Effects of perinatal PCB and dioxin exposure and early feeding mode on child development
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Chapter 2

Polychlorinated biphenyls in adipose tissue, liver, and brain from nine stillborns of varying gestational ages

ABSTRACT

We analyzed polychlorinated biphenyls (PCBs) in subcutaneous adipose tissue, liver, and brain of 9 fetuses who died in utero. Their median (range) gestational ages and birthweights were 34 (17-40) weeks and 2050 (162-3225) g. Three fetuses were small for gestational age. The levels of PCB congener nos. 118, 138, 153, and 180, and the sum of these (ΣPCB), were calculated in terms of tissue total fat content (ng/g fat).

The median (range) ΣPCB (in ng/g fat) amounted to: adipose tissue 235 (97-768), liver 198 (67-362), and brain 50 (22-122). Median (range) ΣPCB levels in liver and brain were 0.8 (0.4-0.9) and 0.2 (0.1-0.3) times, respectively, as high as the ΣPCB levels in adipose tissue. There were strong relations between ΣPCB in adipose tissue and ΣPCB in liver (r=0.98; p<0.01), and between ΣPCB in adipose tissue and ΣPCB in brain (r=0.91; p<0.01). Adipose tissue, liver, and brain did not show differences in the distribution of congeners 118, 138, 153 and 180, and there was no statistically significant association between tissue PCB levels and gestational age (r varied between 0.22 and 0.47). Median ΣPCB levels in fetal adipose tissue proved to be comparable with our previously established ΣPCB levels in mature breast milk of 93 Dutch women (median 414; range 158-969 ng/g fat). The PCB congeneric distribution of fetal adipose tissue was not different from that of human milk.

We conclude that maternal PCBs have a tendency to accumulate notably in fetal tissues with high triglyceride contents. They are easily transferred across the placenta and seem to become equilibrated among the apolar parts of maternal and fetal lipids.
INTRODUCTION

Polychlorinated biphenyls (PCBs) are widespread toxic environmental pollutants. They are polycyclic aromatic compounds, and have a total number of 209 possible congeners which differ in their degree of chlorination and the molecular position of the chlorine atoms. PCBs have extremely long half-lives and are strongly lipophilic. These substances are resistant to high temperatures, they can easily conduct heat, and have electrical insulating properties. PCBs have been used in a wide range of industrial products, including fire retardants, plasticizers, dielectrical fluids in capacitors and transformers, and hydraulic fluids. Their environmental persistence was recognized in the late 1970s, and PCBs were subsequently banned world-wide. PCBs have, however, been produced until the mid-1980s in e.g. Eastern European countries. In the industrialized world residues can at present be found in water, soil, and biological tissue. Due to their chemical stability, these compounds become increasingly concentrated while being transferred through the food chain. High PCB levels can be found in adipose tissue of organisms at the top of the food chain, including human beings.

PCBs enter the fetus via the placenta. In the postnatal period, they are transferred from the nursing mother to her child through breast milk. Exposure to ‘background’ levels of PCBs is known to have adverse effects on child development. Although relatively large amounts of PCBs are ingested with breast milk, greatest risks have been associated with exposure during the prenatal period. Higher levels of intrauterine exposure to PCBs, as determined by PCB levels in maternal and cord plasma, have been found to result in deficits in fetal and postnatal growth, a less optimal neurological development at the ages of two weeks and 18 months, a lower score on psychomotor developmental tests up to the age of two years, and a lower intelligence quotient at 11 years of age.

There are no data on the PCB body burden of the human fetus at different gestational ages and the distribution of these compounds among fetal organs. We analyzed the PCB contents in subcutaneous adipose tissue, liver, and brain of nine stillborns of varying gestational ages.

