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Pollutant concentrations in placenta

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ABSTRACT

Unborn children are exposed to environmental pollutants via the placenta, and there is a causal relationship between maternal intake of pollutants and fetal exposure. Placental examination is an effective way for acquiring data for estimating fetal exposure. We analyzed the concentrations of 104 congeners of persistent organic pollutants, seven organotin compounds, five heavy metals, and methylmercury in 130 randomly selected placentas. Additionally, we examined similarities between pollutant concentrations by analyzing correlations between their placental concentrations. Our results yield new information for conducting contaminant risk assessments for the prenatal period. Out of the 117 individual persistent organic pollutants or metals assayed, 46 could be detected in more than half of the placentas. Moreover, dichlorodiphenyldichloroethylene (*p,p′*-DDE) was found in all placentas. The data indicates that fetal exposure to dioxins and furans (PCDD/Fs), polychlorinated biphenyls (PCBs), *p,p′*-DDE, and methylmercury depends on the mother's parity, and age. We also conclude that sources of the above four pollutants are similar but differ from the sources of polybrominated diphenyl ethers.

1. Introduction

There is plenty of information available on human exposures to various pollutants, and several agencies have established oral reference doses and intake guidelines for pollutants. However, less is known about fetal exposure; this information is available for only a limited number of substances. The contamination of the placenta with environmental pollutants provides an indicator of exposures for both mother and fetus/child later in life. This study brings new information for estimating fetal exposure by presenting a broad spectrum of pollutant concentrations measured in placenta, and calculating correlations between pollutants in placenta for several pollutants known to be toxic, specifically during the prenatal period (e.g. dioxins and methylmercury).

The placenta supplies the fetus with oxygen and nutrients, however, many xenobiotics can be transferred through placenta to the fetal circulation (Lanting et al., 1998). Therefore, placenta can be used as an indicator of both maternal and fetal exposure. Many pollutants that were found in maternal blood are prohibited for use. Due to their persistence, they are still present in the environment, and pose a threat to the child, especially if exposure takes place during pregnancy (Meijer et al., 2008). Pollutants accumulate in fetal organs such as blood, spleen, bone-marrow, brain, and liver. The exact distribution of the pollutants in the organs is a complex toxicological process that depends on several factors, e.g. their lipophilicity and molecular size (Giaginis et al., 2009).

An extensive panel of contaminants were analyzed in placental samples, including 17 polychlorinated dibenzo-*p*-dioxins and

Abbreviations: As, arsenic; BMI, body mass index; DDE, dichlorodiphenyldichloroethylene; DDT, 1,1-bis-(4-chlorophenyl)-2,2,2-trichloroethane; EU, European Union; FFQ, food frequency questionnaire; FCDB, food composition database; GC–MS, gas chromatography–mass spectrometry; Hg, mercury; HIV, human immunodeficiency virus; IARC, International Agency of Research on Cancer; KOH, potassium hydroxide; LOD, limit of detection; LOQ, limit of quantification; MBT, DBT, TBT, MPT, DPT and TPT, different organotin compounds; MeHg, methylmercury; OT, organotin; OTC, organotin compound; Pb, lead; PBB, polybrominated biphenyls; PBDE, polybrominated diphenylethers; PCB, polychlorinated biphenyls; PCDD/F, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; PCN, polychlorinated naphtalenes; POP, persistant organic pollutant; RfD, reference dose; RoHS, Restriction of Hazardous Substances Directive; TEF, toxic equivalency factors; TEQ, toxic Equivalent Quotient; TCDD, tetrachlorodibenzo-*p*-dioxin; US EPA, United States Environmental Pollution Agency; WHO, World Health Organization.

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dibenzofuran (PCDD/F) congeners, 37 polychlorinated biphenyl (PCB) congeners, 16 polybrominated diphenylether (PBDE) congeners, 14 polychlorinated naphthalene (PCN) congeners, 19 polybrominated biphenyl (PBB) congeners, *p,p′*-DDE, seven organotin (OTC) compounds, five heavy metals (Se, As, Cd, Hg, and Pb), and methylmercury (MeHg). Altogether 117 individual compounds were measured. In the following paragraphs we will summarize the reasons these particular pollutants were chosen by introducing some of their physio-chemical and toxicological characteristics, and environmental fate of the pollutant groups.

The goal of the present study was to produce placental concentration data for an extensive number of environmental pollutants relevant to human health. We also calculated correlations between the pollutants to identify quantitative associations between the pollutants. The concentration data and the correlations, provide valuable information for further studies on pollutants' effects on children. Pollutants analyzed are introduced in the following sections.

1.1. Persistent organic pollutants

The Baltic Sea has been a hot spot for several persistent organic pollutants (POPs) for decades, and monitoring of these substances is important. Luckily, pollutant concentrations in fish have been declining (Bignert et al., 1998) but the decline has recently leveled off (Kiviranta et al., 2003, 2005; Karl et al., 2010). PCDD/Fs are perhaps the best known group of environmental pollutants due to their very high toxicity. The dioxin cancer risk is currently under debate (Agency of Research for Cancer, IARC), (Tuomisto et al., 2006; Kogevinas, 2001). The main concerns of exposure to PCDD/F are developmental and reproductional disorders, immune function, and diabetes (The National Academy Press, 2006). The complete list of dioxin congeners examined is presented in Table 3. PCBs share many of the same toxicological features with PCDD/Fs because of their structural similarity with dioxins and furans. Epidemiological studies have reported that PCBs have adverse effects on neurological performance and cognitive development in children 6–11 years of age (Aoki, 2001; Boersma and Lanting, 2000; Chen et al., 1992; Jacobson and Jacobson, 1996; Stewart et al., 2008; Vreugdenhil et al., 2002). Evidence for carcinogenicity is inconclusive (IARC, 2007; US EPA, 2008). The complete list of PCB congeners measured in this study can be seen in Table 4.

Table 1
The distribution (%) of mothers by the number of births and mean (SD) maternal age and the body mass index (BMI).

