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Predictors of Polychlorinated Biphenyl Concentrations in Adipose Tissue in a General Danish Population

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Polychlorinated biphenyls (PCBs) are ubiquitously present in the environment and suspected of carcinogenic, neurological, and immunological effects. Our purpose was to identify predictors of adipose tissue levels of mono-, di-, and tri-ortho-substituted PCBs experienced by a general population and to establish whether predictors vary according to substitution group. In this study of 245 randomly selected persons from a prospective Danish cohort of 57 053 persons, we examined geographical area, age, lactation, BMI, and intake of eight major dietary groups as potential determinants of adipose concentrations of mono-, di-, and tri-ortho-substituted PCBs by linear regression analyses. Lactation, BMI, and intake of fruit, vegetables, and dairy products showed negative associations with PCB concentrations in adipose tissue in all models, and living in Copenhagen city, age, and consumption of fish (particularly fatty fish) were positively associated. The associations between several of the predictors and mono-ortho-substituted PCBs tended to differ from the associations found for di- and tri-ortho-substituted PCBs. In conclusion, geography, age, lactation, BMI, and consumption of fatty fish consistently predicted the concentration of PCBs in adipose tissue. Our results indicate that predictors of PCBs varied according to substitution group, suggesting that ortho-substituted groups should be analyzed separately.

Introduction

Polychlorinated biphenyls (PCBs) were introduced in the late 1920s, and manufacture was stopped in the 1970s due to evidence of environmental build-up (1). During this period more than 1.5 million metric tons were produced worldwide, and it is believed that at least one-third of these PCBs found their way into the natural environment (2, 3) where they are ubiquitously present as a complex mixture of mother compounds and metabolites. PCBs are characterized by high lipid solubility, environmental persistence, and bioaccumulation and their semivolatile characteristics predispose them to long-range transport (4). PCBs are not readily cleared from the body, and half-lives in humans are influenced by ongoing exposure, fluctuations in body weight, and intrinsic elimination. When eliminating the influence of these factors, the estimated half-lives of individual PCB congeners in humans range from 2.6 to 15.5 years (5).

PCBs are carcinogenic in animals (6), and background levels in humans influence the fetal, neonatal, and infant immune systems (7, 8) and have been associated with low sperm counts (9), testicular anomalies (9), and premature delivery of fetus (10). These factors have prompted a growing number of epidemiological studies that have investigated blood or adipose levels of PCBs and their metabolites in association with cancer, neurodevelopmental effects, immunotoxicity, and reproductive outcomes (4, 11). Thus, the identification of factors associated with the body burden of PCBs is important in relation to possible guidelines to reduce population exposure to these compounds.

Bioaccumulation of PCBs through the food chain has resulted in detectable levels of PCBs in meat, dairy, and fish (12), and recent studies have suggested that the ingestion of fish is the strongest predictor of present day body burdens (13). Most previous studies of dietary predictors only consider total fish consumption (14–16). However, lipophilic organic chemicals accumulate in the fatty tissue of fish, and thus, consumption of fish with a high fat content is probably a stronger predictor of PCBs in humans than total fish consumption.

When studying predictors of PCBs, these compounds are often treated as a homogeneous group and total PCBs are typically used. However, environmental persistence, bioaccumulation in food chains, and distribution in human tissue depend on the chemical structure of individual congeners; thus, studying total PCBs may not adequately characterize all substitution groups (1). Only mono-ortho PCBs display dioxin-like properties believed to be important in cancer induction (12, 17), and a few groups have reported that only mono-ortho-substituted congeners were related to cancer risk (18, 19). Therefore, information about predictors of specific PCB congener groups may be valuable but still scarce.

Our objective was to identify predictors of adipose tissue levels of mono-, di-, and tri-ortho-substituted PCBs experienced by the general Danish population.

