Environment International 166 (2022) 107375

Contents lists available at ScienceDirect

Environment International

journal homepage: www.elsevier.com/locate/envint

Full length article

Mixture of environmental pollutants in breast milk from a Spanish cohort of nursing mothers

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ARTICLE INFO

Handling Editor: Adrian Covaci

Keywords:
Human biomonitoring
Breast milk
Endocrine disruptors
Neurotoxicity
Early life exposure
POPs
Pesticides

ABSTRACT

Breastfeeding is one of the most effective ways to ensure child health and survival, with several benefits for both the infants and their mothers. However, breast milk can contain environmental pollutants with endocrine disruption capacity, neurotoxicity and/or potential to alter microbiota. Monitoring breast milk provides information on the current chemical exposure of breastfed infants and, in addition, on the current and historical exposure of nursing mothers. In this study, the levels of a wide range of pollutants were measured in breast milk of Spanish nursing mothers. Target chemicals were dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), oxy-chlordane, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), per- and poly-fluoroalkyl substances (PFASs) (including perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA)), chlorpyrifos, bisphenol A (BPA), tetrabromobisphenol A (TBBPA), and a number of toxic and essential elements. Traces of most chemicals were found. A correlation between the levels of some persistent organic pollutants (POPs) and maternal characteristics (age and body mass index) was observed, while smoking was associated to higher concentrations of some toxic elements. Higher levels of PCBs were detected in samples from Spanish primiparous mothers compared to non-Spanish multiparous women. Breast milk from low-income mothers showed higher content of DDT and DDE than high-income mothers. Although breastfeeding is clearly beneficial for babies, the exposure to this mixture of hazardous substances, as well as their interaction and combined effects must not be disregarded.

1. Introduction

Breastfeeding is one of the most effective ways to ensure child health and survival (WHO, 2022). Besides macronutrients, breast milk has other important constituents, some of them not present in infant formula. These include bioactive components (i.e., secretory immunoglobulin A (sIgA), α-lactalbumin, lactoferrin, and lysozyme), as well as growth factors (i.e., epidermal, neuronal, vascular endothelial growth factors) and immunological factors (Ballard & Morrow, 2013; Binte Abu Bakaret al., 2021). In addition, breast milk composition changes during the lactation period from colostrum and transitional milk to mature milk, as a way to adapt to the needs of the growing infant

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https://doi.org/10.1016/j.envint.2022.107375
Received 25 April 2022; Received in revised form 21 June 2022; Accepted 22 June 2022
Available online 25 June 2022

(Ballard & Morrow, 2013).

The World Health Organisation (WHO) has studied the short and long-term effects of breastfeeding on children's health and has stated that breast milk has a number of important benefits (Horta & Victora, 2013a,b). Breast milk protects from overweight/obesity, having a positive effect on intelligence quotient (IQ), since it increases children's capacity to learn, to think and to react since the neurological development is promoted (Horta & Victora, 2013a,b; Amiel Castro et al., 2021).

Breastfeeding has a preponderant role in child's immune system strengthening, preventing respiratory infections, anaemia, diarrhoea, and hypertension, and reducing colics (Horta & Victora, 2013b; Victora et al., 2016). In addition to facilitate digestion, breastfeeding also reduces malocclusion, since breastfeeding develops and strengthens the baby's oral structure (Victora et al., 2016). Some studies have also shown positive results for women who breastfeed, taking into account that this practice reduces the incidence of breast, uterine and ovarian cancer, osteoporosis, cardiovascular diseases and type 2 diabetes (Victora et al., 2016). Finally, breastfeeding is also positive for the post-partum weight loss (Lambrinou et al., 2019; Jayasinghe et al., 2021).

The composition of breast milk is adapted to the needs of the newborn. Considering that the nutritional needs of the mother during pregnancy and lactation are well covered, breast milk covers also all the nutritional needs of the infant during the first months of life. However, studies over the past four decades have shown that environmental pollutants accumulate in the food chain, remain in our bodies, and consequently, are also present in breast milk. Among these, persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), dioxins and furans, and toxic metals are commonly detected (Schuhmacher et al., 1999, 2013; Cardoso et al. 2014; Anadón et al., 2017; Lakind et al., 2018; Lehmann et al., 2018; Castro et al., 2021). These pollutants are not exclusive of breast milk, as traces of these chemicals can be also found in infant formulas and food (Lehmann et al., 2018; Gardener et al., 2019; Pereira et al., 2020; Macheka et al., 2021). Some substances exogenous to the human body, may have endocrine-disrupting properties, interfering with hormone action and affecting the normal functioning of the endocrine system (WHO, 2013). Exposure to these endocrine disruptors is of special concern in early life stages (Chemeck & Nevoral, 2019). Moreover, these chemicals can also affect infant microbiota and disrupt their essential role for wellbeing (Calatayud Arroyo et al., 2021). Some of these chemicals are also neurotoxic being infants especially vulnerable to their effects due to their immature blood - brain barrier and nervous system (Dórea, 2021).

Monitoring of breast milk provides information on current chemical exposure of breastfeeding infants and, in addition, on current and historical exposures of nursing mothers. Physical-chemical properties, structure and the similarity of these chemicals with endogenous molecules determine their affinity for lipids, as well as their distribution in the organism and capacity for accumulation (Atkinson & Begg, 1990). Diet, habits, lifestyle, and socio-economic status are factors influencing exposure to environmental pollutants (Aerts et al., 2019; Calatayud Arroyo et al., 2021).

The present study aimed at determining the concentrations of a range of environmental pollutants in breast milk samples of a Spanish cohort. The exposure of breastfed new-borns to these pollutants was also evaluated. Target chemicals included several POPs, such as dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), oxy-chlordane, 11 polychlorinated biphenyls (PCBs) congeners, 6 polybrominated diphenyl ethers (PBDEs) congeners, and 7 per- and poly-fluoroalkyl substances (PFASs), as well as chlorpyrifos, bisphenol A (BPA), tetrabromobisphenol A (TBBPA), and a number of toxic and essential elements. The associations between maternal characteristics, such as age, body mass index (BMI), parity, socio economic parameters, and smoking habits and pollutant levels in breast milk were also assessed.

2. Materials and methods

2.1. Participants and sampling

Pregnant women were recruited during the first trimester of pregnancy at Sant Joan University Hospital (Reus, Catalonia, Spain), as part of the HEALS European project (Martínez et al., 2018). In addition, other participants were recruited from breastfeeding support groups of the same medical centre. Milk samples were collected in cases of exclusive breastfeeding during different periods of lactation (<1 month old (n = 22), 1–6 months old (n = 29), >6–9 months old (n = 9)). The recruitment started in March 2016 and ended in June 2019, including a total number of 60 participants. Women were eligible to participate if fulfilling the following inclusion criteria: ≥18 years old, without difficulties of breastfeeding, without health problems or diseases and no communication problems. The Ethical Committee of Clinical Research of the Hospital (No 16–04-28/4aclaproj2) approved the study. A written informed consent was obtained from participants, being pseudo-anonymity assured assigning a participant number. The characteristics

Table 1
Characteristics of the nursing women participating in the study (n = 60).

Maternal age (Mean ± SD) (years)	34 ± 5	**Maternal education**	
18–30	n = 8 (13%)	Primary school	n = 7 (12%)
30–34	n = 21 (36%)	Secondary school	n = 19 (32%)
35–39	n = 23 (38%)	University	n = 34 (56%)
40–45	n = 8 (13%)	**Socio-economic status (€/year)**	
Number of children		Low level (<19,000)	n = 7 (12%)
1 (breastfeeding child)	n = 35 (58%)	Intermediate level (19,000–35,000)	n = 21 (34%)
2	n = 20 (33%)	High level (>35,000)	n = 32 (54%)
>2	n = 5 (9%)	**Maternal country of origin**	
Age of breastfeeding infant (month old)		Spain	n = 51 (85%)
<1	n = 22 (37%)	Other	n = 9 (15%)
1 to 6	n = 29 (48%)	**Active Smoking**	
>6	n = 9 (15%)	Non-active smoker	n = 59 (98%)
Age of the child 1 (years old)		Active smoker	n = 1 (2%)
1 to 2	n = 19 (32%)	**Passive smoking**	
3 to 6	n = 36 (60%)	Non-passive smoker	n = 53 (88%)
>6	n = 5 (8%)	Passive smoker	n = 7 (12%)
Age of the child 2(years old)		**Organic food consumption**	
8–9	n = 36 (60%)	Never	n = 15 (25%)
18–19	n = 24 (40%)	Sometimes	n = 29 (49%)
Maternal pre-pregnancy BMI*		Frequently	n = 10 (16%)
Underweight (<19 kg/m^2)	n = 3 (5%)	Always	n = 6 (10%)
Normal (19–25 kg/m^2)	n = 35 (59%)		
Overweight (>25–30 kg/m^2)	n = 17 (27%)		
Obese (>30 kg/m^2)	n = 5 (9%)		

* BMI: Body mass index. Child 1 is the immediately older child than breastfeeding infant. Child 2 are other children in case of more than two children.

of the selected women are summarized in Table 1. Between 60 and 100 mL of breast milk were obtained from each mother. Samples were collected in polypropylene sterile containers and immediately placed on ice until their arrival to the laboratory, where they were divided in aliquots and frozen at −80 °C.

