

## **Flame retardants, polychlorinated biphenyls and insecticides in pregnant women in the northern part of The Netherlands**

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### **Introduction**

Organohalogens (OHS) are compounds with lipophilic properties, which accumulate in the environment, wildlife and humans. Human exposure is mainly through dietary intake of animal fat<sup>1,2</sup>. After exposure the compounds are slowly metabolised leading to accumulation, mainly in the adipose tissue. During pregnancy the compounds are transported across the placenta to the fetus<sup>3</sup>. OHS have an adverse effect on human health, these adverse effects are more likely to be seen in developing fetus<sup>4</sup>.

In 2001 the Compare project (supported by the European Union) started with the aim to examine the exposure-effect pathways to different kinds of organohalogen compounds. Therefore a cohort was founded in which 90 pregnant women living in the northern part of the Netherlands participated, the Dutch Groningen Infant Cohort. In this cohort *p,p'*-DDE, PCB-153, 4OH-PCB-107, 4OH-PCB-146, 4OH-PCB-187, PCP, BDE-47, 6OH-BDE-47, BDE-99, BDE-100, BDE-153, BDE-154 and HBCDD were determined in blood samples taken in the 20<sup>th</sup> and 35<sup>th</sup> week of pregnancy. Transplacental transfer of these compounds was determined by analyzing the cord blood samples of the infants. The infants were followed for 1½ years to examine the influence of the compounds on their development. In this short paper the results of the levels of the selected OHS in pregnant women are presented.

### **Methods and Materials**

For the Dutch Groningen Infant cohort women were recruited between October 2001 and September 2002. 90 healthy pregnant women, who gave birth to a single healthy child between the 37<sup>th</sup> and 43<sup>rd</sup> week of gestation participated in the study. Blood samples, 30 ml, were obtained at 20<sup>th</sup> and 35<sup>th</sup> week of pregnancy. The blood was centrifugated at 3600 rpm for 10 minutes, after

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which serum was separated and stored in acetone prewashed glass tubes at - 20° Celsius until analysis.

Brominated flame retardants (BFRs) were quantified in 69 randomly selected samples obtained at 35<sup>th</sup> week of pregnancy and in 8 samples of the same women obtained at 20<sup>th</sup> week of pregnancy. Other OHS were analyzed in all 90 samples obtained at 35<sup>th</sup> week of pregnancy.

Lipid content of the samples was based on total cholesterol and triglycerides. Total cholesterol was determined by the CHOD-PAP method (Roche/Hitachi). Triglycerides were determined by the Trig/GB methods (Roche/Hitachi).

Analysis of *p,p'*-DDE, PCB-153, 4OH-PCB-107, 4OH-PCB-146, 4OH-PCB-187, PCP and 6OH-BDE-47, were performed at the Institute for Environmental Studies of the Free University, Amsterdam, The Netherlands, as follows:

### Chemicals

Standard solutions containing the hydroxylated PCB's and both phenols were a kind gift from professor Åke Bergman of the Department of Environmental Chemistry, University of Stockholm. PCB 153, 198 and *p,p*-DDE were purchased from Dr. Ehrenstorfer. Hexane (Ultra-resi, Baker); methyl *tert*-butyl ether (MTBE, Ultra-resi, Baker); 2-propanol (Chromasolv, Riedel-deHaën); dichloromethane (Suprasolv, Merck); Diethylether (p.a. Merck); Silicagel 60 (0.063-0.200mm, Merck); Sulphuric acid (p.a. Fluka); Hydrochloric acid (intra analyzed, Baker); potassium hydroxide (p.a., Riedel-deHaën); potassium chloride (baker-grade, Baker) were used. Ethereal diazomethane, used for the derivatization of phenolic compounds, was generated from N-methyl-N-nitroso-ureum and 50% w/v potassium hydroxide in diethylether according to Vogel<sup>5</sup>.

### Instrumentation

Gas chromatography, was performed on a Agilent 6890 GC, equipped with a HP 7683 autosampler, an Agilent Electron Capture Detector ( $\mu$ ECD) and a Gerstel CIS4 injector with a Siltek baffled glassliner in a splitless mode. The column used was a Varian CP-Sil-8cb (25m x 0.15mm i.d., 0.12  $\mu$ m film thickness) equipped with a retention gap (2m x 0.53mm i.d. Varian 4076) using hydrogen as carrier gas and nitrogen as make-up gas.

The column-oven temperature was programmed as follows: 60°C (2min), 50°C/min up to 200°C (0 min), 1°C/min up to 230°C (0min), 30°C up to 300°C (3min).

