty material and other organic contaminants. Extreme care must also be used to ensure that all reagents and equipment used in analysis are free of CDD contamination. Losses of CDDs can occur as a result of adsorption onto the surfaces of glassware used in sample preparation (EPA 1994c). The routine baking of glassware as a part of the cleaning process should be avoided because this may cause active sites on the glass that will irreversibly adsorb CDDs. The lack of interferences must be demonstrated under the conditions of analysis. Analysts should avoid polyvinyl chloride (PVC) gloves (EPA 1994c). The basic steps of sample preparation include extraction of the sample with a lipophilic organic solvent (e.g., hexane) followed by several evaporation and column chromatography steps to concentrate, clean up, and fractionate the CDDs.

Methods of measuring CDDs in biological samples are very sensitive, generally having method (sample matrix) detection limits in the low- or sub-parts per trillion (ppt) level. If rigorous sample preparation methods are meticulously followed, sensitivity, accuracy, selectivity, and precision can be good. These parameters will vary with the analytical method used, the experience level of the technician, the nature of the sample matrix, the concentrations of the analyte(s) and possible interfering substances, and the specific

homologue/congener being measured. High-resolution gas chromatography (HRGC) is used almost exclusively. The MS method may be low resolution (LRMS), high resolution (HRMS), or tandem LRMS (MS/MS). Individual ionization techniques that have been commonly used with MS to determine CDDs include electron impact ionization (EI), chemical ionization (CI), and negative chemical ionization (NCI). Electron impact ionization instruments are the most common although the least sensitive. The use of CI and NCI methods can improve instrumental sensitivity because less molecular fragmentation occurs, with the resulting ion current concentrated in fewer ions compared to EI. NCI is very selective for those compounds that tend to capture electrons and form negative ions. Both CI and NCI can greatly increase selectivity and sensitivity in complicated matrices. Selected ion monitoring (SIM) is most frequently used for quantitation; however, multiple ion monitoring (MIM), also called multiple ion detection (MID), has also been employed. Isotopically labeled internal standards (such as ^{13}C- or ^{37}Cl-labeled CDDs) are needed both for quantitation and to monitor method performance. Table 6-1 is a summary of some of the most commonly used methods for detecting CDDs in biological samples. Many of the methods for food and wildlife (Table 6-2) could have applicability to CDDs in human samples of similar composition.

HRGC combined with HRMS has been used to determine parts per quadrillion (ppq) levels of CDDs in blood, serum, and plasma (Chang et al. 1993; Nygren et al. 1988; Patterson et al. 1987a, 1989b). Method 8290 (EPA 1994c) is applicable to adipose tissue with a limit of detection of 1 ppt. Method 8290 has also been used to determine CDDs in blood and semen (Schecter 1996). The methods differ in the solvent system used to extract the dioxins and the types of columns used to clean up and fractionate the samples. The method of Chang et al. (1993) used solid phase extraction for the initial step of the isolation. Detection limits were comparable for CDD, but the method used by Patterson et al. (1987a) gave better recovery of the analyte. Precision was similar, with a coefficient of variation (CV) that ranged from 2 to 22% for TCDD.

2,3,7,8-TCDD has been detected (sub-ppt) in human feces using HRGC/LRMS (Wendling et al. 1990). In rodent metabolism studies both parent compound and metabolite were detected in feces and metabolites were detected in urine using GC/LRMS. HRGC/LRMS has also been used successfully in determination of CDDs in rat feces (Abraham et al. 1989a). Adequate comparisons of sensitivity, accuracy, and precision cannot be made because of the lack of these data for several methods and the differences in the media and analytes for the available data.

Table 6-1. Analytical Methods for Determining CDDs in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human serum (CDDs)	Addition of ^{13}C-labeled CDD/CDF mixture to sample; extraction with $(NH_4)_2SO_4$, ethanol, and hexane; washing of hexane layer with distilled water; volume reduction; clean-up with column chromatography	HRGC/HRMS (EI, NCI)	6–150 ppq	No data	Patterson et al. 1989b (CDC method)
Human serum (CDDs)	Addition of ^{13}C-labeled CDD/CDF mixture to sample; extraction with sequential addition of potassium oxalate, ethanol ether, and pentane; remove and washing of pentane layer; clean-up using column chromatography	HRGC/HRMS (EI, NCI)	6–150 ppq	No data	Patterson et al. 1989b
Human serum (2,3,7,8-TCDD)	Addition of [^{13}C]2,3,7,8-TCDD to sample; extraction with $(NH_4)_2SO_4$, ethanol, and hexane; removal of hexane layer and washing with H_2SO_4 and deionized water; volume reduction; clean-up with column chromatography	HRGC/HRMS (SIM)	5 ppq	90–113	Patterson et al. 1987a
Blood (CDDs)	Addition of ^{13}C-labeled CDDs to 100 mL of sample followed by addition of formic acid, equilibration and degassing; passage through C_{18} SPE, elution with hexane and volume reduction; fractionation using benzene sulfonic acid SPE, silica SPE, and Florisil; volume reduction.	HRGC/HRMS (SIM)	<0.005 ng/kg (0.005 ppt)	70–80 at 50 ppq (41% for OCDD)	Chang et al. 1993
Human plasma (CDDs)	Extraction with methanol/chloroform, followed by chloroform/water; removal of chloroform layer and washing with water; evaporation; redissolution in hexane; clean-up on silica gel, elution with hexane; addition of tetradecane and evaporation; redissolution in hexane; separation on Carbopack C®/Celite 545®, elution with toluene; addition of tetradecane followed by solvent evaporation; redissolution in toluene containing ^{13}C-labeled internal standard	HRGC/HRMS (EI/MIM)	3–20 ppq	65–121 (TCDD); 64–135 (CDDs)	Nygren et al. 1988

