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Organochlorine and heavy metals in newborns: Results from the Flemish Environment and Health Survey (FLEHS 2002–2006)

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ABSTRACT

To collect regional information on internal levels of pollutants in humans in Flanders, 1196 mother–child pairs were systematically recruited in 2002–2003 via 25 maternities across Flanders. Cd, Pb, PCB congeners 118, 170, 138, 153 and 180, p,p′-DDE — a key metabolite of DDT- and hexachlorobenzene (HCB) were measured in cord blood or plasma. Cd was detected in 64% of the samples (geometric mean 0.21 µg/L cord blood). p,p′-DDE (110 ng/g plasma lipids) and Pb (14.7 µg/L blood), were measurable in nearly all samples. The individual PCB congeners could be detected in 40 to 81% of the newborns ($138 + 153 + 180 = 64.4$ ng/g plasma lipids). HCB (18.9 ng/g plasma lipids) and dioxin-like compounds measured by DR-CALUX® (23 pg CALUX-TEQ/g lipids) were above detection limit in more than 75% of the samples. Age and smoking habits of the mothers, did not influence the cord blood Pb and Cd levels. The organochlorines increased 4 to 9% per year of the mother's age (partial $R^2 = 0.05$ to 0.22). Mothers had 2.6% less PCBs in cord blood (partial $R^2 = 0.02$) for each unit increase in pre-pregnancy BMI. Season of delivery, breastfeeding previous children or consumption of local dairy products, were minor determinants. Up to 20% of the variability in organochlorine concentrations was explained by residence area. It was concluded that the place of birth in Flanders is an important determinant of the load of pollutants measured at the start of life. This underlines the validity of human biomonitoring on (relatively) small geographical scale.

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1. Introduction

Flanders is the Dutch speaking northern part of Belgium with 5.9 million inhabitants. It is one of the most populated areas in Europe with an average 434 inhabitants/km². To collect data on environmental contamination and related health, a five-year Flemish Environment and Health Survey (FLEHS) (2002–2006) for human biomonitoring in different geographical areas was established. In total, about 4600 participants were recruited in three age groups: newborns and 14–15 years and 50–65 years old men/women. A cohort of about 300 newborns was followed-up till the age of 36 months for either airway or neurodevelopmental problems (Verhulst et al., 2008). This paper presents results on organochlorines (OCs), cadmium (Cd) and lead (Pb) measured in umbilical cord blood/plasma of 1196 Flemish newborns recruited in 2002–2003. Other and additional results are or will be published elsewhere (Bilau et al., 2008a; Keune et al., 2008; Maervoet et al., 2007; Schroijen et al., 2008).

The measured compounds are persistent widespread environmental pollutants. No systematic information on the levels of these pollutants in the Flemish population was available before this study.
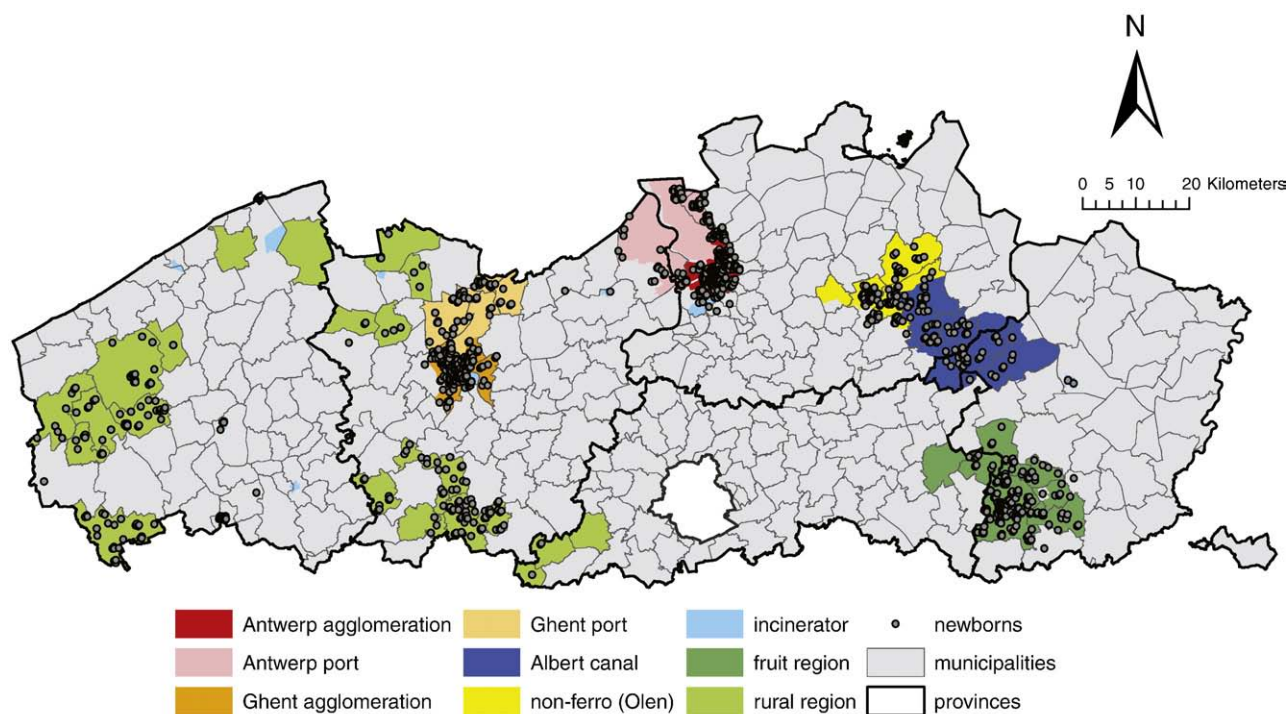
Fig. 1. Geographical distribution of individual newborns (dots) recruited in different areas of Flanders.