MATERIALS AND METHODS

Samples
From March to December 1993, we collected tissue samples of fetuses who died in utero. Each eligible stillborn that was presented for obdution to one of the Public Health Laboratories located within a radius of 60 km...
from Groningen was included in the study. The Groningen region is a semi-urban area in the Northeast of The Netherlands. For inclusion, fetuses had to show no signs of serious chromosomal or congenital malformations. Also, the absence of signs of maceration was a prerequisite for inclusion. From each stillborn we collected 10 grams each of subcutaneous white adipose tissue, liver, and brain. Depending on total brain size, tissue was sampled from the parieto-temporal or parietal area. The samples were stored at -20°C until analysis. Birthweight, gestational age, presumed cause of death, and the age of the mother were recorded. Informed consent was obtained from the parents. The study protocol was approved by the local medical ethics committees, and is in agreement with the Helsinki Declaration of 1975, as revised in 1989.

**Analyses**

Tissue levels of 26 PCB congeners were determined in the TNO Nutrition and Food Research Institute (Zeist, The Netherlands) as described previously. Briefly, tissue samples were weighed, homogenized, and extracted with organic solvent by means of a soxhlet apparatus. Total fat was determined gravimetrically after evaporation of a part of the organic solvent layer to dryness. Another part of the extract was concentrated and purified by column chromatography on basic alumina that was previously deactivated with 10% water. An aliquot was analyzed for PCBs by gas-liquid chromatography/electron capture detection with the use of two capillary columns of different polarity. The recovery amounts typically to >90%, and the variation coefficient is <10%.

**Data analysis and statistics**

PCB levels were expressed on the basis of the extractable tissue fat content (ng/g fat). We report only on the levels of the congeners 118, 138, 153 and 180 (International Union of Pure and Applied Chemistry nomenclature). The levels of these congeners are relatively high and accurately measurable. In Western Europe, they are considered to be markers for the levels of the toxicological most relevant congeners (i.e. the non- and mono-ortho PCBs). The sum of the levels of the congeners 118, 138, 153 and 180 (ΣPCB) was calculated.

For each fetus we established the ratio of ΣPCB in liver and adipose tissue, and the ratio of ΣPCB in brain and adipose tissue. The congenic distribution patterns of the four PCBs in adipose tissue, liver or brain were obtained by normalization to 100% (g/100 g). Spearman rank correlation coefficients were calculated to evaluate the relationships between PCB levels in adipose tissue, liver, and brain, and to investigate associations between tissue PCB levels and gestational age. P-values of
0.05 or less were considered statistically significant.

**RESULTS**

During the study period nine stillborns were found to be eligible for inclusion. The characteristics of the nine stillborns are presented in table 1.

**Table 1: Characteristics of the stillborns.**

<table>
<thead>
<tr>
<th>Fetus nr.</th>
<th>GA* (wks)</th>
<th>Body weight† (g)</th>
<th>Fat content (%)</th>
<th>Maternal age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Adipose tissue</td>
<td>Liver</td>
<td>Brain</td>
</tr>
<tr>
<td>1</td>
<td>17</td>
<td>162</td>
<td>0.8</td>
<td>1.6</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>500</td>
<td>5.3</td>
<td>1.1</td>
</tr>
<tr>
<td>3</td>
<td>28</td>
<td>1220</td>
<td>21.7</td>
<td>4.0</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>2791</td>
<td>26.1</td>
<td>6.0</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>1640‡</td>
<td>46.3</td>
<td>1.2</td>
</tr>
<tr>
<td>6</td>
<td>36</td>
<td>2424</td>
<td>27.7</td>
<td>2.1</td>
</tr>
<tr>
<td>7</td>
<td>36</td>
<td>2050‡</td>
<td>32.9</td>
<td>6.9</td>
</tr>
<tr>
<td>8</td>
<td>38</td>
<td>3225</td>
<td>32.1</td>
<td>6.5</td>
</tr>
<tr>
<td>9</td>
<td>40</td>
<td>2920‡</td>
<td>22.9</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* GA gestational age at death, † body weight at death, ‡ small for gestational age (≤10th percentile, according to Kloosterman*).  

The median gestational age was 34 weeks (range: 17-40), median birthweight was 2050 g (162-3225) and median maternal age was 30 years (18-32). Three stillborns (nos. 5, 7 and 9) were considered small for gestational age, i.e. ≤10th percentile for gestational age according to Kloosterman*.

