PBDEs IN HUMAN PLACENTA - PRELIMINARY CONCENTRATION LEVELS AND CORRELATIONS WITH PHYSIOLOGICAL PARAMETERS

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Introduction

Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants in consumer products e.g. upholstery, furniture, carpets and electronic equipment. Even though the technical penta and octa formulations of PBDEs have been banned in consumer products in the EU since 2004, they will still be present in and evaporate from existing products. Deca-BDE was exempted from the EU-ban in 2005[1], thus at present, there are no restrictions to the use of deca-BDE in Denmark and most other EU countries. Therefore a continuous exposure to BDE-209 makes it highly important to include this congener in human exposure analysis. However, from 30th of June 2008 the exemption of deca-BDE from list of banned substances under the RoHS Directive will end[1].

Placental tissue are being used for human biomonitoring purposes as persistent compounds can accumulate in the placenta during pregnancy while they are often difficult to detect in blood or urine which reflect a more recent exposure[2]. One of the functions of the placenta is to transport nutrients, gases, and waste substrates between the maternal and foetal circulation system. When xenobiotic compounds are present in the placenta, there is a risk that these compounds can be transferred across the placental barrier to the fetus as well. The placenta is an important endocrine organ during pregnancy, thus possibly being more sensitive to e.g. chemicals which are endocrine disruptors. Relatively small, non-ionized, and lipid soluble molecules can be transported across the placenta by passive diffusion[3]. The placenta develops during pregnancy, where the membranes become thinner in near-term placentas in order to facilitate an increased exchange between mother and foetus[4], which also might affect the transport of xenobiotics.

As a part of an ongoing study, PBDEs have been measured in placental tissue of 22 women. The whole study includes samples of placental tissue, breast milk, maternal and umbilical cord plasma from 43 participants and in addition dust and air in their homes have also been sampled. The participants also filled out an extensive questionnaire on lifestyle and exposure pathways. Preliminary results of dust and air samples are presented in another paper in this issue[5].

Materials and Methods

Placenta tissue samples were collected at the maternity ward of Copenhagen University Hospital from April to December 2007. The placentas were received immediately after birth (all Caesarean sections), a blood sample was drawn from the umbilical cord and a Heparin buffer solution was injected into the blood vessels of the placenta. The placenta was transported to the laboratory and a tissue sample was obtained (100-200g) and stored at -20ºC. Prior to analysis, the fluids (maternal blood and remains of buffer) from the samples were discarded, the tissue was homogenised and 20-25 g of homogenate was taken for chemical analysis. In the chemical analysis, the sample was dried with Hydromatrix®, spiked with recovery standards (^{13}C-transchlordan and CB-198), and Soxhlet extracted for 7 hrs with 500 ml hexane:acetone (4:1). The extracts were purified on a packed column (aluminium oxide w. 10% water, silica gel, silica gel w. 40% sulphuric acid, and sodium sulphate). Finally, the cleaned extracts were evaporated to 500 µl and internal standard (BDE-71) was added. The final extracts were analysed by GC-MS (Agilent GC: HP6890 and MS: HP5973) using ECNI.

Pattern recognition was performed by Principal Component Analysis (PCA), where the structure of the variation in the X-data are attempted explained by projection of the original variables in the high-dimensional space onto a lower dimensional hyperspace spanned by few numbers of latent variables called principal components (PC). The strength of the methodology is the capability of including many intercorrelated variables making it possible to elucidate the correlation patterns between the individual BDE congeners and physiological parameters as presented below. For illustrative purposes the fist two latent variables, or Principal Components, with highest explanatory capacity are shown in Figure 1 and 2, The PCA was performed by use of Unscrabler (CAMO ASA, 2005).

Results and Discussion

Concentration levels

Levels of the individual congeners as well as sums are shown in Table 1. The dominating congeners in placental tissue are BDE-47, 99, 153, and 209. One sample has a very high content of BDE-153 (one order of magnitude larger than all other samples). In the same sample other congeners e.g. BDE-154, which is often closely related to BDE-153, were high but not out of range compared with the other samples. This particular sample had been analysed in duplicate which confirmed the original result. This illustrates the variability between the samples, and as indicated by the ranges in Table 1 the difference between low and high level are often about one order of magnitude. BDE-209 was the most dominating congener in the placental tissue, where it accounted for 40-70% of the ΣPBDE in the samples, indicating a relevant exposure and accumulation in the human body. The levels of the tri- to hepta-BDEs found in this study were similar or slightly higher of what was previously found in Finland, Denmark, and Spain[6,7], BDE-209 has only been reported by Gomara et al. (2007), however, the level of BDE-209 seem to be higher in the present study.

Table 1. PBDEs in placental tissue (n = 22), ng/g lw. Values <LOQ are set to ½LOQ in the calculations of ΣPBDE.

