

## 9. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring polybrominated biphenyls (PBBs) and polybrominated diphenyl ethers (PBDEs), its metabolites, and other biomarkers of exposure and effect to PBBs and PBDEs. 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.

PBBs and PBDEs are analyzed in environmental and biological samples by methods quite similar to those used for polychlorinated biphenyls (PCBs) (de Kok et al. 1977; Fries 1985b; Pomerantz et al. 1978). The analytical methods for PBBs were developed primarily in the 1970s, whereas analytical methods for PBDEs were developed very recently. In the 30 years between analytical efforts directed at PBBs and PBDEs, there have been many advances in the technology and costs of analytical instruments. Thus, while gas chromatography-electron capture detection (GC-ECD) with packed columns (i.e., noncongener specific) was the primary analytical technique used in the 1970s for PBBs, gas chromatography-mass spectrometry (GC-MS) with capillary columns (i.e., congener specific) is the primary analytical technique now used for PBDEs. These points should be considered when comparing the differences and quality of analysis for these two classes of compounds.

Covaci et al. (2003) recently reviewed the determination of brominated flame retardants, with emphasis on PBDEs in environmental and human samples. The analysis methodology for PBBs and PBDEs includes several steps: sample collection and storage, sample pretreatment, extraction, cleanup and fractionation, and analytical determination. Care must be taken to assure that the sample collection follows quality-assurance protocols and that equipment and containers are free from contamination. It is important that laboratories utilize blanks when reporting trace levels of PBBs and PBDEs. This practice will minimize the influence of trace contamination samples that can originate from a variety of sources.

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Most sample collections are by grab sampling; however, PBBs and PBDEs may be concentrated from water onto sorbents. Desiccation of solid samples (e.g., soil, sediment, and sewage sludge) is largely done for convenience. Dry samples are more efficiently homogenized, allowing for parallel determination of other analytes (e.g., lipid content) (Covaci et al. 2003).

PBBs and PBDEs are typically separated from the biological and environmental media by extraction with organic solvents. Liquid-solid extraction (e.g., Soxhlet apparatus) remains a widely used technique for solid samples despite recent advances in other extraction techniques. Typical solvents are hexane, toluene, hexane/acetone mixtures, or dichloromethane. New extraction techniques, such as accelerated solvent extraction (ASE) or microwave-assisted extraction (MAE), are also currently used by a number of laboratories. The advantage of these techniques is lower solvent consumption and reduced extraction time. Supercritical fluid extraction (SFE) with solid-phase trapping has been used for the extraction brominated flame retardants from sediment with CO<sub>2</sub> as the supercritical fluid. Extraction with pressurized hot water (PHWE) has been used for the analysis of brominated analytes from sediment. Liquid-liquid extraction has been applied for river and seawater samples, using hexane/acetone mixtures. Solid-phase extraction (SPE) has been used for the analysis of acidic and neutral brominated flame retardants from human plasma (Covaci et al. 2003).

Cleanup steps are necessary to remove compounds that may interfere with the determination (e.g., humic acids, lipids) of PBBs and PBDEs. Lipids (e.g., oils and fats) may be destroyed with concentrated sulfuric acid treatment either directly to the extract or using impregnated silica columns.

Chromatography (e.g., gel permeation, silica gel, Florisil) is used to remove other matrix interferences and to fractionate samples (Covaci et al. 2003).

The identification and quantitation of PBBs and PBDEs are most often accomplished by 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 PBBs and PBDEs 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 highly brominated compounds. The mass-spectrometer detectors have sensitivities somewhat lower than ECD, and they have even greater selectivity for PBBs and PBDEs and can distinguish and individually measure homologs that may co-elute on a particular HRGC column. The use of MS is indispensable in the definitive identification of PBB and PBDE congeners. A recent method of detection is electron-capture negative ionization (ECNI) as an ionization technique in combination with

