Serum levels of organochlorines in Swedish pregnant women are influenced by high exposures during childhood and adolescence: a cross-sectional study

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Abstract

Background. We studied associations between personal characteristics and lipid-adjusted serum concentrations of PCB congeners (IUPAC no 118, 138, 153, 156 and 180), and HCB, β-HCH, *trans*-nonachlor and *p,p´*-DDE, among 323 pregnant primiparous women (age 18-41 years) sampled between 1996 and 1999.

Methods. We performed a cross-sectional study of primiparous women from Uppsala County, Sweden using extensive personal interviews and questionnaires to gather information about personal characteristics of the participants. Organochlorine levels in serum lipids in late pregnancy were analyzed by gas chromatography.

Results. Of the 395 women asked to participate 325 women (82%) agreed to donate a blood sample. Multiple regression analysis showed that levels of all compounds increased with age of the pregnant women and were higher in women sampled early during the 4 year study period 1996-1999. Age-dependency could partly be explained by a longer bioaccumulation time among older women. A birth cohort effect could have contributed to both age-dependency and time trends, since older women and women sampled early in the study had experienced higher exposure levels during childhood and adolescence than younger women and women sampled in the end of the study period. The importance of early exposures during childhood and adolescence was further supported by lower PCB levels and higher β-HCH and *p,p´*-DDE levels among women born in non-Nordic countries. Moreover, serum levels of certain PCBs and pesticide/metabolites were positively associated to consumption of fatty fish, especially fatty fish from the Baltic Sea, during adolescence. Finally, levels of CB 156, CB 180 and *p,p´*-DDE increased significantly with number of months women had been breast-fed during infancy. Short-term changes in bodily constitution may, however, also influence serum levels, as suggested by negative associations between organochlorine levels and BMI before pregnancy and weight change during pregnancy. Regression models including the studied personal characteristics explained 40-70% of the variation in serum levels, showing that there still are unknown factors which have a large impact on serum organochlorine levels.

Conclusion. Although some of the associations found by us could be caused by unknown personal characteristics confounding the results, our findings suggest that organochlorine exposures during childhood and adolescence influence the body burdens of the compounds during pregnancy many years later.

Key words: PCB, DDE, HCB, HCH, age, BMI, weight, temporal trend, breast feeding, fish

Background

Levels of organochlorines, such as polychlorinated biphenyls (PCBs, industrial chemical) and the insecticide DDT and its metabolites, have declined in the environment and foodstuffs in many parts of the world. Background *in utero* exposure to the environmental pollutants may, however, still be a risk factor for neurological, hormonal and immunological effects in infants and children [1-4]. Serum/plasma levels of organochlorines are often used in assessment of organochlorine body burdens among pregnant women, and of fetal exposure [5-8]. Studies of organochlorine levels in serum/plasma of pregnant women often report large inter-individual variations in levels [9]. The reasons behind this variation are still to a large extent unknown, although several studies have investigated if lifestyle/medical factors can explain at least some of the variation [10-12]. Such determining factors may confound results in epidemiological studies. Moreover, a better understanding of determinants of organochlorine body burdens during pregnancy will increase possibilities for future actions and recommendations, with the purpose to lower body burdens during pregnancy.

Here we report determinants of serum concentrations of five PCB congeners (CB 118, CB 138, CB 153, CB 156, CB 180) and four chlorinated pesticides and metabolites, hexachlorobenzene (HCB), β-hexachlorocyclohexane (-HCH), *trans*-nonachlor and *p,p´*-DDE among pregnant primiparous women living in Uppsala County, Sweden, 1996-1999. We selected personal characteristics previously reported to be associated with serum/plasma/breast milk concentrations of organochlorines, such as age, year of sampling, BMI, body weight change, country of birth, smoking and alcohol intake [12-16]. Associations with indices of high organochlorine exposure, due to fish consumption during childhood were also studied, i.e. growing up in a family with a family member involved in commercial fishing or sport-fishing (fisher/sport-fisher family), and growing up along the coast of the contaminated Baltic Sea. Since associations between breast-feeding exposure during infancy and organochlorine blood levels have been found in adolescents [17], we also studied associations between the number of months the woman had been nursed during infancy. Finally, we analyzed associations between organochlorine serum levels and dietary habits the year of pregnancy and the year women attended 7th grade in school (13-14 years of age in Sweden). Influences of dietary habits during the teenage years were studied since levels in food were higher in the 1970s-80s than in the mid-late 1990s when the women got pregnant [18-20]. In an attempt to decrease variation in serum levels ,we only studied women having their first baby. Breast-feeding is a major pathway of organochlorine excretion [21].

The aim of our study was to find major determinants of organochlorine body burden during pregnancy, in order to improve the understanding of reasons behind inter-individual variation in body burdens.

