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Determinants of persistent organic pollutant (POP) concentrations in human breast milk of a cross-sectional sample of primiparous mothers in Belgium

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ABSTRACT

Background: Bio-accumulation of persistent organic pollutants (POPs) in the environment and in the food chain can lead to high pollutant concentrations in human fat-containing tissues and breast milk.
Objectives: We aimed to identify the maternal characteristics that determined POP concentrations in breast milk of primiparous mothers in Belgium.
Methods: Breast milk samples were obtained from a cross-sectional sample of 206 primiparous mothers in 2014. POP concentrations in breast milk samples were determined by GC-ECNI-MS and GC-EI-MS/MS depending on the analytes' sensitivity. Associations between POP concentrations in breast milk and potential determinants were investigated using two-way contingency tables and multivariable generalized linear models.
Results: Fifteen of the 23 screened POPs were detected in the breast milk samples. Four organochlorine compounds (*p,p′*-DDT, *p,p′*-DDE, HCB and β-HCH) and two brominated flame retardant congeners (BDE-47, BDE-153) were detected at concentrations above the limit of quantification in > 50% of the breast milk samples. Maternal age and BMI were usually associated with higher POP concentrations. Rural residency and consumption of home-produced eggs, fatty fish and fish oil supplements were associated with higher concentrations of DDT and DDE. Consumption of fatty fish and being breastfed during childhood were associated with higher concentrations of HCB and β-HCH. Fish oil supplements and home-produced eggs were associated with higher concentrations of BDEs, but for BDE congeners exposure routes other than diet require further investigation.
Conclusions: Dietary and non-dietary determinants predict individual POP concentrations in breast milk.

Abbreviations: BFRs, Brominated flame retardants; CHL, chlordane; DDE, dichlorodiphenyldichloroethylene; DDD, dichlorodiphenyldichloroethane; DDT, dichlorodiphenyltrichloroethane; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; HexaBB, hexabromobiphenyl; OCPs, organochlorine pesticides; (P)BDEs, (poly)brominated diphenyl ethers; PCBs, polychlorinated biphenyls; PeCB, pentachlorobenzene; POPs, persistent organic pollutants; WHO, World Health Organization

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1. Introduction

Persistent organic pollutants (POPs) are organic compounds that include polychlorinated biphenyls (PCBs), brominated flame retardants (BFRs) and organochlorine pesticides (OCPs). These compounds have been used extensively in the industry and in agriculture for several decades and are at present widely distributed in the environment (Jaward et al., 2004). Because of their lipophilic and persistent properties (Fernández-Rodríguez et al., 2015), POPs tend to bioaccumulate in lipid-rich tissues within the body of animals, including humans, and thus accumulate and biomagnify in the food chain (Colles et al., 2008; Govaerts et al., 2018; Imbeault et al., 2018). As a result, the body burden of certain POPs, for instance those used as insecticides or fungicides, is primarily determined by dietary exposure (Bányiová et al., 2017; Gasull et al., 2011; Malisch and Kotz, 2014). For other POPs, in particular BFRs, non-dietary sources such as the inhalation or ingestion of contaminated airborne particles and indoor dust may be the primary route of human exposure (Fromme et al., 2016; Harrad et al., 2010; Jin et al., 2019; Passuello et al., 2010). In addition to external exposure, substantial weight loss may increase internal exposure to POPs by releasing toxicants from adipose tissue (La Merrill et al., 2013; Lignell et al., 2016; Malarvannan et al., 2018) and the POP burden may also differ considerably between socioeconomic groups (Gasull et al., 2013).

Exposure to POPs has been associated with a broad range of negative health effects, which include increased mortality (Fry and Power, 2017), increased risk for type 2 and gestational diabetes (Evangelou et al., 2016; Rahman et al., 2019; Singh and Chan, 2017; Vafeiadi et al., 2017; Zong et al., 2018), hypertension (Lind et al., 2014) and obesity (Yang et al., 2017). Children are extremely sensitive to POPs since they are prenatally exposed through mother-to-fetus POP transfer across the placenta (Vizcaino et al., 2014) and as infant potentially via breast milk (Vukavic et al., 2013). Prenatal and developmental exposure to POPs have been associated with impaired fetal growth (Govarts et al., 2012, 2018), endocrine disruption (de Cock et al., 2017), metabolic diseases (Tang-Péronard et al., 2015), reduced cognitive and neurological development (Berghuis et al., 2018; Kyriklaki et al., 2016; Lam et al., 2017), respiratory health impacts (Gascon et al., 2014), and adiposity and hypertension in early childhood (Vafeiadi et al., 2015). Reducing human exposure to POPs, in particular during early life, is therefore an important public health priority.

The Stockholm Convention on POPs is a global treaty for the control and elimination of POPs. In 2004, the Stockholm Convention banned the production and use of POPs to protect human health and the environment (Stockholm Convention, 2018; www.pops.int). Time series based on the biomonitoring of POP concentrations indicate that the environmental presence and human intake of most POPs are decreasing (Abballe et al., 2008; Bányiová et al., 2017; Colles et al., 2008; Croes et al., 2014; Luzardo et al., 2019; Vukavic et al., 2013). The cumulative present knowledge demonstrates that the benefits of breastfeeding exceed the potential disadvantages associated with exposure to certain POPs via breast milk (van den Berg et al., 2017). Nevertheless, to even further reduce human exposure to POPs and reduce in utero and lactational exposure of infants, the risk factors and determinants for exposure to POPs need to be identified. Therefore, in the present observational study, we analyzed results from the 6th World Health Organization (WHO) coordinated survey on POPs in human milk in Belgium to assess which variables related to personal characteristics, residence and dietary habits of the participating mothers determined POP concentrations in breast milk.