The presumed causes of death were: chronic (nos. 4, 6, 7) and acute (nr. 5) placental insufficiency, abortion (nr. 1), ventricular bleeding...
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(nr. 3), intrauterine pneumonia (nr. 2), or unknown (nos. 8, 9). No adipose
tissue could be obtained from the fetus who died at 17 weeks (nr. 1) and
also its liver PCB congener 180 was found to be below the detection limit.

For each of the fetal compartments, the correlations between the
levels of the PCB congeners 118, 138, 153, and 180, and the \( \Sigma \)PCB on
the one hand, and the gestational age on the other were not significant;
correlation coefficients varied between 0.22 and 0.47. The PCB levels in
adipose tissue, liver, and brain (in ng/g fat) are presented in table 2. The median (range) liver/adipose tissue ratio of \( \Sigma \)PCB amounted to 0.8 (0.4-
0.9) g/g, and the \( \Sigma \)PCB brain/adipose tissue ratio was 0.2 (0.1-0.3) g/g.
There were strong relationships between \( \Sigma \)PCB in adipose tissue and liver
(\( r=0.98; p<0.01 \)), and between \( \Sigma \)PCB in adipose tissue and brain (\( r=0.91;
p<0.01 \)). The congenic distribution patterns of PCBs in subcutaneous
adipose tissue, liver, and brain (in g/100 g) proved similar (table 3).

**Table 2:** PCB levels in fetal subcutaneous adipose tissue, liver, and brain*.

<table>
<thead>
<tr>
<th>Congener†</th>
<th>Adipose tissue (ng/g fat)</th>
<th>Liver (ng/g fat)</th>
<th>Brain (ng/g fat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB 118</td>
<td>20 (11-51)</td>
<td>17 (7-35)</td>
<td>6 (3-22)</td>
</tr>
<tr>
<td>PCB 138</td>
<td>64 (30-189)</td>
<td>58 (20-120)</td>
<td>15 (7-31)</td>
</tr>
<tr>
<td>PCB 153</td>
<td>106 (37-321)</td>
<td>96 (25-154)</td>
<td>20 (9-43)</td>
</tr>
<tr>
<td>PCB 180</td>
<td>51 (9-208)</td>
<td>43 (8-93)</td>
<td>10 (2-30)</td>
</tr>
<tr>
<td>( \Sigma )PCB</td>
<td>235 (97-768)</td>
<td>198 (67-362)</td>
<td>50 (22-122)</td>
</tr>
</tbody>
</table>

ratio to \( \Sigma \)PCB

* data represent median (range), † \( \Sigma \)PCB sum of PCB 118, 138, 153, and 180, \( \Sigma \)PCB
adipose \( \Sigma \)PCB in adipose tissue.
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Table 3: PCB congeneric distribution in fetal subcutaneous adipose tissue, liver, and brain*.

<table>
<thead>
<tr>
<th>Congener†</th>
<th>Adipose tissue (g/100 g)</th>
<th>Liver (g/100 g)</th>
<th>Brain (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB 118</td>
<td>8 (6-21)</td>
<td>9 (3-21)</td>
<td>11 (9-20)</td>
</tr>
<tr>
<td>PCB 138</td>
<td>28 (24-31)</td>
<td>29 (24-33)</td>
<td>29 (25-31)</td>
</tr>
<tr>
<td>PCB 153</td>
<td>44 (39-47)</td>
<td>43 (37-45)</td>
<td>42 (33-48)</td>
</tr>
<tr>
<td>PCB 180</td>
<td>19 (9-27)</td>
<td>21 (13-28)</td>
<td>16 (7-24)</td>
</tr>
<tr>
<td>ΣPCB</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

* data represent median (range), † ΣPCB sum of PCB 118, 138, 153, and 180.

**DISCUSSION**

We report on the levels of the PCB congeners 118, 138, 153 and 180 in subcutaneous adipose tissue, liver, and brain of nine stillborns of 17-40 gestational weeks. PCB levels in The Netherlands are comparable to those found in other parts of the industrialized world, including the United States8-10. Nevertheless, due to the small number of fetuses, the reported levels should rather be regarded as indicative than representative for tissue levels of the general population of unborn children.

