7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring PCBs, its metabolites, and other biomarkers of exposure and effect to PCBs. 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.

Methodology for PCB analysis includes several steps: sample collection and storage, extraction, cleanup, and determination (EPA 1995c, 1999k; Hess et al. 1995). Care must be taken to assure that the sample collection follows quality assurance protocols and that equipment and containers are free from contamination. Most sample collections are by grab sampling; however, PCBs may be concentrated from water or air onto sorbents. PCBs are typically separated from the sample matrix by solid-phase extraction (SPE), separatory funnel extraction, continuous liquid/liquid extraction (CLLE), Soxhlet extraction, or Soxhlet/Dean-Stark extraction. PCBs may be difficult to extract from oily matrices in which they are soluble. Some problems that may occur during extraction include evaporative losses during concentration, sorption onto labware, and contamination of samples. Cleanup steps are necessary to remove compounds that may interfere with the determination. Chromatography (e.g., gel permeation, silica gel, Florisil, activated carbon, high-performance liquid) is often used to remove matrix interferences, and sometimes to fractionate PCBs into several groups. Cleanup by chromatography has been used extensively to separate the non-*ortho* and the mono-*ortho* CBs from the remaining congeners before quantitative analysis (Hess et al. 1995). The identification and quantitation of PCBs are most often accomplished by gas chromatographic (GC) techniques. Capillary or high resolution gas chromatography (HRGC) columns capable of separating a substantial proportion of the congeners are indispensable, and GC detectors possessing high selectivity and sensitivity for the PCBs are required. The more universal and less sensitive flame-ionization detector (FID) is used much less often than the electron capture detector (ECD), which has exceptional sensitivity to multiply chlorinated compounds. The mass spectrometer-selected-ion-monitoring (MS-SIM) or ion-trap mass spectrometer (ITMS) detectors have sensitivities somewhat lower than ECD, and they have even greater selectivity for PCBs and can

distinguish and individually measure homologs that may coelute on a particular HRGC column (EPA 1999k).

Some methods in use are multi-residue methods in which PCBs along with many other analytes such as pesticides, are determined. In general, PCB methods analyze for Aroclor mixtures, PCB homologs, or individual PCB congeners. Until recently, packed column GC/ECD was used most often for the determination of PCBs as Aroclor mixtures. The Webb-McCall technique was used for quantitation. The weight percent and homolog identification were determined for several Aroclors. Response factors were generated to calculate the amount in each sample peak; with packed columns, each peak contains several congeners. The amounts found in each sample peak were then summed (Webb-McCall 1973). Alternately, the total area in the Aroclor region of the chromatogram was used for quantitation. However, Aroclor analyses are estimations that are prone to error as a result of the subjective assignment of Aroclor speciation and response factors. Also, the practice of comparing CB patterns in environmental samples with those of technical mixtures can be misleading since mixtures emanating from different sources are mixed at differing rates by diffusion, evaporation, and adsorption onto solids. Many congeners are metabolized, while others bioconcentrate in lipophilic material. Therefore, the final pattern in the environment is often highly modified and may not resemble the original commercial formulation or mixture of formulations (Draper et al. 1991; Duinker and Hillebrand 1979; Hess et al. 1995). The maximum detection limits (MCLs) for Aroclors vary in the range of 0.054–0.90 µg/L in water and 57–70 µg/kg in soils (EPA 1995c). Another approach is to determine PCBs by level of chlorination (or homolog group). One PCB for each homolog (isomer group) is typically used for calibration. Total PCB concentration is obtained by summing isomer group concentrations (Alford-Stevens et al. 1986). However, since the congener distribution is not determined with this method, an accurate calculation of PCB toxic equivalency (TEQ) can not be accessed. Recently, capillary or HRGC has made it possible to achieve lower detection limits and better separation of individual PCB congeners for quantitation (Frame 1997; Mullin et al. 1984; Newman et al. 1998), although complete separation of all PCB congeners on a single column has not yet been achieved (Duebeleis et al. 1989). The commonly used capillary columns (DB-5, C-18, DB-1701, SE-54, SIL-8, SP-2330, and CP-SIL-8) provide poor or no resolution for the following groups of congeners: 15/18, 28/31, 49/52, 66/95, 77/110, 84/90/101, 118/149, 138/163/164, 105/132/153, 170/190, and 182/187 (Liem 1999; Schantz et al. 1993b). Nevertheless, the trend is toward congener-specific analysis by HRGC. Recent advances include analytical methods that are able to quantify individual PCBs congeners to enable TEQ calculations (EPA 1999k; Frame 1999; Patterson et al. 1994). EPA Method 1668 (Revision A) is the current methodology used to measure individual PCB congeners in water, soil, sediment, and tissue by HRGC/high resolution mass spectrometry (HRMS)