Materials and Methods

Between December 1993 and May 1997, 80 996 men and 79 729 women aged 50–64 years were invited to participate in a prospective study “Diet, Cancer and Health”. The participants had to be born in Denmark, live in the Copenhagen or Aarhus areas, and be without a cancer diagnosis registered in the Danish Cancer Registry at the time of invitation. A total of 57 053 persons, corresponding to 36% of those invited, were enrolled in the cohort. Participation was based on written informed consent. Staff members in the study clinics obtained anthropometric measurements, including height and weight, and took an adipose tissue biopsy from the buttock of each participant using a luer-lock system (Terumo, Terumo Co., Tokyo, Japan), yielding an average of 29 mg (range, 1–97 mg) of tissue. Within 2 h of collection, all samples were frozen at −20 °C and within 8 h put in liquid nitrogen vapor (max, −150 °C) for long-term
storage. The regional ethics committee for human studies in Copenhagen and Aarhus approved the study.

A case-cohort study was conducted to determine risk factors of non-Hodgkin’s lymphoma. A total of 239 cases and 245 gender-matched subcohort members were included; the method of selection has been described elsewhere. Our analyses of predictors of PCB levels were based on the 245 random subcohort members.

Upon enrollment, each participant completed self-administered questionnaires that included questions on dietary habits, health status, family history of cancer, social factors, reproductive factors, and lifestyle habits (20). The food frequency questionnaire included 192 food items and a description of the development and validation of this questionnaire has previously been published (21). Participants were asked how often on average they had consumed the different types of foods during the preceding 12 months. The frequency consumption was categorized into 12 groups ranging from never to 8 or more times daily. A mean daily intake of foods (g/day) was calculated by multiplying the frequencies of intake by a gender-specific portion size using the software Foodcalc version 1.3 (22).

**PCB Analyses.** Ten PCB congeners (International Union of Pure and Applied Chemistry nos. 99, 118, 138, 153, 156, 170, 180, 183, 187, and 201), all with at least five chlorine substitutions, were measured. These 10 were selected as they were among the 26 congeners considered most environmentally threatening due to their prevalence, relative abundance in animal tissues, and potential toxicity (23). Samples were analyzed at the Centre de Toxicologie du Québec, Institut National de Santé Publique du Québec. The laboratory is accredited under ISO 17025 by the Standards Council of Canada and participates in many national and international quality control programs including the Northern Contaminants Program of the Ministry of the Environment of Ontario, the External Quality Assessment Scheme, QUASIMEME http://www.quasimeme.marlab.ac.uk/, as well as the German External Quality Assessment Scheme for Biological Monitoring in Occupational and Environmental Medicine.

The adipose tissue samples were aspirated from the needle into a vacutainer tube under vacuum. The samples were then fortified with internal standards, mixed with dichloromethane, and chemically dried using sodium sulfate. A part of the organic solvent was used to determine the percentage of total lipids in the sample. The remaining fraction was concentrated by evaporation, subsequently purified using gel permeation chromatography, and cleaned up on a florisil column. The extracts were analyzed on a gas chromatography–mass spectroscopy instrument from (Agilent Technologies (Hewlett-Packard; Palo Alto, CA) model 6890/5973) using a DB-5 capillary column. Details of gas chromatography–mass-spectrometry parameters are provided in the Supporting Information.

The total lipid content was determined on the designated extract using a gravimetric method. Two hundred microliters was precisely weighed on an analytic balance, and the solvent evaporated at room temperature in a desiccator. The resulting lipid weight was adjusted to the initial sample weight, and the percentage of lipid content was calculated. The PCB concentrations were expressed in microgram per kilogram of lipids.

Routine checks of the accuracy and precision of the PCB measurements were done using reference materials from the National Institute of Standards and Technology (Gaithersburg, MD) and by participation in quality assessment schemes. The interday precision was between 5.1% and 7.3% for the PCB congeners. On the basis of spiked levels (5 μg/kg in corn oil, n = 3) recovery was between 87% and 96% for the different PCB congeners. Details of analytical quality assurance, quality control, and limit of detection (LOD) are reported in the Supporting Information.

**Statistical Methods.** Potential predictors of the mono-, di-, and tri-ortho PCB groups and total PCB levels were analyzed by generalized linear models using the GLM procedure of SAS (version 9.1; SAS Institute, Cary, NC). To be able to include the PCB levels below the LOD (5.3% of the samples) they were estimated according to the formula LOD/ square root 2, suggested by Hornung and Reed (24). The residuals of the four PCB models were randomly distributed after transformation by the natural logarithm.