Number of births	N (%)	Age (years)	BMI (kg/m^2)
1	59 (46)	27.9 (4.1)	24.8 (4.7)
2	39 (30)	30.6 (4.4)	26.8 (5.5)
3 or more	31 (24)	32.5 (4.9)	24.8 (5.3)
All	129 (100)	29.8 (4.8)	25.4 (5.2)

The wide use of PBDEs as flame retardants in consumer products has led to their accumulation in food, human blood, breast milk and fat tissues (Schecter et al., 2003; Kiviranta et al., 2004). Moreover, PBDEs are potential endocrine disrupters and neurodevelopmental toxicants (de Wit, 2002; Darnerud, 2003; Main et al., 2007; US EPA, 2008). Consequently, the EU has completely banned the use of the so called penta-mixtures since 2004 (European Commission, 2003), and the manufacture of the most potent PBDE congeners have been banned or restricted in several countries. The list of PBDE congeners is presented in Table 5. PCNs are well known to exhibit health effects similar to dioxins and PCBs (Flinn and Jarvik, 1936; Brack et al., 2003; NICNAS, 2002), such as severe skin rashes and liver disease that can lead in the most severe cases to death. Cancer risk evidence of PCNs is inconclusive (Ward et al., 1994). A list of PCN compounds with additional information is presented in Table 6. PBBs (congeners presented in Table 7) are structurally very similar to PCBs, and the clearest evidence for their health effects is their ability to cause skin problems, such as acne (McDonald, 2002) *p,p′*-DDE is a common breakdown product of DDT (1,1-bis-(4-chlorophenyl)-2,2,2-trichloroethane). It is a reproductive toxin in certain bird species (Bowerman et al., 1995). Human endpoints of concern are breast cancer (Crinnion, 2009), Alzheimer's and Parkinson's disease, asthma, immune deficiency, and type 2 diabetes (Crinnion, 2009; Codru et al., 2007; Sunyer et al., 2006). Organotin compounds, typically found in aquatic ecosystems, have been related to endocrine-disrupting effects and acute toxicity after dermal or oral exposure (Fromme et al., 2005). These compounds are presented in Table 8.

1.2. Heavy metals

We examined concentrations of five heavy metals in placenta, i.e. mercury (Hg), selenium (Se), arsenic (As), lead (Pb), cadmium (Cd), and additionally one heavy metal compound, methylmercury (MeHg). Elemental mercury is an ingredient used in dental amalgams, and is also it is present as a preservative in vaccines. The United States Clean Air Act, passed in 1990, placed Hg on a list of toxic pollutants that need to be controlled to the greatest possible extent due to potential adverse human health effects, such as severe central nervous system disturbance (e.g. psychotic reactions characterized by delirium, hallucinations, and suicidal tendencies. tremors, impaired cognitive skills, and sleep disturbance). Occupational exposure to Hg has resulted in a wide range of functional disturbances, including erethism, irritability, excitability, excessive shyness, and insomnia (Liang et al., 1993; Ngim et al., 1992). Se is an essential trace element but toxic if taken in excess (e.g. impaired immune system, diabetes). High exposure to As can damage the human immune system and cause cancer (Li et al., 2011). There is great geographical variation in As concentrations in ground water, and over 40 major arsenic incidents have been reported in Finland (Kurttio et al., 1999). Additionally, a large number of private wells may contain hazardous levels in As-rich areas (Kurttio

Table 2
The results of analytical quality assurance.

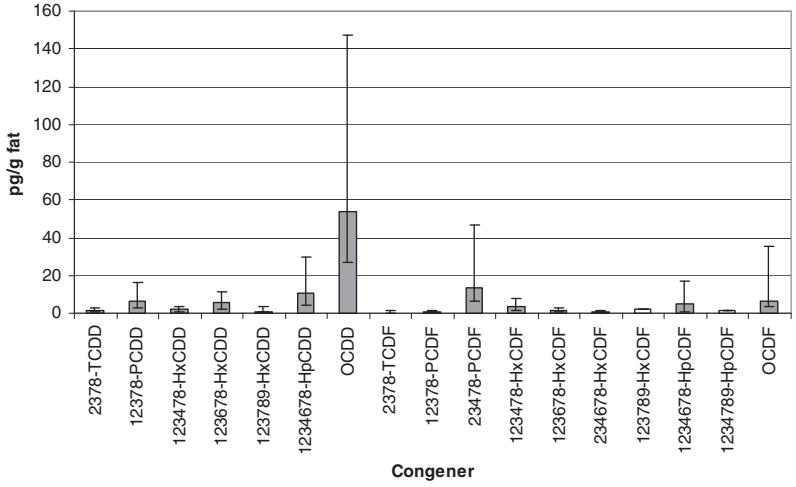
Quality assurance	Unit	Mercury	Selenium	Arsenic	Lead	Cadmium
Isotope	*m/z*	202	78	75	208	111
RM; certified 95% confidence interval	ng/g	7.4–8.2	113–133	11.9–14.5	372–414	5.6–6.4
RM found mean	ng/g	6.6	136	16.0	353	5.7
RM found	ng/g	4.1	6.3	3.4	28	5.6
Number of determinations		10	10	10	10	10
Limit of detection	ng/g	0.7	1.0	0.3	9.0	0.3
Repeatability, relative s_r	%	8	5	7	n.d.*	3

s_r = repeatability standard deviation.
* Pb concentrations in placentaes too low for determination of s_r.

Table 3
Median concentrations of PCDD/F with 90% confidence interval.

PCDD/Fs	pg/g fat	LOQ	Number > LOQ	5th	50th	95th
TCDD	2378-TCDD	0.47	117	<LOQ	1.22	3.16
PCDD	12378-PCDD	0.68	130	2.83	6.66	16
HxCDD_1	123478-HxCDD	0.94	109	<LOQ	1.82	3.58
HxCDD_2	123678-HxCDD	0.92	129	2.27	5.48	11.1
HxCDD_3	123789-HxCDD	0.9	70	<LOQ	1.04	3.23
HpCDD	1234678-HpCDD	1	130	4.59	10.3	29.7
OCDD	OCDD	4.3	130	26.6	53.8	147
TCDF	2378-TCDF	0.32	54	<LOQ	<LOQ	1.43
PCDF_1	12378-PCDF	0.4	46	<LOQ	<LOQ	1.2
PCDF_2	23478-PCDF	0.4	130	6.08	13.6	47
HxCDF_1	123478-HxCDF	0.6	129	1.68	3.39	8.1
HxCDF_2	123678-HxCDF	0.51	118	<LOQ	1.09	2.51
HxCDF_3	234678-HxCDF	0.8	14	<LOQ	<LOQ	1.15
HxCDF_4	123789-HxCDF	2	0	<LOQ	<LOQ	<LOQ
HpCDF_1	1234678-HpCDF	0.42	128	0.704	5.18	16.8
HpCDF_2	1234789-HpCDF	1.5	0	<LOQ	<LOQ	<LOQ
OCDF	OCDF	3.8	84	<LOQ	6.24	35.6
	Sum of all 17 PCDD/F, pg/g fat		130	57	120	256
	$WHO_{PCDD/F}$-TEQ_{1998}, pg/g fat		130	6.93	16.9	44.2

LOQ = Limit of quantification



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et al., 1999). Pb is a well-known toxin, and it affects nearly every organ and system in the human body, causing death after extremely high exposures. Thanks to successful regulations (e.g. it was banned from petrol in many countries at the end of the 20th century), current environmental concentrations are much lower than they used to be. The main health concerns relate to the damage to the nervous system of young children, and its property to cause blood and brain disorders. The main exposure routes of Cd are inhalation and ingestion, and tobacco smoke is the most important single source of Cd exposure. High Cd exposures may lead to chemical pneumonitis, pulmonary edema, cancer, and even death (Ninth Report on Carcinogens, 2000; Hayes, 2007).

