ANALYTICAL METHODS FOR THE DETERMINATION OF POLYBROMINATED DIPHENYL ETHERS IN HUMAN MILK

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ABSTRACT

Polybrominated diphenyl ethers (PBDEs), which are used as flame retardants, are widely used additives so many different kind of materials that are consumed by public. Current researches reveal a significant increase in the levels of PBDEs in human biological fluids all around the world. According to International Agency for Research on Cancer (IARC), PBDEs are declarated as carcinogenic agents and additionally a considerable risks have been shown in animal studies such as liver damage, alteration of thyroid hormone levels, neurotoxicity and hazardous effects on development of fetus. Due to the simplicity of human exposure routes such as ingestion, inhalation or dermal contact, it gains high importance to quantify PBDEs in biological fluids. The exposure to PBDEs is critic aspecially during pregnancy, fetus development and infancy. Excretion of PBDEs in human milk is the focus area for researchers in recent years.

In this study, we reviewed current methodologies for the determination of PBDEs in human milk including sample preparation step, analytical procedure, validation parameters and observed levels of PBDEs in human milk. Because of the high physico-chemical stability of PBDEs, they have long persistence in human body. Besides, they reside in the lipid fractions of tissues because of their hydrophobic characteristics. Human breast milk has high lipid level, so that it is a suitable matrix for the accumulation and determination of PBDEs.

Keywords: PBDE, breast milk, analytical methods, extraction technique, organobromomine compounds, chemical pollutant.

1. INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) are organobromine compounds, with strong lipophilic property, and used as flame retardants since 1960s. PBDEs have been used in so many different kinds of products, such as electronics, furnishing, carpets, mattresses [1]. Due to their hydrophobic features they have long persistance in human body and accumulates in adipose tissues and body fluids which have high lipid content like breast milk.

The chemical structure of PBDEs was given in Figure 1. It is reported that there are 209 PBDE congeners depending on the number and location of bromine atoms in the molecule. Classificiations of commercial mixtures of PBDEs according to their average bromine content, there are three grades of penta-, octa- and decabromo diphenyl ethers (pentaBDE, octaBDE, and decaBDE, respectively) [2]. PentaBDE is the most commonly used as a flame retardant in flexible polyurethane foam for cushioning and mattresses. OctaBDE is used as a flame retardant mainly in plastic industry in electrical and electronic equipment such as computer casings and monitors and decaBDE is widely used in high impact polystyrenes and in electrical and electronic equipment (housings of computers, TV units, music systems etc), in the transportation industry and construction, building (i.e. wires and cables, pipes, carpets etc.) and moreover textile sector [3].
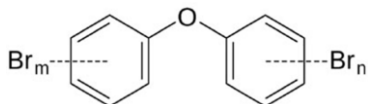
Figure 1. The basic structure of PBDEs.

These lipophilic compounds are the most widely found environmental pollutants. They are harmful for environment, all ecosystems and accumulate in organisms. The researchs indicates that these chemicals have toxicologic effects on liver, thyroid and neurological system [4]. The genotoxicity of decaBDE was conducted on test animals. However there is no enough data on the carcinogenicity of decaBDE in humans, on the basis of evidence for cancer in animals, decaBDE is classified as a possible human carcinogen by EPA [5]. It is also found out that neonatal exposure to decaBDE causes neurodevelopmental toxicity in mice.

PBDEs have long half lives so it is difficult to eliminate them from body. These chemicals accumulate in lipophilic parts of body [6–8]. In the last 30 years PBDE concentrations have increased in human breast milk, human blood and adipose tissues very fast [9]. To criticize the health of different populations, to evaluate environmental pollution and to protect babies from exposure, the concentration levels of these chemicals in breast milk have significant importance.

Biomonitoring/bioanalytical techniques are used to quantify the levels of PBDEs in human tissues and fluids. Because of the high chemico-physical stability of PBDEs, they have long persistence in humans. Besides, they reside in the lipid fractions of tissues because of their hydrophobic characteristics. Human breast milk has high lipid level, so that it is a suitable matrix for the accumulation of PBDEs. Measurements on human breast milk provides information about exposure to chemicals such as PBDEs for mother and baby, especially for nursing infants breastfed is the main source of PBDE exposure. Breast milk sample collection is very easy, and because of high lipid content, very low volumes of milk is sufficient for analysis. For instance, lipid content of breast milk is 10 times higher than blood, accordingly the volume of blood required for analysis is 10 times higher than milk volume.

2. MATERIALS AND METHODS

To determine PBDE in human milk provides clear explanation about human exposure and degree of PBDE intake by infant through breast milk and potential health risks associated with the intake of human milk. Gas chromatography-mass spectrometry (GC-MS) is the major method used for PBDE assays. Some gas chromatographic properties for analysis of PBDEs in breast milk were given in Table 1.

Comprehensive two-dimensional gas chromatography and isotope dilution time-of-flight mass spectrometry (GC-GC-IDTOFMS) for the simultaneous measurement of different types of pollutants including PBDEs in biomonitoring of human serum and milk (selected polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and brominated flame retardants (BFRs)) was developed [10]. Two dimentional GC provides the chromatographic separation of most of the compounds, and TOFMS allows sensitive quantitation with high resolution and high precision by using ^{13}C-labeled internal standards. The percent deviations of the two methods were found less than 20% for most of the analytes with lipid concentrations higher than 1 ng/g.

GC-MS technique for the measurement of PBDEs was developed using two mass spectrometric technologies, one of them combined to quadrupole and the other one with high resolution (HR) time-of-flight (TOF) analyzer and these methods were validated and then applied for the quantitation of nearly 100 human milk samples[11]. HR-TOF analyzer were significantly lower, ranging from 0.002 to 0.005 ng/g lipid weight, that enabled detection of minor PBDE congeners. The major congener in all human milk samples was found as BDE-47. In most of analysed sample levels of this compound ranged from 0.2 to 2 ng/g of lipid weight.

Table 1. Some gas chromatographic properties for analysis of of PBDEs in breast milk.