Methods

Study population. From January 1996 to May 1999, 1037 pregnant women living and seeking prenatal care in Uppsala County were asked to participate as controls in a case-control study of risk factors for early miscarriages [22]. In all, 953 women (92%) accepted this offer. All women were Swedish-speaking, and had completed 6-12 weeks of pregnancy when entering the study. At 32-34 gestational weeks 50 women had been lost from the study due to miscarriage, induced abortions, and due to mothers withdrawing from the study, moving outside Uppsala County or being lost in follow-up [22].

All primiparas recruited between early fall 1996 and spring 1999 were in late pregnancy (week 32-34) asked to participate in the organochlorine study (n=370). In order to increase the number of women living along the coast of the Baltic Sea in the study population, all Swedish-speaking primiparas (n=25) at the prenatal clinic in Östhammar, that were not participating in the case-control study, were in early pregnancy (week 6-12) asked to participate (between fall of 1997 and spring of 1999).

Of the 395 women asked to participate in the organochlorine study, 325 women (82%) agreed to donate a serum sample in late pregnancy (week 32-34) for chemical analysis, 305 women from the case-control study and 20 women from the Östhammar clinic. Two samples were lost before chemical analysis. Age of the participating 323 women ranged from 18 to 41 years (Table 1). Participation did not occur until after informed consent was obtained. The Ethics Committee of the Medical Faculty, Uppsala University, approved the study (dnr 96114).

Interviews and questionnaires. In-person interviews, using a standard structured questionnaire, were conducted at 6-12 and 32-34 completed gestational weeks. Data on maternal characteristics included age, height, weight, pre-pregnancy body mass index (BMI:

weight/height^2 (kg/m^2)), years of education, place of residence, country of birth, alcohol consumption and smoking. Blood samples for cotinine analysis (indicator of smoking habits) were taken on both occasions. After agreeing to participate in the organochlorine study, mothers answered a self-administered questionnaire including questions about dietary habits, delivery, and personal characteristics not covered by the interviews.

Dietary habits. Dietary questions were designed to collect information about consumption of food groups that were major contributors to dietary organochlorine exposure, i.e. meat and meat products (including poultry), dairy products, eggs and egg products, fish and fish products and vegetable oils. Participants were asked how often, on average, they had consumed different types of foods during the year they got pregnant and during the year they attended 7th grade in school. For consumption calculations (g of food stuff/d) we used standard portion sizes [23, 24], and lipid content of dairy products were obtained from the Swedish National Food Administration (NFA) [25].

In questions about fish consumption, single fish species were identified. Concentrations of organochlorines in fish varies considerably, depending on fat content of the fish and place of catch (fresh water fish, Baltic Sea fish, other marine fish) [26]. Consumed fish were divided into three groups: (1) lean fish consisting of cod-like species, flat-fish, canned fish (except herring), pike, pike-perch, perch, burbot and fish products such as fish sticks and fish quenelles; (2) fatty Baltic Sea fish consisting of Baltic herring and wild salmon/trout; (3) other fatty fish consisting of Atlantic herring, mackerel, Pacific salmon, farmed salmon/trout, whitefish and eel.

Blood sampling and chemical analysis. Blood was sampled in late pregnancy (week 32-34). We analyzed the lipid portion of serum samples for chlorinated pesticides/metabolites *p,p´*-

DDT, *p,p´*-DDD, *p,p'*-DDE, *o,p´*-DDT, *o,p´*-DDE, HCB, α-, β- and γ-HCH, *trans*-nonachlor and oxychlordane (Table 2). We chose to analyze IUPAC nos. 28, 52, 101, 105, 118, 138, 153, 156, 167 and 180. The analytical method used is described in detail by Atuma and Aune [27]. After extraction and clean up of samples, they were analyzed on a gas chromatograph with dual capillary columns and electron capture detectors (^{63}Ni). The columns were of different polarity to ease identification of analytes, which was based on retention times relative to internal standards. Quantification was performed using multi-level calibration curves.

Limit of quantifications were 2-4 ng/g lipid. Reproducibility was demonstrated by 21 replicate determinations using an in-house control serum sample, included among analytical batches. Coefficients of variations (CV) were less than 13% for most of the compounds, except for CB 105 (20%) and CB 28 (22%). CV for gravimetric fat content was 4%. Average recoveries of different PCB congeners in spiked serum samples were 98 ± 12% (mean±SD) and 94 ± 8% for 0.1 and 0.8 ppb levels, respectively. Recoveries for the chlorinated pesticides varied from 78 to 118%. This shows that loss of compounds during the analytical process was negligible. Results reported were not corrected for recovery. When concentrations were below LOQ they were set to 50% of that limit in all statistical analysis.

Calculations and statistical analysis. Serum organochlorine concentrations were lipid adjusted and statistical analyses were performed on logarithmically transformed data, since the distribution of data closely followed a log-normal distribution. Level of significance was set to <0.05 in all tests. Levels of CB 28, CB 52, CB 101, CB 167, α- and γ-HCH, *trans*-nonachlor and oxychlordane, *p,p´*-DDT, *p,p´*-DDD, *o,p´*-DDT, and *o,p´*-DDE were in many cases below the LOQ (>20%) and statistical analysis was not performed on these compounds.