Although the vulnerability of the fetal organs might differ, fetal organ-specific PCB levels and the accumulation of congeners in the different fetal organs can be used for risk evaluations. We found that fetal liver and brain ΣPCB levels, expressed on a fat weight basis, are 80% and 20%, respectively, of that encountered in subcutaneous adipose tissue. Congeneric distribution patterns did not differ among the organs. The tissue distribution pattern that we found is in agreement with that reported for other species15, 16.
Different levels of the organs are likely to be caused by differences in the polarity of the fats in each of these organs. More specific it indicates the affinity of the highly apolar PCBs for the highly apolar (storage) lipids notably triglycerides and cholesterol esters, and their moderate affinity for the (structural) amphipathic lipids, notably phospholipids and cholesterol. The cytoplasm of the fat cell is largely composed of a triglyceride droplet. The liver can be regarded as an intermediate storage place for triglycerides after the uptake of fatty acids or lipoprotein remnants from the circulation, or local de novo synthesis of fat from polar precursors. The cytoplasm may also contain some redundant cholesterol esters, but compared with adipose tissue there is a relatively higher contribution from cell membrane phospholipids and cholesterol. The fat in the brain derives merely from membranes and is therefore largely composed of phospholipids and cholesterol.

Since fat from both adipose tissue and human milk is almost exclusively composed of triglycerides, we investigated whether fetal adipose tissue contains, on a fat basis, similar PCB levels compared with human milk. For this we used our previous PCB data of 93 mature milk samples from Dutch mothers who also lived in the Groningen area (table 4). Fetal adipose tissue levels of PCB congeners 118, 138, 153 and 180, and also ΣPCB, (table 2) fell well within the corresponding ranges of milk levels, and also the fetal adipose tissue congenic distribution (table 4) proved to be comparable with that of human milk. The high degree of
similarity between PCBs levels in maternal and fetal fat suggests that PCBs readily cross the placenta, and subsequently equilibrate among lipid compartments according to their high affinity for notably triglycerides and cholesterol esters, and the lower affinity for phospholipids and cholesterol. It should be stressed that, due to ethical reasons, we were not able to draw blood from the mother and investigate the individual ratios for maternal and fetal PCB levels. Therefore, we can not exclude the existence of a partial placental barrier.4

In contrast to postnatal exposure via breast milk, prenatal exposure to PCBs has been found to be the most critical for future growth and development.4-11. During fetal organ development, the transient period of growth spurt is supposed to be one of special vulnerability to adverse influences. The timing and duration of this period of rapid cell multiplication varies between organs. As the fetal PCB levels were found to bear no relationship with the gestational age of the fetus, all of the developing fetal organs are equally at risk of prenatal PCB-induced toxicity. The independency of PCB levels of gestational age reflects a rapid equilibration between the fetal and maternal PCB stores. In the last three months of gestation, an overproportional increase of adipose tissue relative to the total body weight takes place.19. Since the deposited fat is mainly synthesized in the fetus from polar precursors (glucose, lactate), the absence of any dilution effect suggests that this initially PCB-poor fat is rapidly provided with maternal PCBs. It should be kept in mind that, due to the cross-sectional study design and the small number of stillborns, a subtle effect of the gestational age can not be excluded.

We conclude that maternal PCBs have a tendency to accumulate notably in fetal tissues that contain high levels of storage lipids (i.e. especially triglycerides). Consequently, on a fat basis liver contains 80% and brain 20% of the PCB levels encountered in adipose tissue. Fetal tissue PCB levels are not dependent on gestational age, and there are no organ-dependent differences in the distribution patterns of the PCB congeners 118, 138, 153 and 180. Fetal adipose tissue PCB levels compare well with those in human milk. These data suggest that the flux of maternal PCBs across the placenta is of sufficient magnitude to accomplish similar PCB levels in storage fat that is de novo synthesized in the fetus from polar precursors.

ACKNOWLEDGEMENTS

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References


