7. ANALYTICAL METHODS

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

7.1 BIOLOGICAL MATERIALS

Methods for the determination of organochlorine compounds such as hexachlorobenzene generally consist of the following steps: extraction of the analyte from the sample matrix; clean-up to remove interfering compounds; and analysis (separation and quantitation). The primary method of analysis is gas chromatography (GC) coupled with electron capture detection (ECD) or mass spectrometry (MS). Analytical methods have been developed for the determination of hexachlorobenzene in blood or serum, urine, feces, adipose tissue, and breast milk. A summary of methods is shown in Table 7-1.

Several cautions should be noted. Interferences may result from organics of biological origin that are extracted from the sample, and from contaminated glassware, solvent, etc. Sample interferences are usually removed using fractionation and clean-up procedures. Rigorous sample collection and preparation methods must be followed to prevent contamination of the sample. Good quality control procedures must be used to identify and remove interferences caused by sample contamination.

Blood (or serum) is a body fluid often utilized to assess human exposure to chlorinated organics, including hexachlorobenzene. Blood is usually extracted with solvent (Bristol et al. 1982; Burse et al. 1990; EPA 1980b; Langhorst and Nestrick 1979; Mes et al. 1982), and the extract is cleaned up (and sometimes fractionated) by column chromatography utilizing silica gel (Langhorst and Nestrick 1979), Florisil (Mes et al. 1982), a combination of columns (Burse et al. 1990), or by solid-phase extraction (Dmitrovic et al. 2002). Hexachlorobenzene may also be extracted by automated solid-phase extraction

Table 7-1. Analytical Methods for Determining Hexachlorobenzene in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Adipose tissue	Extraction, GPC cleanup, Florisil fractionation, optional additional cleanup	Capillary GC/MS	12 ng/g	No data	EPA 1986c
Adipose tissue	Maceration with sodium sulfate, extraction and back extraction, Florisil fractionation	GC/ECD	No data	No data	EPA 1980b
Adipose tissue	Soxhlet extraction, cleanup on Florisil	Capillary GC/ECD; confirmation on second column	0.001 µg/g	82	Alawi et al. 1992
Adipose tissue	Solvent extraction, filtration, Florisil fractionation	Capillary GC/ECD; confirmation by GC/MS	0.12 ng/g	86	Mes et al. 1982
Adipose tissue	SFE with alumina (to remove lipids, purification by column chromatography	Capillary GC/ECD	10 µg/kg (fatty tissue)	115	Djordjevic et al. 1994
Breast milk	Separation of fat; column cleanup	cap GC/ECD	0.4 ng/g fat	No data	Abraham et al. 1994
Breast milk	Acid treatment, elute from silica gel, concentrate	GC/ECD	0.009 mg/kg	91	Stachel et al. 1989
Breast milk	Solvent extraction, concentration, SPE	GC/MS/MS EI	0.068 ng/mL	63.23–83.07	Chen et al. 2014
Blood	Solvent (hexane) extraction, concentration	GC/ECD	No data	No data	EPA 1980b
Blood	Solvent extraction, cleanup on silica gel, concentration	GC/PID	16 ng/g	79	Langhorst and Nestrick 1979
Blood	Homogenization with benzene, filtration, Florisil fractionation	Capillary GC/ECD; confirmation by GC/MS	0.2 ng/g	80	Mes et al. 1982
Blood	Hexane extraction, concentration	GC/ECD; confirmation by GC/MS	0.16 ng/g	72	Bristol et al. 1982
Serum	Solvent extraction of denatured serum, fractionation on micro-Florisil column, acid treatment/silica gel cleanup	GC/ECD	1 ppb	58–76	Burse et al. 1990
Serum	Solvent extraction, cleanup with solid-phase solvent extraction cartridges, concentration	GC/NICI MS	0.05 ng/mL	~100	Dmitrovic et al. 2002