Multiple regression (MINITAB® For Windows, 12.22) was used to analyze associations between the dependent variable "organochlorine concentration" and independent variables suspected to be determinants of organochlorine concentrations. In the first step all independent variables were included in the regression model. In the next step a "basic" model was used, including independent variables with few missing observations (among <10% of the women), and that were significantly associated with organochlorine levels ($p<0.05$) in the first regression. Independent variables with many missing values were then included one at a time in the basic model. This procedure minimized the loss of power in the statistical analysis. In regression analysis observations with standardized residuals ≥ 3 were omitted because of their exaggerated influence on regression results.

Determinants studied were age of the women, sampling year (starting point Jan. 1 1996), pre-pregnancy BMI, weight change during pregnancy, education level, smoking, alcohol consumption, country of birth, growing up in a fisher/sport-fisher family or along the Baltic Sea coast, and breast feeding during infancy. Weight change during pregnancy was calculated as % per week, from pre-pregnancy weight to weight at blood sampling (week 32-34). Smoking was categorized into three categories, never smoked, stopped smoking before pregnancy, and smoked during pregnancy. Women with cotinine levels >15 ng/ml in early and/or late pregnancy were classified as smokers during pregnancy, even if they reported that they had not smoked during pregnancy [22]. The variable country of birth was divided into two categories, women born in Nordic countries (Sweden, Denmark, Finland, Norway and Iceland) and women born in non-Nordic countries.

Regression analysis of associations between food habits and organochlorine levels only included data on Nordic-born women that had completed the food frequency questionnaire (N=226). The basic regression model, described above, was used, and associations between organochlorine levels and consumption of each food group (g/day) were analyzed one at a time. Consumption was, with two exceptions, divided into four categories, with an effort to have equal number of individuals in the categories. Consumption of fatty Baltic fish was divided into three categories, including women with no consumption in the first category, and remaining women evenly divided into two other categories. Egg consumption was also divided into three categories, with an effort to have equal number of individuals in each category.

Stepwise regression was used to estimate how much of the variation in organochlorine levels that was explained by the different determining variables. Adjusted geometric means of organochlorine levels were calculated using the general linear model (GLM) procedure.

Partial regression coefficients (b) for independent variables age, year of sampling, pre-pregnancy BMI, weight change during pregnancy, and breast feeding during infancy were used in calculation of %change in organochlorine level per unit change of determining variables (eqn. 1).

$$\%\text{change} = (1-\exp(b))*100 \qquad \text{(eqn. 1).}$$

Results

Organochlorine levels. Median serum lipid concentrations were highest for PCB congeners CB 138, CB 153 and CB 180, and for HCB and DDT metabolite *p,p'*-DDE (Table 2). Among PCB congeners, CB 28, CB 52 and CB 101 showed the largest variation (>100-fold), whereas *p,p'*-DDT exhibited the largest variation among chlorinated pesticides/metabolites (approx. 60-fold) (Table 2). A few women had high levels of CB 28, CB 52 and CB 101 (Table 2).

Lifestyle/medical factors. Multiple regression analysis showed statistically significant associations between organochlorine levels and age of the women (all substances, positive), year of sampling (all substances, negative), pre-pregnancy BMI (CB 138, CB 153, CB 156, CB 180, *trans*-nonachlor, negative), and weight change during pregnancy (all substances except β-HCH and *p,p'*-DDE, negative) (Table 3). A regression model including these determinants explained 50-70% (R^2, coefficient of determination) of the variation in organochlorine levels. Associations were strongest for age (R^2:27-52%), whereas year of sampling explained 3-11% of the variation in serum levels. Pre-pregnancy BMI and weight change during pregnancy showed a weaker association, on average explaining 0.5-4% of the variation in organochlorine levels (Table 3).

Regression analysis also showed that women born in countries outside the Nordic region had 1.3-1.6-fold lower adjusted mean levels of CB 138, CB 153, CB 156 and CB 180 than women born in the Nordic countries (R^2: 2-4%) (Fig. 1). On the contrary, higher adjusted mean levels of β-HCH (2.4-fold) and *p,p´*-DDE (3.3-fold) were found among non-Nordic women compared to Nordic women (R^2: 16-17%) (Fig. 1).

A weak (R^2: 1-3%), but statistically significant, positive association was found between number of months women had been breast-fed during their infancy and serum levels of CB 156, CB 180 and *p,p´*-DDE (Table 3). Other potential determining variables (smoking, living in a fishermen/sport fishermen family, living on the east coast for more than 5 years, and alcohol consumption during pregnancy) showed no statistically significant associations to serum organochlorine levels (results not shown), except for alcohol consumption and levels of *trans*-nonachlor. In this case the adjusted mean level was 14.0% (SD:7.4-20.9%, $p \leq 0.05$) higher among women consuming alcohol during pregnancy. Moreover, women with the highest education level had a 13.0 % (SD: 8.5-17.6%, $p \leq 0.05$) higher adjusted geometric mean of HCB than women with lowest level of education.