Sample	GC column	Temperature programme and Injection	LOD	Ref.
PBDE homologues (from mono- to hepta-BDEs)	A HP-5 ms (30 m x 0.25 mm i.d., 0.25µm film thickness) containing 5% phenyl methyl siloxane (model HP 19091S-433) capillary column	110ºC (held for 1 min) to 180ºC (held for 1 min) at 8 ºC/min, then from 180ºC to 240ºC (held for 5 min) at 2ºC/min, and then from 240 ºC to 280ºC (held for 6 min) at 2ºC/min Inj: 2 µL, splitless	The instrumental LODs were of 0.04 and 0.06 µg/l and method LODs were in the order of 0.01 and 0.05 µg kg^{-1}	Lacorte and Guillamon 2008
PBDEs; sum of eight congeners: BDE-28, 47, 99, 100, 153, 154, 183 and 209	A DB-5MS GC column (30-m x 0.25-mm i.d., 0.1-µm film thickness) The same but shorter column for BDE-209	Isothermal at 120ºC for 2 min, 15ºC /min to 230ºC, 5 ºC/min to 270ºC, 10 ºC /min to 330 ºC, which was held for 10 min. For BDE-209, the temperature was programmed as follows: isothermal at 120 ºC for 1 min, 30ºC/min to 330 ºC and held for 10 min. Inj:1 µL, splitless	-	Zhu et al. 2009
PBDEs and 11 hydroxylated (OH-) and methoxylated (MeO-)	For PBDEs, a 30 m DB5 HT column (0.25 mm i.d. -0.25 µ film thickness For OH- and MeO-PBDEs, a 15 m DB-5HT (0.25 mm i.d. -0.1 µm film thickness	For PBDE, The temperature program was: 100ºC (1 min), 2ºC /min to 140ºC, 4ºC /min to 220ºC, 8ºC /min to 330ºC (1.2 min). For OH- and MeO-PBDEs 110ºC (held for 1 min) to 220ºC (held for 1 min) at 18^{0}C /min, then from 220^{0}C to 240ºC (held for 2 min) at 8^{0}C /min, and to 300^{0}C (held for 10 min) at 8ºC /min Inj: 2 µL splitless	LODs calculated at a signal to noise ratio of 3 were between 12.5 and 197 pg g^{-1} lw for PBDEs and 3.59 and 20.4 pg g^{-1}lw for OH- and MeO-PBDEs	Lacorte and Ikonomou 2009
Tri- to deca-polybromodiphenylethers (PBDE)	A capillary column (15 m x 0.25 mm x 0.10 mm) coated with low bleeding diphenyl (5%)-dimethylpolysiloxane (95%) copolymer	The temperature gradient started from 120ºC (2 min), rose to 280ºC (10^{0}C/min) and then to 320^{0}C (20 ^{0}C/min, 8 min) Inj: 2 µL splitless	0.31 to 19.94 ng g^{-1} lw	Antignac et al. 2009
OCP, some nitro musks, indicator PCB, polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF)	A 30 m DB5 column (0.25 mm i.d. -0.25 µ film)	The applied temperature program was as follows: start with 90ºC, held for 1 min, increased up to 250ºC (temperature rate of 20ºC /min), held for 5 min, increased again up to 300ºC (temperature rate of 2ºC /min), and held for 6 min. Inj:-	Method limits of quantitation (LOQ) for PBDEs between 0.8 and 2.7 pg g^{-1}	Raab et al. 2008
PBDEs and hexabromocyclododecane	A DB-5 HT capillary column (15 mx0.25 mm id, 0.1-µm film thickness	60 to 320^{0}C at 150^{0}C/min and the oven from 80^{0}C (1 min), 15^{0}C/ min to 300^{0}C (16 min) Inj:-	The LOQ for BDE-47 (0.041 pmol), BDE-99 (0.01 pmol), BDE-100 (0.002 pmol), BDE-153 (0.005 pmol), BDE-209 (0.019 pmol) and HBCDD (0.007 pmol)	Fangstrom et al. 2009
PBDEs	A 60 m, DB-5 column coated with 5% diphenylpolydi-methylsiloxan (film thickness 0.25 µm, internal diameter 0.25 mm).	90ºC for 2 min, 10ºC/min to 150ºC, 4ºC/min to 310ºC, and held for 20 min Inj: 3 µL splitless	Limits of detection (LOD) and quantification (LOQ) were 0.001 and 0.002 ng/mL, respectively	Carrizo et al. 2007
PBDEs	DB-5-MS (30 m x250 mm i.d.; 0.25 mm film thickness)	Isothermal at 110ºC for 1 min, 15ºC/ min to 210ºC, 5 ^{0}C/min to 250ºC, 0.5 _C/min to 275 ^{0}C, and 15 ^{0}C/min to 295ºC, and held at 295^{0}C for 10 min. Inj:1 µL, splitless	The limit of detection (LOD) of individual PBDE and PCB congeners ranged from 0.01 to 0.07 ng/g for all congeners except for BDE-209. The LOD for BDE-209 was 1 ng/g	Ingelido et al. 2007

GC-HRMS method using a semiautomated extraction and cleanup method was improved for the analysis of some PBDE congeners (triBDEs to heptaBDEs), BB-153, and CB-153 in human milk samples, using a smaller sample size [12]. A relative standard variation of 7.9% was achieved for 130 measurements of the lipid ingredient in a human milk sample. The concentration of BDE-47 from Denver was 230 ng/g of lipid. The concentration of BDE-47 was 280 ng/g of lipid in the sample from California; the corresponding level for North Carolina was 64 ng/g of lipid. The results showed that geographic diversity caused differences in the grades of PBDEs in breast milk.

The levels of 1,1,1-trichloro-bis-2,2V-(4-chlorophenyl)ethane(p,pV-DDT) and its metabolites, hexachlorobenzene (HCB), hexachlorocyclohexane isomers (HCHs), chlordanes (trans- and cis-chlordane, oxychlordane and trans-nonachlor), 11 PCB congeners, and 7 PBDE congeners were determined in 37 individual breast milk obtained from Kahramanmaras region, Turkey [13]. PBDEs were identified just in 3 out of 37 samples, with the highest value being 0.014 ng/g ww (0.40 ng/g lipid weight) and BDE 47 was the predominant congener.

A multi-residue analytical method was improved for the measurement of the main representatives belonging to the three classes of BFRs (Hexabromocyclododecane (HBCD) , tetrabromobisphenol-A (TBBP-A), PBDEs) in different human natural forms such as serum, adipose tissue and milk [14]. For tri- to heptaBDE, the inter-day repeatability was globally in the 6-22%, accuracy deviations were lower than 10%, excepted for some congeners: BDE-25, 75 and 77 in milk. For decaBDE, the inter-day variability was not higher than 5%, accuracy deviation close to 5% was observed for milk.

PBDEs were identified in 2004 in 105 human milk samples obtained from 13 areas of Japan [15]. These 13 areas were determined to indicate present PBDE levels over the entire of Japan. Some congeners (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154) were analyzed by GC/MS. Total PBDE levels ranged from 0.01 to 23.0 ng/g lipid (geometric mean (GM), 1.34 ng/g lipid). BDE-47 (GM, 0.66 ng/g lipid, 59% of PBDE) was the most plentiful congener present in breast milk and was identified in 99% of the samples.

Seven PBDE congeners (BDE-28, -47, -99, -100, -153, -154, and -183) were determined using GC-MS in twenty-one-paired human fetal and maternal serum and 27 breast milk samples at South China [16]. The concentrations of total PBDEs ranged from 1.5 to 17 ng/g. BDE-47 and -153 were the dominant PBDE congeners in all samples and accounted for 60% of the total PBDEs.

The levels of OCPs and 11 PBDE congeners were measured using GC-MS method in the human milk samples in Poland [17]. The median of total PBDEs in human milk was 2.0 ng/g lw. PBDE levels in the Polish milk samples were found lower than those reported studies from other European countries. The gas chromatography with electron capture detection (GC-ECD) method using solid phase microextraction (SPME) technique with multiwalled carbon nanotubes (MWCNTs) as fiber coating was applied for the determination of trace PBDEs in river water, wastewater, and milk samples [18]. The PBDEs were detected in whole fat milk samples, ranging from 13 to 484 ng/mL. In a semi skimmed milk sample, only BDE-47 was found at 21 ng/mL.

Influence of breastfeeding in the accumulation of PBDEs during the first years of child growth were evaluated [19]. The concentrations of PBDEs in children at birth (cord blood sera, $n = 92$) and at the age of 4 years (sera, $n = 244$) from a cohort established in Menorca Island (Balearic Island, Spain) were studied. The higher concentrations of most BDE in breastfed 4 years old children versus those fed with formula indicated that breastfeeding during the nursing period was very relevant for PBDE intake in the first years of growth. The increases of average body burden of total PBDEs between birth and the first 4 years of growth were observed as 65 and 10 ng for breastfed and formula fed children, respectively.

