7. ANALYTICAL METHODS

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

PBDEs are analyzed in environmental and biological samples by methods quite similar to those used for PCBs (de Kok et al. 1977; Fries 1985b; Pomerantz et al. 1978). The analytical methods for PBDEs were developed relatively recently. There have been many advances in the technology and costs of analytical instruments used in the efforts directed at PBDE analysis. GC/MS with capillary columns (i.e., congener specific) is the primary analytical technique now used for PBDEs.

Covaci et al. (2003) and Stapleton (2006) reviewed the determination of brominated flame retardants, with emphasis on PBDEs in environmental and human samples. The analysis methodology for 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 concentrations of PBDEs. 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, 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).

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 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 with solid-phase trapping has been used for the extraction of 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 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 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 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 PBDEs are required. Historically, flame-ionization detectors (FID) or electron-capture detectors (ECD) were used. However, the MS detectors have become the main detection tool for PBDEs. MS detectors have selectivity for 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 PBDE congeners. One method of detection is ECNI as an ionization technique in combination with GC/MS analysis (de Boer et al. 2000a). This method is advantageous because it offers a high sensitivity for compounds with four or more bromine atoms. 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}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 bromine 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 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'-tetraBDE or BDE 47) for other members of its homolog group (e.g., tetraBDEs). This can result in either under- or overestimating concentrations of congeners for which analytical standards are not available (Ikonomou and Rayne 2002). In general, hepta- through decaBDE congeners are difficult to determine accurately by GC analysis, especially in biological samples (Ikonomou and Rayne 2002).

The analysis of BDE 209 and 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 (Beser et al. 2014). In contrast, BDE 154 usually co-elutes 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 concentrations of BDE 209 and BDE 154 in analytical samples, analysts are required perform two separate GC measurements under different operating conditions. The difficulties in the determination of BDE 209 have been addressed through the use of liquid chromatography (LC)/MS/MS (Abdallah et al. 2009). The analytes, BDE 47, BDE 85, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183, BDE 196, BDE 197, BDE 203, BDE 206, BDE 207, BDE 208, and BDE 209, were separated and ionized in a single run using an isotope dilution method for indoor dust samples.

7.1 BIOLOGICAL MATERIALS

Methods for the determination of organobromine compounds such as PBDEs 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 MS. Analytical methods have been developed for the determination of PBDEs in blood or serum, urine, feces, adipose tissue, liver, breast milk, and hair. The methods for determining PBDE residues in biological samples are provided in Table 7-1.

Table 7-1. Analytical Methods for Determining Polybrominated Diphenyl Ethers (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
Animal serum	Extraction with CH_2Cl_2 in hexane; H_2SO_4 to remove lipids; washed with NaOH followed by distilled water, then dehydrated through anhydrous Na_2SO_4	ELISA (specific for BDE 27)	0.2 µg/L	82–138	Ahn et al. 2009
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	Meironyté 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. 1999
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. 2001
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

Table 7-1. Analytical Methods for Determining Polybrominated Diphenyl Ethers (PBDEs) in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human serum	Extraction with ethyl acetate, evaporated to dryness, and dissolved in DMSO	ELISA (specific for BDE 27)	0.2 µg/L	83–90	Ahn et al. 2009
Human serum	Denaturation with formic acid followed by SPE	GC/MS	140–1,300 pg/g (lipid weight)	40–106	Butt et al. 2016
Human hair	Physical extraction followed by washing with 0.3% polyoxyethylene lauryl ether and rinse with tap and distilled water; extraction using hexane/ dichloromethane	GC/MS	0.02 ng/g	No data	Malarvannan et al. 2013
Human hair	Extraction with 4 N HCl and hexane (4:1); clean up on $NaSO_4$/Florisil SPE columns (1:1); elute with hexane	GC/MS (SIM)	0.025 pg/mg hair (lower-brominated) 2.5 pg/mg hair (BDE 209)	70–90	Aleksa et al. 2012a

BDE = brominated diphenyl ether; DMSO = dimethyl sulfoxide; ECD = electron capture detection; EI = electron impact; EILR = electron impact low-resolution; ELISA = enzyme-linked immunosorbent assay; 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 either; NCI = negative chemical ionization; SIM = selected ion monitoring; SPE = solid phase extraction

Determination of hydroxylated PBDEs metabolites were reported by Malmberg et al. (2005). The methylated derivatives of PBDE-OH metabolites were identified by GC-MS ECNI and EI analysis after extraction from the sample matrix, cleanup to remove interfering compounds, and methylation of the hydroxyl group. In plasma samples, the internal standard, BDE 138, had 71 and 98% recovery (relative standard deviation, 8 and 2%) on days 1 and 5 after dosing, respectively.