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GC-MS analysis (de Boer et al. 2000). This method is advantageous because it offers a high sensitivity for compounds with four or more bromine atoms. The sensitivity is approximately 10 times higher than with the use of the ECD. However, ECNI, although generally more sensitive and less costly than other ionization methods for PBDE analysis, does not provide information on the molecular ion cluster (as required for qualitative identification). It is also more subject to brominated interferences and does not allow the use of  $^{13}\text{C}$ -labeled standards for quantification (Ikonomou and Rayne 2002). Conversely, electron ionization (EI) methods suffer from fragmentation of the molecular ions, creating difficulties in both identification and quantitation of congeners in full-scan and single ion monitoring (SIM) modes, respectively. For example, loss of Br atoms from PBDE congeners during EI may lead to incorrect identification of the parent ion as a lower brominated congener. In addition, the relatively unpredictable fragmentation during EI or ECD restricts the utility of applying relative response factors (RRFs) of one congener for which an analytical standard is available (e.g., 2,2,4,4'-tetrabromodiphenyl ether or BDE 47) for other members of its homolog group (e.g., tetrabromodiphenyl ether [tetraBDEs]). This can result in either under- or overestimating concentrations of congeners for which analytical standards are not available (Ikonomou and Rayne 2002b). In general, hepta- through deca-BDE congeners are difficult to determine accurately by GC analysis, especially in biological samples (Ikonomou and Rayne 2002b).

The analysis of decaBDE (i.e., BDE 209) and 2,2',4,4',5,6'-hexabromodiphenyl ether (i.e., BDE 154) has some analytical difficulties. For example, BDE 209 (1) is not stable at high temperatures in the GC injector and GC column; (2) is sensitive to degradation by UV light (i.e., both sunlight and fluorescent light); (3) behaves differently in the MS source from those of chlorinated and lower brominated compounds (de Boer and Cofino 2002); and (4) may easily adsorb to small dust particles in the laboratory, which may result in sample contamination (Covaci et al. 2003). Thermal decomposition of BDE 209 can be avoided using a short GC column and a thermally inert GC injection port. In contrast, BDE 154 usually coelutes from most gas chromatographic columns with 2,2',4,4',5,5'-hexabromobiphenyl (PBB-153). In order to ensure the separation of BDE 154 and PBB 153, analysts need to use a sufficiently long GC column. Thus, in order to accurately determine the levels of BDE 209 and BDE 154 in analytical samples, analysts are required perform two separate GC measurements under different operating conditions.

## 9.1 BIOLOGICAL MATERIALS

Methods for the determination of organobromine compounds such as PBBs and PBDEs generally consist of the following steps: extraction of the analyte from the sample matrix; cleanup to remove interfering

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compounds; and analysis (separation and quantitation). The primary method of analysis is GC coupled with ECD or MS. Analytical methods have been developed for the determination of PBBs and PBDEs in blood or serum, urine, feces, adipose tissue, liver, and breast milk. The methods for determining PBB and PBDE residues in biological samples are given in Tables 9-1 and 9-2, respectively.

***Polybrominated Biphenyls.*** Residues in biological samples can be extracted using hexane/ether, petroleum ether/diethyl ether, toluene/ethyl acetate, or methylene chloride (Burse et al. 1980; Domino et al. 1980; Fawkes et al. 1982; Fehringer 1975b; Wolff et al. 1979b). Elution of samples on a florisil column, which is used for the cleanup of extracts with petroleum ether, separates PBBs from interfering substances (Pomerantz et al. 1978). As in the case of PCBs, the solvent(s) used for the extraction of a sample and the method used for the cleanup of an extract is dependent on the sample matrix (Pomerantz et al. 1978). Quantitation is usually done by GC. The major difference between the methods for the determination of PCBs and PBBs arises from the lower volatility of PBBs compared to PCBs. Due to the lower volatility of PBBs, the GC method is performed at a higher temperature and low liquid-phase load of the stationary phase. Capillary columns are required for the separation of the individual congeners in a mixture (Robertson et al. 1983b). However, decabromobiphenyl is so nonvolatile that a very short capillary column and high carrier gas linear velocity are required, which reduces the advantage of the capillary column over the packed column (Farrell 1980). Peaks from individual congeners of PBBs are detected and quantified with ECD (Robertson et al. 1983b). In general, retention time in gas chromatographic columns and response of ECD increase with increasing bromination. PBB residues in a sample can be confirmed by thin-layer chromatography, photochemical-alteration method, halogen-specific gas-chromatographic detection, or MS (de Kok et al. 1977; Erney 1975; Pomerantz et al. 1978). High recoveries (80–90%) of PBB residues are obtained by the available analytical methods. Typically, the limit of quantitation for PBB residues is about 1 µg/kg in blood serum, 1 µg/kg in human milk, and 0.5 µg/kg in adipose tissue (Eyster et al. 1983; Wolff et al. 1979a). An interlaboratory study is available that validates the precision and accuracy of PBB residue determination in human serum by a commonly used method (Burse et al. 1980).

