ICES TECHNIQUES IN MARINE ENVIRONMENTAL SCIENCES

NO. 46

NOVEMBER 2009

# Determination of polybrominated diphenyl ethers (PBDEs) in sediment and biota

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Our cover photo was taken by N. Penny Holliday aboard the RRS "Discovery" in rough seas in the Rockall Trough.

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Recommended format for purposes of citation:

Webster, L., Tronczynski, J., Bersuder, P. Vorkamp, K., and Lepom, P. 2010. Determination of polybrominated diphenyl ethers (PBDEs) in sediment and biota. ICES Techniques in Marine Environmental Sciences No. 46. 16 pp.



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ISBN 978-87-7482-075-8

ISSN 0903–2606

## Contents

## Abstract

**L. Webster, J. Tronczynski, P. Bersuder, K. Vorkamp, and P. Lepom**

This document provides advice on the analysis of polybrominated diphenyl ethers (PBDEs) in biota and sediment. The determination of PBDEs in sediment and biota generally involves extraction with organic solvents, clean-up, and gas chromatographic separation with mass-spectrometric detection. All stages of the procedure are susceptible to insufficient recovery and/or contamination. Therefore, quality-control procedures are important to check the method's performance. These guidelines are intended to encourage and assist analytical chemists to reconsider their methods and to improve their procedures and/or the associated quality-control measures where necessary.

**Keywords**: polybrominated diphenyl ether, sediment, biota, sample pretreatment, storage, extraction, clean-up, calibration, gas chromatography, mass spectrometry.

# 1 Introduction

Polybrominated diphenyl ethers (PBDEs) constitute a group of additive flame retardants predominately found in electrical equipment, textiles, and furniture. PBDEs are used as additives to polymers and resins and are thought to be released more easily into the environment than reactive flame retardants. PBDEs consist of two phenyl rings, connected by an ether bridge, each ring containing up to five bromine atoms. There are a possible 209 PBDE congeners, depending on the position and number of bromines, with molecular weights ranging from 249 to 960 Da. Congeners are named according to the International Union of Pure and Applied Chemistry (IUAPAC) numbering format, developed for chlorobiphenyl (CB) congeners. However, PBDE technical mixtures used as flame retardants contain only a limited number, ~20, of these congeners. Commercial PBDE mixtures are classified according to the degree of bromination. The penta-mix contains mainly tetra- to hexa-BDEs, the octa-mix mainly hexa- to octa-BDEs, and the deca-mix contains mainly deca-BDE. Penta-BDE is primarily used in furniture and upholstery, octa-BDE in plastics, and deca-BDEs in textiles and polymers. In the European Union (EU), a restriction on the use of the penta- and octa- technical mixtures was put in place on 15 August 2004, limiting the use of the penta- and the octa-technical mixtures to 0.1% by mass for all articles placed on the market[1]. An EU ban on the use of deca-BDE in electronics and electrical equipment became effective on 1 July 2008.

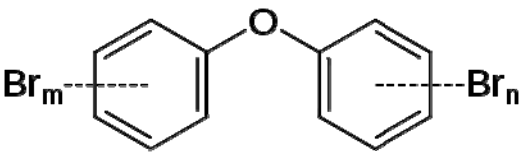


**Figure 1. Chemical structure of PBDEs**

PBDEs may be released into the environment during their production, while manufacturing other products, and during disposal of products containing these chemicals. In addition, PBDEs may leak out of treated material during everyday use of ordinary consumer products. This release has been documented by high PBDE concentrations in the indoor environment. PBDEs have been found to concentrate in the Arctic and bioaccumulate in native animals and humans (de Wit *et al*., 2006), indicating long-range atmospheric transport of PBDEs. Other possible pathways to the marine environment include direct discharge from point sources, such as storm waters and wastewater.

Because PBDEs and CBs have similar structures, PBDEs are expected to persist in the marine environment and exhibit similar toxic properties. PBDEs have high octanol-water partition coefficients ranging from 4.3 for di-BDE to 10.33 for deca-BDE (Table 1 in Section 4.8). PBDEs are hydrophobic, consequently tending to associate with particulate material and accumulate in sediment, particularly sediment with high organic carbon content. PBDEs are readily taken up by marine animals, both across gill surfaces and from their diet. They may bioaccumulate in lipid-rich tissues and biomagnify along the food chain (Law *et al*., 2006, 2008).

