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Organochlorines in Danish women: Predictors of adipose tissue concentrations

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Abstract

Organochlorines (OCs) are ubiquitously present in the environment, and food of animal origin is currently reported as the major source of exposure. Carcinogenicity in animals raises concern, and OCs may also be a risk factor for both neurological and immunological effects. Our primary objective was to study predictors of adipose tissue levels of dichlorodiphenyldichloroethylene (DDE) and polychlorinated biphenyls (PCBs) in Danish women. We showed that adipose tissue concentrations of DDE and PCBs were consistently positively associated with age and the consumption of fish with a high fat content, while total lifetime duration of lactation had an inverse relationship. The direction of the association with body mass index (BMI) depended on the OC studied. The consumption of meat, fruit, lean fish, medium-fat-content fish, poultry, and eggs was not associated with OC concentrations in our study. We classified fish according to fat percentage, which seems more relevant than considering only total fish consumption. When PCBs were subdivided according to their chemical structure, similar results were obtained for the mono-, di-, and tri-ortho PCBs, indicating that PCBs can be treated as a homogenous group when studying predictors of concentrations in humans. In conclusion, the present study shows that age, lactation, and BMI are consistent predictors of human adipose tissue concentrations of DDE and PCBs and that dietary factors other than fish with a high fat content are not important predictors of these concentrations.

Keywords: Organochlorines; DDE; PCB; Adipose tissue; Predictors

1. Introduction

Organochlorines (OCs), such as the insecticide dichlorodiphenyltrichloroethane (DDT) and the industrial chemical polychlorinated biphenyl (PCB) are ubiquitously present as a complex mixture of mother compounds and metabolites in the environment. These compounds are characterized by high lipid solubility, toxicity, environmental persistence, and bioaccumulation in food chains (ATSDR, 1992, 1993). DDT was introduced as an insecticide in the 1940s, but its use is now severely restricted in the Western world. In Denmark, DDT was restricted in 1969 until a total ban enforcement in 1986, but more than 520 metric tons of DDT have been sold in Denmark alone (T & A Nr.4, 1998). One of the primary metabolites of DDT is dichlorodiphenyldichloroethylene (DDE), which is detected at higher levels than DDT in serum, plasma, and adipose tissue samples. PCBs are of particular concern among industrial chemicals, and characteristics, including semivolatility, predispose them to long-range transport and adverse effects in areas removed from point sources (WHO, 2003). These compounds were first manufactured in the 1920s, and more than 1.5 million

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metric tons of PCBs have been produced for a variety of uses (WHO, 2003). Due to evidence of a build-up in the environment and adverse environmental effects such as ozone degradation, manufacture stopped in the 1970s (Schwarzenbach et al., 1993; Seinfeld, 1998). But at least one-third of these PCBs are believed to have found their way into the natural environment (Ahlborg et al., 1995; WHO, 2003).

In countries in which the use and production of DDT and PCBs has been banned for decades, food of animal origin is currently reported as the major source of human exposure (Ahlborg et al., 1995; Duarte-Davidson and Jones, 1994; WHO, 2003). Therefore, the identification of dietary predictors is important, as there are indications that the presence of OCs in food may be a risk factor for both neurological and immunological effects in infants and for endocrine disruption (Gray et al., 1993; Longnecker et al., 1997; Swanson et al., 1995; Tilson et al., 1990; WHO, 2003). In addition, carcinogenicity in animals raises concern about OCs as risk factors for cancer in humans. This has prompted many epidemiological studies of women (Laden et al., 2001; Raaschou-Nielsen et al., 2005; Snedeker, 2001).

Most previous studies of predictors have considered only total fish consumption, and results are inconsistent (DeVoto et al., 1998; Laden et al., 1999; Moysich et al., 2002; Paris-Pombo et al., 2003; Schildkraut et al., 1999). It is widely recognized that lipophilic organic chemicals accumulate in the fatty tissue of fish (Jacobs et al., 1998, 2004), and the amounts of OCs in fish vary according to total fat content. Therefore, it is interesting to consider the predictive value of fish subgroups classified accordingly.