***Polybrominated Diphenyl Ethers.*** Residues in biological samples can be extracted using sulfuric acid, 2-propanol/hexane, methylene chloride, *n*-hexane, formic acid/2-propanol/water, or hexane/methyl *t*-butyl ether (Cramer et al. 1990; Meironyté Guvenius et al. 2001; Ohta et al. 2002a; Sellström et al. 1993; Sjödin et al. 1999; Thomsen et al. 2001b). Samples are cleaned up to remove interferences using Florisil, silica gel, alumina or activated-charcoal column chromatography, gel-permeation chromatography (GPC),

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**Table 9-1. Analytical Methods for Determining PBBs in Biological Materials**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Serum	Extract denatured sample with hexane-ethyl ether; clean up by Florisil column chromatography	GC-ECD	1 µg/L	100.6–106.8 at 100 µg/L	Burse et al. 1980
Serum	Extract denatured sample with hexane-ether; clean up by Florisil column chromatography	GC-ECD	1 ng/g	86–92	Wolff et al. 1979
Plasma	Extract denatured sample with petroleum ether-ethyl ether; clean up by Florisil and silica gel column chromatography	GC-ECD	1.0 µg/L (for hexa)	102 (for hexa)	Willet et al. 1978
Whole blood	Extract denatured sample with petroleum ether-ethyl ether; clean up by Florisil column chromatography	GC-ECD	0.7 ng/g	90–96	Domino et al. 1980
Feces	Extract sample with petroleum ether-ethyl ether; clean up by Florisil and silica gel column chromatography	GC-ECD	1.4 ng/g (for hexa)	61 (for hexa)	Willet et al. 1978
Bile	Extract denatured sample with petroleum ether-ethyl ether; clean up by Florisil and silica gel column chromatography	GC-ECD	0.08 ng/g (for hexa)	92 (for hexa)	Willet et al. 1978
Milk	Extract denatured sample with petroleum ether-ethyl ether; clean up by Florisil and silica column chromatography	GC-ECD	1.4 µg/L (for hexa)	108 (for hexa)	Willet et al. 1978
Milk, human	Extract with potassium oxalate, ethanol/diethyl ether, or hexane	GC-ECD	1 ng/g	No data	Eyster et al. 1983
Liver	Extract sample with methanol-chloroform; clean up by acidic silica column chromatography	GC-ECD	No data	70 (for hexa)	Fawkes et al. 1982
Adipose tissue	Extract sample with methylene chloride; clean up by acidic silica gel column chromatography	GC-ECD	No data	80	Fawkes et al. 1982
Adipose tissue (exposed workers)	Toluene/ethyl acetate (1+3); clean up using GPC/Bio beads	GC-ECD	0.5 ng/g	98	Wolff et al. 1979
Human tissues (post-mortem)	Extract with hexane; clean up using Florisil column	GC-ECD	0.5 ng/g	No data	Micelli et al. 1985