(EPA 1999k). Estimated detection limits (EDL) of selected PCB congeners range from 109 to 193 pg/L for water and 11–19 ng/kg for soil, tissue, and mixed-phase samples. EDLs are listed in Table 7-1 for EPA Method 1668 (Revision A; EPA 1999k). This method has been used to measure specific PCBs in EPA projects such as the assessment of PCBs in fish consumed by four Native American tribes in the Columbia River Basin in Washington state (EPA 1996f). As for all analytical methods, determining the quality and usability of Aroclor, PCB homolog, or specific congener data by formal data validation procedures is recommended; EPA has developed data validation guidelines for HRGC/ECD Aroclor data and HRGC/LRMS (low resolution mass spectrometry) PCB specific congener data (EPA 1994h, 1995g).

7.1 BIOLOGICAL SAMPLES

The quantitation of PCBs in biological samples usually consists of three distinct steps: extraction of PCBs from the sample matrix by a solvent or a combination of solvents; cleanup of PCBs from impurities on single or multiple columns; and finally, quantitation by GC with a suitable detector. A summary of some available methods for biological samples is shown in Table 7-2.

PCBs are extracted from blood or serum by solvent extraction techniques using hexane (EPA 1980; Needham et al. 1980), benzene (Mes et al. 1994, 1995a, 1995b, 1995c), or mixed solvents such as hexane/ethyl ether (Koopman-Esseboom et al. 1994b; Luotamo et al. 1985; Needham et al. 1981), or by solid phase micro-extraction techniques (Poon et al. 1999). A variety of adsorbents may be used for cleanup and/or fractionation of extracts: deactivated silica gel (Burse et al. 1989), Florisil (Mes et al. 1994, 1995a, 1995b, 1995c), alumina (Koopman-Esseboom et al. 1994b), or multiple columns (Patterson et al. 1989). GC/ECD is used most often for determination of biological samples (Burse et al. 1989; Mes et al. 1994, 1995a, 1995b; NIOSH 1984b; Schantz et al. 1994). Confirmation by mass spectrometry is recommended (Burse et al. 1994; Mes et al. 1994). Detection limits are in the low- to sub-ppb range (Luotamo et al. 1985; Mes et al. 1994, 1995a, 1995b; Needham et al. 1981; NIOSH 1984b; Poon et al. 1999). Recovery, where reported, ranges from .80 to 96% (Koopman-Esseboom et al. 1994b; Mes et al. 1994; Needham et al. 1980, 1981; NIOSH 1984b; Poon et al. 1999). The accuracy and precision of the results of PCB analysis in serum using a packed column GC/ECD method were studied in a collaborative study. The mean recovery (for Aroclor 1254) was 82.2%; inter-laboratory precision was <21% for samples spiked at 10–100 ng/mL (Burse et al. 1989). Cord blood, which is the most direct marker of fetal exposure, requires especially sensitive analytical methods for accurate PCB analysis because of its

Table 7-1. EPA Method 1668-Estimated Method Detection Limits (EMDL) and Estimated Minimal Levels (EML) of Selected PCB Congeners[a,b]

	Detection limits and minimal levels-matrix and concentration[c]				
	Water (pg/L)		Other[d] (ng/kg)		Extract (pg/µL)
Congener	EMDL	EML	EMDL	EML	EML
77	169	500	17	50	20
105	109	200	11	20	10
114	120	500	12	50	20
118	193	500	19	50	20
123	150	500	15	50	20
126	136	500	14	50	20
156	132	500	13	50	20
157	132	500	13	50	20
167	115	500	11	50	20
169	161	500	16	50	20
180	136	500	14	50	20
189	177	500	18	50	20

[a]Source: EPA 1999k
[b]for SPB-Octyl gas chromatography column
[c]EMDLs and EMLs with common laboratory interferences present. Without interferences, EMDLs and EMLs will be respectively, 5 and 10 pg/L for aqueous samples, and 0.5 and 1.0 ng/kg for soil, tissue, and mixed-phase samples, and EMLs for extracts will be 0.5 pg/µL.
[d]soil, tissue, and mixed-phase samples

EMDL = estimated method detection limits; EML = estimated minimal levels; EPA = U.S. Environmental Protection Agency

