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Persistent organic pollutants in human milk of Belgian mothers: levels, time trend and exposure assessment for nursing infants

Mirjana Andjelkovic¹ , Ilse Van Overmeire¹, Laure Joly¹, Giulia Poma² , Govindan Malarvannan², Christiane Vleminckx^{1,§}, Svetlana V. Malysheva¹, Martine Vanhouche^{1,§}, Joris Van Loco¹, An Van Nieuwenhuyse^{1,3}, Adrian Covaci²

¹Chemical and Physical Health Risks, Sciensano, Brussels 1050, Belgium.

²Toxicological Centre, University of Antwerp, Wilrijk 2610, Belgium.

³(Present address: Laboratoire National de Santé, Department of Health Protection, Dudelange L-3555, Luxembourg.)

[§]Retired.

Correspondence to: Dr. Mirjana Andjelkovic, Chemical and Physical Health Risks, Sciensano, Juliette Wytsmanstraat 14, Brussels 1050, Belgium. E-mail: mirjana.andjelkovic@sciensano.be; Dr. Giulia Poma, Toxicological Centre, University of Antwerp, Universiteitsplein 1, Wilrijk 2610, Belgium. E-mail: giulia.poma@uantwerpen.be

How to cite this article: Andjelkovic M, Van Overmeire I, Joly L, Poma G, Malarvannan G, Vleminckx C, Malysheva SV, Vanhouche M, Van Loco J, Van Nieuwenhuyse A, Covaci A. Persistent organic pollutants in human milk of Belgian mothers: levels, time trend and exposure assessment for nursing infants. *J Environ Expo Assess* 2024;3:23. <https://dx.doi.org/10.20517/jeea.2024.22>

Received: 20 Jul 2024 **First Decision:** 12 Sep 2024 **Revised:** 6 Nov 2024 **Accepted:** 8 Nov 2024 **Published:** 19 Nov 2024

Academic Editors: Per Ola Darnerud, Stuart Harrad **Copy Editor:** Pei-Yun Wang **Production Editor:** Pei-Yun Wang

Abstract

Human milk samples ($n = 206$) collected in 2014 from Belgian primiparous mothers were analyzed for seven groups of persistent organic pollutants (POPs): dichlorodiphenyltrichloroethane and its metabolites (DDTs), chlordane compounds (CHLs), hexachlorocyclohexane isomers (HCHs), hexachlorobenzene (HCB), polybrominated diphenyl ethers (PBDEs), pentachlorobenzene (PeCB), and hexabromobiphenyl (BB-153). Pooled samples for the analysis of hexachlorobutadiene, heptachlor, chlordecone, dieldrin, and hexabromocyclododecane (HBCD) were prepared. DDTs [median: 41 ng/g lipid weight (lw)], HCB (5.5 ng/g lw), and HCHs (2.4 ng/g lw) were the predominant compounds in all samples. Median levels of PBDEs (0.91 ng/g lw) in Belgian human breast milk samples were lower compared to other European countries. The major PBDE congeners were BDE-47 and BDE-153, and total PBDE levels were low (0.30-4.25 ng/g lw). α -HBCD was the only HBCD stereoisomer found in the pooled milk samples (2.5 ng/g lw). All targeted POPs were determined in a national pooled sample but were lower than levels of most POPs [organochlorine pesticides (OCPs), PBDEs, and polychlorinated biphenyls (PCBs)]



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observed in 2006. The daily dietary intake of POPs via human milk was estimated for nursing infants of 1 month [intake of 260 mL milk/kg body weight (bw)] and compared with either health-based guidance values (HBGV) or the reference point (margin of exposure, MOE). The exposure assessment in a worst-case scenario revealed no concern for most POPs. However, the infants were exposed to levels of 60 pg total WHO2005-TEQ/kg bw/week, indicating a possible risk during their life. Based on the MOE approach, 95th percentile of concentration can result in a health concern for congeners BDE-99 and BDE-153.

Keywords: Human milk, WHO monitoring, persistent organic pollutants, brominated flame retardants, exposure assessment

INTRODUCTION

Human milk is the optimal food for infants, offering practical benefits for mothers as well. Breastfeeding supports maternal health by reducing the risk of ovarian and breast cancer, while also promoting sensory and cognitive development in infants. It protects against infectious and chronic diseases, lowers infant mortality from illnesses such as diarrhea and pneumonia, and aids in faster recovery during sickness^[1]. While breastfeeding provides significant health benefits, it can also serve as a source of exposure to environmental contaminants. Among these, polybrominated diphenyl ethers (PBDEs), which share a structural similarity with polychlorinated biphenyls (PCBs), are notable. PBDEs consist of a diphenyl ether core with various combinations of bromine atoms, though they are believed to degrade more readily in the environment compared to PCBs. In fact, PBDE levels in American women's human milk samples are reported to be up to 100 times higher than those found in European samples^[2]. Similarly, concentrations of other contaminants, such as dichloro-diphenyl-trichloroethane (DDT) and its metabolite dichloro-diphenyl-dichloroethane (DDE), have declined in regions where their use has been banned, particularly in human milk. However, other organochlorine pesticides (OCPs), which were once widely used as agricultural and domestic pesticides, remain present in the environment over time. Although their use is now prohibited in the European Union due to their toxicity and persistence, they are still detected in human milk, particularly in countries where their use has not yet been fully phased out^[3].

These contaminants, commonly grouped as persistent organic pollutants (POPs), are now considered ubiquitous contaminants in human populations worldwide, and recent investigations have revealed significant variability in POP concentrations in human milk, even within the same individual. Studies have shown fluctuations in POP levels on a daily and monthly basis^[4]. Furthermore, geographic location is a major factor influencing the accumulation of POPs. Proximity to agricultural areas, waste incineration sites, and other pollution sources are key factors influencing an individual's body burden of these chemicals. Since humans are at the top of the food chain, POPs gradually build up over time, leading to what is known as a "lifetime body burden". These findings highlight the complexity of human exposure to POPs, which, despite generally being detected at low levels, remains a global concern due to their persistence and bioaccumulation^[2,3,5-11].

The maternal body burden of POPs depends on several factors, including age, geographical location, background exposure, and number of pregnancies and lactations, which are among the most important. Background exposure is commonly the consequence of low-level contamination of food commodities and water. Among women with typical background exposure levels (i.e., no episodes of acute high exposure), age and past lactation are key factors. Age serves as a marker of cumulative exposure, while prior lactation is linked to reduced POP concentrations - particularly for PCBs and DDE - in breast milk^[12].

The World Health Organization (WHO) and various governmental authorities strongly advocate for breastfeeding due to its numerous health benefits for infants^[1]. However, it is also recognized that human milk can contain contaminants, particularly POPs. These include PCBs, dichlorodiphenyltrichloroethane and its metabolites (DDTs), chlordane compounds (CHLs), and hexachlorobenzene (HCB). Due to the high toxicity of POPs, there is significant concern about the risks these chemicals pose to infants through breastfeeding. Therefore, obtaining realistic data on POP exposure in infants via breastfeeding is essential for developing and enforcing future regulations on these pollutants. Under the Global Monitoring Plan for POPs within the Stockholm Convention framework, the WHO conducted surveys to track POP levels in human breast milk. The main objectives of these surveys were to identify global variations in POP contamination in human milk and to establish baseline data for countries lacking prior information. This baseline facilitated the subsequent assessments of the success of measures aimed at reducing POP exposure.

The initial WHO surveys, conducted between 1987 and 1989 and again from 1992 to 1993, primarily examined PCBs, polychlorinated dibenzo-p-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs), focusing mainly on regions in Europe and North America. From 2000 to 2019, the WHO, in partnership with the United Nations Environment Programme (UNEP), executed five global studies assessing the levels of POPs in human milk. The most recent study in this series was conducted from 2016 to 2019, with financial support from the Global Environment Facility (GEF) as part of projects under the POPs Global Monitoring Plan^[13]. Participants included 82 countries from all United Nations regions, with representatives from both the Eastern European Group (EEG) and the Western European and Others Group (WEOG). Among these, 50 countries contributed data in multiple studies.