Table 7-1. Analytical Methods for Determining Hexachlorobenzene in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Serum	Solid-phase extraction, cleanup on acid/silica gel column	GC/MS	0.1–56 pg/g	93	Thomsen et al. 2007
Serum	Rapid headspace solid-phase micro-extraction	GC/MS EI	0.79	103–104	R. Flores-Ramírez et al. 2014
Plasma	SPE, cleanup on small multilayer silica gel columns	HRGC/HRMS	89.1 pg/mL	98.6	Salihovic et al. 2012
Urine	Solvent extraction, cleanup on silica gel, concentration	GC/PID	4.1 ng/g	84	Langhorst and Nestrick 1979
Semen	Solvent extraction, cleanup on Florisil, concentration	Capillary GC/ECD; confirmation by NICI	~0.3 ng/mL	80	Stachel et al. 1989
Feces	Boiling with solvent, cleanup on alumina	Capillary GC/ECD	No data	No data	Abraham et al. 1994

ECD = electron capture detector; EI = electron ionization; GC = gas chromatography; GPC = gel permeation chromatography; HRGC = high resolution gas chromatography; HRMS = high resolution mass spectrometry; MS = mass spectrometry; NICI = negative ionization chemical ionization; PID = photoionization detector; SFE = supercritical fluid extraction; SPE = solid-phase extraction

using a polystyrene-divinylbenzene sorbent, with additional cleanup by column chromatography utilizing a sulfuric acid-silica column (Thomsen et al. 2007). Analysis is usually by GC/ECD (Bristol et al. 1982; Burse et al. 1990; EPA 1980b; Mes et al. 1982), although GC/MS (Thomsen et al. 2007), and GC coupled with photoionization detection (PID) (Langhorst and Nestrick 1979), high resolution gas chromatography (HRGC)/high resolution mass spectrometry (HRMS) (Salihovic et al. 2012) or MS with negative chemical ionization (NICI) (Dmitrovic et al. 2002) may be used as well. Confirmation by GC/MS is recommended (Bristol et al. 1982; Mes et al. 1982). Recovery for all methods is acceptable (≈70–90%) (Bristol et al. 1982; Burse et al. 1990; Dmitrovic et al. 2002, Langhorst and Nestrick 1979; Mes et al. 1982; Thomsen et al. 2007); precision is also acceptable (≤20% relative standard deviation [RSD]) (Bristol et al. 1982; Burse et al. 1990; Langhorst and Nestrick 1979; Mes et al. 1982; Thomsen et al. 2007). Detection limits are in the low-ppb (ng/g) range (Bristol et al. 1982; Burse et al. 1990; Dmitrovic et al. 2002; Langhorst and Nestrick 1979; Mes et al. 1982; Thomsen et al. 2007) and the ppt (pg/g) range (Thomsen et al. 2007).

Adipose tissue is usually solvent extracted (EPA 1980b; Mes et al. 1982), and the hexachlorobenzene is separated from the extracted fat by Florisil column fractionation (Mes et al. 1982). Analysis is by GC/ECD (EPA 1980b; Mes et al. 1982). Confirmation by GC/MS (Mes et al. 1982) or a second GC column is recommended. Recovery is good (82–86%) (Mes et al. 1982); precision is very good (<10% RSD) (Mes et al. 1982). Solvent extraction followed by gel permeation chromatography (GPC) clean-up and Florisil column fractionation was utilized for a large adipose tissue monitoring study (EPA 1986c). Additional clean-up measures may be required if fractions are not clean enough for capillary GC/MS analysis (EPA 1986c). Supercritical fluid extraction (SFE) and treatment with alumina for lipid removal have been combined; additional purification was carried out by column chromatography (Djordjevic et al. 1994). Recovery was 115%, precision 10.5% RSD. Detection limits for all methods are in the low-ppb (ng/g) range (Alawi and Ababneh 1991; Djordjevic et al. 1994; EPA 1986c; Mes et al. 1982).

Few methods are available for monitoring other tissues and fluids. Breast milk has been analyzed with a combination of fat separation, column clean-up, and capillary GC/ECD (Abraham et al. 1994). Detection limits were 0.4 ng/g fat; other performance data were not reported. Methods for urine (Langhorst and Nestrick 1979) and semen (Stachel et al. 1989) have been reported. Both provide good recovery (80–84%). A method for feces has been reported, and involves boiling with solvent and clean-up on alumina followed by capillary GC/ECD analysis (Abraham et al. 1994). Performance data were not reported.