In order to evaluate the levels of infant exposure to PCBs and PBDEs due to breast feeding in Italy, human milk samples in from the Venice and Rome were analysed to measure the levels of selected PCBs and PBDEs by high resolution gas chromatography coupled with low-resolution mass spectrometry (HRGC-LRMS) used in the selected ion monitoring mode (SIM) [20]. 11 PBDEs, comprising the relevant PBDE-47, PBDE-99, and PBDE-153, were determined. Σ_{11}(PBDEs) (ng/g fat) for the areas of Venice and Rome were respectively, 1.6–2.8 and 4.1.

In 2007, it was studied that how PBDEs in breast milk are associated with infant birth outcome and maternal menstruation characteristics [21]. The measurements of PBDEs in breast milk from central Taiwan were compared to those from other countries such as Japan, England, Sweden, the Faroe Islands, and China. Twelve congener levels of PBDEs (BDE-17, 28, 47, 66, 85, 99, 100, 138, 153, 154, 183, 209) in 20 breast milk samples were measured by GC-HRMS. The mean level of PBDEs in breast milk was 3.93 ± 1.74 ng/g lipid. The results indicated that increase in PBDEs was significantly associated with adverse birth outcome. The borderline correlations of PBDEs and the effects of menstruation were found, but there were no significant differences after maternal age, pre-pregnant BMI, and parity were adjusted.

The applicability of two different multidimensional gas chromatography (MDGC) techniques, heart-cut MDGC and GC-GC, both equipped with ECD, for the separation of PBDEs and PCBs has been evaluated [22]. The applicability of both separation techniques was shown using a human breast milk sample with low concentrations of PBDEs (1.2-41 pg/g fresh weight). The levels and accumulation profiles of tri- to deca- BDEs in serum (maternal, paternal, and umbilical cord), placenta, and breast milk samples in the population living in two different areas of Madrid (Spain) were investigated using GC-MS working in the electron capture negative ionization mode (ECNI) [23]. The median PBDE concentrations were found as 2.0-2.1 ng/g lw from tri- to hepta-BDEs and 5.5-6.1 ng/g lw when octa to decaBDEs are included. In breast milk samples, BDE 209 was found a predominant and BDEs 196 and 197 were detected in around 30%. The applicability of two different MS ionisation modes (Electron impact (EI) and ECNI) for the determination of PBDEs at low-trace levels in small-size (up to 1 mL of serum, 1.5 g of breast milk and 5 g of placenta) human samples was compared [24]. GC-ECNI-MS method was found more suitable for the determination of PBDEs, the degree of substitution ranging from tri- to deca-. Among all types of samples investigated, breast milk exhibited the highest median total concentrations (185 pg/g wet weight (w.w.), ranging between 36 and 3328 pg/g w.w.). EI-MS method more selective than the ECNI-MS method. Despite this, the selectivity of the ECNI-MS method was improved by using two ions of the [M−HxBry]⁻ cluster as both qualifier and quantifier ions. Pooled human milk samples (in total, 157 human milk samples collected in 2002 and 2003) obtained from mothers residing in 12 regions of Australia were analysed by HRGC/HRMS for 18 PBDE congener [25]. PBDE concentrations were 11.1 ± 3.2 and 11.0 ng/g lipid, respectively with a range of 6.1-18.7 ng/g lipid. Breast milk samples from 40 first-time mothers from the Pacific Northwest of the US (Montana, Oregon, Washington, and British Columbia) and Canada were analyzed for PBDEs and PCBs [26]. Total PBDEs were calculated by summing values for the 12 PBDEs congeners analyzed, ranged from 6 to 321 ppb (lipid weight). About 40% of the mothers had high (> 100 ppb) levels of PBDEs in their breast milk, and four samples had levels > 250 ppb lw. PBDE 47 was the dominant congener in most samples, whereas PBDE 153 was predominant in a few (3/40). The results showed that the lower brominated PBDEs were surpassing PCBs as major environmental concerns in North America.

PCBs and PBDEs levels were determined by HRGC-HRMS in milk from women living in the vicinity of a new hazardous waste incinerator (HWI) in Catalonia, Spain [27]. Mean PBDE concentrations were 2.2 and 2.5 ng/g fat for women living in urban and industrial zones, respectively. Dietary intake of PBDEs for a standard adult woman samples were 72 and 63 ng/day for residents in urban and industrials areas, respectively. The results showed that dietary intake was more relevant for human exposure to PCBs and PBDEs than living near the HWI.

Human breast milk samples collected during 2003/04 in Buryatia, a Russian autonomous republic, were analyzed in order to assess human exposure to organohalogen compounds including PBDEs [28]. PBDE concentrations were found as 0.46-1.7 ng/g lipid weight. The most common congeners detected were BDE-153, BDE-197, and BDE-207.

The concentrations of the eight PBDE congeners (BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, and BDE-209) in human milk were measured by GC-MS on a 7 m column [29]. The quantitative analysis was performed by isotope dilution method for BDE-209 and internal standard method for the other PBDEs. Lineer calibration range were found as 0.5-50 pg/g and 5-500 pg/g, for tri to heptaBDE standard solutions and BDE-209, respectively.

Temporal changes of OCPs and PCBs in Russian human milk samples were studied [30]. Levels of PBDE were low and dominated by the congeners BDE-47 and BDE-153. The deca brominated BDE-209 was detected in all analysed samples (median concentration 0.19 μg/kg lipid). The levels of 6 PBDEs congeners in 23 pooled breast milk samples from 205 mothers living in Beijing, China, were studied [31]. The breast milk concentration of PBDEs ranged from 0.68 ng/g lipid to 1.86 ng/g lipid. The study was reported to evaluate the association between PBDEs in breast milk and the seafood consumptions in Taiwanese general population [32]. The samples were analyzed using gas chromatograph with a high resolution mass detector. The results showed that body burden of PBDEs was significantly dependent on lower shellfish consumption. The highly frequent consumption of seafood was significantly correlated with the increased ratios of PCB-153/BDE-47, PCB-153/BDE-153, and PCB-153/PBDEs. The ratio of PCB-153/PBDEs might be evaluate as a new indicator of human exposure to these contaminants via seafood. Milk specimens from mothers of the general population of the Venice and Rome areas were collected over the 1998-2001 period, pooled, and analyzed for selected persistent organic pollutants such as PBDEs [33]. Concentration ranges of PBDE congeners 47, 99, 100 and 153 are, respectively, 0.55-1.9, 0.14-0.97, 0.15-0.48, and 0.41-0.60 ng/g lb. The results found to be in the range of values observed in the last decade in other European countries. A different behaviour was noticed for PBDE 153, whose concentration positively correlated with fish consumption as highlighted in other studies.

The concentrations on OCP, some nitro musks, indicator PCB, polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and PCB concentrations in human milk obtained at 12 weeks post-partum of 43 primiparous mothers residing in Bavaria were determined using HRGC-MS method [34]. The average and median value for the total of five PBDE congeners (BDE-47, BDE-99, BDE 100, BDE-153, BDE-183) were 1.90 and 1.62 ng/g lipid at 12 weeks p.p. and 2.03 and 1.64 ng/g lipid at 16 weeks p.p., respectively. Temporal trends of PBDE congeners and HBCD concentrations in human milk from Stockholm from 1980 to 2004 were evaluated using GC-MS method [35]. BDE-153 has continued to increase at least to 2001, after which it has stabilized in the mother's milk but it was not clear how the concentrations of this PBDE congener will increase over the next few years. BDE-47, -99 and -100 peaked 5 years earlier (1995) and are all decreasing. BDE-209 is not a suitable biomarker

for time trend studies according to the present results, showing no changes over time. A GC-MS method with negative chemical ionization were reported for the determination of 40 PBDEs from mono- to hepta-brominated in human milk [36]. The applicability of the method was examined empirically in five mothers milk samples, where only BDE 47 was detected at a maximum concentration of 10.45 µg/kg lipid weight. Levels and congener specific profiles of PBDEs in human breast milk from Nanjing and Zhoushan were evaluated using GC-MS procedure [37]. Residue levels of total PBDEs (sum PBDEs from mono- to deca-BDE) ranged from 1.2 to 15 ng/g lipid wt. with an arithmetic mean and median of 6.1 ng/g lipid wt. and 5.3 ng g¡1 lipid wt. respectively. The congener profiles were different from the general pattern found in human breast milk worldwide, indicating specific exposure of Chinese people to PBDEs.