2.2. Chemical analysis

The list of target organic pollutants determined in breast milked is summarized in Table 2. In turn, the following elements were also determined: aluminium (Al), arsenic (As), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), mercury (Hg), potassium (K), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), tin (Sn), uranium (U), and vanadium (V).

2.2.1. POPs

The sample preparation for POPs determination was performed according to a slightly modified version of the method described by Thomsen et al. (2010a). Briefly, frozen breast milk samples were defrosted and homogenised in an incubator at 37 °C; 2.5 g of sample was added internal standards (^{13}C-labeled) and extracted using a mixture of methanol, diethyl ether and heptane. The organic layer was transferred to a beaker and the extraction procedure was repeated twice. The solvent (combined from the three extractions) was evaporated. After weighing the lipid content, it was re-dissolved in a mixture of dichloromethane and heptane and subjected to clean-up using a sulfuric acid treated silica column. The final extract was concentrated under a gentle stream of nitrogen at 40 °C and recovery standards were added. The analysis was performed as described by Caspersen et al. (2016), except that gas chromatography triple quadrupole mass spectrometry was used for the detection. For dioxin-like PCBs: PCB-105, PCB-118, PCB-156, PCB-157 and PCB-167, toxic equivalents (TEQ) were calculated using the toxic equivalence factors established by the WHO (WHO-TEF) (van den Berg et al., 2006).

The concentrations of PFASs in breast milk were determined as previously reported by Thomsen et al. (2010b). After defrosting and homogenising the samples in an incubator at 37 °C, 200 μL of breast milk were transferred to a centrifugation tube and internal standards (1,2,3,4-^{13}C-PFOA, 1,2,3,4,5-^{13}C-PFNA, 1,2-^{13}C-PFDA, ^{18}O-PFHxS, and 1,2,3,4-^{13}C-PFOS) and acetonitrile were added to make up a total volume of 600 μL for precipitation of proteins. Subsequently, the solution was mixed using a whirl mixer. Samples were subsequently centrifuged, and the supernatant transferred to a glass autosampler vial, 500 μL of 0.1 M formic acid was added and mixed on a whirl mixer. Samples were analysed by column switching liquid chromatography triple quadrupole mass spectrometry. For quantification of PFOS, the total area of the linear and branched isomers was integrated.

Table 2
Organic chemicals determined in breast milk samples.

Abbreviation	Name
DDE	p,p'-Dichlorodiphenyldichloroethylene
DDT	p,p'-Dichlorodiphenyltrichloromethylmethane
HCB	Hexachlorobenzene
Oxy-CD	Octachlor epoxide, Oxychlordane
BDE-28	2,4,4′-Tribromodiphenyl ether
BDE 47	2,2′,4,4′-Tetrabromodiphenyl ether
BDE-99	2,2′,4,4′,5-Pentabromodiphenyl ether
BDE-100	2,2′,4,4′,6-Pentabromodiphenyl ether
BDE 153	2,2′,4,4′,5,5′-Hexabromodiphenyl ether
BDE-154	2,2′,4,4′,5,6′-Hexabromodiphenyl ether
PCB-105	2,3,3′,4,4′-Pentachlorobiphenyl
PCB-118	2,3′,4,4′,5-Pentachlorobiphenyl
PCB-138	2,2′,3,4,4′,5′-Hexachlorobiphenyl
PCB-153	2,2′,4,4′,5,5′-Hexachlorobiphenyl
PCB-156	2,3,3′,4,4′,5-Hexachlorobiphenyl
PCB-157	2,3,3′,4,4′,5′-Hexachlorobiphenyl
PCB-167	2′,3,4,4′,5,5′-Hexachlorobiphenyl
PCB-170	2,2′,3,3′,4,4′,5-Heptachlorobiphenyl
PCB-180	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl
PCB-194	2,2′,3,3′,4,4′,5,5′-Octachlorobiphenyl
PCB-209	Decachlorobiphenyl
PFOA	Perfluorooctanoate
PFNA	Perfluorononanoate
PFDA	Perfluorodecanoate
PFUnDA	Perfluoroundecanoate
PFHxS	Perfluorohexane sulfonate
PFHpS	Perfluoroheptane sulfonate
PFOS	Perfluorooctane sulfonate
BPA	Bisphenol A; 4-[2-(4-hydroxyphenyl)propan-2-yl]phenol
TBBPA	2,2′,6,6′-Tetrabromobisphenol A; 2,6-dibromo-4-[2-(3,5-dibromo-4-hydroxyphenyl)propan-2-yl]phenol
Chlorpyrifos	O,*O*-Diethyl *O*-(3,5,6-trichloropyridin-2-yl) phosphorothioate
MeHg	Methylmercury

2.2.2. Chlorpyrifos

For chlorpyrifos determination, breast milk (50 μL) was mixed with 150 μL of methanol containing chlorpyriphos-d_{10} (Neochemia GmbH, Bodenheim, Germany) as internal standard. The mixture was then vortexed and centrifuged for 5 min at 4 °C and 15,000 rpm, being transferred to a glass vial for analysis. The analytical determination was carried out by Ultra High Perfomance Liquid Chromatography (Agilent Technologies, UHPLC 1290 Infinity II Series) coupled to Triple Quadrupole mass spectrometry (Agilent Technologies QqQ/MS 6490 Series), operating in positive electrospray ionization (ESI). Mobile phase was a gradient between mobile phase A: water (LC-MS grade (Scharlab, Sentmenat, Spain) with 0.1% of formic acid (97.5–98.5%) (LC-MS grade (Sigma Aldrich, Darmstadt, Germany), and mobile phase B: 100% methanol (LC-MS grade (>99.97%) (Merck, Darmstadt, Germany) with 0.1% of formic acid. The column (Kinetex-EVO C18 (150 × 2.1 mm), Phenomenex) temperature was set at 40 °C and the injection volume was 20 μL.

2.2.3. BPA and TBBPA

BPA and TBBPA were determined according to a previously developed procedure (Martínez et al., 2018; González et al., 2019, 2020). For the free fraction of BPA and TBBA, 2 g of homogenized sample were used, while for the total BPA and TBBPA fraction, 2 g of sample were incubated with 40 μL β-glucuronidase solution 20,000 U/mL in 1 M ammonium acetate buffer pH 5.0, overnight at 37 °C. BPA was determined with a gas chromatograph (GC) 6890 (Agilent, Little Falls, DE, USA) coupled to a single quadrupole inert mass selective detector (5975B, Agilent) with an electron ionization (EI) chamber. Regarding TBBPA, a high-performance liquid chromatography (HPLC) system Waters Alliance 2695 (Waters, Milford) interfaced to a Quattro Micro triple quadrupole mass spectrometer (Waters, Manchester, UK) was used. In order to avoid any potential contamination, nitrile plastic gloves were used throughout the analytical work, and the use of plastic materials was avoided. Glass material was heated at 400 °C overnight prior to use. No contamination in analytical blank samples was observed. Relative standard deviations for both compounds were < 18%. Average recoveries were 88.5 ± 15.8% for TBBPA and 88 ± 5.1% for BPA, supporting the efficiency of the method.

2.2.4. Toxic and essential elements

The analyses of toxic and essential elements was described elsewhere (Martínez et al., 2019). Prior to utilization, all laboratory ware was cleaned with nitric acid (20% v/v) for 24 h and rinsed with ultrapure water to avoid contamination. All standards and reagents were of analytical (pro-analysis) or superior grade. Briefly, 0.50 mL of sample was digested with 5 mL of 65% nitric acid in hermetic Teflon for 8 h at room temperature, and 8 h more at 80 °C. The digestion result was filtered and made up to 25 mL with MiliQ water.

The content of most elements (Al, As, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Sb, Sn, Se, Pb, U and V) was determined by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer Elan 6000). The concentrations of Ca, K, Mg and Na were measured by induction coupled plasma with optical emission spectroscopy (ICP-OES, Perkin Elmer Optima 3200RL). Replicates of samples and blanks were also determined. A reference material, whole milk powder (SRM 1549a), was used to verify the accuracy of the method, obtaining recoveries ranging from 90% to 116%.