It is well known that ingestion of hexachlorobenzene can produce porphyria (see Section 3.2.2.2). Urinary porphyrins from humans with porphyria cuntanea tarda (PCT) can be analyzed using thin layer chromatography (TLC). Separation and estimation of porhyrins are carried out on a TLC plate by extraction and esterification of porphyrins, 2-dimensional development, and fluorescent scanning (Miura and Torinuki 1977). Other analysis methods for porphyrins include spectrophotometry. Analysis by this method is carried out by extraction of porphyrins using an anion exchange column, esterification of porphyrins, separation by chromatography, and quantification spectrophotometrically (Grinstein 1977).

7.2 ENVIRONMENTAL SAMPLES

Most environmental analyses have been performed using multiresidue methods involving solvent extract of the analytes from the sample matrix, clean-up to remove interfering compounds, determination by GC with ECD, and confirmation using an ancillary method such as MS. New methods and technologies are evolving, and this has resulted in lower detection limits. For example, detection limits are in the low ppb to ppt range for water matrices and the low ppm to ppb range for food. Analytical methods for the determination of hexachlorobenzene in environmental samples are given in Table 7-2.

Atmospheric hexachlorobenzene is usually sampled by pulling a volume of air through an adsorbent trap (EPA 1988b, 1990b; Hippelein et al. 1993; Langhorst and Nestrick 1979). A filter may be included in the sampling system in order to determine the amount of hexachlorobenzene in particulate (Atlas and Giam 1981; Brorström-Lundén et al. 1994; Farrar et al. 2006; Hippelein et al. 1993). Filters and polyurethane foam (PUF) adsorbent are Soxhlet extracted (EPA 1990b, 1991; Hippelein et al. 1993); filters and sorbent-impregnated polyurethane foam (SIP) adsorbent are Soxhlet extracted (Koblizkova et al. 2012); XAD-2 adsorbent is extracted in a Soxhlet apparatus (Hippelein et al. 1993) or by solvent desorption (Langhorst and Nestrick 1979); polymer-coated glass (POG) adsorbent is extracted by solvent extraction (Farrar et al. 2006). Clean-up on adsorbent columns may be utilized (EPA 1988b; Farrar et al. 2006; Hippelein et al. 1993). A variety of analytical methods are used: GC/ECD (Atlas and Giam 1981; EPA 1991), capillary GC/ECD (Brorström-Lundén et al. 1994; EPA 1990b), GC/PID (Langhorst and Nestrick 1979), and capillary GC/MS (Hippelein et al. 1993; Farrar et al. 2006; Koblizkova et al. 2012). Confirmation on a second GC column or by GC/MS is recommended (Atlas and Giam 1981; EPA 1990b). Reported recovery is good (82–103%) (EPA 1990b; Farrar et al. 2006; Langhorst and Nestrick 1979); precision is also good (<10–20% RSD) (Farrar et al. 2006; Hippelein et al. 1993). Detection limits depend upon the amount of air sampled, but may be in the ppb to sub-ppt range (EPA 1990b; Farrar et al. 2006; Hippelein et al. 1993; Langhorst and Nestrick 1979).

Table 7-2. Analytical Methods for Determining Hexachlorobenzene in Environmental Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collection on PUF; Soxhlet extraction; cleanup on alumina	(EPA Method TO-10) GC/ECD	No data	No data	EPA 1988b
Ambient air	≈2,200 m^3 collected on GFF and XAD-2; Soxhlet extraction; cleanup on layered silica gel; alumina partition	Capillary GC/MS	0.18 pg/m^3 (calculated)	No data	Hippelein et al. 1993
Ambient air	Collection on XAD-2; solvent desorption	GC/PID	70 ppb	95	Langhorst and Nestrick 1979
Ambient air	Collection on PUF; Soxhlet extraction; concentration	dual column megabore GC/ECD or GC/ECD and GC/MS	5 ng/m^3	82–103	EPA 1990b
Ambient air	Collection on SIP; Soxhlet extraction; concentration	GC/MS	No data	No data	Koblizkova et al. 2012
Ambient air	Collection on POG; solvent extraction; clean up on silica gel column	Capillary GC/MS	17 pg/m^3	94	Farrar et al. 2006
Rain, snow	Modified collector; solvent extraction; solvent exchange; cleanup on silica gel	Capillary GC/ECD	0.4 ng/L	No data	Chan et al. 1994
Drinking water	Solid-phase extraction (disk or cartridge)	(EPA Method 525.1) capillary GC/MS	0.111 µg/L	80	EPA 1991
Drinking water	Solvent extraction; solvent exchange	(EPA Method 508) capillary GC/ECD; confirmation using second column	0.077 µg/L (estimated)	68–82	EPA 1988c
Drinking water	Solvent extraction	(EPA Method 505) GC/ECD, confirmation using second column	0.003 µg/L	91–100	EPA 1989b
Drinking water	pH adjustment; concentration on XAD-4; cleanup on silica gel	(Master Scheme) capillary GC/MS	0.1 µg/L (target)	73	Garrison and Pellizzari 1987