The levels of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) were analyzed using GC-MS method in human milk obtained from women residing in the vicinity of a new hazardous waste incinerator (HWI) in Catalonia, Spain [38]. The current concentrations of PCDD/Fs in breast milk varied from 45 to 143 pg/g fat (2.8 to 11.2 pg WHOTEQ/g fat), while entire PCBs varied from 114 to 617 pg/g fat (2.8 to 17.6 pg WHO-TEQ/g fat). PBDE concentrations (sum 15 congeners) varied from 0.57 ng/g fat to 5.9 ng/g fat, with a mean value of 2.5 ng/g fat. The results of the study emphasized that, in terms of exposure to PCDD/ Fs, PCBs, and PBDEs, the HWI of Constantí (Tarragona County, Catalonia) does not have a important effect on the population living in the neighborhood.

Concentrations and congener special profile of DDTs, PCBs and PCDD/Fs have been analyzed in a number of Spanish breast milk samples [39]. The analysis of 9 PBDEs concentrations were performed using GC with an ion-trap detector (ITD)-MS in the MS-MS mode using the isotope dilution technique. The concentrations varied from 0.04 to 1.38 ng/g fat weight. The study was reported to determine the concentrations of OCPs, PCBs and BFRs in human milk of primipara mothers in order to examine the geographic and temporal trends of persistent organic pollutants (POPs) in Norway [40]. The PBDE pattern was dominated by PBDE-47 and PBDE-153. The median level of sum-PBDEs was 4.1 ng/g lw. PBDE-209 was detected in all analysed samples but in low concentrations (median 0.13 ng/g lw). The study was developed to measure concentrations of PBDEs and organochlorines (OCs) in human breast milk from Indonesia covering urban, suburban and rural areas [41]. PBDEs were detected in all the samples of the present study with total concentrations ranging from 0.49 to 13 ng/g lipid wt. Geographical distribution were evaluated, the results indicated that levels of PBDEs were relatively uniform ($p > 0.05$) and the levels were in the same order as those in Japan and some European countries, but were one or two order lower than North America. Three hundred and ninety-three human milk samples from mothers living in various regions throughout Norway were analyzed using GC-MS method [42]. The median concentrations of the sum of the seven most prominent PBDEs (BDE-28, 47, 99, 100, 153, 154 and 183), BDE- 209 and HBCD were 2.1, 0.32 and 0.86 ng/g lipids, respectively. The results show that sources other than the diet were important for the variability seen in breast milk PBDE concentrations.

An integrated evaluation of PBDE exposure and human body burden were conducted using 10 matched samples of human milk, indoor air and dust gathered in 2007-2008 in Brisbane, Australia [43]. PBDEs were detected in all matrices and the median concentrations of BDEs -47 and -209 in human milk, were: 4.2 and 0.3 ng/g lipid.

The breast milk concentrations of PBDEs were identified (by high resolution gas chromatographic high resolution mass spectrometry (HRGC-HRMS)) in samples from primiparous women gathered in 2006 in Tianjin, China [44]. The median total PBDE concentration (including BDE-28, 47, 99, 100, 153, 154, 183) was 2500 pg/g lipid weight, ranging from 1700 to 4500 pg/g lipid weight. There was an important relationship between a woman's PBDE concentration and the time she used electronic appliances. They found that electronics were a potential source of PBDEs to human.

A process-based in a pressurized liquid extraction followed by GC-HRMS was generated to ascertain PBDEs in underivatized milk extracts and OH- and MeO-PBDEs in the same derivatized extracts [45]. PPBDE concentration ranged between 1161 and 1372797 pg/g lw and BDEs 47, 99, 100, 153 and 183 accounted for more than 80% of the total PBDEs.

According to the regular input of PBDEs and OH- and MeOPBDEs, which was between 0.47 and 363 ng/day (excluding a smoking donor), potential health risks associated with these aggregates are evaluated. Simultaneous measurement of tri- to decaBDE from various human biological matrices (serum, adipose tissue and breast milk) were developed using GC-HRMS method [46]. The recommended methodology was utilized to approximately 90 biological samples (maternal and newborn tissues) obtained during caesarean deliveries. The obtained knowledge concerning the occurrence of highly brominated octa- to decaBDE particularly appears of worthy originality, the whole concentration identified for these compounds in adipose tissue and breast milk globally appearing to be in the same range than the concentration measured in the same samples for the lower brominated tri- to heptaBDE.

A study was developed to ascertain concentrations of insistent organohalogens such as PCBs, OC pesticides, PBDEs and HBCDs in human breast milk examples from primipara and multipara mothers [47]. The concentrations of PBDEs in human breast milk examples from the Philippines were higher than those in Japan and other Asian areas. The concentrations of the whole of the 12 congeners (Σ PBDEs) detected in breast milk samples altered widely, from 1.5 to 52 ng/g lipid wt., with an overall mean of 7.5 ng/g lipid wt.

GC-MS-ECNI method was developed for the concentrations of PCBs, non-ortho PCBs, PCDD/Fs, PBDEs and HBCD in individual milk samples and to investigate how the levels of these contaminants have changed among primiparous women between 1996 and 2006 [48]. The levels of PBDEs in mother's milk in Uppsala County in Sweden do not show any consistent trend during 10 years. The concentrations of BDE-47 and BDE-99 decreased, and concentrations of BDE-153 increased during the study period, while concentrations of BDE-100 did not change at all.

A study was developed for determination of the serum and breast milk concentrations of PBDEs in residents of the south coast area of Laizhou Bay and to explore their potential threats to human health [49]. The arithmetic means of ΣPBDE in breast milk was 81.5 ng/g lipid, respectively. BDE-209 was the predominant congener. The results showed that high exposures to PBDEs have led to very high PBDE concentrations in serum and breast milk from the inhabitants living in the south coast area of Laizhou Bay.

Another study was reported for determination of levels of thirty PBDE isomers PBDEs in Taiwanese breast milk [50]. The mean ± standard deviation of Σ PBDEs in breast milk is 3.59 ± 1.07 ng/g lipid. The results indicated that the main factors associated with Σ PBDEs in breast milk were age and education level among the binary variables of demographic, socioeconomic, and lifestyle characteristics in this study.

The concentrations of PBDEs in human milk from mothers in southern Taiwan between 2007 and 2008 were investigated [51]. Thirty congeners of PBDEs were determined to utilize a high resolution gas chromatograph with a high resolution mass spectrometer (HRGC-HRMS). The mean Σ PBDEs was 3.54 ng/g lipid. Σ PBDE levels in human milk were not significantly correlated with age and pre-pregnant body-mass index (BMI) of Taiwanese mothers. The higher hexaBDE level was significantly related to older age (> 29 years). There were no significant differences in PBDE levels among parity and ethnic groups.

Pre-treatment procedures for determination of PBDEs in human milk were given in Table 2.

