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Determination of polybrominated diphenyl ethers in human milk samples in the Czech Republic Comparative study of negative chemical ionisation mass spectrometry and time-of-flight high-resolution mass spectrometry

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Abstract

In the Czech Republic no study on the levels of brominated flame retardants in human milk has been conducted, yet. In the first step analytical method for determination of PBDEs in this bioindicator matrix was implemented. Liquid–liquid extraction (LLE) (hexane, diethyl ether), followed by gel permeation chromatography was employed for isolation of PBDEs. Identification and quantification of PBDEs was carried out by GC–MS operated in negative chemical ionisation (NCI). Two mass spectrometric technologies, one employing quadrupole and the other one high resolution (HR) time-of-flight (TOF) analyzer, etc. were used in our study. Detection limits (LODs) obtained by quadrupole analyzer ranged from 0.02 to 0.05 ng g^{-1} lipid weight, using high resolution time-of-flight analyzer LODs were significantly lower, ranging from 0.002–0.005 ng g^{-1} lipid weight, what enabled detection of minor PBDE congeners.

Within this pilot study 103 breast milk samples, obtained from mothers living in Olomouc region, were examined. Ten PBDE congeners were determined. All samples examined till now contained PBDEs residues, the dominating contaminant representing this group was congener BDE 47. In most of analysed samples levels of this compound ranged from 0.2 to 2 ng g^{-1} of lipid weight. Three exceptionally contaminated samples, containing levels of PBDEs 5–10 times higher than other samples, were found.

Keywords: Brominated flame retardants; Human milk; Analysis; PBDEs; TOF

1. Introduction

During the recent years the list of "classic" persistent halogenated contaminants has been expanded by several "new" compounds. One group of these xenobiotics is represented by brominated flame retardants (BFRs), which have been discovered for recent 25 years in the environment.

Flame retardants (FRs) are incorporated into potentially flammable materials, such as plastics, rubbers and textiles, to slow down and/or inhibit the initial phase of a developing fire [1]. Generally, two types of flame retardants can be distinguished. Reactive FRs, such as tetrabromobisphenol A (TBBPA), are incorporated into polymeric matrix employing covalent binding occurring between FR and matrix, whereas additive FRs represented by polybrominated diphenyl ethers (PBDEs) are only dissolved in polymeric materials [2,3].

Polybrominated diphenyl ethers (PBDEs) are chemicals widely used as flame retardants in many kinds of industrial products [1]. Theoretically 209 congeners of PBDEs exist with specific chemical and physical properties, which leads to various biological and toxicological effects. Only products based on penta-, octa-, and decabromo diphenyl ethers are used in practice [4]. They are currently used in covers of colour TV and personal computers, in electronic parts of the same instruments, in seats of cars and buses and in textiles [1–3]. PentaBDEs are mainly used in textiles and polyurethane foams, whereas decaBDE is used in textiles as well as in many other kinds of synthetic plastics such as polyester used for electronic circuit boards [3,5].

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Alike other organohalogen compounds such as polychlorinated biophenyls (PCBs), DDT and other organochlorine pesticides, PBDEs are lipophilic and very stable and resist to biodegradation [6], hence belong to persistent organic pollutants (POPs) category. Several pentaBDE congeners (BDE 47, 99, 153, etc.) have high potential for bioaccumulation/biomagnification [7]. In the recent years, the flame retardant pentaBDE has been phased out in the European Union (EU) following the results of the EU scientific assessment for pentaBDE [8].

Due to unavoidable emissions into the environment BFRs have been found throughout the world both in abiotic (air, sediment, sewage sludge) and biotic (fish tissue, bird eggs, marine mammals, food crop and terrestrial animals) compartments [5,9–11].

The presence of PBDEs in human milk (breast milk is commonly used to demonstrate exposure of humans to persistent organic pollutants) was reported for example in Sweden [9,12], Canada [13], Finland [14] or Japan [15]. Generally, humans can be exposed to PBDEs not only when working in production of PBDEs, but also on occasion of industrial applications or usage of PBDE-containing products and/or dismantling of electronic goods [16]. Possibly exposure may also occur through skin contact and via inhalation. As other persistent organohalogen compounds, PBDEs may enter the water systems and accumulate in the aquatic food web chain. Consumption of fish may constitute an important source of exposure for the general population [9].