When studying predictors of PCBs, these compounds are often treated as a homogenous group, and total PCB concentrations are typically used. However, environmental persistence, bioaccumulation in food chains, distribution in human tissue, and the dioxin-like effects of PCBs depend on the chemical structure of individual congeners (ATSDR, 1993; Atuma et al., 1998; Laden et al., 1999; McFarland and Clarke, 1989; WHO, 2000, 2003). A few groups (Aronson et al., 2000; Demers et al., 2002) have recently reported that mono-ortho PCB congeners are related to breast cancer risk, and the grouping of PCBs has been suggested (WHO, 2003; Wolff et al., 1997). Information about the determinants of specific congener group concentrations may be valuable but is still scarce (Glynn et al., 2003).

Our objectives were to study predictors of adipose tissue levels of DDE and total PCB congeners in Danish women and to determine whether predictors of PCBs differed according to congener substitution patterns. We selected nondietary variables previously associated with OC concentration such as age, body mass index (BMI), and breast-feeding (Laden et al., 1999; Paris-Pombo et al., 2003; Rogan et al., 1986; Sanz-Gallardo et al., 1999; Soliman et al., 2003) and explored associations with nine dietary variables based on 147 food items.

2. Materials and methods

2.1. Study population

Between December 1993 and May 1997, 79,729 women aged 50–64 years were invited to participate in a prospective cohort study “Diet, Cancer and Health” (Tjonneland and Overvad, 2000; Tjonneland et al., 1999). Eligible cohort members were born in Denmark and had no previous cancers at the time of inclusion. A total of 29,875 women, corresponding to 37% of the women invited, were enrolled in the cohort, representing 7% of the entire Danish population in this age group.

A case-control study nested within the cohort was conducted to determine risk factors for breast cancer among postmenopausal women. A total of 434 case-control pairs were included; the method of selection has been described elsewhere in detail (Tjonneland et al., 2004a,b). Of the 434 control women, 24 did not provide sufficient adipose tissue samples; a laboratory error occurred in 1 tissue sample; and for 7 women inadequate dietary details were available. Our analyses of predictors of OC levels were based on the remaining 402 postmenopausal control women.

2.2. Questionnaire

Data on individual characteristics and dietary patterns were obtained from detailed self-administered, interviewer-checked, food-frequency, and lifestyle questionnaires completed at the time of enrollment. The food-frequency questionnaire included 192 food items, of which 147 were used in the present study. The dietary questions were designed to collect information about dietary habits, and a description of the development and validation of this questionnaire has been published previously (Overvad et al., 1991; Tjonneland et al., 1991). Participants were asked how often on average they had consumed the different types of foods during the preceding 12 months. The frequency of consumption was categorized into 12 groups ranging from never to 8 or more times a day. 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 program FoodCalc Version 1.3 (Lauritsen, 2004).

Staff members in the study clinics obtained anthropometric measures, including height and weight.

2.3. Tissue sampling

An adipose tissue biopsy from the buttock of each participant was obtained by staff members using a...
2.4. Lipid content determinations of adipose tissue

The PCB congeners and DDE were extracted from adipose tissue using dichloromethane (Ryan, 1991a). A fraction of this extract was used to determine the lipid content of the sample and the other fraction was used for quantification. The total lipid content was determined using a gravimetric method (Ryan, 1991b); 200 μL was precisely weighed on an analytical balance and the solvent was evaporated at room temperature in a desiccator. The resulting lipid weight was adjusted to the initial sample weight, and the percentage lipid content was calculated. The OC concentrations were expressed as weight per lipid weight.

2.5. Chemical analysis of organochlorines

The OCs quantified included 10 PCB congeners (IUPAC Nos. 99, 118, 138, 153, 156, 170, 180, 183, 187, and 201) and DDE. Fatty residues were removed using gel permeation chromatography, and clean-up was achieved on a Florisil column. The samples were analyzed using GC/MS (Agilent Technologies, Hewlett Packard Model 6890/5973) using a DB-XLB capillary column and electron-capture 63Ni detection. Quantification was performed using multilevel calibration curves obtained by injection of standard solutions of at least 3 concentrations.