Table 6-1. Analytical Methods for Determining CDDs in Biological Materials (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Rat urine and feces (CDD metabolites)	Homogenization of fecal samples with distilled water; acidifcation of both urine and fecal samples with H_2SO_4 followed by extraction with toluene; centrifugation of fecal sample and removal of aqueous layer; removal of water from extracts with $MgSO_4$ followed by solvent evaporation; redissolution in acetone; methylation with methyl iodide/K_2CO_3; centrifugation to remove excess K_2CO_3; evaporation of acetone; redissolution in toluene; volume reduction; clean-up on silica gel plate using TLC; elution with toluene; volume reduction	GC/LRMS (EI/MIM)	No data	No data	Tulp and Hutzinger 1978
Rat feces (CDDs)	Grinding of sample with Na_2SO_4; addition $^{13}C_{12}$-labeled CDD/CDF mixture; Soxhlet extraction with toluene; volume reduction; clean-up on alumina column, elution with hexane; volume reduction; clean-up on silica gel, elution with hexane; volume reduction; clean-up on alumina B Super®, elution with hexane; solvent evaporation; redissolution in benzene	HRGC/LRMS (EI/MIM)	No data	75–95 (TCDD); >60 (CDD)	Abraham et al. 1989a
Human feces (2,3,7,8-TCDD)	Addition of [^{3}H]2,3,7,8-TCDD to sample; digestion with H_2SO_4; extraction with hexane; clean-up sequentially on silica gel, alumina, and Carbopack C/Celite®; addition of tribromobiphenyl	HRGC/LRMS (EI/SIM)	0.08–0.1 ppt	59–82	Wendling et al. 1990
Human adipose tissue (CDDs)	Addition of [^{37}Cl]2,3,7,8-TCDD to sample; hydrolysis with KOH, ethanol, and heat; extraction with petroleum ether; washing of organic layer with water and H_2SO_4; volume reduction; clean-up on silica gel; elution with hexane; clean-up on alumina; elution with CH_2Cl_2; volume reduction; redissolution in tridecane	HRGC/LRMS (EI/SIM)	10 ppt	No data	Schecter et al. 1985b

Table 6-1. Analytical Methods for Determining CDDs in Biological Materials (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human adipose tissue (CDDs)	Addition of isotope-labeled standards to sample; homogenization and extraction with CH_2Cl_2; clean-up with gel permeation chromatography; clean-up and fractionation on Carbopak® C/Celite® or Florisil®/AMOCO PX-21®	HRGC/MS (SIM)	1 ppb	50–90	Stanley 1986 (EPA method)
Human adipose tissue (CDDs)	Clean-up of sample on potassium silicate/silica gel column, elution with cyclohexane/CH_2Cl_2; clean-up on carbon column, elution with toluene; clean-up sequentially on potassium silicate column in tandem with alumina column, elution with hexane followed by CH_2Cl_2 in hexane	HRGC/HRMS (EI/MIM)	No data	No data	Nygren et al. 1988
Human adipose	Addition of ^{13}C-labeled standards to tissue followed by extraction with methylene chloride, acid-base washing, solvent exchange, treatment with silica gel impregnated with sulfuric acid, column chromatography using acidic silica gel, neutral alumina, and activated carbon; addition of ^{13}C-labeled standards.	HRGC/HRMS (EPA Method 8290)	1 ppt	No data	EPA 1994c
Human lung, liver, kidney, and adipose tissue (CDDs)	Homogenization of sample; saponification with KOH/ethanol; washing with H_2SO_4 and water; extraction with hexane/acetonitrile; clean-up on alumina column, elution with hexane/CH_2Cl_2; addition of [^{13}C]1,2,3,4-TCDD	HRGC/LRMS (SIM)	10 ppt	35–115	Takizawa and Muto 1987
Human adipose, liver, and kidney tissue (CDDs)	Homogenization of tissue; extraction with acetone/hexane,removal of fat with H_2SO_4; clean-up on Florisil® and activated carbon	HRGC/MS/MS (CI)	2 ppt	No data	Ryan et al. 1987a
Human adipose tissue	Homogenization of tissue; extraction on column via elution with cyclohexane/CH_2Cl_2; clean-up with hexane and H_2SO4; re-extraction with pentane/cyclohexane; clean-up on alumina, Florisil®, and silica:carbon column	HRGC/MS	0.2 ppb	86–100	Wagner et al. 1991

Table 6-1. Analytical Methods for Determining CDDs in Biological Materials (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Rat liver and adipose tissue; rat fetuses (CDDs)	Grinding of sample with Na_2SO_4; addition of [^{13}C]2,3,7,8-TCDD and OCDD; Soxhlet extraction with toluene; volume reduction; addition of hexane; clean-up with column chromatography	HRGC/LRMS (SIM)	No data	No data	Van den Berg et al. 1987b
Rat liver tissue (CDDs)	Grinding of sample with Na_2SO_4; addition of [^{13}C]2,3,7,8-TCDD and OCDD; Soxhlet extraction with toluene; volume reduction; addition of hexane; clean-up with column chromatography	HRGC/LRMS (EI/SIM)	100–250 pg	No data	Van den Berg et al. 1989
Human milk (CDDs)	Centrifugation of sample to separate aqueous and lipid fractions; mixing of lipid layer with Na_2SO_4 and washing with hexane; addition of [^{13}C]2,3,7,8-TCDD and [^{13}C]OCDD; shaking with H_2SO_4 and silica; filtration and collection of hexane layer; addition of nonane and volume reduction; clean-up on Super-Macro™, Macro™, and High Aspect™ columns; fractionation on Zorbax™ octadecylsulphate column using HPLC	HRGC/LRMS (SIM)	0.05–50 ppt	No data	Van den Berg et al. 1986b
Human milk (CDDs)	Mixing of sample with formic acid and Lipidex 5000®; transfer of gel mixture into column and elution with acetonitrile; evaporation of solvent; redissolution in hexane; clean-up on aluminum oxide column, elution with hexane; clean-up on silica gel, elution with hexane; clean-up on aluminum oxide, elution with CH_2Cl_2 in hexane; volume reduction	HRGC/HRMS (SIM)	No data	79–91	Noren and Sjoevall 1987

CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; CH_2Cl_2 = dichloromethane (methylene chloride); CI = chemical ionization; EI = electron impact; GC = gas chromatography; HPLC = high-performance liquid chromatography; HRGC = high-resolution gas chromatography; HRMS = high-resolution mass spectrometry; H_2SO_4 = sulfuric acid; K_2CO_3 = potassium carbonate; KOH = potassium hydroxide; LRMS = low-resolution mass spectrometry; $MgSO_4$ = magnesium sulfate; MIM = multiple ion monitoring; MS = mass spectrometry; Na_2SO_4 = sodium sulfate; NCI = negative chemical ionization; $(NH_4)_2SO_4$ = ammonium sulfate; OCDD = 1,2,3,4,5,6,7,8-OCDD = octachlorodibenzo-*p*-dioxin; ppb = parts per billion; ppq = parts per quadrillion; ppt = parts per trillion; SIM = selective ion monitoring; 2,3,7,8-TCDD = tetrachlorodibenzo-*p*-dioxin; TLC = thin layer chromatography