Dietary habits. Consumption of lean fish dominated the fish consumption, and more than 50% of the women did not eat fatty fish from the Baltic Sea. The women reported higher food consumption rates in 7th grade, except for consumption of other fatty fish (Table 4).

Among food groups studied, total fish, fatty Baltic fish and other fatty fish consumption during the year of pregnancy was positively associated to CB 118 serum levels (Table 5, Fig 2), consumption of fatty Baltic fish was positively associated to CB 153 levels, and *trans*-nonachlor levels were positively associated to total fish consumption. Less than 3% of the variation in serum levels was explained by the variation in fish consumption. No other positive or negative associations between food consumption during the year of pregnancy and serum levels were found (results not shown).

Similar results were found for the 7th grade consumption, although results for fish consumption during adolescence was statistically significant for a few more organochlorine compounds than was the case for consumption during the year women became pregnant (Table 6, Fig. 3). Moreover, a significantly higher adjusted mean level of HCB, β-HCH and *trans*-nonachlor was found among women in the highest consumption of eggs and egg products compared to women in the lowest consumption group (Fig. 4). No significant associations were found for other food groups (results not shown).

Discussion

Our results show that lipid-adjusted serum concentrations of PCB and chlorinated pesticides/metabolites among pregnant primiparous women from Uppsala County, Sweden, with a few exceptions, are significantly associated with age (positive), year of sampling (negative), pre-pregnancy BMI (negative), body weight change during pregnancy (negative) and country of birth (Nordic vs. non-Nordic). For CB 156, CB 180 and *p,p´*-DDE we also found significant positive associations with number of months the women estimated that they had been breast-fed during infancy. Consumption of fatty fish, especially fatty fish from the Baltic Sea during teenage years, was positively associated to serum lipid levels of several PCB congeners, *trans*-nonachlor and *p,p´*-DDE many years later.

Regression analysis with independent variables age, year of sampling, pre-pregnancy BMI, weight change during pregnancy, and birth place left 30-60% of the variation in serum organochlorine levels unexplained. The capacity of a regression model to explain the variation in serum organochlorine levels is both dependent on number of variables in the model and number of observations in the analysis. Most determining variables, studied by us, are not the direct cause of the variation in serum levels, instead they are proxy variables for differences in exposures, toxicokinetics, etc. It can therefore be difficult to draw conclusions about the reasons behind the associations.

The influence of some determining factors on serum levels were not the same for all compounds. For instance, the determining factor pre-pregnancy BMI was negatively associated to all substances except CB 118, HCB, β-HCH and *p,p´*-DDE. Weight change during pregnancy was not associated with β-HCH and *p,p´*-DDE, although the rest of the substances were negatively associated to weight change. Moreover, serum levels of HCB and *trans*-nonachlor were not associated with country of birth, whereas women of non-Nordic origin had higher levels of certain pesticides/metabolites and lower levels of certain PCBs than women born in the Nordic countries. This shows that there are differences in sources of exposure and toxicokinetics between some of the compounds that should be accounted for in epidemiological studies.

A few women had high levels of CB 28, CB 52 and CB 101. We hypothesize that exposure to PCBs from building materials in home or working environments could contribute to this observation [28-31].

As evident from other studies [11, 14, 32], age at sampling in late pregnancy was a strong determinant of serum organochlorine levels, after adjustment for other important determinants. For instance, a 6-fold difference in adjusted mean level of CB 153 was found between 18-year-old and 41-year-old women. Age-related bioaccumulation of the persistent and lipophilic compounds probably contributed to this age dependency of serum levels. A birth cohort effect probably also contributed, since environmental and food levels of organochlorines decreased in Sweden during the 1960s-1990s [18, 19, 33]. Women born in the 1960s and early 1970s thus experienced higher levels of exposure during childhood and adolescence than women born in the late 1970s to early 1980s.

Adjusted serum organochlorine levels decreased between 1996-99. This decline was probably mainly caused by the birth-cohort effect. The rate of decline is uncertain due to the short study period (1996-1999). Slower decline rates have been reported in a breast milk trend study in the Stockholm area [16]. Lack of age adjustment of results in the Stockholm study could be one reason for their reported slower declines. Mean age among participating Stockholm women increased from 27-28 years to 30-31 years from early 1970s to late 1990s [16]. Strong positive associations between age and organochlorine levels in humans should be accounted for in time trend studies.

Lower adjusted mean levels of PCBs and higher mean levels of β-HCH and *p,p´*-DDE among women born in non-Nordic countries are further evidence of how important exposures during childhood and adolescence are for body burdens of certain organochlorines during pregnancy. We did not have complete information about time of residence in Sweden, but seven of the sixteen non-Nordic women had lived in Sweden for more than 5 years. These women were born in southern Africa, in the Middle East, in the Balkan region, and in southern Europe, regions where use of insecticides, such as DDT and technical HCH mixtures, has been higher than in the Nordic countries [34-36]. Similar to ours, earlier studies from the USA and Germany have also found elevated levels of β-HCH and *p,p´*-DDE among individuals from countries with extensive use of DDT and HCH products [11, 37]. Moreover, low body burdens of PCBs have been previously found in persons from less industrialized countries [15, 37].