OCs are a diverse group of persistent synthetic compounds, with p,p′-DDE and polychlorinated biphenyls (PCBs) comprising the bulk of OC residues in human tissues (Longnecker et al., 1997). Human tissue levels of OCs clearly indicate, that exposure of the general population declined from the seventies onwards. However, concern remains as the margin of exposure between safe levels and current exposure levels is small with the developing child as most sensitive population group for adverse effects. In Belgium, decreasing levels of OCs have been observed in human milk sampled during successive WHO monitoring campaigns. From the early 1990s to 2006 there was a drop in marker PCBs (from 300 to 80 ng/g lipids), and dioxins/furans (from 30 to 10 pg WHO-TEQ/g lipids) (Colles et al., 2008).

Belgium is the largest producer of Cd in the world. This metal is highly toxic at low concentrations. It is known to accumulate in the placenta and even low levels might be a risk factor for lower birth weight, reflecting developmental impairment in infants (Salpietro et al., 2002).

Pb levels in Europe, North America and Greenland seem to be decreasing during the past two decades (Bjerregaard and Hansen, 2000). However recent epidemiological information emphasized the importance of continued monitoring of blood lead levels prenatally and during early infancy because of the link of elevated blood Pb levels with decreased IQ. The safe threshold level of 10 µg/dL as suggested by the WHO is under discussion as well as the identification of sensitive windows of exposure (Landrigan, 2002).

Cord blood sampling is a non-invasive procedure and is frequently used to assess exposure to persistent pollutants in the earliest stage of life (e.g. Butler Walker et al., 2006; Butler Walker et al., 2003; Dallaire et al., 2003; Hamel et al., 2003; Osman et al., 2000) All of the measured OCs in our study are stored in fat tissue over many years and have fairly long half-lives ranging between 5 and 15 years for PCBs, 4 to 12 years for dioxins/furans (Patandin et al., 1999), 9 years for p,p′-DDE (Hunter et al., 1997) and about 3 to 4 years for HCB (Pierik et al., 2007). In pregnant women, the mobilization of these pollutants can be enhanced. Recently, Bloom et al. (2007) reported a decline in maternal serum PCB concentrations pre- compared to post-conception. Embryonic development or physiological changes accompanying pregnancy, may impact mobilization from lipid reserves. Also Pb, accumulated in bone tissue over more than 20 years, may be mobilized during pregnancy and contribute to fetal exposure (Gulson et al., 1997; Lagerkvist et al., 1996; Raghunath et al., 2000). Cd accumulates in the human body, in particular in the kidney and liver and has an elimination half-life of 10–30 years (Jarup et al., 1998).

Umbilical cord blood is considered to represent the levels of chemicals that have passed through the blood–placenta barrier. OCs are easily transferred to the fetus with rates around 25–30% expressed on fresh weight base (Bjerregaard and Hansen, 2000; Butler Walker et al., 2003; Covaci et al., 2002; Soechitram et al., 2004) or around 80% on lipid weight basis (Jaraczewska et al., 2006). Umbilical cord blood levels of Pb have been reported to be about 85 to 90% of those of the mother (Lagerkvist et al., 1996). For Cd, different transfer percentages of 10% (Osman et al., 2000) to 81% (Raghunath et al., 2000) were observed.

The present campaign was conducted to test the hypothesis that living in different geographical areas in Flanders has a measurable impact on the load of pollutants in newborns. Therefore an inter-regional cord blood monitoring campaign was set up. Determinants of variability were investigated.

2. Materials and methods

2.1. Design

The campaign was approved by the ethical committee of the University of Antwerp. Mothers and their newborns were enrolled via 25 Flemish maternities. They were selected by geographically stratified sampling in two urban areas, two rural areas and four types of industrial areas. All together covering 20% of Flanders' area, and 65 different municipalities (Fig. 1). On average four maternities (viz. one per season) were selected per area. The goal was to recruit

Table 1
Characteristics and self-reported health data of the mother–child pairs.