[1] European Directive 2003/11/EC, 24th amendment of 76/769/EEC.

## 2 Appropriate species for monitoring of PBDEs in the marine environment

For the Northeast Atlantic area, guidance on the selection of appropriate species for contaminant monitoring is given in the OSPAR Joint Assessment and Monitoring Programme (JAMP) guidelines (OSPAR Commission, 1999). Other species, such as sole (*Solea solea*), hake (*Merluccius merluccius*), and oysters (*Ostrea* sp. or *Crassostrea* sp.), may also be appropriate. The HELCOM COMBINE Manual[2] provides advice on appropriate species for monitoring programmes for the Baltic Sea. Existing data indicate that PBDE concentrations for shellfish are very low; therefore, detecting long-term trends may be difficult using these species. High trophic-level organisms and lipid rich tissue, such as fish liver, will accumulate higher levels of PBDEs and, therefore, may be more suitable for temporal trend monitoring.

[2] http://www.helc om.fi/groups/monas/Combine Manual/en_GB/main/

# 3 Pretreatment and storage

## 3.1 Contamination

Sample contamination may occur during sampling, sample handling, pretreatment, and analysis, owing to the environment, the containers, or packing materials used, the instruments used during sample preparation, and from the solvents and reagents used during the analytical procedures. Controlled conditions are therefore required for all procedures, including the dissection of fish organs on board ship. Owing to their widespread use in electrical equipment, plastic materials, textiles, etc., PBDEs may be present in materials and equipment used for sample handling and treatment. It is important that likely sources of contamination are identified and steps taken to preclude sample handling in areas where contamination could occur. One way of minimizing the risk, is to conduct dissection in a clean area, such as within a laminar-flow hood, away from the deck areas of the vessel, and to avoid contact with potentially PBDE-containing materials and products, such as computers and packing materials.

Plastic materials, except polyethylene or polytetrafluorethene, must not be used for sampling, owing to the possible adsorption of contaminants into the container material. Samples should be stored in solvent-washed aluminium cans or glass jars preferably prebaked at 450 °C. Aluminium cans are better, because glass jars are more susceptible to breakage. Sediment samples should be transported in closed containers; a temperature of 25 °C should not be exceeded. If samples are not analysed within 48 h after sampling, they must be stored for the short term at 4 °C. Storage over several months should be limited to frozen (<−20 °C) and dried samples. Biota samples should be kept cool or frozen (−20 °C or lower) as soon as possible after collection. Live mussels should be transported in closed containers, at temperatures between 5 °C and 10 °C.

## 3.2 Shellfish

### 3.2.1 Depuration

Depuration of shellfish may be required to void the gut contents of any associated contaminants before freezing or sample preparation. Depuration is usually applied to shellfish collected close to point sources where the gut contents may contain significant quantities of PBDEs associated with food and sediment particles not assimilated into the tissues of the mussels. Depuration should be undertaken in controlled conditions and in filtered water taken from the sampling site; depuration over a period of 24 h is usually sufficient. The aquarium should be aerated and temperature controlled.

### 3.2.2 Dissection and storage

Mussels should be shucked, live if possible, and opened with minimal tissue damage, by detaching the adductor muscles from the interior of at least one valve. The soft tissues should be removed and homogenized as soon as possible, then frozen in glass jars, prebaked at 450 °C or solvent washed, or in solvent-washed aluminium tins at −20 °C until analysis. When samples are processed, both at sea and onshore, the dissection must be undertaken by trained personnel on a clean bench, wearing clean gloves, and using clean stainless steel knives and scalpels. Stainless steel tweezers are recommended for holding tissues during dissection. After each sample has been prepared, all tools and equipment, such as homogenizers, should be cleaned by

wiping down with tissue and washing with solvent. Knives should only be sharpened using steel, to prevent contamination of the blade from the oils used to lubricate sharpening blocks.

