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DIALYSIS WITH A SEMIPERMEABLE MEMBRANE – A NEW SAMPLE-PREPARATION TECHNIQUE FOR DETERMINATION OF POLYBROMINATED DIPHENYL ETHERS IN FATTY MATRICES

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SUMMARY

Dialysis with a low-density polyethylene semipermeable membrane (SPM) is an efficient means of removal of lipids in the determination of bioaccumulative, persistent, halogenated organic compounds. In this study an SPM was used for preconcentration of polybrominated diphenyl ethers (PBDE) from food samples. The method is useful for determination of PBDE and other halogenated persistent organic pollutants in fatty food samples. In this work chocolate, butter, chicken eggs, pork, and salmon fat were analyzed. Lipid carry-over values (%LC) are reported and recovery of the analytes for the whole procedure was determined by fortifying fish fat with six natural PBDE internal standards which were normally present in the sample at levels below the limit of detection.

INTRODUCTION

Flame-retardant chemicals are added to plastics, textiles, electronic circuitry, and other materials to slow the burning process and prevent fires. Some technical flame-retardant products contain brominated organic compounds. The most widely used brominated flame-retardants (BFR) are polybrominated diphenyl ethers (PBDE), hexabromocyclododecane (HBCD), tetrabromobisphenol-A (TBBP-A), and polybrominated biphenyls (PBB) [1]. Commercial PBDE products consist predominantly of penta, octa, and decabromodiphenyl ether products. Decabromodiphenyl ether is the most widely used PBDE product. Polybrominated diphenyl ethers (PBDE) are added to a wide range of materials at concentrations up to 30% by weight [2]. They are commonly added to high-impact polystyrene, flexible polyurethane foam, textile coatings, cable insulation, and electrical and elec-

tronic equipment [3]. Polybrominated diphenyl ethers are unreactive and so are not incorporated into the polymeric materials by bonding between the polymer and the flame retardant. They may, therefore, leach from products into the environment [4]. PBDE were first discovered in pike, eel, and sea trout from Sweden in 1981. Since then, researchers in Europe, Canada, and the USA have confirmed the presence of PBDE in a variety of environmental samples, primarily detecting the lower brominated isomers, for example tetra and penta-BDE [5]. Polybrominated diphenyl ethers are persistent, lipophilic, and bioaccumulating chemicals. They are toxic to humans and animals [6].

Sample preparation is important in the analysis of PBDE in food samples, because such samples require highly efficient methods of purifycation before final analysis. Dialysis, with a semipermeable membrane (SPM), of solution of an extract in an organic solvent enables separation of organohalogen contaminants, for example PBDE, from lipids. The objective of this study was application of SPM clean-up to the determination of PBDE in animal or plant fat. SPM enable nondestructive separation of the analyte from matrix compounds, which can be useful for preconcentration of organic chemicals in animal or plant fat samples. The membranes are made from low-density polyethylene film with approximately 1-nm pores; these enable permeation of small (analyte) molecules whereas dialysis of molecules of matrix compounds larger than this is not possible [7–9]. The membrane is shaped into a narrow tube which is sealed at one end. The thickness of the polyethylene film used in this work was approximately 80 μm [10]. The method can be used for efficient and inexpensive clean-up of animal fat samples. SPM sample clean-up can be used for determination of other persistent organic pollutants in the environment, for example pesticides [11], polychlorinated biphenyls (PCBs) [12], and polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF) [13].

EXPERIMENTAL

Materials, Reagents, and Apparatus

A standard solution of PBDE containing six native congeners (tri-BDE 28, tetra-BDE 47, penta-BDE 99, hexa-BDE 153 and 154, and hepta-BDE 183; 0.5 μg mL^{-1} of each) was prepared in nonane. Certified PBDE standard solutions BDE-MXA lot MBDEMXA1199 and BDE-MXB lot MBDEMXB0300, containing the same six PBDE congeners at

5 μg mL^{-1} concentration, were obtained from Wellington Laboratories (Ontario, Canada) and used to prepare a labelled standard solution in nonane containing the six congeners at concentrations of 50 ng mL^{-1}.