Belgium, holding the highest participation rate among WEOG countries ($n = 6$), contributed to two early global harmonized human milk studies conducted before 2000, when only 19 countries participated. From 2000 to 2019, Belgium continued its involvement through four WHO-coordinated surveys on POPs in human milk within WEOG^[14]. For each WHO survey, a national pooled sample was analyzed. In the first two surveys, Belgian national samples were composed of fewer individual samples and were not fully regionally representative, consisting of three pooled samples from rural, urban, and industrial areas. In 2001 (third survey), two pooled samples were collected from residents of Liège. In contrast, the fourth survey in 2006 featured a Belgian pooled sample comprised of 178 individual samples distributed proportionally by province. Furthermore, the sixth survey included a larger number of individual samples and covered all three Belgian regions^[15], with sample sizes corresponding to the population of each province. Similarly, in the survey conducted in 2014, a Belgian pooled sample was analyzed. Although the maternal characteristics influencing POP concentrations in the breast milk of primiparous mothers in Belgium have already been published^[16], the specific results of the measured POPs have not yet been published. In 2024, Serreau *et al.* conducted a scoping review, collecting articles (1995-2023) that reported on pollutant levels in breast milk^[17]. Although this review provided a comprehensive overview of contaminants in human milk, data from Belgium were not considered. Therefore, the findings from 2014 still provide valuable insight into the dynamics of POPs and deserve special mention in scientific reviews.

In particular, the study comprised the analyses of 206 milk samples from mothers living in the three Belgian regions (Flanders, Wallonia, and the Brussels Capital Region). All samples were analyzed for the following POPs: hexachlorocyclohexane group [α -hexachlorocyclohexane isomer (HCH), β -HCH, γ -HCH], HCB, DDT group (o,p'-DDD, p,p'-DDD, o,p'-DDE, p,p'-DDE, o,p'-DDT, p,p'-DDT), chlordane-group (oxychlordane, trans-nonachlor, cis-chlordane, and trans-chlordane) PBDEs, pentachlorobenzene (PeCB), and hexabromobiphenyl (BB-153). Pooled samples were also prepared at the provincial level for the analysis of hexachlorobutadiene, heptachlor, chlordecone, dieldrin, and hexabromocyclododecanes (HBCDs). In a

national pooled sample, all POPs included in the Stockholm Convention were determined. The aims of the present study were threefold: (1) to report and discuss the POP levels found both in individual and pooled human milk samples; (2) to compare the POP levels in Belgian human milk samples (individual and national) with results obtained in other countries and with the results from the previous Belgian survey; and finally (3) to estimate the exposure of the nursing infants and perform a risk assessment for the different POPs determinant in the national pooled sample for this age group.

EXPERIMENTAL

Sample collection

Between May and December 2014 a total of 31 hospitals across Belgian provinces (Flanders: Antwerpen, Limburg, Oost-Vlaanderen, West-Vlaanderen, Vlaams-Brabant, and Wallonia: Brabant-Wallon, Hainaut, Liège, Luxembourg, Namur) and the Brussels Capital Region were selected and visited to recruit breastfeeding mothers, with one hospital in the urban and one in the rural area. The recruitment strategy, along with informed consent documents, questionnaires, and all relevant documentation for participating mothers and maternity units, was thoroughly reviewed and approved by several authorities. This included the Commission for the Protection of Privacy (registration number HM002002523) and the Ethical Commission of the Queen Fabiola Children's University Hospital in Brussels, which served as the coordinating ethical body for the multicenter study (registration number CEH 21/14). Additionally, the ethical commissions from eight of the 31 participating maternity hospitals were involved in the process. The other 23 maternity hospitals considered the review and approval from the coordinating ethical commission to be adequate. Details regarding the recruitment and study protocol can be found in^[16], while a summary of the questionnaire is provided in [Supplementary Material 1](#).

Preparation of the individual and pooled milk samples

The samples were registered under a code number to ensure the confidentiality of the data. Most samples (more than 70%) consisted of a minimum of 50 mL, which was sufficient for chemical analyses. The volumes used for each analysis and for preparing the mixed samples, the number of containers per sample, and the province of origin were carefully documented. The sample flow is illustrated in [Figure 1](#).

Before the analyses were conducted, individual samples were homogenized by heating to 38 °C and shaking for 10 min. Necessary aliquots were then taken to create mixed samples that accurately represented each province. The preparation of these samples adhered to a modified WHO protocol, taking into account two key parameters: (1) ensuring adequate representation of each participant in the mixed sample and (2) achieving a final volume of 110 mL for each provincial mixed sample to facilitate the planned analyses [[Supplementary Table 1](#)].

One pooled Belgian sample was prepared following the WHO protocol^[18]. Briefly, 50 individual milk samples were selected according to the geographical distribution [[Supplementary Table 1](#)]. The frozen samples were first left to equilibrate at room temperature. Afterwards, each sample was homogenized by shaking it for 5 min. From each sample, 25 mL was taken and transferred to a 2,000 mL flask, resulting in 1,250 mL milk (50 × 25 mL milk). This was kept at -20 °C and sent frozen to the European reference laboratory for further analyses of the WHO-selected POPs. Analyses of chlorinated and brominated POPs conducted between 2000 and 2019 were carried out at the State Institute for Chemical and Veterinary Analysis of Food; Chemisches und Veterinäruntersuchungsamt (CVUA) in Freiburg, Germany. In contrast, perfluoroalkane substances were analyzed from 2009 to 2019 at Örebro University in Sweden. A high level of reliability in the results was attained^[13].

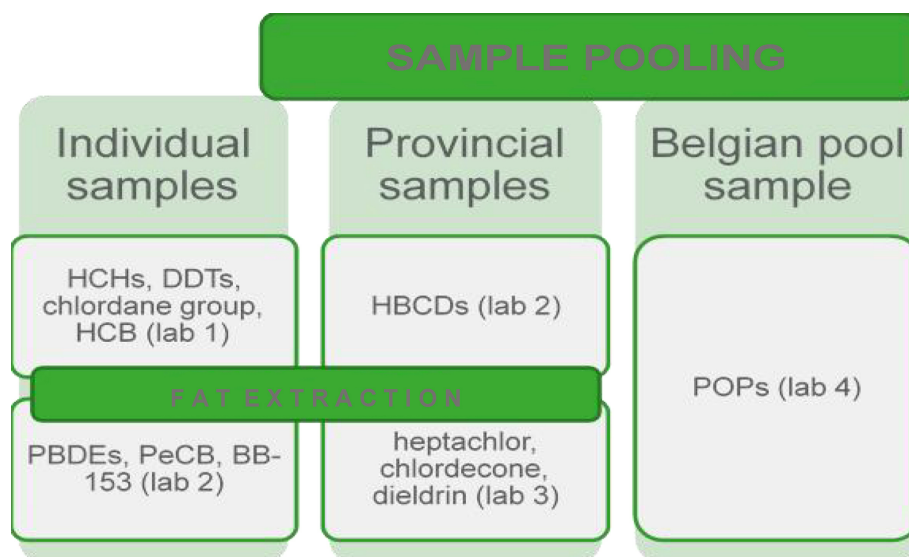


Figure 1. Human milk samples' flow in the study and the analyses of POPs allocated per laboratory where lab 1 is Sciensano, lab 2 is University of Antwerp, lab 3 is Eurofins Food Testing Belgium NV (Brugge, Belgium) which performed the analysis as contractor, and lab 4 is CVUA, Freiburg, Germany. POPs: Persistent organic pollutants; CVUA: State Institute for Chemical and Veterinary Analysis of Food; Chemisches und Veterinäruntersuchungsamt.