Surprisingly, serum levels of CB 156, CB 180 and *p,p´*-DDE were positively associated with number of months the women were breast-fed early in life. It may, however, have been difficult for the participating mothers to get correct information about breast-feeding during their infancy. Moreover, many women did not answer the breast-feeding question (153 women out of 323). Nevertheless, our results suggest that high exposures early in life can be traced in the body several decades later. Other studies have shown that breast milk exposure is associated with serum levels of PCBs, *p,p'*-DDE, HCB and β-HCH in children and adolescents [17, 38-40].

Negative associations between weight increase during pregnancy and serum levels of PCB, HCB and *trans*-nonachlor could be caused by a "dilution" effect of weight gain during pregnancy. Organochlorine levels in lipids ingested during pregnancy were most probably lower than levels in body lipids at the start of pregnancy. Negative associations between pre-pregnancy BMI and serum PCB and *trans*-nonachlor levels could also be due to a dilution effect. Although not studied by us, rapid weight gain before pregnancy could be more common among women with high pre-pregnancy BMI than among those with low BMI. Thus, women with high BMI may have started their pregnancy with more "diluted" serum levels. There are, however, many uncertainties regarding the influence of body composition on blood levels of organochlorines. Diverging results have previously been found for the associations between pre-pregnancy BMI and blood levels of organochlorines during pregnancy [10, 11, 14].

We found no statistically significant associations between organochlorine levels in serum and the potential determinants smoking, alcohol consumption and education level except in a few cases (HCB and education, *trans*-nonachlor and alcohol consumption). Moreover, we found no support for our hypothesis that women growing up in fishermen/sport fisher families, or along the Baltic Sea coast for at least five years, would have higher adjusted mean serum levels than other women due to higher exposures to organochlorines from fish.

Fish consumption is a major source of dietary organochlorine exposure in Sweden [41-43]. Our results confirmed the hypothesis that women reporting high fish consumption, especially contaminated fatty fish from the Baltic Sea, would have higher serum levels than women with low consumption. Associations between consumption of fatty Baltic fish during adolescence and organochlorine body burden were more consistent than associations between body burdens and consumption during the year women became pregnant. Although it can not be excluded that these associations could be due to confounding factors not studied by us, the more consistent associations for consumption during adolescence is supported by higher organochlorine levels in fish during 1960s to 1980s than in the late 1990s when the women got pregnant [44]. Moreover, the women reported a significantly higher consumption of fatty Baltic fish in 7th grade than during the year of pregnancy. Several other studies on pregnant women have shown that fish consumption habits during the time period around pregnancy are positively associated to organochlorine levels in serum/plasma [10, 11, 45, 46].

Median self-reported consumption of meat and fish did not differ between the participating women and 25-34 year old women participating in the national food consumption survey Riksmaten 1997-98 [47]. This suggests that the food consumption reported by the women is representative for the general Swedish population.

Women reporting a high consumption of eggs and egg products during adolescence had higher adjusted mean levels of HCB, β-HCH and *trans*-nonachlor than women reporting the lowest level of consumption. Recent intake calculations show that egg consumption is not a major source of intake of POPs in the Swedish population [24], and results from the food control in the 1970s did not indicate substantially higher levels in eggs than in meat and dairy products [20]. The results could be significant by chance or be due to unknown factors correlated to egg consumption.

Conclusions

Associations between serum organochlorine levels and age of the Uppsala women, sampling year and birth place suggest that long-term cumulative exposures have a major impact on body burdens of organochlorines during pregnancy. Furthermore, high exposures early in life (breast-feeding and fish consumption) may still influenced serum levels during pregnancy decades later. Several of the determining factors studied by us may be important confounders in epidemiological studies of associations between disease and organochlorine exposure, and should be accounted for in such studies. Moreover, adjustment of results temporal changes in age, BMI, etc. is important in time trend studies of body burdens of organochlorines in human populations. For instance, in Sweden the average age of primiparous women has increased from 23.8 yrs to 28.2 yrs between 1973 to 2003 [48]. The ban of production/use of organochlorines in Sweden has resulted in continuously decreasing exposures in Sweden. It can, however, not be excluded that the current body burdens of certain compounds, such as PCBs, in pregnant women could still be a health risk to the developing fetus [9].

The European Food Safety Authority recently published a risk assessment of non-dioxin-like PCBs in food and feed[49]. It was concluded that the average European intake of non-dioxin-like PCBs, dioxin-like PCBs or polychlorinated dibenzo-p-dioxins/dibenzofurans, alone or in combination, results in maternal body burdens that are only slightly lower than the body burdens that have been associated with subtle developmental effects [49]. Continuous efforts should thus be made to identify and remove existing sources of organochlorine contamination, in order to further decrease body burdens among pregnant women in the future.