2. Materials and methods

2.1. Study design and population

Mothers were recruited between May and December 2014 in 31 maternities across the 10 provinces of the Flemish and Walloon Regions and in the Brussels Capital Region of Belgium (see Geospatial Data). The inclusion criteria of the study required that: i) mothers were aged 18–30 years, ii) first-time mothers (primiparae), iii) breast feeding, iv) had a singleton pregnancy, v) had term gestation (37 weeks or more), vi) resided in Belgium for ≥10 years, and (vii) were HIV-negative. The study protocol was based on the protocol of the WHO coordinated surveys on POPs in human milk (WHO, 2007). 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 multicenter 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). Mothers were recruited during the first three weeks after delivery. Informed consent was obtained prior to potential inclusion in the study. Of the 267 mothers that expressed interest, met the inclusion criteria and consented, 206 (77%) were included in the study, i.e. 111 in the Flemish Region, 73 in the Walloon Region and 22 in the Brussels-Capital Region. The main reason for exclusion from the final stage of the study was the inability to deliver a breast milk sample due to insufficient breast milk production or cessation of breastfeeding (Fig. S1). The number of mothers recruited in each province was proportional to the total population of the province. The realized overall sample size was 1.8 mothers per 100,000 inhabitants (Table S1).

2.2. Measurement of outcome variables

2.2.1. Milk sample collection

Participating mothers ($n = 206$) collected milk samples at home using a manual breast milk pump (Manual Breast Pump, Tommee Tippee®, Mayborn USA Inc., Stamford, CT). All samples were taken in the mature milk lactational stage, at three to eight weeks postpartum. As the amount of fat (and hence, POPs) gradually changes during a nursing session, milk samples (≥ 50 ml) were preferentially collected during or after feeding and not at the beginning of the feeding. Mothers were allowed to mix samples from multiple sessions. Milk samples were collected in Pyrex® laboratory bottles with PBT cap and PTFE seal. The bottles and caps had been cleaned, rinsed with demineralized water and oven-dried prior to deployment to remove potentially present analytes of interest and compounds that could cause chromatographic interferences. Participants kept milk samples refrigerated or frozen (max. 72 h at 4 °C, at −18 °C when home storage exceeded 72 h). In the laboratory, milk samples were stored at −20 °C awaiting chemical analysis. All samples were analyzed within a time span of four months.

2.2.2. Sample preparation

All samples were first submitted to fat extraction followed by lipid determination (modified EN 1528-2-1996 method). Briefly, 25 ml milk was spiked with appropriate internal standards and extracted with a solvent mixture of hexane:acetone (1:2, v/v). The organic phase was dried with sodium sulphate. A second extraction with hexane was performed to obtain 200–300 mg fat from each sample. The collected filtrate was concentrated on a Kudern-Danish instrument. The final extract (12 ml) was divided in two aliquots. For the analyses of PBDEs, PeCB and Hexa-BB (UAntwerp), aliquots of 4 ml were used and 8 ml was used for OCPs analyses (Sciensano). In each batch of analyses, a positive control and procedure blank (Milli-Q water) were included. Cow milk was spiked with the same internal standards and used a positive control.

At Sciensano, the extracts were reconstituted in 1 ml hex, vortexed for 20 s, and load onto self-packed 10 g alumina columns. The targeted OCPs analytes were eluted with 80 ml hexane, evaporated to near

dryness, resolubilized in 1 ml hexane and transferred to injection vials for GC-EI-MS/MS analysis.

At UAntwerp, the extracts were reconstituted in 0.5 ml hex:DCM (1:1 v/v), vortexed for 20 s, and load onto 6 ml self-packed acid silica cartridges (44% H_2SO_4; prewashed with 5 ml hexane). The targeted analytes were eluted with 10 ml hex:DCM (1:1, v/v) solvent mixture, evaporated to dryness, resolubilized in 100 µL recovery standard (RS, CB-207) and transferred to injection vials for GC-ECNI/MS analysis.

2.2.3. Determination of POP concentrations

In the framework of WHO-coordinated surveys on POPs in human milk, concentrations of the following POPs were measured: (i) chlordanes; (ii) DDT and its metabolites; (iii) HCB; (iv) hexachlorocyclohexane isomers (HCHs); (v) pentachlorobenzene (PeCB); (vi) hexabromobiphenyl (HexaBB, BB153) and (vii) polybrominated diphenyl ethers (PBDEs) (Table 2). The outcome variables were POP concentrations expressed on a lipid basis (ng g^{-1} lipid). Organochlorine compound concentrations (HCH group: α-HCH, β-HCH and γ-HCH, HCB; DDT group: *o*,p-DDE, *p*,*p*′-DDE, *o*,*p*-DDD, *p*,*p*′-DDD, *o*,*p*-DDT and *p*,*p*′-DDT; and chlordane group: oxychlordane, trans-chlordane, cis-chlordane, trans-nonachlor) were determined by GC-EI-MS/MS. PBDEs, PeCB and HexaBB concentrations were measured by GC-ECNI-MS operated in negative ion mode at temperatures of 170, 150 and 300 °C of ion source, quadrupole and interface, respectively. Details of the analytical methods are described by Dimitriadou et al. (2016).

LOD and LOQ (organochlorine compounds and chlordane group) were determined as levels that correspond to the minimal concentration that could be detected or quantified, respectively, in the blank sample spiked with an analyte demonstrating the chromatographic peak with signal-to-noise (S/N) ratio of 3 or 6, respectively. For the PBDEs, HexaBB and PeCB, LOQ and LOD were assessed at the S/N ratio 10 and 3, respectively.

