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Influence of gestational weight gain on the organochlorine pollution content of breast milk

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ABSTRACT

Background: Transplacental transfer and breastfeeding are the main transport routes of organic pollutants into children at the beginning of life. Although pollutant transmission through these mechanisms primarily depends on the maternal pollution burden, its impact may be modulated by physiological effects.
Objectives: We have examined whether gestational weight gain (GWG) exerts an influence on the content of lipophilic low volatile pollutants in breast milk.
Results: Colostrum from mothers from the INMA cohorts of Sabadell and Gipuzkoa (n = 256 and 119, respectively) with low GWG as defined by the Institute of Medicine (IOM) from the US National Academies of Sciences, Engineering and Medicine had significantly higher concentrations of polychlorobiphenyls (PCBs) and 4,4′-DDE than colostrum in mothers who gained weight within IOM recommendations or in those who exceeded this threshold. Statistically significant differences were also found in the colostrum:maternal serum ratios of these compounds. Women with low GWG retained higher pollutant amounts in colostrum. These observations are consistent with previously described higher concentrations of these pollutants in infant cord blood from mothers with low GWG by IOM standards. They indicate that mobilization of lipophilic organic pollutants by metabolic pregnant changes not only leads to higher fetal transfer but to higher accumulation into the mammary system upon low GWG. **Conclusions**: The present results show that insufficient GWG, besides increasing in utero exposure, also enhances pollutant transfer to infants during breastfeeding which considerably extends the significance of this physiological change for the pollutant children intake in early life.

1. Introduction

Children already incorporate pollutants at the very beginning of life, well before intake of these harmful compounds from direct environmental exposure. Transplacental and breasfeeding transfers constitute the main ways of absorption of lipophilic organic pollutants such as persistent organic pollutants (POPs) in this age period (Carrizo et al., 2006; Gascon et al., 2015; Karmaus et al., 2001; Landrigan et al., 2002; Muckle et al., 2001; Ribas-Fitó et al., 2003; Vizcaino et al., 2014a). Full understanding of the processes influencing the transfer of POPs in these early growth stages is needed.

In the period of organ and metabolism formation there is higher susceptibility to the deleterious effects of POPs and children detoxification potential is weak (Franzek et al., 2008; Selevan et al., 2014; Torgersen and Curran, 2006). Thus, lower capacity of metabolization of DDT and polybromodiphenyl ethers (PBDEs), for instance, has been observed in the fetal period (Vizcaino et al., 2014a). Associations between fetal exposure to some POPs and neurodevelopmental delays (Eskenazi, 2006; Morales, 2008; Ribas-Fitó, 2003, 2006), increases of asthma incidence (Sunyer, 2005, 2006), allergy (higher IgE levels)

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(Reichrtová et al., 1999) and overweight (Smink, 2008; Valvi, 2012) later in life have been documented.

Many of the aforementioned compounds related to these adverse effects are resistant to chemical and environmental degradation, highly lipophilic and bioaccumulate through the food web. Some of them, such as hexaclorobenzene (HCB), DDT and lindane or polychlorobiphenyls (PCBs) were widely used during the second half of the twentieth century as fungicides, pesticides and dielectrics in transformers and other applications, respectively. These uses decreased from the seventies to the nineties (Jones and De Voogt, 1998; Brun et al., 2008; Holoubek et al., 2007), and were finally banned by the Stockholm convention in 2001. However, their strong chemical/environmental stability and continued intentional or non-intentional synthesis and mobilization leads to their occurrence in large amounts in many world areas. Thus, HCB is synthesized as by-product in the manufacture of organochlorine solvents (Barber et al., 2005). Concentration increases of other organochlorine compounds (OCs) in the environment or humans have been observed in some cases *e.g.* as consequence of DDT use against vector diseases (Manaca, 2011, 2012) or PCB remobilization by decrease of mountain snow in temperate areas (Grimalt et al., 2009), involving higher emission of these compounds to the atmosphere and subsequent deposition (Arellano, 2014). Thus, despite regulatory measures, these compounds are still ubiquitous in the environment and detected in a variety of human tissues and fluids (Malisch and Kotz, 2014).

Obviously, the maternal body burden of these compounds constitutes a primary driver for infant accumulation in the first life period (Jaraczewska et al., 2006; Needham et al., 2011; Waliszewski et al., 2001). Gestational weight gain (GWG) as defined by the Institute of Medicine (IOM) (IOM, 2009) was observed to influence the transfer of some organochlorine and organobromine compounds (OCs and OBs, respectively) from mother to fetus, involving higher cord blood serum concentrations in children from mothers with low GWG (Vizcaino et al., 2014b).

As mentioned above, breastfeeding is also a significant way of incorporation of these compounds into children: those who breastfed show higher concentrations of OCs than those who did not. Significant differences between the two groups have been recorded later in life after breastfeeding, involving higher concentrations of OCs in the group of former breastfeeders after 3.5 years (Lanting et al., 1998), 4 years (Carrizo et al., 2006), 7 years (Karmaus et al., 2001) and even 14 years (Gascon et al., 2015).