Characteristics	Percentage or median (IQR[a])
Mothers	
Age (years)	
<25	12.8
25–29	35.6
30–34	33.3
35–39	8.0
≥40	1.2
Pre-pregnancy body mass index (kg m^{-2})	
<19 (underweight)	9.3
19–25 (normal)	64.7
25–30 (overweight)	18.5
≥30 (obese)	7.5
Primiparae	61
% ever smoked	35.8
% smoking during pregnancy	16.2
% alcohol consumption during pregnancy	8.3
% bachelor or master educational level of mother or partner	55.9
% with net family income >2480 €	36.2
% working mothers	79.5
% laboratory work	2.8
% working in metal industry	5.3
% working in chemical industry	2.2
% hobby using dyes/paint	1.9
% consumption of locally grown foods	43.9
Gram animal fat per day	26.7 (19.4–37.9)
Gram fish fat per day	2.1 (0.9–3.7)
Gram fatty fish fat per day	0.9 (0.2–1.8)
Gram chicken fat per day	0.4 (0.2–0.7)
Gram dairy products fat per day	9.2 (5.3–16.2)
% indoor wood/coal heater	13.6
% of dwellings 40 years old	41
% residence at less than 50 m from regular to heavy traffic[b]	42.7
% with passage of more than one bus/truck per 5 min	14.1
Commute distance (km)	6.0 (0–15.0)
Time spent in traffic per week (h)[c]	7.5 (4.5–12.7)
Newborns	
% boys	52.0
Duration of pregnancy in weeks[d]	39.0 (39.0–40.0)
% prematures <37 weeks[d]	4.3
% born in Spring, Summer, Autumn, Winter	26, 21, 28, 25
Total cholesterol in cord plasma (mg/dL)	65 (53–79)
Triglycerides in cord plasma (mg/dL)	33 (24–44)
Total lipids in cord plasma (mg/dL)	203 (162–246)
Ferritin in cord plasma (μg/L)	127 (76–193)

[a] Inter quartile range (P_{25}–P_{75}).
[b] Either passage of more than one car per minute, or residency less than 50 m remote of traffic lights or traffic circle.
[c] On/by foot/bike in urbanized area, or by car, bus or tram/streetcar.
[d] In case of spontaneous delivery ($N = 868$).

200 participants per region, between September 2002 and February 2004. Inclusion criteria were living for at least five years in the area of interest and being able to fill out Dutch questionnaires. Midwives of the maternities selected on residency at the time the women arrived for delivery. Participating mothers filled out an informed consent. Field work nurses visited the women in the maternity to hand over the questionnaires.

2.2. Blood collection

Approximately 30 mL cord blood was collected non-sterile by leaving it run off in 50 mL polypropylene tubes filled with 0.5 mL Na_2EDTA anticoagulants. Some maternities cooperated with stem cell blood banks using venipuncture collection in blood bags. All collection methods were tested on contamination and/or adhesion of the measured compounds. Within one day, cord blood and separated plasma were put in the refrigerator. After transport to the analytical lab, the samples were stored at −20 °C until analysis.

2.3. Questionnaires

The 30 page long questionnaires were filled out by the mothers without supervision. It contained questions on: (i) life style, use of tobacco and alcohol, consumption of local produced foods, occupational and traffic exposure, living environment and housing, hobbies, health, medicine use; (ii) personal perception of local environmental pollution and health risks (Keune et al., 2008) and (iii) a semi-quantitative food frequency consumption questionnaire on food items containing animal fat (meat, fish and seafood, diary products), fruit and vegetables, consumed during the year before pregnancy (Bilau et al., 2008a,b).

2.4. Measurements in cord blood

Pb and Cd concentrations in whole cord blood were determined using acid digestion followed by High Resolution-Inductively Coupled Plasma-Mass Spectrometry (HR-ICP-MS) (Schroijen et al., 2008). Briefly, 200 μL cord blood, 1 mL milli Q water, 1 mL distilled nitric acid and subsequently 1 mL hydrogen peroxide suprapure were mixed in closed vessels and subjected to increased pressure and temperature. The digestion mixture was diluted 28.5 times when analyzed with the HR-ICP-MS. The detection limits for Cd and Pb were 0.09 and 2.0 μg/L, respectively.

PCB 118, 138, 153, 170 and 180 and organochlorine pesticides (OCPs) (p,p′-DDE and HCB) were analyzed in cord blood plasma by two laboratories using two slightly different methods based on those described by Gomara et al. (2002) and Covaci and Schepens (2001). Briefly, 2 to 2.5 mL of blood serum was mixed with formic acid, internal standards were added and the mixture was equilibrated in an ultrasonic bath. The sample was eluted through a solid-phase extraction (SPE) cartridge. Subsequently, the cartridge was washed, dried and placed on top of a multilayer column filled with silica impregnated with sulfuric acid (44% w/w) and topped with anhydrous sodium sulfate. OCPs and PCBs were eluted and concentrated. The extracts were analyzed with gas chromatography combined with mass spectrometric detector (GC–MS) operated in electron capture negative ionization mode. Both laboratories participated three times per year in the AMAP proficiency testing scheme of the Institut National de Santé Publique, Canada (AMAP, 2004, 2005, 2006). The detection limit of all chlorinated compounds in plasma was 0.02 μg/L. This means 10 ng/g lipids for 5 mL plasma, with an average lipid content of 200 mg/dL.

