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Deliverable 19:

Contaminants in placenta

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Deliverable 19: Contaminants in placenta

Introduction

With regard to persistent organic and inorganic pollutants, one of the aims in the BENERIS project and in the following work is to establish the association between external dose (intake) and internal dose (concentrations in the body). In BENERIS, this work is initiated by analysing contaminants from placentas and combining the occurrence data thus obtained with the calculated intake data to find any quantitative or qualitative associations. In this deliverable, the placenta concentrations of POPs are described.

Subjects

Placentas used in BENERIS for contaminant analyses have already been collected for another study, called LUKAS-2. This study was carried out in Kuopio University Hospital area in Finland. It is an extension of the LUKAS-1 study. The LUKAS-1 study is a Finnish cohort of the PASTURE study, which is an international EU-funded birth cohort in Europe ("Protection against Allergy – Study in Rural Environments", PASTURE). All children in the LUKAS-2 study were born between May/2004 - May/2005. The inclusion criteria of LUKAS-2 were: family doesn't live in block of flats; one fetus pregnancy; birth in Kuopio University Hospital; no other siblings in the same research; 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 LUKAS-2 cohort for the BENERIS project. Background data of age and BMI for all participating mothers (except for one mother with missing information) in different parity groups are described in Table 1.

Table 1. The number (N) of mothers with their age and BMI by the number of births. Mean (standard deviation).

Births	N (%)	Age	BMI
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	29.8 (4.8)	25.4 (5.2)

Methods

Preparation of placentas for analysis of POPs and heavy metals

An extensive scheme of contaminant analyses was performed with the placenta samples including 17 polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F), 37 polychlorinated biphenyls (PCB), 16 polybrominated diphenylethers (PBDE), 14 polychlorinated naphtalenes (PCN), 19 polybrominated biphenyls (PBB), p,p'-DDE, 7 organotin (OT) compounds, 5 heavy metals (Se, As, Cd, Hg, and Pb), and methyl mercury (MeHg), altogether 117 individual congeners, compounds or elements were measured (see ANNEX 1, table 1 for individual congeners). Organotin compounds and Se, As, Cd, and Pb were outside the scope of the BENERIS-project DoW. Though, the data are available in detailed in the BENERIS data repository.

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

Before analyses a placentas as a whole were homogenized at THL (KTL) and subsamples for POP (75 g), organotins (OT) (3 g), and heavy metal (10 g) analyses were sampled. Subsamples for heavy metal analyses were delivered to BENERIS partner in Copenhagen (DTU). Placenta subsamples for POPs and OTs were freeze dried before extraction.
The fat content of placentas in BENERIS project was determined separately from the same placenta homogenates after a decision in final meeting of BENERIS in Budapest in spring 2008.

Analysis of POPs

A freeze dried placenta sample was pulverized in a mortar and spiked with a set of ^{13}C-labeled internal standards (sixteen 2,3,7,8-chlorinated PCDD/F congeners; 20 PCB congeners, PCB 30 [^{12}C-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).
The sample was extracted with a mixture of 15% ethanol in toluene for 2 hours using the Twisselman apparatus. After extraction the solvent was exchanged into hexane (40 ml) and 12 ml of sulphuric acid (H_2SO_4) was added in order to remove the fat from the sample. Hexane was then removed on top of the sulphuric acid and 3 g of silica and 3 ml of new sulphuric acid were added into 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 volume of hexane was then evaporated into volume of 1 ml using nonane as a keeper and the sample was applied on top of an alumina column. Sample impurities were eluated with 2 ml of hexane (this eluent was kept until the analyses were finalised). 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 alone was then back eluted with 15 ml of toluene in order to elute PCDD/Fs, non-*ortho*-PCBs, and PCNs.
The first, dichloromethane/hexane, fraction was evaporated into dryness 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 ^{13}C-BDE 126 for PBDEs). The final volume in vial was adjusted into 500 ul of hexane.
Toluene from the second fraction was evaporated into dryness using nonane as a keeper and removed with hexane into an autosampler vial with inserted liner. After addition of recovery standards (^{13}C-1,2,3,4-TCDD and ^{13}C-1,2,3,7,8,9-HxCDD for PCDD/Fs; ^{13}C-PCB 60 for non-*ortho*-PCBs; and PCB 159 for PCNs) hexane was evaporated into dryness and the final volume of sample was adjusted into 20 ul of 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 a Agilent HP 6890 gas chromatographs. Two most intensive ions of the molecular ion patterns were used for the quantification of the analytes. Two µl of sample were injected into a split-splitless injector. The temperature programs of the GC for PCDD/Fs, non-*ortho*-PCBs, mono- and di-*ortho*-PCBs, PBDEs, PBBs, PCNs, and p,p'-DDE were:

start, 140°C (4 min), rate 20°C/min to 180°C (0 min), rate 2°C/min to 270°C (36 min);
start, 140°C (4 min), rate 20°C/min to 180°C (0 min), rate 2°C/min to 270°C (36 min);
start, 100°C (3 min), rate 20°C/min to 180°C (0 min), rate 2°C/min to 270°C (5.5 min);
start, 100°C (3 min), rate 25°C/min to 240°C (0 min), rate 4°C/min to 300°C (25 min),

start, 100°C (2 min), rate 25°C/min to 240°C (0 min), rate 4°C/min to 300°C (20 min),
start, 140°C (2 min), rate 20°C/min to 180°C (0 min), rate 4°C/min to 270°C (17.5 min),
start, 100°C (3 min), rate 30°C/min to 170°C (0 min), rate 4°C/min to 240°C (5 min), rate 12°C/min to 270°C (7 min), respectively.

Limits of quantifications (LOQ) are presented in ANNEX 1, table 1. Recoveries for internal standards were more than 50% for all congeners. Concentrations were calculated with lower bound method, where the results of congeners with concentrations below LOQ were designated as nil.

Analysis of organotin compounds

All weights and concentrations of OTs 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.

To the freeze dried placenta (0.25 g) samples in 12 ml screw capped vials 1 g of solid NaCl and internal standards were added. Samples were lyophilised by sonicating for 1 hour in 5 ml of 25% tetrametylammoniun 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). 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. Organic phase was then evaporated to a volume of about 1 ml of hexane. Small amount of water separating in the end of evaporation process was discarded. Some sodium sulphate was added and hexane phase containing the ethylated OTCs were transferred to a Pasteur pipette containing 3 cm of aluminun oxide. Ethylated OTCs were eluted from the column with 15 ml of 6% diehtylether in hexane. Cleaned sample was evaporated with 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 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 OT were monitored. Two µl of sample were injected into a split-splitless injector. The temperature program of the GC for OTCs 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 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.

Determination of fat content

Hydrochloric acid, HCl, (35 ml) was mixed thoroughly with an amount of 15 g of homogenised placenta. The mixture was placed in a water bath (70 °C) and while stirring the temperature was raised to 100 °C. After heating, the sample was let to cool down into room temperature and an amount of 80 ml of purified water (MilliQ, Millepore, USA) was added. The extraction of fat by shaking in a separation funnel was initiated with diethyl ether (100 ml) and followed by an addition of hexane (100 ml). The organic phase was removed and extraction was repeated two times after which all the organic phases were put together and washed with purified water. The water phase was discarded and the organic phase was dried

overnight by adding 100 g of sodium sulphate (Na_2SO_4). After drying the organic phase was evaporated into small volume after which a known amount of hexane was added into the sample. The fat content of a placenta was determined gravimetrically from the obtained hexane solution.

Quality control and assurance

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 organotin compounds from biological samples.