Table 7-2. Analytical Methods for Determining Polychlorinated Biphenyls in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Serum	Extraction with mixed solvents; treatment with methanolic KOH; extraction with hexane; cleanup on silica gel column	HRGC/ECD	1.0 ng/mL on 1 mL sample	>80 at 25–400 ng/mL	NIOSH 1984b (method 8004)
Serum	Extraction with mixed solvents; cleanup on silica gel column	GC/ECD	No data	82.2 (average)	Burse et al. 1989
Serum	Solvent extraction; cleanup on 10% silver nitrate on silica gel column	GC/ECD	No data	93.7 at 41 µg/L	Needham et al. 1980
Serum	Extraction with mixed solvents; cleanup on hydrated silica gel column for separation of PCBs from PBBs	GC/ECD	2.5 ng/mL	95.3 at 100 µg/L and 105–127 at 10 µg/L	Needham et al. 1981
Serum	Extraction with diethyl ether and hexane; wash of extract with sulfuric acid; cleanup on silica column	HRGC/EC	0.1 ng/mL	85 at 25–125 ng/mL	Luotamo et al. 1985
Serum (congener specific)	Addition of surrogate congener standard PCB 46 and 142, extraction with hexane, cleanup with Florisil.	HRGC/ECD	1 pg/g (PCB 200) - 634 pg/g (PCB 99)	95.1±12.5 (PCB 153)	Greizerstein et al. 1997
Serum	Extraction with SPME; thermal desorption of PCBs into GC column	GC/ECD	1.0 ppb (total PCBs)	<93	Poon et al. 1999
Blood	Solvent extraction; cleanup on Florisil	GC/ECD; confirmation by HRGC/MS-SIM	2 ng/g	81–96	Mes et al. 1994
Plasma	Solvent extraction; cleanup on alumina	Dual column HRGC/ECD	0.01 ng/g	>95	Koopman-Esseboom et al. 1994b

Table 7-2. Analytical Methods for Determining Polychlorinated Biphenyls in Biological Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Serum (congener specific)	Extraction of ^{13}C-labeled PCB-spiked sample with ethanol/ hexane; wash of extract with concentrated sulfuric acid; cleanup and fractionation by multi-column chromatography	HRGC/NICI/MS and IDMS	2 ppq	605 for PCB-77[a], 48 for PCB-126, and 16 for PCB-169	Patterson et al. 1989
Blood	Methanolic KOH hydrolysis; extraction with hexane; cleanup on silica gel and alumina column if necessary	GC/ECD	2 pg	100±4 at 1.09–109 ng/g	Que Hee et al. 1983
Adipose tissue	Solvent extraction; cleanup on sulfuric acid/silica gel and 10% silver nitrate/silica gel columns	GC/ECD	No data	91–93 at 3 µg/g	Smrek and Needham 1982
Adipose tissue	Extraction with acetone/hexane; fractionation by GPC; cleanup on Florisil column	Two dimensional HRGC/MS	No data	>80 at 10–500 ng/g	Le Bel and Williams 1986
Adipose tissue and serum (congener specific)	Extraction of ^{13}C-labeled PCB-spiked sample with ethanol/ hexane; wash of extract with concentrated sulfuric acid; cleanup and fractionation by multi-column chromatography	HRGC/ID/HRMS	No data	No data	Patterson et al. 1994
Human milk	Extraction with mixed solvents; cleanup on Florisil-silicic acid column	HRGC/ECD	No data	94 at ng/mL	Mes et al. 1984; Safe et al. 1985b
Human milk (congener specific)	Extraction with ethanol/hexane; clean up on Florisil column; fractionation on porous graphitic carbon	HRGC/ECD	3 pg/g	90–104	Hong et al. 1992a
Human milk (congener specific)	Addition of surrogate congener standard PCB 46 and 142, extraction with hexane, cleanup with Florisil.	HRGC/ECD	1 pg/g (PCB 200) - 129 pg/g (PCB 48)	No data	Greizerstein et al. 1997

Table 7-2. Analytical Methods for Determining Polychlorinated Biphenyls in Biological Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human milk	Fat separation; cleanup on adsorption columns	HRGC/ECD	0.4 ng/g fat	No data	Abraham et al. 1994
Human hair (congener specific)	Ultrasonic extraction with acetone/hexane; wash of extract with concentrated sulfuric acid and alkaline hydrolysis; cleanup on Florisil column; fractionation on carbon column	HRGC/ECD	No data	No data	Zupancic-Kralj et al. 1992
Liver, kidney, brain tissue (Rhesus monkeys)	Homogenization; solvent extraction; cleanup on Florisil	GC/ECD	12–33 ng/g	78–100 (corn oil)	Mes et al. 1995a, 1995b
Tissue (congener specific)	Homogenized; extracted in methylen chloride:hexane (1:1) using Soxhlet extractor; cleanup using sulfuric acid and chromatography	HRGC/HRMS	See Table 7-1	No data	EPA 1999k (method 1668)

[a]The high recovery for PCB 77 was due to interference from other congeners. The low recovery for PCB 126 and PCB-169 is not critical since ID/MS makes correction for recovery unnecessary.