## 3.3 Fish

### 3.3.1 Dissection and storage

Ungutted fish should be wrapped individually in suitable material, for example, solvent-washed aluminium foil, and stored at <−20 °C. If plastic bags or boxes are used, they should be used as outer containers only, and should not come into contact with tissues. Organ samples, for example, liver, should be stored in solvent-washed containers made of glass, stainless steel, or aluminium, or they should be wrapped in solvent-washed aluminium foil. The individual samples should be clearly and indelibly labelled and stored together in a suitable container at −20 °C until analysis. If the samples are to be transported during this period, for example, from ship to laboratory, the samples should not be allowed to thaw out during transport.

When samples are processed, both at sea and onshore, the dissection must be undertaken by trained personnel on a bench previously washed with detergent, for example, Decon 90, wearing clean gloves and using solvent-washed stainless steel knives and scalpels. Stainless steel tweezers are recommended for holding tissues during dissection. After each sample has been prepared, all tools and equipment (such as homogenizers) should be cleaned by wiping with tissue and rinsing with solvent.

### 3.3.2 Subsampling

When sampling fish muscle, care should be taken that no epidermis or subcutaneous fatty tissue be included in the sample. Samples should be taken underneath the red muscle layer. To ensure uniformity, the right-side dorso-lateral muscle should be sampled. If possible, the entire right-side dorso-lateral fillet should be homogenized and subsamples taken for replicate PBDE determinations. If, however, the amount of material to be homogenized is too large, a specific portion of the dorsal muscle should be chosen. It is recommended that the portion of the muscle lying directly under the first dorsal fin be used in this case.

When dissecting the liver, care should be taken to avoid contamination from the other organs. If bile samples are to be taken, they should be collected first. If the whole liver is not to be homogenized, a specific portion should be chosen to ensure comparability. When pooling of tissues, for example, liver or muscle, is necessary, an equivalent quantity of tissue should be taken from each fish, for example, 10% from each whole fillet.

## 3.4 Sediment

PBDEs should be extracted from dried samples, because storage, homogenization, and extraction are much easier when the samples are dry. Chemical drying can be done by grinding with $Na_2SO_4$ or $MgSO_4$ until the sample reaches a free-flowing consistency. It is essential that at least several hours elapse between grinding and extraction, to allow for complete dehydration of the sample; residual water can decrease extraction efficiency. Freeze-drying is a popular technique for sediment; however, possible losses of analyte or contamination must be checked. Losses through evaporation are diminished by keeping the temperature in the evaporation chamber below 0 °C. Contamination during freeze-drying is reduced by putting a lid,

with a hole of ca. 3 mm in diameter, on the sample container. Before taking a subsample for analysis, the samples should be thoroughly mixed using a metal spatula. Freeze-dried samples should be stored at room temperature and wet sediment frozen at −20 °C or below. More information is provided in the JAMP guidelines for monitoring contaminants in sediment (OSPAR Commission, 1999).

# 4 Analysis

## 4.1 Precautionary measures

Special precautions are required in the laboratory when analysing PBDEs owing to their sensitivity to UV light. PBDEs are prone to photolytic degradation; if exposed to UV light, debromination can occur, especially for BDE209 (Covaci *et al*., 2003; de Boer and Wells, 2006). Therefore, light coming into the laboratory should be minimized by placing UV filters on the windows and over fluorescent lighting, or by not using any artificial lighting within the laboratory. It is recommended that all calibration and spiking standards be prepared and stored in amber glassware.

The use of plastics, in the laboratory as well as during sampling, should be avoided, because they can contain PBDEs. BDE209 can stick to glassware or any other chemically active sites. This can result in contamination of glassware. BDE209 can adsorb onto dust particles, thus being a source of contamination in the laboratory. Therefore, it is recommended that an ionizer be placed in the laboratory and the laboratory be kept as dust free as possible. PBDE-containing computers and packing materials should be avoided in the laboratory. Heating of glassware in an oven, for example, at 450 °C overnight, can also be useful for removing PBDE contamination. In addition, all glassware should be covered with aluminium foil and stored in cupboards to keep out any dust. Old and scratched glassware is more likely to cause blank problems, owing to the larger surface, therefore increasing the chance of adsorption. Moreover, scratched glassware is more difficult to clean. All glassware should be stored in clean cupboards, ensuring dust cannot enter (QUASIMEME, 2007).