A GC–MS–MS experiment was performed with calibration solution set BDE-CVS-A Brominated Diphenyl Ether (Wellington Laboratories) containing five individual solutions each containing twenty individual native brominated diphenyl ethers (mono-BDE 3, di-BDE 7 and 15, tri-BDE 17 and 28, tetra-BDE 47, 49, 66, 71, and 77, penta-BDE 85, 99, 100, 119, and 126, hexa-BDE 148, 153, and 154, hepta-BDE 183, and deca-BDE 209) and ten selected $^{13}C_{12}$-labelled PBDE (mono-BDE 3L, di-BDE 15L, tri-BDE 28L, tetra-BDE 47L, penta-BDE 99L, hexa-BDE 139L, 153L, and 154L, hepta-BDE 183L, and deca-BDE 209L). All native compounds (mono to hepta-BDE) were dissolved in nonane at concentrations of 1.0–400 ng mL^{-1} except for deca-BDE 209, the concentration of which was ten times higher. All labelled congeners were at concentrations of 100 ng mL^{-1}. A certified, labelled PCDD standard solution (DFISS) contained the ^{13}C-labelled PCDD congeners ^{13}C-1,2,3,4-TCDD and ^{13}C-1,2,3,7,8,9-H_6CDD at concentrations of 200 ng mL^{-1}. Each compound was used in the procedure as a syringe standard for gas chromatographic and mass spectrumetric determination.

SPM membranes were purchased from Exposmeter, Sweden. All solvents were purchased from POCh, Gliwice, Poland. Sulphuric acid, sodium hydroxide, and anhydrous sodium sulphate were purchased from J.T. Baker (The Netherlands). Silica gel and aluminium oxide were purchased from Merck and were activated overnight at 130 and 200°C, respectively. Acidic silica gel was prepared by mixing silica gel with 44% of its mass of H_2SO_4 to obtain a uniform powder. Basic silica gel was prepared by mixing silica gel with 33% of its mass of aqueous 1 M NaOH.

Gas chromatographic analysis was performed with a Varian CP3800 instrument equipped with electron-capture detection (GC–ECD). Compounds were separated on a 30 m × 0.32 mm i.d. × 0.25 μm film thickness CP-Sil5 CB capillary column. Splitless injection was used; the split vale was opened 1 min after injection. After injection the column oven temperature was maintained at 90°C for 1 min, then programmed at 10° min^{-1} to 160°C, then at 20° min^{-1} to 250°C, and finally at 10° min^{-1} to 300°C, which was held for 5 min.Gas chromatographic analysis with mass spectrometric detection was performed with a Trace GC 2000 series Thermo Quest coupled with a GCQ Thermo Quest mass spectrometer. Compounds were separated on a 30 m × 0.25 mm i.d. × 0.25 μm film thickness DB5 MS

capillary column. The column oven temperature was maintained at 100ºC for 3 min, then programmed at 80º min^{-1} to 220ºC and then at 3º min^{-1} to 300ºC, which was held for 10 min.

Samples

Salmon and cod originated from the Baltic Sea and carp from the south of Poland. Chicken eggs, pork, beef, chocolate, and butter were bought in Cracow's market. Eggs were cooked and the yolks were separated. Fish tissue samples and egg yolks were extracted with dichloromethane in a Soxhlet apparatus for 8 h then freeze-dried. Butter and adipose tissues of pork and beef were heated for 5 h in glass beaker in 150°C. After the fat had melted it was transferred to a glass bottle, which was weighed, sealed, and stored under refrigeration. The chocolate was dissolved in *n*-hexane in a glass beaker. After deposition of the solid particles (ca 24 h) the upper, transparent yellowish layer was transferred to a rotary evaporation flask and the solvent was removed. The residual fat was weighed.

Procedure

The membranes were pre-washed before use by placing in a bottle containing *n*-hexane for 72 h. Although the highest recovery of PBDE was obtained after washing the membranes for 72 h, acceptable recovery was obtained after washing for 48 h.

