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NERI Technical Report, No. 481

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Data sheet

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Abstract: Analytical methods for the measurement of 12 polybrominated diphenyl ethers (PBDEs) in sewage sludge and wastewater were developed and validated. The congeners were BDE17, 28, 47, 49, 66, 85, 99, 100, 153, 154, 183, and 209. Wastewater was extracted with toluene using magnetic stirring and sewage sludge was soxhlet extracted with hexane:acetone (4:1). Extracts were cleaned on a multilayer column. Tri-hepta BDEs were analysed on GC/MS (NCI) using a 60-m DB5 capillary column and BDE209 on a 15-m DB1. The methods were validated by investigating: a) linear response ranges, LOD, LOQ, interference, recovery, blank values, and precision. A quality control scheme was proposed. Environmental concentrations at Bjergmarken STP, Roskilde, Denmark were 238 (±23) µg/kg for tri-hepta BDEs and 248 (±81) µg/kg for the decabrominated congener in sewage sludge. Total PBDE concentrations were below 1 ng/L in effluent wastewater.

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Sammenfatning

Efter aftale med Miljøstyrelsen har Danmarks Miljøundersøgelser, Afdeling for Miljøkemi og Mikrobiologi udviklet og valideret to analytisk-kemiske metoder til bestemmelse af bromerede flammehæmmere i udløbsspildevand og spildevandsslam. Metoderne omfatter i alt 12 enkeltstoffer af polybromerede diphenyl ethere (PBDE) der er blandt de mest anvendte bromerede flammehæmmere. Det er opgjort at i 2001 var det samlede forbrug på verdensplan 67.390 tons af 3 industrielle produkter indeholdende PBDE.

Afdeling for Miljøkemi og Mikrobiologi har tidligere erfaring med analyse af PBDE i sediment og biota (fx. fisk og muslinger) fra Danmark og Grønland. Metoderne beskrevet i denne rapport er udviklet med udgangspunkt i de eksisterende metoder til sediment og biota.

Følgende enkeltstoffer er analyseret i spildevand og slam: BDE17, 28, 47, 49, 66, 85, 99, 100, 153, 154, 183, og BDE209. Det industrielle produkt PentaBDE indeholder BDE17, 28, 47, 49, 66, 99, 100, 153 og 154, mens BDE183 og BDE209 er hovedbestanddele i henholdsvis OctaBDE og DecaBDE. Spildevandsmetoden består af ekstraktion fra 5 L vand med toluen ved kraftig magnetoprøring, mens ekstraktion fra slam udføres ved soxhlet ekstraktion af 5 gram våd slam med hexane:acetone (4:1). I begge metoder oprenses ekstrakterne i en multikolonne bestående af vandfrit natriumsulfat, silica gel, alumina B og koncentreret svovlsyre. Ekstrakterne analyseres ved gaskromatografi - masse spektrometri (GC/MS) med negativ kemisk ionisering. Udvalgte resultater sammenlignes med resultater fra højt opløsende masse spektrometri. Tri- til hepta-BDE analyses på en 60 m DB5 kolonne mens den decabromerede forbindelse (BDE209) analyses på en 15 meter DB1 kolonne. Dette skyldes at BDE209 er varmefølsom og nedbrydes under langvarig udsættelse for høje temperaturer i kolonnen.

En række aspekter blev undersøgt under metodevalideringen. Dette inkluderer lineære response områder, detektions- (LOD) og kvantificerings grænser (LOQ), interferens, genfinding, præcision, matrix effekter samt blindværdier. For slam blev metodevalideringen udført ved analyse af i alt 15 prøver; 6 slamprøver fra Bjergmarken rensningsanlæg i Roskilde, 3 prøver tilsat 200 nanogram BDE209 inden ekstraktion, 2 blindprøver, 2 prøver uden tilsætning af standarder samt 2 slamprøver fra QUASIMEME 2003. Spildevandsmetoden blev valideret ved analyse af 6 prøver af udløbsspildevand fra Bjergmarken rensningsanlæg.

LOD og LOQ blev bestemt for spildevandsslam til 0.03 – 2 ng/L og 0.1 – 5 ng/L for tri-hepta BDE og 3 henholdsvis 10 for BDE209. Genfindingerne var 106 (±7%) for tri-heptaBDE og 102% for BDE209. Præcision målt som *repeatability* var mellem 9 og 84% for tri-heptaBDE på et 95% konfidens niveau. For spildevand var LOD og LOQ 0.01 – 0.3 og 0.02 - 1 for tri-hepta BDE og 1.4 og 5 for BDE209. Genfindinger var mellem 50 og 103 (tri-heptaBDE), mens præcisionen var mellem 30 og 135% for tri-heptaBDE.