Reagents

Standards of OCPs were obtained from Dr Ehrenstorfer (LGC) as a mix solution (mix71) containing the following pesticides: *cis*-chlordane, *trans*-chlordane, HCB, α -HCH, β -HCH, γ -HCH, heptachlor epoxide, α -endosulfan, β -endosulfan, dieldrin, endrin, *p,p'*-DDD, *p,p'*-DDE, *p,p'*-DDT, *o,p'*-DDT, *o,p'*-DDE, *o,p'*-DDD, and methoxychlor at a concentration of 10 $\mu\text{g/mL}$ and as a neat product for oxychlordane, *trans*-nonachlor, dibromooctafluorobiphenyl (DBOBF) (OCPs surrogate), and mirex (OCPs internal standard). While the internal standard corrects analyte quantification in each sample, the surrogate is used in the control sample to verify the method's recovery performance for the surrogate compound. *n*-Hexane, acetone, sulphuric acid for analysis grade, sodium chloride (NaCl), and silica gel 60 for column chromatography were purchased from Merck (Darmstadt, Germany). Aluminium oxide (Al_2O_3) for chromatography was obtained from Thermo Fisher Scientific (Brussels, Belgium). Analytical grade anhydrous sodium sulfate (Na_2SO_4) was obtained from AnalytiChem Belgium (Zedelgem, Belgium).

Fat extraction and fat content determination

Fat extraction was performed following the prEN1528-2-1996 method^[19]. The details are given in [Supplementary Material 2](#), and the accuracy of the extraction in [Supplementary Table 3](#).

Analysis of OCPs by gas chromatography-tandem mass spectrometry

The analysis of OCPs (α -HCH, β -HCH, γ -HCH; HCB; *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDD, *o,p'*-DDT, *p,p'*-DDD, *p,p'*-DDT, oxychlordane, *trans*-chlordane, *cis*-chlordane, and *trans*-nonachlor) was performed on fat extracts from each individual milk sample by gas chromatography-tandem mass spectrometry (GC-MS/MS) using DBOFB as a surrogate and standard reference material [[Supplementary Table 4](#)]. Two hundred mg of the fat extract was dissolved in 1 mL hexane and 50 μL of surrogate DBOFB (10 $\mu\text{g/mL}$) was added. The mixture was subjected to a clean-up over a 10 g of 8% deactivated Al_2O_3 column topped with 0.5 g Na_2SO_4 . OCPs were eluted with 75 mL of hexane and the eluate was afterwards concentrated with a Kuderna-Danish evaporator and finally under a gentle nitrogen stream to approximately 1 mL. To this, 50 μL of a 1 $\mu\text{g/mL}$ mirex solution was added as internal standard and a volume of 2 μL was injected into the GC-MS/MS system. Analysis was performed on an Agilent 7890B GC coupled with an Agilent 7000C MS (Santa Clara,

California, United States) equipped with a Rxi-XLB capillary column (30 m × 0.25 mm, 0.25 µm). Injection was done in splitless mode. Helium was used as carrier gas (1.2 mL/min). The temperature program was set at 110 °C for 0.5 min, then increased by 25 °C/min to 200 °C, then increased by 10 °C/min to 280 °C, followed and kept for 4 min, and finally by 25 °C/min to 300 °C and held for 3.1 min. The multiple reaction monitoring (MRM) transitions and the MS parameters are given in [Supplementary Table 2](#). As a quality control, a solvent blank, a procedural blank, and a control sample were run within each batch of samples. Average procedural blank levels were then subtracted from the sample results, and a value equal to 3 × SD of the blank measurement was used as the limit of quantification (LOQ). For compounds absent in the blanks, LOQs were based on a signal/noise ratio of 10 (S/N = 10). The LOQ was 2 ng/g lipid weight (lw), and the limit of detection (LOD) was 1 ng/g lw, except for oxychlordan where 5 and 2.5 ng/g lw were LOQ and LOD, respectively).

Analysis of OCPs by GC-MS/MS in provincial samples

Composite samples were created by combining individual samples from each province, yielding a total of 11 provincial samples. Further analyses of hexachlorobutadiene, heptachlor, cis-heptachlor epoxide, trans-heptachlor epoxide, chlordecone, and dieldrin were outsourced to an external lab. The accredited method used for the analyses of the samples was based on GC-MS/MS detection.

Analysis of PBDEs, PeCB and BB-153 by GC-MS

The analysis of PBDEs, PeCB, and BB-153 in human milk samples was conducted in accordance with the protocol outlined by Dimitriadou *et al.* (2016), incorporating minor modifications as detailed in [Supplementary Material 3](#)^[20].

Analysis of HBCDs by LC-MS/MS

Composite samples of human milk were prepared according to the WHO protocol and represented the pooled samples for each Belgian province. These samples were kept in glass containers from the first transport until the analyses and during any further storage. The extraction was done according to the protocol described elsewhere^[20,21]. Details are in [Supplementary Material 4](#).

Analyses of the national pooled sample by the EU reference lab

Out of 1,250 mL prepared to represent the Belgian pooled sample, 450 mL was used for analysis by the CVUA in Freiburg, Germany, as requested by the National Coordinators of the study^[22-24]. CVUA is the WHO Reference Laboratory for the WHO-coordinated human milk study for POPs. All analytical results were reported on a lipid basis and were related to a broad range of POPs, including PCDDs, PCDFs, and dioxin-like PCBs. The whole list of compounds can be found in [Supplementary Material 5](#). The results obtained are discussed in a comparative analysis with findings from other countries.

Quality assurance/quality control

The efficiency of extraction, clean-up, and fractionation steps was evaluated by measurement of the absolute recoveries of the internal standards [[Supplementary Material 6](#)].

Descriptive statistics

The results are outlined and summarized using descriptive statistics. Measures of central tendency (mean and median) and variability (frequency) were calculated for the fat percentage in milk samples and the concentrations of POPs, both overall and by compound group. Two scenarios were analyzed: lower bound (LB) and medium bound (MB), which represent a more conservative approach to minimize the risk of overestimation and, if necessary, to facilitate the development of realistic mitigation strategies. The upper bound was calculated as additional information. The lower and MBs were determined by assigning a value

of zero and $\frac{1}{2}$ LOQ, respectively, to samples where the pollutant concentration was not detected. For the upper bound, the levels of LOQ were assigned.

Exposure assessment and risk characterization

Exposure assessment

The estimated daily intake (EDI) of POPs for infants of 1 month was calculated by assuming a body weight (bw) of 4.3 kg^[25] and a human milk consumption of 260 mL/kg bw/day^[26].

$$EDI_{POP} = \frac{VMI_{day} \times C_{POP/milk}}{1000}$$

where EDI_{POP} is the daily intake ($\mu\text{g/kg bw/day}$), VMI_{day} is the volume of milk an infant of 4.3 kg drinks per day, divided by the bw (mL/day/kg bw) (set to 260 mL/day/kg bw for this worst-case scenario), $C_{pop/milk}$ is the concentration of POP (ng/mL lw) in the consumed milk, measured in lipid weight ($\text{ng}_{POP}/\text{mL}_{milk}$), and 1,000 is a correction for the measurement units. This scenario has been chosen due to the lack of real data on the weight of the infants.

Using the POP concentration, the intake was estimated for the national pooled sample [DDTs, HCB, γ -HCH, BDE-47, BDE-99, BDE-153, heptachlor, PCB+PCDD/F TEQ, HBCDs, perfluorooctane sulfonate (PFOS), and perfluorooctanoic acid (PFOA)]. Both the P50 (median) and the P95 (worst case scenario) concentration values of POPs analyzed in individual samples (DDTs, HCB, γ -HCH, and the PBDE congeners BDE-47, BDE-99, and BDE-153) were used in the estimation.

Risk characterization

The intakes of POP assessed in the national pooled sample were used in risk characterization. Risk was characterized for two clusters of compounds depending on the availability of the toxicological data. The first cluster included the compounds for which the health-based guidance values (HBGV) approach was applied (Sum DDTs, HCB, γ -HCH, Heptachlor, PCB+PCDD/F TEQ, PFOS and PFOA) and the second cluster included the compounds (PBDEs and HBCDs) for which the margin of exposure (MOE) approach was applied.