EC = electron capture detection; GC = gas chromatography; GPC = gel permeation chromatography; hexa = hexa-brominated biphenyl; PBBs = polybrominated biphenyls; SIM = selected ion monitoring

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**Table 9-2. Analytical Methods for Determining PBDEs in Biological Materials**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Animal tissues (muscle, fat, and egg)	Extraction with sulfuric acid; clean up with GPC/silica column/carbon column	GC-MS (NCI)	No data	No data	Sellström et al. 1993
Human adipose tissue	Soxhlet extraction; clean up using 2 solid-phase extraction cartridges	Capillary GC-EILR-MS	0.05–0.30 ng/g lipid	81–103	Covaci et al. 2002
Human adipose tissue	Extraction with methylene chloride; evaporate; clean up on silica gel followed by clean up on alumina and on a carbon/silica gel column	HRGC/HRMS	0.73–120 pg/g	No data	Cramer et al. 1990
Human liver/adipose tissue	Extract with 2-propanol/hexane; clean up with Lipidex 5000, column chromatography/GPC	GC-MS (NCI)	5 pg/g lipids	83 (54–116) liver; 71 (51–95) adipose	Meironyte Guvenius et al. 2001
Human milk	Extract with potassium oxalate/ethanol/diethyl ether/pentane; GPC; clean up on Florisil; elute with hexane	GC/MS (NCI/SIM)	<0.6 ng/g fat	No data	WHO 1994a
Human milk	Extract by column chromatography using hexane/dichloromethane/hexane; clean up using GPC	GC-MS (SIM)	5 pg/g lipids	86–102	Meironyté et al. 1999a, 1999b
Human milk	Extract with n-hexane; clean up using multi-layer column	HRGC-LRMS or LRGC-HRMS (EI-SIM)	No data	>80	Ohta et al. 2002
Human plasma	Extract with formic acid, 2-propanol, and water on a SPE column; derivatized using diazomethane	GC-MS (NCI)	1–10 pg/g plasma	72	Thomsen et al. 2001b
Human serum	Extraction with hexane/MTBE (1:1); clean up silica gel/sulfuric acid column	GC-ECD; GC-MS (NCI)	0.7 ng/g lipid weight	69–104 (low spike); 77–104 (high spike)	Sjödin et al. 1999a

ECD = electron capture detection; EI = electron impact; EILR = electron impact low-resolution; GC = gas chromatography; GPC = gel permeation chromatography; HRGC = high resolution gas chromatography; HRMS = high resolution mass spectrometry; LRGC = low resolution gas chromatography; LRMS = low resolution mass spectrometry; MS = mass spectrometry; MTBE = methyl-tert-butyl ether; NCI = negative chemical ionization; PBDEs = polybrominated diphenyl ethers; SIM = selected ion monitoring; SPE = solid phase extraction

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and/or liquid chromatography (LC) (Sellström et al. 1993; Cramer et al. 1990; Meironyté Guvenius et al. 2001; Sellström et al. 1993; Sjödin et al. 1999). Most techniques are based on analysis by GC with ECD or coupled with MS (WHO 1994a). Capillary columns and temperature programming allow the separation of the different PBDE congeners. High recoveries (69–104%) of PBDE residues are obtained by the available analytical methods. Typically, the limit of quantitation for PBDE residues is about 0.7 ng/g lipid in blood serum, 5 pg/g lipid in human milk, and 0.3 ng/g lipid in adipose tissue (Covaci et al. 2002b; Meironyté Guvenius 1999a, 1999b; Sjödin et al. 1999).

## 9.2 ENVIRONMENTAL SAMPLES

Most environmental analyses have been performed using multiresidue methods involving solvent extraction of the analytes from the sample matrix, cleanup 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 for PBBs are in the low parts-per-billion (ppb) to parts-per-trillion (ppt) range for water matrices and in the low parts-per-million (ppm) to ppb range for food; for PBDEs, detection limits are in the low ppb range for water matrices and in the low ppb to ppm range for fish tissues. Analytical methods for the determination of PBBs and PBDEs in environmental samples are given in Tables 9-3 and 9-4, respectively.