Analysis of mercury, selenium, arsenic, lead and cadmium

3 g (fresh mass) of sample was wet ashed by 4 mL of nitric acid purified by in house subboil distillation and 0.5 ml of hydrogen peroxide in high-pressure quarts pressure bombs (Multiwave, Anton Paar, Austria). The temperature programme applied a ramped input power starting at room temperature from 100 W and up to 800 W during 5 minutes. This power input was maintained for an additional 15 minutes. If the pressure inside the quarts bombs exceeded 70 bar or if the temperature exceeded 300°C the power input was automatically stopped to prevent the risk of explotions to occur. Following the wet ashing the clear acidic solution was diluted to 20 ml by pure water (MilliQ, Millepore, USA). Prior to quantitative determination by ICP-MS, the 3.2 ml of these solution was further diluted to 8.0 ml be water.
The diluted samples were measured using an ELAN 6100 DRC inductively coupled argon plasma mass spectrometer (ICP-MS) instrument (Perkin Elmer/Sciex, Totonto, Canada) equipped with a dynamic reaction cell (DRC) for removal of argon based interferences. The instrument was run in conventional and in DRC mode 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 table below gives further instrumental details:

		Mercury	Selenium	Arsenic	Lead	Cadmium
Isotope	m/z	202	78	75	208	111
Cell mode/conventional		Conventional	Cell mode	Cell mode	Cell mode	Conventional
Cell gas				Methane		
Standard curve	ng/ml	1; 2; 3	10; 20; 30	10; 20; 30	10; 20; 30	1; 2; 3
Internal standard	ng/ml	Bi; 10 ng/ml	Rh; 10 ng/ml	Rh; 10 ng/ml	Bi; 10 ng/ml	Rh; 10 ng/ml
ICP-MS						
Dwell time	ms	50	50	50	50	50
Sweeps/replicate		20	20	20	20	20
Replicates		6	6	6	6	6
RF power	W	1100	1100	1100	1100	1100

The analyses of samples were carried out in batches each comprising 10 placenta samples (single determinations), 2 analytical blanks, 1 double determination (one of the 10 repeated) and one certified reference material (CRM). The CRM used was Seronorm Whole Blood, L-2. Art. no.: 201605. Lot no.: 0503109 (Seronorm, Norway).

The analytical quality assurance gave the following results:

		Mercury	Selenium	Arsenic	Lead	Cadmium
Isotope	m/z	202	78	75	208	111
CRM; certified 95% confidence interval	ng/g	7,4-8,2	113-133	11,9-14,5	372-414	5,6-6,4
CRM found mean	ng/g	6,6	136	16,0	353	5,7
CRM 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
Comment to s_r					Pb concentrations in placentae too low for determination of s_r	

Quality control and assurance

The CRM was determined in parallel with the unknown samples and the results obtained for the 5 elements are in accordance with the certified confidence intervals. The criterion used in the evaluation was that the mean and standard 2 times the deviation of the analyses overlapped with the confidence intervals.
The limits of detection was estimated for the 5 elements in the samples (fresh mass) on the basis of three times the standard deviation of the blank determinations, and based on a sample intake of 3 g and a final volume of ashed residue of 50 ml.
The repeatablility (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.

Analysis of methyl mercury

The placenta sample was 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 as 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 whereby equilibrium between the enriched and the natural content of MeHg was attained. Then 0.6 ml of nitric acid was added together with 0.5 ml of 4 M ammonium acetate buffer in water. The acidity around pH 5 was checked by indicator paper. Following this, 0.4 ml hexane and 0.5 ml of 10% water-free tetraethylborate in methanol was added and the mixture was rotated for 20 minutes at room temperature. The mixture was then centrifuged at 4700 rpm at 10°C to establish phase separation. About 300 µl of the hexane supernatant was pipetted off and taken to about 50 µl under a flow of nitrogen in a GC vial. The concentrated hexane was analysed by GC-ICP-MS and the chromatographic areas at m/z 198 (enriched isotope) and at m/z 200 (reference isotope) were recorded using the settings in the following table:

ICP-MS

Power	700 W	
Plasma Ar	15 l/min	
Auxiliary Ar	1,2	
Sampling depth		Optimised by Xe in Ar
Integration time per isotope	50 ms	
Points per mass peak	1	