ECD = electron capture detector; GC = gas chromatography; GPC = gel permeation chromatography; HRGC = high resolution gas chromatography; HRMS=high resolution mass spectroscopy; ID/HRMS = isotope dilution high resolution mass spectrometry; IDMS = isotope dilution mass spectrometry; KOH = potassium hydroxide; MS = mass spectrometry; MS-SIM = mass spectrometer-selected-ion-monitoring; NICI/MS = negative ion chemical ionization mass spectrometry; PBBs = polybrominated biphenyls; PCBs = polychlorinated biphenyls

relatively low fat content. Analysis of breast milk does not present this difficulty. While analytical techniques have improved enormously in recent years, the low concentration of fat in cord blood may still present difficulties in achieving accurate and reliable PCB levels.

Methods for determining PCBs in adipose tissue are similar to those for blood. Solvent extraction is used to separate the PCBs along with other soluble organics from the tissue. The PCBs are then separated from the lipids, usually by column chromatographic techniques. Most procedures include a step for determining the percent fat since results are often reported on a percent fat basis. Very little performance data are available for PCBs in adipose tissue. Detection limits of 51–144 ng/g have been reported for adipose tissue from Rhesus monkeys (Mes et al. 1994, 1995b).

A congener-specific analysis of a commercial PCB preparation and the PCB composition of a human milk sample were reported originally by Safe et al. (1985b). Recent studies have demonstrated the analysis of non-*ortho* coplanar and mono-*ortho* coplanar PCBs in breast milk (Dewailly et al. 1991) and coplanar PCBs in serum and adipose tissue (Patterson et al. 1994). Determination of these congeners (PCBs 77, 126, 169) is useful in assessing the toxic potential of breast milk for infants.

Recently, supercritical fluid extraction (SFE) has been utilized for extraction and cleanup of biological samples. The procedure is quick and avoids the use of flammable or toxic organic solvents (Anitescu and Tavlarides 1998; Djordjevic et al. 1994). Packed-column GC techniques are still widely used; however, HRGC has made it possible to achieve better separation of PCB congeners for quantitation (Ballschmiter and Zell 1980; Mullin et al. 1984).

The congeners to be determined in samples may be selected on the basis of their abundance in the samples, their toxicity, or the availability of analytical standards. The coplanar, non-*ortho*-substituted congeners are PCB-77, PCB-126, and PCB-169. The mono-*ortho*-substituted congeners, PCB-28, PCB-74, PCB-105, PCB-118, and PCB-156, are also frequently determined, along with the non-*ortho*-substituted PCB congeners. PCBs 138, 153, and 180 are frequently measured in higher amounts than other congeners (Safe 1993; Schecter et al. 1994) and are often included in sets of congeners for quantitation.

Variables in sampling methods greatly influence results. For example, PCB levels in milk fat may decrease during lactation, with maternal age and weight, and with number of children born (Jensen 1987). It has been shown by Lawton et al. (1985b) that random error, inter-laboratory variations in procedure,

and methods used for reporting data may have considerable impact on the reported PCB levels in human tissues. Caution should be exercised when comparing exposure estimates or health effect studies reported by different investigators unless similar analysis methodologies are employed. Also, without the separation and quantitation of individual PCB congeners during analysis, PCB concentrations can not be directly correlated to toxic equivalency. Currently, EPA Method 1668 (Revision A) is a standard method for analysis of individual PCB congeners in biological tissues (EPA 1999k).

7.2 ENVIRONMENTAL SAMPLES

An overview of PCB analysis, including sampling technique, extraction, cleanup procedures, and quantification is reported in EPA Method 1668 (Revision A; EPA 1999k). A summary of representative methods is shown in Table 7-3. The table includes methods that have been standardized by NIOSH, EPA, American Society for Testing and Materials (ASTM), AOAC, and Food and Drug Administration (FDA). Most of these methods were developed for the determination of Aroclors (noncongener-specific PCBs) in environmental samples.

Air samples are usually collected by pumping air through a sampler containing a glass fiber filter and adsorbent trap to separate the particle bound and vapor phase fractions. Adsorbents used most often include Florisil (Lin and Que Hee 1985, 1987; NIOSH 1984a), XAD-2 (EPA 1988b; Hippelein et al. 1993), and polyurethane foam (PUF) (Bremle and Larsson 1998; EPA 1988b). Florisil traps are solvent desorbed (Lin and Que Hee 1985, 1987; NIOSH 1984a) and XAD-2 traps are Soxhlet extracted (Bremle and Larsson 1998; EPA 1988b; Hippelein et al. 1993). PCBs are determined by GC/ECD (Bremle and Larsson 1998; EPA 1988b; Irvine and Loganathan 1998; Lin and Que Hee 1985, 1987; NIOSH 1984a) or HRGC/MS (Hippelein et al. 1993). Detection limits depend upon the volume of air sampled; however, detection limits in the low ng/m^3 (EPA 1988b) to low pg/m^3 (Hippelein et al. 1993) have been reported. Recovery, where reported, is good (>80%) (Bremle and Larsson 1998; Brownlow and Que Hee 1985; EPA 1988b; Irvine and Loganathan 1998; Lee et al. 1996; Lin and Que Hee 1985, 1987).

