CONCENTRATION LEVELS OF ENVIRONMENTAL PERSISTENT ORGANIC POLLUTANTS IN BREAST MILK FROM DANISH AND FINISH POPULATIONS

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Introduction

It is widely accepted that foetal events can predispose an individual to develop certain health disorders in adulthood; indeed, the commonest disorders of European and Western adults today (obesity, type 2 diabetes, CVD), which are increasing progressively in incidence, are well-established to have prenatal determinants and are termed metabolic syndrome. Good biomarkers for developing these disorders have not yet been established, which prevents effective prevention. Equally, it is increasingly clear that the commonest disorders of male reproductive health in newborn (cryptorchidism, hypospadias) and young adult human males (low sperm counts, testis germ cell cancer) may also be caused by adverse events in foetal (and perhaps neonatal) life. These reproductive disorders are thought to comprise a testicular dysgenesis syndrome (TDS), which arises because of a cascade of changes triggered by maldevelopment of the foetal testis. Several pieces of evidence suggest that common environmental chemicals, probably acting together in mixtures or in combination with other factors (genetic, lifestyle) could contribute causally to TDS.

In this context, the EU FP7 DEER project aims to add epidemiological and mechanistic information for reproductive and developmental effects of several environmental contaminants, helping to identify connections between exposures, disrupted reproductive maturation and mis-programming of metabolic homeostasis. One part of the work was to investigate potential correlation between contamination levels in breast milk and some reproductive troubles observed in the corresponding breastfed infants. In the present study, 261 breast milk samples collected in the frame of a Danish-Finish mother-child cohort were analysed for determination of a large panel of environmental persistent organic pollutants (POPs) including dioxins (PCDDs), furans (PCDFs), polychloro biphenyl (PCBs), polybrominated diphenyl ethers (PBDEs), polybromobiphenyls (PBBs), hexabromocyclododecane (HBCDs) and perfluorinated compounds (PFCs). These breast milk samples were collected from Danish or Finish mothers that have given birth to boys with hypospadias cases versus controls, but also to normal boys with large versus small testes, as well as normal boys with high versus low serum levels of particular hormones (LH/testosterone ratio and inhibin B).

The purpose of the present study was to describe and discuss the contamination levels observed for each class of targeted pollutant in these analysed breast milk samples. A second objective of the project will be to rely theses observed concentration levels to the previously mentioned biological and clinical outcomes (not presented here).

Materials and methods

Samples

All biological samples analyzed in this study were collected by the Department of Growth and Reproduction of the Copenhagen University Hospital (Rigshospitalet), in a frame of a Danish-Finish Mothers/Child cohort in relation with reproductive disorders observed on new-borns.

Analytical method for dioxins, PCBs and BFRs [1,2]

Before extraction, ^{13}C-labelled congeners were added to each sample for quantification according to the isotopic dilution method. For dioxins, PCBs and BFRs, samples were first submitted to a liquid/liquid extraction with pentane. Resulting extracts were weighed to measure fat content, and reconstituted in hexane for further sample clean-up. For dioxins, PCBs, PBDEs and PBBs, three purification steps were then performed, using successively acid silica, florisil and celite/carbon columns. HBCDs stereoisomers were purified on acidified silica column. dioxins, PCBs, PBDEs and PBBs measurements were performed by GC-HRMS using an Agilent 7890A gas

chromatograph coupled to a JEOL JMS 700D or 800D high resolution mass spectrometers. The spectrometric resolution was set at 10,000, and the signal acquisition was performed in the Single Ion Monitoring (SIM) mode. HBCD stereoisomers were analyzed by LC-MS/MS on an Agilent 6410 triple quadrupole instrument, after negative electrospray ionisation and using the MRM acquisition mode.

Analytical method for PFCs [3]
Before extraction, ^{13}C-labelled congeners were added to each sample for internal standard calibration. Samples were first submitted to a liquid/liquid extraction with acetone. Extracts are then acidified (formic acid) and purified on two successive SPE cartridges (Oasis HLB and ENVI-Carb). Final extracts are evaporated to dryness and reconstituted in methanol/water mixture to be injected in LC-MS/MS. Negative electrospray ionisation is applied, and two diagnostic signals (MRM transitions) were monitored for each target compound.