2.3. Definition of potential predictor variables

A structured questionnaire was used to obtain information on potential predictor variables. The potential non-dietary predictor variables included in this study were the mothers' age, BMI (kg m^{-2}), income (EUR $month^{-1}$), education level (no higher education vs. higher education), nursing history (breastfed vs. not breastfed or not sure), birth order (mother first-born child vs. not first-born child) and residence [urban (≥600 inhabitants km^{-2}) vs. rural (< 600 inhabitants km^{-2})]. In the context of dietary advise, fish species with a fat content exceeding 5% are classified as fatty or oily fish (examples include eel, trout, sardines, mackerel, halibut, salmon, tuna and herring). The potential dietary predictor variables included in this study were the frequency of fish consumption [less than recommended (< 2 times $week^{-1}$) vs. recommended or more (≥2 times $week^{-1}$)], the frequency of consumption of other fish products [none, less than once per week (< 1 time $week^{-1}$) or at least once per week (≥1 time $week^{-1}$)], the frequency of consumption of fish oil supplements (omega-3 fatty acids) [none, not daily (1–6 times $week^{-1}$) or daily (7 times $week^{-1}$)], the inclusion of fatty fish in the diet (diet includes fatty fish vs. diet does not include fatty fish), the frequency of the consumption of milk products [not daily (0–6 times $week^{-1}$) vs. daily (7 times $week^{-1}$)], the frequency of the consumption of meat and poultry products [not daily (0–6 times $week^{-1}$) vs. daily (7 times $week^{-1}$), the frequency of the consumption of eggs and egg products [less than once per week (< 1 times $week^{-1}$), once per week (1 times $week^{-1}$), more than once per week (> 1 times $week^{-1}$) and the type of eggs that are consumed (commercially produced, home-produced, or both types of eggs) (Table 1).

2.4. Statistical analysis

For POPs that were quantified in only 10–50% of the participants' milk samples, associations between POP concentrations in breast milk and potential determinants were investigated using unadjusted odds ratios (ORs) and their asymptotic 95% confidence interval (CI). To that end, POP concentrations were reclassified as present/absent data (concentrations ≥ LOQ = present), and ORs were calculated for two-way contingency tables of POPs (outcomes) and binary determinant (exposure) factors.

For POPs quantified in at least half of the milk samples (number of samples above LOQ ≥ 50%), association between POP concentrations in breast milk and potential determinants was investigated using multiple generalized linear models for log-transformed POP concentrations. Four cases had missing data and were excluded (cases included in linear model analysis: $n = 202$). Prior to log-transformation, POP concentrations < LOQ, including concentrations < LOD and non-detections, were assigned the arbitrary value of ½ LOQ. This enables the inclusion of non-detections (zeros) in the models for log-transformed concentrations (the log of zero is undefined). Main effects and an intercept were included in these models. To be able to compare results across all analytes of interest, we always included all potential determinants in the models, except income because income was correlated to age (Pearson $r = 0.215$; $p = 0.007$). We calculated fully adjusted exponential parameter estimates (odds ratios) and their 95% Wald confidence intervals. Linear models of not log-transformed POP concentrations were used to calculate estimated marginal mean POP concentrations adjusted for BMI and age in study population subgroups. Age and BMI were entered as covariables in these analyses due to the

Table 1
Characteristics of 206 Belgian first-time mothers included in the sixth WHO-coordinated survey of human milk for persistent organic pollutants (POPs).

		N	%
Age (years)	< 20	1	0.5
	20–25	48	23.3
	> 25	157	76.2
BMI	< 18.5 (underweight)	7	3.4
	18.5–24.9 (normal)	140	68.0
	25–29.9 (overweight)	39	18.9
	> 30 (obese)	16	7.8
	Missing	4	1.9
Education	No higher education	40	19.4
	Higher education	166	80.6
Income (EUR/month)	P50	3300	
	IQR	803	
Nursing history	Not breastfed (or not sure)	80	38.8
	Breastfed	126	61.2
Mother first-born	First-born child	90	43.7
	Not first-born child (or missing)	116	56.3
Residence	Urban (≥600 inh km^{-2})	98	47.6
	Rural (< 600 inh km^{-2})	108	52.4
Diet			
Fish servings per week	Less than recommended	162	78.6
	Recommended or more (≥2×/week)	44	21.3
Other fish products	none or missing	27	13.1
	< 1×/week	162	78.6
	≥1×/week	17	8.3
Fish oil supplements	None or missing	157	76.2
	Not daily	21	10.2
	Daily	28	13.6
Fatty fish (> 5% fat)	Diet includes fatty fish	154	74.8
	Diet excludes fatty fish or missing	52	25.2
Milk products	Not daily	65	31.5
	Daily	141	68.4
Meat and poultry	Not daily	87	42.2
	Daily	119	57.8
Eggs (egg products)	< 1×/week	78	37.9
	1×/week	73	35.4
	> 1×/week	55	26.7
Egg types	Commercially produced	95	46.1
	Home-produced	33	16.0
	Both types	74	35.9
	Missing	4	1.9

bioaccumulation and lipophilic nature of POPs (Fernández-Rodríguez et al., 2015). Subgroups were based on type of eggs consumed (H, home-produced eggs; C, commercially produced eggs; H + C, both egg types), consumption of fatty fish (FF, fatty fish included in diet; NFF, only non-fatty fish included in diet), being breastfed (BF, breastfed; NBF, not breastfed), and residence (R, rural residence, < 600 inhabitants km^{-2}; U, urban residence). All analyses were carried out with IBM SPSS Statistics Subscription 11-2018 (IBM Corporation, Armonk, NY). The reporting of this study conforms to the STROBE statement for cross-sectional studies (von Elm et al., 2007).

3. Results

3.1. Population characteristics

The participating women were between 19 and 30 years old, with an average age of 26.9 years (95% CI 26.6–27.2 years) and an average BMI of 23.5 (95% CI 22.9–24.0 kg/m^2) indicating a normal BMI (BMI range 18.5–24.9). In Belgium, mothers are on average 28.7 years old when they have their first child (STATBEL, 2018). The majority of the participating mothers had completed higher education (80.6%), consumed less fish servings than recommended (< 2×/week; 78.6%); consumed other fish products less than once a week (78.6%); did not use fish oil food supplements (73.8%); included fatty fish in their diet (74.8%); consumed milk products (68.4%) and meat/poultry products (57.8%) on a daily basis; consumed eggs less than once (37.9%) or once a week (35.4%); consumed home-produced eggs or a mix of home-produced and commercially produced eggs (51.9%); and was breastfed during own childhood (61.2%).