Efforts to decrease these initial pollution stresses require understanding the maternal metabolic and physiological processes influencing these transfers. The present study aims to determine hydrophobic organochlorine pollutants in colostrum and to elucidate the effect of GWG in the maternal transfer of these compounds to their newborns through breastfeeding. The results of the present study might provide useful guidelines for decreasing the impact of environmental pollutants in the context of the recommendations of the Institute of Medicine.

2. Methods

2.1. Study population and sampling

The study is based on the INMA (Childhood and Environment) research network, specifically on the cohorts of Sabadell (Catalonia) and Gipuzkoa (Basque Country). A total of 1267 pregnant women were recruited during the first trimester of pregnancy in public health centers or referral hospitals of the two cities, and followed up during the entire period of pregnancy until discharge after delivery. The recruitment period was from July 2004 and July 2006 for the Sabadell cohort, and from May 2006 to February 2008 for the Gipuzkoa cohort. A total of 376 women provided blood and colostrum samples: 256 from Sabadell and 120 from Gipuzkoa. Blood samples were collected by venipuncture under fasting conditions in the 12th week of pregnancy. Colostrum samples were taken in the 3rd day after delivery. All samples were stored at −20 °C until analysis.

2.2. Extraction and analysis

The serum samples were blindly analyzed at the Gipuzkoa Public Health Laboratory, following a previously described method (Goñi et al., 2007), namely solid phase extraction of 500 μL of serum mixed with 5% Na_2SO_4 on C_{18} plates, purification by adsorption on silica/H_2SO_4 columns and quantification by gas chromatography (GC) coupled to electron capture detection (ECD). PCB-46 (for HCB, β-HCH, γ-HCH, PCB-28 and PCB-52) and PCB-143 (for 4,4′-DDE, 4,4′-DDT, PCB-101, PCB-118, PCB-138, PCB-153 and PCB-180) were used as internal standards and added when mixing with 5% Na_2SO_4. Confirmation of compound identification and lack of coelutions were assessed by GC coupled to mass spectrometry. In each batch of samples, two blanks were included as well as a control serum sample and a NIST Standard Reference Material (SRM) 1589a (National Institute of Standards and Technology, Gaithersburg, MD, USA). The results of the control serum and SRM 1589a samples were monitored in control charts for ten months of performance. The dispersion values, expressed as relative standard deviation, were <15% for all compounds. The limits of detection (LOD) and quantification (LOQ) were calculated from the blank averages plus three or five times the standard deviation of the measurements, respectively. The LOQ were 0.10 and 0.25 ng/mL of serum for PCBs and organochlorine pesticides, respectively.

Venous lipid weight was determined by enzymatic methods (Phillips et al., 1989).

The colostrum samples were blindly analyzed at IDAEA-CSIC laboratory in Barcelona. Approximately 1 mL of colostrum was introduced into a 10 mL centrifuge glass tube and spiked with tetrabromobenzene (TBB) and PCB-209 as surrogate standards. 3 mL of n-hexane and 3 mL of concentrated H_2SO_4 were added, mixed in a vortex (ca. 1500 rpm, 30 s) and centrifuged (ca. 3500 rpm, 10 m). The supernatant was transferred to a new centrifuge tube. Two mL of hexane were added to the previously extracted acid fraction for further recovery of analytes. The content was again vortex mixed, centrifuged and the upper n–hexane layer transferred to the tube collecting the solvent fractions. The procedure was repeated again until the final collected n-hexane volume was 7 mL. Then, 3 mL of concentrated H_2SO_4 were added, vortex mixed (ca. 1500 rpm, 90 s) and centrifuged (ca. 3500 rpm, 20 m). The clean extract was evaporated under a gentle N_2 stream to near dryness, transferred to a vial for GC and dissolved with isooctane. Prior to injection, the content of the vial was evaporated under a gentle N_2 stream and a specific volume of PCB-142 was added as internal standard.

The colostrum extracts were injected automatically in a GC/ECD (Model 6890, Agilent; Palo Alto, CA) in spitless mode (2 μL). The analytical column was a 60 m DB-5 (J&W/Agilent) and helium was used as carrier gas (1.5 mL/min). The temperature program started at 90 °C (held 2 min) increased at 15 °C/min to 130 °C and finally at 4 °C/min to 290 °C, were it was held for 15 min. Quantification was performed using PCB-142 as an internal standard for volume correction. The recoveries of TBB and PCB-209 (70–85%) were used for the final calculation of concentrations. Mean LOD and LOQ were 0.06 ng/mL and 0.09 ng/mL respectively. This analytical method performed satisfactorily in repeated international intercalibration exercises within the AMAP Ring Test Proficiency Program for POPs (Centre de Toxicologie Institut National de Santé Publique du Québec, Québec, Canada).

Lipid content was measured by the creamatocrit technique (Lucas et al., 1978; Wang et al., 1999), determining lipid content as the proportion of cream in a microcapillar after centrifugation.