For each of the compounds, the limit of detection (LOD) was determined by first estimating its value equivalent to a signal to noise ratio of 3. Then, 10 replicates of a sample containing the compounds at a concentration from 4 to 10 times the estimated detection limit were measured. The LOD became the value equivalent to 3 times the standard deviation of these 10 replicates. For each sample, the detection limit was adjusted with respect to the weight of the sample and the lipid content. The median (5th, 95th percentiles) LOD was 8.5 (4.5; 41.8) μg/kg lipids for DDE and PCB congener no. 99 and 2.8 (1.5; 13.9) μg/kg lipids for the remaining congeners. Routine checks of the accuracy and precision of the OC measurements were made using reference materials from the National Institute of Standards and Technology and by participation in the German External Quality Assessment Scheme for Proficiency Testing in Biological Monitoring and Occupational and Environmental Medicine. The coefficients of variation were 5–7% for the PCB congeners and 7% for DDE. The loss of compounds during the analytical process was negligible, and average recoveries of the different OCs in spiked corn oil (5 μg/kg in corn oil, N = 3) were between 87% and 96%.

2.6. Statistical analysis

Predictors for OC concentrations were analyzed in 5 separate regression models. The 5 dependent variables included DDE, total PCBs, and the sums of mono-, di-, and tri-ortho PCB groups, and compound concentrations below the LOD were treated as zero. The distributions of the PCBs and DDE lipid-adjusted tissue concentrations were skewed; therefore, all statistical analyses were performed on the natural logarithm of these data. The influence of the explanatory variables on OC concentrations was calculated by exponential transformation of regression estimates and expressed as percentage change in OC concentrations per unit change in the explanatory variable.

The explanatory variables used in all 5 models were age at the time of clinic visit, BMI, total lifetime duration of breast-feeding, and dietary factors, including both the consumption of foods of animal origin (dairy products, eggs, fish, meat, poultry) and the consumption of fruit and vegetables (see Table 1). The analyses were conducted using the PROC GLM procedure of SAS v8.2, (SAS Institute Inc., Cary, NC, USA). We used the following approaches to study predictors: (i) a univariate approach including each explanatory variable individually, (ii) a multiple regression approach including all explanatory variables, mutually adjusted, and (iii) a backward stepwise linear regression approach. In the last approach the exclusion of variables continued in each model until the P value of all the included explanatory variables was below 0.15.

3. Results

3.1. Descriptive statistics

Table 1 shows the baseline characteristics of the women and the median consumption of the foods included in this study. Fruit, vegetables, and dairy products were the food groups consumed in the largest amounts, and the consumption of fat originating from fish with a high fat content was a factor 4.5 times greater...
than the consumption of fat originating from either lean or medium-fat-content fish, respectively.

Table 2 shows compound Cl-substitution patterns and tissue lipid-adjusted concentrations of DDE and the PCB congeners. The concentration of DDE was quantified in 401 of the 402 adipose samples, and the median tissue concentration was 505 mg/kg lipid.

### 3.2. Regression analyses

Among the 12 explanatory variables, age, lifetime duration of lactation, BMI, and the consumption of fish with a high fat content had the lowest P values in both the DDE and the total PCB models (Table 3). The associations with the OC levels were positive for age and the consumption of fish with a high fat content, while total lifetime duration of lactation showed inverse relationships. BMI was positively associated with DDE concentrations but negatively associated with total PCBs. The effect estimates for the explanatory variables shown in Table 3 resembled those found in univariate models in which each explanatory variable was entered alone (results not shown). None of the effect estimates obtained in the univariate models differed by more than 1 standard error from the estimates we report in Table 3. When considering total PCBs, the reduced model included the same 4 above-mentioned explanatory variables, all with \( P \leq 0.06 \) and similar effect estimates (results not shown). For DDE, a similar approach resulted in a model also including the same 4 explanatory variables; additionally, dairy and vegetable intake was included. All of these variables had \( P \) values below 0.11, and effect estimates were also similar to those reported in Table 3 (results not shown). The models presented in Table 3 explain 6% of the variation in DDE concentrations and 9% of the variation in total PCB concentrations (\( R^2 \) values).