3.2. Detected POPs

Fifteen of the 23 screened POPs were detected in concentrations above the level of quantification (LOQ) in the 206 breast milk samples: *p,p′*-DDD, γ-HCH (Lindane), HexaBB and BDE-28 were detected in < 10% of the samples; oxychlordane, PeCB, BDE-99, BDE-100 and BDE-154 were detected in 10–50% of the samples; and *p,p′*-DDT, *p,p′*-DDE, HCB, β-HCH, BDE-47 and BDE-153 were detected and quantified in > 50% of the participants' milk samples (Table 2). The most prevalent POPs were *p,p′*-DDE, HCB and BDE-153: *p,p′*-DDE was detected in all samples (100%); HCB in 97.6% and BDE-153 in 92.7% of all samples (Table 2).

3.3. Determinants of POPs in breast milk

The prevalence of *p,p′*-DDD, γ-HCH, HexaBB and BDE-28 was too low (< 10% of the samples) to perform statistics for these POPs. For PeCB, BDE-99 and BDE-154 the unadjusted odds ratios were not significantly different from one (no associations between detection and potential determinants). There was a positive association between detection of oxychlordane and being breastfed as a child (unadjusted OR = 4.47, 95% CI 1.49–13.42, $P = 0.008$). There was a positive association between the detection of BDE-100 and consumption of fatty fish (OR = 2.79, 95% CI 1.03–7.58, $P = 0.044$). Results from the fully adjusted multivariable analyses for the POPs quantified in > 50% of the samples (*p,p′*-DDT, *p,p′*-DDE, HCB, β-HCH, BDE-47 and BDE-153) are presented in Tables 3, 4 and 5.

3.3.1. DDT group

The log-transformed concentration of *p,p′*-DDT increased with BMI [adjusted odds ratio (95% CI): OR = 1.01 per 1 unit increase (1.00–1.03)] and was higher in rural areas [OR = 1.09 (0.98–1.20]). The log-transformed concentration of *p,p′*-DDT was higher in milk of mothers that used supplements based on fish oil [on a daily basis vs. not: OR = 1.19 (1.03–1.37); not on a daily basis vs. not: OR = 1.17 (0.99–1.39)] and in the milk of mothers that consumed home-produced eggs [only home-produced vs. only commercially produced eggs: OR = 1.22 (1.06–1.41); both types of eggs vs. only commercially produced eggs: OR = 1.16 (1.04–1.29)]. The log-transformed concentration of *p,p′*-DDE increased with age [OR = 1.02 per year increase (1.00–1.04)] and was higher in milk of mothers that included fatty fish in their diet [OR = 1.09 (0.99–1.20)] and in milk of mothers that consumed home-produced eggs [only home-produced vs. only commercially produced eggs: OR = 1.20 (1.06–1.35)] (Table 3).

3.3.2. HCH group

The log-transformed concentration of HCB was higher in milk of mothers that were breastfed as a child [OR = 1.10 (1.04–1.16)] and in milk of mothers that that included fatty fish in their diet [OR = 1.06 (1.00–1.13)]. The log-transformed concentration of β-HCH increased with age [OR = 1.03 per year increase (1.01–1.05)] and with BMI [OR = 1.01 per 1 unit increase (1.00–1.02)]. The log-transformed concentration of β-HCH was higher in milk of mothers that were breastfed as a child [OR = 1.12 (1.03–1.22)] and in milk of mothers that included fatty fish in their diet [OR = 1.09 (0.99–1.21)] (Table 4).

3.3.3. PBDEs

The log-transformed concentration of BDE-47 increased with age [OR = 1.03 per year increase (1.00–1.06)] and with BMI [OR = 1.02 per 1 unit increase (1.00–1.03)]. The log-transformed concentration of BDE-47 was higher in milk of mothers that used supplements based on fish oil [not on a daily basis vs. not: OR = 1.21 (0.97–1.49)]. The log-transformed concentration of BDE-154 decreased with BMI [OR = 0.98 per 1 unit increase (0.97–1.00)] and was lower in rural environments [OR = 0.90 (0.82–0.98)]. The log-transformed concentration of BDE-154 was higher in milk of mothers that consumed home-produced eggs [both types of eggs vs. only commercially produced eggs: OR = 1.20 (1.06–1.35)] (Table 3).

4. Discussion

4.1. Key results

In a survey of POPs in human breast milk in a cross-sectional sample of 206 primiparous mothers in Belgium, the organochlorine pesticides *p,p′*-DDT, HCB and β-HCH, the organochlorine pesticide metabolite *p,p′*-DDE, and the polybrominated diphenyl ether congeners BDE-47 and BDE-153 were the most prevalent POPS. Fatty fish, fish oil supplements and home-produced eggs were the primary dietary determinants of DDT/DDE concentrations. Fatty fish and reception of breastfeeding were the main dietary determinants of HCH/HCB concentrations. Fish oil supplements and home-produced eggs were also determinants of BDE concentrations but the models for BDE congeners were statistically weak. Maternal age and BMI were usually associated with higher POP concentrations. Rural residency was associated with higher DDT concentrations, while urban residency was associated with higher BDE-154 concentrations.

4.2. Comparison with other studies

4.2.1. Diet

4.2.1.1. Eggs. The consumption of home-produced eggs was associated with higher *p,p′*-DDT, *p,p′*-DDE and BDE-154 concentrations (Fig. 1; Tables 3, 5).These results are in line with an earlier study that compared free-range eggs (from commercial companies) with home-produced free-range eggs in Belgium. Home-produced eggs contained significantly higher levels of dioxins, marker PCBs and ΣDDTs (Van Overmeire et al., 2006). Studies in areas where DDT is still used for disease control purposes (e.g. for the control of mosquito vectors of malaria) have also documented substantial contamination of free-range eggs by DDT and DDT congeners (Thompson et al., 2017). POPs have ample opportunity to enter and accumulate in the food chain in

Table 2
POPs concentrations (in ng g^{-1} lipid basis) determined in individual breast milk samples of 206 Belgian primiparous mothers.