### Clean-up and analysis

The serum samples ( $\pm$  5 ml) were extracted based on a previously described method by Hovander et.al<sup>6</sup>. Prior to extraction PCB198 (2,2',3,3',4,5,5',6-octachlorobiphenyl), 2,4,5-trichlorophenol and 4OH-PCB193 (4OH-2,3,3',4',5,5',6-heptachlorobiphenyl) were added as internal standards. The neutral compounds were subsequently separated from the phenolic compounds by a liquid/liquid partitioning with potassium hydroxide (0.5 M in 50% Ethanol). After adding 2M HCl, the phenolic compounds were extracted from the potassium hydroxide with a mixture of MTBE:hexane (1:9 v/v). The phenolic fraction was methylated overnight with ethereal diazomethane at 4 °C. Both neutral and phenolic fractions were shaken with concentrated sulphuric acid to remove lipids. A second clean-up step was performed by using a sulphuric acid-silica gel column (1 g of concentrated sulphuric acid: silicagel (1:2 w/w)). The neutral fraction was eluted with hexane and the phenolic fraction with hexane:dichloromethane (8:2 v/v). After elution the extracts were quantitatively transferred into a autosampler vial for measuring on the GC-ECD. With every 10 samples one blank (water, HPLC-grade, Baker) and one reference sample (serum, Academic Hospital, Vrije Universiteit Amsterdam) was included. The levels of the compounds were corrected

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for the blank values. Recovery of the internal standards 2,4,5-trichlorophenol, PCB 198 and 4OH-PCB 193 was 98% (SD 25%, n=98), 89% (SD 10%, n=97) and 89% (SD 20%, n=96) respectively.

Analysis of BDE-47, BDE-99, BDE-100, BDE-153, BDE-154 and HBCDD, were performed at the Department of Environmental Chemistry of the Stockholm University, Stockholm, Sweden. The details of analytical methods are described in the report of Weiss et.al in the current edition of Organohalogen Compounds.

### Results and Discussion

In table 1 anthropometric measurements of the women are presented. All women met the inclusion criteria and resemble the normal Dutch population.

	<i>Median</i>	<i>Range</i>
<b>Females</b>		
Age	32 years	24-42
Height	171 cm	154-183
Weight	68 kg	49-109

Table 1. Characteristics of the cohort, in median value with range.

Blood levels of OHS determined in the 35<sup>th</sup> week of pregnancy are shown in table 2. Except for 6OH-BDE-47 almost all levels were above the detection limits. A wide range in blood levels was observed for all the compounds.

To our knowledge this is the first study describing the levels of PBDEs (except for BDE-47) and HBCDD in pregnant women.

We compared the levels found in our cohort to levels found in pregnant women from cohorts in other countries, which were founded no more than 5 years prior to our cohort. Comparison was made between levels determined in the last trimester of pregnancy. BDE-47 levels found in our cohort are 7 times lower compared to levels reported from the USA<sup>7</sup> and 5 times higher compared to levels reported from Sweden<sup>8</sup>. In pregnant women from Belgium<sup>9</sup> and Spain<sup>10</sup> the levels of *p,p'*-DDE and PCB-153 were higher compared to the levels in our study. The levels of PCB-153 and the hydroxylated metabolites in the cohort from Sweden<sup>8</sup> were lower than in our cohort and the levels of PCP were higher than in our cohort.

Compared to a cohort founded in The Netherlands 10 years prior to our cohort the level of PCB-153 were lower by 50% (830 pg/g serum<sup>11</sup> compared to 444 pg/g serum).

In our cohort BDE-153 had in the highest level of the BFRs, both at 20<sup>th</sup> and 35<sup>th</sup> week of pregnancy, in contrast to studies done in breast milk where BDE-47 accounted for the highest PBDE levels found<sup>12,13</sup>.

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	<b>Median</b>	<b>Range</b>	<b>n.d.</b>	<b>(N)</b>
<i>P,p</i> -DDE*	134.8	26.7-579.6		90
PCB-153*	94.5	28.8-351.9		90
PCP	970.7	296.6-8531.5		90
4-OH-PCB-107	25.8	n.d.-119.9	3	90
4-OH-PCB-146	103.3	36.3-695.5		90
4-OH-PCB-187	79.5	35.8-477.8		90
BDE-47*	4.1	0.9-25.6		69
6-OH-BDE-47	n.d.	n.d.-n.d.	90	90
BDE-99*	1.2	n.d.-8.7	3	69
BDE-100*	0.9	0.1-5.8		69
BDE-153*	5.6	1.2-73.2		69
BDE-154*	1.8	0.6-13.6		69
HBCDD*	1.3	n.d.-11.8	1	69

Table 2. Levels of organohalogens determined at 35<sup>th</sup> week of pregnancy. Data are median and range. N.d. = none detected. (N) = number of samples. Reported levels are pg/g serum or \*ng/g lipid.

The BFRs were analysed in 8 samples obtained at 20<sup>th</sup> week of pregnancy. BDE-153 was the main BFR (5.5 ng/g lipid), followed by BDE-47 (4.7 ng/g lipid), BDE-154 (3.1 ng/g lipid), HBCDD (1.4 ng/g lipid), BDE-99 (1.3 ng/g lipid) and BDE-100 (1.0 ng/g lipid). The median values are presented.

The levels at the 20<sup>th</sup> and 35<sup>th</sup> week of pregnancy were highly comparable.

In this short paper an overview of levels of some selected OHS in the 20<sup>th</sup> week and 35<sup>th</sup> week of pregnancy has been given. The selected OHS could be found in the almost all blood samples in this cohort except for 6OH-BDE-47 which could not be detected in any of the samples. Most of the levels of OHS determined in our cohort are higher than levels found in a similar cohort in Sweden and lower than levels found in similar cohorts in Belgium and Spain. BDE-47 levels in our cohort are much lower compared to levels found in the USA. Of the BFRs BDE-153 was the main congener. These differences reflect different magnitudes of exposure to these compounds in the food chain. The lower levels of PCB-153 in our cohort compared with previous Dutch cohort reflect the ban on the use of the compounds in industry.

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