## 4.2 Solvent purity and blanks

For work at low concentrations, the use of high-purity solvents is essential particularly when large solvent volumes are being used for column clean-up. All batches of solvents should be checked for purity by concentration of an aliquot of solvent by at least the same volume factor as used in the overall analytical procedure. Batches that demonstrate significant contamination and might interfere with the analysis should be rejected.

## 4.3 Preparation of materials

In addition to solvents, reagents and adsorptive materials must be "free" of PBDEs and other interfering compounds. Therefore, precleaning of all reagents, for example, alumina, silica, sodium sulphate, hydromatrix, etc., is essential, either by solvent extraction and/or by heating in a muffle oven as appropriate. Glassfibre materials, for example, Soxhlet thimbles and filter papers used in pressurized liquid extraction (PLE), should be cleaned by solvent extraction or prebaked at 450 °C overnight. Clean materials could be recontaminated by exposure to laboratory air; therefore, the method of storage after cleaning is of critical importance. Ideally, materials should be prepared immediately before use, but if they are to be stored, the conditions should

be considered carefully. All containers that come into contact with the sample should be made of glass or aluminium, and should be cleaned before use.

## 4.4 Lipid determination

The lipid content of tissues can be of use in characterizing the samples and reporting concentrations in biota on a wet-weight or lipid-weight basis. The total lipid content of fish or shellfish should be determined using the method of Bligh and Dyer (1959) as modified by Hanson and Olley (1963), or an equivalent method, such as Smedes (1999). Extractable lipid may be used, particularly if the sample size is small and lipid content is high. It has been demonstrated that if the lipid content is high (>5%), this will be comparable with the total lipid. If extraction techniques are applied that destroy or remove lipid materials (e.g. PLE with fat retainers), the lipid content should be determined on a separate subsample of the tissue homogenate.

## 4.5 Dry weight determination

The dry weight of shellfish or sediment samples should be determined gravimetrically, so that concentrations can also be expressed on a dry-weight basis.

## 4.6 Extraction and clean-up

The similarity in structure of the PBDEs to CBs means that extraction and clean-up techniques used for the analysis of CBs can also be applied to the analysis of PBDEs (de Boer *et al*., 2001). A range of extraction methods have been used for the extraction of PBDEs from sediment and biota. These include the more traditional methods, such as Soxhlet extraction, and the newer automated methods, such as pressurized liquid extraction (PLE). Supercritical fluid extraction (SFE) has also been applied to PBDE extractions, although reproducibility was poor compared with Soxhlet extraction (Covaci *et al*., 2003). For Soxhlet extractions, hexane/acetone mixtures or toluene (particularly for BDE209) have been demonstrated to give the best recoveries of PBDEs combined with an extraction time of 6–24 h. Hexane/acetone mixtures or toluene are also used with PLE, if no fat retainers are used, with an extraction time of ~10 min per sample. PLE or Soxhlet extraction are therefore the preferred methods with PLE, having the advantage of using less solvent, being fully automated, and taking less time than Soxhlet extraction.

Tissue or sediment extracts will always contain many compounds besides PBDEs, and a suitable clean-up is necessary to remove the compounds that can interfere with the subsequent analysis. Different techniques can be used, either singly or in combination; the choice will be influenced by the selectivity and sensitivity of the final measurement technique, as well as by the extraction method used. PBDEs are stable under acid conditions; therefore, treatment with sulphuric acid or acid-impregnated silica columns may be used in the clean-up. If Soxhlet extraction is used for biota, there is a much greater quantity of residual lipid to be removed than when using PLE with fat retainers. An additional clean-up stage may therefore be necessary. The most commonly used clean-up methods involve the use of alumina or silica adsorption chromatography, but gel permeation chromatography (GPC) is also used. When using GPC, the elution of PBDEs should be carefully checked, particularly for BDE209. When applying GPC, two serial columns are often used for improved lipid separation. Solvent mixtures, such as dichloromethane/hexane or cyclohexane/ethyl acetate, can be used as eluents for GPC. However, a second clean-up step is often required to separate the PBDEs from other organohalogenated compounds.