MeHg is formed from inorganic mercury by anaerobic organisms inhabiting aquatic systems; lakes, rivers, ocean, sediments, and soils (Ullrich et al., 2001). In Finland, predatory fish species such as pike, pike-perch, and perch, usually contain the highest concentrations of MeHg (Björnberg et al., 2005). In fish MeHg binds to proteins and is thus significantly distributed into edible parts (fillet) of the fish. After being ingested, MeHg is readily absorbed and distributed throughout the human body and it easily crosses the placenta and consequently accumulates in the fetus. Typically, MeHg concentration in fetal blood is equal or higher than the maternal blood concentration (Iyengar and Rapp, 2001). Following *in utero* exposure, the nervous system and fetal brain are the principal target tissues for the health effects of MeHg.

2. Methods

2.1. Study subjects

This study was carried out in Kuopio University Hospital in Finland. Placentas were collected for studies called LUKAS-1 and LUKAS-2 (Opasnet, 2010a,b). Originally, LUKAS-1 was the Finnish cohort of the PASTURE (von Mutius and Schmid, 2006) study, an EU-funded birth cohort study in Europe ("Protection against Allergy – Study in Rural Environments", PASTURE). All children in the LUKAS-2 study were born during May 2004–May 2005. Inclusion criteria included singleton pregnancy; birth in the Kuopio University Hospital; no other siblings in the same study; gestational age >35 weeks at delivery; mother's native language Finnish; and family has no plans to move away from Kuopio area.

A total of 130 placentas were randomly selected from the study cohort for the further analysis. Descriptive background data of age and BMI for all participating mothers (except for one mother with missing information) in parity groups are described in Table 1.

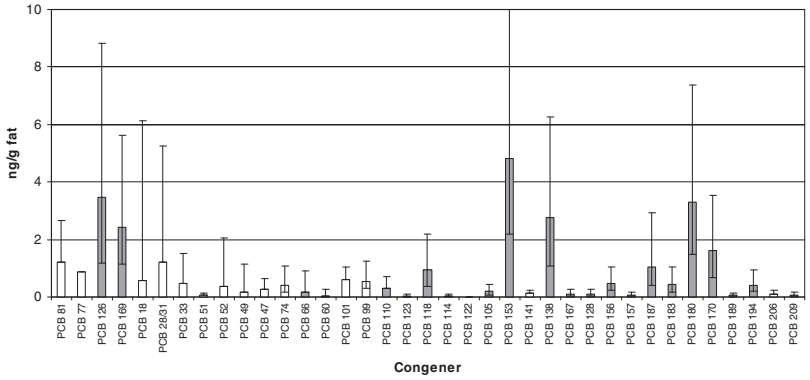
2.2. Preparation of placentas for analysis of POPs and heavy metals

An extensive scheme of contaminant analyses was performed on the placental samples including: 17 congeners of PCDD/F, 37 congeners of PCB, 16 congeners of PBDE, 14 congeners of PCN, 19

Table 4
Median concentrations of PCBs with 90% confidence interval.

PCBs	LOQ	Number > LOQ	5th	50th	95th
PCB 81, pg/g fat	1.2	15	<LOQ	<LOQ	2.66
PCB 77, pg/g fat	0.86	52	<LOQ	<LOQ	53.6
PCB 126, pg/g fat	0.45	130	1.17	3.48	8.83
PCB 169, pg/g fat	0.34	130	1.16	2.42	5.63
PCB 18, ng/g fat	0.56	30	<LOQ	<LOQ	6.13
PCB 28/31, ng/g fat	1.2	21	<LOQ	<LOQ	5.25
PCB 33, ng/g fat	0.46	21	<LOQ	<LOQ	1.53
PCB 51, ng/g fat	0.051	35	<LOQ	<LOQ	0.14
PCB 52, ng/g fat	0.36	25	<LOQ	<LOQ	2.05
PCB 49, ng/g fat	0.16	26	<LOQ	<LOQ	1.14
PCB 47, ng/g fat	0.28	19	<LOQ	<LOQ	0.63
PCB 74, ng/g fat	0.095	128	0.17	0.39	1.07
PCB 66, ng/g fat	0.17	43	<LOQ	<LOQ	0.92
PCB 60, ng/g fat	0.039	58	<LOQ	<LOQ	0.27
PCB 101, ng/g fat	0.6	21	<LOQ	<LOQ	1.06
PCB 99, ng/g fat	0.064	130	0.29	0.54	1.26
PCB 110, ng/g fat	0.29	28	<LOQ	<LOQ	0.72
PCB 123, ng/g fat	0.011	126	0.014	0.041	0.1
PCB 118, ng/g fat	0.16	129	0.38	0.95	2.2
PCB 114, ng/g fat	0.012	129	0.021	0.046	0.093
PCB 122, ng/g fat	0.012	0	<LOQ	<LOQ	<LOQ
PCB 105, ng/g fat	0.03	128	0.074	0.19	0.45
PCB 153, ng/g fat	0.53	129	2.2	4.81	10.9
PCB 141, ng/g fat	0.12	18	<LOQ	<LOQ	0.22
PCB 138, ng/g fat	0.4	129	1.08	2.76	6.27
PCB 167, ng/g fat	0.013	129	0.038	0.1	0.27
PCB 128, ng/g fat	0.042	84	<LOQ	0.1	0.27
PCB 156, ng/g fat	0.028	130	0.23	0.48	1.05
PCB 157, ng/g fat	0.013	129	0.036	0.075	0.17
PCB 187, ng/g fat	0.1	129	0.4	1.04	2.92
PCB 183, ng/g fat	0.065	129	0.17	0.43	1.06
PCB 180, ng/g fat	0.14	130	1.48	3.31	7.38
PCB 170, ng/g fat	0.077	130	0.69	1.63	3.52
PCB 189, ng/g fat	0.013	130	0.026	0.056	0.12
PCB 194, ng/g fat	0.027	130	0.2	0.4	0.94
PCB 206, ng/g fat	0.095	26	<LOQ	<LOQ	0.22
PCB 209, ng/g fat	0.014	130	0.024	0.065	0.18
Sum of all 37 PCB, ng/g fat		130	8.67	20.2	48.5
WHO_{PCB}-TEQ_{1998}, pg/g fat		130	0.36	0.8	1.7

LOQ = Limit of quantification



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congeners of PBB, *p,p′*-DDE, seven OTC, five heavy metals (Se, As, Cd, Hg, and Pb), and MeHg were measured.

Toxic equivalents (TEQ) for PCDD/Fs and PCBs were calculated with the set of toxic equivalency factors (TEF) proposed by the WHO in 1998 (van den Berg et al., 1998).

Before analyses, placentas were homogenized, and subsamples for POP (75 g), OTC (3 g), and heavy metal (10 g) analyses were sampled. Subsamples for heavy metal analyses were delivered to the Technical University of Denmark (DTU). Placental subsamples for POPs and OTCs were freeze dried before extraction. The fat content of placentas were determined separately from the same placental homogenates.