Generelt er begge analysemetoder robuste med høje genfindinger, lave usikkerheder og detektionsgrænser, der er miljømæssigt relevante. Koncentrationer målt i slam er 238(±23) µg/kg for triheptaBDE og 248(±81) µg/kg for BDE209. Dette er højere eller sammenligneligt med koncentrationer målt i Sverige og Holland, men under niveaurene målt i Nordamerika. Der eksisterer ingen eller meget få målinger af PBDE i udløbsspildevand. Under metodeudviklingen blev der målt PBDE i slam fra Avedøre rensningsanlæg som modtager mere industrispildevand end Bjergmarken. Her var koncentrationerne af PBDE da også højere end i slam fra Bjergmarken (ca. 1/3 højere).

Ved den seneste Quasimeme 2002 blev det konkluderet, at analyserne af BDE209 ikke er under kontrol i de deltagende laboratorier, hvilket blandt andet skyldes at BDE209 er både fotonedbrydelig og varmefølsom. Vores metode er meget robust med forholdsvis lav detektionsgrænse og høj reproducerbarhed (33% på et 95% konfidens niveau). Den høje analytiske kvalitet for bestemmelsen af BDE209 skyldes sandsynligvis at vi anvender ^{13}C-mærket BDE209 som intern standard i modsætning til hovedparten af laboratorierne i verden der måler BDE209.

1 Introduction

Flame retardants are added to materials such as electrical and electronic equipment, paint and textiles to prevent them from catching fire and to slow down the burning process. Different groups of flame retardants exist: inorganic chemicals, organic phosphate esters and chlorinated and brominated organic compounds. Brominated flame retardants are a chemically diverse group of organic brominated compounds that are used to increase the flame retardancy of e.g. polyurethane foam, backcasings of TVs, printed circuit boards, and electronic component encapsulates. Some of the most important brominated flame retardants are polybrominated diphenyl ethers (PBDEs), produced as three industrial products; PentaBDE, OctaBDE and DecaBDE. On 30 January 2001, the European Commission has issued a proposal to ban the production and use of PentaBDE.

Bromine Science and Environmental Forum added up the total market demand in 2001 in Europe, America, Asia, and the rest of the world. The worldwide demand was 67390 metric tonnes whereas the demand in Europe was 150, 610, and 7600 metric tonnes for PentaBDE, OctaBDE and DecaBDE, respectively (www.bsef.com). The main constituents of the three products are BDE47, BDE99, and BDE100 (PentaBDE), BDE183 (OctaBDE), and BDE209 (DecaBDE).

There is no production of PBDEs in Denmark and no point sources have been located. In the Danish environment PBDEs are entered by long range transport and by emission due to washout, evaporation and incineration of products such as textiles, televisions, computers and from polyurethane foam applications.

The individual congeners of PBDE are ubiquitous pollutants and have been detected in the environment since the late 1970s (Andersson and Blomkvist, 1981;Covaci et al., 2003;de Wit, 2002;Watanabe et al., 1983). Only few measurements of PBDEs in fish, sediment and soil have been performed in Denmark (Christensen et al., 2002). However, from 2004, PBDEs becomes a part of NOVANA (http://ovs.dmu.dk/) and will be measured in sediments, mussels, sewage sludge and wastewater.

As PBDEs are hydrophobic and resistant to degradation they will enter sewage treatment plants (STPs) and will subsequently be concentrated in sewage sludge. Thus, monitoring of PBDEs in sludge and wastewater will give indications of the environmental discharge. Levels of PBDEs in sewage sludge have been measured in a few studies. Hale *et al* (2003) compared levels of PentaBDE congeners (e.g. BDE47, BDE99 and BDE100) and BDE209. Levels from four regions of the US ranged from 1000 to 2290 µg/kg dry weight (tri-hexa) and 84.8 – 4890 µg/kg dry weight for BDE209 with mean values of approx. 1600 and 1000 µg/kg. Concentrations of BDE47, 99, 100 and 209 exceeded those of the major PCB congeners and other halogenated contaminants present (Hale et al., 2003). The North American levels are substantially higher than those reported in European sludge. Sellström *et al* (1999) found levels of tri-hexa BDEs between 98 and 238

µg/kg and for BDE209 between 140 and 350 µg/kg dry weight in sludge from three plants in Stockholm (Sellstrom, 1999). De Boer *et al* (2000) found PBDE levels of 11 – 35 (tri-hexa BDEs) and 310 – 920 µg/kg (BDE209) in STP effluent, residues after filtration (de Boer et al., 2003).

Here, methods for the chemical analyses of 12 BDE congeners, BDE17, 28, 47, 49, 66, 85, 99, 100, 153, 154, 183, and 209 in sewage sludge and wastewater (effluent) were developed. The methods were validated for use at the Department of Environmental Chemistry and Microbiology, National Environmental Research Institute, Denmark.