Table 7-2. Analytical Methods for Determining Hexachlorobenzene in Environmental Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Drinking water	Functionalized polysulfone membrane extraction (membrane); solvent desorption	GC/MS	1.3 ng/L	72.9	Nuhu et al. 2011
Groundwater	Solvent extraction; solvent exchange	(National Pesticide Survey Method 2) capillary GC/ECD, confirmation using second column	0.12 µg/L	96	Munch et al. 1990
River water	Centrifugation; chromic acid digestion; extraction	Capillary GC/ECD	No data	97.5	Driscoll et al. 1991
Drinking water and river water	DLLME; phase separation	Capillary GC/ECD	0.0005–0.05 µg/L	71.1–81.3	Kozani et al. 2007
Tap water	Add CH_3OH 5% (v/v) and NaCl 5% (w/v) to sample; sorptive extraction by rotation of C18 extraction disk and Teflon disk	GC/ECD	0.08 µg/L	84±2% (C18 phase); 85±3% (PDMS phase)	Canas and Richter 2012
Municipal and industrial waste	Solvent extraction; solvent exchange; optional cleanup on Florisil	(EPA Method 612) GC/ECD	0.05 µg/L	95	EPA 2012a
Municipal and industrial waste	pH adjustment; solvent extraction; concentration	(EPA Method 625) GC/MS	1.9 µg/L	79	EPA 1984
Waste water, soil, sediments, solid wastes	Solvent extraction	(EPA Method 8410) capillary GC/FTIR	20 µg/L	Not applicable	EPA 1986a
Groundwater, soils, solid wastes	Various extraction; cleanup methods	(EPA Method 8270B) capillary GC/MS	660 µg/kg (soil, sediment); 10 µg/L (groundwater)	72.6 (automated Soxhlet extraction)	EPA 1994
Soil, sediment, solid waste	Liquid-liquid extraction	HPLC	0.3 µg/L	97	Khan et al. 2011
Soil	Solvent extraction; liquid-liquid partition; cleanup by sulfuric acid treatment	GC/ECD	No data	98	Waliszewski and Szymczyn-ski 1985

Table 7-2. Analytical Methods for Determining Hexachlorobenzene in Environmental Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil	Soxhlet and sonication extraction; acetylation; solvent extraction; fractionation on silica gel	dual column capillary GC/ECD	No data	83–106	Ojala 1993
Soil	Homogenization with solvent; microwave-assisted solvent extraction	GC	No data	27–115	de Andrea et al. 2001
Sediments	Microwave extraction centrifugation; filtration	Capillary GC/ECD	No data	91.7	Onuska and Terry 1993
Fish tissue	Grind with sodium sulfate; extract with hexane/acetone	GC/ECD	No data	No data	Oliver and Nicol 1982b
Fish	Homogenization; Soxhlet extraction; GPC fractionation; silica gel fractionation	Capillary GC/MS	12.5 ng/g	96	Tiernan et al. 1990
Fish	Maceration; Soxhlet extraction; cleanup with sulfuric acid/silica gel	Dual capillary GC/ECD	5 ng/g (lipid basis)	95	Rahman et al. 1993
Fish	Solvent extraction; column clean-up	GC/IDMS	3.7 ng/g	90–110	Majoros et al. 2013
Fish, aquatic biota	Homogenization with solvent; solvent exchange; cleanup on Florisil	Capillary GC/ECD, confirmation on second column	0.01 mg/kg	~94	Miskiewicz and Gibbs 1994
Aquatic organisms	Homogenization; Soxhlet extraction; GPC fractionation; SPE fractionation; solvent exchange	(USGS method) capillary GC/ECD	No data	50–75	Shan et al. 1994
Butterfat, fish	Isolation on Florisil column; solvent partition; partition on Florisil	GC/ECD	No data	95–98 (fish), 99–104 (butterfat)	Bong 1975
Fatty foods	SFE/SFC (on-line cleanup)	Capillary GC/ECD	4 ppb	85	Nam and King 1994
Fatty foods	Extraction and pretreatment; Florisil cleanup	(DFG Method S9) GC/ECD; confirmation by TLC	0.01 mg/kg	90	Thier and Zeumer 1987b
Milk	Extraction; silica, alumina, and carbon column cleanup	GC/HRMS	1.86 pg/g fat	No data	Kim et al. 2013
Milk	Solid-phase extraction	GC/ECD	No data	88–94	Manes et al. 1993