Table 6-2. Analytical Methods for Determining CDDs in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (hazardous waste site) (2,3,7,8-TCDD)	Collection of sample onto glass fiber filter/polyurethane foam cartridge; add [$^{37}Cl_4$]2,3,7,8-TCDD and [$^{13}C_{12}$]2,3,7,8-TCDD; Soxhlet extraction with CH_2Cl_2; clean-up with acid/base sequentially on silica gel, modified silica gel, alumina, and carbon	HRGC/LRMS	No data 0.02 pg/m^3	91–112 74–112	Fairless et al. 1987 Harless et al. 1992
Air (CDDs)	Drawing of approximately 325 m^3 of air through quartz fiber filter/polyurethane foam; Soxhlet extraction with benzene, volume reduction; clean-up using silica, alumina, activated carbon; volume reduction; addition of [$^{13}C_{12}$]2,3,7,8-TCDD.	HRGC/HRMS; MID (EPA TO-9)	1–5 pg/m^3	68–140 from ultrapure, filtered air	EPA 1988g
Air (CDDs)	Collection of sample onto glass fiber filter/polyurethane foam cartridge; addition of internal standard; Soxhlet extraction with toluene; volume reduction; clean-up and fractionation on Florisil®, elutiion with toluene/diethylether, evaporation and redissollution in cyclohexane; clean-up on modified silica gel using HPLC and hexane/diethyl ether; volume reduction	HRGC/LRMS (EI/SIM); HRGC/LRMS (NCI/SIM)	0.1–1 pg/m^3 0.2–3 pg/inj (0.01–0.1 pg/m^3)	80–122 86–102	Oehme et al. 1986
Air (CDDs)	Collection of sample onto quartz fiber/polyurethane foam plug; Soxhlet extraction with acetone; clean-up with hexane and sulfuric acid followed by silica gel and alumina columns	HRGC/MS (SIM)	0.5 pg/m^3	70–90	Kuwata et al. 1993
Air (CDDs)	Collection of sample onto glass fiber filter/XAD-2® cartridge with $^{13}C_{12}$- labeled CDD mixture added; Soxhlet extraction with toluene and tetradecane; evaporation and redissolution in hexane; clean-up on silica, elution with hexane; evaporation and redissolution in hexane; clean-up on Carbopack C®/Celite 545®, elution with toluene	HRGC/LRMS (EI)	0.01–0.05 pg/m^3	≤5	Rappe et al. 1988

Table 6-2. Analytical Methods for Determining CDDs in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Standards	Coating wells of microtiter plate with TrCDD-hapten-protein conjugate; blocking with ovalbumin; solubilization of CDD or other test compound in BSA using ultrasonication; application of test compound to wells of microtiter plate; addition of hybidoma antibody and incubation; washing with Tween 20®/water; addition of peroxidase-conjugated goat anti-mouse antiserum; addition of 2,2-azino-di-3-ethyl benzthazoline sulfonic acid	ELISA/UV	0.5 ng	No data	Stanker et al. 1987
Drinking water (CDDs)	Addition of ^{13}C-labeled CDD internal standards; extraction with organic solvent; volume reduction; clean-up on multiple columns of silica gel/basic silica/acidic silica, $AgNO_3$-silica/basic alumina, and HPLC	HRGC/LRMS (SIM); HRGC/MS/MS (SIM)	No data	No data	McCurvin et al. 1989
Drinking water (CDDs)	Filtration of sample and collection of CDDs on Separalyte™ cartridge using HPLC; elution from cartridge with acetone; solvent exchange with hexane; water removal using Na_2SO_4; concentration and exchange with benzene; Soxhlet extraction of filters with benzene and addition to cartridge extract; volume reduction; sequential clean-up on acid alumina, graphitized carbon on Celite 545®, and neutral alumina columns	HRGC/LRMS (SIM)	0.5–1.1 ppq	86–124	O'Keefe et al. 1986
Fog (water and particulates) (CDDs)	Collection of sample on Teflon® screen collector; extraction with CH_2Cl_2; solvent evaporation and redissolution in hexane; clean-up on silica gel column, elution with CH_2Cl_2; clean-up on alumina column, elution with hexane/CH_2Cl_2; volume reduction; addition of ^{13}C-labeled CDD/CDF standards	HRGC/HRMS (MIM)	No data	No data	Czuczwa et al. 1989

Table 6-2. Analytical Methods for Determining CDDs in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Landfill leachate (oil extract and bottom layer) (CDDs)	Homogenization of oil sample and dissolution in benzene; addition of ^{13}C-labeled CDD standards; homogenization of bottom sample and dissolution in toluene; addition of ^{13}C-labeled CDD standards followed by reflux and filtration; volume reduction and addition to benzene; for both sample types, clean-up on alumina/Na_2SO_4 column, elution with hexane/CH_2Cl_2; volume reduction; clean-up on silica gel/H_2SO_4/Na_2SO_4 column, elution with hexane; volume reduction; clean-up on Bio-Beads S-X3® column, elution with cyclohexane/ethylacetate; solvent evaporation; redissolution in benzene; clean-up on alumina/Na_2SO_4 column, elution with hexane/CH_2Cl_2; addition of [$^{13}C_6$]1,2,3,4-TCDD; volume reduction	HRGC/LRMS (MIM)	0.02 ppb	60–80	Först et al. 1988
Groundwater, soil, sediment (HxCDD, HpCDD, OCDD)	Extraction of soil and sediment samples with Na_2SO_4/acetonitrile/CH_2Cl_2; centrifugation; removal of organic supernatant and filtration into sampling vial; extraction of water samples with CH_2Cl_2; washing with KOH and water removal with Na_2SO_4; vlume reduction	HRGC/LRMS (CI/SIM); HRGC/MS/MS	No data No data	No data No data	Pereira et al. 1985
	Mixing of sediment with Na_2SO_4, oven drying overnight, and soxhlet extraction with hexane:acetone (1:1) for 16 hours; washing of extract with saturated NaCl, solvent volume reduction, sulfur removal, column clen-up, solvent exchange to DMSO; Extraction of water with hexane and solvent exchange to DMSO.	Chemical-Activated Luciferase Gene Expression (CALUX)	<1 pM per well (<0.5 fmol/well; 32 fg/well)	No data	Murk et al. 1996
Water, soil (2,3,7,8-TCDD equivalents)	Details for sample preparation were not reported by the authors.	Enzyme induction assay (EROD)	62.5 pg/L	No data	Schuman and Hunter 1988
Water, soil, sediment, fly ash, fuel oil, sludge, still bottoms, fish, adipose	Addition of ^{13}C-labeled standards followed by solvent extraction (exact method depends on matrix), acid-base washing treatment, solvent exchange, and cleanup using alumina, silica gel, and activated carbon, addition of ^{13}C-labeled internal standards.	HRGC/HRMS (MIM); (EPA Method 8290)	10 ppq for water to 1 ppt for other matrices (depending on complexity)	No data	EPA 1994c

