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Fetus contaminants from mothers' diet

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Fetus contaminants from mothers' diet (D36)

Introduction

In the BENERIS project, one of the aims was to establish the association between the external dose (intake) of persistent organic pollutants (POPs) and their concentrations in the body.

The primary persistent organic pollutant group was polychlorinated dibenzo-*p*-dioxins (PCDD/F), but in this deliverable also polychlorinated biphenyls (PCB) and polybrominated diphenylethers (PBDE) were included when determining the associations. The association work was initiated by analysing these contaminants from 130 placentas and by combining this occurrence data with calculated intake data based on food frequency questionnaires (FFQs).

This deliverable describes the associations between the placental concentrations of POPs and the estimated intakes of these pollutants.

Material and Methods

Placentas

Placentas originated from mothers (n=129) that are participants of the LUKAS2 study (n=228). The study was carried out in Kuopio University Hospital area. It is extension of LUKAS1 study. LUKAS1 study is 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 LUKAS2 study were born between 5/2004 - 5/2005. Inclusion criteria of the study were: family doesn't live in block of flats; one fetus gravidity; 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.

POP occurrence data in food

The occurrence data of PCDD/Fs, PCBs, and PBDEs originated from the annual national food monitoring program run by the Finnish Food Safety Authority (EVIRA) and from a specific research project on Finnish fish, the "EU-fish" project (Hallikainen et al. 2004; Isosaari et al. 2006; Parmanne et al. 2006; Kiviranta et al. 2006). The samples included in this study, covering years 2002-2005, were meat, (n=29), liver (n=5), milk (n=18), egg (n=16), oil and fat (n=7), fish (n=175), and other food (n=8) samples (Wiborg et al. 2008).

Chemical analyses of PCDD/Fs, PCBs, and PBDEs from food and placental samples

The occurrence of seventeen 2,3,7,8-chlorine substituted PCDD/F congeners, of four non-*ortho* (PCB 77, 81, 126, and 169), eight mono-*ortho* (PCB 105, 114, 118, 123, 156, 157, 167, and 189), of 24 di-*ortho* (PCB 18, 28, 33, 49, 51, 52, 60, 66, 74, 99, 101, 110, 122, 128, 138, 141, 153, 170, 180, 183, 187, 194, 206, and 209) PCB congeners, and of fifteen PBDE congeners (BDE 28, 47, 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 183, and 209) were measured in both food and placenta samples. For PCDD/Fs and PCBs, toxic equivalents quantities (WHO-TEQ) were calculated with toxic equivalency factors (TEF) recommended

by WHO in 1998 (van den Berg et al., 1998). All analyses were performed in the Chemical Exposure Unit at the National Institute for Health and Welfare (THL).

Food samples

A previously published method was used in all food samples (Kiviranta et al. 2004). In brief, samples were spiked with ^{13}C-labeled PCDD/F, PCB, and PBDE standards and fat was extracted. Samples were defatted in a silica gel column, after which PCDD/Fs were separated from PCBs and PBDEs on a carbon column. Both fractions were further cleaned by passing through an activated alumina column. The PCB-PBDE fraction was further fractionated in order to separate the non-*ortho* PCBs from mono- and di-*ortho* PCBs and PBDEs. The quantification was performed with HRGC/HRMS instrument. Concentrations were calculated with both lower bound and upper bound methods. In the lower bound method, the results of congeners with concentrations below limit of quantification (LOQ) were designated as nil, while in the upper bound method they were denoted as the LOQ.

Placenta samples

A freeze-dried placenta sample (75 g) was pulverized in a mortar and spiked with a set of ^{13}C-labeled internal standards. 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, and PBDEs. The carbon column alone was then back eluted with 15 ml of toluene in order to elute PCDD/Fs and non-*ortho*-PCBs.

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 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 and ^{13}C-PCB 60 for non-*ortho*-PCBs) 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.

Determination of fat content in placentas

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 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 methods 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-compounds from food and biological samples.

Assessing contaminant intake

A background questionnaire was sent to mothers in LUKAS2 study about 6 weeks before birth. It contained questions about the mothers' allergies and respiratory symptoms, number of children, children’s allergies, residential environment, pets, smoking, education, and marital status.

The diet during pregnancy was assessed using a validated FFQ (Erkkola et al. 2001), comprising a list of 181 food items. The FFQ assessed the use of foods and food groups by frequency of consumption during 1 month. The FFQ focused on past diet, i.e. on the diet during the eighth month of pregnancy, before the working mothers were supposed to be on maternity leave. The food consumption data were entered into a dietary database by a software program of the National Institute for Health and Welfare, THL (former National Public Health Institute, KTL) and an in-house program was used to calculate daily nutrient intakes and food amounts.

In addition, fish consumption was investigated with a questionnaire addressing 11 fish items (frozen fish (ocean), canned fish (ocean), rainbow trout, Baltic herring, inner lake predatory fish, vendace, other inner lake fish, Baltic salmon or trout, other Baltic sea fish, other ocean fish, shellfish). The fish questionnaire data was transformed into daily intake frequencies and then multiplied by portions estimated from FINDIET2002 (Männistö et al. 2003). Thereafter, fish intakes calculated from the FFQ were replaced by intakes from fish questionnaire. Daily intakes of compounds (PCDD/Fs, PCBs and PBDEs) were calculated using the mean values of the compounds of single foods.