Dioxin-like compounds in cord plasma were determined by the dioxin-responsive DR-CALUX® assay (BioDetection Systems, The Netherlands) as previously described in Koppen et al. (2001). Briefly, the compounds were extracted from 5 to 10 mL cord plasma with n-hexane liquid/liquid extraction. The extracts were eluted through a silica column containing (33%, w/v) H_2SO_4, and concentrated. H4IIE rat hepatoma cells were dosed for 24 h with 100 μL sample extracts and TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) standards in triplicate. This DR-CALUX® assay cell line is transfected with an aryl hydrocarbon receptor (AhR)-controlled luciferase reporter gene construct. If dioxin-like compounds bind to the receptor, luciferase is produced and its activity is measured by a Luminometer. The limit of detection was 0.03 pg CALUX-TEQ/mL or 14 pg CALUX-TEQ/g lipids for 5 mL plasma, with a lipid content of 200 mg/dL.

Plasma total lipid (TL) concentrations were determined gravimetrically during fact extraction for the Calux® bioassay. Additionally, triglyceride (TG) and cholesterol (CH) levels were measured individually by spectrophotometry on a Modular analyzer (Roche diagnostics, Belgium). The combination of both methods allowed to derive the following formula: $TL = 1.33 \times (TG + CH) + 50.5$ mg/dL.

Table 2
Levels of pollutants measured in cord blood of all neonates recruited in 2002–2003.

	N	% >DL[a]	Ref. mean	Ref. P_{90}	Mean	Geometric mean (95% CI)	Min	P_{10}	P_{25}	Median	P_{75}	P_{90}	Max
Organochlorines (/g plasma lipid)													
PCB 138 (ng)	1054	71			21.3	15.3 (14.6–16.2)	2.3	4.5	7.5	17.4	28.8	43.2	156.7
PCB 153 (ng)	1065	81			37.7	25.9 (24.5–27.5)	2.3	5.2	14.9	31.3	52.4	78.0	230.2
PCB 180 (ng)	1071	75			26.0	20.4 (19.5–21.3)	2.1	6.2	13.9	22.5	33.3	47.3	153.1
PCB 118 (ng)	1051	60			13.8	10.5 (10.1–11.0)	1.9	4.0	5.5	11.1	18.4	26.6	195.1
PCB 170 (ng)	1050	40			9.6	7.7 (7.5–8.0)	1.1	3.9	4.8	6.9	12.2	18.7	62.5
PCB 138 + 153 + 180 (ng)	1054		64 (61–68)	166	85.0	64.8 (61.8–67.9)	7.0	21.3	39.7	69.4	112.7	166.7	528.1
$\sum$ all PCBs (ng)	1047				108.3	85.9 (82.3–89.6)	11.7	33.5	52.9	89.3	141.8	205.9	617.7
Dioxin-like compounds (pg CALUX-TEQ)	871	89	23 (21–24)	55	27	22 (21–23)	4	9	12	23	37	52	158
p,p′-DDE (ng)	1112	99	110 (104–116)	332	163.9	109.3 (103.6–115.4)	4.4	36.6	61.9	108.5	188.4	328.9	1815.5
HCB (ng)	1044	77	19 (18–20)	48	25.4	18.4 (17.4–19.3)	2.0	4.8	10.9	21.1	32.9	47.9	310.7
Metals (µg/L blood)													
Cd	1116	64	0.21 (0.19–0.23)	1.28	0.50	0.21 (0.19–0.23)	0.05	0.05	0.05	0.23	0.65	1.32	13.87
Pb	1112	96	14.7 (14.0–15.5)	42.6	19.2	13.1 (12.4–13.8)	1.0	4.3	7.9	14.1	24.3	38.4	177.6

All values are rough data except for the Flemish reference geometric mean (Ref. mean) and reference 90th percentile (Ref. P_{90}), which were adjusted for smoking before and during pregnancy, age of the mother and number of inhabitants in the different regions (population weighted).
  [a] Above detection limit.

2.5. Statistical analysis

Database management, data quality control and statistical analysis were performed using Statistica version 7, and SAS software, version 9.1.

Cord blood concentrations below detection limit were set at half of this limit. The natural logarithmic transformation was used in the analysis. A Flemish reference mean of each pollutant was calculated based on a linear regression model, with smoking (before and during pregnancy), mother's age and area of residence, included as independent variables. For the covariate 'area of residence', weights proportional to the number of inhabitants were used. Similarly, adjusted 90th percentile (P_{90}) reference values for all pollutants were calculated using quantile regression. ANCOVA analysis was used to test geographical differences in the pollutant concentrations.