The HBGV approach, which uses values such as the acceptable daily intake (ADI), tolerable daily intake (TDI), provisional tolerable daily intake (PTDI), or tolerable weekly intake (TWI), is designed to assess chronic intake over the entire lifespan, excluding the breastfeeding period. However, HBGVs such as TWI and TDI cannot be directly applied to breastfed infants for two main reasons: first, these values are established for the general population and reflect lifelong exposure, and second, they do not account for the unique developmental stage of infants. In the evaluated scenario - exposure of 1-month-old breastfed infants - it was assumed that their diets consisted primarily of human milk or formula, along with tap or mineral water. The potential introduction of small amounts of infant-specific foods was not considered at this age. Although breastfeeding for six months represents less than 1% of the average human lifespan, it is crucial to recognize that HBGVs established for the general population are not suitable for infants, whose organ systems are still developing. Therefore, HBGV values alone are insufficient to adequately assess health risks for infants during breastfeeding. Nonetheless, they can function as a general indicator of potential health risks. If the EDI of substances through breastfeeding is calculated to be below or within the HBGV, it can be inferred that the likelihood of adverse health effects is minimal.

For the second cluster, MOE method was utilized. In line with EFSA's guidelines^[27], it was assumed that human exposure to PBDEs and HBCDs primarily occurs through ingestion of food. This approach enabled

the estimation of chronic dietary intake ($D_{r,h}$), which reflects the body burden in an average individual. Another assumption made is that PBDEs and HBCDs predominantly accumulate in adipose tissue, suggesting that a one-compartment model is adequate for kinetic analysis. As a result, determining chronic dietary intake in humans ($D_{r,h}$) necessitates accounting for the steady-state body burden at the BMDL10, the proportion of daily intake that is absorbed by the body, and the rate constant governing the elimination of the compound.

Therefore, $D_{r,h}$ can be calculated as follows:

$$D_{r,h} = \frac{BB_a * k_{el,h}}{F_{abs,h}}$$

where $D_{r,h}$ represents the chronic daily dietary intake for humans (amount/kg bw per day); BB_a denotes the body burden in the experimental animal (amount/kg bw); $k_{el,h}$ is the elimination rate constant from the human body (day^{-1}); and $F_{abs,h}$ refers to the fraction of the chemical in food that is absorbed into the human body (dimensionless).

Finally, the MOE was calculated as the ratio of the $D_{r,h}$ and the estimated daily milk intake (EDI_{POP}).

$$MOE = \frac{D_{r,h}}{EDI_{POP}}$$

where $D_{r,h}$ is expressed in ng/kg bw/d and EDI_{POP} in ng/kg bw/d. EFSA calculated the MOE based on the average intake of breastfed infants, focusing on those around three months old (weighing approximately 6.1 kg). They estimated an average daily intake of around 800 mL of human milk, with higher consumption reaching 1,200 mL. The higher intake closely aligns with the consumption levels estimated in our study. For BDE-99 and BDE-153, an MOE of 2.5 or lower was observed, while for BDE-47, values below 12 might indicate potential health risks. In a later opinion, EFSA^[28] applied the combined margin of exposure (MOET) method for a mixture of BDE-47, -99, -153, and -209, in accordance with their guidance on mixture risk assessment.

For HBCDs, a MOE lower than 8 indicated a health concern^[29]. These reference points were used for risk characterization in our study. It should be mentioned that in 2021, EFSA^[30] issued an update opinion where, on the basis of new toxicological data, the EFSA Contam Panel identified a LOAEL of 0.9 mg/kg bw as the Reference Point for HBCDs, corresponding to a body burden of 0.75 mg/kg bw with an additional factor of 3. The chronic intake required to result in an equivalent body burden in humans was estimated at 2.35 µg/kg bw per day. An MOE of 24 or higher was considered to indicate a low level of concern.

RESULTS AND DISCUSSION

Lipid levels in milk samples

Almost half of all measured samples had 2%-4.2% fat (group 2), with an average of 3.9% fat, whereas only exceptionally (7.3% of all samples), the percentage of fat was above 6.2% (group 4) [Figure 2]. This was comparable to some reported data in other European countries^[16]. While 3.8% is considered the average fat content in Europe, the percentage in primiparous mothers can range from 1.6% to 4.7%^[31], with values in Italy ranging between 2.6% and 3.1%^[32], and a maximum of 9.6% observed in Norway^[33], where the average was 3.6%^[4].

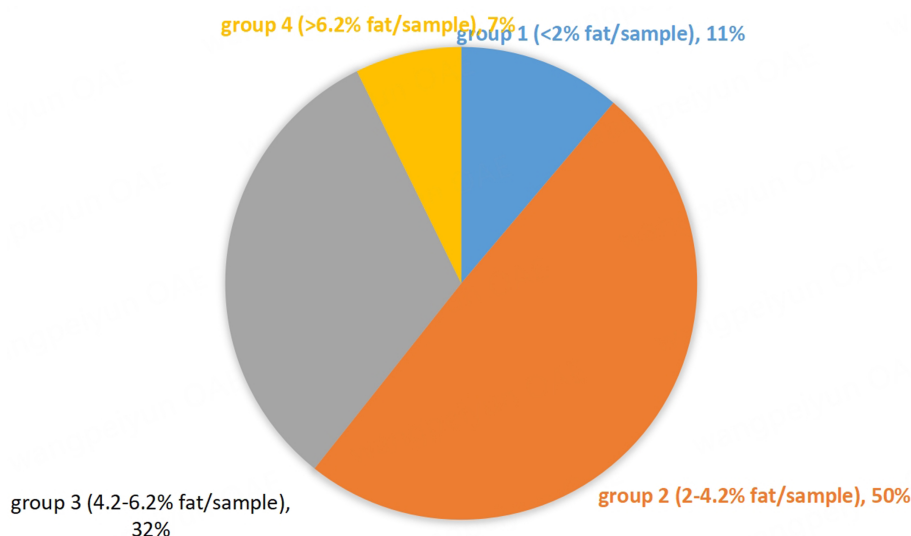


Figure 2. Frequency (%) of fat percentage in the human milk samples ($n = 206$).

POP levels in individual samples

In 206 individual samples, several POPs were quantified with various frequencies, which are presented in a heatmap [Supplementary Figure 1]. In more than 50% of the samples, concentrations of HCB, β -HCH, p,p'-DDE, p,p'-DDT, BDE-47, and BDE-153 were above the LOQ. The mean values using MB concentrations for these six POPs were 5.57, 2.91, 52.23, 4.40, 0.24, and 0.46 ng/g lw, respectively. Contrastingly, oxychlordane, BDE-28, BDE-99, BDE-100, BDE-154, PeCB, and BB-153 values above the LOQ were found in less than 50% of the total number of samples. Cis- and trans-chlordane, trans-nonachlor, α -HCH, o,p'-DDD, o,p'-DDT, o,p'-DDE, and BDE-183 were not found at levels above LOQ. Independently of the percentage of non-detects, for each POP, the mean results of LB, MB, and upper bound were calculated and presented in Table 1. Relevant median values were also calculated and used for comparison to other studies [Table 2].

OCPs

In all samples, p,p'-DDE was detected with the highest mean concentration of 52.2 ng/g lw, comparable to levels previously measured in Belgium^[34], France^[35], and the Netherlands^[36]. Two studies from Croatia^[37,38] reported lower p,p'-DDE, but additionally revealed that p,p'-DDE was a predominant organochlorine contaminant with a descending concentration pattern of p,p'-DDE > p,p'-DDT > HCB > γ -HCH > β -HCH > α -HCH > p,p'-DDD. This pattern was also found in our study and in the previous national WHO survey of 2006, and in Tunisia^[42], while in Turkey^[39], China^[43], France^[35] and Flanders region^[34], β -HCH was the most prevalent POP contaminant. The major OCPs were p,p'-DDE, HCB and β -HCH. Table 2 lists the concentrations of p,p'-DDT, p,p'-DDE, β -HCH, and HCB in milk samples obtained during the last 10 years in some European countries. A large part of the exposure to DDT is probably due to its historical use. The ratio DDT/DDE, which indicates the time-span exposure (recent or historical exposure for high or low value, respectively), was 0.075, and it was similar to the results of WHO-2006 survey and the report of Croes et al.^[34], indicating a rather historical exposure^[20].