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. 2002; Sellström et al. 1993; Sjödin et al. 1999a; Thomsen et al. 2001). Samples are cleaned up to remove interferences using Florisil, silica gel, alumina or activated-charcoal column chromatography, gel permeation chromatography (GPC), and/or LC (Cramer et al. 1990; Meironyté Guvenius et al. 2001; Sellström et al. 1993; Sjödin et al. 1999a). Most techniques are based on analysis by GC 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, 0.3 ng/g lipid in adipose tissue, and 0.025 pg/mg in hair (Aleksa et al. 2012a; Covaci et al. 2002; Meironyté Guvenius et al. 1999; Sjödin et al. 1999a). Additionally, a selective competitive enzyme-linked immunosorbent assay (ELISA) has been developed to detect BDE 47 (Ahn et al. 2009). This method also reports high recoveries (83–90%) and a limit of quantitation for BDE 47 in blood of 0.2 µg/L.

7.2 ENVIRONMENTAL SAMPLES

Most environmental analyses have been performed using multi-residue methods involving solvent extraction of the analytes from the sample matrix, cleanup to remove interfering compounds, determination by GC with confirmation using MS. New methods and technologies are evolving, and this has resulted in lower detection limits. Analytical methods for the determination of PBDEs in environmental samples are given in Table 7-2.

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 (Dodder et al. 2000; 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. Beser et al. (2014) discussed a GC/MS method to quantify PBDEs that used microwave-

Table 7-2. Analytical Methods for Determining Polybrominated Diphenyl Ethers (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. 2000
Air	Samples collected using a large-volume active sampler at 30 m^3/hour for 24 hours onto filters followed by microwave assisted extraction using hexane/acetone.	GC/MS	0.063–0.210 pg/m^3	80–120%	Beser et al. 2014
Air	Passive samplers set out with PUF disks; PUF disks were collected at 10-day intervals over 50 days; active samplers had low volume pump with PUF plugs housed in a glass holder; solvent extraction (collected hexane layer); washed with H_2SO_4 back extraction using dimethyl sulfoxide followed by column elution containing Florisil, hexane, and anhydrous Na_2SO_4	GC/MS	No data	41–78% (passive samplers) 42–80% (active samplers)	Hazrati and Harrad 2007
Dust	Samples collected from vacuum cleaner bags; extracted using hexane by an accelerated solvent extraction system, concentrated, treated with H_2SO_4, liquid/liquid back extraction using dimethyl sulfoxide, column elution	GC/MS	0.03 ng/g	No data	Harrad et al. 2006
Dust	Samples collected from vacuum cleaner bags; extracted using microwave-assisted solvent extraction (collected hexane layer); washed with H_2SO_4, then deionized water, and dried with anhydrous Na_2SO_4	ELISA (specific for BDE 47)	0.2 µg/L	105±22.7	Ahn et al. 2009

Table 7-2. Analytical Methods for Determining Polybrominated Diphenyl Ethers (PBDEs) in Environmental Samples

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Clean up by GFF followed by PUF plugs; extraction with dichloromethane	GC/MS	0.2–1.4 pg/L	No data	Yang et al. 2014
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	Soxhlet extraction acetone:hexane (1:1, v/v), clean-up with liquid-liquid extraction with fuming sulfuric acid, GPC and silica gel column, and a basic alumina column	HRGC/MS	No data	No data	Zennegg et al. 2013
Sewage	Extract with chloroform; evaporate and dissolve residue in ethanol	GC/MS	0.06 µg/g	No data	WHO 1994a
Sediment	Extract with hexane and dichloromethane (1:1 v/v), treat with copper, sulfonate with sulfuric acid, clean and fraction using neutral, acid, and alkaline silica gel chromatography	GC/MS	1.93–227 pg/g	88.8−138	Tang et al. 2014
Sediment	Clean up by cartridge-type Florosil 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 dichloromethane:n-hexane (1:1, v/v) column chromatography on silica and Al_2O_3	GC/MS	No data	58–106	Yang et al. 2008
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	Andersson and Blomkvist 1981

Table 7-2. Analytical Methods for Determining Polybrominated Diphenyl Ethers (PBDEs) in Environmental Samples

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Fish	SE, ASE, and MAE performed followed by evaporation to dryness; dissolved in n-hexane; added silica gel, column filtered; concentrated; silica gel column followed by a basic alumina column eluted with n-hexane/ dichloromethane (1:1, v/v)	HRGC/ HRMS	24.8 pg/g	79–118 (SE) 50–96 (MAE)	Wang et al. 2010
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 chromato-graphy; 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 or silica gel chromatography or activated charcoal	GC/MS (NCI)	10 pg/g	No data	Jansson et al. 1991
Vegetables	Homogenize; MAE; extract clean up with Florisil or silica cartridge; elute with n-hexane:toluene (80:20, v/v)	GC/MS	1-3 ng/g	99–106	Bizkarguenaga et al. 2014
Vegetables	Extract with acetone/ n-hexane (1:1 v/v)	GC/MS (ion trap)	1 ng/kg dry weight	82–98 (mean)	Parolini et al. 2012