The explanatory variables used in all models were geographical area, gender, age at time of clinic visit, total lifetime duration of lactation, BMI, consumption of foods of animal origin (dairy products, eggs, fish, meat, poultry), and consumption of fruit and vegetables (Table 1). The following approaches were used: (i) univariate regression analyses, where each explanatory variable was included individually in the model, and (ii) a multiple regression approach, including all explanatory variables with mutual adjustment. We also tested whether age (above and below median age of 56.3 years) or gender modified the association between the PCBs and the variables that seemed to predict the PCB concentration: geography, lactation, BMI, and consumption of fatty fish, fruit, and vegetables.

**Results.**

The baseline characteristics, daily intake of major dietary groups, and lipid-adjusted concentration of PCBs in adipose tissue are presented in Table 1. With regard to geographical area, 59, 114, and 72 of the participants lived in Copenhagen, suburban Copenhagen, and the Aarhus area, respectively, and this was equally distributed according to gender. The PCB congeners with a di-ortho substitution pattern (with the exception of PCB 99) had the highest median concentrations among the PCBs, representing 82% of the total sum of the measured PCBs.

Both the uni- and multivariate analyses (Tables 2 and 3) showed that living in the Aarhus area was associated with lower adipose levels of all PCB substitution groups compared with living in Copenhagen. People living in suburban Copenhagen had intermediate concentrations. We also found that total lifetime lactation and consumption of fruit, vegetables, and dairy products were inversely associated, while age and consumption of fish, regardless of fat content, was positively associated in both models. In the multivariate models consumption of fish with a high fat content had a stronger association with PCB concentrations than lean fish or fish with a medium fat content.

Estimates for the determinants of mono-ortho-substituted PCBs differed from the estimates obtained in the di-ortho-substituted PCBs, tri-ortho-substituted PCBs, and total PCB models, the last three challenge the very similar estimates. This difference was most apparent in the multivariate models (Table 3). We found that BMI was inversely associated with all PCB substitution groups and that this association was insignificant for the mono-ortho-substituted PCBs and highly significant (P ≤ 0.0004) for the other substitution groups. Consumption of fruit and vegetables was also inversely associated with all substitution groups, but this association was only significant for the mono-ortho-substituted PCBs.

We found a statistically significant interaction with gender (P = 0.04) with regard to the association between the tri-ortho-substituted PCBs and consumption of fatty fish, with a stronger association among women (14.9; 95% CI: from 3.49 to 27.7) than among men (1.74; 95% CI: from −6.28 to 10.4), and this modification was borderline significant for the mono- (P = 0.05) and di-ortho (P = 0.07) substituted PCBs. Gender did not modify the associations between the
PCBs and geography, BMI, and consumption of fruit and vegetables \( (p \) for all interactions >0.41). Also, age did not modify the associations between the PCBs and geography, lactation, BMI, and consumption of fatty fish, fruit, and vegetables \( (p \) for all interactions >0.30).

The effect estimates for all variables except the intake of meat and eggs were similar in the univariate and multivariate models. The multivariate models presented in Table 3 explained 17%, 36%, 28%, and 34% of the variation in the concentration of the sum of the mono-, di-, and tri-ortho-substituted PCBs and total PCBs, respectively \( (R^2 \) values). Dietary variables were important and explained over 50% of the above variations for all substitution groups, and fish with a high fat content was the most important dietary factor considered, explaining almost one-half of the variation originating from all dietary variables.

### Discussion

Lactation, BMI, and consumption of fruit, vegetables, and dairy products showed negative associations with PCB concentrations in adipose tissue, whereas living in Copenhagen city, age at time of enrollment, and consumption of fish (particularly fatty fish) showed positive associations. Men had higher concentrations of di- and tri-ortho-substituted PCBs. Estimates of the mono-ortho-substituted PCBs differed from the estimates of the other substitution groups for gender, BMI, and intake of fruit and vegetables.