POP	Min	P10	P50	P90	Max	Mean (SE)	LOD	LOQ	N (%) > LOQ
Chlordane group*[A]									
cis-Chlordane							1.0	2.0	0 (0)
trans-Chlordane							1.0	2.0	0 (0)
Oxychlordane	< LOQ	< LOQ	< LOQ	5.30	10.80	< LOQ	1.0	5.0	28 (13.6)
trans-Nonachlor							1.0	2.0	0(0)
DDT group*[B]									
o,p′-DDT							1.0	2.0	0 (0)
p,p′-DDT	< LOQ	< LOQ	2.80	8.40	65.50	4.40 (0.43)	1.0	2.0	144 (70.0)
o,p′-DDD							1.0	2.0	0 (0)
p,p′-DDD	< LOQ	< LOQ	< LOQ	< LOQ	3.10	< LOQ	1.0	2.0	2 (1)
o,p′-DDE							1.0	2.0	0 (0)
p,p′-DDE	7.80	17.40	36.95	116.12	256.00	52.23 (3.10)	1.0	2.0	206 (100)
HCB*[A,C]	< LOQ	3.25	5.50	8.50	16.60	5.57 (0.15)	1.0	2.0	201 (97.6)
HCH group*[A]									
α-HCH							1.0	2.0	0 (0)
β-HCH	< LOQ	< LOQ	2.40	4.83	46.30	2.91 (0.27)	1.0	2.0	126 (61.2)
γ-HCH (Lindane)	< LOQ	< LOQ	< LOQ	< LOQ	4.00	< LOQ	1.0	2.0	4 (1.9)
PeCB*[A,C]	< LOQ	< LOQ	< LOQ	0.84	3.39	< LOQ	0.2	0.5	30 (14.6)
HexaBB*[A]	< LOQ	< LOQ	< LOQ	< LOQ	0.58	< LOQ	0.03	0.1	1 (0.5)
PBDE congeners*[A]									
BDE-28	< LOQ	< LOQ	< LOQ	< LOQ	0.55	< LOQ	0.03	0.1	12 (5.8)
BDE-47	< LOQ	< LOQ	0.13	0.63	2.42	0.24 (0.02)	0.03	0.1	110 (53.4)
BDE-99	< LOQ	< LOQ	< LOQ	0.22	0.85	0.10 (0.01)	0.03	0.1	53 (25.7)
BDE-100	< LOQ	< LOQ	< LOQ	0.22	0.80	< LOQ	0.03	0.1	51 (24.8)
BDE-153	< LOQ	0.16	0.38	0.89	1.87	0.46 (0.02)	0.03	0.1	191 (92.7)
BDE-154	< LOQ	< LOQ	< LOQ	0.27	1.07	0.13 (0.01)	0.03	0.1	79 (38.3)
BDE-183							0.06	0.2	0 (0)

Values are minimum, 10th percentile, median, 90th percentile, maximum, mean (and standard error of mean), limit of detection (LOD), limit of quantification (LOQ) and number (and %) of samples above LOQ. Results below the LOQ were given half the LOQ value and values under LOD were given zero value in the calculation of mean concentrations and percentiles.
*Chemicals targeted by the Stockholm Convention and listed in the Elimination (A), Restriction (B) and/or Unintentional Production (C) annex of the convention text.

uncontrolled, non-professional free-range farming activities. In a study in northern Belgium, POP concentrations (DDT, PCBs and PBDEs) were measured in soil and in earthworms that were collected in grassland and open woodland (Vermeulen et al., 2010). Earthworms accumulated POPs; free ranging chicken feeding on earthworms are therefore likely to be exposed to higher POP levels. The positive association between DDT/DDE and the consumption of home-produced eggs that was recorded in this study, may therefore be related to POP bio-accumulation in the soil-earthworm-chicken-egg-human food chain. Although technically the consumption of home-produced eggs qualifies as a dietary exposure route to POPs, it also represents environmental exposure, as contamination of consumed eggs occurred in the environment. Thus, the present study suggests that consumption of home-produced free-range eggs may contribute to the body burden of

Table 3
Adjusted associations between determinants and DDT/DDE concentrations (log-transformed, ng g^{-1} lipid basis) determined in individual breast milk samples of 202 Belgian primiparous mothers.

Predictor	*p,p′*-DDT		*p,p′*-DDE	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Age in years	1.01 (0.99–1.03)	0.34	1.02 (1.00–1.04)	0.04
BMI	1.01 (1.00–1.03)	0.04	1.00 (0.99–1.02)	0.41
Higher education	1.04 (0.91–1.19)	0.54	1.07 (0.96–1.19)	0.22
Mother breastfed	1.00 (0.90–1.11)	1.00	1.07 (0.98–1.17)	0.12
Mother firstborn	0.99 (0.90–1.09)	0.85	1.00 (0.92–1.09)	0.98
Rural residence	1.09 (0.98–1.20)	0.10	1.06 (0.98–1.15)	0.17
Fish consumption (≥ 2 servings/week)	1.00 (0.87–1.15)	0.99	1.00 (0.89–1.12)	1.00
Consumption of fish products (≥ 1 time/week)	0.98 (0.77–1.24)	0.84	0.97 (0.80–1.19)	0.80
Use of fish oil supplements (vs. none)				
Daily	1.19 (1.03–1.37)	0.02	1.02 (0.90–1.14)	0.81
Not daily	1.17 (0.99–1.39)	0.06	1.06 (0.92–1.22)	0.43
Fatty fish in diet	1.02 (0.90–1.14)	0.84	1.09 (0.99–1.20)	0.10
Daily consumption of milk products	1.00 (0.90–1.14)	0.96	1.06 (0.97–1.16)	0.23
Daily consumption of meat products	1.06 (0.96–1.18)	0.24	1.02 (0.93–1.11)	0.70
Egg consumption (vs. less than once/week)				
> Once/week	1.08 (0.95–1.23)	0.22	1.08 (0.97–1.20)	0.19
Once/week	1.09 (0.97–1.22)	0.14	1.03 (0.94–1.14)	0.53
Type of eggs (vs. commercially produced)				
Home-produced	1.22 (1.06–1.41)	0.006	1.20(1.06–1.35)	0.004
Mixed	1.16 (1.04–1.29)	0.008	1.04 (0.95–1.14)	0.40

Model fit: log-transformed *p,p′*-DDT: AIC = 180, Omnibus *p* (against intercept only model) = 0.007; log-transformed *p,p′*-DDE: AIC = 109, Omnibus *p* = 0.025.