2 Materials and methods

2.1 Sample collection

Wastewater (effluent) was collected in 5-L pyrex containers at Bjergmarken sewage treatment plant (STP), Roskilde, Denmark and adjusted to 5-L in the laboratory. 100 ml of toluene was added and the containers were refrigerated until extraction (within 2 days of sampling).

Sewage sludge was collected at Bjergmarken STP, which is an average Danish provincial plant receiving both urban and agricultural wastewater. The sludge was homogenised and divided into portions of approx. 100 gram. Samples were stored in a freezer (-20°C) in glass jars until extraction and were used in the method development, the validation process and for quality control (QC) samples. Sewage sludge from Avedøre STP was used in the method development as a representative of heavily polluted urban waste.

2.2 Chemicals

Table 2.1 lists the materials and chemicals used in the two analytical methods. Glasswool, Alumina, silica, sodium sulphate and hydromatrix were precleaned in methylene-chloride. Copper plates were cut in pieces (approx. 1 cm^2) and cleaned for 12 hours (at least) in concentrated sulphuric acid prior to use.

Table 2.1 List of materials and chemicals used

Materials and Chemicals	Supplier and purity
Acetone	Rathburn, Glass distilled grade
n-dodecane	BDH, purity 99%
n-hexane	Rathburn, Glass distilled grade
Toluene	Rathburn, Glass distilled grade
Isooctane	Merck, p.a.
Methylene-chloride	Rathburn, Glass distilled grade
Sulfuric acid	Merck, p.a. purity 95-97%
Anhydrous sodium sulphate	Merck, p.a.
Alumina B	ICN, Akt. 1, Ecochrom
Silicagel 60	Merck, 0.063-0.200 mm
Copper plates	Merck, purity 99.7%, 0.1 mm
Chem Tube-Hydromatrix	Varian
Single congeners of PBDEs	CIL 50 µg/ml in nonane, purity 99%

2.3 Wastewater extraction

PBDEs were extracted from 5-L water samples (pyrex flasks) including particles. The extraction procedure was as follows:

I. Samples were spiked with internal standards dissolved in acetone. The standards were 200 µL 24.76 ng / ml BDE77 and 200 µL 60 ng / ml $^{13}C_{12}$BDE209.

II. After ½ h of equilibration, samples were extracted with 3 × 100 ml toluene using vigorously magnetic stirring (note: The stirring is increased until emulsification starts).

III. Between each extraction, samples were transferred stepwise to a 1-L separation funnel. Water was poured into a new 5-L pyrex flask and the organic phase transferred to a 500 ml flask through glasswool and an excess of anhydrous sodium sulphate.

IV. Extracts were spiked with 100 µL of *n*-dodecane and concentrated to approx. ½ ml on a rotary evaporator.

Other solvents were tested during the method development. Results showed that methylene-chloride:hexane (1:1) and toluene gave comparable extraction efficiencies. However, the latter was preferred because methylene-chloride:hexane gave poor recovery in the extraction of sludge (<80%). Probably the extraction efficiency is less affected by increased amounts of particles using toluene than with methylene-chloride:hexane.

De Boer et al (2003) presented another approach for the analysis of wastewater, where the influent and effluent were filtered and only the residues analysed (de Boer et al., 2003). The analytical methods for sewage sludge described below could easily be extended to the analysis of residues after filtering. Because PBDEs are highly hydrophobic with log K_{ow} of 5.9-10 (tetra to deca-BDE), this would probably not change the analytical results significantly.

2.4 Sewage sludge extraction

The extraction procedure described by Christensen *et al* (2001) for sediment analysis were used with some modification for the extraction of sewage sludge (Christensen and Platz, 2001).

I. 5-g of sewage sludge (water content of approx. 80%) were mixed with sufficient Chem Tube-Hydromatrix (approx. 5 g) and allowed to dry for 2-h. Chem Tube-Hydromatix were added until a free flowing dry powder was obtained.

II. The dried samples were quantitatively transferred to a Soxhlet thimble and internal standards were added. 400 µL of 24.76 ng / ml BDE77 and 200 µL 300 ng / ml $^{13}C_{12}$-BDE209.

III. Samples were allowed to stand for 6 hours and was then soxhlet extracted for 24 hours at 75°C using 400 ml of hexane:acetone (4:1).

IV. 100 µL of toluene was added as keeper and the extract was concentrated to approx. ½ ml on a rotary evaporator.

During method development the extraction efficiencies were compared for several solvents. Toluene and hexane:acetone (4:1) gave comparable extraction efficiencies for tri-decaBDEs and sludge from Bjergmarken and Avedøre, respectively. The latter was preferred because it is used regularly in our laboratory for the analysis of PBDEs in biota and sediments (Christensen et al., 2002;Christensen and Platz, 2001).