Table 4 shows results from similar multiple regression models for the 3 PCB congener groups. Again, age, lifetime duration of lactation, BMI, and the consumption of fish with a high fat content had the lowest \( P \) values (all \( P \leq 0.15 \)). The effect estimates for age, lactation, and the consumption of fish with a high fat content were similar in these 3 models, whereas the results for BMI showed a weaker association with mono-ortho PCBs than the other 2 substitution groups. Stepwise model reductions until \( P \) values were below 0.15 confirmed this pattern, and the same 4 explanatory variables were included in each of the 3 reduced models, all with \( P \leq 0.10 \). The only other explanatory variable included was dairy intake, which occurred in the reduced mono-ortho PCB model with an inverse association with the mono-ortho PCB level and \( P = 0.09 \). Effect estimates similar to those presented in Table 4 were obtained in the univariate models and the reduced multiple regression models (results not shown).
4. Discussion

The present study showed that age, total lifetime duration of lactation, and the consumption of fish with a high fat content were all consistently associated with DDE and PCB levels in the adipose tissue of Danish women. Associations for BMI were not consistent; we found a positive association with DDE levels, while the
## 4. General considerations

Examining each PCB congener individually was not considered in our study, as it would lead to the testing of numerous associations, where even if true associations did not exist, some associations would appear significant by chance. Adipose tissue levels used in this study are regarded a good indicator for the evaluation of long-term human exposure to OCs (Anderson, 1985; Archibeque-Engle et al., 1997).

Our participants were 50- to 65-year-old postmenopausal Danish women, and caution should be taken when generalizing our group-specific results to other populations, as subgroup composition may influence the strength of associations.

It can be argued that the registration of dietary intake at the time of enrollment used in this study did not adequately reflect previous dietary intake, which may be important for accumulated body concentrations of persistent OCs. This possible misclassification could mask true associations and may, in part, explain the relatively low $R^2$ values of the regression models.

### 4.2. Nondietary variables

#### 4.2.1. Age and total lifetime lactation

The adipose tissue concentrations of DDE and PCBs were higher in older women and lower in women with the longest duration of lactation; both associations are well established (Laden et al., 1999; Moysich et al., 2002; Paris-Pombo et al., 2003; Rogan et al., 1986; Soliman et al., 2003). The association with age may be related to higher cumulative exposures caused by an age-dependent bioaccumulation of lipophilic compounds. In addition, the older women in the study have historically been exposed to higher concentrations because they were alive for more of the period before the ban of these OCs in Denmark. Lactation mobilizes body stores of fat, thus reducing the body burden of OCs, and is, therefore, considered the most important route of OC excretion for women (Jensen, 1987).

#### 4.2.2. BMI

In this study, BMI was positively associated with the adipose tissue concentrations of DDE, which is comparable with the results of some (Glynn et al., 2003; Hunter et al., 1997; Sanz-Gallardo et al., 1999; Schildkraut...
et al., 1999; Wolff et al., 2000a) but not with those of other studies (Laden et al., 1999; Moysich et al., 2002; Wolff et al., 2000b). A positive association could be expected in relation to prolonged high consumption of fatty food, which is often associated with being overweight and possibly also with a high intake of lipophilic OC compounds accumulated through the food chain (Blundell and King, 1996; Cox et al., 1999; Drewnowski et al., 1992; Macdiarmid et al., 1996), but this is a controversial argument (Blundell and Cooling, 1999; Macdiarmid et al., 1998). Conversely, a high BMI and body fat content could result in a dilution effect, and we found an inverse relationship between BMI and total PCBs, which is in agreement with 1 study (Wolff et al., 2000b) but not with others (Glynn et al., 2003; Laden et al., 1999; Paris-Pombo et al., 2003; Sanz-Gallardo et al., 1999; Schildkraut et al., 1999). The estimated effect of BMI on the concentrations of the mono-ortho-substituted congener group was a factor 2 weaker than the effects on the di- and tri-ortho PCB congener groups, which may be explained by the mono-ortho-substituted congeners being less lipophilic than PCB congeners from the other substitution groups (WHO, 2000).