Congener	Mean	Median	Range
BDE-17	< 0.0489	< 0.0439	< 0.0308 - 0.1424
BDE-28	< 0.1082	< 0.0975	< 0.0625 - 0.3005
BDE-49	0.0605	0.0550	< 0.0250 - 0.1591
BDE-47	0.8253	0.7371	0.2942 - 2.4427
BDE-66	< 0.0514	< 0.0442	< 0.0308 - 0.1580
BDE-100	0.1413	0.0920	0.0602 - 1.0496
BDE-99	0.4452	0.3905	0.1464 - 0.7468
BDE-85	< 0.0426	< 0.0412	< 0.0178 - 0.0743
BDE-154	0.1138	0.0986	0.0594 - 0.2256
BDE-153	0.8365	0.3761	0.2254 - 9.4579
BDE-183	< 0.0924	< 0.0889	< 0.0626 - 0.1314
BDE-209	2.6329	2.2984	1.1670 - 6.2161
ΣPBDE (17-183)	2.6425	1.9745	1.3180 - 14.2861
ΣPBDE (all)	5.2754	4.8554	2.4948 - 18.4582
Lipid content	1.31%	1.24%	1.11 - 1.80%

Relation to physiological parameters

Principal Component Analyses (PCA) was performed on log-transformed data to investigate PBDE correlation patterns within and between placental tissues from the individual mothers and possible relations to physiological parameters such as body mass index (BMI). The sample IDs were designed to include information on the number and intensity of potential emissions of PBDEs from electronic products in the respective homes. The sample ID was assigned as follows: xx.a.b.c.d. The first part of the text string, i.e. 'xx', is a randomized identification for the participant or MotherID ranging from 51 to 93. The letter 'a' refers to BMI_{preg}, i.e. BMI at the ultimo stage of pregnancy. The letter 'b' refers to BMI prior to pregnancy. The BMI has been categorized into 0: <18.5 (underweight); 1:18.5-24.9 (normal); 2: 25-29.9 (overweight); 3: ≥30 (obese). The letter 'c' refers to the intensity of exposure to electronic equipment grouped into number of electronic products and rooms

included electronic devices. Lastly, the letter 'd' refers to the presence of electric components in the bedroom (0 = no, 1 = yes).

Original variables included in the PCA are listed in Table 1 and visualised in the resulting loading plot in Figure 1. Original variables in terms of PBDE-congeners were excluded in cases where more than 50% of the samples was below the limit of quantification (LOQ); i.e. BDE 17, 28, 66, 85 and 183. Physiological parameters like BMI, age, months of breastfeeding and number of children as well as the fraction of BDE-209 compared to the total sum of PBDEs in the samples were included in the preliminary analysis. Age of the mother, the number of children, and months of previous breastfeeding were close to the centre of loading plot, and thus had little or no explanation power in the model. Therefore they were removed during optimisation of the model performance parameters. In addition, one sample with very high level of BDE-153 had to be removed as it completely dominated the model; i.e. had high leverage

The resulting loading plot (Figure 1) shows that all original variables have positive loadings in PC1, which represents an explanatory capacity of 39%, thus concentration levels increase from left to right. Furthermore the loading plot showed that the BMIs have insignificant loadings in PC1. The original variables BDE-209, fraction(209) and the BMI-variables have the highest loading in PC2 and explaining 31% of the variation in the data. The second PC reveals a correlation pattern of the original variables BDE-209 and fraction(209), as well as BMI, being inversely correlated to the lower PBDEs.

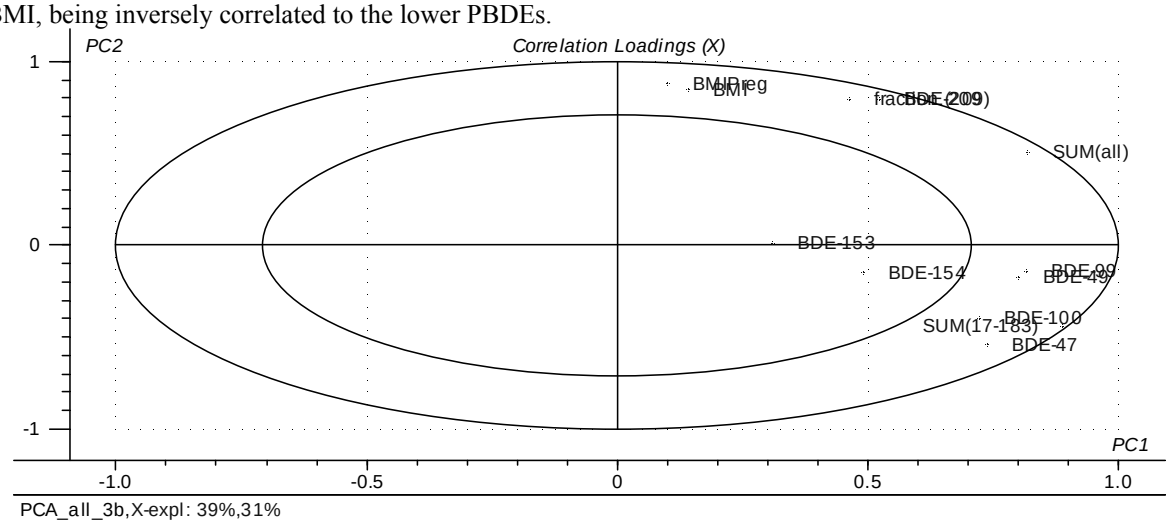

Figure 1. Loading plot of the variables: 7 PBDE congeners 47, 49, 99, 100, 153, 154, 209, Sum(17-183), Sum(all), BMI_{preg}, and BMI. The model has a total explained variance of 70%. The outer ellipse indicates 100% variance and the inner ellipse indicates 50% of explained variance.