EPA Method 1668 (Revision A) is the current methodology used to measure specific toxic, dioxin-like PCB congeners in surface, ground, and drinking water by HRGC/HRMS (EPA 1999k). Drinking water samples are typically extracted with solvent prior to analysis by GC/ECD, HRGC/ECD, and HRGC/HRMS (EPA 1989c, 1999k). Detection limits are in the sub-ppb range and recovery is good (>80%) (EPA 1989c). Preconcentration techniques may be used for extraction of large water volumes, thus lowering the method detection limit (Leister and Baker 1994; Swackhamer and Armstrong 1987).

Table 7-3. Analytical Methods for Determining Polychlorinated Biphenyls in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (occupational)	Adsorption on glass fiber filter and Florisil; hexane desorption	GC/ECD	0.0006 mg/m^3 for 50 L sample	No data	NIOSH 1984a (method 5503)
Air	Adsorption on water-deactivated Florisil; hexane desorption; perchlorination	GC/ECD	No data	84–103 at 4–49 µg/m^3	Lin and Que Hee 1985, 1987
Air	Adsorption on Florisil or Chromosorb 102 or Tenax GC or XAD-2; hexane desorption	GC/ECD	10 µg/m^3 for 4 L sample	>80 at 300 µg/m^3	Brownlow and Que Hee 1985
Ambient Air	Sample collection on glass fiber filter and PUF cartridge; Soxhlet extraction; alumina column cleanup	GC/ECD	>1 ng/m^3	36–94	EPA 1988b (method TO-4)
Ambient air (target congeners)	Sample collection on glass fiber filter and XAD-2 trap; Soxhlet extraction; adsorption column cleanup and fractionation	HRGC/MS	low pg/m^3 (calculated)	No data	Hippelein et al. 1993
Water (congener specific)	Extracted using SPE, SFE, CLLE; cleanup using sulfuric acid and chromatography	HRGC/HRMS	See Table 7-1	No data	EPA 1999k (method 1668)
Drinking water	Extraction with hexane	HRGC/ECD	0.08–0.15 µg/L	84–97 (tap water)	EPA 1989c (method 505)
Finished drinking water and groundwater	Extraction with methylene chloride; solvent exchange to methyl tert-butyl ether	GC/ECD or HRGC/ECD	No data	No data	EPA 1989c (method 508)
Drinking water (screening)	Extraction with methylene chloride; solvent exchange to chloroform; perchlorination to decachlorobiphenyl	GC/ECD or HRGC/ECD	0.14–0.23 µg/L	82–136 ng/g	EPA 1989c (method 508A)
Drinking water	Extraction on SPE cartridges or disks; elution with methylene chloride	HRGC/MS	0.045–0.24 µg/L	65–100	EPA 1987f (method 525)
Drinking water (congener specific)	Sample spiked with ^{13}C-labeled PCBs; solvent extraction of sample (filtered water and particles); cleanup and fractionation by adsorption chromatography	HRGC/HRMS	0.02–0.04 pg/L	No data	Miyata et al. 1993