PBDEs are suspected to be transferred via placenta and breast milk from the mother to the offspring in mammals [14] and the available data suggest that some of these congeners are potential thyroid disruptors and developmental neurotoxicants [17]. Other endocrine and genetic effects have also been observed. Therefore, mainly occurrence of PBDEs in mothers is of great concern relating to the healthy growth of foetuses and infants.

The reported methods used for isolation of PBDEs in human milk involve liquid–liquid extraction (LLE) using a mixture of solvents, such as hexane/acetone [12,13] or hexane/diethyl ether [8]. Fractionation of extract can be performed on silica gel [12,13] or multi-layer column [8]. In some studies sulphuric acid was used for purification of the extracts [12]. Extraction of PBDEs from human milk was also carried out with a mixture of lipophilic gel and formic acid [9]. The milk samples were incorporated into the gel (Lipidex 5000) by shaking the sample with the gel and formic acid for 2.5 h. The mixture was transferred to a glass column, and after elution of interfering compounds with water–methanol and methanol–dichloromethane–hexane mixtures, the analytes were then recovered with acetonitrile. For identification and quantification of PBDEs usually GC–MS technique, operated in electron impact (EI) or negative chemical ionisation, is used [8,9,12,13].

The main objective of this study was implementation and validation of analytical method suitable for determination of PBDEs in human milk samples. The performance characteristics of procedures employing either negative chemical ionisation (NCI) quadrupole MS or time-of-flight (TOF) high-resolution (HR) were critically assessed. Altogether 103 human milk samples were examined.

2. Experimental

2.1. Chemicals

PBDE standards, all with declared 99% purity, were purchased from Cambridge Isotope Labs. (USA). Working standard mixtures in isooctane contained following congeners: 2,4,4′-triBDE (BDE 28), 2,2′,4,4′-tetraBDE (BDE 47), 2,2′,4,5′-tetraBDE (BDE 49), 2,3′,4,4′-tetraBDE (BDE 66), 2,2′,3,4,4′-pentaBDE (BDE 85), 2,2′,4,4′,5-pentaBDE (BDE 99), 2,2′,4,4′,6-pentaBDE (BDE 100), 2,2′,4,4′,5,5′-hexaBDE (BDE 153), 2,2′,4,4′,5,6′-hexaBDE (BDE 154) and 2,2′,3,4,4′,5′,6-heptaBDE (BDE 183). PCB 112 was received from Dr. Ehrenstorfer GmbH (Germany).

Organic solvents (hexane, ethanol, cyclohexane, diethyl ether, ethyl acetate, and isooctane) were of HPLC gradient grade (Merck). Sulphuric acid (98%, Merck), Bio Beads S-X3 (200–400 mesh) were purchased from Bio-Rad Labs. (Richmond, USA).

2.2. Instruments

GC–MS analyses were performed on an HP 6890 gas chromatograph (Hewlett-Packard, CA, USA) coupled to an HP 5973 mass selective detector (Hewlett-Packard) equipped with quadrupole analyzer. The analyses were also performed on an HP 6890 gas chromatograph (Hewlett-Packard) coupled to a mass selective detector (GCT high-resolution time-of-flight mass spectrometer, Micromass, Manchester, UK).

2.2.1. GC–MS system with quadrupole analyzer

The GC conditions were as follows: DB-XLB capillary column (30 m × 0.25 mm i.d., 0.1 μm film thickness, J & W Scientific, Folsom, USA); column temperature program: from 110 °C (hold for 2 min) to 300 °C at 30 °C min^{-1}, and hold for 5 min; carrier gas: helium with constant flow 1.5 ml min^{-1}; injection temperature: 280 °C; injection volume: 2 μl using the pulsed splitless injection mode (splitless time: 2 min).