Table 4
Adjusted associations between determinants and HCB/β-HCH concentrations (log-transformed, ng g^{-1} lipid basis) determined in individual breast milk samples of 202 Belgian primiparous mothers.

Predictor	HCB		β-HCH	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Age in years	1.01 (0.99–1.02)	0.08	1.03 (1.01–1.05)	0.004
BMI	1.00 (1.00–1.01)	0.20	1.01 (1.00–1.02)	0.05
Higher education	1.02 (0.95–1.09)	0.65	1.03 (0.92–1.15)	0.61
Mother breastfed	1.10 (1.04–1.16)	0.001	1.12 (1.03–1.22)	0.01
Mother firstborn	0.99 (0.94–1.05)	0.79	1.01 (0.93–1.10)	0.76
Rural residence	1.02 (0.97–1.07)	0.47	0.98 (0.91–1.07)	0.69
Fish consumption (≥2 servings/week)	1.03 (0.96–1.11)	0.42	1.01 (0.90–1.13)	0.89
Consumption of fish products (≥1 time/week)	1.04 (0.91–1.18)	0.57	0.91 (0.74–1.11)	0.34
Use of fish oil supplements (vs. none)				
Daily	0.98 (0.91–1.06)	0.62	0.98 (0.87–1.10)	0.69
Not daily	0.97 (0.89–1.06)	0.48	0.96 (0.84–1.11)	0.59
Fatty fish in diet	1.06 (1.00–1.13)	0.05	1.09 (0.99–1.21)	0.07
Daily consumption of milk products	1.03 (0.97–1.09)	0.29	1.06 (0.97–1.16)	0.22
Daily consumption of meat products	0.97 (0.93–1.04)	0.60	0.97 (0.89–1.06)	0.53
Egg consumption (vs. less than once/week)				
> Once/week	0.98 (0.92–1.05)	0.61	1.01 (0.91–1.12)	0.86
Once/week	1.01 (0.95–1.08)	0.70	0.96 (0.87–1.06)	0.42
Type of eggs (vs. commercially produced)				
Home-produced	1.04 (0.96–1.12)	0.32	0.98 (0.87–1.10)	0.73
Mixed	1.00 (0.95–1.06)	0.95	0.94 (0.86–1.03)	0.21

Model fit: log-transformed HCB: AIC = −84, Omnibus *p* (against intercept only model) = 0.033; log-transformed β-HCH: AIC = 107, Omnibus *p* = 0.020.

POPs including DDT.

4.2.1.2. Fish and fish oil supplements. Inclusion of fatty fish in the diet was associated with higher HCB, β-HCH and *p,p′*-DDE concentrations (Fig. 1; Tables 3, 4). Fatty fish, in particular predatory fish, have increased odds to be contaminated with POPs (see e.g. Vukovic et al., 2018). In Flanders, the consumption of wild eal is highly discouraged for that reason (Vanhaeren, 2005). A number of previous studies similarly demonstrated that fatty fish intake is a likely dietary source of POPs, resulting in higher levels of organochlorine compounds in breast milk (Behrooz et al., 2009; Ennaceur et al., 2008; Leng et al., 2009; Lu et al., 2015). Other studies reported that fish consumption was associated with elevated DDT concentrations in breast milk (Lu et al., 2015) and with higher serum concentrations of *p,p′*-DDE (Cao et al., 2011; Rivas et al., 2007). DDT and BDE-47 concentrations were also higher in the milk of mothers that used dietary supplements based on fish oil. These results are in line with previous studies that detected DDT in fish oil food supplements (Rawn et al., 2008; Storelli et al., 2004; Smutna et al., 2009). In our study population, only few participants consumed fish very frequently (> 2×/week; 5.3%) and only 13.6% of the mothers used fish oil supplements on a daily basis. Populations that include more fish, fish products (or marine mammals that eat fish) in their diet may be at higher risk for dietary exposure to POPs than our study population (Bjermo et al., 2013; Singh and Chan, 2017).

4.2.1.3. Milk, dairy and meat. In the multiple linear regression models of the present study, no significant associations between POP levels in

Table 5
Adjusted associations between determinants and BDE-47/BDE-153 concentrations (log-transformed, ng g^{-1} lipid basis) determined in individual breast milk samples of 202 Belgian primiparous mothers.

Predictor	BDE-47		BDE-153	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Age in years	1.03 (1.00–1.06)	0.04	0.99 (0.97–1.01)	0.38
BMI	1.02 (1.00–1.03)	0.07	0.98 (0.97–1.00)	0.006
Higher education	1.03 (0.88–1.22)	0.70	1.08 (0.96–1.21)	0.23
Mother breastfed	1.00 (0.87–1.14)	0.98	1.01 (0.91–1.11)	0.90
Mother firstborn	0.98 (0.86–1.11)	0.77	1.02 (0.93–1.12)	0.63
Rural residence	0.94 (0.82–1.06)	0.47	0.90 (0.82–0.98)	0.019
Fish consumption (≥2 servings/week)	1.03 (0.86–1.22)	0.78	1.08 (0.95–1.22)	0.25
Consumption of fish products (≥1 time/week)	1.06 (0.78–1.44)	0.73	1.00 (0.80–1.25)	1.00
Use of fish oil supplements (vs. none)				
Daily	0.99 (0.82–1.18)	0.87	0.93 (0.81–1.05)	0.24
Not daily	1.21 (0.97–1.49)	0.09	0.99 (0.85–1.15)	0.86
Fatty fish in diet	1.07 (0.92–1.24)	0.37	1.08 (0.97–1.20)	0.16
Daily consumption of milk products	1.00 (0.87–1.14)	0.96	0.97 (0.88–1.07)	0.50
Daily consumption of meat products	1.01 (0.88–1.15)	0.92	1.01 (0.92–1.11)	0.90
Egg consumption (vs. less than once/week)				
> Once/week	0.93 (0.79–1.10)	0.41	0.95 (0.85–1.07)	0.43
Once/week	1.02 (0.88–1.19)	0.76	1.02 (0.92–1.13)	0.73
Type of eggs (vs. commercially produced)				
Home-produced	1.05 (0.87–1.26)	0.62	1.03 (0.91–1.18)	0.64
Mixed	1.04 (0.91–1.20)	0.55	1.10 (1.00–1.21)	0.06

Model fit: log-transformed BDE-47: AIC = 278, Omnibus *p* (against intercept only model) = 0.497; log-transformed β-HCH: AIC = 141, Omnibus *p* = 0.130.