Total Hg and methyl mercury (MeHg) were determined according to the procedure described by González et al. (2021). Briefly, MeHg was extracted from 1 mL samples (only those with total Hg levels above 0.100 mg/kg ww)) with 10 mL of hydrobromic acid (47% wet weight (w/w)) and 35 mL toluene (99.8% w/w). After centrifugation (10 min; 10,000 rpm; 10 °C), toluene was removed with 6 mL cysteine aqueous solution (1% L-cysteinium) chloride in 12.5% anhydrous sodium sulphate and 0.775% sodium acetate (Merck, Darmstadt, Germany). Total Hg and MeHg were determined in samples of 100–300 µL by atomic absorption spectrometry (AAS), following the method 7473 of the US EPA (2007), using an automatic Hg analyzer (AMA 254, LECO, St. Joseph, Michigan, USA). Mercury concentrations were calculated from linear calibration (using at least five different standard concentrations), with an Hg (II) nitrate standard solution (1000 mg/L) dissolved in nitric acid (0.5 mol/L, Merck). The detection limit was 0.004 mg/kg ww., while the limit of quantification was 0.011 mg/kg ww. Accuracy was checked through the analysis of the certified reference material DORM-4 (fish protein certified reference material for trace metals, National Research Council Canada, Canada), obtaining recoveries of 88–89%, within the certified range. A minimum of three measurements (replicates) was performed per sample, being the results reported as mg/kg ww, according to sample moisture.

2.3. Chemical exposure trough breastfeeding

The exposure via breastfeeding of new-borns and infants to the environmental pollutants analysed in breast milk was calculated by applying equation (1), obtained from Martínez et al. (2019).

$$DailyIntake_{i,p} = C_i \times Imilk_p \quad (1)$$

where Daily intake$_{i,p}$ is the daily intake of the chemical *i* in the breastfeeding period *p*, C_i is the concentration of the chemical *i* in milk, and Imilk$_p$ is the daily amount of milk ingested per body weight in each period (mL/day/kg$_{bw}$). Data on milk intake were obtained from the US EPA exposure handbook (US EPA, 2011), with a monthly temporal resolution. The daily amount of ingested milk varied from 75 to 150 mL/day/kg$_{bw}$, for babies of < 1 month old, to infants of 9 months old, respectively.

The daily intake for infants was calculated for three periods (<1 month old, 1–6 months old, and 6–9 months old) in a probabilistic way using a Monte-Carlo simulation, propagating the variability and uncertainty of each parameter until 100,000 iterations (Martínez et al., 2018). Oracle Crystal Ball© software (version 11.1.2.4.850) was used.

2.4. Statistics

For data analysis, Statistical Package for the Social Sciences software (IBM SPSS; version 26) was used. Statistically significant differences of chemicals and elements in breast milk according to maternal characteristics of the cohort were set at a level of significance of 0.05 ($p <$ 0.05). To check the homogeneity of variances, the Levene test was performed. Subsequently, ANOVA or Kruskal-Wallis were applied. The relationships between chemical pollutants were conducted through a Spearman's correlation (bilateral) test. For calculations, when the concentration of a specific pollutant in human milk was below the limit of detection (LOD), a value of LOD/2 was assumed.

Lineal regression models of pollutant levels in breast milk according to maternal characteristics (mother age, mother nationality, passive smokers, BMI, educational level, annual income, organic products intake, and multiparous women) were assessed by using R software (version 4.1.1). General lineal regression models (GLM) were applied considering, as an independent variable, the most commonly detected chemicals in the breast milk samples from our cohort study population. A total of 58 participants out of 60 were included in this evaluation, as the information regarding 2 mothers was not complete. Fourteen GLM were constructed by including the following chemicals separately: DDE, DDT, HCB, OXY-CD, ΣBDEs, ΣPCBs, PFOS, chlorpyrifos, total BPA, total TBBPA, As, Cr, Mn, and Hg. The logarithm (log) of each chemical was used to perform the GLM. Independent variables -log(chemicals)- were considered normally distributed according to the central limit theorem (Kwak and Kim, 2017). Each GLM was adjusted through the Akaike information criterion (AIC), using the R package leaps (version 4.1.1). The β coefficient and confidence interval (CI), standard deviation (SD) and P-value were provided for each GLM.

3. Results and discussion

3.1. Pollutant levels in breast milk

The concentrations of all the target chemicals in breast milk samples given by volume and by amount of fat are shown in Tables 3 and 4, respectively. DDT and its metabolite DDE were detected in all samples, with concentrations ranging from 9.1 to 878 pg/mL (DDT) and 226 to 61,789 pg/mL (DDE). In turn, based on the fat content, the median concentrations of DDT and DDE were 2.0 ng/g fat (range: 0.5–50 ng/g fat) and 68 ng/g fat (range: 12–3,511 ng/g fat), respectively. Similar levels of DDT and DDE have been reported in other European countries (Table 5). An exception is Slovakia, where higher median levels of both compounds were detected (8.7 and 167 ng/g of fat for DDT and DDE, respectively) (Čechová et al., 2017). The UNEP/WHO Human Milk Survey Global Monitoring Plan (UNEP/WHO-GMP) for POPs under the Stockholm Convention reported median values of DDT and DDE of 11.13 and 242 ng/g fat, respectively, in Spain, in 2002 (Hůlek et al., 2014). HCB was also detected in all the analysed samples, with a median concentration of 308 pg/mL (11 ng/g fat) and a range of 64–1,254 pg/mL (2.9–42 ng/g fat). As for DDT and DDE, similar levels of HCB in breast milk were recently found in other European countries (Sweden, Norway and Slovakia) (Table 5). In Spain, the UNEP/WHO-GMP for POPs found in 2002 higher HCB levels (75 ng/g of fat) (Hůlek et al., 2014) than those observed in the present study. However, HCB levels in Spain found in the current investigation were higher than those found in Slovenia, Belgium and the Netherlands (Čechová et al., 2017; Aerts et al., 2019; Runkel et al., 2021). Traces of oxy-CD were detected in 48% of the samples, with concentrations ranging between < 16 and 231 pg/mL (<0.2 and 6.7 ng/g fat). These levels were low compared to those found in other recent studies from Norway and Sweden, where oxy-CD maximum concentrations of up to 30 ng/g fat were reported (Iszatt et al., 2019; Lenters et al., 2019; Gyllenhammar et al., 2021). In Spain, the median oxy-CD level found by the UNEP/WHO-GMP for POPs in 2002 (7.5 ng/g fat) was higher (Hůlek et al., 2014) than that observed in the current study (median: <0.2 ng/g fat)).

Regarding PBDEs, the detection rates varied depending on the specific congener. Percentages ranged between 5% for BDE-154, and 100% for BDE-47. Median concentrations were: 0.3 pg/mL (0.01 ng/g fat), 3.7 pg/mL (0.1 ng/g fat), <0.8 pg/mL (<0.01 ng/g fat), 2.6 pg/mL (0.1 ng/g fat), 9.5 pg/mL (0.3 ng/g fat) and < 0.8 pg/mL (<0.01 ng/g fat) for BDE-28, BDE-47, BDE-99, BDE-100, BDE-153 and BDE-154, respectively. With respect to PCBs, 9 of the 11 analyzed congeners were detected in all the samples, while for PCB-157 and PCB-209 the detection rates were 95% and 28%, respectively. In general terms, these concentrations were lower than those reported in previous studies conducted in Northern European countries, such as Norway and Sweden (Iszatt et al., 2019; Lenters et al., 2019; Gyllenhammar et al., 2021), but similar to the levels reported in France and Slovenia (Cano-Sancho et al.,

Table 3
Levels (per volume of milk) of organic pollutants, and toxic and essential elements determined in breast milk samples.