Mass selective detector with quadrupole analyzer was operated in a selected ion-monitoring mode (SIM) under NCI. The monitored ions (*m*/*z*) were 79, 81, 159 and 161. Ion at *m*/*z* 79 was used for quantification. Methane which was used as a reagent gas (purity 99.995%, Linde, Germany) was set at a pressure 2×10^{-4} mbar. Ion source temperature was 150 °C and quadrupole temperature 105 °C.

2.2.2. GC–MS system with high-resolution TOF analyzer

The GC conditions were as follows: DB-XLB capillary column (30 m × 0.25 mm i.d., 0.1 μm film thickness, J &

W Scientific, Folsom, USA); column temperature program: 110 °C (hold for 1 min) to 250 °C at 45 °C min^{-1}, then to 300 °C at 20 °C min^{-1} and hold for 5 min; carrier gas: helium with constant flow 1.5 ml min^{-1}; injection temperature: 280 °C; injection volume: 2 μl using the pulsed splitless injection mode (splitless time: 1 min).

Mass selective detector with high resolution time-of-light analyzer was operated in negative chemical ionization and ions at *m/z* 79.918 and 80.916 were used for identification and quantification of PBDEs. Methane was set at a pressure 2×10^{-4} mbar Ion source temperature was 220 °C.

2.3. Sampling

Samples of breast milk were obtained from 103 Czech women, living in Olomouc region, situated in the north–east part of the Czech Republic, in spring and summer 2003 in the co-operation with the Gynaecological-maternity Clinic, Faculty Hospital in Olomouc. The participating mothers in this study were from 17 to 39 years old (mean 28 years, median 26.5 years). The breast milk was expressed manually into the glass bottles and stored at −10 °C until analysis according to WHO methodology (WHO/EURO 1991) [18]. All the sample donors were healthy women and the breast milk was collected within the period of Day 3–8 from their first parturition.

2.3.1. Validation samples

Homogenized cow milk (2.6% fat) was fortified with the appropriate amount of PBDE congeners (in isooctane solution) in the preparation of validation samples. Spiked cow milk was used within the validation study instead of breast milk because of difficulty in obtaining larger volume of the latter matrix. Although some differences exist between these two matrices (e.g. in protein composition) no significant influence on the analytes in terms of their recovery and subsequent analytical steps were assumed. Actually, this generic approach is quite common, see e.g. study [19] concerned with occurrence of PCBs in breast milk.

Six spiked cow milk samples at level 1 ng g^{-1} lipid weight and 6 spiked lipid extracts (at level 1 ng g^{-1} lipid weight) isolated from cow milk were used for precision and recovery studies. For linearity study samples of lipid extracts were spiked at the levels 10, 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05, 0.025 and 0.01 ng g^{-1} lipid weight.

2.4. Analytical method

About 10 ml of human milk was weighted and transferred into 100 ml separating funnel. One millilitre saturated potassium oxalate was then added together with 10 ml of ethanol and 20 ml of hexane/diethyl ether (1:1, v/v). The extraction of target analytes was carried out according to the European Standard EN 1528-2:1996, Part 2 [20] which describes extraction of fat, pesticides and PCBs, and determination of fat content. The mixture was shaken for about 15 min and the bottom layer was removed into another separating funnel and another 10 ml of hexane/diethyl ether (1:1, v/v) together with 5 ml of ethanol was added. The lower layer was removed out and upper layer was put together with upper layer from the first funnel. Ten millilitre of water was added to this organic phase and lower layer was again removed. Organic phase was filtrated through anhydrous sodium sulphate and then the lipid content was gravimetrically determined.

Gel permeation chromatography (GPC) employing 500 cm × 8 mm i.d. column, containing Bio Beads S-X3 and mobile phase cyclohexane/ethyl acetate (1:1, v/v) with flow rate 0.6 ml min^{-1} was used for fractionation of lipids and target analytes. Approximately 150 mg of lipids dissolved in 2 ml of mobile phase were loaded into the column. Fraction 15–28 ml was collected and the eluate was evaporated by rotary evaporation and the remaining solvent was carefully removed by a gentle stream of nitrogen gas.