2.5 Cleanup

The crude extracts require a cleanup procedure because other compounds, e.g. humic acids, polars, lipids are co-extracted with the PBDE analyts. The cleanup procedure for wastewater and sewage sludge was identical. Cleanup was performed through a multilayer glass column (1 (i.d.) × 20 cm) packed from the bottom with:

- 5 g alumina B activated at 160°C for 24 hours and deactivated with 10% water
- 1 g silicagel activated at 160°C for 24 hours
- 5 g silicagel activated at 160°C and impregnated with concentrated sulfuric acid (30 g silicagel + 10 mL 98% sulfuric acid)
- 1 cm anhydrous sodium sulphate.

Each sample was applied to the column with 3 × 1-ml hexane and eluted with 200-ml hexane. The extract was concentrated to approx. ½ mL on a rotary evaporator with 300-µL toluene added as keeper. Wastewater extracts were adjusted to 1-ml and sewage sludge extracts to 2-ml with toluene. 100 µL of 80.3 ng / ml BDE71 was added to the wastewater extracts as external standards, and the double amounts to the sewage sludge extracts.

Amber glassware or glassware wrapped in alumina were used throughout the analyses. UV-radiation was blocked by installing UV-filtering foils on the windows and on lamps in the laboratory. This was done to prevent photodegradation of BDE209. Recent laboratory studies have shown that BDE209 in toluene and applied to silica gel is photolytically debrominated by UV-light to lower brominated PBDE congeners. The half-life in toluene was less than 15 min (Sellstrom et al., 1998).

2.6 Gas chromatography - mass spectrometry (GC/MS)

Extracts were analysed by a HP-6890 GC (Agilent Technologies) coupled to a HP-5973 quadropole mass spectrometer (Agilent Technologies). Tri-hepta BDEs were analysed using a DB-5 (methyl + 5% phenyl) 60 m × 0.25 mm × 25 µm high-resolution capillary column (J&W Scientific). Splitless injection with inlet temperature of 270 °C was used. Selected samples were analysed by high resolution MS using the same GC parameters and MS parameters by (Vikelsøe et al., 2002).

Temperature program: Tri-hepta BDEs (60 m DB5 column)

Rate (°C/min)	Temp (°C)	Time (min)
	90	2
50	220	0
5	300	20

The decabrominated congener (BDE209) was analysed on a DB-1 (dimethylpolysiloxane) 15 m × 0.25 mm × 10 µm high-resolution capillary column (J&W Scientific). Helium was used as carrier gas with a flow of 1.2 ml/s.

Temperature program: BDE209 (15 m DB1 column)

Rate (°C/min)	Temp (°C)	Time (min)
	90	1
30	220	0
10	300	8

A short capillary column is necessary to minimise thermal degradation of BDE209 by long exposures to elevated temperatures in the GC oven (de Boer et al., 2003). The mass spectrometer operated in negative chemical ionisation mode (NCI). Ion source temperature was 200°C and the quadropole temperature 150°C. Methane was used as the chemical reaction gas. The mass fragments were monitored by selected ion monitoring (SIM).

PBDEs listed in Table 2.2 were analysed as a part of the method development. Table 2.3 lists the internal and external standards used. Calibrations with 5-10 standards were applied for quantification of tri-hepta and BDE209. The concentration intervals depended on the matrix. For tri-hepta BDEs the calibration interval was 0.1 – 10 ng/ml for wastewater and 0.1 – 50 ng/ml for sewage sludge. For BDE209 it was 2 – 10 ng/ml for wastewater and 10 – 400 ng/ml for sewage sludge. Figure 2.1 shows the chromatogram of tri-hepta BDEs, and BDE209 including internal and external references.

Table 2.2 List of PBDEs. Quant. and Qual. denote the quantifying and qualifying ions used in the analyses

Compound	Abbr.	Quan.	Qual.
2,2',4-tribromodiphenylether	BDE17	79	159,161
2,4,4'-tribromodiphenylether	BDE28	79	159,161
2,2',4,4'-tetrabromodiphenylether	BDE47	79	159,161
2,2',4,5'-tetrabromodiphenylether	BDE49	79	159,161
2,3',4,4'-tetrabromodiphenylether	BDE66	79	159,161
2,2',3,4,4'-pentabromodiphenylet.	BDE85	79	159,161
2,2',4,4',5-pentabromodiphenylet.	BDE99	79	159,161
2,2',4,4',6-pentabromodiphenylet.	BDE100	79	159,161
2,2',4,4',5,5'-hexabromodiphenylet.	BDE153	79	159,161
2,2',4,4',5,6'-hexabromodiphenylet.	BDE154	79	159,161
2,2',3,4,4',5',6-heptabromodiph.	BDE183	79	159,161
Decabromodiphenylether	BDE209	486.5	484.5

Table 2.3 List of internal and external standards.