2.3. Covariates of study

Individual and socio-economic data, including anthropometric measures, were obtained from questionnaires administered by trained interviewers as well as in health visits during the first trimester of

pregnancy and at delivery. Specifically, the following variables were collected: maternal educational level (3 categories: primary, secondary and university); social class (divided into 3 categories: least affluent (V and IV), affluent (III) and most affluent (II and I); smoking status at the end of pregnancy (3 categories: never smoked, smoked until the beginning of pregnancy, and smoked during all pregnancy); past accumulated breastfeeding (4 categories: never breastfed (including women without previous children), <3 months, 3–12 months, and >1 year); place of birth (3 categories: born in Spain, born in the rest of Europe, and born in South America). GWG was defined as the difference between the last recorded weight during pregnancy and the mother's self-reported pre-pregnancy weight (see Vizcaino et al., 2014a,b). In addition, we calculated GWG as the difference between first trimester weight and delivery weight, following the same procedure. The total GWG was converted to a categorical variable with three categories (recommended, inadequate or low, and excessive or high), following the IOM definition (Rasmussen et al., 2009). Informed consent forms were signed from all women participating in the cohort. The study was approved by the Clinical Research Ethical Committee of the Municipal Institute of Health Care (Sabadell cohort) and the Ethics Committee of the Donostia Hospital (Gipuzkoa cohort).

2.4. Data analysis

Data analysis and graphics were performed using Stata (version 12.0) and R (version 4.0.4). For descriptive analysis, means and medians, as well as the percentile 90, were reported in the tables. The colostrum:maternal serum concentration ratios were calculated for each subject. Possible associations between colostrum:serum ratios and these variables were calculated using Spearman's correlations, simple generalized additive models (GAM) and multivariate linear regression models adjusted for potential confounding factors. For multivariate regression models, compound concentrations and continuous GWG were transformed into logarithmic scale. One model was constructed for each compound, either in lipid adjusted colostrum concentration or for Colostrum:Serum ratio. GWG was the dependent variable (in log continuous scale), and the models were adjusted by maternal age, cumulative breastfeeding, social class, maternal educational level and country of origin. Sensitivity analysis was performed, by switching cumulative breastfeeding by parity (since both variables were highly correlated and in order to avoid multicollinearity, they could not be added together in the models). Graphical representation was performed using ggplot package. Graphical comparisons of the median ratios and log(Kow) (36 °C), log(Koa) (36 °C) and molecular weight were also performed.

3. Results

We studied two cohorts of volunteer mothers from Sabadell (Catalonia; July 2004–2006) and Gipuzkoa (Basque Country; May 2006–February 2008) that belong to the INMA Spanish research network (Guxens et al., 2012). The average age of the participants providing colostrum and serum (n = 376) was 31 years (range 18–42 years) (Table 1). Fifty-two percent of the women were primiparous and 41% had a previous child. The average period of past accumulated breastfeeding in the latter group was 37 weeks (range 0–508 weeks). Most volunteer women were non-smokers (57%), 30% smoked until the beginning of pregnancy and a 13% of women smoked throughout the pregnancy (Table 1).

HCB, hexachlorocyclohexanes (HCHs), PCBs, DDT and metabolites were analyzed in paired colostrum and maternal venous serum samples from the 1st trimester of pregnancy. Similar compound distributions in maternal serum and colostrum were found (Table 2). 4,4′-DDE, β-HCH, HCB, PCB138, PCB153 and PCB180 exceeded the detection limits in more than 50% serum and colostrum samples. 4,4′-DDE was the compound with the highest concentrations, with median levels of 104 ng/g lw and 98 ng/g lw in serum and colostrum, respectively (Tables 2 and 3). HCB had median concentrations of 38 ng/g lw and 29 ng/g lw in serum and colostrum, respectively. β-HCH was the dominant HCH isomer, with median concentrations of 26 ng/g lw and 21 ng/g lw in serum and colostrum, respectively. PCB-153 was the most predominant PCB congener, with medians of 38 ng/g lw and 40 ng/g lw in serum and colostrum, respectively, followed closely by PCB congeners 138 and 180. Congeners 28, 52 and 101 were below 50% of LOD in both serum and colostrum samples. PCB-118 and 4,4′-DDT were below LOQ in more than 50% of serum. When non-lipid adjusted values were considered, the median concentrations of all OCs were higher in colostrum than in serum (Table 2). The OC concentrations in both colostrum and serum were similar to those reported in earlier studies from non-polluted areas (Table 3).

The lipid-normalized concentrations of all OCs (ng/g lw) in serum and colostrum were significantly correlated ($p < 0.001$; Table 4), demonstrating a strong internal consistency of the database which was obtained from the independent analyses of the Gipuzkoa and Barcelona teams (see Methods Summary). The correlation coefficients were similar for most compounds, ranging between 0.67 and 0.74, except in the case of β-HCH, which was 0.49. Significant correlations between OC concentrations in maternal serum and breast milk have also been identified in earlier studies (Jaraczewska et al., 2006; Needham et al., 2011; Waliszewski et al., 2001). These correlations excluded the occurrence of

Table 1
Maternal and sociodemographic characteristics of the volunteers.