4.3. Dietary variables

4.3.1. Fish

The consumption of fish with high a fat content was identified as a predictor of adipose tissue concentrations of the OCs in all models, while lean and medium-fat-content fish were not. The strength of the association was similar for the 3 PCB congener groups. When considering total PCBs, only 1 previous study has investigated fish with a high fat content (Glynn et al., 2003), and this group reported a weak positive association with PCBs. All remaining studies consider total fish consumption: 2 groups found no associations between total fish consumption and concentrations of PCBs in plasma (Laden et al., 1999) and adipose tissue (Schildkraut et al., 1999), while 3 other groups reported weak positive associations in breast adipose tissue (Paris-Pombo et al., 2003), serum (Moysich et al., 2002), and plasma (DeVoto et al., 1998). For DDE, 1 group (Sanz-Gallardo et al., 1999) reported weak associations between a dietary biomarker of total fish consumption (docosahexaenoic acid) and DDE levels in adipose tissue, while another (Glynn et al., 2003) found no association between fish with high fat contents and DDE levels. The inconsistency in the previously reported results indicates that the method of classifying fish according to fat content used in the present study is more informative. However, the inconsistencies may also reflect different OC levels in fish or different consumption patterns in some study populations. The consumption of fish with a high fat content is relatively high among Danish women, whereas the consumption of total fish is relatively normal for European populations (WHO, 2002).

Numerous other studies reported strong associations between fish consumption and body burdens of OCs within special exposure populations, such as sport/professional fishermen or individuals who consume relatively high amounts of contaminated fish (Asplund et al., 1994; Fiore et al., 1989; Hovinga et al., 1993; Kreiss, 1985; Lommel et al., 1992; Sjodin et al., 2000); it was not possible to identify whether the fish consumed by our study population originated from contaminated waters.

4.3.2. Dairy products

We found weak and insignificant inverse relationships between the total consumption of dairy products and DDE concentration, which may have been due to chance. The results of the few studies that have previously investigated dairy products vary. One group (Moysich et al., 2002) reported a positive association with DDE levels, while another (Laden et al., 1999) reported null associations. Additionally, another group (Paris-Pombo et al., 2003) reported a negative association between dairy products and PCB levels, which is as surprising as the results we report for DDE levels.

4.3.3. Other dietary variables

Our results indicate that the other included dietary variables were not substantial predictors of DDE and PCB concentrations in Danish women. This may be because these products originate from countries in which DDE and PCBs have been banned for decades, resulting in low levels of DDT, DDE, and PCBs in the food supply. Only a few previous studies have included an extensive list of dietary variables (Laden et al., 1999; Moysich et al., 2002; Paris-Pombo et al., 2003; Schinas et al., 2000) comparable to that in our study. One group (DeVoto et al., 1998) reported that red meat intake was the strongest predictor of DDT plasma levels among 297 elderly German residents, while 2 others (Laden et al., 1999; Moysich et al., 2002) reported no associations between red meat and DDE or PCBs. In another study (Paris-Pombo et al., 2003), inverse associations with poultry and PCBs were reported, while a fifth group (Moysich et al., 2002) found null associations in-line with ours. Our null results for total egg consumption are in contrast to those of 2 other studies (Laden et al., 1999; Moysich et al., 2002), both of which report positive associations between eggs and PCB levels.

5. Conclusion

Age, lactation, BMI, and the consumption of fish with a high fat content were all consistently found to be associated with concentrations of DDE and PCBs in
adipose tissue; however, these factors explained only a minor part of the variation in concentrations.

The results of our study suggest that dietary factors, other than fish with a high fat content, are not important predictors of human concentrations of DDE and PCBs in Denmark. The findings of consumption of fish with a high fat content but not the consumption of lean and medium-fat-content fish as a predictor for OC adipose concentrations may have implications for food recommendations. Finally, our results indicate that there is only a limited variation in the identified predictors of PCB groups with different chemical structure.

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