***Polybrominated Biphenyls.*** Residues in environmental samples can be extracted using hexane-ether, petroleum ether-ether, toluene-ethyl acetate, or methylene chloride (Burse et al. 1980; Domino et al. 1980; Fawkes et al. 1982; Fehring 1975b; Wolff et al. 1979b). As for biological samples, quantitation of environmental samples is also usually done by GC. Capillary columns are required for the separation of the individual congeners in a mixture (Robertson et al. 1983b). High recoveries (74–98%) of PBB residues in environmental samples are obtained by the available analytical methods. Typically, the limit of quantitation for PBB residues is about 0.1 µg/kg in soil and 0.7 µg/kg in sediment (Jacobs et al. 1976, 1978; Kuosmanen et al. 2002).

***Polybrominated Diphenyl Ethers.*** Like PCBs, air samples containing PBDEs 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, respectively (Dobber et al. 2000a; Hillery et al. 1997). The filters and adsorbents are then Soxhlet extracted with acetone/hexane, and the extracts are cleaned up and analyzed by high-resolution GC techniques.

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**Table 9-3. Analytical Methods for Determining PBBs in Environmental Samples**

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Commercial	Sample dissolved in benzene FireMaster BP-6	GC-ECD	1.6 ng (EC) GC-PED	Not applicable 2.8 ng (PED)	Mulligan et al. 1980
Soil	Extract sample with hexane- acetone; clean up by Florisil column chromatography	GC-ECD	0.1 ng/g	74.2–83.2 (for hexa)	Jacobs et al. 1976, 1978
Soil	Extraction using hexane/acetone; clean up using Florisil column	GC- FID/ECD	No data	No data	Hill et al. 1982
Plant tissue	Extract macerated sample with hexane-acetone; clean up by Florisil column chromatography	GC-ECD	0.3 ng/g	No data	Jacobs et al. 1978
Effluent and river water	Extract sample with hexane-ethyl ether	GC-ECD	0.1 ng/g	90	Hesse and Powers 1978
Sediment	Extract sample with hexane-acetone	GC-ECD	No data	No data	Hesse and Powers 1978
Sediment	Pressurized hot water extraction coupled with clean up by LC	LC-GC-MS/ FID	0.71 ng/g	No data	Kuosmanen et al. 2002
Fish	Extract homogenized sample with hexane-water; clean up by acidic and basic silica columns	GC-ECD	No data	98 (for hexa)	Gobas et al. 1989
Fish	Extract homogenized sample with hexane-methylene chloride; clean up by gel permeation and silica gel chromatography	HRGC- HRMS	No data	No data	Kuehl et al. 1991
Fish	Extract homogenized sample with hexane-acetone; clean up by gel permeation chromatography	HRGC-MS/ NCI and HRGC-ECD	No data	No data	Jaffe et al. 1985
Terrestrial, fresh water, and marine samples	Extraction with diethyl ether/hexane; hydrolysis with 98% sulfuric acid/bio beads/silica gel/activated charcoal	MS (NCI)	No data	No data	Jansson et al. 1991, 1993
Dolphin fat	Soxhlet extraction using hexane- methylene chloride; clean up using GPC, silica gel	MS	No data	No data	Kuehl et al. 1991
Animal feeds	Elute ground sample containing celite with methylene chloride; clean up by Florisil column chromatography	GC-ECD	8 ng/g (for hexa)	98 (for hexa)	Fehringier 1975b
Dairy products	Fat extracted by methanol/ether; clean up by GPC, 25% toluene in ethyl acetate	GC-ECD	7 ng/g	No data	Fehringier 1975
Plants	Cut, extracted with hexane/acetone; clean up with Florisil column	GC-ECD	0.3 ng/g wet basis	No data	Chou et al. 1978