GC

Column	HP-5
Carrier gas, He	4 ml/min
Make-up Ar for ICP-MS	125 ml/min

Oven programme

	Temp 1 °C	Temp 2 °C	Hold	Gradient °C/min
Step 1	50		1 min	0
Step 2	50	260	5 min	30
Step 3	50			Cooling
Inj volume			1 ul	
Inj temperature			250 °C	

Preparation of the $Me^{198}HgCl$ spike solution.
An ampoule of isotopically enriched methylmercury chloride was obtained as a gift from Dr. Zoltan Mester of National Research Council of Canada. The isotopic purity was given at a value of 96.385 %. 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.
A placenta sample with a natural content of MeHg of natural isotopic abundance was taken through the procedure and the resulting extract 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. Hereby 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) 1,134 +/- 0.087.

Quality control and assurance

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).
Precision.
The within-day repeatability was estimated from double determinations of placentas taken through the entire procedure. Only 4 sets of double results were just 2 times above the LOD

of 0.8 ng/g and their mean value was 1.8 ng/g. Based on their differences, these double determinations translated into a value of sr of 31 % RSD.

Accuracy
The Seronorm Whole Blood CRM, which is not certified for MeHg, but had a relevant matrix in relation to placenta samples, was analysed repeatedly (N=8). The mean and standard deviation of the analyses was 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.

Results and discussion

Out of the 117 individual persistent organic congeners or metals 46 were measured in more than half of the placentas suggesting that developing fetus is exposed to a diverse mixture of chemicals.

The occurrence of individual congeners in placentas was the most abundant within the group of PCDD/F of which 12 out of 17 were measured in more than half of the placentas. With in the group of PCDD/Fs the most abundant congeners in placentas were PCDDs of which all 7 measured congeners were present in more than half of the placentas. The most interesting congeners/variables of PCDD/Fs according to previous knowledge are those with the longest half lives or substantial dietary exposure. Congeners like these are 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 2,3,7,8-PCDF, and $WHO_{PCDD/F}$-TEQ.

Among PCB compounds the higher chlorinated congeners dominated due to higher bioaccumulation efficiency and longer half lives in humans. The correlations between these higher chlorinated PCB compounds were ranging from 0.6 to 0.9. Hence it is possible to use a few of them to represent the whole group of PCBs in following tables below. Those PCB congeners were PCB 126, PCB 153, and also the toxic equivalency concentration, WHO_{PCB}-TEQ.

Among the group of PBDEs those congeners present in technical mixtures (especially so called penta mixture) of these flame retardants dominated also in placentas. Such congeners being BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, and BDE 154.

Although the manufacturing and use of the pestiside DDT is banned in Finland and also in EU for many years even decades this pesticide or it's metabolite p,p'-DDE can still be found frequently in all kinds of biological matrices. Also in BENERIS placentas p,p'-DDE was measurable in all samples demonstrating a continuous exposure of population to this very persistent organic pollutant.

The two other groups of POPs, PCNs and PBBs showed very little occurrence in the placentas and it can be concluded that the exposure of fetus to these compounds is irrelevant and hence these compounds were excluded from the following tables. The manufacturing and usage of PCNs and PBBs has been stopped/banned for at least two decades in many countries.

After analysing 73 of the 130 BENERIS placentas for organotin (OT) compounds the procedure was stopped due to non existence of these compounds in placentas.

Among metals the occurrence of methyl mercury (MeHg) was the most interesting in respect of BENERIS. The exposure to MeHg is predominantly due to consumption of fish.