PBDEs

All PBDE congeners were quantified in the human milk samples, except BDE-183 [Table 1]. The highest concentration was observed for BDE-153, with a mean of 0.50 ng/g lipid weight, detected in 93% of the samples. Other congeners frequently quantified included BDE-47 (53%), BDE-154 (38%), BDE-99 (26%),

Table 1. POP levels (ng/g lw) in human milk samples* (n = 206) collected in Belgium in 2014

POP	Mean			LOQ [*]	Occurrence frequency (%) [*]
	LB	MB [*]	UB		
Chlordane group					
cis-Chlordane	0.00	1.00	2.00	2.0	0
Trans-Chlordane	0.00	1.00	2.00	2.0	0
Oxychlordane	0.88	3.04	5.20	5.0	13.6
Trans-Nonachlor	0.00	1.00	2.00	2.0	0
DDT group					
o,p'-DDD	0.00	1.00	2.00	2.0	0
p,p'-DDD	0.03	1.02	2.01	2.0	1
o,p'-DDE	0.00	1.00	2.00	2.0	0
p,p'-DDE	52.23	52.23	52.23	2.0	100
o,p'-DDT	0.00	1.00	2.00	2.0	0
p,p'-DDT	4.10	4.39	4.69	2.0	70.0
HCB	5.54	5.56	5.59	2.0	97.6
HCB group					
α-HCH	0.00	1.00	2.00	2.0	0
β-HCH	2.52	2.91	3.30	2.0	61.2
γ-HCH	0.06	1.04	2.02	2.0	1.9
PeCB	0.24	0.45	0.67	0.5	14.6
BB-153	0.00	0.05	0.10	0.1	0.5
PBDE group					
BDE-28	0.01	0.06	0.11	0.1	5.8
BDE-47	0.22	0.24	0.27	0.1	53.4
BDE-99	0.06	0.10	0.14	0.1	25.7
BDE-100	0.06	0.09	0.13	0.1	24.8
BDE-153	0.46	0.46	0.47	0.1	92.7
BDE-154	0.10	0.13	0.16	0.1	38.4
BDE-183	0.00	0.10	0.20	0.2	0

*The values were also reported by Aerts et al. and may be used for comparison^[16]. POP: Persistent organic pollutant; LB: lower bound; MB: medium bound; UB: upper bound; LOQ: limit of quantification; DDT: dichloro-diphenyl-trichloroethane; DDD: dichloro-diphenyl-dichloroethylene; DDE: dichloro-diphenyl-dichloroethane; HCB: hexachlorobenzene; HCH: hexachlorocyclohexane isomer; PeCB: pentachlorobenzene; BB-153: hexabromobiphenyl; PBDE: polybrominated diphenyl ether; BDE: brominated diphenyl ether.

and BDE-100 (25%). Conversely, BDE-28 exhibited the lowest detection frequency at 6%, and BDE-183 was absent from all samples.

In other European countries, the BDE congener pattern was also dominated by BDE-47, BDE-153, BDE-99, and BDE-100 [Table 3]. The highest median levels of BDE-153 were reported in France, the Netherlands, and Germany, while the highest median levels of BDE-47 were found in milk from Norway, Greece, the UK, Denmark, Finland, and Slovakia. The detection of BDE-47 in human milk and tissues is generally associated with exposure to commercial formulations of Penta-BDE, in which BDE-47 is a primary component. In contrast, the occurrence of BDE-153 is likely related to exposure to Octa-BDE products, along with its increased persistence; multiple studies have identified BDE-153 as the most abundant PBDE congener. Previous research conducted in Belgium^[15,34], including the present study, has noted elevated levels of BDE-153 in human milk compared to BDE-47, as well as in cord blood, serum, and human milk samples collected from Flanders^[47].

Table 2. Concentrations (ng/g lw) of OCPs in human milk from various countries

Country	Sampling year	β -HCH mean	β -HCH median	p,p'-DDE mean	p,p'-DDE median	p,p'-DDT mean	p,p'-DDT median	HCB Mean	HCB median	Ref.
Belgium	2014	2.91	2.40	52.2	37.0	4.40	2.80	5.57	5.50	current study [*]
France	2011-2014		15.0		60.1		2.2		10.3	[35]
the Netherlands	2011-2014			69.2	49.4	2.0	1.5	6.5	6.2	[36]
Israel (1 pooled sample)	2012	10.8		147		4.3				[40]
Slovakia	2010-2012			266	167	10.6	18.7	13.3	12.9	[36]
Tunisia	2010	28.1		371		271		203		[38]
Croatia	2011	1.7		14.2		8.1		3		[37]
Croatia (Zadar)	2009-2011		2.3		23.5		1.9		2.0	[37]
Croatia (Zagreb)	2009-2011		3.1		21.0		1.5		2.5	[37]
Belgium (rural Flanders)	2009-2010		6.1		56.9		2.6		6.4	[34]
Turkey	2009	36.3		325.1		10.5		5.4		[39]
Belgium	2006		nd		95.9		nd		15.5	[15]
Norway	Birth cohort	4.70	4.32	66.0	53.5	2.65	2.11	11.1	10.5	[41]

^{*}For current study: medians and means = medium bound results. OCPs: Organochlorine pesticides; HCH: hexachlorocyclohexane isomer; DDE: dichloro-diphenyl-dichloroethane; DDT: dichloro-diphenyl-trichloroethane; HCB: hexachlorobenzene; nd: not determined.

Table 3. PBDE levels (ng/g lw) in human milk from Europe

Country	Sampling year	BDE-28	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	Ref.
Belgium	2014	0.06	0.24	0.10	0.09	0.46	0.13	< LOQ	Current study [*]
France	2011-2014	0.04	0.43	0.10	0.10	0.54	0.03	0.05	[35]
the Netherlands	2011-2014	0.02	0.20	0.08	0.06	0.48		0.04	[36]
Slovakia	2011-2012		0.17	0.07	0.04	0.12			[36]
UK	2011-2012	0.09	1.92	0.88	0.64	1.01	0.07	0.05	[44]
UK	2010		2.80	0.69	0.38	0.91	0.21		[45]
Belgium	2009-2010	< LOQ	0.16	0.06	0.06	0.29	0.07	< LOQ	[34]
Germany	2009		0.24	0.08	0.08	0.47			[46]
Norway	2003-2006	0.08	1.10	0.10	0.10	0.25			[36]
Greece	2004-2005	< 0.10	0.48	0.27	0.19	0.30	< 0.10	< 0.10	[20]
Denmark	1997-2002	0.19	1.99	0.76	0.45	1.20	0.07	0.07	[35]
Finland	1997-2001	0.33	3.12	0.54	0.42	0.77	0.05	0.03	[35]
Norway	Birth cohort	0.16	1.10	0.26	0.26	0.50	0.03		[40]

^{*}Mean MB values. PBDE: Polybrominated diphenyl ether; BDE: brominated diphenyl ether; LOQ: limit of quantification; MB: medium bound.

POP levels in regional pooled samples

The analyses of the mixed samples per each Belgian region ($n = 10$) did not reveal the presence of hexachlorobutadiene (LOQ = 50 pg/mL), chlordecone (LOQ = 10 pg/mL), dieldrin (LOQ = 5 pg/mL), cis-heptachlor (LOQ = 5 pg/mL), trans-heptachlor epoxide (LOQ = 5 pg/mL), or heptachlor (LOQ = 4 pg/mL). However, heptachlor was detected in the pooled Belgian sample at 3.5 ng/g lw [Supplementary Table 5]. Only α -HBCD could be quantified in comparable levels as in the pooled Belgian sample (0.9 to 4.97 ng/g lw). Despite α -HBCD's minor contribution to global HBCD production (technical mixture: α -HBCD of around 10% vs. γ -HBCD of > 70%), it is the dominant congener found in most biotic samples worldwide. Its predominance is hypothesized by the different toxicokinetics and persistency of the HBCD stereoisomers^[48].