The high median concentrations of the di-ortho-substituted PCBs compared with the other substitution groups are in accordance with previous studies and probably reflect the slower human metabolism of these congeners \( (25) \). The generally lower PCB concentrations in women may be explained by elimination via lactation among women or may be due to the higher body fat percentage and lower muscle mass generally observed among women when compared to men, resulting in a dilution effect. Consistent with this dilution theory we found an inverse relation between PCBs and BMI. Although correlations vary slightly across studies, our finding supports the general picture of a negative association between PCBs and BMI \( (14, 15, 26) \).

In our study, geography accounted for a large proportion of the total variation in our models and we found that people living in the Aarhus area of Denmark had lower levels of adipose PCB concentrations than people living in Copenhagen while those in the suburban Copenhagen had intermediate levels. Three studies have previously reported geographical variations in total PCB adipose/blood concentrations in nonoccupationally exposed persons \( (14, 27, 28) \). However, these three studies covered larger geographical areas than the present study, and the variations reported...
TABLE 3. Multivariate Associations between Explanatory Variables and Adipose Tissue Concentrations of PCB Sum and Congener Groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>mono-ortho PCBs % differencea (95% CI)</th>
<th>P value</th>
<th>di-ortho PCBs % difference (95% CI)</th>
<th>P value</th>
<th>tri-ortho PCBs % difference (95% CI)</th>
<th>P value</th>
<th>ΣPCBs %b difference (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>gender (reference men)</td>
<td>-12.5(-21.4; -2.63)</td>
<td>0.02</td>
<td>-14.9(-21.7; -7.38)</td>
<td>0.0002</td>
<td>-8.71(-17.3; 0.77)</td>
<td>0.07</td>
<td>-14.0(-21.0; -6.42)</td>
<td>0.01</td>
</tr>
<tr>
<td>Aarhus area</td>
<td>-18.7(-27.4; -9.89)</td>
<td>0.0004</td>
<td>-19.7(-26.6; -12.2)</td>
<td>&lt;0.0001</td>
<td>-10.4(-19.3; -0.48)</td>
<td>0.04</td>
<td>-18.7(-25.7; -11.1)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>age at time of enrolment (per year)</td>
<td>1.84(0.85; 2.84)</td>
<td>0.0003</td>
<td>1.71(0.93; 2.49)</td>
<td>&lt;0.0001</td>
<td>27.3(-37.4; -15.7)</td>
<td>&lt;0.0001</td>
<td>20.1(-29.7; -9.27)</td>
<td>0.001</td>
</tr>
<tr>
<td>total lifetime duration of lactation (per month)</td>
<td>-0.25(-1.08;0.58)</td>
<td>0.55</td>
<td>-0.51(-1.16;0.14)</td>
<td>0.12</td>
<td>-0.65(-1.42;0.11)</td>
<td>0.09</td>
<td>-0.51(-1.17;0.05)</td>
<td>0.13</td>
</tr>
<tr>
<td>body mass index (per unit kg/m²)</td>
<td>-0.09(-1.15;0.98)</td>
<td>0.87</td>
<td>-1.61(-24.4; -0.06)</td>
<td>0.0002</td>
<td>-1.78(-2.74; -0.81)</td>
<td>0.0004</td>
<td>-1.54(-2.37; -0.71)</td>
<td>0.0004</td>
</tr>
<tr>