For clean-up also adsorption chromatography was tested. One hundred fifty milligram of lipids were applied on column filled with basic alumina and eluted with 190 ml of hexane. Eluate was evaporated to 2 ml and this volume was applied to second silica column. Elution was carried out with 11 ml of isooctane and 10 ml of mixture isooctane/diethyl ether (85:15, v/v).

The analytes were identified by retention times and by presence of *m/z* ions 79, 81, 159 and 161. For quantification multilevel calibration curve was used (at least five points for each analyte). The concentrations have been corrected for blanks and recovery (by using of PCB 112 added before GPC/adsorption chromatography as an internal standard). PCB 112 was used as the recovery standard because to our experience this compound has similar analytical properties as PBDEs involved in this study (e.g. the same elution profile on GPC). In addition, under normal circumstances PCB 112 does not occur in milk samples at detectable levels. Its use as recovery standard was reported also in other studies e.g. [22].

Recovery of analytical method was investigated with six spiked cow milk samples at level 1 ng g^{-1} lipid weight (recovery of whole analytical method) and six spiked lipid extracts (at level 1 ng g^{-1} lipid weight) isolated from cow milk (recovery of clean-up). The spiked samples were submitted to the same procedure, described above. Precision of analytical method (repeatability) was also determined, expressed as relative standard deviation, from analyses of 12 samples of spiked cow milk. Repeatability of GC–MS was estimated by six times injection of spiked lipid extract (1 ng ml^{-1} lipid weight) isolated from cow milk.

3. Results and discussion

3.1. Implementation and validation of analytical method

For isolation of PBDEs from human milk liquid–liquid extraction by hexane/diethyl ether mixture was used. Similar

extraction procedure was employed in other studies [8,12,13] concerned with determination of PBDEs in human milk.

A major concern in the gas chromatographic analysis of PBDEs is efficient removal of coextracted lipids from the crude extract. Two alternative techniques: gel permeation chromatography (GPC) and adsorption chromatography were tested. Adsorption chromatography is often used for fractionation of PBDEs [12,13] and in this study was tested the use of basic alumina, which is able to retain the most of lipids, residual lipids were removed in the second step on column filled with neutral silica. Although clean extracts were obtained in this way, the realisation of this step is laborious and time consuming, more than 2 h are needed for processing of one sample. In addition, the consumption of solvents is relatively high (about 200 ml). For these reasons we preferred the use of GPC, which is easily automated, only 40 min are needed for one sample and for particular size of column (500 cm × 8 mm i.d.) the consumption of mobile phase (cyclohexane/ethyl acetate, 1:1, v/v) was only 25 ml. Since residual lipids may penetrate into GPC fraction of analytes, their removal by concentrated sulphuric acid was needed. No decomposition of target analytes under these conditions was observed.

The precision of measurements (repeatability, expressed as relative standard deviation ($n = 12$)) and recovery of the overall analytical procedure and individual analytical steps is shown in Table 1. The concentrations of PBDEs congeners in (i) spiked cow milk samples (determination of repeatability and recovery of the overall method) and (ii) spiked lipid extracts (determination of repeatability and recovery of clean-up) were 1 ng g^{-1} lipid weight. In the determination of recovery, the spiked samples were extracted according to the described procedure (see Section 2.4).

As shown in Table 2 excellent linearity was obtained for all the analytes in the tested range, calibration points encompassed typical concentrations of PBDEs occurring in human milk samples.

The limits of detection (LODs) were determined from injection of spiked lipid extract (1 ng g^{-1} lipid weight) obtained from cow milk and are shown in Table 2.