2.3. Pollutant analyses

2.3.1. Analysis of POPs

The placenta samples were freeze dried to remove water, to prevent bias in sample analysis. A freeze dried placental sample was pulverized in a mortar and spiked with a set of 13C-labeled internal standards (sixteen 2,3,7,8-chlorinated PCDD/F congeners; 20 PCB congeners, PCB 30 [12C-labeled], 28, 52, 77, 80, 81, 101, 105, 118, 123, 126, 138, 153, 156, 157, 169, 170, 180, 194, and PCB 209; nine PBDE congeners, BDE 28, 47, 77, 99, 100, 153, 154, 183, and BDE 209; seven PCN congeners, PCN 27, 42, 52, 64, 67, 73, and PCN 75; two PBB congeners, PBB 52 and PBB 153; and *p,p′*-DDE).

Table 5
Median concentrations of PBDE congeners with 90% confidence interval.

PBDEs	LOQ	Number > LOQ	5th	50th	95th
BDE 28, ng/g fat	0.012	111	<LOQ	0.039	0.16
BDE 75, ng/g fat	0.0035	2	<LOQ	<LOQ	<LOQ
BDE 71, ng/g fat	0.0045	2	<LOQ	<LOQ	<LOQ
BDE 47, ng/g fat	0.058	130	0.19	0.39	2.2
BDE 66, ng/g fat	0.0046	118	<LOQ	0.0099	0.033
BDE 77, ng/g fat	0.0035	3	<LOQ	<LOQ	<LOQ
BDE 100, ng/g fat	0.012	129	0.028	0.082	0.52
BDE 119, ng/g fat	0.01	1	<LOQ	<LOQ	<LOQ
BDE 99, ng/g fat	0.041	102	<LOQ	0.1	0.91
BDE 85, ng/g fat	0.015	33	<LOQ	<LOQ	0.066
BDE 154, ng/g fat	0.031	65	<LOQ	<LOQ	0.25
BDE 153, ng/g fat	0.051	122	<LOQ	0.19	0.65
BDE 138, ng/g fat	0.065	2	<LOQ	<LOQ	<LOQ
BDE 183, ng/g fat	0.16	0	<LOQ	<LOQ	<LOQ
BDE 190, ng/g fat	0.8	0	<LOQ	<LOQ	<LOQ
Sum of 15 PBDE, ng/g fat		130	0.42	0.89	4.75
BDE 209, ng/g fat	0.92	23	<LOQ	<LOQ	<LOQ
Sum of 15 PBDE + BDE 209, ng/g fat		130	0.44	1.04	6.75

LOQ = Limit of quantification

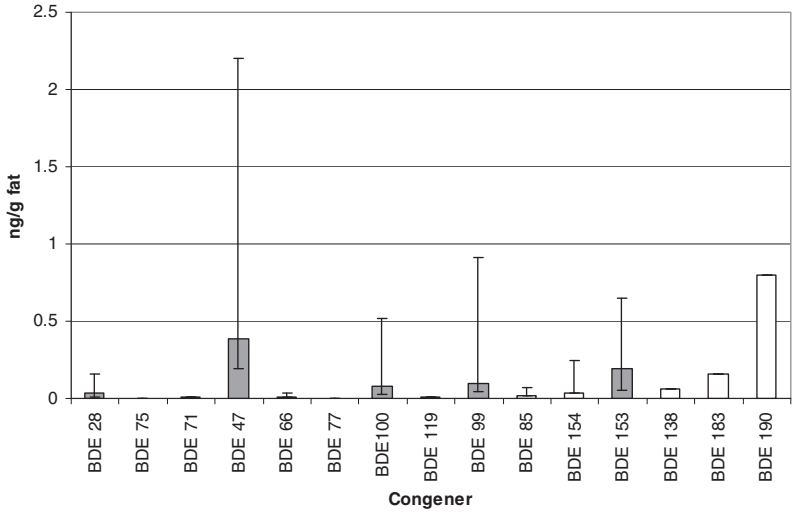

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Table 6
Limits of quantification (LOQ), number of placentas with concentrations >LOQ, congener specific PCN concentrations with 5th, 50th, and 95th percentiles, and sum concentrations of PCNs.

PCNs	LOQ	Number > LOQ	5th	50th	95th
PCN 42, ng/g fat	0.052	13	<LOQ	<LOQ	0.072
PCN 36, ng/g fat	0.011	25	<LOQ	<LOQ	0.023
PCN 27, ng/g fat	0.012	11	<LOQ	<LOQ	0.017
PCN 48, ng/g fat	0.27	0	<LOQ	<LOQ	<LOQ
PCN 52, ng/g fat	0.0045	57	<LOQ	<LOQ	0.0097
PCN 54, ng/g fat	0.00054	26	<LOQ	<LOQ	0.0073
PCN 53, ng/g fat	0.031	15	<LOQ	<LOQ	0.049
PCN 66/67, ng/g fat	0.0025	126	0.0044	0.01	0.022
PCN 68, ng/g fat	0.0025	1	<LOQ	<LOQ	<LOQ
PCN 71/72, ng/g fat	0.003	0	<LOQ	<LOQ	<LOQ
PCN 70, ng/g fat	0.003	0	<LOQ	<LOQ	<LOQ
PCN 73, ng/g fat	0.0084	0	<LOQ	<LOQ	<LOQ
PCN 74, ng/g fat	0.00099	0	<LOQ	<LOQ	<LOQ
PCN 75, ng/g fat	0.013	0	<LOQ	<LOQ	<LOQ
Sum of 14 PCN, ng/g fat		129	0.0052	0.014	0.14

LOQ = Limit of quantification.

The sample was extracted with a mixture of 15% ethanol in toluene for 2 h using the Twisselman apparatus. After extraction, the solvent was exchanged into hexane (40 ml) and 12 ml of sulfuric acid (H_2SO_4) was added in order to remove the fat from the sample. Hexane was then removed from the top of the sulfuric acid and 3 g of silica and 3 ml of new sulfuric acid were added to the hexane. The sample was placed on top of a silica gel column containing acidic and neutral layers of silica, and all target analytes were eluted with 200 ml of hexane. The aliquot of hexane was then evaporated to a volume of 1 ml using nonane as a keeper and the

Table 7
Median concentrations of PBB congeners with 90% confidence interval.