Table 6-2. Analytical Methods for Determining CDDs in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil, sediment (CDDs)	Addition of isotopically labeled internal standards to sample; addition of Na_2SO_4 and extraction with hexane/methanol or Soxhlet extraction with toluene; clean-up using column chromatography if needed; volume reduction	HRGC/MS (EI/SIM)	No data	No data	Eschenroeder et al. 1986
Soil, sediment (2,3,7,8-TCDD)	Soxhlet extraction of sample; volume reduction; clean-up on basic silica/acidic, silica/alumina, elution with CH_2Cl_2 in hexane; analysis; clean-up on silver nitrate silica or 2,3,7,8-TCDD-specific alumina, elution with CH_2Cl_2 in hexane; analysis; repeating of clean-up or extraction if needed	HRGC/LRMS (SIM); HRGC/MS/MS (SIM)	1 ng/g <1 ng/g	40–90 57–102	Simon et al. 1989
Soil, sediment (CDDs)	Mixing of Na_2SO_4 and sample; elution with acetone/ethyl acetate/CH_2Cl_2; evaporation and redissolution in hexane	HRGC/ECD	No data	92–100	Jasinski 1989
Soil (CDDs)	Sieving of sample; addition of [$^{13}C_{12}$]2,3,7,8-TCDD and [$^{13}C_{12}$]1,2,3,4,6,7,8-HpCDD; Soxhlet extraction with hexane/acetone; removal of organic layer and clean-up on Na_2SO_4/H_2SO_4/silica/$NaHCO_3$; volume reduction of eluate and clean-up on Florisil®, elution with CH_2Cl_2; volume reduction; addition of dodecane and hexane; clean-up on porous graphite column using HPLC and elution with hexane; addition of dodecane and volume reduction	HRGC/MS (SIM)	1 ng/kg	53–86	Creaser and Al-Haddad 1989
Soil (CDDs)	Soxhlet extraction with toluene; addition of [$^{13}C_{12}$]2,3,7,8-TCDD and [$^{13}C_{12}$]OCDD; volume reduction; clean-up on silica and alumina columns	GC/MS/MS GC/LRMS GC/HRMS	2–38 pg 5–20 pg 1–5 pg	No data	Bobbie et al. 1989
Soil (from hazardous waste site) (CDDs)	Addition of [^{13}C]-2,3,7,8TCDF and OCDD and [^{37}Cl]2,3,7,8-TCDD; extraction and clean-up using column chromatography	HRGC/LRMS (EI/SIM)	No data	No data	Stalling et al. 1986

Table 6-2. Analytical Methods for Determining CDDs in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil, fly ash, pottery clay, still bottoms, sludges (CDDs)	Addition of [$^{13}C_{12}$]2,3,7,8-TCDD to sample and, if not a soil sample, extraction as in EPA method 8280; if soil sample, addition of Na_2SO_4 and extraction with petroleum ether and methanol; filtration of soil sample extract into Kuderna-Danish concentrator and addition of petroleum ether; volume reduction, addition of hexane, and volume reduction again; washing of all extracts (soil, chemical waste, etc.) sequentially with KOH, distilled water, H_2SO_4, and distilled water; clean-up on alumina, elution with CH_2Cl_2 in hexane; volume reduction, addition of isooctane during evaporation; just prior to analysis, dilution with isooctane or tridecane; if extra clean-up is necessary, use of HPLC	HRGC/LRMS (SIM) HRGC/ECD	3 ng/g (solid samples); 3 ng/L (liquid samples) No data	60–96 (soil); 62–90 (pottery clay); 68–104 (still bottom) 40–106 (pottery clay)	Donnelly et al. 1986 (modified EPA method 8280)
Soil, water, still bottoms, fuel oils, sludges, fly ash, reactor residues (CDDs, CDFs)	Addition of [$^{13}C_{12}$]CDDs and [$^{13}C_{12}$]CDFs followed by extraction (matrix specific); washing with 20% KOH, 5% NaCl, concentrated sulfuric acid, 5% NaCl, water removal, solvent exchange, and fractionation on alumina; collection of fraction eluted by 60% methylene chloride/hexane; clean-up on carbon column; addition of [$^{13}C_{12}$]1,2,3,4-TCDD.	HRGC/LRMS; MID (EPA 8280)	2 ppb (soils), 10 ppb (other solid wastes), 10 ppt (water)	54–125 (depends on matrix, isomer)	EPA 1986k
Water, soil, sediment, sludge, fish, tissues (tetra- through octa-CDDs and CDFs)	Addition of ^{13}C analogs of 15 of the 2,3,7,8-CDDs/CDFs to sample; filtration, homogenization, acid digestion (depending on matrix) followed by SPE for water samples and liquid/liquid extraction for others; addition of [$^{37}Cl_4$]2,3,7,8-TCDD and clean-up using back extraction with acid and/or base, gel permeation, alumina, silica gel, Florisil, and activated carbon, depending on matrix; volume reduction; addition of internal standard	HRGC/HRMS (EPA 1613)	4 ppq for 2,3,7,8-TCDD in water; 1 ppt in solid	25–164	EPA 1994a
Foods (CDDs)	Homogenization of sample; saponification with KOH/ethanol; washing with H_2SO_4 and water; extraction with hexane/acetonitrile clean-up on alumina column, elution with hexane/CH_2Cl_2; addition of [^{13}C]1,2,3,4-TCDD	HRGC/LRMS (SIM)	10 ppt	35–115	Takizawa and Muto 1987