<td>fruit and vegetables including juices (per 100 g/day)</td>
<td>-2.18(-4.02; -0.30)</td>
<td>0.02</td>
<td>-1.08(-2.55;0.41)</td>
<td>0.16</td>
<td>1.24(-2.96;0.51)</td>
<td>0.17</td>
<td>2.84(-4.90;2.76)</td>
<td>0.27</td>
</tr>
<tr>
<td>red meat (per 100 g/day)</td>
<td>-1.68(-9.93;7.32)</td>
<td>0.71</td>
<td>1.15(-5.58;8.38)</td>
<td>0.75</td>
<td>-0.23(-7.57;6.67)</td>
<td>0.96</td>
<td>0.86(-5.92;13.13)</td>
<td>0.81</td>
</tr>
<tr>
<td>poultry (per 25 g/day)</td>
<td>-0.40(-5.89;5.42)</td>
<td>0.89</td>
<td>-0.43(-8.32;0.25)</td>
<td>0.07</td>
<td>0.65(-4.48;0.87)</td>
<td>0.81</td>
<td>-3.13(-7.40;1.13)</td>
<td>0.17</td>
</tr>
<tr>
<td>fish with a medium fat content (per 20 g/day)</td>
<td>6.33(-9.95;25.5)</td>
<td>0.47</td>
<td>4.20(-8.58;18.5)</td>
<td>0.54</td>
<td>4.36(-10.5;21.7)</td>
<td>0.59</td>
<td>4.33(-8.57;19.0)</td>
<td>0.53</td>
</tr>
<tr>
<td>fish with a high fat content (per 20 g/day)</td>
<td>7.19(-0.00;16.7)</td>
<td>0.05</td>
<td>5.61(-0.65;12.3)</td>
<td>0.08</td>
<td>6.13(-1.22;14.0)</td>
<td>0.11</td>
<td>5.75(-0.57;12.5)</td>
<td>0.08</td>
</tr>
<tr>
<td>dairy products (per 100 g/day)</td>
<td>-0.37(-1.66;0.93)</td>
<td>0.57</td>
<td>-0.69(-1.70;0.33)</td>
<td>0.19</td>
<td>-0.45(-1.83;0.75)</td>
<td>0.46</td>
<td>-0.60(-1.62;0.43)</td>
<td>0.25</td>
</tr>
<tr>
<td>eggs (per 25 g/day)</td>
<td>-0.27(-5.30;5.02)</td>
<td>0.21</td>
<td>4.46(-1.83;6.72)</td>
<td>0.24</td>
<td>0.47(-1.75;2.54)</td>
<td>0.83</td>
<td>0.96(-2.13;6.25)</td>
<td>0.35</td>
</tr>
<tr>
<td>R² for model</td>
<td>0.17</td>
<td>0.36</td>
<td>0.28</td>
<td>0.34</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* Mutually adjusted. * Percentage (%) difference in mean PCB concentrations for each given increment in the predictor variable, e.g., a percent difference of 1.84 for age is interpreted as a 1.84% increase in mono-ortho PCB concentration per year of age. For the class variables (geographical area and gender) the value reflects the difference according to the reference group. * * Sum of PCB no.s 99, 118, 138, 153, 156, 170, 180, 183, 187, and 201.
probably reflected the large differences in the previous manufacture of these compounds. In Denmark, PCBs have never been produced. Other explanations for geographical differences include variation in lifestyle, the occurrence of dietary sources with varying levels, and differences in local environmental contamination. Urban air concentrations of volatile (lower chlorinated) PCBs in several European countries have been reported to be consistently higher than the respective suburban and rural levels in these countries (29–31), reflecting variation in PCB sources, population density, and industrialization. Since PCBs were banned in Denmark in 1977, our result suggests that there is regional variation in exposure to these compounds even in a small and relatively homogeneous country such as Denmark.