PBBs	LOQ	Number > LOQ	5th	50th	95th
PBB-18, ng/g fat	0.007	0	<LOQ	<LOQ	<LOQ
PBB-29, ng/g fat	0.0026	0	<LOQ	<LOQ	<LOQ
PBB-31, ng/g fat	0.0026	0	<LOQ	<LOQ	<LOQ
PBB-22, ng/g fat	0.0026	0	<LOQ	<LOQ	<LOQ
PBB-38, ng/g fat	0.0026	0	<LOQ	<LOQ	<LOQ
PBB-37, ng/g fat	0.0026	0	<LOQ	<LOQ	<LOQ
PBB-53, ng/g fat	0.007	0	<LOQ	<LOQ	<LOQ
PBB-52, ng/g fat	0.0081	0	<LOQ	<LOQ	<LOQ
PBB-49, ng/g fat	0.008	1	<LOQ	<LOQ	<LOQ
PBB-75, ng/g fat	0.0037	0	<LOQ	<LOQ	<LOQ
PBB-80, ng/g fat	0.0043	0	<LOQ	<LOQ	<LOQ
PBB-56, ng/g fat	0.0045	0	<LOQ	<LOQ	<LOQ
PBB-77, ng/g fat	0.005	0	<LOQ	<LOQ	<LOQ
PBB-103, ng/g fat	0.017	0	<LOQ	<LOQ	<LOQ
PBB-101, ng/g fat	0.017	0	<LOQ	<LOQ	<LOQ
PBB-155, ng/g fat	0.035	1	<LOQ	<LOQ	<LOQ
PBB-154, ng/g fat	0.043	0	<LOQ	<LOQ	<LOQ
PBB-153, ng/g fat	0.047	6	<LOQ	<LOQ	0.021
PBB-169, ng/g fat	0.17	0	<LOQ	<LOQ	<LOQ
Sum of 19 PBB, ng/g fat		7	<LOQ	<LOQ	0.038

LOQ = Limit of quantification.

Table 8
The Spearman correlation coefficients and regression model result between mother's age and contaminant concentration for the primipara mothers ($N = 59$) in BENERIS project.

Congener	R	R^2
2378-TCDD	0.579	0.356
12378-PeCDD	0.723	0.523
23478-PeCDF	0.615	0.378
WHO-PCDD/F-TEQ	0.671	0.451
PCB 126	0.524	0.274
PCB 153	0.622	0.387
WHO-PCB-TEQ	0.611	0.374
BDE 47	0.031	0.001
BDE 153	0.086	0.007
Sum of BDEs	0.077	0.006
p,p′-DDE	0.346	0.119
MeHg	0.071	0.005

sample was pipetted on top of an alumina column. Sample impurities were eluted with 2 ml of hexane (this eluent was kept until the analyses were finalized). A second, activated carbon column, was then placed below the alumina column and elution of the sample through this series of two columns was continued with 6 ml of 20% dichloromethane in hexane. This fraction included mono- and di-ortho-PCBs, PBDEs, PBBs, and *p,p′*-DDE. The carbon column on its own was then back eluted with 15 ml of toluene in order to elute PCDD/Fs, non-ortho-PCBs, and PCNs.

The first fraction, i.e. dichloromethane/hexane, was evaporated, until dry, under a gentle stream of nitrogen in an autosampler vial using nonane as a keeper and recovery standards were added (PCB 159 for mono- and di-ortho-PCBs, PBBs, and *p,p′*-DDE; and 13C-BDE 126 for PBDEs). The final volume in the vial was adjusted to 500 μl with hexane.

Toluene from the second fraction was evaporated, until dry, with N_2 using nonane as a keeper and transferred in hexane to an autosampler vial with an inserted liner. After addition of recovery standards (13C-1,2,3,4-TCDD and 13C-1,2,3,7,8,9-HxCDD for PCDD/Fs; 13C-PCB 60 for non-ortho-PCBs; and PCB 159 for PCNs) hexane was evaporated, until dry, and the final volume of sample was adjusted to 20 μl with nonane.

The quantification was performed by selective ion recording using VG-70 250 SE and Autospec Ultima (both from Waters) high resolution mass spectrometers (HRMS) (resolution 8000) equipped with Agilent HP 6890 gas chromatographs. The two most intensive ions of the molecular ion microliters of patterns were used for the quantification of the analytes. Two samples were injected into a split–splitless injector.

Limits of quantifications (LOQ) are presented later in result tables. Recoveries for internal standards were more than 50% for all congeners. Concentrations were calculated with the lower bound method, where the results of congeners with concentrations below LOQ were designated as nil.

2.3.2. Analysis of organotin compounds

All weights and concentrations of OTCs are expressed as organotin cations. Perdeuterated analogs of MBT, DBT, TBT, MPT, DPT, and TPT were used as internal standards for the respective native 1H-compounds. Perdeuterated DPT was used as an internal standard for DOT. 1 g of solid natrium chloride and internal standards were added to the freeze dried placenta samples (0.25 g) in 12 ml screw capped vials. Samples were lyophilized by sonicating for 1 h in 5 ml of 25% tetramethylammoniun hydroxide and after sonication acidified with 2 ml of glacial acetic acid. OTCs were then extracted twice with 3.5 ml of 0.02% tropolone in ether/hexane (8:2 v/v). The organic phase was separated and OTCs ethylated with 1.5 ml of 1% sodiumtetraethylborate. To clean up the derivatized extract, the organic phase was first washed with 2 ml of 2 M KOH. The organic phase was then evaporated to a volume of about 1 ml of hexane. The small amount of water remaining at in the end of evaporation process was discarded. Some sodium sulfate was added, and hexane phase containing the ethylated OTCs were transferred to a Pasteur pipette containing 3 cm of aluminum oxide. Ethylated OTCs were eluted from the column with 15 ml of 6% diethylether in hexane. The cleaned sample was evaporated under a gentle stream of nitrogen to a volume of 0.5 ml, and transferred to an autosampler vial for GC–MS analysis.

The GC–MS analysis was performed with an Agilent HP 6890 Gas Chromatograph connected to Waters Autospec Ultima high resolution mass spectrometer operated in the selected ion recording mode. The two most intensive fragment ions of each ethylated OTC were monitored. Two were injected into a split–splitless injector. The temperature program of the GC for OTC was:

start, 50 °C (1 min), rate 15 °C/min to 245 °C (0 min), rate 40 °C/min to 300 °C (1 min).

LOQs for OTCs were from 0.1 ng/g fw to 0.5 °C/min to 300°40 ng/g fw for butyltins and phenyltins and 1.1 ng/g fw for DOT. Recoveries for internal standards were more than 50% for all OTCs.

2.3.3. Determination of fat content

Hydrochloric acid, HCl, (35 ml) was mixed thoroughly with 15 g of homogenate. The mixture was placed in a water bath (70 °C) with stirring while the temperature was raised to 100 °C. After heating, the sample was allowed to cool to room temperature, and 80 ml of purified water (MilliQ, Millipore, USA) was added. The extraction of fat was initiated by shaking with diethyl ether (100 ml) and followed by addition of hexane (100 ml) in a separation funnel. The organic phase was removed, and the extraction was repeated two times after which all the organic phases were combined and washed with purified water. The water phase was discarded, and the organic phase was dried overnight by adding 100 g of sodium sulfate (Na_2SO_4). After drying, the organic phase was evaporated to a small volume after which a known amount of hexane was added to the sample. The fat content of the placenta was determined gravimetrically from the obtained hexane solution.