To evaluated the temporal and spatial differences of contaminant profiles of PBDEs and PCBs, 42 human milk samples collected from three geographical places in Ghana (Accra (coastal) (n= 16); Kumasi (forest zone) (n= 14) and Tamale (savannah) (n= 12) in 2009 were analyzed by GC–MS [52]. The levels of PBDEs and PCBs ranged from 0.86–18 ng g^{-1} lw and 15–160 ng g^{-1} lw respectively in the human milk samples and the mean value of PBDEs and PCBs were of 4.5 ng g^{-1} lw and 62 ng g^{-1} lw respectively. Although Ghana was a non-industrialized country compared to many Asian and European countries, these results were unexpectedly high. The concentrations of PCBs and PBDEs were not statistically different between urban and rural locations in Ghana.

The analyses of seven PBDEs congeners in 24 pooled human milk samples comprised of 1237 different specimens from living in 12 provinces in China were implemented using HRGC/HRMS [53]. BDE-28, BDE-47 and BDE-153 were the dominant PBDE congeners, which accounted for approximately 70% of the total PBDEs, while the median value of $\sum_7$PBDEs concentrations were 1.49 ng g^{-1} lw. The consequences of the studies indicated that PBDE concentrations in human milk did not show a significant relationship with maternal age, food consumption and geographical location.

Table 2: Pre-treatment procedures for determination of PBDEs in breast milk.

Methods	Extraction	Clean-up	Recovery	References
Gas chromatography coupled to mass spectrometry with negative chemical ionization (GC–NCI–MS)	Pressurized liquid extraction (PLE) was used PLE parameters pressure = 1500 psi, temperature = 100 ^{0}C, preheat time = 0 min, heat time = 5 min, static time = 5 min, flush volume = 60%, purge time = 90 s, static cycles = 2 and hexane:CH_2Cl_2 (1:1)	The clean-up step was optimized using alumina SPE disposable cartridges of 2 and 5 g	Mono-BDEs were recovered in 60 % whereas from di to hepta BDE, acceptable recoveries from 70% to 131% were obtained with 5 g alumina clean-up.	Lacorte and Guillamon 2008
High resolution gas chromatographic high resolution mass Spectrometry (HRGC-HRMS)	The sample was Soxhlet extracted for 24 h with 250 mL of 1:1 hexane–acetone	The extracts were treated with acidified silica (50 g) and cleaned up by the Power-Prep system, a commercially available automated multi-column clean-up system	The average recoveries of each ^{13}C-labeled PBDE congener were about 80%, except for ^{13}C-BDE-209, which was 67%.	Zhu et al. 2009
GC–HRMS	PLE hexane:CH_2Cl_2 (2:1 v/v) at 2000 psi and at a temperature of 100 ^{0}C (100% flush volume) with a heat-up time of 5 min. Two cycles of extraction were performed during 5 min in static mode. The purge time was of 100 s and the extraction cell volume was of 11 mL.	Sulfuric acid	For PBDEs congeners, the average recovery was of 91%. For OH- and MeO-PBDEs, recoveries were between 71% and 102%.	Lacorte and Ikonomou 2009
GC-HRMS	For milk samples, liquid/liquid extraction using a dichloromethane/ acetone mixture	The PBDE fraction was further purified according to two SPE steps (Oasis_ HLB cartridge followed by a multilayer H_2SO_4 activated silica column).	70–120% range	Antignac et al. 2009
HRGC-MS	Homogenized breast milk samples were dried with 150 g anhydrous sodium sulfate in a glass column, which was filled with 1 cm sea sand layer. After 2 h, the lipid portion was extracted from the solid phase using a pentane/acetone mixture (50/50) (v/v)	The lipid was redissolved in 10 ml of n-hexane, The solution then was treated with sulfuric acid at 60ºC for 1 h. additionally purified with 1 g of silica gel in another micro column using n-hexane	The average recovery rate of PBDE congeners 45-98%.	Raab et al. 2009
GC-MS	Formic acid (1 mL) and 2-propanol (6 mL) were added to a milk sample (5 g), subsequently extracted with a mixture of n-hexane/diethyl ether (1:1)	Lipids were removed with concentrated sulfuric acid, cleanup was performed on two subsequently applied sulfuric acid/silica gel columns	The overall recovery for BDE-47, BDE-99, BDE-100 and BDE- 153 were about 90%. The overall recoveries of BDE-77 were 84%.	Fangstrom et al. 2008
GC-MS	Sera from cord blood was analyzed n-Hexane (3 mL) and concd H_2SO_4 (2 mL) were used for the extraction.	-	-	Carrizo et al. 2007
GC-MS	Hexane/methyl t-butyl ether (MTBE; 1:1)	Liquid-solid chromatography using a silica/sulfuric acid column (silica/sulfuric acid 2:1 by weight; 1 g). PBDEs were eluted from this column employing hexane (8 mL) as the mobile phase.	91% to 110%	Bi et al. 2006
GC-HRMS	Diethyl ether and hexane after addition of sodium oxalate and ethanol.	Cleaned by an activated alumina column Elution of PBDEs from the alumina column was done with 2% dichloromethane in n-hexane	Recoveries for internal standards were more than 60% for all congeners	Schuhmacher et al. 2009
GC–MS	Column extraction on the absorber, 40 g pre-cleaned diatomite earth and eluted with 250 ml of diethyl ether.	The extract was then subjected to gel permeation chromatography for lipid removal and eluted with mixture of 50% hexane/dichloromethane (1:1).	60% and 120%.	Malarvannan et al. 2009
GC-an ion trap detector (ITD)-MS in the MS-MS mode	Extraction step involved a matrix solid-phase dispersion of the samples in anhydrous sodium sulphate and silica gel	Acid and basic impregnated silica gel multilayer columns. n-Hexane was used as elution solvent.	For PBDEs recoveries were higher than 65%.	Bordajandi et al. 2008
GC–MS	Matrix solid-phase dispersion (MSPD)	For cleanup and lipid removal was performed by using acid and basic impregnated silica gel multilayer columns	Higher than 65%	Gomara et al. 2007a

Table 2: (Continued).