	n	>LOD (%)	Mean	SD	Minimum	Median	P75	P95	Maximum
Organic pollutants (pg/mL)									
DDE	60	100	4,050	8,798	226	1,695	3,205	10,334	61,789
DDT	60	100	91	125	9.1	56	95	251	878
HCB	60	100	367	236	64	308	473	820	1,254
Oxy-CD	60	48	30	42	<16	<16	29	85	231
BDE-28	60	55	0.5	0.5	<0.2	0.3	0.9	1.5	1.7
BDE-47	60	100	6.2	7.1	0.9	3.7	5.7	19	41
BDE-99	60	38	1.9	3.8	<0.8	<0.8	1.2	9.5	22
BDE-100	60	66	3.6	3.4	<1.6	2.6	5.2	9.3	16
BDE 153	60	92	12	9.4	<1.6	9.5	16	27	54
BDE-154	60	5	0.5	0.3	<0.8	<0.8	<0.8	<0.8	2.1
PCB-105	60	100	29	19	7.1	25	35	60	108
PCB-118	60	100	136	76	40	120	175	246	410
PCB-138	60	100	437	281	112	360	567	900	1,510
PCB-153	60	100	1,012	655	250	798	1,385	2,092	3,495
PCB-156	60	100	48	35	4.8	35	76	106	152
PCB-157	60	95	8.9	6.4	<0.4	6.8	13	20	29
PCB-167	60	100	17	13	3.1	13	23	44	52
PCB-170	60	100	275	221	13	190	437	685	861
PCB-180	60	100	643	507	33	447	967	1650	2036
PCB-194	60	100	57	51	3.9	37	85	165	231
PCB-209	60	28	1.1	1.4	<0.8	<0.8	1.4	3.8	7.8
dl-PCBs (fg TEQ/mL)	60	–	7.2	4.5	1.7	6.0	9.7	14	23
PFOA	60	12	8.2	12	<10	<10	<10	19	86
PFNA	60	25	6.8	3.6	<10	<10	10	13	25
PFUnDA	60	5	5.3	1.2	<10	<10	<10	<10	11
PFOS	60	87	31	18	<10	28	43	60	76
Chlorpyrifos	57	39	18	25	<13	<13	24	64	149
Other organic pollutants – BPA and TBBPA (ng/mL)									
Free BPA	40	85	0.6	0.5	<0.02	0.6	0.9	1.5	1.7
Total BPA	40	88	1.8	1.5	<0.02	1.6	2.4	4.5	6.8
Free TBBPA	40	35	0.03	0.05	<0.04	<0.04	0.05	0.1	0.2
Total TBBPA	40	50	0.08	0.1	<0.04	0.02	0.1	0.3	0.4
Toxic and essential elements (μg/mL)									
Al	60	40	7.6	8.8	<3.5	<3.5	16	20	39
As	60	2	0.02	0.00	<0.03	<0.03	<0.03	<0.03	0.03
Ca	60	100	276	50	163	270	311	345	399
Co	60	28	0.04	0.04	<0.04	<0.04	0.1	0.1	0.1
Cr	60	8	0.02	0.01	<0.03	<0.03	<0.03	0.05	0.09
Cu	60	53	0.2	0.2	<0.20	0.1	0.3	0.6	0.7
Hg	60	12	0.01	0.00	<0.01	<0.01	<0.01	0.01	0.01
K	60	100	488	71	325	478	521	599	741
Mg	60	100	54	24	26	44	82	95	108
Mn	60	15	0.1	0.5	<0.09	<0.09	<0.09	0.3	4.1
Na	60	100	126	122	39	98	126	333	800
Ni	60	48	1.9	6.9	<0.2	<0.2	0.8	3.9	47
Sn	60	12	0.02	0.01	<0.03	<0.03	<0.03	0.04	0.06
U	60	28	0.003	0.003	<0.002	<0.002	0.01	0.01	0.01
% Lipid	60	100	3.2	1.6	1.0	3.2	4.0	6.0	8.0

PFDA, PFHxS, PFHpS, MeHg, Cd, Se, Pb, V, Sb, and Mo levels were below detection limit in all samples analysed.
SD: Standard deviation; P75 and P95: Percentile 75th and 95th, respectively; dl-PCB: dioxin-like PCBs.

2020; Runkel et al., 2021) (Table 5). The PCB congener profile showed the highest contribution for PCB-153, PCB-180, PCB-138, PCB-170 and PCB-118, with median concentrations of 798 pg/mL (33 ng/g fat), 447 pg/mL (19 ng/g fat), 360 pg/mL (14 ng/g fat), 190 pg/mL (8.3 ng/g fat) and 120 pg/mL (4.3 ng/g fat), respectively. Recently, similar concentrations have been also reported for various European countries (Table 5), with the exception of the levels found in Slovenia, where lower concentrations have been observed (Runkel et al., 2021).

Previous investigations performed also in the current study area (Schuhmacher et al., 1999a,b, 2004, 2009), as well as in other areas of Spain (Gómara et al.,2007; Lacorte and Ikonomou 2009) showed higher levels of PBDEs than those found in the present study. Average sum ± standard deviation (SD) PBDEs (Σ_{15}PBDE congeners: BDE 28, 47, 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 183, 190) concentrations were 2.4 ± 1.67 ng/g fat (Schuhmacher et al., 2004) and 2.5 ± 1.6 ng/g fat (Schuhmacher et al., 2009) in Tarragona, while the sum of PBDE (Σ_{11}PBDE congeners: BDE 15, 28/33, 47, 49, 66, 74, 75, 77, 99, 100, 119) concentrations ranged between 1.16 and 18.6 ng/g fat in Barcelona (Lacorte and Ikonomou 2009). The current mean ± SD concentration of sum PBDEs was 0.84 ± 0.74 ng/g fat. However, it is important to highlight that the former investigations were performed in 2002 (Schuhmacher et al., 2004), 2007 (Schuhmacher et al., 2009) and 2000–2003 (Lacorte and Ikonomou 2009), while our sampling was conducted in the period 2016–2019. Similarly, concentrations of dioxin-like PCBs showed a significant decrease during the last two decades, when the results were compared with previous data. In the present study, the following PCBs were considered in order to calculate the TEQ values: PCB-105, PCB-118, PCB-138, PCB-153, PCB-156, PCB-157, PCB-167, PCB-170, PCB-180, PCB-194 and PCB-209. The mean ± SD concentration of dioxin-like PCBs were estimated to be 0.2 ± 0.1 pg $WHO_{2005}TEQ\ g^{-1}$ fat, a value substantially lower than those found in 1998 (17.6 ± 5.8 pg $WHO_{1998}TEQ\ g^{-1}$ fat), 2002 (9.4 ± 3.1 pg

Table 4
Levels (per mass of fat) of organic pollutants, and toxic and essential elements determined in breast milk samples.

	n	>LOD (%)	Mean	SD	Minimum	Median	P75	P95	Maximum
Persistent organic pollutants (ng/g of fat)									
DDE	60	100	153	453	12	68	96	377	3511
DDT	60	100	3.3	6.3	0.5	2.0	3.4	6.6	50
HCB	60	100	13	7.4	2.9	11	16	27	42
Oxy-CD	60	48	0.9	1.1	<0.2	<0.2	1.0	2.7	6.7
BDE-28	60	55	0.02	0.02	<0.003	0.01	0.03	0.05	0.08
BDE 47	60	100	0.2	0.2	0.05	0.1	0.2	0.8	1.4
BDE-99	60	38	0.1	0.1	<0.01	<0.01	0.004	0.3	0.8
BDE-100	60	66	0.1	0.1	<0.03	0.1	0.2	0.5	0.6
BDE 153	60	92	0.4	0.3	<0.11	0.3	0.5	1.0	1.7
BDE-154	60	5	0.02	0.02	<0.01	<0.01	<0.01	<0.01	0.1
PCB-105	60	100	1.0	0.5	0.2	0.9	1.3	1.8	3.0
PCB-118	60	100	4.8	2.1	1.0	4.3	6.4	8.4	11
PCB-138	60	100	15	7.2	2.4	14	19	28	35
PCB-153	60	100	35	18	3.9	33	43	65	93
PCB-156	60	100	1.5	0.9	0.2	1.5	2.0	3.1	4.7
PCB-157	60	95	0.3	0.2	<0.01	0.3	0.4	0.6	0.9
PCB-167	60	100	0.6	0.3	0.08	0.5	0.7	1.1	1.6
PCB-170	60	100	9.0	6.3	0.6	8.3	12	21	34
PCB-180	60	100	21	15	1.2	19	28	46	77
PCB-194	60	100	2.0	1.8	0.1	1.5	2.4	5.2	10
PCB-209	60	28	0.04	0.1	<0.01	<0.01	0.04	0.1	0.3
dl-PCBs (pg TEQ/g)	60		0.2	0.1	0.04	0.2	0.3	0.5	0.6
PFOA	60	12	0.3	0.2	<0.1	<0.1	<0.1	0.6	1.4
PFNA	60	25	0.3	0.2	<0.1	<0.1	0.3	0.5	0.8
PFUnDA	60	5	0.2	0.1	<0.1	<0.1	<0.1	0.5	0.5
PFOS	60	87	1.2	1.0	<0.1	1.0	1.4	2.9	5.7
Chlorpyrifos	57	39	0.9	1.7	<0.2	<0.2	0.7	3.2	11
Other organic pollutants – BPA and TBBPA (ng/g of fat)									
Free BPA	40	85	28	26	<0.3	25	37	68	143
Total BPA	40	88	77	71	<0.3	63	105	234	286
Free TBBPA	40	35	1.1	1.8	<0.7	<0.7	1.8	4.5	7.8
Total TBBPA	40	50	2.8	3.9	<0.7	13	4.3	11	14
Toxic and essential elements (µg/g of fat)									
Al	60	40	314	445	<44	<44	347	1663	1737
As	60	2	0.6	0.4	<0.4	<0.4	<0.4	<0.4	1.7
Ca	60	100	11,660	7,428	3,168	9,114	15,160	24,239	40,223
Co	60	28	2.0	2.5	<0.5	<0.5	2.0	6.3	11
Cr	60	8	0.9	1.3	<0.4	<0.4	<0.4	1.7	9.4
Cu	60	53	8.8	7.6	<3.2	6.6	11	26	31
Hg	60	12	0.2	0.16	<0.1	<0.1	<0.1	0.53	0.61
K	60	100	20,592	12,700	5427	16,363	24,796	49,644	62,959
Mg	60	100	2,325	1,967	407	1,503	2,756	6,831	9,982
Mn	60	15	6.1	28	<1.1	<1.1	<1.1	7.3	216
Na	60	100	5,428	7,956	990	3,339	5,430	12,154	57,110
Ni	60	48	53	146	<3.5	<3.5	23	252	983
Sn	60	12	0.7	0.40	<0.5	<0.5	<0.5	1.6	1.8
U	60	28	0.1	0.2	<0.02	<0.02	0.1	0.5	0.8

PFDA, PFHxS, PFHpS, Cd, Se, Pb, V, Sb, MeHg and Mo levels were below detection limit in all samples analysed.
SD: Standard deviation; P75 and P95: Percentile 75th and 95th, respectively; dl-PCB: dioxin-like PCBs.