Results and discussion
The first part of the work consisted to determine the qualitative and quantitative contamination profiles in the 261 analysed breast milk samples. The obtained results are sumarized in Table 1. For dioxins (Figure 1), the observed contamination levels ranged from 3.3 to 72.1 OMS_{1998}-TEQ pg/g of lipid, with mean and median values found at 16.0 and 15.4 OMS_{1998}-TEQ pg/g of lipid, respectively. The global distribution of these data appeared quite normal. For dioxin-like PCBs (Figure 2), the observed contamination levels ranged from 3.3 to 79.6 OMS_{1998}-TEQ pg/g of lipid, with mean and median values found at 12.7 and 11.5 OMS_{1998}-TEQ pg/g of lipid, respectively. The global distribution of these data also appeared quite normal. For non dioxin-like PCBs, the observed contamination levels (sum of the 7 PCB congeners #28, 52, 101, 118, 138, 153 and 180) ranged from 47.7 to 1054.4 ng/g of lipid, with mean and median values found at 190.1 and 169.9 ng/g of lipid, respectively. The global distribution of these data appeared quite normal again. For polybromodiphenylethers (Figure 3), the observed contamination levels (sum of the 7 main marker PBDE congeners #28, 47, 99, 100, 153, 154 and 183) ranged from 1.5 to 111.1 ng/g of lipid, with mean and median values found at 6.6 and 4.9 ng/g of lipid, respectively, and with some particularly elevated values. For polybromobiphenyls, the observed contamination levels (sum of the 3 main marker PBB congeners #25, 101 and 153) ranged from 0.1 to 1.3 ng/g of lipid, with mean and median values found at 0.3 ng/g of lipid for both. For hexabromocyclododecane, the observed contamination levels (sum of the α, β and γ HBCD isomers) ranged from 0.0 to 49.6 ng/g of lipid, with mean and median values found at 0.8 and 0.4 ng/g of lipid, respectively. For PFCs (Figure 4), the observed contamination levels for the sum of PFOA and PFOS ranged from 0.01 to 1.90 ng/mL, with mean and median values found at 0.6 and 0.6 ng/mL, respectively. The global distribution of these data appeared quite normal.
Comparing the results obtained for the Danish versus Finish sub-populations, levels were found statistically higher in Danish breast milk samples for dioxins, PCBs and PFCs (Student t-test or Mann Whitney test). But these differences have to be moderated considering (1) the very limited number of analysed Finish samples compared to the number of Danish ones (at least10 times more samples for the Danish sub-population) and (2) several very high values found in these Danish samples (maximum values) that may participate to the observed difference. These data are now on track for further more completed and advanced statistical analysis. The relation between these contamination levels and some biological/clinical outcome are also currently investigated

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References
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Table 1: Concentrations levels in the analysed breast milk samples from Finish (FIN) and Danish (DK) mothers (pg/g of lipid for PCDD/Fs and dl-PCBs, ng/g lipid for ndl-PCBs, PBDEs, PBBs and HBCDs, ng/mL for PFCs).

		FIN	DK	Total	p (Student t-test)	p (Mann-Whitney)
OMS_{1998}-TEQ PCDD/F	N	22	239	261	0.0033**	0.0007***
	Mean	11.6	16.4	16.0		
	Median	10.9	15.5	15.4		
	Minimum	4.0	3.3	3.3		
	Maximum	21.7	72.1	72.1		
	s.d.	5.1	7.3	7.2		
OMS_{1988}-TEQ dl-PCB	N	22	238	260	0.0022**	0.0001***
	Mean	8.2	13.2	12.7		
	Median	7.8	11.8	11.5		
	Minimum	3.3	3.8	3.3		
	Maximum	14.4	79.6	79.6		
	s.d.	3.1	7.5	7.4		
Sum 7 ndl-PCBs	N	22	238	260	0.0001***	0.0000***
	Mean	113.0	197.2	190.1		
	Median	115.8	174.3	169.9		
	Minimum	47.7	62.9	47.7		
	Maximum	197.5	1054.4	1054.4		
	s.d.	45.8	100.0	99.4		
Sum 7 marker PBDEs	N	22	239	261	0.8515	0.6034
	Mean	7.0	6.6	6.6		
	Median	5.2	4.9	4.9		
	Minimum	1.5	1.5	1.5		
	Maximum	19.0	111.1	111.1		
	s.d.	5.0	9.0	8.8		
Sum 3 PBBs	N	10	223	233	0.0625	0.0019**
	Mean	0.2	0.3	0.3		
	Median	0.1	0.3	0.3		
	Minimum	0.1	0.1	0.1		
	Maximum	0.6	1.3	1.3		
	s.d.	0.2	0.2	0.2		
Sum α,β,γ-HBCD	N	21	236	257	0.8989	0.3843
	Mean	0.9	0.8	0.8		
	Median	0.4	0.4	0.4		
	Minimum	0.03	0.05	0.03		
	Maximum	7.5	49.6	49.6		
	s.d.	1.6	3.2	3.1		
Sum 2 PFCs (PFOS+PFOA)	N	22	199	221	0.0000***	0.0000***
	Mean	0.3	0.7	0.6		
	Median	0.3	0.6	0.6		
	Minimum	0.1	0.1	0.1		
	Maximum	0.8	1.9	1.9		
	s.d.	0.2	0.3	0.3		