We found that PCB levels were highest among the oldest participants, which is probably caused by the longer period of exposure to these bioaccumulating compounds. Additionally, during the 1960s and 1970s environmental concentrations of the PCBs were much higher than they are today, leading to much higher body burdens in older people. Indeed in a recent paper Ritter and co-workers find that body burdens in persons 50 years and older do in fact reflect past exposures due to a ‘memory effect’ (5). Finally, age may have an independent effect through an age-related reduction in elimination capacity (32).

Concentrations of PCBs were lowest in women with the longest duration of lactation. This association is well established (15, 33), as lactation mobilizes body stores of fat, thus reducing the body burden of lipophilic compounds. Our results suggest that the tri-ortho-substituted PCBs are eliminated most effectively in breast milk, followed by the di- and then the mono-ortho-substituted PCBs. This seems logical as the partitioning of PCB congeners into breast milk is thought to be dependent on molecular weight, such that the PCBs with higher molecular weight and greater lipophilicity such as the tri- and di-ortho-substituted PCBs partition more readily in breast milk (33, 34).

Fish with a high fat content was most strongly associated with the sum of all PCB concentrations, and when comparing the three substitution groups we found a slightly higher association between all fish groups and the mono-ortho-substituted PCBs. The strong association we found between fatty fish and PCBs is most likely explained by the more efficient accumulation of PCBs in fish with a high fat content. Interestingly, we found that the association between consumption of fatty fish and PCBs was only important among women. This is interesting as in our study women consumed slightly less fatty fish than men. We have no specific explanation for the stronger associations we find among women, but differences in metabolism and elimination of the compounds may play an important role.

In our study the concentration of fish with a high fat content was highest among the oldest participants, which is probably caused by the longer period of exposure to these compounds even in a small and relatively homogeneous country such as Denmark.

Interestingly, we found that the association between consumption of fatty fish and PCBs was only important among women. This is interesting as in our study women consumed slightly less fatty fish than men. We have no specific explanation for the stronger associations we find among women, but differences in metabolism and elimination of the compounds may play an important role. Three studies have previously investigated the predictive effect of fruit and vegetables. The results are, however, inconsistent, as one study reports results in accordance with ours (35), while the second study found no associations (14), and the third found positive associations between consumption of fruit and vegetables and total PCB levels (15). In contrast to our study, these three studies all considered only women. However, in our study we found no indications that the association between the PCBs and intake of fruit and vegetables varied between the genders.

We are one of the first groups to determine the concentrations of specific PCB congeners in adipose tissue. Adipose tissue is the principal storage medium for lipophilic compounds such as PCBs in the human body (38), and we would, therefore, expect concentrations measured in adipose tissue to be the best estimate of body burden (39–41). Many previous predictor studies have used blood samples, and all have argued that the equilibrium between PCBs in adipose tissue and blood lipids is rapidly established. However, correlation studies of PCBs in serum/plasma and adipose tissue show the majority of correlations for individual PCB congeners between 0.1 to 0.3 and some varying from negative to 0.48 (41, 42). Thus, plasma/serum is not methodologically equal to adipose tissue.

We investigated PCB congeners grouped according to substitution pattern rather than treating the PCBs as a homogeneous group. Data regarding predictors of PCBs according to substitution groups seems most important as dioxin-like effects and cancer risk have only been reported for the mono-ortho PCBs and not the di- and tri-ortho-substituted groups (18, 19). In our study the concentration of the di-ortho-substituted PCBs represent over 80% of the total sum of PCBs, and we show that the information provided from studying total PCBs does not adequately reflect the body burdens of all substitution groups and underscores the importance of congener substitution group-specific analyses.

To reduce the number of statistical tests and thereby the potential for chance findings we investigated major dietary groups instead of specific food items and did not examine each congener individually. However, if a specific dietary food item is highly contaminated with PCBs the other dietary items in the same major food group may have diluted the effect of this food item. Similarly, grouping congeners in substitution groups may also mask the effect of one specific congener. Multiple statistical tests were carried out in this study. We included 4 compound groups and 13 predictors, equivalent to 52 tests in the adjusted analyses. Thus, we would expect approximately three statistically significant results by chance alone. In this study, we found 18 significant results in the adjusted analyses; thus, these findings are not attributable to random correlations.

Our participants represented older Danish persons with a median age of 56, and caution should be taken when generalizing our results to other populations including teenagers and infants (5). It can also be argued that registration of dietary intake at enrollment used in this study may not adequately reflect the previous dietary intake, which may be most relevant for body burden of these compounds. This possible misclassification could mask true associations...
and, in part, explain the relatively low $R^2$ values of some of the regression models.

In conclusion, geography, age, lactation, BMI, and consumption of fish with a high fat content were all consistently found to be associated with concentrations of PCBs in adipose tissue. When searching for potential predictors of PCBs it might be important to study the different substitution groups separately, as our study indicated that predictors varied according to substitution group.

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Supporting Information Available

Details of gas-chromatography—mass-spectrometry parameters, analytical quality assurance, quality control, and LOD. This material is available free of charge via the Internet at http://pubs.acs.org.

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