2.3.4. Quality control and assurance for POPs and OTCs

The laboratory reagent and equipment blank samples were treated and analyzed with the same method as the actual samples, one blank for every ten samples. These analyses did not reveal substantial background levels of target analytes. The Chemical Exposure Unit of the National Institute for Health and Welfare is an accredited testing laboratory (No. T077) in Finland (current standard: EN ISO/IEC 17025). The scope of accreditation includes POP and OTC from biological samples.

2.3.5. Analysis of heavy metals

Three grams (fresh mass) of sample were wet washed in 4 mL of nitric acid purified by an in house subboil distillation and 0.5 ml of hydrogen peroxide in high-pressure quartz pressure bombs (Multiwave, Anton Paar, Austria). The temperature program involved a ramped input of power starting at room temperature from 100 W and up to 800 W during 5 min. This power input was maintained for an additional 15 min. If the pressure inside the quartz bombs exceeded 70 bar or if the temperature exceeded 300 °C, the power input was automatically shut down to prevent explosions. Following the wet ashing the clear acidic solution was diluted to 20 ml with pure water (MilliQ, Millepore, USA). Prior to quantitative determination by ICP-MS, the 3.2 ml of the solution was further diluted to 8.0 ml of water.

The diluted samples were measured using an ELAN 6100 DRC inductively coupled argon plasma mass spectrometer (ICP-MS) instrument (Perkin Elmer/Sciex, Toronto, Canada) equipped with a dynamic reaction cell (DRC) for removal of argon based interferences. The instrument was run both in conventional and in DRC mode using methane as cell gas, according to the manufacturer's specifications. The instrument was equipped with a Miramist nebulizer (Burgener Research, Canada) using a liquid uptake rate of 1 ml/minute.

The analyses of samples were carried out in batches each comprising of 10 placenta samples (single determinations), two analytical blanks, one double determination (one of the 10 repeated), and one reference material (RM) (Table 2). The RM used was Seronorm Whole Blood, L-2. Art. No.: 201605. Lot No.: 0503109 (Seronorm, Norway). Whole blood RM was used because the target values were close to the expected physiological level in the samples. Hereby the major requirement of matching the analyte level in RM and sample was met.

2.3.6. Analysis of methyl mercury

The placenta samples were homogenized using an Ultra Turrax laboratory blender. A subsample of 0.5 g (fresh weight) was weighed into a 15 ml polyethylene centrifuge tube. Then 15 ng Hg of the enriched $Me^{198}HgCl$ (96.385% pure) was added followed by 3 ml of 25% tetramethylammonium hydroxide solution. The mixture was placed in a rotating wheel overnight at room temperature. The placenta became almost completely dissolved such that equilibrium between the enriched and the natural isotopic composition content of MeHg was achieved. Then 0.6 ml of nitric acid was added together with 0.5 ml of 4 M ammonium acetate buffer in water. Acidity of around pH 5 was checked by indicator paper. Subsequently, 0.4 ml hexane and 0.5 ml of 10% water-free tetraethylborate in methanol were added, and the mixture was rotated for 20 min at room temperature. The mixture was then centrifuged at 4700 rpm at 10 °C to achieve phase separation. About 300 µl of the hexane supernatant was reduced to about 50 µl under a flow of nitrogen in a GC vial. The concentrated hexane was injected into and analyzed by GC–ICP-MS and the chromatographic areas at m/z 198 (enriched isotope) and at m/z 200 (reference isotope) were recorded.

Preparation of the $Me^{198}HgCl$ spike solution was performed by the following procedure. An ampoule of isotopically enriched methylmercury chloride was obtained as a gift from Dr. Zoltan Mester of National Research Council of Canada and its isotopic purity was given as value of 96.4%. The concentration of this solution was determined by ICP-MS following dissolution in 1% nitric acid solution and calibrated against an external standard curve constructed by a certified standard of natural isotopic composition. The slope was corrected by the ratio of the natural and enriched isotopic abundance ratio of 9.97/96.39 = 0.103.

Correction for mass bias was done by measuring MeHg in unspiked placenta. The resulting extract was used repeatedly for the correction, which was carried out before and after each batch of 10 unknown placenta samples. The mean bias value was used for correction of the ratio of GC areas in the unknowns. In this way the influence of the biological matrix on the estimation of the mass bias was taken into account. The mass bias value was (mean and standard deviation, $N = 16$) 1134 ± 0.087.

2.3.7. Quality control and assurance for heavy metals

The CRM was determined in parallel with the unknown samples, and the results obtained for the five elements are in accordance with the certified confidence intervals. The criterion used in the evaluation was that the mean and two times the standard deviation of the analyses overlapped with the confidence intervals. The limits of detection were estimated for the five elements in the samples (fresh mass) on the basis of three times the standard deviation of the blank determinations, and based on a sample size of 3 g and a final volume of ashed residue of 50 ml.

The repeatability (within day uncertainty) was calculated from the standard deviation estimated from double determinations of samples. Only placenta samples with a concentration of at least 10 times the LOD were included in these calculations.

2.3.7.1. Limit of detection for MeHg. The LOD was based on the standard deviation of blanks ($N = 8$) taken through the entire procedure and has been estimated at 0.8 ng Hg/g sample (fresh weight).

2.3.7.2. Precision for MeHg. The within-day repeatability was estimated from double determinations of placentas taken through the entire procedure. Based on their differences, these double determinations translated into a value of s_r of 31% RSD.

2.3.7.3. Accuracy for MeHg. The Seronorm Whole Blood CRM, which is not certified for MeHg, but had a relevant matrix in relation to placenta samples, was analyzed repeatedly ($N = 8$). The mean and standard deviation of the analyses was 1.5 ± 0.8 ng Hg/g as MeHg. The interpretation of this value in relation to accuracy is, however, not possible without any established reference value.

2.3.8. Statistical analysis

Intake distributions and statistical analysis were estimated using C-SIDE® (version 1.0; Department of Statistics, Center for Agricultural and Rural Development – CARD, Iowa State University, Ames), which implements the Nusser method (Nusser et al., 1996).

3. Results

Out of the 117 individual persistent organic congeners or metals, 46 were measured in more than half of the placentas. The most abundant congeners in the placentas were PCDD/Fs of which 12 out of 17 were measured in more than half of the placentas (Table 3). Within the group of PCDD/Fs, the most abundant congeners in placentas were PCDDs, of which all seven measured congeners were present in more than half of the placentas.