Table 7-2. Analytical Methods for Determining Hexachlorobenzene in Environmental Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Milk	Solvent extraction; solvent partition; solvent exchange; GPC cleanup; optional alumina cleanup	GC/ECD, confirmation on second column	<0.5 ppb	88–91	Trotter and Dickerson 1993
Vegetable oils, oil seeds	Sandwich-type extraction fractionation	GC/ECD	1–2 ppb	80–100	Seidel and Linder 1993
Fruits, vegetables	Chop and blend; blend with solvent; partition with water; dry	GC/ECD, confirmation by GC/MS	0.002 ppm	93	Pylypiw 1993
Crops and foods	Solvent extraction; GPC cleanup; optional silica gel cleanup	(DFG Method S19) dual GC/ECD	No data	>70	Thier and Zeumer 1987a
Tobacco	Solvent extraction; SPE	GC/MS/MS	5 µg/kg	72–95	Chen et al. 2014
Pine needles	Dry and mince; homogenization; Soxhlet extraction; sulfuric acid cleanup; fractionation on Florisil	Capillary GC/ECD	0.1 ng/g (dry weight)	80–100	Calamari et al. 1994

DLLME = dispersive liquid-liquid microextraction; ECD = electron capture detector; EPA = Environmental Protection Agency; FTIR = Fourier transform infrared spectrometry; GC = gas chromatography; GFF = glass fiber filter; GPC = gel permeation chromatography; HPLC = high performance liquid chromatography; HRMS = high resolution mass spectrometry; IDMS = isotope dilution mass spectrometry; MS = mass spectrometry; PDMS = polydimethylsiloxane; PID = photoionization detector; POG = polymer-coated glass; PUF = polyurethane foam; SFC = supercritical fluid chromatography; SFE = supercritical fluid extraction; SIP = sorbent-impregnated polyurethane foam; SPE = solid phase extraction; TLC = thin-layer chromatography; USGS = U.S. Geological Survey

Hexachlorobenzene is usually extracted from water with organic solvents for analysis (EPA 1988c, 1989b; Kozani et al. 2007; Munch et al. 1990). Hexachlorobenzene may also be extracted and concentrated by adsorption on adsorbent cartridges, disks, or membranes, with subsequent solvent desorption (EPA 1988a; Nuhu et al. 2011). Clean-up of the extracts is usually not necessary; however, methods are available for samples that contain interfering compounds (Chan et al. 1994; Driscoll et al. 1991; Garrison and Pellizzari 1987). Analysis is usually by capillary GC/ECD (Chan et al. 1994; Driscoll et al. 1991; EPA 1988c; Kozani et al. 2007; Munch et al. 1990). Confirmation using a second method is recommended (EPA 1988c, 1989b; Munch et al. 1990). Capillary GC/MS and GC/MS are also utilized for analysis (EPA 1991; Garrison and Pellizzari 1987; Nuhu et al. 2011). Accuracy ranges from acceptable (≈60–80%) (EPA 1988a; Garrison and Pellizzari 1987; Kozani et al. 2007; Nuhu et al. 2011) to excellent (>90%) (Driscoll et al. 1991; EPA 1989b, 1991; Munch et al. 1990). Precision is rarely reported; 16% RSD was reported for the Master Scheme (Garrison and Pellizzari 1987), 0.52–2.8% RSD was reported for dispersive liquid-liquid microextraction (DLLME) (Kozani et al. 2007), and 9.2% RSD was reported for functionalized polysulfone membrane extraction (Nuhu et al. 2011). Detection limits are in the low- to sub-ppb range (EPA 1991; Garrison and Pellizzari 1987; Kozani et al. 2007; Munch et al. 1990). Detection limits in the ppt range have been achieved by methods utilizing solvent extraction and functionalized polysulfone membrane extraction with capillary GC/ECD and GC/MS analysis, respectively (Chan et al. 1994; EPA 1989b; Nuhu et al. 2011). Waste water is solvent extracted with analysis by GC/ECD (EPA 2012a) or GC/MS (EPA 1989b). Reported recovery is good (79–95%) (EPA 1984, 2012a). Detection limits are in the low-ppb range, with lower detection limits reported for the GC/ECD analysis (EPA 2012a).