EC = electron capture detection; FID = flame ionization detector; GC = gas chromatography; hexa = hexabrominated biphenyl; HRGC = high resolution gas chromatography; HRMS = high resolution mass spectrometry; LC = liquid chromatography; MS = mass spectrometry; NCI = negative chemical ionization; PED = plasma emission detection; PBBs = polybrominated biphenyls



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**Table 9-4. Analytical Methods for Determining PBDEs in Environmental Samples**

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Air pumped through glass fiber filter and adsorbent trap; filters and adsorbents are Soxhlet extracted with acetone/hexane; cleaned-up by column chromatography	GC/MS	No data	No data	Dodder et al. 2000a
Water	Clean up by disk-type C18 solid-phase extraction	Capillary GC-ECD	0.12 pg/L	103±8.6 (river water); 87±10.7 (sea water)	Yamamoto et al. 1997
Sewage	Extract with chloroform; evaporate and dissolve residue in ethanol	GC/MS	0.06 µg/g	No data	WHO 1994a
Sediment	Clean up by cartridge-type Florisil extraction	Capillary GC-ECD	9.7 ng/g	91±6.3	Yamamoto et al. 1997
Sediment	Pressurized hot water extraction coupled with clean up by LC	LC-GC-MS/FID	0.71 ng/g	No data	Kuosmanen et al. 2002
Sediment	Extract with acetone; clean up on Florisil	NAA; GC/EC	<5 ng/g; <5 ng/g	No data	Watanabe et al. 1987
Fish	Extract with acetone-hexane + hexane-ethyl ether; treatment with sulfuric acid or clean up on alumina; chromatography on silica gel	GC/EC; GC/MS	0.1 µg/g fat	No data	Anderson and Blomkvist 1981
Fish	Extract with dichloromethane on chromatography column; clean-up using GPC; fractionation using silica gel column	GC-HRMS (NCI)	5–93 pg/g	No data	Alaee et al. 2001
Fish	Extract clean up with GPC and mini-column chromatography; concentration	GC-MS (NCI)	0.01–0.2 ng/g lipid	88–128	Akutsu et al. 2001
Animal tissues	Homogenize; extract with n-hexane-acetone; treatment with sulfuric acid; GPC; chromatography on silica gel chromatography or activated charcoal	GC/MS (NCI)	10 pg/g	No data	Jansson et al. 1991

ECD = electron capture detection; GC = gas chromatography; GPC = gel permeation chromatography; HRMS = high resolution mass spectrometry; LC = liquid chromatography; MS = mass spectrometry; NAA = neutron activation analysis; NCI = negative chemical ionization; PBDEs = polybrominated diphenyl ethers

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Residues in environmental samples can be extracted using chloroform, acetone, acetone-hexane, hexane-acetone, and hexane-ether (Anderson and Blomkvist 1981; Jansson et al. 1991; Watanabe et al. 1987; WHO 1994a). Samples are cleaned up to remove interferences using Florisil, silica gel, alumina or activated charcoal column chromatography, gel permeation chromatography (GPC), and/or liquid chromatography (LC) (Akutsu et al. 2001; Alaei et al. 2001; Anderson and Blomkvist 1981; Jansson et al. 1991; Watanabe et al. 1987; Yamamoto et al. 1997). As for biological samples, quantitation of environmental samples is also usually done by GC. Capillary columns are required for the separation of the individual congeners in a mixture (WHO 1994a). High recoveries (88–128%) of PBDE residues in environmental samples are obtained by the available analytical methods (Akutsu et al. 2001). Typically, the limit of quantitation for PBDE residues is about 0.12 ng/mL in water, 9.7 µg/kg in sediment, and 0.2 µg/kg lipid in fish (Akutsu et al. 2001; Yamamoto et al. 1997). The first inter-laboratory study on PBDEs in environmental samples showed that there is good agreement for quantification of BDE 47 and 2,2',4,4',6-pentabromodiphenyl ether (BDE 100) congeners. However, improved methods are required for analysis of 2,2',4,4',5-pentabromodiphenyl ether (BDE 99), 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE 153), 2,2',4,4',5,6'-hexabromodiphenyl ether (BDE 154), and BDE 209 congeners (de Boer 2000).