List of abbreviations

BMI, body mass index; CB, chlorinated biphenyl; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; IUPAC, International Union of Pure and Applied Chemistry; PCB, polychlorinated biphenyl; SD, standard deviation.

Competing interests

The authors declare that they have no competing interests.

Authors' contribution

AG participated in the planning of the study, was responsible for data collection, did the data analysis and wrote the first draft of the manuscript. MA was involved in the planning of the study and was responsible for organochlorine analysis. POD participated in the planning of the study and in data collection. SC took part in the planning of the study and helped with data collection. RB was involved in data collection and analysis. WB helped with planning of the study and data analysis. SL helped with data analysis. All authors participated in the preparation of the final manuscript and approved the submission.

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Figure legends

Figure 1. Adjusted geometrical means (±SD) of PCB and chlorinated pesticide/metabolite levels in serum lipids (ng/g lipid) among primiparous women born in Nordic (N=307) or non-Nordic countries (N=16). For details about statistical analysis, see Methods. Results shown are for organochlorines with significantly different adjusted means between Nordic (N=307) and non-Nordic women (N=16) ($p \leq 0.05$).

Figure 2. Adjusted geometrical means (±SD) of PCB and chlorinated pesticide/metabolite levels in serum lipids (ng/g lipid) among primiparous women with different consumption rates (g/day) of herring and wild salmon/trout from the Baltic Sea (fatty Baltic fish) the year they became pregnant. For details about statistical analysis, see Methods. Results shown are for organochlorines with significantly different adjusted means between women with the lowest and highest consumption levels ($p \leq 0.05$, N=226).

Figure 3. Adjusted geometrical means (±SD) of PCB and chlorinated pesticide/metabolite levels in serum lipids (ng/g lipid) among primiparous women with different consumption rates (g/day) of herring and wild salmon/trout from the Baltic Sea (fatty Baltic fish) the year they attended 7th grade in school. For details about statistical analysis, see Methods. Results shown are for organochlorines with significantly different adjusted means between women with the lowest and highest consumption levels ($p \leq 0.05$, N=226).

Figure 4. Adjusted geometrical means (±SD) of PCB and chlorinated pesticide/metabolite levels in serum lipids (ng/g lipid) among primiparous women with different consumption rates (g/day) of eggs the year they attended 7th grade in school. For details about statistical analysis, see Methods. Results shown are for organochlorines with significantly different adjusted means between women with the lowest and highest consumption levels (except for CB 180).

Table 1. Personal characteristics of the participating primiparous pregnant women.

Variable	N	Mean (SD)			
Age (yr)	323	28 (4)			
Pre-pregnancy BMI (kg/m^2)	315	23.2 (3.7)			
Weight increase during pregnancy (%/week)	315	0.61 (0.25)			
Breast fed during infancy (months)	170	4 (3)			
		(%)			
Smoking during pregnancy	321	20			
Alcohol during pregnancy	323	17			
Childhood in a fisher/sportfisher family	213	12			
Lived on east coast of Sweden ≥5 yr	223	25			
Country of birth	323	Nordic: 95	Non-Nordic: 5		
Years of education	323	≤11: 28	12-13: 21	14-16: 25	≥16: 25

SD, standard deviation

Table 2. Serum concentrations of PCB congeners and chlorinated pesticides/metabolites[a].

PCB (ng/g lipid)		Pesticide/metabolite (ng/g lipid)	
	Median (range)		Median (range)
CB 28	1 (1-432)	Hexachlorobenzene	23 (12-163)
CB 52	1 (1-166)	γ-Hexachlorocyclohexane	1 (1-8)
CB 101	1 (1-183)	α-	1 (1-7)
CB 105	1 (1-24)	β-	9 (3-60)
CB 118	11 (3-93)	Oxychlordane	3 (1-22)
CB 138	29 (6-100)	*trans*-Nonachlor	5 (6-23)
CB 153	59 (14-179)	*o,p'*-DDT	2 (2-2)
CB 156	4 (1-27)	*o,p´*-DDE	2 (2-2)
CB 167	1 (1-9)	*p,p´*-DDT	5 (2-124)
CB 180	38 (8-139)	*p,p´*-DDD	2 (2-19)
		p,p´-DDE	88 (2-622)

[a] N=321-323

Table 3. Percent change in serum concentrations of PCB congeners and chlorinated pesticides/metabolites per unit change of determining factors[a].