Fat samples (5 g) were dissolved in 15 mL dichloromethane–*n*-hexane, 10:90 (*v*/*v*), and transferred quantitatively into the SPM membrane by use of a Pasteur pipette (Fig. 1) The membrane was then sealed at its

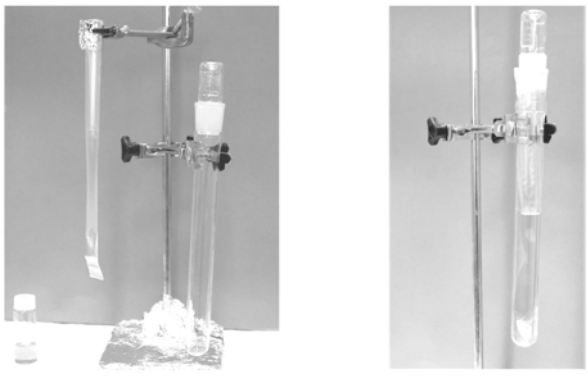

Fig. 1

Photograph of sample preparation by SPM

open end and placed in a bottle containing 80 mL *n*-hexane. Dialysis was conducted for 24 h, after which the dialysate was concentrated to ca 2 mL by rotary evaporation. A new portion of 80 mL *n*-hexane was then added to the bottle containing the membrane and dialysis was conducted for another 24 h. The procedure was repeated a third time to give three samples of dialysate from a total dialysis time of 72 h.

Further clean-up was achieved by column chromatography in a glass column containing 0.5 g anhydrous Na_2SO_4, 2 g basic silica gel, 0.5 g neutral silica gel, 8 g acidic silica gel, and 0.5 g anhydrous Na_2SO_4, from bottom to top (Fig. 2). Concentrated dialysate (2 mL) was applied to the silica column. The analytes were eluted from this column with 60 mL *n*-hexane and the sample was evaporated to ca 1 mL by rotary evaporation.

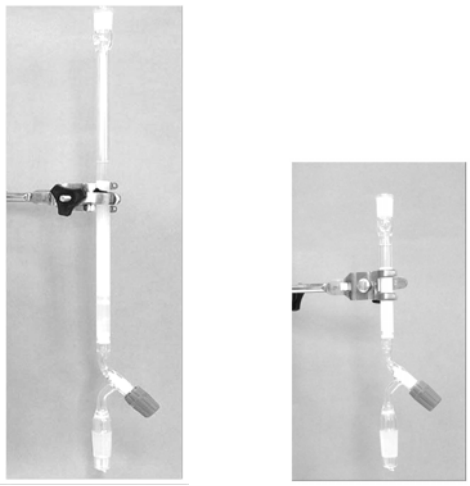

Fig. 2

Photograph of silica gel and alumina columns

Final clean-up was achieved on neutral alumina in a column containing 0.5 g anhydrous Na_2SO_4, 5 g aluminium oxide, and 0.5 g anhydrous Na_2SO_4 (Fig. 2). After transfer of the sample to the column it was eluted with 15 mL *n*-hexane and 30 mL dichloromethane–*n*-hexane, 2:98 (*v*/*v*). These fractions were discarded. The analytes were eluted with 50 mL 1:1 (*v*/*v*) dichloromethane–*n*-hexane, to furnish a third fraction. Finally, 50 µL *n*-decane, as a keeper, and 2 µL DFISS standard were added to the third fraction and excess solvent was evaporated with a stream of inert gas.

The $[M - 2Br]^+$ ion was monitored for quantitative and qualitative determination of PBDE by GC–MS–MS. The internal standard method was used for calculation of results from the appropriate PBDE congener peak areas in the sample analysed and the peak area for the corresponding PBDE congener in the BDE-CVS-A standard solution.

To monitor analyte recovery, the chocolate and butter fat solutions before SPM dialysis were fortified by addition of 2.5 ng of each of the selected six native PBDE congeners – BDE28, BDE47, BDE99, BDE153, BDE154, and BDE183. Amounts of these compounds in the samples investigated were below the detection limit. In this work we selected PBDE congeners from tri to heptabrominated diphenyl ethers because these groups of congeners are the most abundant in animal adipose tissue.