	N (%)
Maternal characteristics	
Age	**Mean:** 30.9 years/**Range:** 18.5–42
<25	27 (7.2)
25-29	134 (35.8)
30-35	152 (40.6)
>35	61 (16.3)
Pre-pregnancy body mass index	**Mean:** 23.44 kg/m^2/**Range:** 16.2–41.0
<18.5	17 (4.5)
18.5–25	261 (69.6)
25-30	73 (19.5)
≥30	24 (6.4)
Number of children	
0	196 (52.4)
1	152 (40.6)
≥2	26 (7.0)
Cumulative breastfeeding	**Mean:** 37.0 weeks/**Range:** 0-508
Never	5 (2.8)
<3 months	35 (19.9)
3–12 months	104 (59.0)
<1 year	32 (18.2)
Gestational weight gain (GWG)	
Total weight gain	**Mean:** 13.9 kg/**Range:** 1.37–30.43
Weight gain rate	**Mean:** 0.34 kg/week/**Range:** 0.03–0.78
IOM[a] adequate	134 (36.2)
IOM[a] Low	98 (26.5)
IOM[a] High	138 (37.3)
Socio-demographic characteristics	
Maternal education level	
Primary	81 (22)
Secondary	163 (44)
University	128 (34)
Socio-economic level	
I + II + III (most affluent)	191 (51)
IV + V (least affluent)	184 (49)
Smoking status	
Never	184 (57)
Until the beginning of pregnancy	97 (30)
During all pregnancy	43 (13)
Origin	
Spanish	338 (91)
Other European Countries	8 (2)
Latin American	24 (7)

[a] Institute of Medicine of the US National Academies of Sciences, Engineering and Medicine.

Table 2
Concentrations of organochlorine compounds found in serum and colostrum.

	Serum (N = 361)				Colostrum (N = 368)			
	>LOQ	Arithmetic mean (SD)	Median	P90	>LOQ	Arithmetic mean (SD)	Median	P90
ng/mL								
HCB	87%	0.27 (0.21)	0.22	0.50	96%	1.2 (1.2)	0.78	2.5
β-HCH	75%	0.24 (0.59)	0.15	0.40	97%	1.2 (3.5)	0.59	2.3
4,4′-DDE	98%	1.4 (7.1)	0.65	2.0	100%	5.9 (10)	2.9	13
PCB-153	90%	0.26 (0.19)	0.23	0.48	98%	1.6 (1.7)	0.99	3.5
PCB-138	77%	0.15 (0.12)	0.12	0.30	98%	0.99 (0.98)	0.66	2.2
PCB-180	83%	0.19 (0.15)	0.15	0.36	98%	1.4 (1.6)	0.88	3.3
ng/g lw								
HCB	87%	45 (34)	38	86	96%	40 (89)	29	89
β-HCH	75%	39 (108)	26	65	97%	39 (108)	21	75
4,4′-DDE	98%	220 (941)	104	316	100%	206 (517)	98	370
PCB-153	90%	44 (31)	38	80	98%	52 (48)	40	110
PCB-138	77%	25 (21)	20	49	98%	33 (29)	27	68
PCB-180	83%	32 (25)	26	62	98%	47 (45)	34	99

Table 3
Concentrations of organochlorine pollutants in breast milk (ng/g lw) and venous serum (ng/mL, between brackets ng/g lw) in the present and previous studies.