Table 2
Linearity and detection limits (LODs) of GC–MS method using quadrupole analyzer (ng g^{-1} lipid weight)

Analyte	LOD	Range of linearity (regression coefficient)
BDE 28	0.02	0.06–10 (0.999)
BDE 47	0.02	0.06–10 (1.000)
BDE 49	0.02	0.06–10 (1.000)
BDE 66	0.02	0.06–10 (0.999)
BDE 85	0.03	0.09–10 (0.999)
BDE 99	0.02	0.06–10 (1.000)
BDE 100	0.02	0.06–10 (1.000)
BDE 153	0.03	0.09–10 (1.000)
BDE 154	0.02	0.06–10 (0.999)
BDE 183	0.05	0.15–10 (1.000)

Compared to major PCBs and some organochlorine contaminants (such as DDE) levels of PBDEs in human milk samples are typically lower by one order of magnitude. The detection sensitivity of conventional low resolution mass spectrometers, when operated in electron impact ionisation mode is, under common chromatographic conditions (splitless injection, capillary column 15–60 m, 0.1–0.32 mm i.d., 20–60 min temperature program), insufficient regardless of this selected ions are monitored (quadrupole analyzer) or MS/MS measurement is carried out (ion trap analyzer). Utilizing negative chemical ionization technique, monitoring of selective bromine ions *m/z* 79 and 81 generated from parent compounds enable significant decrease of detection limits. To get narrower peaks, hence increased S/N ratio, faster temperature program was set up. Further, decrease of GC analysis time was limited by potential co-elution of bromine-containing compounds as well as by detector acquisition rate; at most 3 scans/s can be obtained by quadrupole employed in this study. Chromatogram of milk sample obtained under optimised conditions is shown in Fig. 1a. Chemical noise in the proximity of target peaks (see e.g. BDE 49) does not allow lower LODs.

As a part of optimisation of PBDE analysis high-resolution TOF-MS (GCT, Micromass, UK) was used for quantification of target analytes. While for obtaining high signal

Table 1
Selected performance characteristics of analytical method using quadrupole analyzer

Analyte	GPC		Removal of residual lipids		GC–NCI–MS	Overall analytical method	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
BDE 28	90.9	4.9	100.5	2.5	4.5	90.5	7.3
BDE 47	89.9	6.1	100.0	2.7	5.4	85.3	9.5
BDE 49	93.3	4.7	99.1	3.6	3.1	95.6	7.9
BDE 66	94.3	4.5	99.6	3.2	5.1	86.1	4.8
BDE 85	89.6	7.9	99.5	3.0	2.6	97.6	12.2
BDE 99	93.4	4.7	100.5	2.4	6.8	90.7	5.3
BDE 100	98.4	4.9	99.5	2.4	4.8	110.9	9.5
BDE 153	91.9	10.1	100.0	3.3	3.2	91.4	8.2
BDE 154	98.5	7.9	100.7	3.2	3.2	105.1	9.1
BDE 183	94.4	6.1	99.1	3.0	4.6	93.0	6.2