Lipid carry-over (%LC) through the SPM membrane was investigated. Butter, chocolate, chicken egg, salmon, and pork lipid samples were prepared as described above and dialysed for 24 h. After this time the dialysate was transferred to a round-bottomed flask and concentrated to 1 mL by rotary evaporation. The residual solvent was finally evaporated by a stream of inert gas in a small conical flask and the fat obtained was weighed.

RESULTS AND DISCUSSION

Recovery of PBDE after dialysis for 24, 48, and 72 h is depicted in Fig. 3. Approximately 50% recovery of PBDE standards was obtained in

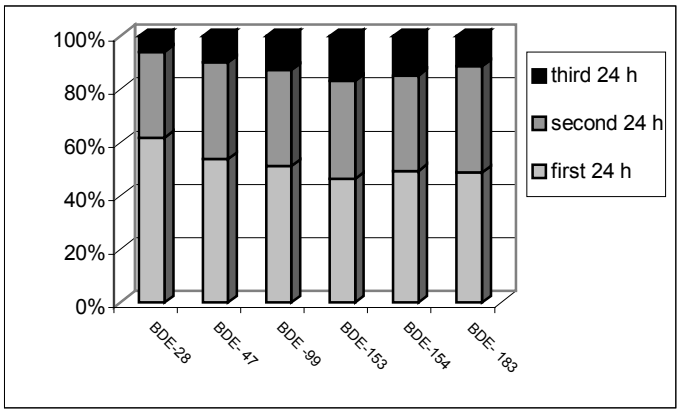

Fig. 3

Dependence of PBDE recovery on dialysis time

the first day of dialysis. In the third day recovery for all six PBDE congeners was below 10%. These studies indicated that acceptable recovery of PBDE standards could be obtained by dialysis of food samples for 48 h. To investigate recovery chocolate and butter fat were used. For both matrices analyte recovery after SPM dialysis for 48 h was in the range 50–70% (Table I).

Table I

Recovery of PBDE standards from butter and chocolate fat (dialysis for 48 h)

Congener	Recovery of PBDE standards (%)	
	Butter fat	Chocolate fat
2,4,4′-TriBDE (28)	64	70
2,2′,4,4′-TeBDE (47)	66	70
2,2′,4,4′,5-PeBDE (99)	67	66
2,2′,4,4′,5,5′-HxBDE (153)	64	54
2,2′,4,4′,5,6′-HxBDE (154)	69	62
2,2′,3,4,4′,5′,6-HpBDE (183)	51	57

Use of pre-washed and unwashed membranes was investigated. Dialysis was conducted with a standard solution containing six PBDE congeners and the dialysate was analysed after dialysis for 24 h. Comparison of the PBDE chromatograms (Fig. 4) reveals the signal-to-noise ratio was better for dialysate obtained by use of pre-washed membranes. All subsequent dialysis was therefore conducted with membranes pre-washed by placing in bottles filled with *n*-hexane for three days or more.

All fat samples were dissolved in 10:90 dichloromethane–hexane and dialysed with *n*-hexane. The results obtained showed that the best recovery of PBDE standards was obtained by use of this solvent mixture for dialysis. If pure dichloromethane was used inside and outside the membrane, recovery of the standards was between 34 and 50% after dialysis for 24 h. With *n*-hexane inside and outside the membrane recovery was between 76 and 97% but addition of 10% dichloromethane to fat samples improved solvation of the lipids.

Lipid carry-over values (%LC) obtained for animal and plant fats are listed in Table II.