Methods	Extraction	Clean-up	Recovery	References
heart-cut MDGC-ECD and GC-GC-lECD	Extraction of breast milk was done by matrix solid phase dispersion (MSPD). between sample were homogenised with 1:1 w/w silica gel/anhydrous sodium sulphate. The mixture was ground to a fine powder, loaded into a column and extracted with a mixture of acetone/n-hexane (1:1 v/v).	The clean-up step consisted in two multilayer columns filled with neutral silica, silica impregnated with sulphuric acid and silica modified with KOH.	-	Gomara et al. 2007b
GC–MS	Matrix solid phase dispersion (MSPD) was used. Fatty extract, containing PBDEs, was eluted using a mixture of acetone:n-hexane (1:1, v:v).	Clean-up and lipid removal was achieved by using acid and basic impregnated silica gel multilayer columns and n-hexane was used as elution solvent.	-	Gomara et al. 2007c
HRGC-HRMS	Hexane after the addition of acetone	A multi-layered silica column with a plug of glass wool, an activated silica gel, a silver nitrate silica gel (10%, w/w), and anhydrous granular sodium sulfate was used, and then passed through an alumina oxide column.	70 and 130%.	Koh et al. 2010
High resolution gas chromatography—high resolution mass spectrometry (HRGC-HRMS)	n-hexane	Silver nitrite silica gel chromatography (from the bottom 0.5 g of silica, 2 g of 10% AgNO₃– silica, 0.5 g of silica n-hexane and 10 % methylene chloride/n-hexane (150 ml) were used as elution solvents.	over 80%	Eslami et al. 2006
GC-MS	solid/liquid extraction with acetone/dichloromethane 1:1 (v/v) (12 + 6 mL) was realized Three milliliters of acetonitrile were added after same volume of n-hexane, and in total n-hexane extraction was repeated three times (3×3 mL).	Two SPE purification steps. First, a HLB cartridge was used in normal phase with 5mL n-hexane for the washing step and 5mL n-hexane/dichloromethane 1:1 (v/v) for elution. Secondly, a multilayer column packed with 1 g, 3 g and 4 g of neutral, 22% and 44% of sulfuric acid activated G60 silica, respectively, between two layers of anhydrous sodium sulfate was used only with n-hexane as washing and eluting solvent	For tri- to heptaBDE congeners, 60–110%	Cariou et al. 2005
Two-dimensional gas chromatography and isotope dilution time-of-flight mass spectrometry (GC-GC-IDTOFMS)	Matrix solid-phase dispersion using diatomaceous earth	A two-layered custom-made SPE cartridge (3 mL) packed with 100 mg of silica and 1000 mg of sulfuric acid silica. The elution of analytes was obtained by passing 11 mL of hexane through the cartridge.	-	Focant et al. 2004
GC-MS using two mass spectrometric technologies, one employing quadrupole and the other one high resolution (HR) time-of-flight (TOF) analyzer	Saturated potassium oxalate was then added together with ethanol and hexane/diethyl ether (1:1, v/v).	Gel permeation chromatography (GPC) employing 500 cm × 8mm 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 was used for fractionation of lipids and target analytes. Elution was carried out with isooctane and mixture isooctane/diethyl ether (85:15, v/v).	Ranged from 89.9 to 98.5	Kazda et al. 2004
GC-HRMS	Extraction is t performed using an automated modular solid-phase extraction system. The extraction procedure includes drying the sample on diatomaceous earth by pressurized nitrogen and eluting target analytes and lipids with dichloromethane.	A two-layered SPE cartridge (3 mL) packed with silica (0.1 g; activated 250ºC; top layer) and silica/sulfuric acid (1 g; 2:1 by weight; bottom layer); the two layers were separated by a polypropylene frit. The cartridges were conditioned with hexane	60 to 89% for the eight PBDE congeners; 74 and 113% were recovered for BB-153 and CB-153	Sjodin et al. 2004

Table 2: (Continued).

Methods	Extraction	Clean-up	Recovery	References
GC-MS	The extraction was performed with n-hexane / dichloromethane (5:1, v/v).	The organic layer was subjected to clean up onto a cartridge containing 8 g silica impregnated with concentrated sulphuric acid (44%, w/w).	75% and 85%	Erdogrul et al. 2004
GC-MS	0.15 M sodium oxalate solution, methanol, diethyl ether and n-heptane The upper, organic layer was transferred to a pre-weighted beaker that had been heated at 110°C for 1 h and then left to cool for 1 h in a desiccator prior to weighing.	The clean-up was performed on an automated solid phase extractor	Above 40% for all the individual samples (mean ~80%, RSD ~ 10%) above 20% for 13CBDE-209 in the subset of 46 samples (mean 53%, RSD ~30%).	Thomsen et al. 2010
GC-MS	The samples were subsequently extracted twice with hexane/methyl t-butyl ether (MTBE; 1:1).	The serum extract samples were treated with concentrated sulfuric acid and cleaned up by liquid–solid chromatography using a silica/sulfuric acid column (silica/sulfuric acid 2:1 by weight; 1 g). PBDEs were eluted from this column employing hexane as the mobile phase.	86.9%±4.4%	Jin et al. 2009
HRGC-HRMS	Breast milk lyophilizates were extracted by accelerated solvent extraction using the ASE 200TM.	Samples were clean up in two steps mixed silica gel column cleanup, and GPC column cleanup.	-	She et al. 2007
HRGC-HRMS	The proteins in the sample were denatured using potassium oxalate added directly to the sample. Liquid– liquid extraction was performed using 2:1 acetone:hexane	The extracts were then concentrated prior to clean-up on the Power-PrepTM system.	25–150%	Toms et al. 2009
GC-HRMS	A mixture of 45 mL of acetone and 15mL of milk sample was extracted with 15mL of n-hexane,	The extract was cleaned up as follows. The first cleanup involved treatment with concentrated sulfuric acid. The next cleanup procedure involved a multi-layered silica column packed sequentially with a plug of glass wool, 0.3mL of activated silica gel, 0.5mL of silver nitrate (AgNO₃) silica gel (10%, w/w), 0.3 mL of activated silica gel, 0.5mL of basic silica gel, 0.3mL of activated silica gel, 6.2 mL of acid silica gel, and 2.0 mL of anhydrous granular sodium sulfate.	70% and 130%.	Chao et al. 2009
GC-MS	hexane/methyl t-butyl ether (MTBE; 1:1).	Liquid-solid chromatography using a silica/ sulfuric acid column (silica/sulfuric acid 2:1 by weight; 1 g) was used for clean-up. PBDEs were eluted from this column employing hexane (8 mL) as the mobile phase	91% to 110%	Bi et al. 2006
GC-MS	cyclohexane and acetone (3:2)	ultra clean (purity 98.8%) concentrated H₂SO₄	83–109%	Polder et al. 2008
GC-MS	Diethyl ether.	The gel permeation chromatography (GPC) fraction (for lipid removal) containing PBDEs was concentrated and passed through 1.5 g of activated silica gel column with 5% dichloromethane in hexane for clean up.	60% and 120%.	Tsydenova et al. 2007
GC-MS	Hexane/methylene chloride (1:1, v/v)	A multilayer silica-alumina column (i.d. 1 cm), which consisted of sodium sulfate (2 cm height), 6 g alumina, 5 g acidified silica gel, and 2 g activated silica gel, and sodium sulfate (2 cm height)	61.5% and 108%	Liu et al. 2008

Table 2: (Continued).

Methods	Extraction	Clean-up	Recovery	References
GC-MS	Human milk sample was lyophilized and extracted with a solvent extractor.	Fat content was determined gravimetrically from an aliquot of the extract. The remaining extract was spiked with $^{13}C_{12}$-labeled PCBs, $^{13}C_{12}$-labeled PBDEs (5 ng each) and 10 ng of $^{13}C_{12}$-labeled HBCDs as surrogates and then subjected to gel permeation chromatography (GPC) for fat removal and eluted with a mixture of hexane/dichloromethane (1:1).	For 13C-labeled surrogates; 65–110% for PCBs, 78–110% for PBDEs and 103–112% for HBCDs.	Asentha et al. 2011
HRGC/HRMS	The samples were extracted with a mixture of 50% hexane/dichloromethane (1:1), using Soxhlet apparatus for 24 h. Gravimetric lipid determination was performed after solvent evaporation.	The lipid fraction was dissolved in hexane and removed by shaking with acid-modified silica gel at 60 ℃. The hexane extracts were concentrated prior to clean-up on a instrument with multiple silica columns and alumina columns.	–	Zhang et al. 2011
GC-ECD	Liquid–liquid extraction with hexane, acetonitril and ethanol.	The extract was cleaned up in a chromatographic mini-column 40 cm x 0.5 cm id. The column was packed with 2 g of activated florisil and topped with 1 g of anhydrous sodium sulfate. The extract was eluted with 30 mL of dichloromethane and n-hexane (1:9; v/v).	–	Hassine et al. 2012
LC-MS-MS and GC-NCI-MS	Extraction was performed by the pressurised liquid extraction method on a fully automated accelerated solvent extraction system.	Extraction techniques were applied as follow: dichloromethane/hexane (2:1) mixture as solvent extractor, temperature of 100 ℃. After extraction, the crude extracts were concentrated to 3 mL and then subjected to a purification step via acid attack with concentrated H_2SO_4.	-	Toms et al. 2012
UPLC-MS/MS and GC/MS	The samples were applied Soxhlet extraction with a mixture of n-hexane and acetone (1:1, v/v) for 20–24 h. After Soxhlet extraction, the crude extract was evaporated to dryness and the lipid content was determined by gravimetry.	The hexane extract was purified by a multi-layer silica column packed withanhydrous sodium sulfate, activated silica gel and acid silica gel.	75 %-125 %	Shi, Jiaoo et al. 2013
UPLC-MS/MS and GC/MS	Milk and serum samples were extracted using expedited solvent extraction procedure with acetone/hexane (1:1, v/v) and liquid-liquid extraction with methyl-tert-butyl ether/hexane (1:1, v/v), respectively.	The removal of co-extracted biogenic materials was succeed by gel permeation chromatography followed by sulfuric acid treatment. The fractionation of the PBDEs and HBCD/TBBPA was performed using a Supelco LC-Si SPE cartridge.	78.6 %-108.8%	Shi, Wang et al. 2013
LC-MS/MS and GC-MS	A mixture of acetone-hexane was used for extraction of milk samples. Then, solvent was evaporated. The lipid content was determined gravimetrically. The extract was then redissolved in hexane and the lipid was degraded and removed by partitioning with strong sulfuric acid.	In the cleanup procedure, the fraction not absorbed by the carbon column contains both the PBDEs and HBCDs although the HBCDs are a little more polar.	-	Ryan, Rawn et al. 2014
GC-MS	Sonication were used to homogenize for milk samples.	For cleanup step, potassium oxalate solution (8%, w/w), ethanol, and diethyl ether were added before extraction.	74.6 % ± 22.6%	Zhang et al. 2014
GC–NCI-MS	Extraction was applied three times with 20 mL of n-hexane, 5 mL of acetonitrile, and 1 mL of ethanol.	The extract was cleaned up in a Florisil mini-column chromatography and eluted with dichloromethane/hexane (1:9; v/v) mixture.	53 %-90 %	Ben Hassine et al. 2015