Reference	Sampling years	Country/city/region	N	HCB	β-HCH	PCB-138	PCB-153	PCB-180	4,4′-DDE
COLOSTRUM/BREAST MILK									
This study[a b]	*2004–2006*	*Sabadell/Gipuzkoa*	*256*	*29*	*21*	*27*	*40*	*34*	*98*
Yu et al. (2007) [a b]	2003	Slovaquia	14	8.0	15	121	184	153	537
Ribas-Fitó et al. (2005) [a b]	1997–1999	Flix (Spain)	92	910	…	…	…	…	1030
Polder et al. (2008) [a b]	2000	Russia	23	55	157	39	46	13	711
Cioroiu et al. (2010) [a b]	2007	Romania	51	195	14	…	…	…	96
Waliszewski et al. (2002) [e b]	1998–99	Mexico	100	40	80	…	…	…	3220
Riva et al. (2004) [a b]	2000	Italy	25	…	…	136	95	78	…
Jaraczewska et al. (2006) [a b]	2004	Poland	22	29	11	23	35	27	817
Shen et al. (2007) [c]	1997–2001	Finland	43	8.4	12	…	…	…	77
Shen et al. (2007) [c]	1997–2001	Denmark	43	12	18	…	…	…	137
Paumgartten et al. (2000) [d]	1992	Rio de Janeiro	40	12	270	35	37	18	1520
Ennaceur et al. (2007) [b]	2002–03	Tunisia	87	114	27	…	…	…	1389
Greizerstein et al. (1999) [c]	1991–94	New York	7	28	…	51	71	31	300
Colles et al. (2008) [b]	2006	Belgium	197	16	<10	31	43	23	124
LaKind et al. (2009) [b]	nr	USA	10	8.0	nq	20	23	10	142
VENOUS SERUM									
This study [b]	*2004–2006*	*Sabadell/Gipuzkoa*	*361*	*0.22 (38)*	*0.15 (26)*	*0.12 (20)*	*0.23 (38)*	*0.15 (26)*	*0.65 (104)*
Jaraczewska et al. (2006) [b]	2004	Poland	18	(15)	(3.9)	(12)	(21)	(15)	(343)
Sandanger et al. (2003) [c]	1996	Russia	27	0.57	3.6	0.53	0.49	0.17	5.4
Bergonzi et al. (2009) [b]	2006	Brescia (Italy)	70	(20) [c]	…	(35)	(54)	(49)	(126)
Greizerstein et al. (1999) [c]	1991–94	New York	7	(15)	…	(44)	(69)	…	(259)
Guvenius et al. (2003) [b]	2000–01	Sweden	…	…	…	(39)	(56)	(29)	…
Waliszewski et al. (2001) [c]	nr	Mexico	100	80	1.4	…	…	…	2770
Walker et al. (2003) [b]	nr	Arctic Canada	385	0.22	0.08	0.16	0.24	0.12	1.1
LaKind et al. (2009) [a]	nr	USA	10	(6.9)	…	…	(16)	(9.8)	(79)
Covaci et al. (2002) [b]	1999	Belgium	44	0.18	…	0.42	0.58	0.31	1.6
Adetona et al. (2013) [e]	2004–05	Peru	79	(2.8)	…	(6.8)	(18.8)	(6.9)	(413)

[f] Only primiparous women.
[a] Colostrum samples.
[b] Median.
[c] Mean.
[d] Pooled sample.
[e] GM.

Table 4
Colostrum-serum correlations and median ratios calculated after normalization to lipid weight (ng/g lw), and dependence of these ratios from gestational weight gain (GWG). The physical-chemical properties of each compound are given for comparison.

Compound	Colostrum-serum		Colostrum-serum and GWG[b]		Log(K_{ow})	Log(K_{oa})	MW
	Correlations[a]	Median ratios	All pregnancy	From 12th week on	25 °C (36 °C)	25 °C (36 °C)	
HCB	0.69***	0.82	−0.17**[b]	−0.16**	5.48 (5.38)	7.35 (6.95)	284.8
β-HCH	0.49***	0.77	−0.17**	−0.16**	3.8 (3.7)	8.88 (8.26)	290.8
4,4′-DDE	0.67***	1.01	−0.22***	−0.22***	5.95 (5.90)	9.33 (9.33)	318
PCB-153	0.74***	1.05	−0.20***	−0.18***	6.75 (6.65)	9.62 (9.06)	360.9
PCB-138	0.71***	1.27	−0.15*	−0.15*	6.16 (6.10)	9.99 (9.43)	360.9
PCB-180	0.74***	1.36	−0.15**	−0.13*	7.16 (7.15)	9.71 (9.16)	395.3

[a] Spearman's rank calculated over all paired data.
[b] Spearman's correlation rates. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

major changes in OC body burden in the last months of pregnancy, which is in agreement with some studies of OC serum composition in diverse pregnancy periods (Bloom et al., 2007; Jarrell et al., 2005; Waliszewski et al., 2001).

Calculation of the lipid normalized OC colostrum:serum ratios for each pair of samples showed median ratios ranging from 0.77 for β-HCH to 1.36 for PCB-180 (Table 4). GWG showed statistically significant negative correlations with the colostrum:serum ratios of all compounds (Fig. 1).

Higher GWG involved lower colostrum:serum ratios (Fig. 1) and the relationships were statistically significant ($p < 0.001$ for 4,4′-DDE and PCB-153; $p < 0.01$ for HCB and β-HCH; $p < 0.05$ for PCB-138 and PCB-180; Table 4). These significant correlations were observed when GWG was calculated for the period between venous blood collection and delivery (about 6 months) and when considering the whole GWG during pregnancy (data not shown). Multivariate linear regression models adjusting for potential confounding factors confirmed the aforementioned association (Fig. 4). The increase in the GWG during the pregnancy resulted in a lower POP concentration, which was statistically significant for all the compounds, except HCB. When the models were applied to the Colostrum:Serum ratios instead of Lipid adjusted Colostrum concentrations, we obtained similar results, although only 4,4′-DDE, β-HCH and PCB-153 were statistically significant at 95% CI (data not shown).