Lipid masses obtained after dialysis were negligible and caused no problems with subsequent clean-up steps. Use of SPM as an efficient method for lipid removal thus makes the whole procedure for clean-up of fat

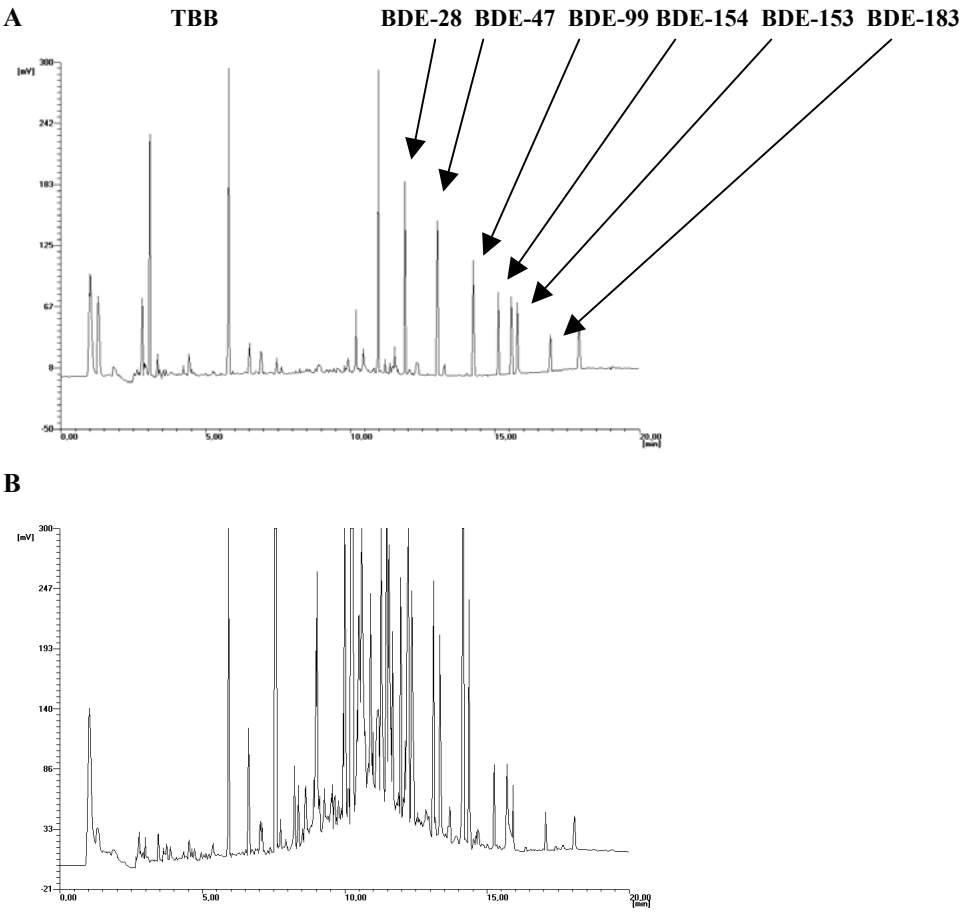

Fig. 4

GC–ECD chromatograms obtained after dialysis with (A) a membrane pre-washed in *n*-hexane for 7 days and (B) an unwashed membrane

Table II

Percentage lipid carry-over (%LC) for 5 g animal fat using the SPM method

Food sample	Fat mass breakthrough (g)	%LC	Average %LC
Butter	0.0162	1.31	1.10±0.30
Chocolate	0.0079	0.88	
Chicken egg	0.0120	1.15	
Salmon	0.0102	0.83	
Pork	0.0170	1.35	

samples more effective. Results obtained from real animal fat and plant fat samples indicated that all the samples investigated contained PBDE. Analytical results from determination of PBDE in fat samples are presented in Fig. 5.