Statistical analysis

The correlations between fetus concentrations (per fat grams) and compound intakes from food were estimated with Spearman's rank correlation. In addition, regression models were used to estimate the relationship between fetus concentrations as the dependent variable and food intake, number of births, mother's age, mother's weight at the first maternity clinic visit and weight difference between first and last maternity clinic visits proportion to weeks between visits (kg/week) as the independent variables.

Fish consumption was tested with repeated measures analysis of variance. The sums of PCDD/Fs, PCBs, and PBDEs, WHO_{PCDD}-TEQ and WHO_{PCB}-TEQ between high fat fish and low fat were tested with paired t-tests.

Results

Background data of age and BMI for all participating mothers in different parity groups are described in Table 1.

Table 1. Summary table, mothers by births, mean (standard deviation).

Births	N (%)	Age	BMI
1	59	27.9 (4.1)	24.8 (4.7)
2	39	30.6 (4.4)	26.8 (5.5)
3 or more	31	32.5 (4.9)	24.8 (5.3)
All	129	29.8 (4.8)	25.4 (5.2)

Fish intakes

Calculated fish intakes are presented in Table 2. Mother's high-fat fish consumption (grams/day) was lower than low-fat ($p<0.0001$). The parity of mothers did not have any significant effect on fish consumption.

Table 2. Fish intake of mothers during pregnanacy (calculated from additional fish-FFQ, g/d), mean (sd) [%]

Births	Total intake of fish	Low-fat fish* [%]	High-fat fish* [%]
1	19.1 (10.8)	10.7 (7.2) [56.0]	8.4 (8.2) [44.0]
2	17.1 (11.4)	9.9 (7.3) [57.6]	7.3 (7.6) [42.4]
3 or more	19.1 (12.0)	11.7 (10.6) [61.4]	7.4 (6.0) [38.6]
Overall mean	18.5 (11.2)	10.7 (8.1) [57.8]	7.8 (7.5) [42.2]

*low-fat fish = frozen fish (ocean), canned fish (ocean), perch (IL), pike (IL), burbot (IL), pike-perch (IL), other IL fish, shellfish
**high-fat fish = salmon, rainbow trout, Baltic herring, vendace, Baltic salmon

Intake calculations of PCDD/Fs, PCBs, and PBDEs along with TEQs based on FFQ and contaminant occurrence data are presented in table 3.

Table 3. PCDD/F, PCB, and PBDE intakes from low-fat and high-fat fishes (mean (sd) [%],pg/kg_bw/d, for Sum PCDD/Fs and TEQs, ng/kg bw/d for Sum PCBs and Sum PBDEs).

Compound	**Total intake from fish**	**Low-fat fish (%)**	**High-fat fish (%)**	**p-value**
Sum(PCBs)	6.16 (4.07)	3.29 (3.00) [53]	2.87 (2.91) [47]	0.27
Sum(PCDD/Fs)	0.45 (0.45)	0.13 (0.18) [29]	0.32 (0.36) [71]	<0.0001
Sum(PBDEs)	0.27 (0.23)	0.06 (0.07) [23]	0.21 (0.21) [77]	<0.0001
WHO_{PCB}-TEQ	0.26 (0.18)	0.11 (0.09) [41]	0.15 (0.15) [59]	0.003
$WHO_{PCDD/F}$-TEQ	0.13 (0.13)	0.04 (0.05) [29]	0.08 (0.10) [71]	<0.0001

Models and correlations

Results of regression models are shown in Table 4 on dioxins 2378TCDD, 23478PCDF, 12378PCDD, $WHO_{PCDD/F}$-TEQ; PCB's PCB118, PCB126, PCB156, sum of 37 PCB's and WHO_{PCB}-TEQ.
Correlations on 17 PCDD/F compounds were between [-0.11, 0.27], on 37 PCB's between [-0.04, 0.34] and on 14 PBDE compounds between [-0.13, 0.17] (not shown in the table). Correlations on WHO_{PCB}-TEQ and $WHO_{PCDD/F}$-TEQ were 0.23 and 0.25 respectively. On sum of 14 BDE compounds correlation was – 0.06 (not shown).

The number of births has a negative effect in all chosen models. In contrast, mother's age has a positive effect in models. The weight difference has a negative effect in all models, except for 23478PCDF, where it is not significant (p=0.14). Mother's weight at the first maternity clinic visit does not have a significant effect in models, except for PCB 156 (p=0.04).

In PBDE models, there were no statistically significant effects.

Table 4. Correlations between fetus concentrations and food intakes and regression models, fetus concentration as the dependent variable.