4. Discussion

4.1. Pollutant concentrations in colostrum and serum

The OC concentrations in both colostrum and serum were similar to those reported in previous studies from non polluted areas (Table 3). Thus, the colostrum concentrations of HCB are 30 times lower than in Flix (Catalonia, Spain) where the population was under the emissions of a chlor-alkali plant (Ribas-Fitó et al., 2005). In this factory, high amounts of DDT were produced in the past and the 4,4′-DDE colostrum of the population from this site are also much higher than those in Sabadell/Gipuskoa (Table 3). Comparison with the colostrum concentrations of other sites shows that in general the concentrations of HCB, β-HCH and PCBs from the study area range among the high limit of the concentration intervals of general populations not receiving pollution inputs from specific sources. In contrast, the concentrations of 4,4′-DDE in the study populations show that they range close to the low end of the literature descriptions for general population (Table 3). The concentrations of these compounds in maternal venous serum are in agreement with these differences.

4.2. Distributions of pollutants between colostrum and serum

The above reported data shows that fat formation during pregnancy (Zafon, 2007) exerts an influence on the distribution of OCs between colostrum and serum. The inverse relation with body weight increases indicates that most fat incorporated through the diet during pregnancy has negligible OC concentrations. These pollutants have been described to be preferentially stored in the abdominal adipose tissue (Pelletier et al., 2003) and their clearance rates decrease when adiposity levels are high. In these conditions, an increase in weight and fat has an effect of diluting OC concentrations.

This dilution effect is more significant for the OCs in colostrum than in serum. Sixty percent of lipids in breast milk originate from the mother adipose tissue (Hachey et al., 1987), which constitutes the predominant OC source in breast milk (LaKind et al., 2009) during late pregnancy. Furthermore, lipids in milk may attract lipophilic chemicals from blood,

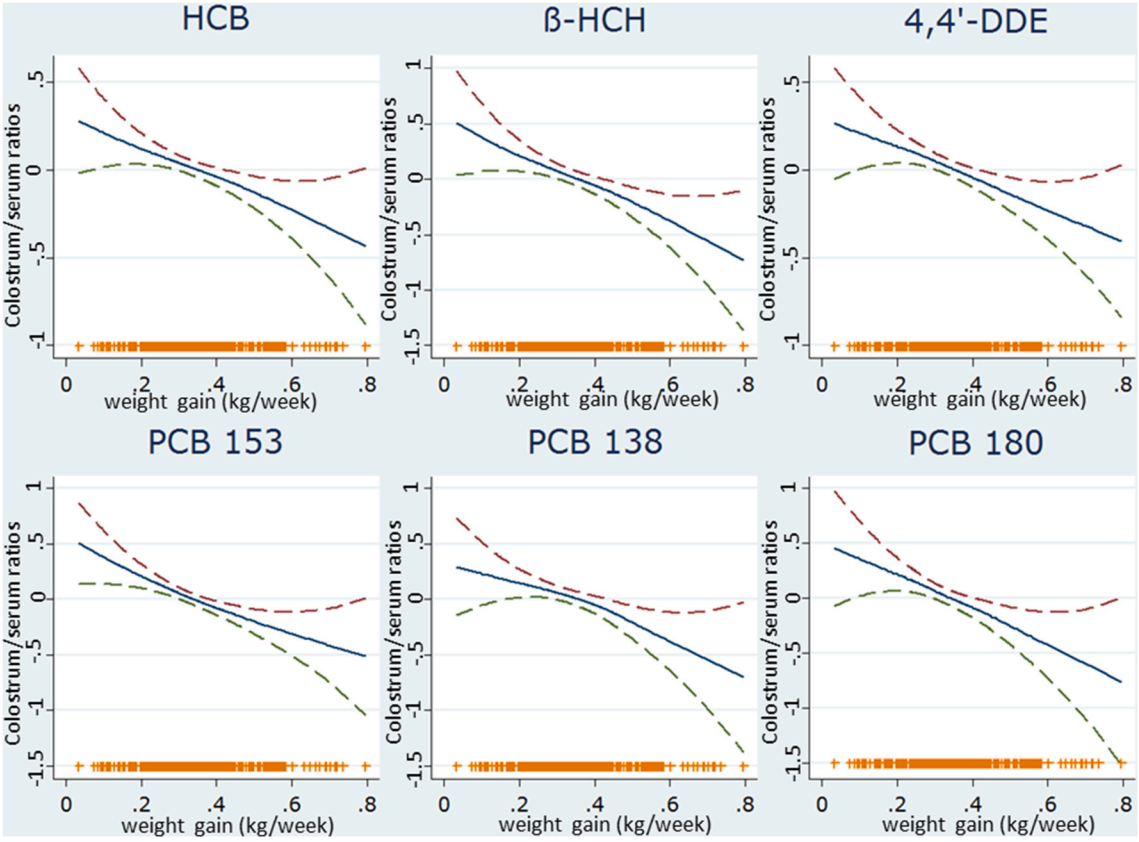

Fig. 1. Representation of general additive models showing the relationship ($p < 0.05$) of individual colostrum:serum ratios (calculated by normalization to lipid weight) of each compound (Y-axis) and gestational weight gain (X-axis; total weight gain normalized to 39 weeks). The symbols (+) on the X-axis show the gestational weight gain of each subject (weight gain during pregnancy/number of weeks).

migrating through the alveolar cell by passive diffusion and achieving equilibrium between both fluids (Wang and Needham, 2007). Accordingly, women who accumulate more fat, *e.g.* weight, during pregnancy will transfer smaller proportions of OC per unit of energy to their children upon breastfeeding.