Table 6-2. Analytical Methods for Determining CDDs in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Foods (CDDs)	Homogenization of sample, addition of 1,3,7,8-TCDD, and digestion with KOH/ethanol solution; extraction with hexane; washing of organic phase with water and H_2SO_4; clean-up on acid-silica column/Florisil® column, elution with hexane followed by CH_2Cl_2; evaporation and redissolution in acetonitrile-CH_2Cl_2; clean-up using HPLC	HRGC/ECD	No data	85–106	Jasinski 1989
Beef fat CDDs, CDFs)	Addition of ^{13}C analogs of 15 of the 2,3,7,8-CDDs/CDFs to sample; filtration, homogenization, acid digestion (depending on matrix) followed by SPE for water samples and liquid/liquid extraction for others; addition of $^{37}Cl_4$-2,3,7,8-TCDD and clean-up using back extraction with acid and/or base, gel permeation, BioSil, PX-21 carbon cleanup; volume reduction; addition of internal standard	HRGC/HRMS (Modification of EPA 1613)	0.05 ppt (wt:wt) for TCDD	56-96 (±20%)	Ferrario et al. 1996
Crab tissue (CDDs, CDFs)	Addition of ^{13}C-labeled standards, digestion with 30% ethanolic KOH; extraction with hexane; washing with sulfuric acid; column chromatography using silica gel, neutral alumina, activated carbon/silica; volume reduction	HRGC/HRMS (MID)	3–15 ppt	40–110	Cai et al. 1994
Fish (CDDs, CDFs)	Blending of sample with anhydrous sodium sulfate, addition of ^{13}C-labeled standards followed by Soxhlet extraction with hexane/methylene chloride (1:1); volume reduction and sovent exchange to isooctane; columen chromatography on silica gel/potassium silicate/sodium sulfate/celite/sulfuric acid/sodium sulfate; volume reduction and solvent exchange to isooctane; clean-up using Florisil, carbon/silica; volume reduction and addition of internal standard	HRGC/HRMS (MID)	1 ppt (2,3,7,8-TCDD)	94–109	Marquis et al. 1994
Fish tissue (CDDs)	Homogenization of sample; digestion with HCl; extraction with hexane; clean-up on glass column containing H_2SO_4; addition of [$^{13}C_{12}$]2,3,7,8-TCDD, [$^{13}C_{12}$]OCDD, and 2,3,7,8-TCDD; volume reduction; clean-up on silica and alumina columns; clean-up with HPLC	HRGC/MS/MS HRGC/LRMS HRGC/HRMS	2–38 pg 5–20 pg 1–5 pg	85–12,500 105–110 ND–95	Bobbie et al. 1989

Table 6-2. Analytical Methods for Determining CDDs in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Fish (2,3,7,8-TCDD equivalents)	Homogenization of sample; digestion with HCl; extraction with pentane; filtration of pentane extract through Na_2SO_4; evaporation; redissolution with pentane/toluene and washing with H_2SO_4; removal of organic layer and clean-up on Na_2SO_4/basic silica gel/acidic silica gel column, elution with pentane; evaporation and redissolution in pentane; clean-up on Carbopak C®/Celite 545® column, elution with toluene; evaporation and redissolution in DMSO; addition to cells	Enzyme induction assays (EROD and AHH)	No data	No data	Zacharewski et al. 1989
Herring gull eggs (CDDs)	Homogenization of sample and extraction; clean-up on Biobeads SX-3® using gel permeation chromatography, elution with CH_2Cl_2/hexane; clean-up by sequential carbon, Florisil®, and alumina column chromatography	HRGC/LRMS (EI/SIM)	10 pg/g	No data	Stalling et al. 1986
Fish, birds, seals (CDDs)	No methods details; extraction and clean-up on silica, modified silica, and alumina columns used; internal standards added	HRGC/LRMS (EI/SIM)	1–50 pg	No data	Buser et al. 1985
		HRGC/LRMS (NCI/SIM)	0.01–0.1 pg		
Wipe and liquid samples from pyrolized transformer oil (CDDs)	Extraction with organic solvent and washing of organic layer sequentially with base and acid; separation on neutral silica gel; clean-up and fractionation on carbon/silica column	HRGC/LRMS	No data	83–134 (wipe); 19–70 (liquid)	Hardin et al. 1989
Wipe and liquid samples from pyrolized transformer oil (CDDs)	Extraction with organic solvent; clean-up on neutral silica/basic silica/acidic silica column; clean-up and fractionation by sequential chromatography on basic alumina, carbon/silica gel, and Sepralyte® columns	HRGC/HRMS	No data	58–151 (wipe) 51–136 (liquid)	Hardin et al. 1989 (ASME method)
Liquid and gaseous waste effluents (CDDs)	Collection of gaseous samples on XAD-2 cartridge followed by Soxhlet extraction with benzene; addition of internal standards to liquid samples; clean-up and sequential fractionation on basic silica gel/neutral silica gel/acidic silica gel columns and alumina column; addition of $[^{37}CL_4]$2,3,7,8-TCDD	HRGC/HRMS (MIM)	1–7.8 pg/m^3 (gases); No data (liquids)	94–101 (gases); No data (liquids)	Cooke et al. 1988

Table 6-2. Analytical Methods for Determining CDDs in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
PCP (CDDs)	Fractionation of sample on Na_2SO_4/neutral alumina column, elutiion with benzeneand on basic alumina, elutiion with CH_2Cl_2-hexane; evaporation and redissolution in toluene	GC/LRMS GC/HRMS	No data No data	>90 >90	Singh et al. 1985
Dust and swab samples (HpCDD and OCDD)	Collection of swab samples on cyclohexane-soaked gauze; extraction with hexane; collection of dust into vials followed by homogenization and Soxhlet extraction with hexane; evaporation to dryness and redissolution in hexane; addition of KOH to both sample types followed by centrifugation; removal of aqueous phase and washing of organic layer with deionized water; water removal using Na_2SO_4 column, elution with hexane; evaporation of sample and redissolution in cyclohexane/CH_2Cl_2; clean-up on activated carbon/silica column using HPLC, elution with CH_2Cl_2/methanol/benzene and toluene; evaporation and redissolution in hexane; removal of aliquot of sample to be analyzed, evaporation, and redissolution in *n*-hexadecane	HRGC/ECD	0.2–4 µg/m^2	61–90	Korfmacher et al. 1985
Cigarettes, and cigarette smoke and ash (CDDs)	Collection of smoke on glass fiber filter/polyurethane foam/XAD-II® cartridges; washing of ash samples with H_2SO_4; Soxhlet extraction of all samples with benzene; volume reduction; addition of hexane and [$^{13}C_6$]1,2,3,4-TCDD; washing with H_2SO_4; volume reduction, addition of hexane; clean-up on alumina, elution with CH_2Cl_2 in hexane; volume reduction; clean-up on Zolbax SIL®, elution with hexane; volume reduction; addition of benzene	HRGC/LRMS (SIM)	0.5 pg/g (cigarettes, ash); 0.22 ng/m^3 (smoke)	No data	Muto and Takizawa 1989
Incinerator stack emission; air from contaminated building (CDDs)	Addition of ^{13}C-labeled TCDD to collection tube followed by collection of sample; addition of internal standards; Soxhlet extraction; clean-up and sequential fractionation on acidic silica/potassium, silicate/silica gel, acidic alumina, carbon, neutral alumina columns; volume reduction	HRGC/HRMS (MIM)	1 pg/m^3	No data	Smith et al. 1986b