$WHO_{1998}TEQ\ g^{-1}$ fat) and 2009 (9.0 ± 4.4 pg $WHO_{2005}TEQ\ g^{-1}$ fat) (Schuhmacher et al., 2014). However, to calculate the TEQ values from 1998, 2002 and 2009, the PCB congeners considered were: PCB 18, 28/31, 33, 47, 49, 51, 52, 60, 66, 74, 77, 81, 99, 101, 105,110, 114, 118, 122, 123, 126, 128, 138, 141, 153, 156, 157, 167, 169, 170, 180, 183, 187, 189, 194, 206, and 209). Nevertheless, the current levels are well in agreement with the decreasing trend observed in previous studies, being similar to other updated concentrations of PCBs detected in other European studies (Table 5). In addition, similar trends have also been observed for dioxins and furans (Schuhmacher et al., 1999a; 2019).

Regarding PFASs, the levels of PFDA, PFHxS, and PFHpS were below their respective detection limits (10 pg/mL) in all samples. A higher detection frequency was observed for PFOS (87%) when compared to PFOA (12%). PFOS concentrations ranged from < 10 to 76 pg/mL, while PFOA levels were between < 10 and 86 pg/mL. The detection rates for PFNA and PFUnDA were 25% and 5%, respectively, with concentrations ranging from < 10 to 25 pg/mL and < 10 to 11 pg/mL, respectively. The levels observed in the present study were again relatively low compared to those of other studies found the scientific literature (Table 5). Similar values of PFOS, PFOA, PFNA, and PFUnDA have previously been found in several Spanish areas (Kärrman et al. 2010; Llorca et al., 2010; Guzmán et al., 2016; Serrano et al., 2021), which is also in the same order of magnitude as those from the Czech Republic and Ireland (Abdallah et al., 2020; Cerná et al. 2020).

Chlorpyrifos was detected in 39% of the breast milk samples, with levels ranging from < 13 to 149 pg/mL (<0.2 to 11 ng/g fat) (mean: 18 ± 25 pg/mL or 0.9 ± 1.7 ng/g fat). No recent data on levels of chlorpyrifos in human milk from European mothers are available in the scientific literature. However, the levels found in the present study were similar or slightly lower than those found in other non-European countries, such as the USA, where median values ranging between 20 and 60 pg/g have been reported (Weldon et al., 2011; Chen et al., 2014; Hartle et al., 2018). In contrast, substantially higher levels of chlorpyrifos in human milk from Iran and India have been found, with mean

Table 5
Levels of POPs and PFASs in breast milk samples from various European countries recently reported in the scientific literature.

Study	Runkel et al., 2021	Aerts et al., 2019	Grešner et al. 2021	Čechová et al., 2017			Iszatt et al., 2019	Lenters et al., 2019	Gyllenhammar et al., 2021	Cano-Sancho et al., 2020	Hernández et al., 2020	**Present study**
Sampling year	2011–2014	2014	2007–2011	2011–2014	2010–2012	2001–2006	2002–2005	2002–2009	1996–2017	2011–2016	2015	2016–2020
Country	Slovenia	Belgium	Poland	Slovakia	Netherlands	Norway	Norway	Norway	Sweden	France	Spain	Spain
n=	448*	206	110	37	120	388	267	1199	539	58	75	60
DDE	50 (120)	37 [8–52]		167 (670)	50 (122)	52 (185)	54 [5.4–617]	48 (160)	73 [9.2–649]			68 (377)
DDT	ND	2.8 [ND-66]		8.7 (26)	1.5 (3.9)	1.8 (5.5)	2.1 [0.04–35]	2.0 (5.6)	4.5 [<0.6–240]]			2.0 (6.6)
HCB	5 (10)	5.5 [ND-17]		13 (19)	6.2 (9.7)	7.7 (14)	10 [1.7–49]	9.8 (18)	11 [3.9–29]			11 (27)
Oxy-CD							3.3 [0.5–30]	3.1 (7.3)	3.1 [0.5–11]			<0.2 (2.7)
BDE-28	0.05 (0.17)	ND [ND-0.6]					0.2 [0.01–5.6]	0.1 (0.5)	0.08 [<0.03–1.8]	0.03 (0.18)		0.01 (0.05)
BDE 47	0.5 (1.2)	0.1 [ND-2.4]					1.1 [0.2–59]	1.0 (5.5)	1.0 [<0.04–16]	0.4 (2.0)		0.1 (0.8)
BDE-99	0.09 (0.4)	ND [ND-0.9]					0.3 [0.04–15]	0.3 (1.4)	0.2 [<0.1–5.2]	0.08 (0.3)		<0.01 (0.3)
BDE-100	0.09 (0.3)	ND [ND-0.8]					0.3 [0.01–7.5]	0.3 (1.0)	0.2 [<0.04–5.1]	0.1 (0.3)		0.1 (0.5)
BDE 153	0.2 (0.8)	0.4 [ND-1.9]					0.5 [0.05–4.03]	0.5 (1.4)	0.5 [<0.9–4.7]	0.5 (1.6)		0.3 (1.0)
BDE-154	0.01(0.05)	ND [ND-1.1]					0.03 [ND-1.2]	0.03 (0.1)	0.07 [<0.03–0.9]	0.02 (0.3)		<0.01 (<0.01)
PCB-105	0.6 (1.8)		0.6 [0.09–3.0]				1.4 [0.4–13]	1.3 (3.2)	0.9 [<0.5–31]	1.8 (6.3)	0.4 (1.0)	0.9 (1.8)
PCB-118	2.7 (8.4)		3.1 [ND-9.6]	3.5 (10)	3.6 (7.3)	5.2 (14)	6.8 [2.0–62]	5.9 (14)	6.7 [1.0–64]	8.2 (22)	1.8 (4.5)	4.3 (8.4)
PCB-138	0.01 (0.03)			31 (92)	11 (23)	17 (43)	20 [4.4–145]	19 (41)	21 [2.7–94]	18 (46)		14 (28)
PCB-153	0.01 (0.05)			59 (133)	16 (31)	27 (67)	35 [7.3–296]	31 (68)	39 [5.3–186]	35 (83)		33 (65)
PCB-156	1.0 (3.1)		1.0 [ND-7.2]				3.3 [0.6–23]	3.2 (7.6)	3.4 [0.8–24]	2.6 (7.9)	1.2 (2.4)	1.5 (3.1)
PCB-157	0.2 (0.7)		0.2 [ND-0.8]				0.6 [0.1–4.9]	0.7 (1.8)	0.4 [<0.2–2.5]	0.5 (1.4)	0.2 (0.5)	0.3 (0.6)
PCB-167	0.3 (0.8)						0.8 [0.2–8.0]	0.8 (1.8)	0.9 [<0.2–5.7]	0.8 (2.1)	0.3 (0.8)	0.5 (1.1)
PCB-170							6.9 [1.2–46]	6.2 (14)				8.3 (21)
PCB-180	0.01 (0.02)			44 (98)	11 (22)	14 (36)	18 [4.2–142]	15 (35)	19 [2.2–84]	18 (57)		19 (46)
PCB-194							1.4 [ND-12]	1.4 (3.2)				1.5 (5.2)
PCB-209							0.1 [ND-0.8]	0.1 (1.-0)				<0.01 (0.1)
Study	Fiedler & Sadia, 2021 *				Cerná et al. 2020	Abdallah et al., 2020	Iszatt et al., 2019	Lenters et al., 2019	**Guzmán et al., 2016**	Kärrman et al. 2010	Serrano et al., 2021	**Present study**
Sampling year	2017–2019	2017–2019	2017–2019	2016–2019	2017		2002–2005	2002–2009	2014	2007		2016–2020
Country/ Area	Africa	Asia-Pacific	Latin America	Europe	Czech Rep.	Ireland	Norway	Norway	Spain	Spain	Spain	Spain
n=	14	13	9	8	232	16 pool	267	1199	67	10	82	60
Units	pg/g milk	pg/g milk	pg/g milk	pg/g milk	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL	pg/mL
PFOA	13 [6.2–18]	15 [10–32]	16 [7.8–19]	31 [18–37]	24 (58)	100 (350)	51 [2.2–183]	40 (110)	[ND-211]	ND	7.17 (55.1)	<10 (19)
PFNA					7 (12)	14 (75)			[ND-70]	ND	2.59 (25.5)	<10 (13)
PFUnDA									[ND-57]	ND	<0.74 (3.29)	<10 (<10)
PFOS	10 [0–22]	17 [0–212]	12 [0–41]	18 [12–51]	22 (78)	20 (85)	117 [23–371]	117 (260)		110 [80–220]	<0.86 (26.0)	28 (60)
PFDA					ND				[ND-34]	ND	<0.72 (23.0)	<10 (<10)
PFHxS	ND	ND [ND-111]	ND	ND [ND-17]		<40 (80)				40 [20–110]	<0.66 (16.0)	<10 (<10)
PFHpS										ND		<10 (<10)

DDT, DDE, HCB OXy-CD PBDE and PCB reported in ng/g of fat.