The medians of the OC colostrum:serum ratios (Table 4) were significantly different ($p < 0.001$; Friedman's test) and correlated significantly with the octanol-water partition coefficient (K_{ow}) (Fig. 2), which is consistent with the observed fat dependence of the OC distributions in the present study. Higher colostrum:serum ratios were observed at higher log(K_{ow}) (Fig. 2). Earlier studies on the distributions of 4,4′-DDE and HCB in breast milk (Greizerstein et al., 1999; Jarrell et al., 2005; LaKind et al., 2009; Waliszewski et al., 2002) or colostrum (Waliszewski et al., 2002) and serum found higher dependence of 4,4′-DDE than HCB on breast milk or colostrum than serum, which agrees with the trend described in Fig. 2.

Other properties besides log(K_{ow}) may also modulate these distributions. Molecular weight also shows a remarkable consistency with the median OC colostrum:serum ratios (Fig. 2), which suggests that molecular size may also modify the transport capacity of these pollutants through membrane permeability (Wittsiepe et al., 2007) and therefore the colostrum:serum partitioning.

4.3. Influence of gestational weight gain in the distributions of pollutants

Grouping by the IOM GWG recommendations (Table 1) showed that the mean colostrum concentrations of 4,4′-DDE, PCB-153, PCB-138 and PCB-180 in the low GWG group were significantly higher than in the groups of adequate or high GWG (Fig. 3). Comparison of the mean values of the colostrum:serum ratios of these IOM groups showed similar results. The ratios of 4,4′-DDE, PCB-153 and PCB-180 of the low GWG group were significantly higher than those of the adequate and high GWG groups. No differences were found between mothers with recommended or excessive GWG. Women not meeting the IOM weight recommendations may lose weight during the formation of pregnancy-related organs such as the placenta, the amniotic fluid, the uterus, maternal breast tissue or the fetus. An inadequate level of maternal body fat may result in higher mobilization rates of maternal fat stores in the last trimester of pregnancy (Haggarty, 2010), which may trigger the release of OCs into bloodstream and ultimately into colostrum and breast milk.

These results are consistent with a previous study in which mothers who put on less weight during pregnancy than recommended by the IOM were found to transfer higher burdens of OCs to their newborns through the placenta (Vizcaino et al., 2014b). This earlier study and the present results provide compelling evidence of the lower maternal OC retention and the higher transference to newborns when the GWG is lower than that recommended by the IOM. In the previous study (Vizcaino et al., 2014b) only transplacental transport was considered. The present results show that low GWG plays a more significant role in OC transfer through breastfeeding.

The most important limitation of our study is the different time of sampling. While most studies are related to serum collected just before labour or during the first days after birth, our samples were collected during the 12th week of pregnancy. Another limitation is the use of different methodologies to calculate the lipid content in serum and colostrum samples. However, we have been able to find a good agreement between both matrices, with high correlations for the different compounds. Among the strengths of our study, the present results increase the relevance of GWG to ensure a healthy life of newborns. While too large GWG are not recommended to avoid too big fetus and delivery problems, too low GWG, besides other issues, involve higher pollutant transfers of OCs and compounds of similar properties through placenta and, as shown in the present study, through breastfeeding. To the best of our knowledge, this breastfeeding effect is described here for the first time. In the context of the strong pregnancy metabolic changes of the maternal-fetal consortium, it could be anticipated that low GWG may involve higher transfer of chemically stable lipophilic pollutants to the fetus as consequence of low maternal retention. However, the higher association of these pollutants to the breastfeeding glands upon low GWG constitutes a new finding that extends the significance of this parameter for pollution transfer to the first months of life after delivery.

The OCs showing enhanced maternal-newborn transfer are highly lipophilic (log(K_{ow}) > 5) and are within a 300–400 molecular weight interval. The good agreement of the OCs studied with some defined physical-chemical properties indicates that the correlations could also be observed in other pollutants. Compounds with similar properties will also show increases in maternal-newborn transfer in mothers with low GWG according to IOM recommendations.

5. Conclusions

The present data show for the first time that GWG exerts a considerable influence on the content of lipophilic and low volatile pollutants in breast milk. Colostrum from mothers with low GWG as defined by the IOM had significantly higher concentrations of polychlorobiphenyls (PCBs) and 4,4′-DDE than colostrum from mothers who gained weight within IOM recommendations or those exceeding this threshold. Statistically significant differences were also found in the colostrum: maternal serum ratios of these compounds. Women with low GWG retained higher pollutant amounts in colostrum. Accordingly, low GWG involves higher mobilization of stored organochlorine pollutants in pregnant women which, besides leading to higher transplacental transfer, results into higher accumulation in breast milk. This accumulation pattern extends considerably the early life period of incorporation of these pollutants into children.