Predictor variables (listed in Table 1) for the cord blood pollutants, which were significant at the 10% level in a univariate regression analysis, were introduced in a forward stepwise multiple regression model. In the regression models, levels of OCs were expressed per mL serum, with plasma lipids included as a predictor (Schisterman et al., 2005). The partial R^2 indicates the % of variability explained by the covariate in the model. The effect size was calculated from the regression coefficient, for a unit change in predictor variable.

3. Results

3.1. Recruitment and study participants

We recruited 1196 neonates and their 1186 mothers, over a period of about 1.5 years, this means 6% of all Flemish births during this period. They were enrolled equally spread over all seasons of the year. During the first months of the campaign, midwives recorded the reason of non-participation. The mean reasons were (in descending importance): not asked for participation, living less than 5 years in the region, not having enough knowledge of Dutch, or complications in delivery. The overall participation rate of eligible mothers was 98%.

The median age of the mothers was 29.6 ranging from 18.1 to 44.0. They had a median pre-pregnancy body mass index (BMI) of 22.4 (IQR = 20.3–25.1) kg/m². Respectively 18.5 and 7.5% of the mothers were overweighed (25<BMI<30) or obese (BMI>30) before pregnancy (Table 1). Sixteen % of the mothers smoked during pregnancy, 35.8% ever smoked and 8.3% consumed alcohol during pregnancy. The majority were working mothers (79.5%), who lived on average 6 km (IQR = 0–15 km) from their working places. They spent about 8 h per week (IQR = 4.5–12.7 h) in traffic.

3.2. Cord blood pollutant levels

For most of the pollutants there was a broad interindividual variability resulting in a factor three difference between the 25th and 75th percentile (Table 2). Two pollutants, p,p′-DDE and Pb, were above the detection limit in nearly all neonates. Cd levels were measurable in 64% of the newborns. The individual PCB congeners could be detected in

40 to 81% of the cases and in increasing order for PCB 170<118<138<180<153. HCB, dioxin-like compounds and p,p′-DDE were measurable in respectively 77%, 89% and 99% of the neonates.

Table 3
Data on European cord blood levels measured in the time period of our study.

| Country	N	Average (SD or 95%CI)	Ref.
Organochlorines (ng/g plasma lipid)			
$\sum$ 6 marker PCB			
Belgium (Flanders)	1054	64 (61–68)	This study
Spain	69	105	Sala et al. (2001)
Belgium	44	$P_{50} = 150$	Covaci et al. (2002)
The Netherlands	51	229	Soechitram et al. (2004)
Germany	200	$P_{95} = 700$	Lackman (2002)
p,p′-DDE			
Belgium (Flanders)	1054	110 (104–116)	This study
The Netherlands	27	$P_{50} = 145$	Peters (2005)
Belgium (Antwerp)	44	$P_{50} = 245$	Covaci et al. (2002)
Spain	69	415	Sala et al. (2001)
HCB			
Belgium (Flanders)	1054	19 (18–20)	This study
Belgium	44	$P_{50} = 35$	Covaci et al. (2002)
The Netherlands	27	$P_{50} = 35$	Peters (2005)
Germany	100 rural/100 urban	$P_{50} = 80/130$	Lackman (2002)
Metals (µg/L blood)			
Cd			
Belgium (Flanders)	1107	0.21 (0.19–0.23)	This study
Sweden	101	$P_{50} = 0.02$	Osman et al. (2000)
Italy	45	0.13 (0.99)	Salpietro (2002)
Poland	83	0.28	Durska (2001)
The Netherlands	25	0.06 (0.01) non-smokers 0.07 (0.02) smokers	Godschalk et al. (2005)
Pb			
Belgium (Flanders)	1107	14.7 (14.0–15.5)	This study
Sweden	101	$P_{50} = 11.2$	Osman et al. (2000)
Greece	25 rural/25 urban	12.9 (3.6)/ 20 (3.4)	Dussias et al. (1997)
Poland	83	21.4	Durska (2001)
France	206	32 (20)	Smargiassi et al. (2002)
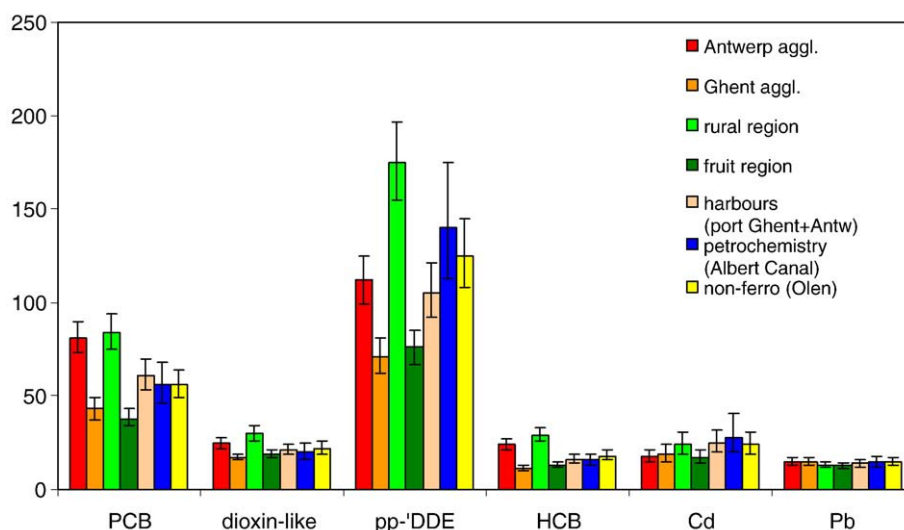
Fig. 2. Regional differences in cord blood pollutants in Flanders. PCB: PCB138 + 153 + 180 (ng/g lipid), Dioxin-like: CALUX-TEQ (pg/g lipid), ppDDE (ng/g lipid), HCB (ng/g lipid), Cd (E^{-2} µg/L),Pb (µg/L). All pollutant levels were adjusted for age of the mothers and for smoking habits before and during pregnancy.