Compound	Food	Number of births (p-value)	Weight difference* (p-value)	mother's weight** (p-value)	mother's age (p-value)	R^2***	Correlation****
2378TCDD	13.4 (0.03)	-0.24 (<0.001)	-1.21 (0.003)	0.0003 (0.94)	0.08 (<0.001)	0.322	0.24
23478PCDF	15.0 (0.01)	-3.16 (<0.001)	-7.49 (0.14)	-0.01 (0.86)	1.09 (<0.001)	0.317	0.27
12378PCDD	11. 8 (0.29)	-1.34 (<0.001)	-4.26 (0.02)	0.001 (0.96)	0.48 (<0.001)	0.376	0.19
$WHO_{PCDD/F}$-TEQ	11.6 (0.05)	-3.45 (<0.001)	-10.8 (0.03)	0.001 (0.98)	1.20 (<0.001)	0.369	0.23
PCB126	269 (0.06)	-0.74 (<0.001)	-2.74 (0.02)	0.021 (0.12)	0.24 (<0.001)	0.33	0.23
PCB118	237 (0.11)	-178.2 (<0.001)	-624 (0.01)	5.46 (0.06)	65.0 (<0.001)	0.407	0.18
PCB156	632 (0.22)	-95.2 (<0.001)	-356 (0.002)	-2.76 (0.04)	36.4 (<0.001)	0.451	0.26
Sum of PCBs	466 (0.09)	-3406 (<0.01)	-11724 (0.05)	2.92 (0.97)	1397 (<0.001)	0.312	0.2
WHO_{PCB}-TEQ	0.31 (0.06)	-0.16 (<0.001)	-0.58 (0.004)	0.001 (0.61)	0.056 (<0.001)	0.43	0.25

* Weight change between first and last maternity clinic visits (kg/wk)
** at first maternity clinic visit
*** multiple coeffecient of determination
**** Spearman's rank correlation between fetus concentration and food intake

Table 5. Fish and shellfish intake in the general population of Finland.

Sex, age (years)	Mean intake (g/day)	Standard deviation (g/day)
Male, 1	4.67	7.1
Male, 3	8.14	12.14
Male, 6-9	12.04	17.14
Male, 25-34	20.54	34.06
Male, 35-44	27.26	42.81
Male, 45-54	33.5	56.63
Female, 1	4.79	7.4
Female, 2	8.16	12.39
Female, 3	8.16	12.39
Female, 6-9	9.6	14.53
Female, 25-34	17.91	29.26
Female, 35-44	19	33.06
Female, 45-54	19.72	30.16

Discussion

Pregnant mothers ate a fair amount of fish, Table 2. It was very close to the intake of the general population of the same age and sex, Table 5. The Finnish fish intake is higher than in many other countries in Europe. Finnish mothers consumed both high-fat fish and low-fat fish, low-fat fish being somewhat more common.

Pollutant intakes were mostly due to fatty fish, as expected. However, also the low-fat fish was a significant source of PCBs, PCDDs, and PBDEs, Table 3. The total PCDD/F and PCB intakes were lower than in a study on the general population in Finland (Kiviranta et al. 2005). PCBs were more important a source of dioxin-like pollutants than PCDD/Fs, which might be due to a high consumption of farmed salmon/rainbow trout. In those fish species the WHOPCB-TEQ dominates over the WHOPCDD/F-TEQ (Isosaari et al 2006).

The regression analyses showed that the most significant predictor of the fetus pollutant concentration was mother's age. This was true for all the pollutants analysed. Another important predictor was the number of the birth, in such a way that fetal concentrations were lower if the number was higher. This is along with previous observations and it is due to the fact that a part of mother's body burden is transported to the fetus, and later to the child during breast feeding.

Two other factors had somewhat lower but still significant impact on the pollutant concentrations in the fetus. These were the estimated pollutant intake from food, and mother's weight difference between the measuring points, a higher difference predicting a lower pollutant concentration. The rank correlations between the estimated pollutant intakes and the actual concentrations in the fetus were between 0.18 and 0.27. These numbers are lower

than what would be expected *a priori*, because food is obviously the source of these pollutants. This results deserves careful discussion.

One clear result from this study was that the pollutant concentration in the fetus is due to a long exposure history of the mother, as can be seen from the importance of mother's age and number of births. The food intake is based on the previous months and only indirectly tells about previous exposures.

Uncertainties in the intake estimates also dilute the importance of this determinant. The food intake estimate is based on self-reporting, which is not an ideal way because people e.g. forget what they have eaten and tend to under-report unhealthy food items. In addition, the pollutant concentrations in food are based on a limited number of analyses and on national averages and not on the concentrations of the actual food items eaten. Therefore, local variation in e.g. pollutants in fish is ignored.

In any case, these results show that the pollutant intake estimates based on diet studies are an important source of information also in predicting individual fetal concentrations. It was also very important to find out that the fetal concentrations should be measured per fat and not per wet weight, as the latter did not correlate with food intake predictors at all.

Overall, the predictors studied were collectively able to explain between 31 % and 45 % of all the variation in the fetal concentrations. Although this is far from perfect, these numbers are clearly promising for benefit-risk assessment. Useful models can be built in such a way that they provide guidance about policies to avoid pollutant exposures.

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