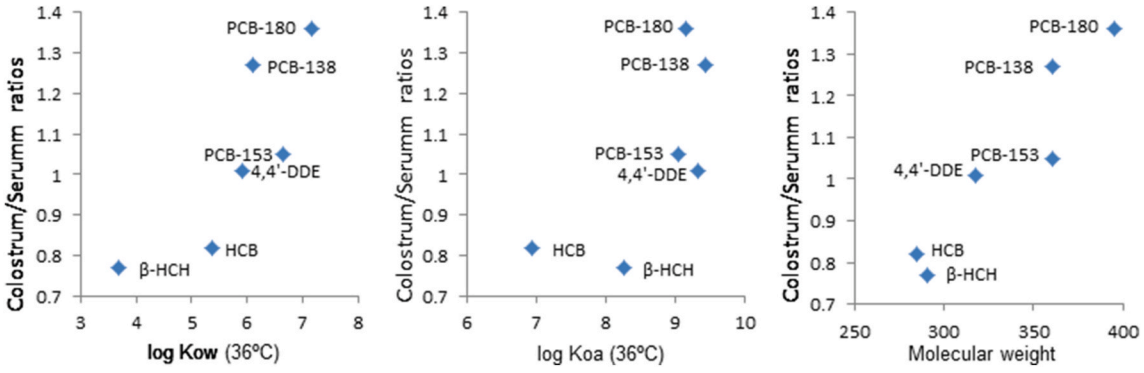

Fig. 2. Median colostrum:serum ratios (calculated by normalization to lipid weight) of the distributions of the organochlorine compounds in the mothers from the present study represented vs log(Kow) (36 °C), log(Koa) (36 °C) and molecular weight.

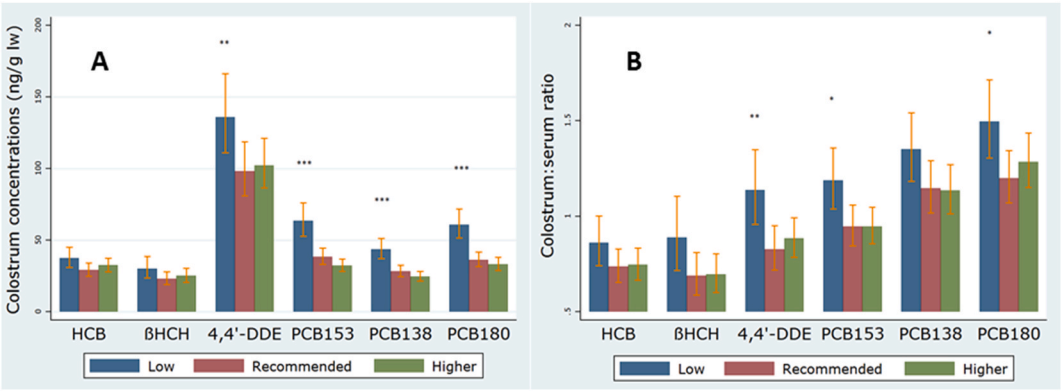

Fig. 3. Representation of the mean colostrum concentrations (A) and colostrum:serum ratios (B; calculated by normalization to lipid weight) of each pollutant after grouping by the gestational weight gain (GWG) of the volunteer mothers defined from the Institute of Medicine (IOM) of the US National Academies of Sciences, Engineering and Medicine. The intervals indicate standard deviation. The significant differences between IOM GWG groups are indicated (*p < 0.05, **p < 0.01, ***p < 0.001).

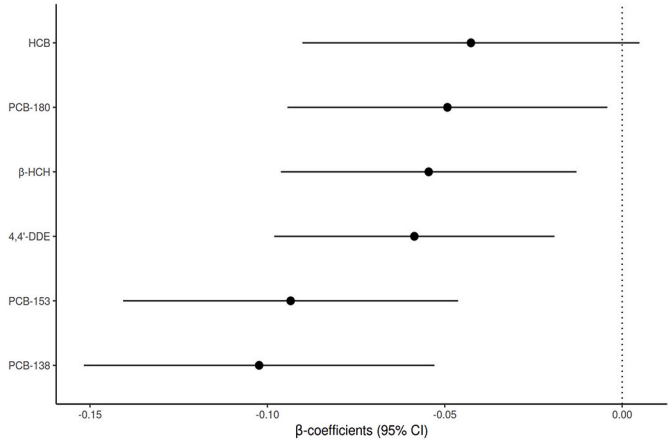

Fig. 4. Beta-coefficients from multivariate regression models for the pollutants' effect on the GWG. Models were adjusted by maternal age, cumulative breastfeeding, social class, maternal educational level and country of origin. Lipid adjusted Colostrum concentrations and GWG were log-transformed.

Credit author statement

JOG designed the study, interpreted the results and wrote the paper. MG performed the statistical analyses and contributed to paper writing. L.M.-S. organized the sampling in the Gipuzcoa cohort and interpreted the results. J.I. organized the sampling in the Gipuzcoa cohort and interpreted the results. J.S. organized the sampling in the Sabadell cohort and interpreted the results. All authors discussed the health implications of the results.

Declaration of competing interest

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

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