Internal standards	Abbr.	Quant	Qual
3,3',4,4'-tetrabromodiphenylether	BDE77	79	159,161
$^{13}C_{12}$-Decabromodiphenylether	$^{13}C_{12}$BDE209	494.5	496.5
External standards			
2,3',4',6-tetrabromodiphenylether	BDE-71	79	159,161

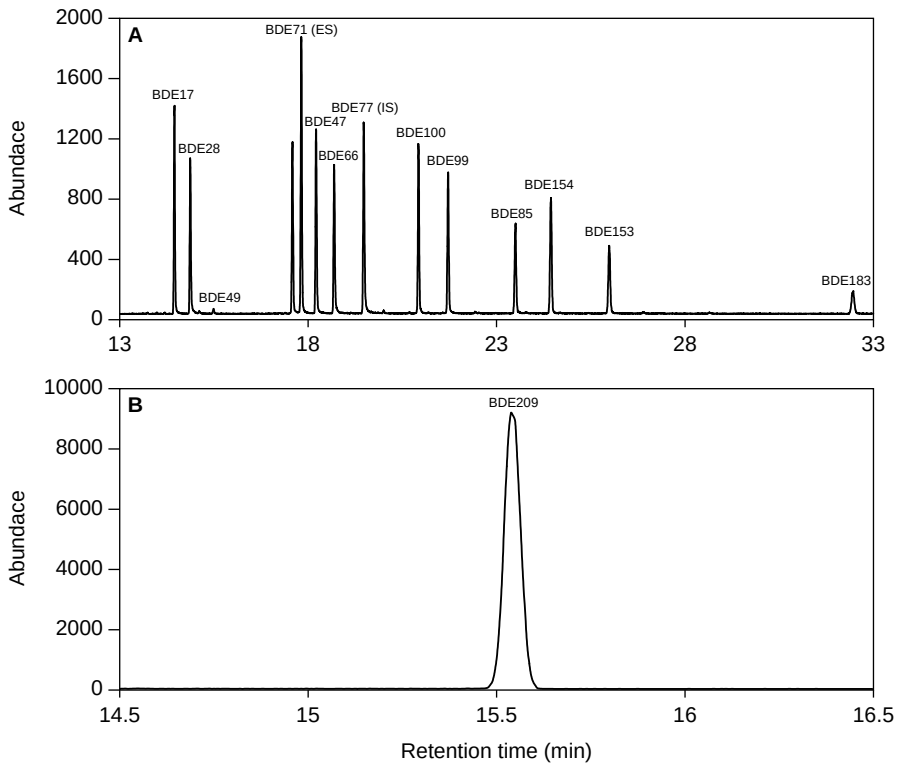

Figure 2.1 NCI-MS chromatogram of PBDEs; a) 60 m × 0.25 mm × 25 µm DB5 5 ng / ml solution of tri-hepta congeners (m / z 79), and b) 15 m × 0.25 mm × 10 µm DB1, extract of sewage sludge (m / z 486.5).

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3 Method validation

The two analytical methods; PBDEs in wastewater and PBDEs in sewage sludge are validated in this section. The linear response ranges for each compound listed in Table 2.2 were tested by duplicate analyses of 5-10 calibration standards. In Table 3.1 the linear response ranges and r^2 are listed. Because the GC/MS was unstable at the time of the method validation for tri-hepta BDEs the calibration standards were divided into two calibration curves. For quality control, each standard must be within 7.5% of the nominal value. In some calibrations the hexa and hepta congeners did not fulfil this criterion, which decreased the analytical quality of these congeners. A higher quality (lower repeatability) can be obtained with a more stable analysis. The variations were probably due to discrimination in the inlet, which is a general obstacle when analysing the heavy BDE congeners. Throughout the analysis, values were corrected for recovery, and blind values were not subtracted.

Table 3.1 Linear response ranges and r^2 for each of the 12 PBDE congeners. Picogram (pg) corresponds to ng/ml in injected vial because 1 µL was injected.

PBDE congener	Linear response ranges Wastewater / Sludge picogram injected (pg)	r^2
BDE-17	0.1 – 10 / 0.1 – 25	1.00
BDE-28	0.1 – 10 / 0.1 – 25	1.00
BDE-47	0.1 – 10 / 0.1 – 50	1.00
BDE-49	0.1 – 10 / 0.1 – 25	1.00
BDE-66	0.1 – 10 / 0.1 – 25	1.00
BDE-85	0.1 – 10 / 0.1 – 25	0.993 – 0.999
BDE-99	0.1 – 10 / 0.1 – 50	0.997 – 0.999
BDE-100	0.1 – 10 / 0.1 – 25	0.999 – 1.00
BDE-153	0.25 – 10 / 0.25 – 25	0.991 – 1.00
BDE-154	0.1 – 10 / 0.1 – 25	0.997 – 1.000
BDE-183	----- / 0.5 – 10	-----
BDE-209	2 – 10 / 10 – 400 ng/ml	1.00 / 0.99

The issues listed below were investigated for a) the wastewater method and b) the sewage sludge method.