The results for HBCDs in regional samples are comparable to those reported by Roosens *et al.*, who also detected HBCDs in samples from Flanders, Belgium (0.6-5.7 ng/g lw)^[47]. Several studies in Flanders have

evaluated HBCD presence, particularly suggesting that the elevated values in eels from Flemish rivers could be linked to the textile industry present in this region^[21,41,49]. **Supplementary Table 5** highlights the variation in HBCD concentration across the provinces, with the highest concentration measured in East Flanders, confirming regional differences in Belgium. Additionally, Goscinny *et al.* detected HBCDs in 80% of analyzed foods, with only 8 out of 43 samples showing levels below the LOD^[50]. They found the highest concentration of Σ HBCD in fish and fishery products, followed by meat and meat products and dairy products. This indicated that dietary sources might have partially contributed to external exposure metrics.

Levels of POPs in the Belgian national pooled sample and time trends

Trend analysis of POP concentrations

Belgium participated in six surveys. While the first three campaigns included limited geographical coverage and fewer individual samples, the fourth and sixth surveys incorporated a greater number of samples from all three regions. The fourth survey in 2006 featured a single pooled Belgian sample comprising 178 individual samples, with sizes proportional to each province's population^[15]. A similar method was applied in this study (see section "Preparation of the individual and pooled milk samples"). **Figure 3** shows a downward trend in the concentration of most POPs between 2006 and 2014.

A substantial decrease was observed for p,p'-DDE from 132 to 74 ng/g lw, for HCB from 15 to 10.8 ng/g lw and for β -HCH from 12 to 4.3 ng/g lw. The main reason for this decrease is probably linked to the reduced use of these POPs and the time-related reduction of their environmental levels. More than 10 years passed since the last Belgian WHO-coordinated survey on POPs in human milk^[15]. As the first human biomonitoring survey in Flanders (2002-2006) demonstrated an increased exposure to p,p'-DDE in some parts of Flanders, the Flemish government took several policy actions to limit the exposure to DDT. The results of the different surveys in Flanders indicated that consumption of locally-produced vegetables and home-produced eggs was associated with a higher body burden of p,p'-DDE^[51]. Measurements in eggs confirmed the elevated p,p'-DDE levels in home-produced eggs compared to levels in eggs from retail shops^[52-54]. The Flemish government issued guidelines for healthy gardening and safe consumption of locally grown food. In addition, an action was organized whereby individuals could return residues of banned pesticides such as DDT to container parks^[55]. The presence of DDT in human milk is a worldwide phenomenon, with higher levels in milk from countries where DDT use is still allowed for some applications^[56]. During the survey in 2014, a reduction of half of the burden of p,p'-DDE was observed over a 10-year period. In Flanders, the human biomonitoring surveys also demonstrated a significant decrease in p,p'-DDE levels in cord blood samples. Both results indicate that the measures taken by the government to reduce the presence of POPs had a positive impact. The concentrations of PCDD/Fs have decreased substantially since the first survey in which Belgium participated. In the initial four WHO milk surveys, PCDD/F levels in Belgian samples ranged from the highest among participating countries to those comparable to other industrialized nations. Importantly, PCDD/F concentrations in human milk collected in Belgium reflected the international downward trend. In the first four WHO milk surveys, Belgium had PCDD/F levels ranging from the highest level of all participating countries to levels comparable with other industrialized countries. The PCDD/F concentrations in Belgian human milk followed the international downward trend. The concentrations decreased from 37.5 pg TEQ/g lw (1988-1989) to 24.8 pg TEQ/g lw (1991-1992) and further to 10 pg WHO TEQ/g lw (2006) with a substantial decrease to 4 pg TEQ/g lw (current study). The concentration for the sum of 6 marker PCBs decreased from around 200 ng/g lw in 2001 (for 2 samples from Liège) to 80 ng/g lw (in the pooled Belgian sample from 2006) and 37.5 ng/g lw in the present study. The concentrations for dioxin-like PCBs have been determined only since 1991. In 2006, similar levels as in the years '90 were found. However, in the present study, a decrease from 7 to 2.5 pg WHO TEQ/g lw was observed, marking a threefold decrease between 2006 and 2014. These results are not unexpected, as many European countries have reported a general decrease in dietary exposure to dioxins