Table 6-2. Analytical Methods for Determining CDDs in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Car exhaust (CDDs)	Addition of ^{13}C-labeled CDD standards to XAD-2® resin of an EPA MM5 sampling train; collection of sample; Soxhlet extraction with toluene; clean-up and fractionation on acid- and base-modified silica; further fractionation on basic alumina; clean-up on activated carbon; evaporation and redissolution in isooctane	HRGC/HRMS (EI, SIM)	No data	36–165	Bingham et al. 1989

$AgNO_3$ = silver nitrate; AHH = aryl hydrocarbon hydroxylase; ASME = American Society for Mechanical Engineering; BSA = bovine serum albumin; CDD = chlorinated dibenzo-*p*-dioxin; CDF = chlorinated dibenzofuran; CH_2Cl_2 = dichloromethane (methylene chloride); CI = chemical ionization; DMSO = dimethylsulfoxide; ECD = electron capture detection; EI = electron impact; ELISA = enzyme-linked immunosorbant assay; EPA = Environmental Protection Agency; EROD = ethoxyresorufin O-deethylase; GC = gas chromatography; HCl = hydrochloric acid; HPLC = high-performance liquid chromatography; HRGC = high-resolution gas chromatography; HRMS = high-resolution mass spectrometry; H_2SO_4 = sulfuric acid; HpCDD = heptachlorodibenzo-*p*-dioxin; HxCDD = hexachlorodibenzofuran; inj = injection; KOH = potassium hydroxide; LRMS = low-resolution mass spectrometry; MIM = multiple ion monitoring; MM5 = modified method 5; MS = mass spectrometry; $NaHCO_3$ = sodium bicarbonate; Na_2SO_4 = sodium sulfate; NCI = negative chemical ionization; OCDD = octachlorodibenzo-*p*-dioxin; PCP = pentachlorophenol; ppq = parts per quadrillion; SIM = selective ion monitoring; 2,3,7,8-TCDD = tetrachlorodibenzo-*p*-dioxin; 2,3,7,8-TCDF = tetrachlorodibenzofuran; TrCDD = trichlorodibenzo-*p*-dioxin; UV = ultraviolet detection

HRGC has been combined with LRMS, HRMS, and MS/MS for the detection of CDDs in tissues. Sensitivity is generally in the ppt range with the best sensitivity (2 ppt) reported with MS/MS using CI (Ryan et al. 1987a). The limit of detection was higher for MS than for MS/MS (Schecter et al. 1985b; Stanley 1986; Takizawa and Muto 1987). No recovery data were given for HRMS (Nygren et al. 1988). Precision for these methods is usually <20% (Takizawa and Muto 1987; Van den Berg et al. 1989).

CDDs have been measured in breast milk using HRGC/MS in the SIM mode. Reported detection limits are in the low- to sub-ppt (Van den Berg et al. 1986b), and recovery (75–89%) is good (Noren and Sjoevall 1987).

An additional screening test for TCDD-like (aryl hydrocarbon receptor, AhR, active) chemicals has been developed (Garrison et al. 1996) and is available commercially (Anonymous 1997). Dubbed the CALUX (for chemically activated luciferase gene expression) system, the assay is based on recombinant cell lines into which researchers have inserted a firefly luciferase gene. When exposed to dioxin-like compounds, the recombinant cells luminesce. The method is sensitive to ppt levels of 2,3,7,8-TCDD equivalents in blood, serum, and milk (Anonymous 1997). Samples testing positive can be subjected to more definitive and specific analytical testing.

6.2 ENVIRONMENTAL SAMPLES

As with biological samples, the most common method of determining CDDs in environmental samples is HRGC/HRMS. Other methods, including enzyme bioassays, and monoclonal antibody-based enzyme-linked immunosorbent assays (ELISAs) have also been used or are under development. Even in relatively simple matrices, such as air and water, detection and quantitation of CDDs require rigorous sample preparation procedures. Methods used to prepare environmental samples are similar to those used for biological samples: organic solvent extraction of CDDs from the sample and concentration, clean up, and fractionation of the dioxins using evaporative and column chromatography techniques. The same MS techniques described for biological samples are available for environmental samples, with essentially the same results and limitations. Table 6-2 describes some of the most common methods that have been used to determine CDDs in environmental samples, with specific MS techniques listed when known. The following section describes the methods available for the different types of environmental samples.

HRGC/LRMS and HRGC/HRMS have been used to analyze for CDDs in ambient and hazardous waste site air, cigarette smoke, car exhaust, and gaseous waste emissions. Sample preparation steps for gaseous samples are very similar for these two analytical methods. The steps consist of collection of sample contaminants on a filter/trapping cartridge apparatus, organic solvent extraction of the cartridge, and clean up and fractionation of the extract using column chromatography (Bingham et al. 1989; Cooke et al. 1988; Fairless et al. 1987; Harless et al. 1992; Muto and Takizawa 1989; Oehme et al. 1986; Rappe et al. 1988; Smith et al. 1986). A quartz fiber filter and polyurethane foam plug are commonly used to collect air samples (EPA 1988g; Harless et al. 1992; Kuwata et al. 1993), although XAD-2 has also been used (Hippelein et al. 1993). The sensitivity of these methods is in the low- to sub-pg/m^3 range. Reported recovery and precision were generally good for measurements in air and gaseous waste emissions (Cooke et al. 1988; Fairless et al. 1987; Oehme et al. 1986), but severe sample loss can occur (Bingham et al. 1989; Rappe et al. 1988). Electron capture, negative ionization, low resolution MS has also been used to quantify CDDs in ambient air; however, 2,3,7,8-TCDD is difficult to detect using this method and results must be confirmed with HRGC (Koester et al. 1992).