Soxhlet or sonication extraction is most commonly used to extract hexachlorobenzene from solid matrices such as soils and sediments, and wastes (EPA 2012a; Ojala 1993). Solvent extraction (Waliszewski and Szymczynski 1985) and microwave extraction techniques (de Andrea et al. 2001; Onuska and Terry 1993) may be used as well. Clean-up is usually required for the extracts (EPA 1994; Ojala 1993; Waliszewski and Szymczynski 1985), with subsequent analysis by GC (de Andrea et al. 2001), GC/ECD (Waliszewski and Szymczynski 1985), capillary GC/ECD (Ojala 1993; Onuska and Terry 1993), or capillary GC/MS (EPA 1994). Reported recovery is good (73–106%) (de Andrea et al. 2001; EPA 1994; Ojala 1993; Onuska and Terry 1993; Waliszewski and Szymczynski 1985). Precision, where reported, is acceptable (≤20% RSD) (de Andrea et al. 2001; EPA 1984, 2012a; Ojala 1993). Little information is available on detection limits. Detection limits of 660 µg/kg (ppb) have been reported for automated Soxhlet extraction with capillary GC/MS analysis (EPA 1994). A high performance liquid chromatography (HPLC) based method for the determination of hexachlorobenzene and its possible

metabolites, including chlorophenolic and chloroquinolic intermediates, by liquid-liquid extraction has been developed (Khan et al. 2011). Sample cleanup or derivatization is not needed. Analysis is done by HPLC with a reported recovery of 97%. Precision is acceptable (2.1% RSD). A detection limit of 0.3 µg/L is reported (Khan et al. 2011).

Fish and aquatic organisms are homogenized, then extracted with solvent (Miskiewicz and Gibbs 1994; Oliver and Nichol 1982b), isolated on Florisil columns (Bong 1975), or Soxhlet extracted (Rahman et al. 1993; Shan et al. 1994; Tiernan et al. 1990). Clean-up is usually necessary to remove lipids and interfering substances (Bong 1975; Miskiewicz and Gibbs 1994; Rahman et al. 1993; Shan et al. 1994; Tiernan et al. 1990). Capillary GC/ECD analysis is used most often (Miskiewicz and Gibbs 1994; Rahman et al. 1993; Shan et al. 1994). Capillary GC/MS (Tiernan et al. 1990) and GC/ECD (Bong 1975; Oliver and Nichol 1982b) are also utilized. Reported recovery ranges from moderate (50–75%) (Shan et al. 1994) to excellent (>90%) (Bong 1975; Miskiewicz and Gibbs 1994; Rahman et al. 1993; Tiernan et al. 1990). Precision is usually not reported; however, 4–6% RSD has been achieved (Shan et al. 1994). Detection limits, where reported, are in the low-ppb range (ng/g) (Miskiewicz and Gibbs 1994; Rahman et al. 1993; Tiernan et al. 1990).