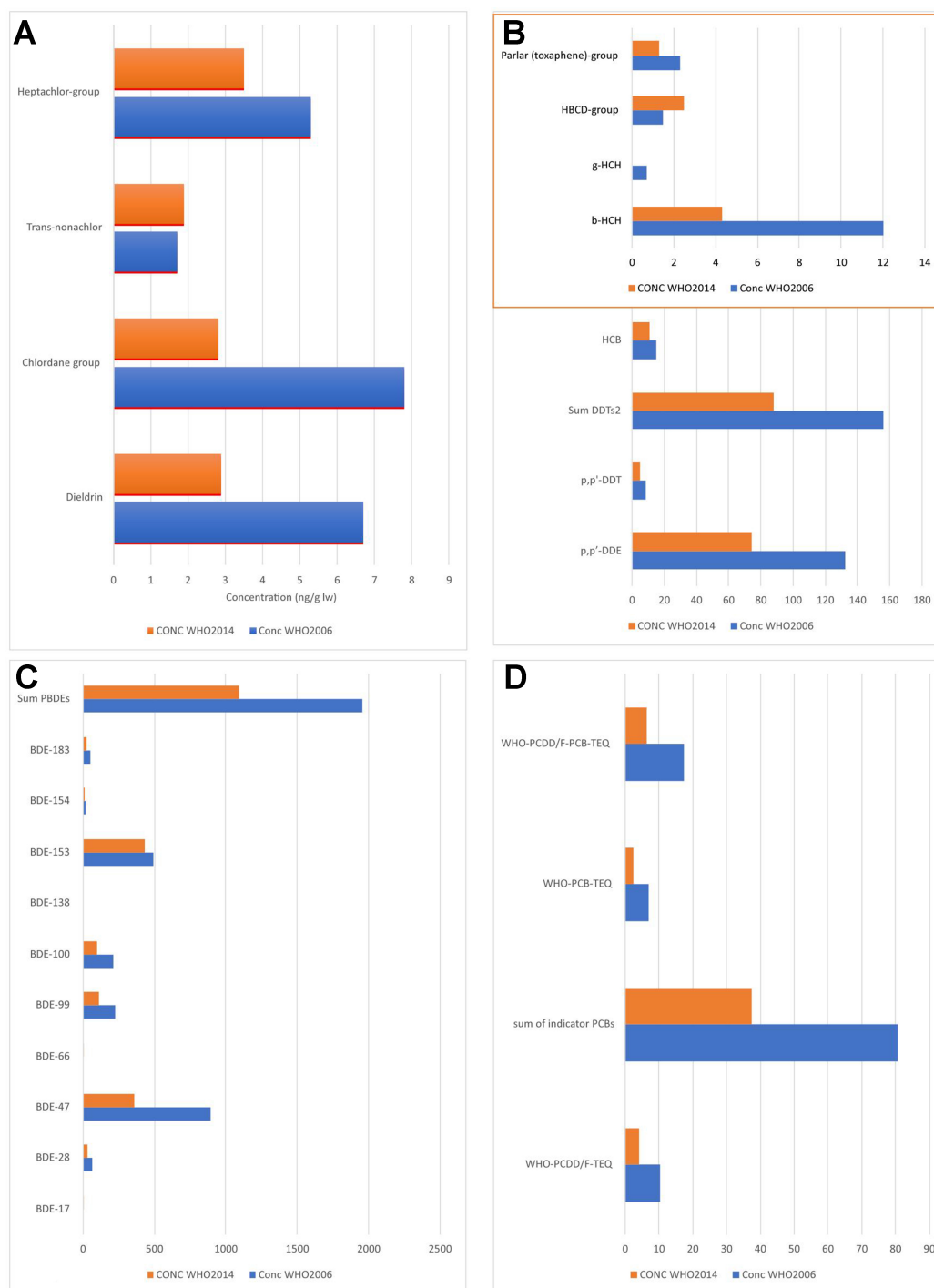


Figure 3. Concentration levels of POPs measured in the Belgian pooled national samples from the WHO 2006 and 2014 studies. (A) chlordane group is expressed as the sum of α -chlordane, β -chlordane and oxchlordane; (B) DDT group as the sum of o,p'-DDT, p,p'-DDT, p,p'-DDE, and p,p'-DDD; heptachlor group as the sum of heptachlor and heptachlor-epoxide (cis/trans), toxaphene is expressed as the sum of Parlar 26, Parlar 50, and Parlar 62 and the sum of α -HBCD, β -HBCD, and γ -HBCD [HBCD group: LOQ = 0.8 (yr 2006) and 0.1 (yr 2014)]; (C) individual PBDE congeners and (D) various sums of PCDD/Fs and PCBs expressed as WHO2005 TEQ. POPs: Persistent organic pollutants; WHO: World Health Organization; DDT: dichloro-diphenyl-trichloroethane; DDE: dichloro-diphenyl-dichloroethane; DDD: dichloro-diphenyl-dichloroethylene; HBCD: hexabromocyclododecane; LOQ: limit of quantification; PBDE: polybrominated diphenyl ether; PCDD: polychlorinated dibenzo-p-dioxin; PCBs: polychlorinated biphenyls; TEQ: total toxic equivalence.

and dioxin-like PCBs between 2002 and 2010^[57].

In general, a decrease in all POPs included in the Stockholm Convention, except for HBCD, was observed in the Belgian pooled sample compared to the results from 2006. The sum of PBDE congeners in the Belgian samples has decreased from 2.1 ng/g lw in 2006^[15] to 1.1 ng/g lw in the present study. The concentrations of only two congeners (BDE-17 and BDE-66) increased. These two congeners are, however, less abundant and may be present in low concentrations.

In a later study from 2012, Croes *et al.* reported concentrations of α -HBCD (3.2 ng/g lw) and dieldrin (7.2 ng/g lw) for rural areas in Flanders (a pooled sample)^[34]. The values in the Belgian pooled sample taken in 2014 were lower. The explanation is that Croes *et al.* aimed to follow the elevated exposure to POPs in the Flemish rural area determined in the human biomonitoring campaign of the first generation Environment and Health (2002-2006)^[34]. The initial study revealed significantly elevated levels of POPs in the cord blood of newborns, as well as in the blood of adolescents (ages 14-15) and adults (ages 50-65), compared to seven other regions in Flanders. Additionally, a downward trend was observed in rural areas, consistent with the overall findings for Belgium. In that first study, significantly high levels of POPs were observed in the cord blood of newborns and in the blood of adolescents (14-15 years old) and adults (50-65 years old) compared to 7 other areas in Flanders. Hence, a downward trend was also observed in rural areas, as in the whole of Belgium.

For the first time, BB-153, PeCB, chlordecone, and short-chain chlorinated paraffins (SCCPs) were analyzed, but not detected in the national sample. The decreasing trend in concentrations of POPs in human milk is a major conclusion from this study and confirms the decreasing concentrations of POPs observed in the previous surveys. On the other hand, the WHO report^[13] indicated that POP concentrations in certain areas remained elevated, with DDT continuing to represent the largest average proportion, followed by SCCPs and PCBs.

In the same report, the high levels of the new POPs listed in the Stockholm Convention, such as SCCPs and PFASs, were reported, pointing to the need for further monitoring. In the national sample, PFAS compounds (PFOS and PFOA) and related polyfluoroalkyl compounds were also measured. PFOA was not detected (LOQ = 80 ng/L), while PFOS and PFHxS were measured at low concentrations (64 and 15 ng/L, respectively).

POP levels-country comparisons

Compared to levels measured in other countries, the indicator PCB value (37 ng/g lw) was higher than that in an Israeli national sample (23.9 ng/g lw)^[41], but the p,p'-DDE level in the pooled sample from Israel (147 ng/g lw) was twice that found in the Belgian sample (74.2 ng/g lw). Likewise, the level of β -HCH for Israel (10.8 ng/g lw) exceeded the level in the Belgian sample from the present study (4.3 ng/g lw).

Fluorinated compounds found in the present study in the pooled Belgian sample (< 80 ng/L PFOA and 64 ng/L PFOS) were comparable to the concentrations reported in human milk from France (40 ng/L PFOS and 41 ng/L PFOA)^[58], Spain (54 ng/L PFOA)^[59], and The Czech Republic (33 ng/L PFOS and 50 ng/L PFOA)^[60]. No trend could be established since the fluorinated compounds were not determined in the Belgian national pooled sample from the year 2006. However, Croes *et al.* reported higher concentrations of PFOS (130 pg/mL) and PFOA (80 pg/mL) in human milk samples collected in Flanders (Belgian region) during 2009-2010^[34]. On average, for the period 2000-2019, WEOG countries showed a gradual downward trend for many POPs^[14,18,22-24]. This comparative analysis of all surveys shows that next to Belgium, also the

Netherlands, Czechia, Slovakia, Austria, and Lithuania, among WEOG countries, achieved reductions in the range of 85% to 95% between the late 1980s/early 1990s and the period from 2012 to 2019. For example, DDT concentrations, with levels ranging from 29 µg DDT complex/kg lipid in Finland (2007) to 615 µg DDT complex/kg lipid in Australia (2010), followed by a threefold decrease in Australia in subsequent surveys. DDT concentrations in Belgium during the 2014 survey fell on the lower end of the spectrum. The relatively low DDT levels across WEOG countries are likely attributed to the early adoption of bans on its agricultural use.

Exposure assessment for nursing infants

The exposure of nursing infants to the POPs measured in national human milk samples ranged between 3.5×10^{-3} µg/kg bw/day (BDE-99) and 1.7 µg/kg bw/day (ΣDDTs). In [Tables 4](#) and [5](#), the estimated dietary exposure to various POPs via human milk is shown and compared with their respective reference values.

Out of ten PBDE congeners, the highest dietary exposure was for BDE-47 and BDE-153 (the most predominant PBDE congeners). The average dietary exposure of an infant to total HBCDs via human milk was estimated at 23 ng/kg bw/day, which was comparable to or lower than some previously reported levels^[29]. The reference values in [Table 4](#) are HBGV and in [Table 5](#) are reference points in case data from toxicological studies were lacking or were insufficient to establish a HBGV. Furthermore, some HBGV, like TWI and TDI, could not be directly used to evaluate the exposure of breastfed infants. In the assessed scenario involving one-month-old breastfed infants, it was assumed that their diets were specialized, primarily consisting of human milk or formula, along with tap or mineral water. The possible gradual introduction of small amounts of a limited number of foods that may be specifically designed for infants was not considered for this age.

The estimated exposure to PFOS/PFOA was higher than the established TWI^[66]. As said previously, this is a protective value for children at later ages, describing that the risk of adverse effects increases if TWI and TDI are exceeded. Moreover, the concentration of PFOS/PFAS was comparable across various European countries.

The current TWI for the PCB+PCDD/F TEQ was 2 pg total WHO2005-TEQ/kg bw/week^[67]. Since this study assessed exposure solely in infants through breastfeeding, without accounting for maternal exposure via diet or other sources, the TWI can only serve as an indicator. The infants were exposed to 60 pg total WHO2005-TEQ/kg bw per week, suggesting a potential risk over their lifetime; however, exposure levels throughout their lives may change. Additionally, PCDD/F concentrations showed a downward trend over time. In 2004, Belgium had some of the highest PCDD/F levels among participating countries, but by 2014, these levels had become comparable to those in other industrialized nations.