- ✓ Limit of detection (LOD) and limit of quantification (LOQ)
- ✓ Precision
- ✓ Outline of QC procedure
- ✓ Recovery
- ✓ Interference
- ✓ Matrix effects
- ✓ Blank values

The limit of detection (LOD) and the limit of quantification (LOQ) as defined by IUPAC (1976) can be estimated in different ways. Here, LOD is defined as the signal to noise ratio (S/N) equal to 3 and LOQ as S/N equal to 10. The values were found by extrapolating results from, calibration standards, low level concentrations and spiked samples. The procedure was recommended by (Covaci et al., 2003). If the congeners were present in blanks LOD and LOQ were increased accordingly. Precisions are considered at a) repeatability conditions (i.e. same operating conditions over a short time) and b) at intermediate precision (within several laboratory variations). Here, the precision is given under repeatability conditions. A QC procedure was outlined (section 3.3) for the sewage sludge method using sludge from Bjergmarken STP collected in this study. The procedure can be used to determine the intermediate precision.

Recoveries for tri-hepta BDEs were determined from the internal standard BDE77 assuming that this congener is a good representative for the tri-hepta BDEs. In the sludge method the recovery of BDE209 was determined by standard addition. Here, 200 ng of the congener (corresponding to 100 ng/ml in the final extract) was added to the soxhlet thimble prior to extraction.

Interferences were investigated in two ways: a) by the use of qualifying ions and b) by the analyses of samples without addition of internal and external standards. In the first approach deviations in the isomer ratio of 20% was accepted. In the latter, interferences on the standards were investigated. Matrix effects were analysed by adding a calibration mixture to the extracts after extraction and cleanup.

3.1 Method validation - wastewater

Seven 5-L wastewater samples (effluent) from Bjergmarken STP and two blanks (deionised water) were used in the validation. Table 3.2 shows LOD and LOQ. Furthermore, the precision (under repeatability conditions) is shown together with recoveries determined from the recovery of BDE77. Blank values and possible matrix effects on BDE209 are shown in Table 3.3. Matrix effects have been proposed in the literature by Christensen et al (2001) (Christensen and Platz, 2001).

Table 3.2 LOD, LOQ, the relative precision, RV95% (95% CL with t = 2.447 for tri-hepta BDEs and 3.182 for BDE209 using a 2 tailed probability), and the recovery in % (Rec%).

PBDE congener	LOD (ng/L)	LOQ (ng/L)	RV95%	Rec%
BDE-17	0.015	0.02	66%	50-103
BDE-28	0.015	0.04	99%	50-103
BDE-47	0.08	0.1	30%	50-103
BDE-49	0.015	0.02	124%	50-103
BDE-66	0.015	0.02	-----	50-103
BDE-85	0.015	0.05	-----	50-103
BDE-99	0.04	0.05	43%	50-103
BDE-100	0.015	0.02	49%	50-103
BDE-153	0.015	0.05	-----	50-103
BDE-154	0.01	0.02	135%	50-103
BDE-183	0.3	1	-----	50-103
BDE-209	1.4	5	-----	-----

Three of the replicates were stored for 5 days prior to analysis. Hence, these samples were excluded from the validation of BDE209 since the results were strange.

Table 3.3 Blank values and possible matrix effects on BDE209.

PBDE congener	Matrix Rec%	Blank (ng/L)
BDE-17	-----	0.01
BDE-28	-----	0.02
BDE-47	-----	0.05
BDE-49	-----	0.01
BDE-66	-----	0.01
BDE-85	-----	0.005
BDE-99	-----	0.03
BDE-100	-----	0.01
BDE-153	-----	0.005
BDE-154	-----	0.005
BDE-183	-----	< 0.3
BDE-209	102 (±8)	1

The level of PBDEs in wastewater effluent was low and only BDE47, BDE99, and BDE100 were above the LOD in all replicate samples (see Table I in the appendix). Two samples had recoveries of approx. 50% whereas the remaining 5 samples had recoveries close to 100%. There was no interference on the standards.

3.2 Method validation - sewage sludge

Eleven sludge samples (5 g wet weight) from Bjergmarken STP, 2 blanks, and 2 sludge samples (2 g dry weight) from the 2003 QUA-

SIMEME exercise on brominated flame retardants were used in the validation. Three replicate samples from Bjergmarken were spiked in the soxhlet thimble with 200 ng BDE209 prior to extraction. These were used to determine the recovery of BDE209. In two samples standards were not added. These were used to determine possible interference on standards.