Methods have been developed for detecting CDDs in liquid samples including drinking water (McCurvin et al. 1989; O'Keefe et al. 1986), groundwater (EPA 1986k, 1994a, 1994c; Pereira et al. 1985), fog (Czuczwa et al. 1989), liquid waste effluents (Cooke et al. 1988), an oil extract of landfill leachate (Först et al. 1988), pentachlorophenol (Singh et al. 1985), fuel oils, still bottoms, and reactor residues (EPA 1986k, 1994a), and pyrolyzed transformer oil (Hardin et al. 1989). HRGC was combined with either LRMS, HRMS, or MS/MS in these methods. Not all methods reported on recovery, precision, and sensitivity, so it is difficult to compare these parameters. Based on the data available, sensitivities range from sub-ppq (O'Keefe et al. 1986) to low-ppt levels (Först et al. 1988). Recoveries were usually >60% (Först et al. 1988; O'Keefe et al. 1986), although some lower values were reported (Hardin et al. 1989).

HRGC/LRMS, HRGC/HRMS, HRGC/MS/MS, and HRGC/ECD have been used to analyze for CDDs in soils and/or sediments (Bobbie et al. 1989; Creaser and Al-Haddad 1989; Donnelly et al. 1986; EPA 1986k, 1994a, 1994c; Eschenroeder et al. 1986; Jasinski 1989; Pereira et al. 1985; Simon et al. 1989; Stalling et al. 1986), solid wastes (Donnelly et al. 1986; Först et al. 1988; Popp et al. 1997), and other solid materials (Donnelly et al. 1986; Hardin et al. 1989; Korfmacher et al. 1985; Muto and Takizawa 1989). Detection limits for the MS methods range from low-ppt to low-ppb levels. The sensitivity cannot be compared to ECD because no detection limits were reported for the ECD methods. For soil/sediments, recovery seemed to be better for GC/ECD (92–100%) (Jasinski 1989) than for the HRGC/MS methods

(40–102%) (Creaser and Al-Haddad 1989; Donnelly et al. 1986; Simon et al. 1989). Polychlorinated biphenyls, polychlorinated diphenyl ethers, polychlorinated naphthalenes, and polychlorinated alkydibenzofurans may be found at concentrations several orders of magnitude higher than the analytes of interest (EPA 1994a) and could thus interfere with the CDDs. Retention times must be verified using reference standards.

A method for determining CDDs in municipal incinerator fly ash has been reported (Alexandrou and Pawliszyn 1990). The method uses supercritical fluid extraction (SFE) to recover CDDs from fly ash samples prior to GC. Supercritical fluid extraction is faster and less expensive than the typically used Soxhlet extraction and gives quantitative removal of CDDs and CDFs from fly ash. Extracts obtained using SFE will still require additional clean-up steps prior to analysis. Supercritical CO_2 has also been used to assist solvent-based extraction of CDDs from soils (Friedrich and Kleiböhmer 1997). In this case, the supercritical fluid was combined with accelerated solvent extraction (liquid extractions conducted under elevated temperature and pressure) to provide good recoveries relative to Soxhlet extractions.

TCDD and other CDDs have been measured in foods (Jasinski 1989; Schecter et al. 1994; Takizawa and Muto 1987) and wildlife (birds and bird eggs, fish, and seals) (Bobbie et al. 1989; Buser et al. 1985; EPA 1994a; Stalling et al. 1986) using HRGC/ECD or HRGC/LRMS. Schecter et al. (1994) reported data as TCDD toxic equivalents with detection limits of approximately 0.01 ppt. Ferrario et al. (1996) reported a new modification of EPA Method 1613 (EPA 1994a) for use in measuring CDDs and CDFs in beef fat; an LOD of 0.05 ppt was shown. A comparison of HRGC/LRMS methods conducted using samples from fish, birds, and seals showed that NCI was substantially more sensitive than EI for some, but not all, congeners (Buser et al. 1985). A within-lab comparison of fish tissue analysis using HRGC combined with either LRMS, HRMS, or MS/MS showed HRMS to be the most sensitive of the three methods (Bobbie et al. 1989). However, the large variations in recovery obtained with these methods also demonstrated the significance of the problems of sample loss and sample contamination that can occur in the analyses of CDDs. The data were not sufficient to permit a comparison of methods among different laboratories.

Bioassays using induction of the enzymes ethoxyresorufin *o*-deethylase (EROD) and/or arylhydrocarbon hydroxylase (AHH) in rat hepatoma H-4-IIE cells (Zacharewski et al. 1989) and modified mouse liver cells (Schuman and Hunter 1988) have been developed and tested on water, soil, and fish samples. The bioassays are based on induction of AHH or EROD enzymatic activity in the cell cultures. Since the cells used in the bioassays are most sensitive to induction by 2,3,7,8-TCDD, this dioxin is used to generate a

standard curve for the bioassays, and induction of activity is expressed as TCDD equivalents. These bioassays are highly sensitive to concentrations of Ah receptor-mediated cytochrome P-450 inducers (Holcomb et al. 1988; Zacharewski et al. 1989), and could be used to rapidly pre-screen environmental samples for 2,3,7,8-TCDD toxicity equivalents. A major drawback to these assays is that they are not highly selective. A number of halogenated aromatics other than CDDs can induce AHH and EROD activity (e.g., chlorinated dibenzofurans, polychlorinated biphenyls, and polychlorinated phenols), although none to the extent of TCDD induction. There is also a question about the possible effects of chemical mixtures, such as might be found in contaminated soil or fish, on the assay results (Zacharewski et al. 1989). An ELISA based on derivation of monoclonal antibodies specific to CDDs has also been investigated as a means of screening environmental samples for chlorinated dioxins (Stanker et al. 1987). Monoclonal antibodies (MAbs) developed using 1-amino-substituted 3,7,8-TrCDD derivatives could detect sub-ng levels of TCDD standards. The derived antibodies had a stronger affinity for CDDs substituted at the 1 position and for CDFs substituted at the 2, 3, 7, and 8 positions than for other CDDs including 2,3,7,8-TCDD. However, development of MAbs more specific for CDDs, especially 2,3,7,8-TCDD would provide a rapid, inexpensive, sensitive, and reasonably selective method for screening samples for CDD contamination. Sugawara and coworkers (Sugawara et al. 1998) have recently described an ELISA-based method for polychlorinated dibenzo-p-dioxins that can detect as little as 0.5 pg/well of 2,3,7,8-TCDD and shows great promise as a screening tool. The cross reactivity for octachloro-dibenzo-p-dioxin is very low (<0.1%), but it is much higher for compounds with three, four, or five chlorine atoms in a substitution pattern similar to the of 2,3,7,8-TCDD. As with all screening approaches, more accurate chemical analysis would be needed to confirm the compounds present.