Table 2: (Continued).

Methods	Extraction	Clean-up	Recovery	References
GC–MS	The samples were transferred into Soxhlet apparatus through 2 h extraction with 100 mL hexane/acetone (1:2, v/v). The lipid content was identified gravimetrically on an aliquot of the extract.	The rest of the extract was purified by acidified silica (44%, w/w) and eluted with 20 mL hexane: dichloromethane (1:1, v/v).	For PCB 143, 86 % ± 6% ; For BDE 77, 93 % ± 10%,	Dimitriadou et al. 2016
GC-HRMS	Milk samples were extracted by using accelerating solvent extractor and the mixture of n-hexane and dichloromethane (1:1, v/v) was applied as extraction solvent.	The Power Prep instrument with multiple commercial columns was used for further cleanup.	44%–98% for PBDEs; 50 %–94% for PCBs.	Zhang et al. 2017
GC-HRMS	Pressurized liquid extraction (PLE)	A two-stage clean-up was applied: dialysis with semipermeable membrane (2 × 20 mL of hexane, 48 h duration with the exchange of the solvent after 24 h) and sorbent chromatography.	Recoveries for PBDEs 91 % - 115 % (RSD 2–17%); for AFRs 40% - 121% (RSD 8–21%).	Čechová et al. 2017
GC–MS	Soxhlet extraction was applied	GPC clean-up and sulfuric acid treatment	50 %–120%	Chen et al. 2019
GC-MS	Milk sample was put into extraction tube. 13C-labelled congener in toluene and ethanol was added in and the mixture vortxed.	Solid-phase extraction (SPE) glass column was preferred for clean-up step.	-	Matovu et al. 2019
GC-EI/MS	Milk samples were extracted through Pressurized Liquid Extraction using hexane and dichloromethane (3:1 v/v) at a temperature of 90 °C.	SPE cartridge (500 mg/3 mL Sigma Aldrich) was used for cleanup and target compounds eluted with 12 mL of 1:1 hexane/DCM.	-	Wemken et al. 2020

The levels of 10 PBDE congeners concentrations were measured using GC-MS method in the human milk samples (n = 36) collected in 2010 from primipara and multipara mothers from Bizerte [54]. The concentrations of all PBDE ranged from 2.5 to 22.6 ng g^{-1} lw in the human milk samples and the value of the mean and median were of 10.7 and 9.8 ng g^{-1} lw respectively. According to the results of the studies, there was no significant correlation between age of primiparous mothers and the concentrations of total PBDE.

To evaluated temporal trends of PBDE concentrations, 12 pooled human milk samples (in total, 4-10 human milk samples per pool gathered in 1993 and 2009) obtained from primiparae mothers living in Australia were examined by LC-MS-MS [55]. The concentrations of PBDE in human milk samples indicate a peak around 2002/03 (mean $\sum_4$PBDEs = 9.6 ng g^{-1} lw) and 2003/04 (12.4 ng g^{-1} lw) followed by a reduce in 2007/08 (2.7 ng g^{-1} lw) and 2009 (2.6 ng g^{-1} lw).

A study was developed to ascertain concentrations of BFRs such as TBBPA, PBDEs and HBCD in 103 human breast milk examples obtained from Beijing in 2011 [56]. The personal information of the donors from which the milk samples were obtained, such as dietary habits, socioeconomic and lifestyle factors, was obtained through surveys. The analyzes of the concentrations of PBDEs were performed using GC-MS and the values obtained from ∑PBDE ranged from 0.22 to 135.41 ng g^{-1} lw and the value of the median was 3.24 ng g^{-1} lw. BDE-209 was the predominant PBDE in the majority of the breast milk samples. There was no important relationship between BFR levels in milk and the mother's diet, place of residence, smoking habits, nursing time or computer use habits. Also, There was no meaningful relationship between PDFEs levels in milk samples and dietary habits, socioeconomic and lifestyle factors.

In another study, the level of PBDEs, HBCD and TBBP were analyzed by GC-MS and UPLC-MS/MS from 13 breast milk samples obtained from Beijing in 2011 [57]. ∑PBDE concentrations ranged from 0.41 to 8.98 ng/g lw in breast milk, which is higher than the TBBPA and HBCD concentrations. This is probably due to the higher amount of PBDE used in China. BDE-209 was the predominant congener in PBDEs and the median concentration in milk samples was 2.26 ng/g lw.

The method of GC-MS was developed for the measurement of concentrations of BFRs, PBDEs and HBCD in breast milk samples were obtained from persons residing in diverse areas across Canada mostly in the years 1992 to 2005 [58].

More than 300 milk samples were analyzed, with comparative samples obtained from inhabitants of Austin, Texas, USA in 2002 and 2004. The total PBDE values obtained from the milk samples collected from Canada in 2002-2005 show median levels of 20 μg/kg on a lipid basis, which is importantly higher than the 1980s and 1990s. In Canadian, the level of PBDEs appear to have reached a plateau in the 2000s and are presently exceeded only by the slightly higher levels in the USA, especially the state of California.

Another study was reported for temporal movements determination of PBDE concentrations obtained from human milk samples in 2006 (n = 16), 2008 (n = 13), 2010 (n = 21), and 2012 (n = 30) in Shanghai [59]. The PBDE concentrations were determined using GC-MS. PBDE concentrations reduced from 14.8 to 4.85 pmol /g lw in the period 2006-2012.

Human milk samples (in total, 36 human milk samples gathered in 2010) obtained from healthy mothers residing in Bizerte country from Tunisia were analyzed by GC–MS for 8 MeO-PBDEs congeners [60]. ∑MeO-PBDEs levels obtained from the milk samples ranged from 0.23 to 4.70 ng g^{-1} lw in the samples, with a mean and median value of 1.52 and 1.11 ng g^{-1} lw respectively. Although MeO-PBDEs levels were higher in primipara mothers compared to multipara mothers, no statistically significant difference was observed.