Table 3.4 shows LOD, LOQ, and the precisions. Blank values and possible matrix effects on BDE209 are listed in Table 3.5.

Table 3.4 LOD, LOQ, the relative sampling (S) and analytical (A) precision denoted as RV95% (95% CL with t = 2.571 using a 2 tailed probability), and the recovery in % (Rec%).

PBDE congener	LOD (µg/Kg)	LOQ (µg/Kg)	RV95% (S)	RV95% (A)	Rec%
BDE-17	0.03	0.1	51%	7%	106 (±7%)
BDE-28	0.15	0.2	84%	12%	106 (±7%)
BDE-47	0.8	1	11%	12%	106 (±7%)
BDE-49	0.03	0.1	12%	7%	106 (±7%)
BDE-66	0.03	0.1	19%	2%	106 (±7%)
BDE-85	0.08	0.25	46%	75%	106 (±7%)
BDE-99	0.03	0.1	9%	9%	106 (±7%)
BDE-100	0.03	0.1	9%	10%	106 (±7%)
BDE-153	0.08	0.25	44%	33%	106 (±7%)
BDE-154	0.03	0.1	36%	17%	106 (±7%)
BDE-183	0.5 – 2*	1 – 5*	-------	15%	106 (±7%)
BDE-209	3*	10*	33%	2%	102%

* These values are conservative and are only guidelines.

Table 3.5 Blank values, and possible matrix effects on BDE209. The blank values are calculated from 1-g dry weight.

PBDE congener	Matrix Rec%	Blank (µg/Kg)
BDE-17	------	< 0.1
BDE-28	------	0.1
BDE-47	------	0.5
BDE-49	------	< 0.1
BDE-66	------	< 0.1
BDE-85	------	< 0.25
BDE-99	------	< 0.1
BDE-100	------	< 0.1
BDE-153	------	< 0.25
BDE-154	------	< 0.1
BDE-183	------	< 5
BDE-209	107(±16)	1.2

The level of PBDEs in sewage sludge was high and both the analytical and sampling variation (95% confidence limit) was low especially for BDE47, BDE49, BDE66, BDE99, BDE100, and BDE209. The recoveries were close to 100%, and there was no interference on the standards. However, BDE71 could not be used as external standard for calculating the concentration of BDE209, because its response varied independently from that of BDE209 when there was a matrix present. Thus, it is essential that $^{13}C_{12}$BDE209 is used in the quantification of BDE209. Otherwise a large and variable bias will affect the data analysis.

3.3 Quality Control

Wastewater or sewage sludge samples are divided into batches with 10-13 samples. In each batch, one blank and two samples of laboratory reference material (LRM) are included. The LRM is sewage sludge from Bjergmarken STP collected in this study. More than twenty 100-g portions of the LRM are kept in the freezer in glass jars. Furthermore, in each batch a sample is analysed in duplicate. Intermediate precision is monitored for each congener by plotting the results of the LRM in control charts with warning and action limits (95% and 99.5% confidence limits).

The accuracy and overall quality of the analyses is monitored by regular participation in QUASIMEME intercalibration exercises on PBDEs in sewage sludge and other matrixes. Two sewage sludge samples from QUASIMEME 2003 on PBDEs in sewage sludge were included in the validation. However, the results from the exercise have not been received at the time of publication.

The use of GC/MS-NCI for detection of PBDEs demands thorough quality control and maintenance. The analytical quality (e.g. sensitivity, stability and chromatographic separation) is highly dependent on the cleanness of especially the inlet and the ion-source. Before each batch, the quality should be tested by a standard solution of tri-hepta BDEs and BDE209, respectively.

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4 Environmental PBDE concentrations

The PBDE concentrations measured in effluent wastewater and sewage sludge from Bjergmarken STP are shown in Table 4.1. Results from NCI and high resolution (HR) EI analysis were compared. Only the concentration of BDE66 was different on a 95% confidence limit. BDE209 was not measured in the HR method. Table 4.2 shows the concentration of BDE209 in the 2003 QUASIMEME sewage sludge.

Table 4.1 Environmental concentrations of the 12 PBDE congeners in effluent wastewater (ng/L) and sewage sludge (µg/kg on a dry weight basis). 95% confidence limits are given. Not analysed (n.a.).