* p<0.05 ; ** p<0.01; *** p<0.001

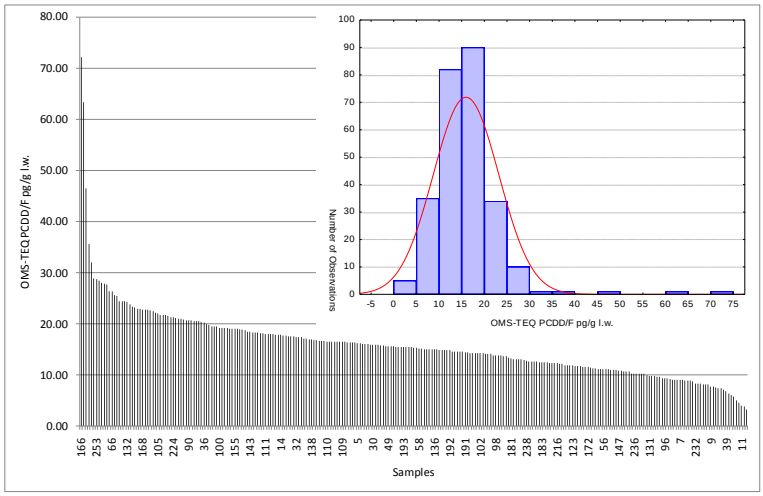

Figure 1: dioxin (PCDD/F) contamination levels measured in the analysed breast milk samples.

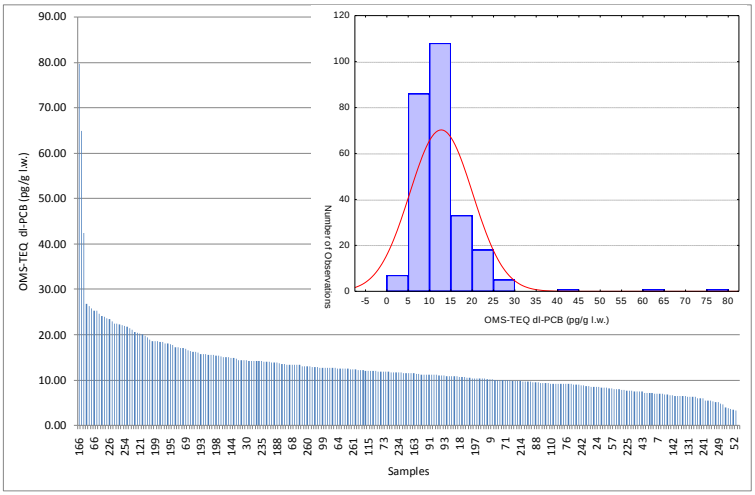

Figure 2: dl-PCBs contamination levels measured in the analysed breast milk samples.

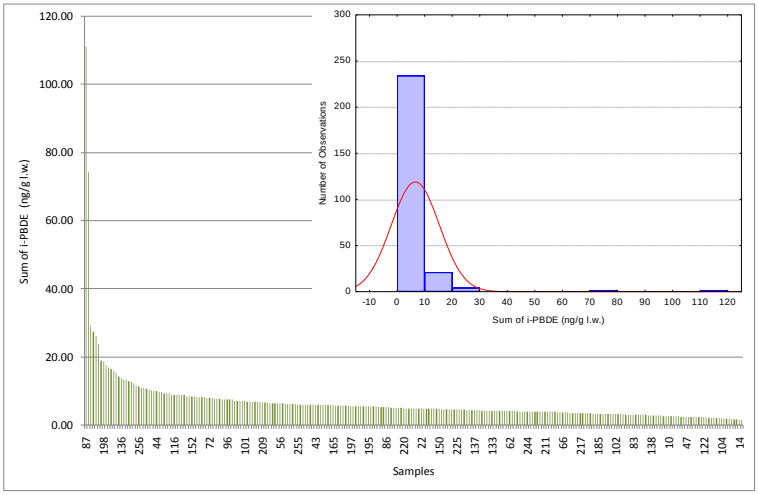

Figure 3: PBDEs contamination levels measured in the analysed breast milk samples.

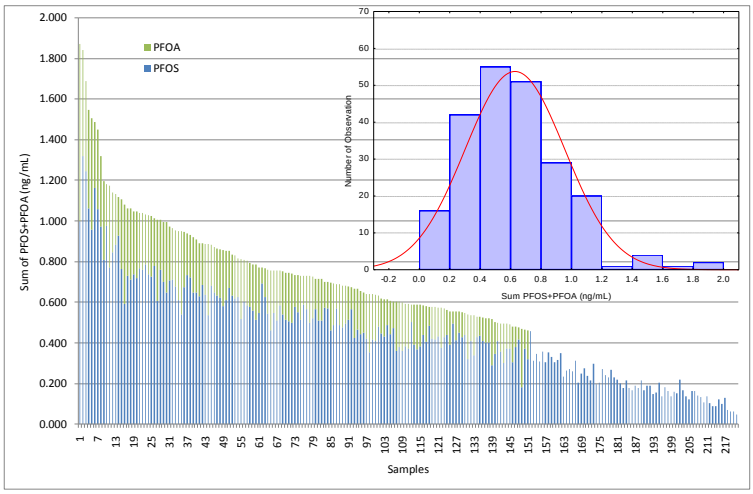

Figure 4: PFCs contamination levels measured in the analysed breast milk samples.