A group of individual, representative to a certain group, congeners of all the measured ones was selected in order to describe the occurrence of pollutants in placentas and to give data for the exposure assement of fetus to this kind of hazardous compounds for risk assessment purposes. The selected individual congeners were: 2378-TCDD, 12378-PCDD, 23478-PCDF, $WHO_{PCDD/F}$-TEQ, PCB 126, PCB 153, WHO_{PCB}-TEQ, BDE 47, BDE 153, sum of BDEs, p,p'-DDE, and MeHg. The exposure to these pollutants might be affected by the parity of mothers due to lactational elimination of these fat soluble contaminants and hence we calculated the concentrations of placentas in parity wise in order to describe the exposure of fetus in different parity classes. The age of the mother can have a crucial effect of the exposure of the fetus since almost all these pollutants have a long half lives and due to that they bioaccumulate into mothers for a long time before the pregnancy. The age correlation of pollutants was conducted with the mothers of first pregnancy in order to find out the effect of age to exposure of fetus to these compounds. A third descriptive of exposure is the source of contaminants. The correlations between selected congeners were calculated in order to reveal possible differences in exposure sources of these compounds/respective groups of contaminants. The source of a very many fat soluble organic contaminant in Finland is fish and therefore the occurrence of target congeners in placentas was calculated in increasing fish usage classes.

In table 2 the concentrations of selected congeners in different parity groups are presented. A continuing downward trend according to increasing parity was observed with highly persistent organic pollutants PCDD/F and PCBs. The concentrations in placentas of mothers having three or more children were on average 16% lower than in placentas with primipara mothers. With p,p'-DDE the trend was similar though not continuing. Methyl mercury also showed a decreasing trend along with increasing parity, but one must be cautious in respect of fetus exposure with this result due to much shorter half life of MeHg when compared to PCDD/Fs, PCbs or p,p'-DDE. The most controversial result was obtained with the trend of PBDEs with parity. It seemed like exposure of fetus to PBDEs was increasing with increasing parity. The standard deviations of PBDEs were wide indicating a very skewed distributions of these compounds. Nevertheless these results indicated that as a group PBDEs are different from the other POPs, like PCDD/Fs, PCbs or p,p'-DDE.

Table 2. The mean (standard deviation) concentrations of selected congeners in different parity groups in BENERIS placentas.

	Parity		
Congener	**1 (n=59)**	**2 (n=39)**	**3 or more (n=31)**
2378-TCDD, pg/g fat	1.43 (0.92)	1.40 (0.83)	1.24 (0.85)
12378-PeCDD, pg/g fat	7.69 (3.64)	7.75 (4.39)	6.56 (4.21)
23478-PeCDF, pg/g fat	19.0 (12.2)	16.3 (9.45)	15.1 (10.2)
WHO-$_{PCDD/F}$-TEQ, pg/g fat	20.3 (11.0)	19.2 (10.7)	16.8 (10.3)
PCB 126, pg/g fat	4.62 (2.93)	3.98 (2.15)	3.66 (1.80)
PCB 153, ng/g fat	5.43 (2.47)	5.55 (3.12)	5.13 (3.16)
WHO-$_{PCB}$-TEQ, pg/g fat	0.98 (0.50)	0.91 (0.47)	0.82 (0.38)
BDE 47, ng/g fat	0.49 (0.46)	0.69 (0.73)	1.61 (4.83)
BDE 153, ng/g fat	0.41 (1.50)	0.25 (0.21)	0.49 (1.18)
Sum of BDEs, ng/g fat	2.19 (8.38)	1.44 (1.23)	4.88 (18.3)
p,p'-DDE, ng/g fat	9.08 (5.22)	9.48 (6.47)	7.70 (3.64)
MeHg, ng/g fat	187 (125)	125 (110)	132 (128)

In order to obtain an estimate of the effect of age of mother to the exposure of fetus to contaminants the correlation and regression model between mother's age and contaminant concentration for the primipara mothers were calculated. The results are in table 3.

Table 3. The correlation and regression model result between mother's age and contaminant concentration for the primipara mothers 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

The groups correlating the most with age of mother's were PCDD/Fs and PCBs indicating their long half lives in humans. Age is a very strong variable, according to R^2 figures, when considering estimating concentrations of PCDD/Fs and PCBs without actually measuring them. P,p'-DDE showed an intermediated correlation with age and PBDEs together with MeHg showed poor correlation with primipara mother's age.