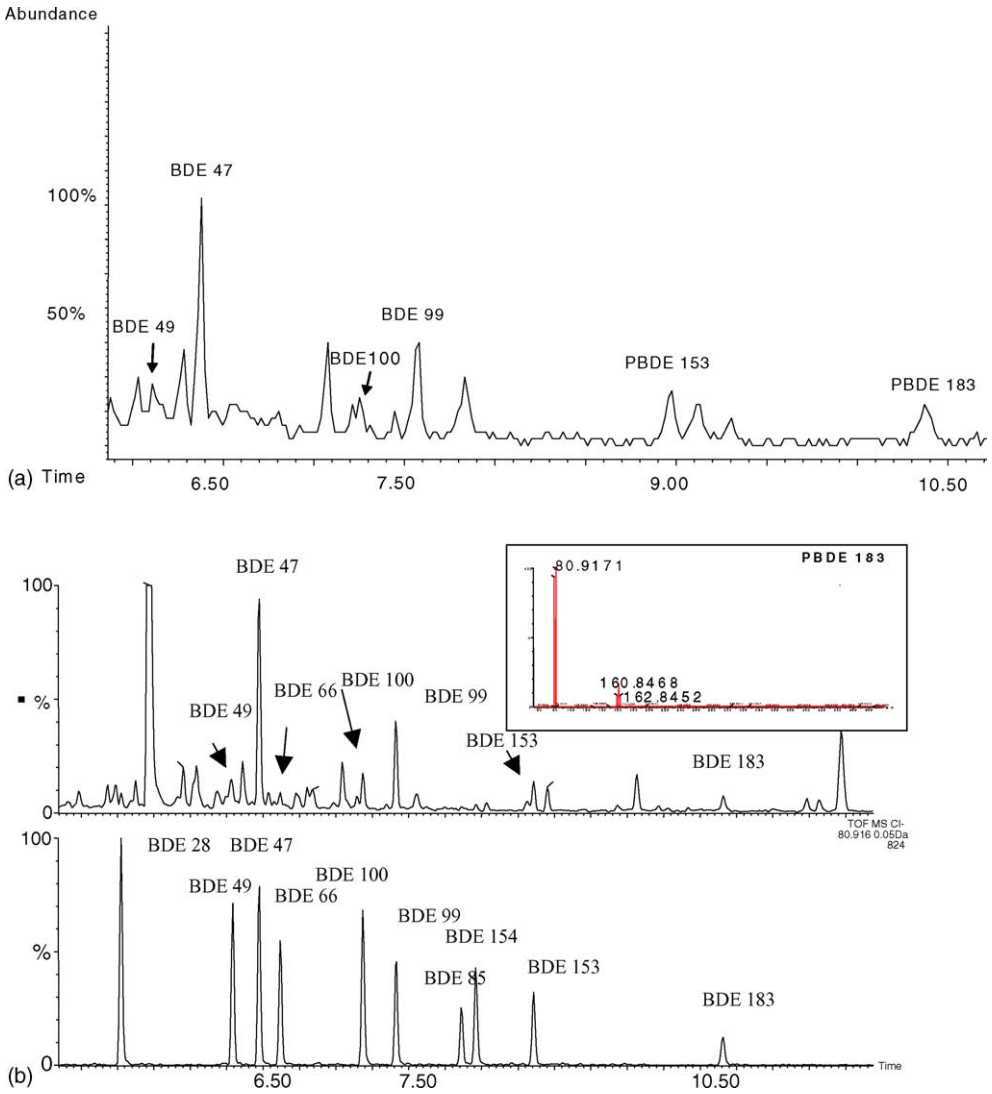

Fig. 1. (a) Chromatogram of milk sample (M 61) analysed by GC–MS with quadrupole analyzer operated in the NCI mode (injection equivalent to 1.2 mg of lipids); (b) chromatogram of milk sample (M 61) analysed by GC–MS with TOF analyzer operated in the NCI mode (injection equivalent to 0.6 mg of lipids) with NCI spectrum of BDE 183, time scale in minutes.

to noise ratio, hence low LODs by low (unit) resolution quadrupole MS analyzers, their operation in the SIM mode is needed, TOF as a non-scanning MS analyzer provides full spectral information even at very low concentration levels of analytes. Both electron impact and negative chemical ionization were tested. In the latter case significantly lower LODs were obtained, when negative bromine ion (*m*/*z* 80.916) was used for quantification of PBDEs.

In the case of high-resolution TOF-MS instrument the acquisition rate 2 spectra/s was selected as a compromise to achieve good detection performance characteristics together with sufficient repeatability of measurement (generally, the higher acquisition rate, the higher LODs).

Compared to routine measurements that were conducted by MS detector with quadrupole analyzer, high-resolution TOF-MS GCT instrument enabled achieving LODs by one order of magnitude lower (Table 3) and also minor

Table 3
Detections limits (LODs) (ng g^{-1} lipid weight) obtained by TOF-MS analyzer operated in negative chemical ionisation mode (NCI)

Analyte	LOD
BDE 28	0.002
BDE 47	0.002
BDE 49	0.002
BDE 66	0.003
BDE 85	0.003
BDE 99	0.003
BDE 100	0.002
BDE 153	0.002
BDE 154	0.002
BDE 183	0.005