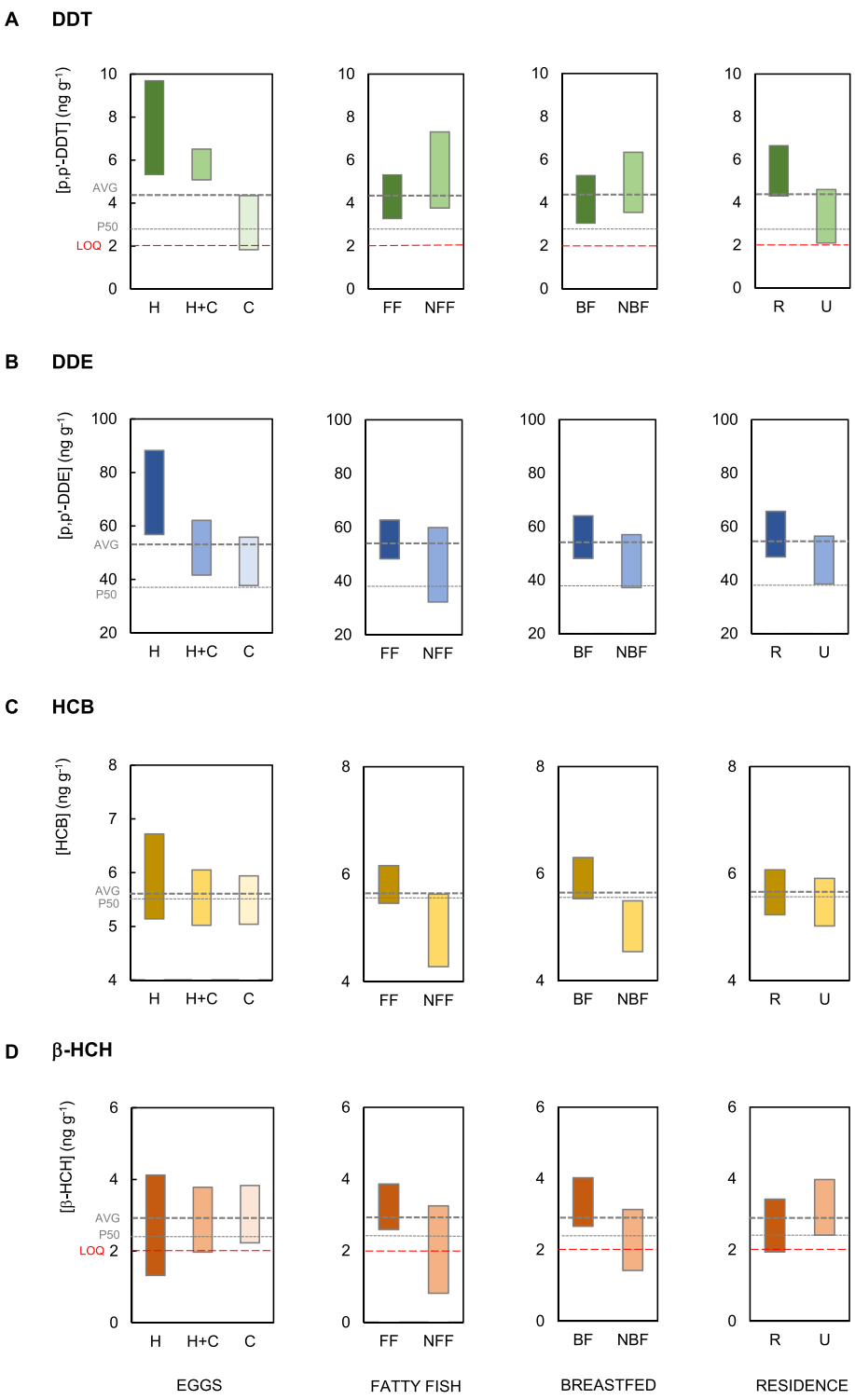

Fig. 1. Mean (95% CI) concentrations (ng g^{-1} lipid basis) of *p,p′*-DDT, *p,p′*-DDE, HCB and β-HCH (A to D) in breast milk of primiparous mothers in Belgium (n = 202), adjusted for BMI (23.5) and age (26.9 years), in function of personal and environmental determinants: type of eggs consumed (H, home-produced eggs; C, commercially produced eggs; H + C, both egg types), consumption of fatty fish (FF, fatty fish included in diet; NFF, only non-fatty fish included in diet), being breastfed (BF, breastfed; NBF, not breastfed), and residence (R, rural residence, < 600 inhabitants km^{-2}; U, urban residence). Reference lines are limit of quantification (LOQ), median (P50) and overall mean (AVG) POP concentration.

breast milk and the consumption of milk and dairy products or meat were found. An earlier study performed in Flanders found that consumption of milk and dairy products was associated with DDT concentrations in breast milk (Croes et al., 2012) and a correlation between the consumption of meat and serum levels of *p,p′*-DDE has also been reported previously (Cao et al., 2011). The cumulative evidence of earlier research and the present study confirms that the consumption of food of animal origin (eggs, milk, diary, meat) is an important dietary exposure route to POPs.

4.2.2. Childhood breastfeeding history of the mother

There were positive associations between concentrations of HCB and β-HCH and maternal breastfeeding history. Breast milk is rich in fat and POPs are lipophilic, and therefore the transfer of POPs from mother to breast milk to the infant is plausible (Vukavic et al., 2013). It has been reported that mothers who breastfeed during 12 months of lactation or more had lower serum levels of POPs than non-breastfeeding mothers (Bjermo et al., 2013; Thomsen et al., 2010a), suggesting a release of POPs through lactation (Zong et al., 2016). The elimination of POPs from the body through breastfeeding also results in lower levels of

POPs in the breast milk of multiparae mothers (Ennaceur et al., 2008; Hassine et al., 2012; Klinčić et al., 2016; Polder et al., 2009; Zietz et al., 2008). This suggests that the firstborn child experiences the highest POP transfer, and mothers that were firstborn child may have been exposed to higher concentrations of POPs in breast milk than their younger siblings. In our sample, concentrations of POPs were not higher in milk of mothers that were firstborn themselves (Tables 3–5).