In Finland the main source of persistent organic pollutants or methyl mercury is fish. Therefore the effect of fish consumption to placenta concentrations was examined with primipara mother's. Mothers were divided into two groups according to fish consumption and placenta results were compared (Table 4).

Table 4. Mean (min-max) of mother's age and contaminant concentration according to fish consumption for the primipara mothers in BENERIS project.

	Fish consumption	
Congener	**0-18 g/day**	**18.1-47 g/day**
Age	**26.8** *(19.5-35.3)*	**28.7** *(20.4-35.9)*
2378-TCDD, pg/g fat	**1.13** *(< LOQ-3.14)*	**1.64** *(< LOQ-4.82)*
12378-PeCDD, pg/g fat	**6.32** *(2.87-14.4)*	**8.70** *(2.37-16.7)*
23478-PeCDF, pg/g fat	**15.3** *(6.91-48.8)*	**21.7** *(5.21-53.2)*
WHO-$_{PCDD/F}$-TEQ, pg/g fat	**16.7** *(7.28-44.4)*	**22.9** *(6.31-48.1)*
PCB 126, pg/g fat	**3.81** *(1.40-9.52)*	**5.22** *(1.0-16.3)*
PCB 153, ng/g fat	**4.55** *(2.17-8.20)*	**6.08** *(2.17-13.1)*
WHO-$_{PCB}$-TEQ, pg/g fat	**0.79** *(0.40-1.62)*	**1.12** *(0.30-2.89)*
BDE 47, ng/g fat	**0.60** *(0.18-3.31)*	**0.41** *(0.19-1.22)*
BDE 153, ng/g fat	**0.70** *(< LOQ-11.7)*	**0.20** *(< LOQ-0.52)*
Sum of BDEs, ng/g fat	**3.90** *(0.36-65.1)*	**0.93** *(0.37-3.42)*
p,p'-DDE, ng/g fat	**8.88** *(4.06-25.39)*	**9.23** *(2.44-26.1)*
MeHg, ng/g fat	**140** *(< LOQ-487)*	**220** *(< LOQ-764)*

Mothers consuming more fish had their placenta concentration higher when compared to less fish consuming mothers. This was true for all the other congeners with the exception of PBDEs. Those showed no increasing concentrations with increasing fish consumption.

Tables 2, 3, and 4 already indicated that flame retardants PBDEs deviate as a group from the rest of the measured groups. This was also seen from the table 5 in where concentrations of selected pollutants were correlated. Table 5 indicated that PBDEs must have a different exposure sources and it might be that food is not the predominant source of exposure to these flame retardants. The correlation of MeHg between the other POPs was also poor, but that most likely is due to it's shorter half life in humans when compared to PCDD/F and PCBs.

Conclusions

The data in Deliverable 19 of the BENERIS project indicated that the exposure of the fetus to the persistent organic pollutants PCDD/Fs, PCBs, p,p'-DDE, and MeHg was dependent on three key variables:

a) Mother being a primipara mother
b) The age of the mother
c) Fish consumption of the mother

The data also indicated that the sources of these four pollutants are similar but differ from the sources of flame retardants, PBDEs.

References

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Table 5. Correlations of selected pollutants in BENERIS placentas.

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**	**0.80**	**0.03**	**0.05**	**0.02**	**0.65**	**0.07**
23478-PeCDF			**1**	**0.97**	**0.68**	**0.80**	**0.79**	**-0.03**	**0.06**	**0.01**	**0.61**	**0.23**
WHO-$_{PCDD/F}$-TEQ				**1**	**0.70**	**0.83**	**0.82**	**0.00**	**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**	**0.00**	**0.72**	**0.28**
BDE 47								**1**	**0.48**	**0.83**	**0.00**	**-0.05**
BDE 153									**1**	**0.87**	**-0.01**	**-0.13**
Sum of BDEs										**1**	**-0.03**	**-0.10**
p,p'-DDE											**1**	**0.19**
MeHg												**1**

ANNEX 1

Table 1. Limits of quantification (LOQ), number of placentas with concentrations > LOQ, 5th, 50th, and 95th percentiles of concentrations of individual compounds of POPs and metals in 130 placentas in BENERIS. Sum concentrations of analyte groups along with toxic equivalencies (TEQ) of PCDD/Fs and PCBs are also presented.