Compound	Age (%change)	Sampling yr (%change)	BMI (%change)	Weight gain (%change)	Breast fed (%change)
CB 118	7.9 (7.4, 8.5)	-16 (-18, -13)	ns	-28 (-34, -22)	ns
CB 138	8.0 (7.5, 8.6)	-13 (-15, -11)	-1.3 (-1.9, -0.7)	-16 (-22, -8)	ns
CB 153	8.5 (8.1, 9.0)	-12 (-14, -11)	-2.9 (-3.4, -2.4)	-24 (-30, -18)	ns
CB 156	13 (12, 13)	-16 (-19, -14)	-4.5 (-5.3, -3.8)	-35 (-42, -27)	2.3 (1.2, 3.5)
CB 180	9.0 (8.6, 9.4)	-12 (-14, -10)	-3.6 (-4.1, -3.2)	-32 (-36, -27)	1.4 (0.7, 2.0)
HCB	3.6 (3.3, 4.0)	-11 (-13, -9)	ns	-22 (-26, -18)	ns
β-HCH	6.4 (5.9, 6.9)	-16 (-18, -14)	ns	ns	ns
trans-Nonachlor	7.6 (7.0, 8.3)	-18 (-20, -15)	-3.0 (-3.6, -2.4)	-38 (-44, -32)	ns
p,p´-DDE	8.8 (7.8, 9.3)	-13 (-16, -10)	ns	ns	3.4 (2.2, 4.7)

[a] Adjusted geometric means (-standard deviation, +standard deviation) calculated from the partial regression coefficients. Determinants were age at sampling (years), sampling year with Jan. 1 1996 as starting point, pre-pregnancy BMI (kg/m^2), weight gain during pregnancy (% per week), and breast feeding during infancy (months). For details about multiple regression analysis see Methods. ns=p>0.05, N=170-312.

Table 4. Self-reported food consumption among pregnant primiparous women.

Food products	Pregnancy year (g/day) Mean (SD)	Adolescence (g/day)[a] Mean (SD)	Difference (g/day)[b] (Mean (SD)
Meat, meat products	103 (47)	113 (49)	12 (49)*
Milk fat	26 (14)	31 (20)	6 (17)*
Vegetable fat	13 (10)		
Eggs	12 (9)	14 (9)	3 (9)*
Fish total	24 (20)	32 (24)	8 (21)*
Lean fish	18 (15)	23 (18)	6 (17)*
Fatty Baltic fish	1 (3)	3 (5)	2 (5)*
Other fatty fish	5 (6)	5 (6)	-0.3 (6)

SD, standard deviation

[a] The year women attended 7th grade in school. In Sweden 13-14 years old.

[b] Consumption during adolescence-consumption during the year they became pregnant.

*Wilcoxon Signed Rank Test, $p \leq 0.05$, N=222-226.

Table 5. Serum concentrations of PCB and chlorinated pesticides/metabolites in primiparous pregnant women with different self-reported consumption of fish the year they became pregnant[a].

Consumption (g/day)	N	CB 118 (ng/g lipid)	*trans*-Nonachlor (ng/g lipid)
Fish total			
0-8.0	56	10.6 (10.1-11.1)	5.2 (4.9-5.5)
8.1-21.4	57	11.5 (11.0-12.0)	5.5 (5.3-5.8)
21.5-32.2	56	11.5 (11.3-12.0)	5.2 (4.9-5.4)
32-3-106.7	57	12.1 (11.6-12.7)*	6.0 (5.7-6.3)*
Other fatty fish			
0-0.9	58	10.1 (9.6-10.6)	
1.0-2.4	55	11.8 (11.2-12.4)*	
2.5-6.9	53	11.8 (11.3-12.8)*	
7.0-30.0	60	12.1 (11.6-12.7)*	

[a] Geometric mean (±standard deviation) adjusted for age, year of sampling, pre-pregnancy BMI (not CB 118), and weight change during pregnancy (not CB 118). Statistical analysis performed only on women born in Nordic countries.