In earlier studies, organochlorine compounds (HCB, β-HCH, p,p′-DDE and 4,4′-DDE) in serum of adolescents who were breastfed as child were reported to be higher than in the serum of children that were not breastfed (Den Hond et al., 2009; Gascon et al., 2015). This suggests that postpartum exposure to POPs via breast milk may still be detectable at adolescent and adult age, but the evidence from other studies is not conclusive. Some authors also found positive associations between receipt of breastfeeding and POP exposure (Gallo et al., 2011; Hsu et al., 2014) but others found negative associations (Fernández-Rodríguez et al., 2015). The present generation of breastfed mothers received breast milk from mothers (present day grandmothers) that grew up in a period when OCP use was widespread (and not yet regulated). Those grandmothers were therefore more likely to be exposed to higher POP concentrations in the environment than the present mothers and their children, including the present-day mothers, may have been exposed to higher POP concentrations in breast milk.

4.2.3. Age and BMI

Age and BMI usually had positive associations with POP concentrations (Tables 3–5). Positive associations between age and levels of OCPs in breast milk have been found previously in several studies (Colles et al., 2008; Dimitriadou et al., 2016; Ennaceur et al., 2008; Hassine et al., 2012; Lignell et al., 2009; Lu et al., 2015; Polder et al., 2009; Sudaryanto et al., 2006; Zietz et al., 2008). Age was also positively associated with serum levels of POPs in earlier studies (Cao et al., 2011; Luzardo et al., 2019; Pirard et al., 2018). Some studies found an effect of age for some PBDE congeners (Haraguchi et al., 2009; Lignell et al., 2009; Lee et al., 2013) while others did not (Lacorte and Ikonomou, 2009; Dimitriadou et al., 2016). Also for BMI previous studies have reported positive associations with organochlorine compounds (Lu et al., 2015; Luzardo et al., 2019; Mikeš et al., 2012; Pirard et al., 2018), PBDE congeners (BDE-47, BDE-99 and BDE-100) (Croes et al., 2012; Hoopmann et al., 2012).

Higher age may be associated with higher cumulative exposure to POPs, and relative body fat content increases with BMI. Positive associations between age/BMI and POP concentrations are therefore plausible. However, in our study population, the age and BMI ranges were small, and while effects were in most cases statistically significant, effects on POP concentrations were small.

4.2.4. Residence

DDT concentrations were higher in the breast milk of mothers residing in rural areas (Table 3). BDE-154 concentrations were higher in the breast milk of mothers residing in urban areas (Table 5). These results are in line with results from an earlier biomonitoring study performed between 2002 and 2006 in the Flemish region of Belgium, where a higher body burden of *p,p′*-DDE was found in rural areas (Koppen et al., 2009; Schroijen et al., 2008). The rural population might be exposed to higher background concentrations of legacy POPs from agriculture (insecticides, fungicides) or be engaged in activities or have dietary habits that result in higher exposure to POPs (Luzardo et al., 2019). Urban populations may be more at risk to be exposed to flame retardants, either at home or in occupational settings, resulting in higher PBDE concentrations.

4.3. Limitations

The concentrations of POPs in breast milk may vary over the duration of the lactation. Some studies find evidence of declining concentrations with duration of active lactation (Vigh et al., 2013) while others find evidence for relatively constant or varying concentrations without typical pattern (Kakimoto et al., 2018) or increasing concentrations with increasing maternal weight loss (Lignell et al., 2016). The timing of the collection of the breast milk sample in terms of duration of lactation (number of days) may therefore have an impact on the POP concentrations. In future studies it is recommended to adjust statistical analyses for the timing of sample collection.

The amount of time that present-day mothers were breastfed may or may not have impacted their exposure to POPs. Therefore, in future studies, it would be interesting to adjust statistical analyses for the length of time that the mothers were breastfed, although such data may be prone to high uncertainty.

Other studies have reported effects of education level or occupation on PBDE levels in breast milk (Dimitriadou et al., 2016; Cui et al., 2012). Women working in office environments had higher PBDE concentrations in their breast milk than others, indicating potential occupational exposure to flame retardants (Dimitriadou et al., 2016). Exposure routes other than diet, such as household dust and indoor air, may thus be more important than diet in determining exposure to PBDEs and other BFRs (Abdallah and Harrad, 2014; Thomsen et al., 2010b; Toms et al., 2009). Such exposure routes deserve more attention in future research on human POP exposure and bioaccumulation (Thomsen et al., 2010b).

Finally, we need to acknowledge that there were many POPs and potential determinants to be compared. This increased the statistical probability of obtaining false significant results.

5. Conclusions

A decade after the Stockholm Convention entered into force, 15 of the 23 banned POPs that were screened were detected in the breast milk of primiparous mothers in Belgium. Our findings highlight that both diet and exposure routes other than diet (childhood nursing history) predict individual POP concentrations in breast milk. Exposure routes other than diet deserve more attention in future research on human POP exposure and bioaccumulation.

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Ethical approval

This multicenter study was registered and approved by the Data Protection Authority (Registration Nr. HM002002523), by the Ethical Committee of the Hôpital Universitaire des Enfants Reine Fabiola (coordinating center; Registration Nr. CEH 21/14) and by eight local Ethical Committees of collaborating hospitals.

Declaration of Competing Interest

None of the authors declares an actual or potential conflict of interest.

Participating mothers received a manual breast milk pump (Manual Breast Pump, Tommee Tippee®, Mayborn USA Inc., Stamford, CT) in return for participation to the study.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at https://doi.org/10.1016/j.envint.2019.104979. These data include the Google map of the most important areas described in this article.

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