When looking at the score plot (Figure 2), the patterns in placental tissue samples in horizontal direction is explained by an increase in BDE-47, BDE-100, BDE-49 and BDE-99, and to a lesser extent BDE-154, BDE-153 and BDE-209, from the left towards the right. As such placenta tissue from mother 52, 55, and 63 have highest concentration of BDE-47 and BDE-100, which is correlated to SUM(17-183). In general, samples with high negative score values in PC1, i.e. being positioned in the left of Figure 2, are characterised by low contamination levels of PBDEs in the placental tissues

The vertical direction, i.e. PC2, samples with high score values have a high level of contamination SUM(all) and as may be observed, samples with high positive score values in PC2 are described by high BMI values, explained by the original variables BMI and BMI_{preg}, which is visualised marked by the highest numbers; e.g. '3.2' following the first two mortherID characters. These high BMI samples have tendency for being placed in the first quadrant and explained therefore mainly by high BDE-209 contamination levels decreasing in diagonal direction towards the third quadrant.

There are patterns of placenta tissue of mothers with lower BMI to have increased contamination levels of tri- to hepta-BDEs compared to mothers with higher BMI, who on the other hand have higher BDE-209 levels. To assure the result, the model was recalculated without the BMI-variables, however, it did not change the model significantly. The mothers with high BMI were still showed tendency for high levels of BDE-209.

Whether this correlation of BMI with BDE-209 and inverse correlation of the lower brominated PBDEs are caused by differences in distribution between lipid depots with increasing weight is unknown. The data has been normalized to lipid content of the tissue, however, correlation between BMI and lipid content of placenta was examined, and no correlation was found.

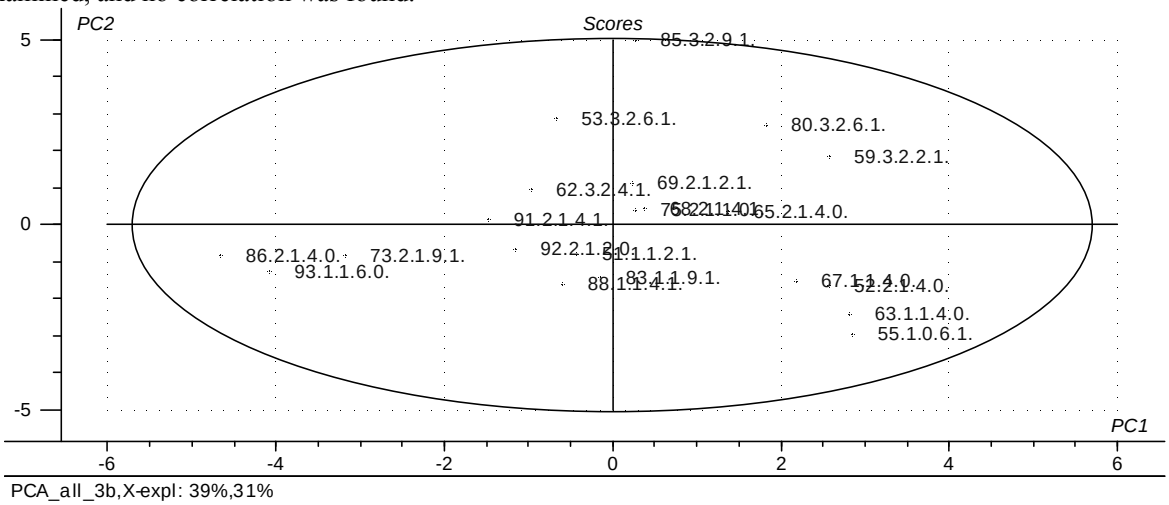

Figure 2. Score plot of the placenta samples (n=21). One sample has been excluded from the model. The sample IDs refer to: motherID. BMI_{preg} . BMI . electronic equipment. electronic eq. in bedroom. as described in the text. The Hotelling T2 ellipse is indicative of outliers in the sample set.

This data set comprises 22 of 52 placenta samples collected. Further information on risk of exposure as well as exposure pathways is expected from the exploration of the questionnaire data on the participants' lifestyle and potential contact with PBDEs and combining this information with measurements on a full data set. In addition analyses of the plasma, milk dust and air samples from the same individuals will contribute to a better understanding of human exposure to PBDEs. For now the present data set, some interesting groupings in the score plot are observed indicating significant differences in exposure sources. The placenta data are in this respect themselves very interesting and provide an idea of the capability of the entire data set regarding the elucidation of varying source intensities and exposure patterns with regard to potential pre-and neo-natal exposure of the child via placenta.

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