Table 7-3. Analytical Methods for Determining Polychlorinated Biphenyls in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Rain water (congener specific)	Passage through filter and XAD-2 resin; solvent extraction; cleanup on Florisil column	2-dimensional HRGC/ECD	<1–30 pg/L	79–83	Leister and Baker 1994
Waste water	Extraction with methylene chloride; exchange to hexane; cleanup on Florisil column; removal of elemental sulfur if necessary	GC/ECD	0.065 µg/L (PCB-1242)	88–96 at 25–110 µg/L	EPA 1982a, 1988b (method 608)
Waste water	Extraction with methylene chloride	GC/MS	30–36 µg/L (PCB-1221, 1254)	77–80 at 5–2,400 µg/L	EPA 1982a (method 625)
Lake water	Passage through glass fiber filter and XAD-2; Soxhlet extraction; cleanup on alumina and silica gel column	HRGC/ECD	No data	93	Swackhamer and Armstrong 1987
Sea water (congener specific)	Collection of particulate and filtered water in a pressurized extraction-filtration system; cleanup with sodium hydroxide, alumina, and silica column	HRGC/ECD	0.1–3.0 ng/L	67–106	Kelly et al. 1993
Soil, sediments, and other solid sample matrices	Extraction with hexane/acetone; cleanup on Florisil column; desulfurization if necessary	GC/ECD	<1 µg/g	No data	EPA 1994f (method 8080A)[c]
Soil, sediments, and other solid sample matrices (congener specific)	Filtered and homogenized; extracted using Soxhlet/Dean-Stark extractor; cleanup using sulfuric acid and chromatography	HRGC/HRMS	See Table 7-1	No data	EPA 1999k (method 1668)
Solid wastes (Aroclors or congeners)	Soxhlet extraction; sulfuric acid/potassium permanganate cleanup	HRGD/ECD; confirmation on second column	57–70 µg/kg (soil)	62–125 (multiple lab)	EPA 1995c (Method 8082)[c]
Hazardous wastes	Extraction with hexane/acetone; cleanup on silica gel column; desulfurization by copper or mercury if necessary	HRGC/ECD	60–70 µg/kg	104–107 (for soil)	Lopez-Avila et al. 1988

Table 7-3. Analytical Methods for Determining Polychlorinated Biphenyls in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil/sediment (low level)	Extraction with methylene chloride/acetone (1:1); cleanup by gel permeation and micro-alumina column	GC/ECD	80 µg/kg (required quantitation limit)	No data	EPA 1987a (CLP)[b]
Sediment (congener specific)	Ultrasonic extraction with acetone/hexane; sulfur removal; cleanup on Florisil; fractionation by HPLC	HRGC/ECD	No data	70–93	Fuoco et al. 1993
Sediment	Supercritical fluid extraction; mini-Florisil column cleanup sulfur removal	HRGC/ECD; confirmation by MS	No data	.90	Lee and Peart 1994
Railcar paint scrapings	Extraction with 90% methylene chloride/10% methanol; cleanup on Florisil column	HRGC/ECD	1 mg/kg	74–86	Welsh 1995
Fly ash	Soxhlet extraction; optional column cleanup	GC/ECD or GC/MS-SIM	No data	80–100	Ko..an et al. 1994
Fish (congener specific)	Extraction of homogenized tissue with petroleum ether/ethyl acetate; cleanup by gel permeation chromatography	HRGC/NICI/MS	0.2–3 pg	65–115	Schmidt and Hesselberg 1992
Fish (congener specific)	Homogenized; extracted in methylene chloride:hexane (1:1) using Soxhlet extractor; cleanup using sulfuric acid and chromatography	HRGC/HRMS	See Table 7-1	No Data	EPA 1999k (method 1668)
Fish, fish egg, and bird egg (congener specific)	Extraction of homogenized ^{13}C-PCB labeled tissues with methylene chloride; removal of lipid by gel permeation or dialysis; cleanup by multi-layer and multiple chromatography; fractionation by HPLC	HRGC/ECD	0.1–0.73 ng/g (lipid)	62–92	Schwartz et al. 1993
Mammal blubber	Sample ground; solvent extraction; micro-Florisil column cleanup	dual column HRGC/ECD	30 µg/kg	95.2 (mean)	Newman et al. 1994
Marine animals	Microextraction	GC/ECD	1 ng	95 (Aroclor 1254)	Wirth et al. 1994
Cow's milk (congener specific)	Mixing of sample fortified with ^{13}C-labeled PCBs with sodium oxalate and methanol; solvent extraction; cleanup and fractionation by porous carbon and alumina	HRGC/MS	0.1–0.5 pg/g (fat) for tetra- to hexa-congeners of PCB	50–60	Van der Velde et al. 1994

Table 7-3. Analytical Methods for Determining Polychlorinated Biphenyls in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Eggs, fish	Supercritical fluid extraction combined with Florisil separation	GC/ECD	No data	91–95	Alley and Lu 1995
Fatty foods	Solvent extraction; liquid-liquid partitioning; cleanup on Florisil column	GC/ECD	No data	No data	AOAC 1990

[a]This method converts the different congeners to decachlorobiphenyl and cannot differentiate between commercial mixtures (e.g., Aroclor 1242, 1260).
[b]As required by Contract Laboratory Program
[c]Method 8080A is proposed for deletion from SW-846; method 8082 is proposed for inclusion in SW-846.

