7. 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) , its metabolites, and other biomarkers of exposure and effect to PBBs. 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 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 and the primary analytical technique was gas chromatography-electron capture detection (GC-ECD) with packed colums (i.e., noncongener specific).

Covaci et al. (2003) reviewed the determination of brominated flame retardants in environmental and human samples. The analysis methodology for PBBs 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. This practice will minimize the influence of trace contamination samples that can originate from a variety of sources.

Most sample collections are by grab sampling; however, PBBs 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 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 extractions 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_2 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. 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 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 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 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 congeners.

7.1 BIOLOGICAL MATERIALS

Methods for the determination of organobromine compounds such as PBBs generally consist of the following steps: extraction of the analyte from the sample matrix; cleanup to remove interfering 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 in blood or serum, urine, feces, adipose tissue, liver, and breast milk. The methods for determining PBB residues in biological samples are given in Table 7-1.

Table 7-1. 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 chromato-graphy	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. 1979a
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

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).

7.2 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. Analytical methods for the determination of PBBs in environmental samples are given in Table 7-2.

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; Fehringer

Table 7-2. 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 applic-able 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)	Fehringer 1975b
Dairy products	Fat extracted by methanol/ether; clean up by GPC, 25% toluene in ethyl acetate	GC-ECD	7 ng/g	No data	Fehringer 1975a
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

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).

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 PBBs 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.

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.

Exposure. Methods used as biomarkers for exposure to PBBs 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 in adipose tissue, serum, and breast milk (Burse et al. 1980; Covaci et al. 2002b; Domino et al. 1980; Fawkes et al. 1982; Fehringer 1975a; Meironyté Guvenius 1999a, 1999b; Sjödin et al. 1999; Willet et al. 1978; Wolff et al. 1979a, 1979b). Additional congener standards are needed for PBBs analysis. Metabolites are also important biomarkers for exposure to PBBs. However, these compounds are mostly unknown, and standards are not available.

Effect. No studies have been conducted to determine if known effects of PBBs exposure can be quantitatively correlated with PBB 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 in environmental samples (Akutsu et al. 2001; Andersson and Blomkvist 1981; Covaci et al. 2003; Fehringer 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 biodegrade to debrominated products under anaerobic conditions in polluted environments (Morris et al. 1992).

7.3.2 Ongoing Studies

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