Fatty foods, including milk, have been extracted with solvent to remove the fat, and then cleaned up to separate the hexachlorobenzene from the fat (Bong 1975; Thier and Zeumer 1987b; Trotter and Dickerson 1993). Newer methods for combined separation and clean-up are supercritical fluid techniques (Nam and King 1994), solid-phase extraction (SPE) (Manes et al. 1993), and a sandwich system (Seidel and Linder 1994). Analysis is by GC/ECD (Bong 1975; Manes et al. 1993; Seidel and Linder 1993; Thier and Zeumer 1987b; Trotter and Dickerson 1993). Confirmation on a second GC column (Trotter and Dickerson 1993) or by TLC (Thier and Zeumer 1987b) is recommended. Capillary GC/ECD has also been utilized (Nam and King 1994). Reported recoveries are good (>80%) (Bong 1975; Manes et al. 1993; Nam and King 1994; Seidel and Linder 1993; Trotter and Dickerson 1993). Precision, where reported, is very good (<15% RSD) (Bong 1975; Nam and King 1994; Thier and Zeumer 1987b; Trotter and Dickerson 1993). Limit of detection, where reported, is in the low-ppb (ng/g) range (Nam and King 1994; Seidel and Linder 1993; Thier and Zeumer 1987b; Trotter and Dickerson 1993).

Fruits, vegetables, and crops are blended, solvent extracted, and then cleaned up and fractionated (Pylypiw 1993; Thier and Zeumer 1987a). Capillary GC/ECD is the analytical method. Recovery is acceptable (>70%) (Pylypiw 1993; Thier and Zeumer 1987a). Precision was not reported. The reported detection limit is 2 ppb (Pylypiw 1993).

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 hexachlorobenzene is available. Where adequate information is not available, ATSDR, in conjunction with 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 hexachlorobenzene.

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. Methods exist for measuring hexachlorobenzene in blood (Bristol et al. 1982; Burse et al. 1990; Langhorst and Nestrick 1979; Mes et al. 1982) and adipose tissue (Alawi and Ababneh 1991; Djordjevic et al. 1994; EPA 1980b, 1986c; Mes et al. 1982). The methods for blood and adipose are sensitive (low-ppb range), but improved accuracy is needed for blood analysis. The data on determination of hexachlorobenzene in urine, breast milk, and tissues are limited, and the methods may not be sufficiently sensitive. Methods that could be used to measure low levels in human tissues would be useful for determining the relationship between chronic low-level exposure and the effects observed in specific tissues. Improved methods to detect phenolic metabolites are not needed since these metabolites are not unique to hexachlorobenzene. Representative methods for determining pentachlorophenol and other phenolic metabolites using GC/ECD and GC/MS are shown in Table 7-3.

Biomarkers for effects of hexachlorobenzene are porphyric symptoms and increased gamma-glutamic transferase activity. Since these effects are also indicative of exposure to other toxicants, additional studies are needed for more specific biomarkers for effects of hexachlorobenzene exposure.

Table 7-3. Analytical Methods for Determining Biomarkers of Hexachlorobenzene

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood (pentachloro-phenol)	pH adjustment, solvent extraction, derivatization	GC/ECD	10 ppb	92	EPA 1980b
Urine (chlorinated phenol metabolites)	Hydrolysis; solvent extraction, derivatization	GC/ECD, confirmation by GC/MS	No data	>90 (PCP); most other metabolites >80	EPA 1980b

ECD = electron capture detector; GC = gas chromatography; MS = mass spectrometry; PCP = pentachlorophenol

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining hexachlorobenzene in air (EPA 1988b, 1990b; Hippelein et al. 1993; Langhorst and Nestrick 1979) and water (Chan et al. 1994; EPA 1988c, 1989b, 1991; Garrison and Pellizzari 1987), the media of most concern for human exposure, are reliable, but may not be sensitive enough to measure background levels in the environment. Limited performance data are available for methods for soil and other solid media. In addition, there is insufficient performance information for methods for determining hexachlorobenzene in media such as shellfish, fish, and plants. Some exposure to hexachlorobenzene may occur via ingestion of food and standardized methods for foods are needed. Methods with sufficient sensitivity for measuring background levels in foods would be helpful as well.

7.3.2 Ongoing Studies

No ongoing studies regarding analytical methods sponsored by NIH or EPA were identified for hexachlorobenzene.