For biota, destructive methods for lipid removal, such as saponification, have also been investigated; however, this method can result in the degradation of the higher brominated PBDEs and is therefore not recommended.

One advantage of using PLE extraction is that it is possible to combine the clean-up with the extraction, especially when mass spectrometry will be used as the detection method. Methods have been developed for online clean-up and fractionation of dioxins, furans, and CBs with PLE for food, feed, and environmental samples (Sporring *et al*., 2003). These methods utilize a fat retainer for the online clean-up of fat. Silica impregnated with sulphuric acid, alumina, and Florisil has been used as fat retainers. A non-polar extraction solvent, such as hexane, should be used if fat retainers are used during PLE. This method can also be applied to the extraction of PBDEs. However, problems have been highlighted with BDE209, which can be lost during PLE extraction through adsorption onto the extraction system tubing. However, with careful optimization, it is possible to use PLE for BDE209. Losses of BDE209 may be accounted for using labelled BDE209 as an internal standard.

Sulphur should be removed from sediment extracts, to reduce interferences and to protect the detectors. This can be achieved by the addition of copper powder, wire, or gauze during or after Soxhlet extraction. Sulphur can also be removed by GPC. Copper can also be added to the PLE cell; however, this is not always sufficient, and further treatment with copper may be required following extraction. Ultrasonic treatment might improve the removal of sulphur. As an alternative to copper, other methods can be used (Smedes and de Boer, 1997).

## 4.7 Preconcentration

TurboVap sample concentrators can be used to reduce solvent volume. This is a rapid technique, but it should be carefully optimized and monitored to prevent losses, both of volatiles and solvent aerosols, and cross-contamination. The use of rotary-film evaporators is more time-consuming, but more controllable. However, evaporation of solvents using this technique should be done at low temperature, water bath temperature of ≤30 °C, and under controlled pressure conditions to prevent losses of the more volatile PBDEs. For the same reasons, evaporation to dryness should be avoided. Buchi Syncore parallel evaporators can be used with careful optimization of the evaporation parameters. The Buchi Syncore also uses glass tubes, but the system is sealed, avoiding contamination from the laboratory air during evaporation. It does not use a nitrogen stream, thus reducing the loss of volatiles, and, if the flush-back module is fitted, the sides of the tubes are rinsed automatically thus reducing the loss of the heavier components. Again, water-bath temperatures should be minimized to prevent losses. When reducing the sample to the required final volume, solvents can be removed by a stream of clean nitrogen gas. Suitable solvents for injection into the gas chromatograph (GC) include hexane, heptane, toluene, and isooctane.

## 4.8 Selection of PBDEs to be determined

PBDE technical mixtures used as flame retardants contain only a limited number of the possible 209 congeners (~20). There are three technical mixtures: penta-, octa-, and deca-. Nine BDE congeners have been detected in the penta-mix, the major ones being BDE47 (37%) and BDE99 (35%). The octa-mix contains hexa- to octabrominated congeners, with the main congener being BDE183, a heptabrominated congener. The deca-mix contains 98% decaBDE (BDE209).

PBDE congeners currently analysed vary considerably, however, the congeners found in environmental samples are relatively consistent. Most laboratories analyse for the penta-mix compounds, i.e. tetra- to hexa-BDEs. In addition, these congeners are thought to be the most toxic and most likely to bioaccumulate. In biota, the dominant congeners are usually BDE47, 99, 100, 153, and 154. BDE209 is less frequently measured, owing to analytical difficulties, but can be the predominant congener in sediment samples. Law *et al.* (2006) proposed a minimum congener set for use when determining BDEs to cover all three technical mixtures and those congeners commonly found in biota and sediment. This list included BDE28, 47, 99, 100, 153, 154, 183, and 209, consistent with the congeners required by the QUASIMEME[3] schemes for biota and sediments routinely measured by most laboratories. However, it is apparent that other congeners are found in marine samples, for example, BDE66 and 85; therefore, they should also be analysed. In 2007, OSPAR's Working Group on Concentrations, Trends, and Effects of Substances in the Marine Environment (SIME) recommended that the PBDE congeners measured should include nine compounds (BDE28, 47, 66, 85, 99, 100, 153, 154, 183; OSPAR Commission, 2007). BDE209 should be monitored if possible, but not included in any total, owing to the analytical difficulties associated with the analysis of this congener.