The CALUX assay described in Section 6.1 has been applied to Ah receptor-active compounds (not limited to dioxins) in sediments and pore waters (Murk et al. 1996) and to blood with mixed results. Sensitivities as low as 0.5 fmol of 2,3,7,8-TCDD were reported. Two polychlorinated terphenyl mixtures, the PCB-substituted Ugilec 141, polybrominated diphenyl ethers, and the PCB mixture Clophen 150 were tested in the CALUX assay and had induction potencies that were 10^{-4} to 10^{-7} compared to TCDD. Thus, this assay is more selective than earlier, induction-based assays, although clearly not as selective as GC/MS.

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 CDDs 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 CDDs.

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. Methods exist for determining CDDs in human serum and plasma, feces, biological tissues, and milk (Abraham et al. 1989a; Anonymous 1997; Chang et al. 1993; EPA 1994a, 1994c; Noren and Sjoevall 1987; Nygren et al. 1988; Patterson et al. 1987a, 1987b; Ryan et al. 1987a; Schecter et al. 1985b; Stanley 1986; Takizawa and Muto 1987; Van den Berg et al. 1989; Wendling et al. 1990). These methods have been used to determine ppq to ppt levels of CDDs in biological samples. The commonly used methods are sensitive enough to detect background levels of CDDs in most media, especially adipose tissue. The background concentration for non-occupationally-exposed people has been reported to be on the order of 4 ppt in lipid (Michalek et al. 1998). Improved clean-up and instrument sensitivity could make blood a more useful monitoring medium, although it is usually reagent and background contamination that is most problematic; CDD concentrations in blood tend be quite low. Improvements in current methods or development of new methods to increase sensitivity and selectivity would help to decrease the time involved in sample preparation, and would reduce the high cost ($800–$1,000 per sample) and possible errors associated with current methods of determining exposure to CDDs.

Several effects such as chloracne and alterations in hepatic metabolism have been associated with exposure to 2,3,7,8-TCDD in humans. However, these effects are not specific for 2,3,7,8-TCDD or other CDDs, but may be induced by numerous other chlorinated hydrocarbons. Determination of specific biomarkers of effect for CDD and development of reliable methods to quantify these effects would be useful in assessing the effects associated with exposure to CDDs.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods exist for measuring CDDs in a variety of environmental media, including air, water, sediment, soil, chemical waste, foods, fish, and other solid matrices (Bingham et al. 1989; Bobbie et al. 1989; Buser et al. 1985; Cai et al. 1994; Cooke et al. 1988; Creaser and Al-Haddad 1989; Donnelly et al. 1986; EPA 1986k, 1988g, 1994a, 1994c; Fairless et al. 1987; Jasinski 1989; Marquis et al. 1994; McCurvin et al. 1989; Muto and Takizawa 1989; Oehme et al. 1986; O'Keefe et al. 1986; Pereira et al. 1985; Rappe et al. 1988; Smith et al. 1986a). Of the EPA methods, Method 8280 (EPA 1986k) and 8290 (EPA 1994a) are both commonly used; Method 8290 is approximately three orders of magnitude more sensitive. Assuming an acute oral MRL of 20 pg/kg/day, an intermediate oral MRL of 7 pg/kg/day, and a 70-kg individual, the limit of detection needed for water (2 L/day consumption) is 770 ppq for acute and 245 ppq for intermediate exposure. The methods of O'Keefe et al. (1986) (LOD reported to be 0.5–1.1 ppq) and EPA (1994a, 1994c) (LODs reported to be 4 ppq to 10 ppq) are adequate for detecting CDDs in drinking water. If a 2 kg/day consumption of food is assumed, the needed method LODs will be 700 ppq for acute and 245 ppq for intermediate exposure. Of those method reporting LODs in foods, the methods of Bobbie et al. (1989) and of Ferrario et al. (1996) have the required LODs. Since CDDs are typically determined on a fat weight basis, the method of Ferrario et al. (1996) should be suitable for most food types once the fat is extracted. The sensitivity of the HRGC/MS methods is excellent, but because of the very low levels of these chemicals in the environment, increased sensitivity may be desirable in order to obtain detectable values. Increased accuracy and selectivity would help make analyses more reliable and possibly reduce the costly and time-consuming sample preparation steps that are currently required. Additional development of bioassays to detect CDDs could provide screening methods with sufficient sensitivity to detect the very low concentrations of toxicological importance.

6.3.2 Ongoing Studies

A collaborative study was identified in which researchers at CDC, NIEHS, University of Mainz in Germany and the German Cancer research Center in Heidelberg are studying biochemical markers of exposure and susceptibility to dioxin in human peripheral blood lymphocytes (Yang et al. 1997).

The following information was obtained from a search of Federal Research in Progress (FEDRIP 1998).

Under an SBIR (Small Business Innovative Research) grant, Xeonobiotic Detection Systems, Inc. of Durham, NC, is marketing the CALUX assay (Anonymous 1997) described in Section 6.1. Hybrizyme

Corp, of Raleigh, NC, is working on a new test method for dioxins in human and animal samples. This work is also being performed under an SBIR. No other details were available. Antibody-based methods for 2,3,7,8-TCDD analysis is the subject of a project lead by R. Carlson of Ecochem Research, Inc. (another SBIR) during which methods for gases will be developed. Finally, G. Wheelock, Paracelsian, Inc., Ithaca, NY, is using SBIR funding to develop an Ah receptor-based assay for the determination of toxic equivalency factors.