Concentrations of PCBs are presented in Table 4. There was a tendency for higher chlorinated congeners to be above the limit

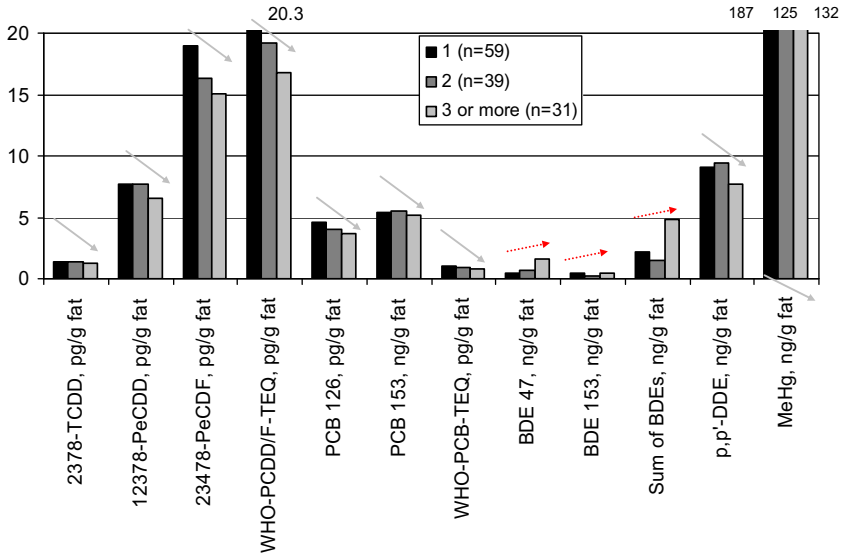

Fig. 1. The mean concentrations of selected congeners in the placentas with increasing parity.

of quantification compared to their less chlorinated counterparts. PCB 122 was the only one with the 95th percentile below the LOQ (0.012 ng/g fat).

Concentrations of PBDEs are presented in Table 5. Congeners present in technical mixtures (so called penta-mixture, in particular) were also found in the placentas. These congeners include BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, and BDE 154. The occurrence of PCNs is shown in Table 6. Concentrations of PBBs in placentas (Table 7) were very low, only one (PBB-153) out of 19 compounds resulted in a sample value above the LOQ level (0.047 ng/g fat). The median concentration of *p,p′* DDE was 7.79 ng/g fat (90% confidence interval 3.58–17.5 ng/g fat). There are several potential explanations for the difference. First, comparing mean and median concentrations causes bias in a way that if there are high individual measurements, the mean concentration is higher than median. Second, variation in fish consumption patterns between the cities of Turku and Kuopio may partly explain the difference. Third, Shen et al. (2007) placentas were analyzed using a slightly different methodology.

A group of individual congeners, representative of a particular pollutant group, out of all the measured ones was selected in order to describe the occurrence of pollutants in placentas and to provide data for the fetal exposure assessment to these compounds. This information can be used for further risk assessment purposes. The selected congeners were: 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 2,3,4,7,8-PCDF, WHO PCDD/F-TEQ, PCB 126, PCB 153, WHO PCB-TEQ, BDE 47, BDE 153, sum of BDEs, *p,p′*-DDE, and MeHg.

Fig. 1 illustrates the concentrations of selected congeners in the different parity groups. A continuing downward trend according to increasing parity, described as descending gray arrows in the figure, was observed with the highly persistent organic pollutants PCDD/F and PCBs. Their concentrations in placentas of the mothers having three or more children were on average 16% lower than in placentas of primipara mothers. With *p,p′*-DDE, the trend was similar though not continuing. Methyl mercury also showed a decreasing trend with increasing parity. However, PBDEs showed a rising trend with parity, described as ascending red arrows in Fig. 1.[1] Importantly, the trends became clear in the third or later parities but not necessarily in the second.

In order to obtain an estimate for the effect of mother's age on the fetal exposure to contaminants, a regression model between mother's age and contaminant concentration for the primipara mothers was calculated. The results are presented in Table 8. Table 9 presents heavy metal concentrations and MeHg in placenta. Se, As, and Cd were measured above LOQ in all samples, and Hg in almost every sample, whereas Pb was observed only in 25% of the analyzed placentas. Table 10 describes the correlation coefficients between the studied pollutants. The Figure shows correlation in three classes, with high correlations between PCDD/Fs, PCBs, and DDE having high correlations and BDEs and MeHg without any correlation with the other pollutants. Organotin concentrations were very low. In fact, after analyzing 73 of the 130 placentas for organotin (OT) compounds, the procedure was terminated due to the failure to find even one placenta containing a concentration above the LOQ (Table 11).

4. Discussion

The human fetus is exposed to a diverse mixture of chemicals. Out of the 117 individual compounds analyzed in this study, 46 were measured in more than half of the placentas. The tested compounds have several special characteristics affecting their concentrations. The most relevant PCDD/F congeners are those with the longest half lives, high Toxic Equivalent Factors (TEF), or substantial dietary exposure. This list includes congeners such as 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 2,3,7,8-PCDF. With respect to the PCB compounds, the higher chlorinated congeners were predominant due to higher bioaccumulation efficiency and longer half lives in humans. Correlations between the higher chlorinated PCB compounds ranged from 0.6 to 0.9 (Table 10), meaning that it would be acceptable to use just a few of them to represent the whole group of PCBs. The two most representative PCB congeners were PCB 126 and PCB 153.

Although manufacturing and the use of the pesticide DDT has been banned in Finland and also in the EU for many years, its metabolite *p,p′*-DDE, can be frequently found in all kinds of biological matrices, even decades after the prohibition came into force. This can be considered as evidence of continuous exposure of the population to this very persistent organic pollutant. Shen et al. (2007) found higher *p,p′* DDE concentrations (mean 21.23 ng/g fat) in Finnish placentas compared with the ones found in the

[1] For interpretation of color in Fig. 1, the reader is referred to the web version of this article.

Table 9
Median concentrations (ng/g placental fresh mass) of metals with 90% confidence interval.

Metals	Limit of detection	Number of samples > LOQ	5th	50th	95th
Selenium	1	130	180	212	260
Arsenic	0.3	130	4.40	5.68	10.0
Cadmium	0.3	130	1.86	3.70	7.13
Mercury	0.4	128	0.76	2.31	5.69
Lead	9	33	9.20	13.1	56.9
MeHg	60	108	<LOQ	130	414

LOQ = Limit of quantification.

Table 10
Spearman correlations coefficients of selected pollutants in placentas of the BENERIS study population BENERIS placentas, and average Spearman correlation coefficients between the pollutants. Dark gray background color represents high correlation (>0.70), light gray color medium correlation ($0.50 < x < 0.70$), and white color low correlation (<0.50).

Congener	2378-TCDD	12378-PeCDD	23478-PeCDF	WHO-$_{PCDD/F}$-TEQ	PCB126	PCB153	WHO-$_{PCB}$-TEQ	BDE47	BDE153	Sum of BDEs	p,p'-DDE	MeHg
2378-TCDD	1	0.85	0.87	0.91	0.66	0.72	0.75	0.09	0.08	0.09	0.56	0.08
12378-PeCDD		1	0.87	0.96	0.67	0.81	0.8	0.03	0.05	0.02	0.65	0.07
23478-PeCDF			1	0.97	0.68	0.8	0.79	-0.03	0.06	0.01	0.61	0.23
WHO-$_{PCDD/F}$-TEQ				1	0.7	0.83	0.82	0	0.07	0.03	0.65	0.15
PCB 126					1	0.71	0.94	0.04	-0.01	0.01	0.63	0.32
PCB 153						1	0.88	0.03	-0.02	-0.02	0.75	0.15
WHO-$_{PCB}$-TEQ							1	0.04	-0.01	0	0.72	0.28
BDE 47								1	0.48	0.83	0	-0.05
BDE 153									1	0.87	-0.01	-0.13
Sum of BDEs										1	-0.03	-0.1
p,p'-DDE											1	0.19
MeHg												1

Table 11
Limits of quantification (LOQ), number of placentas with concentrations >LOQ, compound specific OT concentrations with 5th, 50th, and 95th percentiles, and sum concentrations of OTs.