Pb and Cd were significantly correlated in all areas with Spearman rank correlation coefficient varying between 0.37 and 0.72. The correlation of PCB138 + 153 + 180 with p,p′-DDE and HCB varied between the regions from 0.40 to 0.81. For dioxin-like compounds the degree of correlation with other OCs, was even more regionally dependent ($r = 0.05$–0.53).

3.3. Determinants of pollutant levels in cord blood

Area of residence determined up to 20% for p,p′-DDE, HCB and marker PCBs and only 0.9% of the variation in Pb levels (Table 3). Indeed, the mean concentrations of chlorinated compounds differed by a factor 2 to 3 between the areas, whereas the average metal levels only differed slightly (Fig. 2). OC levels were highest in participants from the rural area and from Antwerp city, while participants from Ghent city and the fruit area had relatively lower levels. In the rural area there were clearly more than 10% of participants with OC levels above the reference P_{90}, namely between 16.5 to 26.4% for the different OCs. The industrial area near petrochemical industry had significantly more participants (25%) with pp′-DDE levels above the reference P_{90}.

Age and smoking habits before or during pregnancy, did not influence the levels of Pb and Cd measured in cord blood. Age was a determinant for the marker PCBs, HCB and p,p′-DDE levels in cord blood (Table 4). They increased 4 to 9% if the mother's mean age increased with one year. Age of the mother and area of residence contribute equally to the variability in PCBs with a partial R^2 of respectively 22 and 20% (Table 4).

The heavy metal levels were higher in women delivering in summer compared to women delivering in winter: respectively 0.32 vs. 0.17 µg/L for Cd ($p < 0.001$) and 15.1 vs. 12.0 µg/L for Pb ($p = 0.07$). On the contrary, marker PCBs and HCB levels were higher when the delivery took place in winter, compared to spring or summer: respectively 67 vs. 60 ng/g lipids for PCBs ($p > 0.05$), and 23 vs.17 ng/g lipids for HCB ($p < 0.001$).

For all chlorinated compounds there was a decrease in cord blood concentrations, if the mother had breastfed previous children (from −0.28 till −0.32% per week of breastfeeding ever). Mothers who consumed local dairy products had 10% more PCBs or 18% more p,p′-DDE in cord blood, compared to non-consumers.

4. Discussion

In several European countries, OCs and/or metals are monitored in cord blood: e.g. Faroes cohorts ('86–), Spain ('97–'99, '04–'06), Slovakia ('03–'05), and French cohorts in Brittany ('02–'05) and Guadeloupe ('05–'06) (Kogevinas et al., 2004). In the seventies, heavy metals were measured in a large group of nearly 500 cord blood samples originating from all over Belgium (Buchet et al., 1978). Since then, no other large cord blood survey has been done in Belgium.

4.1. Cord blood pollutant levels

All measured pollutants are known to pass through the placenta into the umbilical cord (Butler Walker et al., 2003; Osman et al., 2000). Lead can easily cross the placental barrier (Raghunath et al., 2000). The Flemish average concentration of 14.7 µg/L was in the range of median/mean levels of other European studies in the time period of the current study (Table 3). The observed average Cd levels of 0.21 µg/L cord blood, were comparable to Italian and Polish newborns (0.13 and 0.28 µg/L respectively), and clearly higher than average levels observed in some other Western countries (Table 3). Background exposure to Cd might be relatively high in Flanders, since there is some historical contamination originating from several zinc or lead smelters (Van Meirvenne and Goovaerts, 2001). However, adolescents recruited from the same areas as the current study population, did not show elevated serum cadmium levels compared with data from other countries (Schroijen et al., 2008). The sum of the six marker PCBs (64 ng/g lipids), were in line with European and Canadian data, where average values ranged between 52 and 272 ng/g lipids (Table 3). The same was true for p,p′-DDE and HCB measured in those studies.