### 9.3 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 PBBs and PBDEs 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 PBBs and PBDEs.

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.

### 9.3.1 Identification of Data Needs

#### Methods for Determining Biomarkers of Exposure and Effect.

**Exposure.** Methods used as biomarkers for exposure to PBBs and PBDEs are available (Brilliant et al. 1978; Covaci et al. 2002b; Eyster et al. 1983; Landrigan et al. 1979; Meironyté Guvenius 1999a, 1999b; Sjödin et al. 1999; Wolff et al. 1982). Analytical methods of sufficient precision and accuracy are presently available for the determination of PBBs and PBDEs in adipose tissue, serum, and breast milk (Burse et al. 1980; Covaci et al. 2002b; Domino et al. 1980; Fawkes et al. 1982; Fehring 1975a; Meironyté Guvenius 1999a, 1999b; Sjödin et al. 1999; Willet et al. 1978; Wolff et al. 1979a, 1979b). Additional congener standards are needed for PBB and PBDEs analysis. Only 30–40 congener standards are currently available for identification and quantification of PBDEs (Eljarrat et al. 2002; Sjödin et al. 1998). Metabolites are also important biomarkers for exposure to PBBs and PBDEs. However, these compounds are mostly unknown, and standards are not available.

**Effect.** No studies have been conducted to determine if known effects of PBBs and PBDEs exposure can be quantitatively correlated with PBB or PBDE exposure.

#### Methods for Determining Parent Compounds and Degradation Products in Environmental

**Media.** Analytical methods of sufficient sensitivity are presently available for the determination of PBBs and PBDEs in environmental samples (Akutsu et al. 2001; Anderson and Blomkvist 1981; Covaci et al. 2003; Fehring 1975b; Hesse and Powers 1978; Jacobs et al. 1976, 1978; Yamamoto et al. 1997).

It would be helpful to develop data determining the detection limit and accuracy of PBBs determinations in fish and other aquatic animals (e.g., seals) and in sediment (Gobas et al. 1989; Jaffe et al. 1985; Kuehl et al. 1991). Analytical methods for determining lower brominated PBBs in environmental samples are available (Morris et al. 1992). An analytical method to determine PBB metabolites in fish would be helpful. A method for determining of 6-hydroxy-2,2',4,4',5,5'-hexabromobiphenyl, a metabolite of 2,2',4,4',5,5'-hexabromobiphenyl, in dog feces is available (Gardner et al. 1979). Photochemical degradation leads to the formation of lower brominated products, which are the only environmental degradation products identified for PBBs. Analytical methods are presently available for the determination of these compounds in environmental samples (De Kok et al. 1977; Hill et al. 1982; Robertson et al. 1983b). There is no evidence in the literature of detectable biodegradation of PBBs in the environment under aerobic conditions (Griffin and Chou 1981a, 1981b), but the compounds may

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biodegrade to debrominated products under anaerobic conditions in polluted environments (Morris et al. 1992).

It would be helpful to develop data determining the accuracy of PBDE determinations (e.g., percent recovery) in environmental samples. Methods for determining degradation products and metabolites of PBDE are needed. There is no information in the literature of detectable biodegradation of PBDEs in the environment under aerobic or anaerobic conditions. The analysis of PBDE pyrolysis degradation products, such as polybrominated dibenzo-*p*-dioxins and dibenzofurans (PBDD/Fs), is often disturbed by the presence of PBDEs. Ebert et al. (1999) demonstrated that by using a Florisil column in a sample clean-up process, almost complete separation of PBDEs and PBDDs/PBDFs is achieved before analysis by GC-MS.

### 9.3.2 Ongoing Studies

No ongoing studies regarding analytical methods for determining PBBs and PBDEs residues or metabolites were located as a result of a search of the Federal Research in Progress Database (FEDRIP 2003).