Organotins	LOQ	Number > LOQ	5th	50th	95th
MBT, ng/g fat	37	2	<LOQ	<LOQ	<LOQ
DBT, ng/g fat	22	1	<LOQ	<LOQ	<LOQ
TBT, ng/g fat	15	1	<LOQ	<LOQ	<LOQ
MPhT, ng/g fat	30	0	<LOQ	<LOQ	<LOQ
DPhT, ng/g fat	15	0	<LOQ	<LOQ	<LOQ
TPhT, ng/g fat	7.5	0	<LOQ	<LOQ	<LOQ
DOT, ng/g fat	82	3	<LOQ	<LOQ	<LOQ
Sum of 7 OTs, ng/g fat		6	<LOQ	<LOQ	280

study (median 7.79 ng/g fat). Conversely, some of the other compounds, such as PBB, exhibited very low concentrations. Manufacturing and use of PCNs and PBBs has been halted/banned for at least two decades in many countries, and this was seen in the low occurrence of PCN (Table 6) and PBBs (Table 7) in placenta further suggesting that fetal exposure to these compounds is not significant. Similarly, organotin concentrations were very low.

PBDEs deviate from the rest of the measured groups. This can be seen in Table 10 where correlations of marker pollutant concentrations are presented. This further suggests that the exposure source of PBDEs is different from the other pollutants, almost certainly not fish, perhaps not even food at all, which is the main source of the other pollutants (EVIRA, 2009). The correlation of MeHg between the other POPs was also weak which can be explained by two facts. First, MeHg has a shorter half life compared to PCDD/F and PCBs. Second, these pollutants are present in different fish species. MeHg is predominantly found in large lean predator fish species (in Finland pike, pike-perch, perch) whereas fatty fish, such as salmon and herring, contain the highest concentrations of PCDD/Fs.

Iyengar and Rapp (2001), Al-Saleh et al. (2010) and Gundacker et al. (2010) have reviewed the concentrations of several heavy metals in placenta, including As, Cd, Hg, and Pb. The cadmium, mercury, and lead concentrations measured in the present study were at the low end of the scale compared to the studies reviewed by Iyengar and Rapp (2001) and Gundacker et al. (2010). The heavy metal and metalloid concentrations in the present study were comparable to those values described in thee study of Ward et al. (1987), with the exception of the arsenic concentration which was approximately one order of magnitude lower than in the present study. The lower arsenic concentration may be explained by the generally lower content of arsenic in seafood collected from brackish waters such as the Baltic Sea (Larsen and Francesconi, 2003). These findings suggest that heavy metals or metalloids are not of greatest concern in Finland. Leung et al. (2010) measured placental PBDE concentrations in women working at an electronic waste recycling site in China. The results can be considered as a high exposure scenario, and concentrations in this study were an order of magnitude lower.

The analysis contains several potential confounding factors. First, exposure to these pollutants might be affected by the parity of the mothers due to lactational elimination of fat soluble contaminants. Second, the age of the mother may have a crucial effect on the exposure of the fetus because almost all of these pollutants have long half lives, and they accumulate in the mother for many years before she becomes pregnant. Third, the source of contaminants has an effect on the exposure.

The most controversial result was obtained with the relationship between PBDEs and parity. It shows that exposure of fetus to PBDEs tended to increase with parity. The standard deviations of PBDEs were wide, indicating skewed distributions. Therefore, PBDEs are different from the other POPs examined in this study. The pollutant groups correlating clearly with mother's age were PCDD/Fs and PCBs, reflecting their long half lives in humans. Age was a rather significant variable for most of the compounds. *p,p′*-DDE showed moderate correlation with age whereas the PBDEs together with MeHg showed a poor correlation with primapara mother's age. Ultimately, risks from pollutant toxicity to the fetus are naturally far greater than the risk to the mother.

Fish is the major source for a number of fat soluble contaminants in Finland. Also, exposure to MeHg is known to be predominantly

attributable to fish consumption. This fact indicates that further studies should be conducted utilizing maternal fish intake data. Our findings suggest that mothers consuming more fish had higher placental pollutant concentrations than the less fish consuming mothers. This was true for all the other compounds with the exception of PBDEs, which showed no elevated concentrations with increasing fish consumption.

Doucet et al. (2009) found that total PBDEs in fetal liver increased over time, whereas placental levels were generally lower, without any clear trend. This suggests that concentrations in placenta do not necessarily reflect fetal concentrations. There are several other factors which need to be controlled, e.g. low maternal weight gain mobilizes lipid soluble pollutants exposing the fetus to an additional burden, while in contrast, a high maternal weight gain seems to protect the fetus (Alexander and Slay, 2002; Windham and Fenster, 2008).

Placental concentrations measured on one occasion only during the pregnancy cannot be considered as completely relevant predictors for evaluating fetal exposure to environmental pollutants. There are several reasons for this statement. First, the placenta itself possesses metabolic potential, and thus fetal exposure and toxicity can be modified on many occasions during the course of pregnancy by placental biotransformation of toxicants. Second, not only the fetus but also the placenta is a target organ for toxicity, which makes analyzing the mother–placenta–fetus complex even more difficult. Furthermore, during the whole pregnancy significant alterations take place in many physiological factors between the mother, placenta and fetus that affect the toxicokinetic behavior of toxicants between these three compartments. Finally, environmental pollutants have biological half-lives that are, to some extent, typical for each compound groups but there are notable congener-specific variations, especially in the PCDD/Fs. This also makes estimation of the fetal body burden a very challenging task. MeHg for instance has a much shorter biological half-life compared to that of PCDD/Fs, PCBs or *p,p′*-DDE. Despite these uncertainties, we do not have any reason to assume that fetal exposure to the detected contaminants would not last the whole pregnancy. Woodruff et al. (2011) analyzed pollutants similar to those in the present study in pregnant women and found that generally levels were similar to or lower than levels in nonpregnant women.

5. Conclusions

The association found between placental concentrations of the analyzed pollutants revealed that there are differences in how pollutants from various compound groups are transferred to or remain in the placenta.

The data indicated that fetal exposure to the persistent organic pollutants PCDD/Fs, PCBs, *p,p′*-DDE, and MeHg was dependent on at least two key variables (1) Parity: primaparas being at highest risk, and (2) Maternal age (higher risk with higher maternal age). Further, the results indicated that the exposure sources for humans are similar for PCDD/Fs, PCBs, *p,p′*-DDE, and MeHg but differ from the sources of the brominated flame retardants, PBDEs.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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