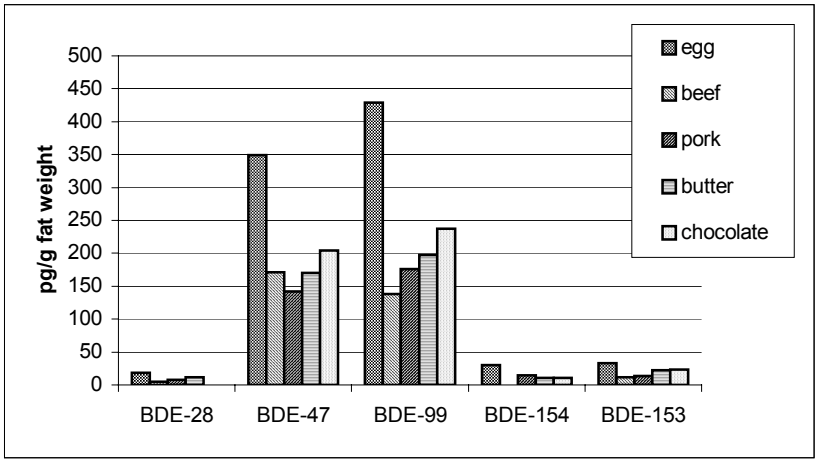

Fig. 5

PBDE concentrations in five types of food

The concentrations of PBDE in beef, pork, butter, and chocolate fat samples, were similar for each congener. For egg samples PBDE concentrations were approximately twice as high. Among the PBDE congeners determined in food samples, BDE-47 and BDE-99 were approximately in 90% of the total mass of PBDE. The concentrations of PBDE in edible fillet tissue from three species of fish are presented in Fig. 6. The highest concentrations were measured in salmon tissue. The figure shows there are large differences between PBDE concentrations in the two types of marine fish. The greater fat content of salmon tissue compared with cod tissue is the reason for this difference. PBDE are very lipophilic and poorly water soluble and therefore tend to accumulate in marine, fatty fish.

The dominance of BDE-47 and BDE-49 (tetra) over the other PBDE congeners may indicate that tetrabrominated diphenyl ethers are bioaccumulated to a greater extent than the more brominated congeners [12]. Although the deca-BDE mixture constitutes 82% of global PBDE production, BDE-209 has rarely been reported in wildlife [13]. The PBDE

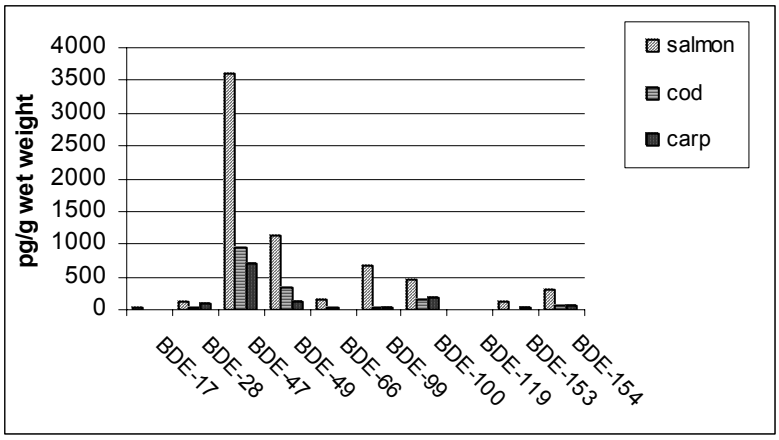

Fig. 6

PBDE concentrations in three types of fish

congeners most often detected in food and fish samples are the tri to hexa-BDE congeners [14]. In our research only tri to hexa-BDE congeners were determined in the samples collected; BDE-209 was not detected in these samples.

CONCLUSIONS

PBDE were found in all the samples investigated. Tetra to hexa-BDE were detected in all the samples; among these BDE-47 was most abundant. Qualitative determination of PBDE in fish and food products indicates that tetra and penta-BDE congeners are the most abundant in fatty matrices [1]. PBDE concentrations were highest in marine fish samples. PBDE tend to accumulate in marine, fatty fish [15].

Dialysis with organic solvent using polyethylene membranes is a good method for cleaning animal fat samples. The SPM technique is easy to handle and requires no advanced or costly equipment. Sample preparation with the SPM technique and silica gel and alumina chromatography takes approximately 3 days. This seems a long time in contrast with other methods, but in serial analysis use of a SPM results in good time saving.

The SPM technique can be recommended for routine determination of PBDE in fatty matrices. Recovery for this method is between 50 to 70%. These recovery values are not acceptable for residue analysis, for which isotope dilution should be used.

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