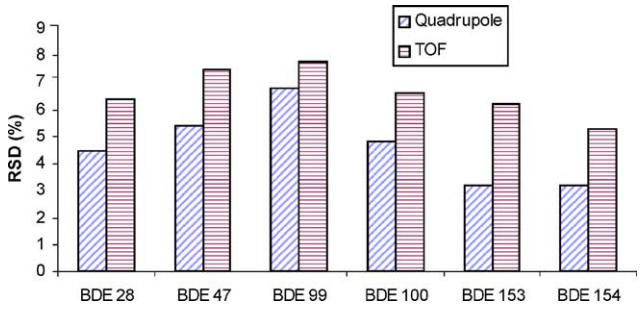

Fig. 2. Comparison of intra-day repeatability of GC–TOF-MS and GC–MS quadrupole analysis.

congeners, such as BDE 100, 153 and 183 could be quantified by using high-resolution TOF-MS (Fig. 1b). However, as shown in Fig. 1b, in spite of setting very narrow mass window (0.05 u), due to higher sensitivity of high-resolution TOF-MS technique many signals were still present in chromatogram. Examination of their spectra showed, that these signals correspond to brominated compounds, however their identification was, under our experimental conditions (NCI), impossible.

As regards to repeatability of measurements employing high-resolution TOF-MS, which was comparable with conventional GC–MS system (see Fig. 2) and was in range 4.9–8.2% for PBDEs at concentration level 1 ng g^{-1} lipid weight.

3.2. Human milk samples

In Tables 4 and 5, there are summarised levels of PBDEs obtained by GC–MS (quadrupole) analysis of 103 breast milk samples. The major congener in all human milk samples was BDE 47. This tetrabromo congener typically contributes with about 40–70% to the total content of PBDEs and was found in concentrations above LOD in all samples. This fact is not surprising, because BDE 47 is the main component of various technical mixtures used in industry as FRs and is also the major PBDE congener occurring in the environmental samples.

Other relatively abundant congeners presented in human milk samples were BDE 99, 100 and 153. Concentrations of these contaminants were above detection limit in more than 60% of the samples. Congeners BDE 49, 66, 85, 154 and 183 were detected randomly, approximately in 20% of the samples. BDE 28 was above the LOD only in seven samples.

The highest contamination of milk samples was found particularly in three samples (M 50, M 70 and M 85), in which the levels of PBDEs were 5–10 times higher than the median of data set. By using of *F*-test, these samples were identified as outliers.

No correlation was observed both for the lipid content ($r = 0.149$, $\alpha = 0.05$) or age of mothers ($r = 0.059$, $\alpha = 0.05$) and the levels of PBDEs in breast milk samples.

An inspection of individual PBDE patterns shows large inter-individual differences (an example is shown in Fig. 3). These findings are rather different from other environmental contaminants (e.g. PCBs), where those patterns exhibit only small variation through the general population [21]. While for many other halogenated POPs dietary intake is dominating, in the case of PBDEs dermal and/or inhalation exposures might be of great importance. Due to lack of information on particular habits of donors it is rather difficult to identify the major source of nursing mother exposure.

The absence of the mathematical relationship between the PBDEs content and age of the donors indicates that individual habits rather than a general exposure determine the body burden by PBDEs.

Table 4
Mean and median concentration levels of PBDEs determined in breast milk (ng g^{-1} lipid weight)

	Mean (range)	R.S.D. (%)	Median (5% percentile–95% percentile)
BDE 28	0.15 (<0.02–0.16)	76.1	0.07 (0.06–0.15)
BDE 47	0.86 (0.16–8.13)	159.9	0.61 (0.30–1.43)
BDE 49	0.08 (<0.02–0.18)	50.9	0.06 (0.06–0.15)
BDE 66	0.09 (<0.02–0.13)	29.9	0.08 (0.08–0.13)
BDE 99	0.28 (<0.02–2.21)	119.9	0.22 (0.10–0.52)
BDE 100	0.17 (<0.02–1.54)	136.8	0.12 (0.07–0.31)
BDE 153	0.19 (<0.03–1.14)	77.6	0.15 (0.12–0.35)
BDE 154	0.11 (<0.02–0.22)	65.5	0.09 (0.06–0.14)
BDE 183	0.28 (<0.05–0.61)	61.1	0.24 (0.18–0.42)

Table 5
Mean and median concentration levels of PBDEs determined in breast milk, three seriously outliers contaminated samples were excluded (ng g^{-1} lipid weight)