Standards are available for all of these congeners. Table 1 lists the PBDEs most commonly monitored.

**Table 1. Congeners commonly monitored in environmental samples along with their degree of bromination, chemical name, and the octanol water partition coefficient (Log KOW), where available (Braekevelt *et al.*, 2003).**

| PBDE congener | Number of Br | Name | Log $K_{OW}$ |
|---|---|---|---|
| BDE17 | 3 | 2,2',4-tribromodiphenyl ether | 5.74 |
| BDE28* | 3 | 2, 4,4'-tribromodiphenyl ether | 5.94 |
| BDE75 | 4 | 2, 4,4', 6-tetrabromodiphenyl ether | |
| BDE49 | 4 | 2, 3,4, 5'-tetrabromodiphenyl ether | |
| BDE71 | 4 | 2, 3', 4', 6-tetrabromodiphenyl ether | |
| BDE47* | 4 | 2, 2',4, 4'-tetrabromodiphenyl ether | 6.81 |
| BDE66 | 4 | 2, 3',4, 4'-tetrabromodiphenyl ether | |
| BDE77 | 4 | 3, 3',4, 4'-tetrabromodiphenyl ether | |
| BDE100* | 5 | 2, 2',4, 4', 6-pentabromodiphenyl ether | 7.24 |
| BDE119 | 5 | 2, 3',4, 4', 6-pentabromodiphenyl ether | |
| BDE99* | 5 | 2, 2',4, 4', 5-pentabromodiphenyl ether | 7.32 |
| BDE85 | 5 | 2, 2',3, 4, 4'-pentabromodiphenyl ether | 7.37 |
| BDE154* | 6 | 2, 2',4, 4', 5, 6'-hexabromodiphenyl ether | 7.82 |
| BDE153* | 6 | 2, 2',4, 4', 5, 5'-hexabromodiphenyl ether | 7.90 |
| BDE138 | 6 | 2, 2',3, 4, 4', 5'-hexabromodiphenyl ether | |
| BDE183* | 7 | 2,2',3,4,4',5',6-heptabromodipheny l ether | 8.27 |
| BDE209* | 10 | Decabromodiphenyl ether | 10.33 |

*** Congeners proposed by Law *et al.* (2006) as a minimum congener set for use when determining BDEs; they are also included in the QUASIMEME scheme.**

[3] Publications related to QUASIMEME schemes are available at www.quasimeme.org/ to registered QUASIMEME participants.

## 4.9 Instrumental determination of PBDEs

Automatic sample injection should be used wherever possible to improve the reproducibility of injection and the precision of the overall method. Splitless, pulsed-splitless, programmed temperature vaporizer (PTV), and on-column injectors have been used for the determination of PBDEs. All of these are capable of yielding good results. For PBDE analysis, the cleanliness of the liner is very important, if adsorption effects and discrimination are to be avoided. The analytical column should not contain active sites onto which PBDEs, particularly BDE209, can be adsorbed. Helium is the preferred carrier gas and only capillary columns should be used. Mainly non-polar columns are used, e.g. HT-8, DB-5, and STX-500; DB1 is usually used for BDE209. Korytár *et al*. (2005) provide comprehensive information on various capillary columns used for PBDE analysis. Baseline separation should be achievable for all BDEs listed in Table 1. However, BDE33 may coelute with BDE28. Because of the wide boiling range of the PBDEs to be determined and the surface-active properties of the higher PBDEs, the preferred column length is 25–50 m, except for BDE209, with an internal diameter of 0.1–0.3 mm. Film thicknesses around 0.2 μm are generally used.