For the first time, persistent compounds having dioxin-like activity were determined in Belgian cord blood plasma using the DR-CALUX® bioassay. We only found one Japanese study reporting on ongoing measurements in cord blood using a similar assay (Nakai et al., 2004). The DR-CALUX-assay has the advantage over the classical chemical dioxin/furan analysis, that only a small volume of 5 mL cord serum/ plasma is needed. Moreover, the bioassay 'integrates' the mixture of all compounds, which can activate the Aryl hydrocarbon receptor (AhR) pathway and which resist the clean-up procedure: dioxins/ furans, HCB, PCBs, acid-stable polycyclic aromatic hydrocarbons (PAHs), polybrominated biphenyls (PBBs), polychlorinated naphtha-lenes (PCNs), azo and azoxybenzenes (Schecter et al., 1999; Van Wouwe et al., 2004; Windal et al., 2003). In most of our study regions, DR-CALUX values were moderate to well correlated with the other measured OCs ($r = 0.21$–0.47). However, this correlation was low or absent in newborns from the non-ferro region of Ghent (data not shown). This could indicate a different pollutant profile of organoha-logenated compounds compared to the other regions.

Limited data are available allowing to evaluate trends of con-taminants in Belgian cord blood. In the seventies, the median cord blood lead level in 474 women was around 77 µg/L (Lauwerys et al., 1978). This means about 5 times higher as the current levels measured in the present study. Covaci et al. (2002) reported a median level of 150 ng/g lipids for six marker PCBs in cord blood collected in 1999 from 44 Flemish women living in Antwerp. This is substantially higher than the median of this region (81 ng/g lipids) measured 3–4 years later, during the current campaign. A similar decline was observed for HCB and p,p′-DDE. In 2001 OC levels in Belgian mother milk, were

Table 4
Determinants of the pollutants in cord blood, assessed via multiple forward regression analysis.

Exposure marker	Determinants	Effect size[a]	Partial R^2	p
Organochlorines (/mL plasma)				
Marker PCBs (ng)	Area of residence	Non linear	0.20	<0.001
	Age of mother (years)	9 (8–10)	0.22	<0.001
	Plasma lipids (g/L)	30 (23–38)	0.05	<0.001
	Pre-pregnancy BMI (kg/m^2)	−2.6 (−3.5–1.7)	0.02	<0.001
	Breastfeeding (weeks in total)	−0.32 (−0.49–0.14)	0.008	<0.001
	Consumption of local dairy products (no/yes)	9.8 (0.5–19.9)	0.003	0.04
	Summer season[b]	−10 (−20–0.8)	0.002	0.07
CALUX-TEQ (pg)	Area of residence	Non linear	0.10	<0.001
	Plasma lipids (g/L)	13 (6–20)	0.02	<0.001
	Consumption of animal fat (g/day)	0.14 (0.01–0.26)	0.005	0.03
p,p′-DDE (ng)	Area of residence	Non linear	0.18	<0.001
	Age of mother (years)	6 (5–7)	0.07	<0.001
	Plasma lipids (g/L)	25 (17–35)	0.03	<0.001
	Breastfeeding (weeks in total)	−0.28 (−0.51–0.05))	0.004	0.02
	Summer season[b]	−20 (−31–8)	0.007	0.003
	Spring season[b]	−24 (−34–13)	0.01	<0.001
	Consumption of local dairy products (no/yes)	18 (5–33)	0.006	0.004
HCB (ng)	Area of residence	Non linear	0.19	<0.001
	Age of mother (years)	4 (3–6)	0.05	<0.001
	Plasma lipids (g/L)	0.12 (0.05–0.19)	0.01	<0.001
	Breastfeeding (weeks in total)	−0.29 (−0.49–0.14)	0.007	0.006
	Spring season[b]	−19 (−28–7)	0.008	0.002
Metals (µg/L blood)				
Cd	Area of residence	Non linear	0.04	<0.001
	Summer season[b]	71 (31–122)	0.02	<0.001
	Working in metal industry (months)[c]	−1.2 (−2.4–0.01)	0.004	0.04
Pb	Area of residence	Non linear	0.009	<0.001
	Summer season[b]	24 (3–49)	0.006	0.03
	Age of mother (years)	1.5 (−0.1–3.0)	0.004	0.06

[a] Percentage change (95% confidence interval) in dependent variable associated with a one-unit change in the independent variable.
[b] Compared to winter period.
[c] Scored as follows: <6 months = 3 months, 6 months–1 years = 9 months, 1–5 years = 36 months, >5 years = 60 months.

among the highest in European industrialized countries (Van Leeuwen and Malisch, 2002). However, mother milk samples collected during the last WHO campaign of 2006, showed over a five-year-interval a decrease of 30–60% to 10 pg WHO-TEQ/g lipids for PCDD/Fs and 80 ng/g lipids for the sum of six marker PCBs (PCB 28 + 52 + 101 + 138 + 153 + 180) (Colles et al., 2008).