Finally, regarding BDE congeners, no health risk concern was estimated for BDE-47, but for the congeners BDE-99 and BDE-153 at their 50th and 95th percentile concentration (worst case scenario) for both individual samples, as well as for pooled samples, MOE was below 2.5, indicating a health concern. In addition, the derived MOET value for the three BDE congeners was below 25, indicating a possible health concern. Although this approach slightly deviates from the EFSA's recommended PBDE mixture approach^[28], which also includes BDE-209, the derived values may still imply a health concern.

Table 4. Estimated daily exposure and risk characterization for breastfed 1-month-old infants in Belgium to POPs measured in the national pooled sample for which HBGV is known

Compound	Estimated exposure* (ng/kg bw/d)			Risk characterization	
	Individual samples		Pooled sample	HBGV (ng/kg bw/d)	Ref.
	50th percentile	95th percentile			
Sum DDTs	419	1,707	823	10,000 (TDI)	[61]
HCB	56	94	101	170 (PTDI)	[62,63]
γ-HCH	10	10	Nd	5,000 (ADI)	[64]
Heptachlor	nd	nd	33	100 (TDI)	[65]
PCB+PCDD/F TEQ	nd	nd	0.061	0.002 (TWI)	[58]
PFOA, PFNA, PFHxS, PFOS	nd	nd	17	4.4 (TWI)	[57]
PFOA	nd	nd	21		

POPs: Persistent organic pollutants; HBGV: health-based guidance value; DDTs: dichlorodiphenyltrichloroethane and its metabolites; TDI: tolerable daily intake; HCB: hexachlorobenzene; PTDI: provisional tolerable daily intake; HCH: hexachlorocyclohexane isomer; nd: not determined; ADI: acceptable daily intake; PCB: polychlorinated biphenyl; PCDD: polychlorinated dibenzo-p-dioxin; TEQ: total toxic equivalence; TWI: tolerable weakly intake; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid; PFHxS: perfluorohexanesulfonic acid; PFOS: perfluorooctane sulfonate.

Table 5. Estimated daily exposure and risk characterization for breastfed 1-month-old infants in Belgium to POPs measured in the national pooled sample for which MOE was applied

Compound	Estimated exposure* (ng/kg bw/d)			MOE			Ref.
	Individual samples		Pooled sample	Individual samples		Pooled sample	
	P50	P95		P50	P95		
BDE-47 ^a	1	9	3	172	19	57	[27]
BDE-99 ^b	0.5	3.5	1	8.4	1.2	4.2	[27]
BDE-153 ^c	4	12	4	2.4	0.8	2.4	[27]
HBCDs ^d	nd	nd	23	Nd	nd	130	[28]

*calculated for the scenario (infant of 4.3 kg consuming 260 mL milk/kg bw/d) with P50 or P95 concentration for individual samples. ^aReference point 232,000 ng/kg bw/d Body Burden at BMDL10 (dr h: 172 ng/kg bw), ^bReference point 9,000 ng/kg bw/d Body Burden at BMDL10 (dr h: 4.2 ng/kg bw), ^cReference point 62,000 ng/kg bw/d Body Burden at BMDL10 (dr h: 9.6 ng/kg bw), ^dReference point 790,000 ng/kg bw/d Body Burden at BMDL10 (dr h: 3,000 ng/kg bw). The average percentage of fat was 3.9% in individual samples and 3.6% in the pooled sample. POPs: Persistent organic pollutants; MOE: margin of exposure; BDE: brominated diphenyl ether; HBCD: hexabromocyclododecane; nd: not determined; BMDL: lower confidence limit of benchmark dose.

CONCLUSIONS

The majority of the analyzed POPs were either detected at very low levels or not detected at all in the Belgian milk samples from mothers with their first child. HCB, β-HCH, p,p'-DDE, p,p'-DDT, BDE-47, and BDE-153 were quantifiable in 50% of the individual samples analyzed. Particularly, the concentrations of BDE-47 and BDE-153 were either lower than or comparable to those observed in samples from other European countries collected during the same timeframe. Among the regional samples, only α-HBCD was detected among the examined POPs. The analysis of the national Belgian sample revealed a decrease in the presence of all POPs listed in the Stockholm Convention, except HBCDs, since the last measurements in 2006. Furthermore, PFOS and PFOA, newly measured in national samples, were found at levels similar to those reported in other European nations. Overall, a significant conclusion of this study is the observed declining trend of POPs in Belgian human milk.

The exposure assessment for nursing infants indicated no health risk for most POPs, but a possible health concern was identified for two PBDE congeners (BDE-99 and BDE-153). This study underscores the necessity of ongoing measures to control POPs and the importance of international human biomonitoring initiatives conducted by the WHO. Although the use of many POPs has been prohibited for decades in Western European countries like Belgium, these substances remain in the environment and food chain,

resulting in their continued detection in human milk. It is also essential to emphasize that the WHO advocates for breast milk as the optimal nutrition for infants, as it contains antibodies that protect against various common childhood illnesses and delivers all the energy and nutrients required during the initial months of life.

DECLARATIONS

Acknowledgments

The authors thank the Belgian Federal Public Service of Public Health, Safety of Food Chain and Environment for giving us the trust to conduct the study (DG5/AMSZ/DA/14002) and Aurelie Dussart for ensuring the sample flow. We thank the team of Sciensano, namely Tim Reyns (currently working elsewhere), Philippe Szternfeld, Jean-Yves Michelet, Khariklia Tsilikas, Martine Deridder (retired), Jessica Marchi, Karoline Witpas (currently working elsewhere), and Patricia Ntarima (currently working elsewhere) for performing part of analyses. We thank Sophie Carbonnelle for reading the analytical protocols and ensuring the quality of the analyses according to the performance criteria set in ISO 17025, as evaluated by the Belgian accreditation body BELAC. Finally, we thank all the mothers who participated in this study. We also acknowledge the support of the staff at the participating maternities.

Authors' contributions

Conceptualization, methodology, formal analysis, investigation, writing - original draft preparation, review and editing, visualization, funding acquisition, supervision, project administration, funding acquisition: Andjelkovic M

Methodology, formal analysis, investigation, writing - original draft preparation, review and editing, visualization: Van Overmeire I

Conceptualization, methodology, formal analysis, investigation, writing - review and editing: Joly L

Conceptualization, methodology, writing - review and editing. Visualization: Poma G

Conceptualization, formal analysis, methodology, resources, validation, writing - review and editing: Malarvannan G

Writing - review and editing, methodology, formal analysis: Vleminckx C

Resources, validation, writing - review and editing: Malysheva SV

Investigation, formal analysis: Vanhouche M

Funding acquisition, conceptualization: Van Nieuwenhuyse A

Conceptualization, writing - review and editing: Van Loco J

Conceptualisation, funding acquisition, methodology, writing - review and editing, supervision: Covaci A

Availability of data and materials

Data not shown here are reported in [Supplementary Materials](#) or can be made available upon reasonable request to the corresponding author.

Financial support and sponsorship

Funding source: Belgian Federal Public Service of Public Health, Safety of Food Chain and Environment (study DG5/AMSZ/DA/14002).

Conflicts of interest

Poma G and Covaci A are Editorial Board members of *Journal of Environmental Exposure Assessment*, while the other authors have declared that they have no conflicts of interest.

Ethical approval and consent to participate

The recruitment strategy, informed consent documents, questionnaires, documentation for participating mothers and maternities, sampling protocol, and insurance documents were reviewed and approved by the

Commission for the Protection of Privacy (registration number HM002002523), the Ethical Commission of the Queen Fabiola Children's University Hospital in Brussels (acting as the coordinating ethical commission of the multicentre study; registration number CEH 21/14), and the ethical commissions of eight of the 31 participating maternities (the 23 other maternities considered the review and approval of the coordinating ethical commission sufficient).

Consent for publication

Not applicable.

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