BDE209 can be measured with the other PBDEs, but will give a smaller and broader peak. Detection limits will be approximately tenfold greater for BDE209. Because the retention time is long, the determination of BDE209 is often done separately, using thinner films (0.1 μm) and/or a shorter column, both of which have been found to improve the detection of BDE209.

### 4.9.1 Detection methods

Either gas chromatography–mass spectrometry (GC-MS) or GC-MS-MS (ion trap or triple quadrupole) should be used. GC-ECD is rarely used, owing to its limited linear range and lack of selectivity. If GC-ECD is used, the clean-up will have to separate out all other organohalogenated compounds that may give coelution problems. Both high- and low-resolution GC-MS can be used in conjunction with either electron ionisation (EI) or electron-capture negative ionization (ECNI). Although gas chromatography high-resolution mass spectrometry with electron impact ionization (GC-HRMS) is the best method to unambiguously identify and quantify PBDEs in environmental samples, the expense and limited availability of this instrumentation means that most laboratories use low-resolution GC-MS usually in ECNI mode. Lower brominated PBDEs and mono- and di-BDEs display better sensitivity in EI mode. However, the higher brominated PBDEs, >3 bromines, give better sensitivity using the ECNI mode; limits of detection for these congeners are approximately tenfold lower in ECNI compared with EI. ECNI demonstrates improved sensitivity compared with positive-impact chemical ionization (PCI). Either ammonia or methane may be used as the reagent gas when using chemical ionization.

### 4.9.2 Gas chromatography–mass spectrometry

For the tri- to hepta-BDEs, the base ions detected using ECNI are the bromine ions (m/z = 79/81). BDE congeners demonstrate the typical $^{79}Br$ (50.5%) and $^{81}Br$ (49.5%) isotope distribution pattern. One of the drawbacks of the ECNI mode is that isotopically labelled standards ($^{13}C$) cannot be used as internal standards for quantification purposes when only the bromide ions are monitored. However, mono fluorinated BDEs, such as FBDE28, 100 and 160, may be used as internal standards, though they should be checked for the presence of PBDEs. Alternatively, when using GC-ECNI-MS, a recovery standard can be added before extraction. CB198 and other

halogenated compounds not present in environmental samples can also be used as recovery standards. Larger-fragment ions, necessary for confirmation, are only found for BDE209. These are formed by the cleavage of the ether bond to give the pentabromo phenoxy ion (m/z = 484/486). An internal standard method, including a labelled BDE209 standard, should be used for the quantification of BDE209.

One advantage of using EI is that $^{13}C$ labelled internal standards may be used. The major ions formed in EI mode are the molecular ions that can be used for identification and quantification purposes. Other fragment ions that can be used as confirmatory ions are also formed in EI mode. A range of $^{13}C$-labelled PBDEs is available for use as internal standards in PBDE analysis using GC-EIMS.

#### 4.9.3 Possible pitfalls and solutions

Degradation of PBDEs, particularly BDE209, can occur on the GC column. The presence of a hump or rising baseline before BDE209 is an indication of degradation during injection, whereas the presence of nona-, octa-, and eventually other lower brominated BDEs, indicates possible degradation during extraction and clean-up. To minimize this, the GC liners and injection syringe should be changed regularly. Silanizing both the syringe and liner may help. When using on-column injection, the choice of retention gap can also have an effect on the degradation of BDE209 during analysis. Deactivated fused silica retention gaps are often used. The QUASIMEME external quality assurance scheme has also highlighted the difficulties with the analysis of BDE209, with the CV% for this congener ranging from 40 to 256%. As a result, many laboratories do not analyse for BDE209.

## 5 Calibration and quantification

### 5.1 Standards

Standard solutions of known purity should be used for the preparation of calibration standards. If the quality of the standard materials is not guaranteed by the producer or supplier, as for certified reference materials, it should be checked by GC-MS analysis. In addition, certified standard solutions are available from QUASIMEME and other suppliers for cross-checking. Calibration standards should be stored in the dark, because some PBDEs are photosensitive, and ideally, solutions to be stored should be sealed in amber glass ampoules. Solutions to be used regularly can be stored in a refrigerator in stoppered measuring cylinders or gas tight flasks, to minimize evaporation of the solvent during storage. Dates of preparation should be noted and solutions should not be used for longer than 1–2 months without verifying their concentrations.