ASE = accelerated solvent extraction; BDE = brominated diphenyl ether; EC = electron capture; ECD = electron capture detection; ELISA = enzyme-linked immunosorbent assay; GC = gas chromatography; GFF = glass fibre filter; GPC = gel permeation chromatography; HRGC = high resolution gas chromatography; HRMS = high resolution mass spectrometry; LC = liquid chromatography; MAE = microwave-assisted extraction; MS = mass spectrometry; NAA = neutron activation analysis; NCI = negative chemical ionization; PUF = polyurethane foam; SE = soxhlet extraction

assisted extraction (MAE) rather than the traditional Soxhlet extraction technique in order to shorten the extraction time and quantity of solvents used. For a sampling rate of 30 m^3 per hour over a sampling duration of 24 hours, they achieved low limits of quantification (0.063 pg/$m^3$for BDE 28, BDE 49, BDE 47, BDE 66, and BDE 100; 0.105 pg/m^3 for BDE 119 and BDE 99; and 0.210 pg/m^3 for BDE 155, BDE 154, BDE 153, BDE 139, and BDE 183). Sampling of PBDEs is also performed using passive or diffusive samplers (Covaci et al. 2003). Hazrati and Harrad (2007) describe passive sampling with polyurethane foam disks (PUF) of BDE 28, BDE 47, BDE 99, and BDE 100 with mean recoveries of 57 and 62% for passive and active samplers, respectively. Harrad and Hunter (2006) performed passive air sampling with PUF disks in the United Kingdom. BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, and BDE 154 were detected using GC/MS. The detection limit for individual BDEs were approximately 0.05 pg/m^3.

Passive air sampling techniques have been developed to monitor both vapor- and particulate-phase PBDEs in indoor air through the use of a PUF disk and glass fiber filter (GFF) sampling media (Abdallah 2010). The PUF disks and GFFs were evaluated independently by initial soxhlet extraction with dichloromethane, concentration of extracts, and purification with SPE cartridge. Elution with hexane:dichloromethane (1:1, v/v), evaporation, reconstitution in methanol, and analysis using LC/MS/MS followed. BDE 47, BDE 85, BDE 99, BDE 100, BDE 153, BDE 154, BDE 183, BDE 196, BDE 197, BDE 203, BDE 206, BDE 207, BDE 208, and BDE 209 were evaluated using this technique. The octaBDE to decaBDE analytes were detected on the GFF media only, indicating that these congeners are expected to primarily be found in the particulate phase.

Residues in environmental samples can be extracted using chloroform, acetone, acetone-hexane, hexane-acetone, and hexane-ether (Andersson 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, GPC, and/or LC (Akutsu et al. 2001; Alaee et al. 2001b; Andersson and Blomkvist 1981; Jansson et al. 1991; Watanabe et al. 1987; Yamamoto et al. 1997). Vegetable and soil samples have been prepared for analysis using, focused ultrasound solid-liquid extraction (Bizkarguenaga et al. 2014).

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 BDE 100 congeners. Additionally, a selective competitive ELISA has been developed to detect BDE 47 in dust (Ahn et al. 2009). This method also reports high recoveries (105%) and a limit of quantitation for BDE 47 in blood of 0.2 µg/L.

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 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 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.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. Methods used as biomarkers for exposure to PBDEs are available (Ahn et al. 2009; Aleksa et al. 2012a; Brilliant et al. 1978; Covaci et al. 2002; Eyster et al. 1983; Landrigan et al. 1979; Meironyté Guvenius et al. 1999; Sjödin et al. 1999a; Wolff et al. 1982). Analytical methods of sufficient precision and accuracy are presently available for the determination of PBDEs in adipose tissue, serum, breast milk, and hair (Ahn et al. 2009; Aleksa et al. 2012a; Burse et al. 1980; Covaci et al. 2002; Domino et al. 1980; Fawkes et al. 1982; Fehringer 1975a; Meironyté Guvenius et al. 1999; Sjödin et al. 1999a; Willet et al. 1978; Wolff et al. 1979a, 1979b). Additional congener standards are needed for 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 PBDEs. Ryden et al. (2012) discussed a GC/MS method for the analysis of hydroxylated PBDE metabolites in

human blood. GC/MS has been used to identify hydroxylated-BDE metabolites from recombinant cytochrome P450 by Simpson et al. (2015), and Gross et al. (2015) used GC/MS and GC/MS/MS.

Effect. No studies have been conducted to determine if known effects of PBDEs exposure can be quantitatively correlated with 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 PBDEs in environmental samples (Akutsu et al. 2001; Andersson and Blomkvist 1981; Covaci et al. 2003; Stapleton 2006; Yamamoto et al. 1997).

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 PBDD/PBDF, 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.

7.3.2 Ongoing Studies

Analysis of PBDEs and other anthropogenic pollutants in marine mammals by a novel application of comprehensive two-dimensional gas chromatography with time- of-flight mass spectrometry (GCxGC/TOF-MS) is being studied at the University of California, San Diego (RePORTER 2016).