	Mean (range)	R.S.D. (%)	Median (5% percentile–95% percentile)
BDE 28	0.06 (<0.02–0.07)	18.2	0.07 (0.06–0.07)
BDE 47	0.65 (0.16–2.02)	48.2	0.58 (0.30–1.23)
BDE 49	0.06 (<0.02–0.08)	16.5	0.06 (0.06–0.06)
BDE 66	0.08 (<0.02–0.11)	11.9	0.08 (0.08–0.11)
BDE 99	0.24 (<0.02–0.70)	50.3	0.22 (0.10–0.44)
BDE 100	0.13 (<0.02–0.35)	42.4	0.12 (0.07–0.25)
BDE 153	0.17 (<0.03–0.54)	50.6	0.15 (0.12–0.34)
BDE 154	0.08 (<0.02–0.12)	27.1	0.07 (0.06–0.11)
BDE 183	0.26 (<0.05–0.41)	41.4	0.24 (0.18–0.34)

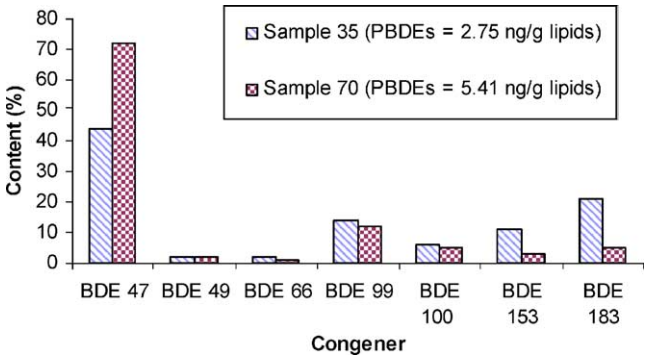

Fig. 3. Illustration of differences in PBDEs pattern determined in human milk samples.

Table 6
Comparison of mean levels of some PBDEs in human milk from Czech Republic with similar studies (ng g^{-1} lipid weight)

	BDE 47	BDE 99	Ref.
Sweden, 1998	2.52 (0.33–6.1) ($n = 39$)	0.72 (0.18–4.47)	[12]
Canada, 2000	3.39 (0.31–18.72) ($n = 10$)	1.19 (0.10–5.63)	[13]
Finland, 2000	1.31 (0.3–4.25) ($n = 11$)	0.39 (0.14–0.94)	[14]
Japan, 2000	0.48 (0.22–0.57) ($n = 6$)	0.11 (0.09–0.13)	[15]
Czech Republic, 2003	0.86 (0.16–8.13) ($n = 103$)	0.28 (<0.02–0.70)	This study

Since this is the first study concerned with levels of PBDEs in breast milk from the Czech Republic, no assessment of the time trends can be made. In Table 6, there are shown some results reported in similar recent studies carried out in other countries. The extent of contamination by PBDEs in Czech breast milk samples collected in 2003 is comparable to those reported in Japanese or Finland studies and is slightly lower than levels determined in the Swedish and Canadian samples.

4. Conclusions

In the present work analytical method for determination of PBDEs was implemented, validated and then applied for examination of about 100 human milk samples. Liquid–liquid extraction (hexane, diethyl ether), followed by GPC was employed for isolation of PBDEs. Identification and quantification of PBDEs was carried out by GC–MS operated in the NCI mode. Two mass spectrometric technologies, one employing quadrupole and the other one high-resolution TOF mass analyzer, were used in our study. Detection limits obtained by high-resolution TOF analyzer were by one order of magnitude lower than those obtained by unit resolution quadrupole analyzer. Detection of even minor congeners was possible under high-resolution TOF-MS conditions.

The present study confirms that several PBDEs accumulate in human body. The contamination of human milk with PBDEs is of great concern since these compounds may be transferred to an infant in this way. Assuming that an infant weighing 5 kg consumes 700 ml breast milk per day the average daily intake of PBDEs via milk can be estimated to be approximately 32 ng. Due to lack of comprehensive toxicological the health risk for infants can not be assessed at present.

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