Ideally, internal standards should fall within the concentration range of the compounds to be determined and should not include compounds that may be present in the samples.

### 5.2 Calibration

Multilevel calibration with at least five calibration levels is preferred to define the calibration curve adequately. In general, GC-MS calibration is linear over a considerable concentration range, but exhibits non-linear behaviour when the mass of a compound injected is low owing to adsorption. The use of a syringe standard is recommended, for example, BDE190. Quantification should be conducted in the linear region of the calibration curve; otherwise, the non-linear region must be well characterized during the calibration procedure.

## 6 Analytical quality control

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination that they consider acceptable. Achievable limits of determination based on a 10 g sample[4], on a wet-weight basis for biota and a dry-weight basis for sediment, for each individual component, are as follows:

- GC-ECNI-MS: 0.05 $\mu g\,kg^{-1}$ for tri- to hepta-BDEs and 0.50 $\mu g\,kg^{-1}$ wet weight for BDE209;
- GC-EIMS: 0.5 $\mu g\,kg^{-1}$ wet weight;
- High-resolution GC-MS: 0.02 $ng\,kg^{-1}$ wet weight for biota for tri- to hepta-BDEs and 0.5 $ng\,kg^{-1}$ wet weight for BDE209, and the same for sediment, but on a dry-weight basis for sediment.

A procedural blank should be measured with each batch of samples and should be prepared simultaneously using the same chemical reagents and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will result in errors in quantification. Recoveries should be checked for all samples. Recoveries should be between 70% and 120%, otherwise samples should be repeated. The procedural blank is also very important in the calculation of limits of detection and limits of quantification for the analytical method. In addition, a laboratory reference material (LRM) or certified reference material (CRM) should be analysed within each sample batch. The LRM must be homogeneous and well characterized for the determinands of interest within the analytical laboratory. Ideally, the LRM or CRM should be of the same matrix type, for example, liver, muscle, or mussel tissue, because the samples and the determinand concentrations should be in the same range as those in the samples. The data produced for the LRM or CRM in successive sample batches should be used to prepare control charts. It is also useful to analyse the LRM or CRM in duplicate from time to time, to check within-batch analytical variability. The analysis of an LRM is primarily intended as a check that the analytical method is under control and yields acceptable precision. A CRM may be analysed periodically to check the method bias. CRMs certified for PBDEs are available (Wise *et al*., 2006). At regular intervals, the laboratory should participate in an intercomparison or proficiency exercise where samples are circulated without knowledge of the determinand concentrations, to provide an independent check on performance.

## 7 Data reporting

The calculation of results and the reporting of data can represent major sources of error. Control procedures should be established to ensure that data are correct and to obviate transcription errors. Data stored in databases should be checked and validated, and checks are also necessary when data are transferred between databases. If possible, data should be reported in accordance with the latest ICES reporting formats.

[4] Detection limits will be higher if smaller sample sizes are used.

## Acknowledgements

These guidelines were prepared by ICES Marine Chemistry Working Group. The authors acknowledge the assistance of the chair, Evin McGovern, members of the working group, and members of the ICES Working Group on Marine Sediments in Relation to Pollution for their input.

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## 9 Abbreviations and technical terminology

| | |
|---|---|
| BDE | brominated diphenyl ether |
| CB | chlorobiphenyl |
| CRM | certified reference material |
| ECD | electron-capture detection |
| ECNI | electron-capture negative ionization |
| EI | electron ionization |
| FBDE | fluorinated brominated diphenyl ether |
| GC | gas chromatograph |
| GPC | gel-permeation chromatography |
| HRMS | high-resolution mass spectrometry |
| LRM | laboratory reference material |
| MS | mass spectrometry |
| PBDE | polybrominated diphenyl ether |
| PCI | positive-impact chemical ionization |
| PLE | pressurized liquid extraction |
| PTV | programmed temperature vaporizer |
| SFE | supercritical fluid extraction |

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