PBDE congener	Wastewater (±95% CL) (ng/L)	Sewage sludge (±95% CL) NCI (µg/Kg)	Sewage sludge (±95% CL) HR (µg/Kg)
BDE-17	0.02 (±0.01)	3.0 (±1.5)	3.3 (±1.5)
BDE-28	0.02 (±0.02)	1.9 (±1.6)	2.2 (±0.9)
BDE-47	0.42 (±0.13)	96.8 (±10.7)	109.2 (±14.6)
BDE-49	0.02 (±0.03)	10.7 (±1.3)	11.5 (±2.6)
BDE-66	< 0.02	1.7 (±0.3)	3.1 (±0.9)
BDE-85	< 0.05	3.1 (±1.4)	2.3 (±0.6)
BDE-99	0.25 (±0.11)	86.2 (±7.7)	61.9 (±29.7)
BDE-100	0.06 (±0.03)	19.1 (±1.7)	13.8 (±5.4)
BDE-153	< 0.05	7.8 (±3.2)	5.7 (±0.5)
BDE-154	0.02 (±0.02)	6.1 (±1.7)	4.0 (±1.1)
BDE-183	< 1.0	2.0	< 5
Total tri-heptaBDEs	< 1.0	238 (±23)	217 (±24)
BDE-209	<1.4	248 (± 81)	n.a.

Table 4.2 The level of PBDEs in QUASIMEME sediment. The level of tri-hepta BDEs was generally, below the LOQ.

PBDE congener	Sewage sludge (Low-High) (µg/Kg)
BDE-209	149 (143-154)

The sewage sludge method is robust with high recoveries and precisions, and low detection limits that are relevant in environmental samples. The wasterwater method is less robust and only three congeners were found at levels well above the LOQ. The sum of triheptaBDE and BDE209 in sludge from Bjergmarken was 238 (±23) µg/Kg dry weight and 248 (±81) µg/Kg dry weight, respectively. These values are comparable or higher than those measured in Sweden and The Netherlands but below the values measured in North America. In the method development, PBDEs were measured in samples from Avedøre that receive more industrial wastewater compared to Bjergmarken. PBDE levels were as expected higher (approx. 1/3) in sludge from Avedøre than in sludge from Bjergmarken.

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7 Appendix

Table I Levels of 12 PBDEs (ng/L) in seven replicate samples of wastewater from Bjergmarken STP. Three replicates (*) was not analysed for BDE209

	A1	A2	A3	A4	A7	A6	A8
BDE17	0.020	0.020	<0.02	0.02	0.03	0.02	0.02
BDE28	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04	<0.04
BDE47	0.50	0.43	0.39	0.42	0.42	0.45	0.34
BDE49	0.020	<0.02	0.02	0.02	0.05	0.02	0.02
BDE66	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02
BDE85	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
BDE99	0.32	0.25	0.22	0.24	0.26	0.27	0.17
BDE100	0.08	0.05	0.05	0.06	0.06	0.06	0.04
BDE153	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
BDE154	0.02	< 0.02	< 0.02	0.02	0.03	0.02	0.01
BDE183	<1	<1	<1	<1	<1	<1	<1
BDE209	<5	<5	<5	<5	*	*	*

Table II Levels of 12 PBDEs (µg/Kg dry weight) in six replicate samples of sewage sludge from Bjergmarken STP

	A1	A2	A3	A4	A5	A6
BDE17	2.8	2.7	3.0	2.7	2.6	4.2
BDE28	1.6	1.6	2.1	1.6	1.6	3.2
BDE47	101.4	99.0	91.3	98.6	91.9	98.5
BDE49	10.8	10.8	10.3	10.7	10.2	11.6
BDE66	1.7	1.7	1.7	1.7	1.6	2.0
BDE85	3.3	3.6	2.9	3.6	2.2	2.7
BDE99	85.7	85.6	87.6	85.5	82.0	91.1
BDE100	19.4	19.1	18.9	19.2	17.9	19.9
BDE153	8.1	7.4	9.3	10.4	6.4	8.4
BDE154	6.0	5.8	7.1	7.7	5.4	6.9
BDE183	<5	<5	2.0	<5	<5	<5
BDE209	245	239	269	291	248	197

Table III Levels of 11 PBDEs (µg/Kg dry weight) in six replicate samples of sewage sludge from Bjergmarken STP measured by HR- MS.

	A1	A2	A3	A4	A5	A6
BDE17	2.8	4.4	3.3	3.2	2.8	3.2
BDE28	1.9	2.8	2.2	2.1	2.0	2.0
BDE47	118.0	110.7	112.1	104.5	102.0	109.5
BDE49	13.3	11.7	11.7	10.4	10.7	11.2
BDE66	3.2	2.7	3.7	3.0	3.2	3.1
BDE85	2.0	2.0	2.4	2.4	2.4	2.6
BDE99	48.6	47.4	61.5	69.8	74.2	69.9
BDE100	11.4	11.1	14.3	15.9	15.9	14.2
BDE153	5.4	5.6	5.9	5.8	5.5	5.9
BDE154	3.9	3.5	4.0	3.9	4.1	4.8
BDE183	< 5	< 5	< 5	< 5	< 5	< 5
BDE209	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

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