CLLE = continuous liquid/liquid extraction; ECD = electron capture detection; GC = gas chromatography; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; HRMS = high resolution mass spectrometry; MS = mass spectrometry; NICI/MS = negative ion chemical ionization mass spectrometry; ng = nanogram (10^{-9} g); pg = picogram (10^{-12} g); PUF = polyurethane foam; SIM = selected ion monitoring; SFE= separatory funnel extraction; SPE = solid phase extraction

Detection limits in the low pg/L range have been reported (Leister and Baker 1994). Some of the methods are noncongener-specific; that is, the results are reported as PCB mixtures (Aroclors) (EPA 1982a, 1989c), and some are congener-specific (EPA 1987f, 1999k; Kelly et al. 1993; Leister and Baker 1994; Miyata et al. 1993). EPA Method 508A, which converts all of the PCBs to decachlorobiphenyl, is a screening method for quantifying total PCBs (EPA 1989c). The method is likely to show interference due to perchlorination of biphenyl or related compounds (EPA 1991b) and the method cannot quantify individual commercial Aroclors in a PCB mixture. Some waters, particularly surface and waste waters, may require cleanup on adsorption columns prior to analysis (EPA 1982a, 1988b; Miyata et al. 1993). SPE media may be used, reducing the use of flammable or toxic solvents (EPA 1987f).

Soil, sediment, and solid waste samples are usually Soxhlet extracted (EPA 1994f, 1995c, 1999k). Ultrasonic extraction with various solvent combinations (Fuoco et al. 1993) and SFE (Lee and Peart 1994) are utilized as well. Recoveries using these methods are comparable to Soxhlet extraction (80–100%). Cleanup procedures include sulfur removal (EPA 1994f; Fuoco et al. 1993; Lee and Peart 1994; Lopez-Avila et al. 1988) and separation on adsorbent columns (Bandh et al. 1996; EPA 1994f; Fuoco et al. 1993; Lee and Peart 1994; Lopez-Avila et al. 1988). HRGC/ECD is used most often for determination of PCBs (Fuoco et al. 1993; Lee and Peart 1994; Lopez-Avila et al. 1988). Detection limits are generally in the ppb range (60–80 µg/kg) (EPA 1987a, 1995c; Lopez-Avila et al. 1988). Recovery of 62–125% of PCBs in clay and soil samples has been reported for a multiple lab study (EPA 1995c). Methods using the enzyme-linked immunosorbent assay are commercially available for screening PCB contamination in soils (Baek 1993; EPA 1995d). These methods are inexpensive and have a fast turnaround time.

Methods are available for measuring the concentration of PCBs in fish and animal tissues. Tissues are homogenized, and then dried by blending with anhydrous sodium sulfate prior to Soxhlet or column extraction (EPA 1999k; Newman et al. 1994). Direct extraction has also been utilized (Schwartz et al. 1993). After cleanup, PCBs are determined by HRGC/ECD (Schwartz et al. 1993), dual column HRGC/ECD (Newman et al. 1994), or HRGC/MS (Schmidt and Hesselberg 1992). A micro extraction method for very small sample masses (25 µg) has been developed (Wirth et al. 1994). A limit of detection of 1 ng/kg sample, and good recovery (95%) and precision were reported (Wirth et al. 1994). Few methods are available for the determination of PCBs in foods. Little performance data have been reported as well. A method is available for the determination of Aroclors in poultry fat, fish, and dairy products (AOAC 1990).

A number of Standard Reference Materials (SRMs) with certified PCB congener concentrations are available from the National Institute of Standards and Technology (NIST); these include SRM 1588, PCBs in Cod Liver Oil; SRM 1939, PCBs in River Sediment; SRM 1941, PCBs in Marine Sediment; and SRM 1974, PCBs in Mussel Tissue (Schantz et al. 1993a, 1993b). These SRMs are useful in validating the accuracy of methods for the determination of PCBs, and for verifying that the method remains within acceptable levels of error in during analysis. A summary of available SRMs with certified PCB concentrations is shown in Table 7-4. SRMs with non-certified concentration data for PCBs are included in the table as well.

7.3 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 PCBs is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (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 PCBs.

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.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Several investigators have used whole blood, serum, breast milk, and hair as biomarkers for environmental exposure to PCBs (Brown and Lawton 1984; Fait et al. 1989; Furst et al. 1994; Luotamo 1988; Safe et al. 1985b; Zupancic-Kralj et al. 1992). Consequently, levels of PCBs in these media can provide estimates of exposure to PCBs. Analytical methods of satisfactory accuracy are available for determining congener- and noncongener-specific PCBs in blood, serum, breast milk, and human hair; these methods are shown in Table 7-2. Some methods for determining biomarkers are shown in Table 7-5. The method developed by several investigators can be used for the determination of the three non-*ortho* substituted PCB congeners

Table 7-4. NIST Standard Reference Materials for the Determination of Polychlorinated Biphenyls (PCBs)[a]