* Per- and polyfluoroalkyl compounds reported in pg/mL with the exception of Fiedler and Sadia, 2021 that are reported in pg/g of milk. Levels reported as median (95th percentile); median [range] or mean ± standard deviation. ND: not detected.

± SD concentrations of 2100 ± 1400 pg/mL, and 37.3 ± 67.1 ng/g fat, respectively (Srivastava et al., 2011; Brahmand et al., 2019).

Total (conjugated + unconjugated) BPA was detected in 88% of the samples. The median total BPA concentration was 1.6 ng/mL, with one third of (0.6 ng/mL) being present in the unconjugated form (BPA free). The current study found similar levels of BPA to those reported in recently published studies. For example, free and total BPA levels in two Spanish (Valencia and Madrid) studies were 0.10 and 0.26 ng/mL, respectively (Dualde et al., 2019), and 0.26 and 1.30 ng/mL (free and total, respectively) (Martínez et al., 2019). Low levels of free BPA (0.11 ng/mL) were detected in Turkey (Sayıcı et al., 2019), while in the USA, 6.5 ng/g milk were observed (Hartle et al. 2018). In a recent review of 50 scientific articles world-wide, a mean of total BPA concentration of 1.4 ng/mL was reported, with a range between 0.1 and 3.9 ng/mL (Iribarne-Durán et al., 2022). BPA concentration in breast milk from Tarragona mothers agree well with that mean BPA concentration. Total TBBPA (conjugated + unconjugated) was detected in half of the breast milk samples, with a median concentration of 0.02 ng/mL (13 ng/g fat). The mean concentration of unconjugated TBBPA was 0.03 ng/mL (1.1 ng/g fat). Few studies have reported TBBPA in human milk. In UK, Spain and France, mean values of 0.06 ng/g of milk, 0.58 ng/mL, and 0.48 ng/g of milk, respectively, have been reported (Cariou et al., 2008; Abdallah & Harrad, 2011; Martínez et al., 2019).

A number of essential and toxic elements were also determined. Cd, Mo, Pb, Se, Sb, and V were all below their respective LODs, while As, Cr, Hg, Mn and Sn could be detected in some samples, although at low detection percentages (2%-15%). Total mercury was detected in only 12% of samples, with a maximum concentration close to the detection limit. Nevertheless, MeHg was not detected in any sample. The detection frequency of MeHg varies widely, with 10% detection in samples of Brazil up to 60% detection in samples of Italy (Miklavčič et al., 2013; Rebelo et al., 2017). Essential elements were detected in all the samples, with a median concentration (range) of 270 μg/mL (163–399 μg/mL) for Ca, 478 μg/mL (325–741 μg/mL) for K, 44 μg/mL (26–108 μg/mL) for Mg, and 98 μg/mL (39–800 μg/mL) for Na. Similar levels of these elements have been also observed in other Spanish studies (Martínez et al. 2019; Mandiá et al. 2021).

Environmental pollutants (pesticides, PFASs PCBs, PBDEs, BPA, TBBPA, toxic metals) are not exclusively present in human breast milk. In fact, they also occur in infant formulas and baby food (Mezcua et al., 2007; Pandelova et al., 2011; Chen et al., 2014; Kilic et al., 2018; Martínez et al., 2019). These contaminants can be incorporated to infant food from feed, raw material, production chain or even from packaging materials (Pereira et al., 2020).

Spearmans' correlations between pollutants concentrations in breast milk were assessed (Fig. 1). Several significant positive correlations were detected. DDT and DDE were highly correlated with a Pearson coefficient of 0.53 ($p < 0.001$), while various PBDE congeners were also significantly correlated ($p < 0.001$) to each other, and PCB congeners were also highly correlated. This was especially evident for PCB congeners with the same number of chlorine atoms. For example, PCB-105 and PCB-118, both with 5 chlorine atoms, had a correlation coefficient of 0.96 ($p < 0.001$). In turn, PCB-170 and PCB-180, both with 7 chlorine atoms, had a correlation coefficient of 1.0 ($p < 0.001$). High correlations between different PCB congeners could be explained by co-occurrence in food, environment, and consumers goods, as well as common exposure pathways. As expected, total and free BPA, as well as total and free TBBPA were also significantly positively correlated ($p < 0.01$). Finally, HCB showed high correlations (>0.5; $p < 0.01$) with all PCBs, with the exception of PCB-209.

3.2. Contaminants and maternal characteristics

Interestingly, breast milk concentrations of pollutants varied depending on maternal characteristics (Tables S1 to S8 in the supplementary materials). POPs levels were higher in older than in younger women. However, significant ($p < 0.001$) differences were only found for HCB, with mean levels of 9.7, 12.3, 11.5 and 23.6 ng/g fat in < 30 years old, 30–34 years old, 35–39 years old, and > 40 years old, respectively. In addition, maternal age showed a significant ($p < 0.01$) positive high correlation with HCB and PCBs. These associations and trends, which have been observed elsewhere (Colles et al., 2008; Dimitriadou et al., 2016), could be related to the cumulative lifetime exposure to POPs. Notwithstanding, the relatively low number of analysed samples could explain why statistical significance was only reached for HCB. In contrast, PFASs showed higher levels in younger

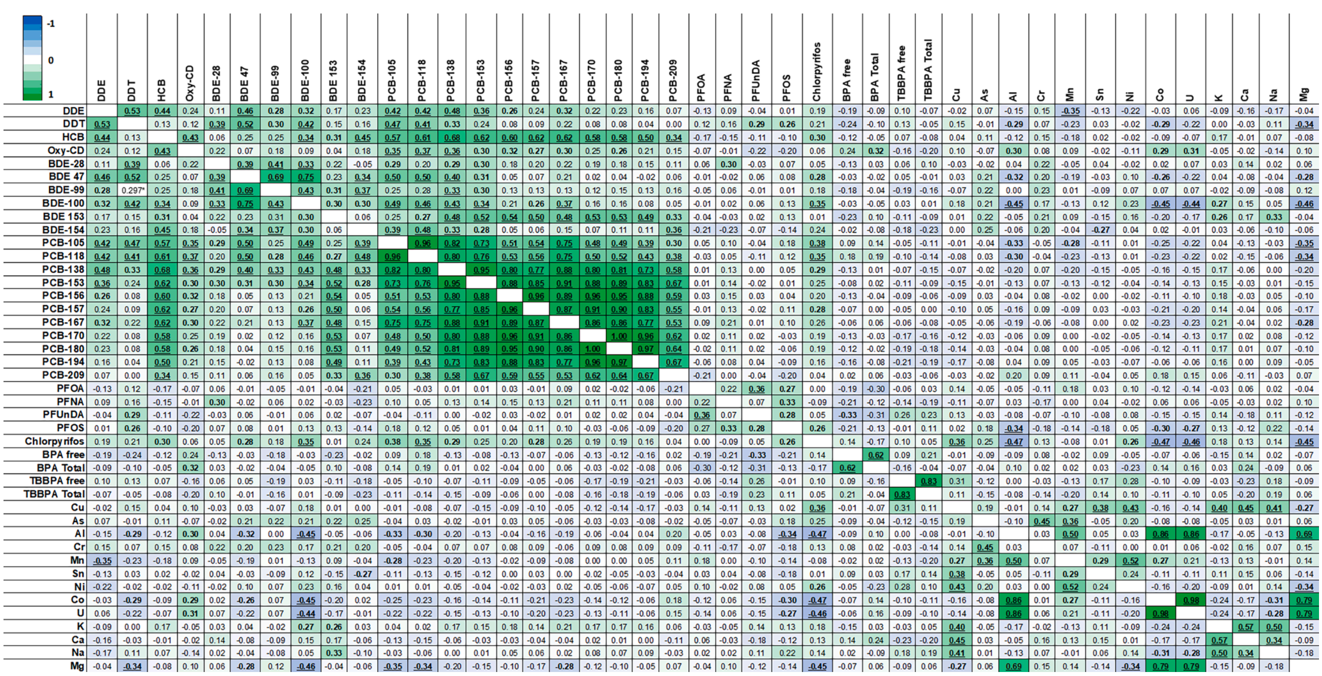

Fig. 1. Spearman correlations' coefficients. Bold numbers indicate statistical significance at $p < 0.05$; bold plus underlined numbers indicate statistical significance at $p < 0.01$.