PCDD/Fs	**LOQ**	**Number > LOQ**	**5th**	**50th**	**95th**
2378-TCDD, pg/g fat	0.47	117	< LOQ	1.22	3.16
12378-PCDD, pg/g fat	0.68	130	2.83	6.66	16.0
123478-HxCDD, pg/g fat	0.94	109	< LOQ	1.82	3.58
123678-HxCDD, pg/g fat	0.92	129	2.27	5.48	11.1
123789-HxCDD, pg/g fat	0.90	70	< LOQ	1.04	3.23
1234678-HpCDD, pg/g fat	1.0	130	4.59	10.3	29.7
OCDD, pg/g fat	4.3	130	26.6	53.8	147
2378-TCDF, pg/g fat	0.32	54	< LOQ	< LOQ	1.43
12378-PCDF, pg/g fat	0.40	46	< LOQ	< LOQ	1.20
23478-PCDF, pg/g fat	0.40	130	6.08	13.6	47.0
123478-HxCDF, pg/g fat	0.60	129	1.68	3.39	8.10
123678-HxCDF, pg/g fat	0.51	118	< LOQ	1.09	2.51
234678-HxCDF, pg/g fat	0.80	14	< LOQ	< LOQ	1.15
123789-HxCDF, pg/g fat	2.0	0	< LOQ	< LOQ	< LOQ
1234678-HpCDF, pg/g fat	0.42	128	0.704	5.18	16.8
1234789-HpCDF, pg/g fat	1.5	0	< LOQ	< LOQ	< LOQ
OCDF, pg/g fat	3.8	84	< LOQ	6.24	35.6
Sum of all 17 PCDD/F, pg/g fat		130	57.0	120	256
$WHO_{PCDD/F}$-TEQ_{1998}, pg/g fat *		130	6.93	16.9	44.2

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	0.60	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	0.10
PCB 118, ng/g fat	0.16	129	0.38	0.95	2.20
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	0.030	128	0.074	0.19	0.45
PCB 153, ng/g fat	0.53	129	2.20	4.81	10.9
PCB 141, ng/g fat	0.12	18	< LOQ	< LOQ	0.22
PCB 138, ng/g fat	0.40	129	1.08	2.76	6.27
PCB 167, ng/g fat	0.013	129	0.038	0.10	0.27
PCB 128, ng/g fat	0.042	84	< LOQ	0.10	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	0.10	129	0.40	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	0.20	0.40	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	0.80	1.70

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	2.20
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	0.010	1	< LOQ	< LOQ	< LOQ
BDE 99, ng/g fat	0.041	102	< LOQ	0.10	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	0.80	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

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	0.010	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		0 129	0.0052	0.014	0.14

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
		0			
Sum of 19 PBB, ng/g fat		7	< LOQ	< LOQ	0.038

DDE	LOQ	Number > LOQ	5th	50th	95th
p,p'-DDE, ng/g fat	0.58	130	3.58	7.79	17.5

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		6.0	< LOQ	< LOQ	280

Metals	LOD	Number > LOQ	5th	50th	95th
Se, ng/g fw	1	130	180	212	260
As, ng/g fw	0.3	130	4.40	5.68	10.0
Cd, ng/g fw	0.3	130	1.86	3.70	7.13
Hg, ng/g fw	0.4	128	0.76	2.31	5.69
Pb, ng/g fw	9	33	9.20	13.1	56.9

Methyl mercury	LOD	Number > LOQ	5th	50th	95th
MeHg, ng/g fat	60	108	< LOQ	130	414