SRM	Description	PCBs certified	PCBs quantified
1589 PCBs (as Aroclor 1260) in human serum		1[b]	
1588 Organics in cod liver oil	Surrogate for a tissue extract with high lipid content	4	43
1649 Urban dust/organics	Air particulate material		10
1939 PCB congeners in river sediment	Sediment with high levels of PCB congeners	3	17
1941a Organics in marine sediment	Collected in Baltimore Harbor		15
1974 Organics in mussel tissue	Frozen powder-like homogenate		13
1945 Whale blubber	Frozen blubber homogenate		26

[a]Source: Schantz et al. 1993a
[b]certified as Aroclor 1260

NIST = National Institute of Standards and Technology

Table 7-5. Analytical Methods for Determining Biomarkers for Polychlorinated Biphenyls

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Liver and adipose tissue of gray seal (methyl sulfone metabolite)	Homogenization; extraction with methylene chloride/cyclohexane; removal of lipids by dialysis; cleanup by gel permeation chromatography; fractionation on carbon and Florisil columns	HRGC/ECNI/MS	No data	No data	Buser et al. 1992
Serum (major metabolites of PCB-77)	Solvent extraction; methylation; partition with H_2SO_4	HRGC/ECD and HRGC/MS	No data	low nmol/mL	Morse et al. 1995

ECD = electron capture detection; ECNI = electron capture negative ionization; HRGC = high resolution gas chromatography; MS = mass spectrometry

(77, 126, and 169) in human milk, adipose tissue, and serum at levels normally found in these tissues of control population (Harrad et al. 1992; Hong et al. 1992a; Patterson et al. 1989, 1994).

While blood, serum, breast milk, adipose tissue, and hair have been used as biomarkers of exposure, the possible equivalency of these biomarkers has not been intensively investigated. At least two of these biomarkers, serum and breast milk, did not appear to give equivalent measures of exposure even when expressed on a lipid basis. (The theory of equivalency is based on the assumption that the steady state concentration of a persistent lipophilic substance in different body compartments is the same when expressed on a lipid basis.) Greizerstein et al. (1999) compared levels of PCB congeners between serum and milk from seven women in the New York Angler Study. The congener profiles for serum and milk samples were similar for each individual, but different among all subjects. The sum of the congener concentrations was used to estimate the total PCB concentration. The ratio of serum to milk concentrations in the women ranged from 0.18 to 1.66 with a mean of 0.65±0.49, showing no consistency among individuals. Considerable differences were also found in the lipid-adjusted concentrations of PCBs among individuals. The range of lipid-adjusted serum-to-milk ratios was 1.1–2.8 with a mean of 1.9±0.5. The lipid-adjusted serum levels were also >1 for the most abundant congeners, PCBs 118, 153, 138, and 180. The lipid-adjusted ratios of these four non-planar congeners in serum and milk were similar to those found by Koopman-Esseboom et al. (1994b) in a study involving 418 mother-infant pairs. This latter study found that correlation coefficients between PCB congener levels (PCBs 118, 138, 153, and 180) in maternal plasma, human milk (lipid-basis), and cord plasma were highly significant within one biological sample (0.71–0.98) as well as between different biological samples. However, the correlation between other PCB congeners in human milk varied considerably. The study by Greizerstein et al. (1999) was small and collection of blood and milk samples was not uniform for all subjects.

Therefore, more data are needed to establish whether equivalency factors can be established between various measures of body burden so as to allow normalization of measurements between different studies.

Biomarkers of effects of exposure to PCBs are detailed Chapter 3 (Section 3.8.1). No single effect or combination of effects that could be used specifically as an indicator of exposure to PCBs are being developed to screen large numbers of food samples for PCBs and related compounds (J.K. Huwe et al. of Agricultural Research Service, Fargo, North Dakota). New screening methods for trace detection of PCBs in the environment and feeds are being developed by M. Franek et al. (Ministerstvo Zemedelstvi, Czech Republic). Development and application of semipermeable membrane devices (SPMDs) as environmental dosimeters for PCB contaminants in water, air, sediment, and soil is the subject of ongoing

research by Huckins and Petty at Columbia Environmental Research Center in Missouri. Also at the Columbia Environmental Research Center, C. Orazio et al. are developing analytical methods for determining PCBs in environmental matrices. A reliable method for continuous monitoring of PCBs in incinerator stack gas emissions using resonance-enhanced multiphoton ionization spectroscopy in conjunction with time-of-flight mass spectroscopy (REMPI/TOFMS) is the topic of current research by T.A. Cool at Cornell University. No additional information or ongoing studies regarding analytical methods for determining PCBs in environmental and biological samples resulting from exposure were located. Accordingly, new and improved analytical methods for trace detection of PCBs that could be used specifically as indicators of exposure in environmental and biological samples to PCBs are needed.