mothers. However, the difference only reached a level of statistical significance ($p < 0.05$) for PFOA (mean:19 pg/mL in women aged < 30 years, and < 10 pg/mL in mothers who were ≥ 40 years old), while PFOS was only detected in 12% of samples. A similar trend was also found for chlorpyrifos, whose levels ranged from a mean of 23.6 pg/mL in breast milk of younger women, to 9.9 pg/mL, for women ≥ 40 years old. It should be noticed that more than one-half of samples showed concentrations of PFASs and chlorpyrifos below their respective detection limits. In the scientific literature, age has been identified as a relevant factor modulating breast milk levels, with higher PFASs levels in old mothers (Lee et al., 2018). However, this is not in agreement with the results of other recent studies (Serrano et al., 2021). With respect to smoking, only one mother reported to smoke during pregnancy and lactation. Therefore, no statistical analysis was possible. Regarding passive smokers, significant ($p < 0.05$) differences were detected for As, Cr, and Mn levels, being all higher in the passive smoking group. It is well known that tobacco smoke contains metals such as Cd, Cr, Cu, Ni or Pb, at high levels (Bernhard et al., 2005). Other studies also found differences in Cd, Mn and Pb levels between passive smokers and non-smokers (Olowoyo et al., 2021; Szukalska et al., 2021).

In relation to BMI, no significant ($p < 0.05$) differences were detected for any of the chemicals or elements measured. Nevertheless, significant ($p < 0.01$) positive correlations were observed between BMI and DDT, DDE and HCB. Various studies have reported higher levels of lipophilic contaminants in people with a high BMI (Luzardo et al., 2019), possibly due to the accumulation of lipophilic organic compounds in fat. Nonetheless, in the present study this association was not found, which is in agreement with other investigations (Aerts et al., 2019). With respect to the citizenship of the mother, significantly ($p < 0.05$) higher levels of PCBs (157, 170, 180 and 194) were found in Spanish mothers when compared to mothers of other countries. Although the same trend was found for HCB, Oxy-CD, PBDEs, DDT and DDE, the difference was not significant ($p > 0.05$). Non-significant ($p > 0.05$) lower levels of DDT, DDE and chlorpyrifos were observed in mothers born in Spain. Although there is a clear decreasing trend in POPs and PFASs blood levels with increasing number of children -which is in line with previous studies (Brantsæter et al., 2013)- differences were only significant ($p < 0.05$) for PCB-156 and PCB-157. No other significant ($p < 0.05$) differences were noted regarding parity. In relation to the maternal educational status, non-significant differences were observed for all the compounds analysed in breast milk. Regarding the annual income, significantly higher DDT and DDE levels were observed in mothers with a low annual income (602 and 10.3 ng/g fat for DEE and DDT, respectively) than in those with a high annual income (10.3 and 2.6 ng/g fat for DEE and DDT, respectively). The results regarding socioeconomic position and exposure are in accordance with those of previous studies (Fisher et al., 2016; Montazeri et al., 2019).

No significant differences for any compound analysed in human milk were observed in relation to food (salad, vegetables, milk, meat, fish eggs, and seafood) consumption, or dietary habits (organic food consumption) collected from the food frequency questionnaires (FFQ) filled out during the breastfeeding periods. Similarly, no significant ($p < 0.05$) differences in breast milk pollutant levels were observed according to the use of personal care products (PCPs), which included body creams, makeup and hair products. Our results disagree with those recently published by Serrano et al. (2021) and Thépaut et al. (2021), who reported that PFASs exposure could be influenced by the use of PCPs. However, the lack of association between PFASs body burdens and PCPs use could be due to low detection rates of PFASs, and more specifically of PFOA, PFNA, PUnDA, PFDA, PFHxS and PFHpS. Moreover, it might be also due to the sample size analysed.

The current results from the 14 differently GLM (Table S9 in the supplementary materials), considering the chemical levels in breast milk and the maternal characteristics, revealed different expected relationships. Overall, most models were consistent with higher annual income, non-Spanish and multiparous women and higher BMI, being significantly associated with lower levels of chemicals in breast milk. The only exception corresponded to TBBPA, whose GLM indicated that higher levels of this substance were associated with a higher annual income. On the other hand, age and passive smoking were related with higher chemicals levels in breast milk. The GLM conducted strengthens our results in terms of socioeconomic status and exposure to different chemicals. Significantly lower levels were observed in mothers with a high annual income, in agreement with the findings of Fisher et al. (2016) and Montazeri et al. (2019). In addition, the results from the GLM were also complemented by the significantly associations previously observed through Levene test, where higher levels of chemicals were detected in samples from Spanish primiparous mothers compared to non-Spanish multiparous women. Furthermore, regarding passive smokers, the GLM used for Mn was again in line with previous significant associations observed in the current paper and in concordance with different recent studies (Olowoyo et al., 2021; Szukalska et al., 2021). Finally, women's aged was related to higher chemicals levels in breast milk. Most of the chemicals analyzed are lipophilic or amphipathic in nature; therefore, they tend to bioaccumulate in the human body, being eventually found at higher levels in older women. These results strengthen the previously found associations regarding POPs, in line with the observations of Colles et al. (2008) and Hůlek et al. (2014).

GLM also showed unpredicted results. Regarding As model, women with secondary studies had higher levels of As in breast milk compared with women with primary studies. However, women with university degrees compared with women with primary studies presented lower levels of As in breast milk, but not at a significantly level. In addition, the As detection ration was very low compared with the other chemicals determined. For this reason, it may not be a very strong result to consider despite being significant. Another unpredictable result of the GLM was the relationship between higher BMI and lower levels of some chemicals, such as ΣBDE, ΣPCB, and PFOS. However, these results could be explained by the fact that a higher BMI enhances a greater accumulation of these chemicals in body fat rather than in breast milk. In any case, more observational studies to identify the role of different body compartments for the storage of these chemicals are clearly necessary.

These results should be interpreted with caution, since the limited sample size or the cross-sectional design of the study could lead to some unexpected observations. In addition, it the observational design of the study did not allow to determine any cause-effect.

3.3. Exposure to pollutants through breastfeeding

The early exposure of infants at 3 stages (<1 month old, 1–6 months old and > 6–9 months old) was evaluated by considering exclusive breastfeeding, being calculated in a probabilistic way (Table 6). Because of the relative reduction of the amount of ingested breast milk per body weight through time, the daily intake of toxic substances decreased with infant age. A decreasing concentration of contaminants in breastmilk during the course of lactation can also result in a reduction in the exposure of children (Thomsen et al., 2010b). Exposure levels of BPA and PFASs (PFOS and PFOA) in all age categories exceed the most updated tolerable daily or weekly intake (TDI or TWI) values set by the European Food Safety Agency (EFSA) at 0.04 ng/kg/day for BPA (EFSA, 2022) and 4.4 ng/kg bw/week for the sum of PFOA, PFNA, PFHxS and PFOS (EFSA, 2020). Similarly, the infant exposure to dioxin-like PCBs also exceeded -for all the lactation periods- the tolerable weekly intake (TWI) established by the EFSA (2018a) in 2 pg TEQ/kg bw/week. However, these TDI and TWI should prevent those mothers reach a body burden, which can result in breastmilk levels that could lead to serum levels in the infant associated with a decrease in vaccination response. Nevertheless, the pollutant intake by infants should therefore not be directly compared with these TDI and TWI set by the EFSA (EFSA, 2020). It is basic to better understanding the pollutants toxicokinetics during the first stages of life, including breastfeeding period, in order to refine and establish tolerable intakes for that periods of life (VKM, 2013).

Table 6
Exposure to organic pollutants, and toxic and essential elements trough exclusive breastfeeding depending on the specific lactation period.