4.2. Determinants of pollutant levels in cord blood

The variance in the concentration of heavy metals could hardly be predicted by information from the questionnaires (7 and 2% for Cd and Pb, respectively). However, for the OCs, the explained variance was higher and ranged from 13 to 50% (Table 3).

There was no influence of smoking on the level of Cd or Pb in cord blood. Similarly, Galicia-Garcia et al. (1997) reported that previous smoking habits of the mother increased the concentration of Cd in maternal blood, but not in cord blood or venous blood of the newborn.

In our study, minor influential factors on the Pb concentrations in cord blood were age of the mother and season of birth. For Cd, the season of birth was also an explanatory variable. Delivering in the summer caused somewhat higher metal concentrations compared to winter. Soils in many areas in Flanders are contaminated with Cd.

Consumption of locally produced food in spring and summer, might be an explanation for the higher summer concentrations. Seasonal variations in blood Pb levels have been observed in venous blood of adults and children (Farias et al., 1996; Laidlaw et al., 2005). In venous blood of 513 pregnant women of Mexico City (where climate and nutrition are quite different from Belgium): higher levels were measured during fall and winter, compared to spring and summer (Farias et al., 1996).

BMI before pregnancy was a weak ($R^2 = 0.02$) negative determinant of PCBs in cord blood of the Flemish newborns. This dilution of OC levels via increased weight was reported by Herbstman et al. (2007) analyzing 297 cord blood samples. An inverse association between p,p′-DDE and BMI was also observed in studies analyzing maternal blood or human milk (Laden et al., 1999; Sarcinelli et al., 2003; Schildkraut et al., 1999; Torres-Arreola et al., 1999). At the time of delivery, mothers were mostly about 6–8 h without consumption of food. After fasting (meaning that there are no triglycerides of recent food consumption in the serum/plasma), the concentration of OCs in serum/plasma lipids give a good reflection of the concentration in the fat tissue of the body (Schisterman et al., 2005). Indeed, we observed that the OC – present in plasma lipids – if expressed per wet weight (ng/mL), were positively associated with the plasma lipid concentration.

As observed in other studies, one of the most important factors influencing the levels of OCs, was age of the mother (Bjerregaard and Hansen, 2000; Covaci et al., 2002; Huisman et al., 1995; Laden et al., 1999). Our cohort included women from 18 to 44, with an average of 29 years old. OCs do not only accumulate with age, older mothers were also exposed to higher levels, since the levels of the measured OCs declined in time.

Area of residence explained most of the variation of the markers, especially for the OCs (between 10 and 22%). The influence of area of living is reported in many other studies (Bjerregaard and Hansen, 2000; Butler Walker et al., 2006; Butler Walker et al., 2003; Laden et al., 1999; Lagerkvist et al., 1996) It was not expected that the residents of the rural areas would show the highest levels of OCs in cord blood (Fig. 2). These rural areas were selected in our campaign because of what we considered 'low pollution pressure' i.e. low density of population (<250 inhabitants/km^2) less than 5% of the area occupied by industry, no registered pollution sources, no major highways. The main route of intake of these OCs is supposed to be consumption of animal/fish products (Fries, 1995; Liem et al., 2000; Parzefall, 2002). However, intake of animal fat-containing food items showed little variation across the participating mothers (P_{25}–P_{75} = 22 to 42 gram animal + fish fat per day) and did therefore not explain the higher cord blood levels of OCs in participants from the rural area. OCs accumulate in body fat over many years. The food frequency questionnaire focussed on the dietary intake of the year before pregnancy, which is a relatively short period compared to the age of the participants. Huisman et al. (1995) indicated this as one of the reasons why food intake explained only a small fraction of the variance of cord blood PCB and dioxin levels in 355 women. Recently, Glynn et al. (2007) found OC levels measured in Swedish pregnant women, were indeed influenced by fatty fish consumption years before pregnancy, namely during adolescence. We noticed that significantly more residents from rural areas reported consumption of local diary products (25%) and of locally produced animal fat (41%) compared with the whole population (respectively 13% and 17%). Table 3 indicates a weak but significant positive relationship between consumption of local diary/animal fat with levels of PCBs, pp′-DDE and CALUX-TEQ in cord blood. We have no systematic information on OC levels in regional food samples. But studies on free range chicken eggs suggest that concentrations in animal fat may be high (Schoeters and Hoogenboom, 2006).

5. Conclusion

The present campaign showed that in cord blood levels of widespread environmental pollutants like PCBs, pp′-DDE, HCB, Pb

were comparable with available European data. Only the Cd levels tended to be in the higher range compared to other European studies. The individual variability of all pollutant levels was high. The main determinants for the levels of OCs present in cord blood were area of residence and age of the mother. Obviously area of residence is a broad 'umbrella', that covers differences in: habits, consumption, of locally grown foods, degree of urbanization, way of housing, traffic density, etc. In conclusion, even in a small territory as Flanders, the place of birth determines the load of pollutants at the start of life. This underlines the validity of human biomonitoring on (relatively) small geographical scale.

Acknowledgments

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