LC-MS/MS technique was produced for the measurement of concentrations of PBDEs, HBCDs, the HBCD degradation outcomes tetrabromocyclododecenes (TBCDs), and TBBP-A in human milk gathered in 2010−2011 from 10 first-time mothers from Birmingham, UK [61]. A total of 120 samples, 12 samples from each mother, were collected to assess whether the concentrations changed significantly in the first 12 months after birth. While the concentrations of PBDE, HBCD and TBBP-A in breast milk do not alter importantly during the first year of breastfeeding, the concentrations of the more insistent BDE-153 congener and HBCD degradation outcome TBCDs show a notable rise in the same period.

The levels of PBDEs and PCBs were measured using GC-MS method in the human milk samples (n = 87) collected between July 2004 and July 2005 from primipara (n = 34) and multipara (n = 53) mothers from Thessaloniki, Greece [62]. The median values of PBDEs and PCBs were determined 1.5 ng/g lw and 90 ng/g lw, respectively. There is a correlation between detecting PBDEs (especially BDE 47 and BDE 153) and women working in office environments.

The analyses of eight PBDE congeners in 63 breast milk samples were obtained from 43 healthy volunteer donors living in Shijiazhuang region, Hebei province in north China from 2014 to 2015 were implemented using GC-MS [63]. Breastfeeding from donors aged 21 to 39 years were collected in four specific periods: birth, after 1 month, after 3 months and after 6 months. The concentrations of ΣPBDEs ranged from 0.04 to 19.93 ng g^{-1} lw, with median and mean value of 1.21 and 2.72 ng g^{-1} lw. BDE-209 was the predominant congener. There is no correlation between the age of mothers and PBDEs concentrations. The median levels of all PBDEs were 0.74, 2.80, 2.43 and 0.90 ng g^{-1} lw in colostrum, milk sampled at 1, 3 and 6 months after birth, respectively. It is thought that excessive consumption of foods of animal origin after birth may cause an increase in PBDEs concentration in human milk.

A study was developed to determine concentrations of the legacy flame retardants (LFRs) such as PBDEs and hexabromocyclododecane (HBCDD) in human milk samples obtained in the UK in 2010 (n=25) and 2014-15 (n=10) [64]. The analyses of the samples were implemented using GC/MS followed by LC-MS/MS. The concentrations of BDE 47 and α-HBCDD, which are the most frequently LRFs, were determined as 2.8 and 2.1 ng/g lw, respectively (geometric mean = 2.1 and 1.7). According to the results obtained from the analyses, there was no significant temporal difference between BDE 209 and HBCDD concentrations in human milk sampled in 2010 and those obtained in 2014–15.

In another study, the analyses of 10 pPBDEs and 19 alternative halogenated flame retardants (AFRs) in 450 human milk samples across three European countries, representing northern, western and eastern Europe were performed using a GC [65]. Sums of median concentrations of the most commonly analyzed PBDEs were 2.16, 0.88 and 0.45 ng g^{-1} in Norway, the Netherlands and Slovakia, respectively. The sum of the concentrations of AFRs ranged from 0.14 to 0.25 ng g^{-1} lw in all countries.

Seven congeners of PBDEs and six indicator PCBs in 32 regional pooled human milk samples originating from 1760 volunteering primiparous mothers were analyzed by GC-HRMS [66]. The median of total PBDEs in human milk were in the range of 0.3 to 4.0 ng g^{-1}. The mean level of PBDEs was 1.5 ng g^{-1}. The total PCBs levels in human milk were in the range of 2.3 to 19.0 ng g^{-1} with a mean of 6.6 ng g^{-1}.

Level of 23 chemicals including the POPs, PCBs, PBDEs, DDT and dichlorodiphenyldichloroethylene (DDE) isomers concentrations in 21 breast milk samples collected from a different unidentified mother were analyzed using GC-MS [67]. To interpret the impact of pasteurization on human milk chemical contaminants, raw and pasteurized samples of breast milk were analyzed separately. 19 of 23 chemicals in all of prepasteurized milk and 18 of 23 chemicals in all of the pasteurized milk were detected, indicating that pasteurization had no effect presence of most of the chemicals.

In another study, the level of eight PBDEs and six novel brominated flame retardants (NBFRs) were tested by GC-MS from 111 human milk examples obtained from 37 Beijing mothers [68]. The total PBDE levels in human milk were in the range of 0.288 to 22.2 ng g^{-1} lw. BDE-209 was the predominant congener. Decabromodiphenyl ethane (DBDPE), as an NBFR, was detected to be the most plenty BFR in all tested human milk (median:5.96 ng g^{-1} lw), indicating that predominantly consumed BFRs in China have altered from PBDEs to PBDE substitutes. Despite these results, the risk evaluation assessed by the edge of exposure approach showed that dietary BFR input for nursing infants is unlikely to produce important health risks.

The levels of PBDEs concentrations were measured using GC-MS method in the human milk examples (n = 50) from healthy primiparous mothers who had lived in Kampala capital city (urban area) and Nakaseke district (a rural area) for the last five years [69]. The concentrations of all PBDEs ranged from 0.59 to 8.11 ng g^{-1} lw. PBDE levels in examples taken from the Kampala capital city were remarkably higher than those in Nakaseke.

To make a comparison with a study by Pratt et al [70], which included the determination of PBDEs concentration in breast milk obtained from Ireland in 2011, the human milk concentrations of eight PBDEs and BFRs were determined by using GC-MS in 16 pooled samples of human breast milk obtained from 92 Irish primiparas between October 2016 and April 2018 [71]. BDE-209 identified in 81% of the samples was the predominant PBDE congener in concentration.

The concentrations of BDE-47, -99, -100, -153 were statistically significantly lower than the Irish breast milk obtained in 2011. In opposition, BDE-209 concentrations were higher than in 2011.

3. CONCLUSION

Analysis of PBDE levels in breast milk provides a good marker of exposure. The aim of this review is to introduce the methods for PBDE analysis in human breast milk which were reported over the period 2004-2020. Studies for the determination of PBDE in human milk have been conducted worlwide. The results of the analyses provide information about the amount of exposure in a time period and geographical region. Besides, according to those analyses, it is possible to realize which factors effect human milk exposure to PBDEs.

GC is the most commonly used method for PBDE analysis because of its high resolution. Recently, two-dimensional GC technique has been prefable because this method provides much more information than frequently used (single-column) GC. Combination of single MS, tandem MS, and hybrid MS with GC provides succesful analysis in milk samples. Capillary GC offers high resolution because of high number of theoretical plates. With the use of multidimensional mode the resolution increases substantially. Despite the fact that ECD provides high sensitivity, a clear identification is not possible and misidentification easily occurs. MS detection strongly provides selectivity, notably clear identification and high resolution (with MS-MS, TOF MS and HRMS). Extraction, sample clean-up and fractionation, are important procedures before starting GC analysis of PBDEs. A currently developed technique called pressurised liquid extraction (PLE) is an effective, efficient and selective extraction and clean-up technique that provides processing of multiple samples in a short period of time (less than 1 h).

Existence of PBDE in human milk instruct to understand the relationship between the amount of chemicals in human milk and effective factors of exposure. Further researches are required to examine this relationship. Human breast milk is tend to be exposed to relatively low levels of PBDEs when compared with air, water or other environmental samples. It is required to develop rapid, sensitive, feasible, cost efficient methods for the analysis of PBDEs in human milk.

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