	<1 month old			1–6 months old			>6–9 months old			TDI*
	P50	P75	P95	P50	P75	P95	P50	P75	P95	
DDE	452	759	1589	377	619	1262	221	371	781	
DDT	9.5	17	39	7.9	14	31	4.6	8.3	19	5000
HCB	45	69	128	38	56	100	22	34	63	170
Oxy-CD	2.9	5.4	13	2.5	4.4	10.4	1.4	2.7	6.4	
BDE-28	0.1	0.1	0.2	0.04	0.1	0.2	0.02	0.05	0.1	
BDE 47	0.6	1.1	2.9	0.5	0.9	2.3	0.3	0.6	1.4	
BDE-99	0.1	0.3	1.0	0.1	0.2	0.8	0.1	0.1	0.5	
BDE-100	0.4	0.7	1.4	0.3	0.5	1.1	0.2	0.3	0.7	
BDE 153	1.5	2.3	4.2	1.2	1.8	3.3	0.7	1.1	2.0	
BDE-154	0.06	0.09	0.17	0.05	0.07	0.13	0.03	0.04	0.08	
PCB-105	3.8	5.5	9.4	3.2	4.5	7.3	1.9	2.7	4.6	
PCB-118	18	25	39	15	20	30	9.0	12	19	
PCB-138	56	82	142	47	67	111	27	40	70	
PCB-153	130	190	330	108	154	257	63	93	162	
PCB-156	5.9	9.0	17	4.9	7.3	12.9	2.9	4.4	8.1	
PCB-157	1.1	1.7	3.1	0.9	1.4	2.4	0.5	0.8	1.5	
PCB-167	1.9	3.3	6.8	1.6	2.7	5.4	0.9	1.6	3.4	
PCB-170	31	52	107	26	42	85	15	26	52	
PCB-180	71	121	254	59	99	203	35	59	126	
PCB-194	5.8	11	25	4.8	8.7	20	2.8	5.2	12	
PCB-209	0.1	0.2	0.6	0.1	0.1	0.5	0.0	0.1	0.3	
dl-PCBs	0.9	1.3	2.3	0.8	1.1	1.7	0.5	0.6	1.1	0.29[A]
PFOA	1.0	1.5	2.9	0.8	1.2	2.3	0.5	0.8	1.4	0.63[B]
PFNA	0.9	1.3	2.0	0.8	1.0	1.5	0.4	0.6	1.0	0.63[B]
PFUnDA	0.8	0.9	1.3	0.6	0.7	0.9	0.4	0.5	0.6	0.63[B]
PFOS	4.0	5.7	9.5	3.3	4.6	7.4	2.0	2.8	4.7	0.63[B]
Chlorpyrifos	1.3	3.0	9.7	1.1	2.5	7.8	0.6	1.5	4.7	0[c]
Free BPA	77	121	230	65	99	181	38	59	113	0.04
Total BPA	207	343	700	173	278	552	101	168	343	
Free TBBPA	1.8	4.6	17	1.5	3.8	14	0.9	2.2	8.5	
Total TBBPA	4.0	11	44	3.4	8.9	36	2.0	5.3	22	$1{\cdot}10^3$
Al	0.8	1.4	3.1	0.7	1.2	2.5	379	595	1129	0.29
As	0.002	0.003	0.003	0.002	0.002	0.002	0.001	0.001	0.002	
Ca	41	48	61	33	37	43	20	24	30	
Co	0.005	0.008	0.016	0.004	0.007	0.013	0.003	0.004	0.008	
Cr	0.002	0.004	0.007	0.002	0.003	0.006	0.001	0.002	0.004	0.3
Cu	0.02	0.04	0.09	0.02	0.03	0.08	0.01	0.02	0.05	0.5
Hg	0.001	0.001	0.002	0.001	0.001	0.001	0.000	0.001	0.001	
K	72	85	106	59	65	75	35	42	52	
Mg	7.4	10.0	15.2	6.2	8.0	11.6	3.6	4.9	7.5	
Mn	0.02	0.03	0.04	0.01	0.02	0.03	0.01	0.01	0.02	
Na	14	24	51	11	19	41	6.7.	12	25	
Ni	0.24	0.35	0.61	0.20	0.28	0.48	0.12	0.17	0.30	0.003
Sn	0.002	0.003	0.006	0.002	0.003	0.005	0.001	0.002	0.003	2
U	0.0003	0.0005	0.0011	0.0002	0.0004	0.0009	0.0001	0.0003	0.0006	

Units: DDT, DDE, HCB, Oxy-CD, PBDE, PCB, per- and polyfluoroalkyl compounds, chlorpyrifos, BPA and TBBPA in ng/kg/day. dl-PCB: sum of dioxin like PCBs in pg TEQ/kg/day; and toxic and essential elements in mg/kg/day. SD: Standard deviation; P50, P75 and P95: Percentile 50th, 75th and 95th, respectively. *TDI (tolerable daily intake) values taken from WHO (2018), (EFSA (2006a,b; 2014; 2015a,b;2018a,b; 2019). According to EFSA PFFAs and dl-PCBs TDI were not applicable to infants.

[A] 2 pg/kg/week dioxin-like PCBs.

[B] TWI of 4.4 ng/kg/week for the sum of PFOA, PFNA, PFHxS and PFOS.

[c] no safe exposure level can be set for the substance.

3.4. Strengths and limitations of the study

The current study has some limitations that deserve to be discussed. Firstly, the results cannot be generalized to other populations, since the participants included in the analysis were exclusively breastfeeding and samples were collected during different periods of lactation (<1 month old, 1–6 months old, >6–9 months old). Secondly, the assessment of food intake through a FFQ is prone to possible measurements errors. However, despite this limitation, food-based FFQs have been widely used in epidemiological studies since the 1990 s (Shim et al., 2014). Thirdly, the design of the study, being an observational study with a small sample size might partially justify the absence of significant associations for any analysed compound with food consumption, dietary habits, or the use of personal care products. Notwithstanding, the significant associations regarding maternal characteristics (age, BMI) and smoking with environmental pollutants exposure were in the expected direction. Repeating these analyses with a longer duration of follow-up, such as three-time sampling collection during the course of lactation and FFQ corresponding to each lactation period, and with an representative population cohort, could allow to validate the present findings. It could even reveal other associations, in particular between food consumption and chemical exposure, taking into account that diet is the main source of exposure to these environmental pollutants.

The present study also has strengths that we would like to emphasize. We assumed that the milk samples were difficult to obtain since a volume of>50 mL was required to carry out the different determinations of the pollutants. A total of 31 organic contaminants and 14 widely distributed toxic and essential elements were determined in human breast milk. The results from the current study suggest that the presence of this mixture of toxic substances in human milk leads to a direct implication of the exposure of infants to these chemicals. Thus, these results support the need for biomonitoring breast milk for chemical exposure of exclusively breastfed infants. In addition, chemical biomonitoring and administration of FFQs might help to improve dietary background of nursing women.

4. Conclusions

The occurrence of DDT, DDE, HCB, Oxy-CD, PCBs, PBDEs, PFASs, chlorpyrifos, BPA, TBBPA, as well as a series of toxic and essential elements was determined in 60 breast milk samples from a cohort of Spanish nursing mothers. Traces of most environmental pollutants could be detected. Maternal characteristics, such as age and BMI, seem to be linked to higher levels of POPs (DDT, DDE and PCBs). Higher concentrations of PCBs were detected in Spanish and primiparous mothers. Breast milk of low-income mothers had higher DDT and DDE levels than high-income mothers, evidencing the influence of the socio-economic status on the women's exposure to environmental pollutants. Despite that breastfeeding is essentially beneficial for infants, the effects of the chemical mixture in breast milk should not be disregarded. Human milk contains traces of environmental pollutants, whose co-exposure may have an evident impact on the children's development and their human health. This cocktail of toxic substances is not exclusive of breast milk, since chemicals can be also found in infant formulas. Biomonitoring and food monitoring studies are clearly required, not only to control the presence of pollutants in children's food, but also to raise awareness of competent inspection authorities.

CRediT authorship contribution statement

Joaquim Rovira: Conceptualization, Formal analysis, Investigation, Writing – original draft. **María Ángeles Martínez:** Formal analysis, Investigation, Writing – original draft. **Montse Mari:** Investigation, Writing – review & editing. **Sara Cristina Cunha:** Investigation, Writing – review & editing. **Jose Oliveira Fernandes:** Investigation, Writing – review & editing. **Isa Marmelo:** Investigation, Writing – review & editing. **António Marques:** Investigation, Writing – review & editing. **Line Småstuen Haug:** Investigation, Writing – review & editing. **Cathrine Thomsen:** Investigation, Writing – review & editing. **Martí Nadal:** Conceptualization, Writing – review & editing. **José L. Domingo:** Conceptualization, Writing – review & editing, Funding acquisition. **Marta Schuhmacher:** Conceptualization, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

We would like to thank all women who participated in this study. Grant PCIN-2017-012 funded by MCIN/AEI/ 10.13039/501100011033 and cofunded by the "European Union". JR acknowledge Grant IJC 2018-035126-I funded by MCIN/AEI/ 10.13039/501100011033 and by "ESF Investing in your future". MAM was granted with Sara Borell fellowship by Instituto de Salud Carlos III (CD21/00045). We thank Antonio Pino, from the Metabolomics facility of the Centre for Omic Sciences (COS) Joint Unit of the Universitat Rovira i Virgili-Eurecat, for their contribution to chlorpyrifos analysis. The Portuguese Foundation for Science and Technology supported the Ph.D. Grant of IM (DFA/BD/4413/2020). This work was supported by UIDB/04423/2020 and AgriFood XXI R&D&I project, operation No. 708 NORTE-01-0145-FEDER-000041, co-financed by the European Regional Development Fund 709 (ERDF) through NORTH 2020 (Northern Regional Operational Program 2014/2020). Sara C. Cunha acknowledges FCT for IF/01616/2015 contract.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107375.

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