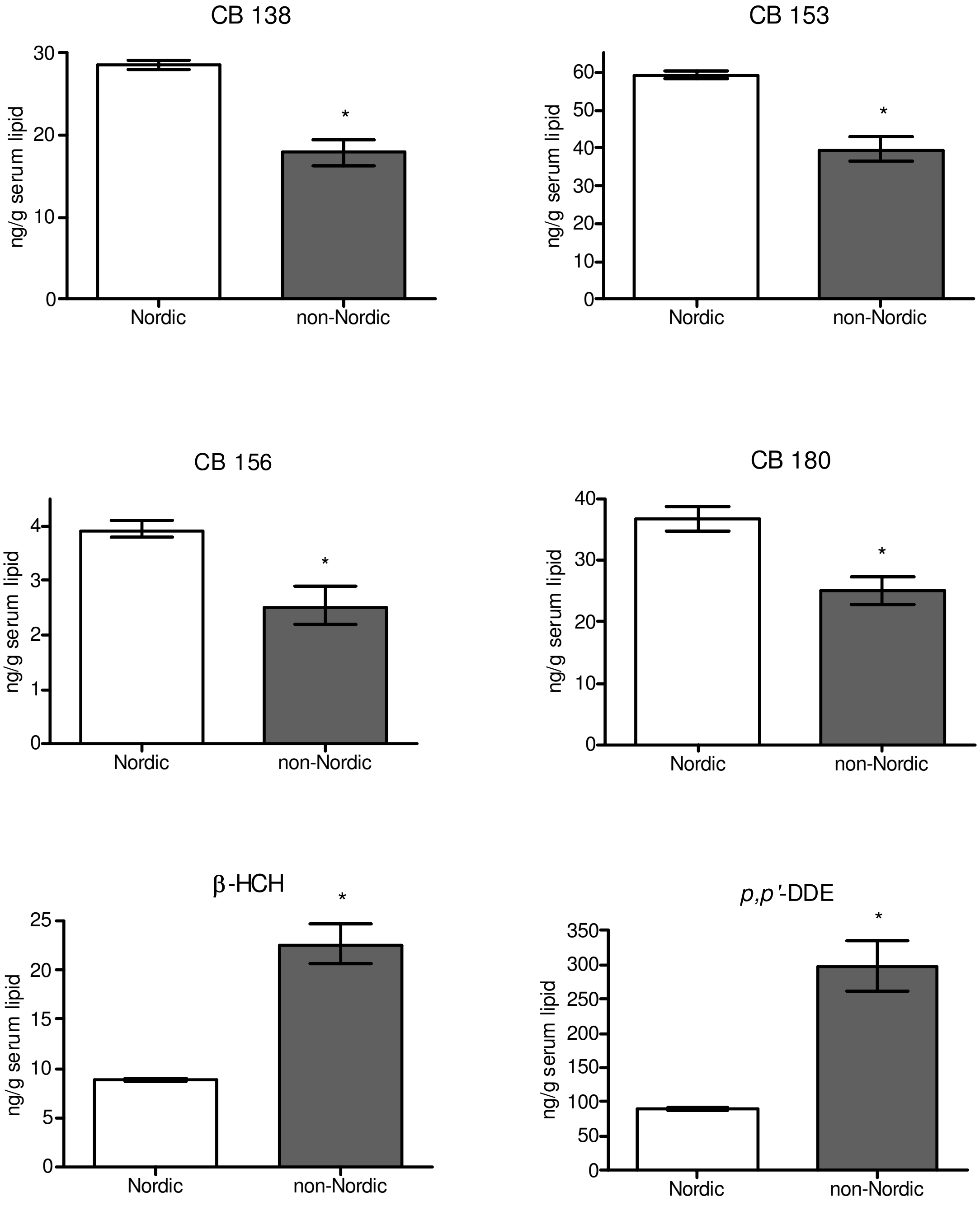
*Significantly different from the group with lowest consumption, $p \leq 0.05$. Results are shown for organochlorines where adjusted means in the groups with lowest and highest consumption were significantly different.

Table 6. Serum concentrations of PCB and chlorinated pesticides/metabolites in primiparous pregnant women with different self-reported consumption of fish the year they attended 7th grade in school[a].

Consumption (g/day)	N	CB 118 (ng/g lipid)	CB 156 (ng/g lipid)	HCB (ng/g lipid)	*trans*-Nonachlor (ng/g lipid)
Fish total					
0-16.6	55	10.4 (9.9-10.9)	3.7 (3.5-4.0)	21.3 (20.6-21.9)	5.1 (4.8-5.3)
16.7-26.2	57	11.4 (10.8-11.9)	4.1 (3.8-4.3)	24.5 (23.8-25.3)*	5.3 (5.0-5.6)
26.3-40.1	56	11.5 (11.0-12.0)	4.4 (4.2-4.7)*	23.3 (22.6-24.0)*	5.5 (5.2-5.8)
40.2-297.1	57	12.4 (11.8-12.9)*	4.4 (4.2-4.7)*	24.1 (23.4-24.8)*	6.0 (5.7-6.3)*
Other fatty fish					
0-0.9	55	10.5 (10.0-11.1)			
1.0-2.4	56	11.4 (10.9-11.9)			
2.5-6.9	56	11.4 (10.9-12.0)			
7.0-30.0	57	12.3 (11.7-12.9)*			

[a] Geometric mean (±SD) adjusted for age, year of sampling, pre-pregnancy BMI (not CB 118 and HCB), and weight change during pregnancy (not CB 118). HCB results were also adjusted for education level. Statistical analysis performed only on women born in Nordic countries.

*Significantly different from the group with lowest consumption, $p \leq 0.05$. Results are shown for organochlorines where adjusted means in the lowest and highest consumption were significantly different.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ng/g serum lipid
0
10
20
30
Nordic
non-Nordic
*
CB 153
ng/g serum lipid
0
10
20
30
40
50
60
Nordic
non-Nordic
*
CB 156
ng/g serum lipid
0
1
2
3
4
Nordic
non-Nordic
*
CB 180
ng/g serum lipid
0
10
20
30
40
Nordic
non-Nordic
*
β-HCH
ng/g serum lipid
0
5
10
15
20
25
Nordic
non-Nordic
*
p,p'-DDE
ng/g serum lipid
0
50
100
150
200
250
300
350
Nordic
non-Nordic
*

Figure 1

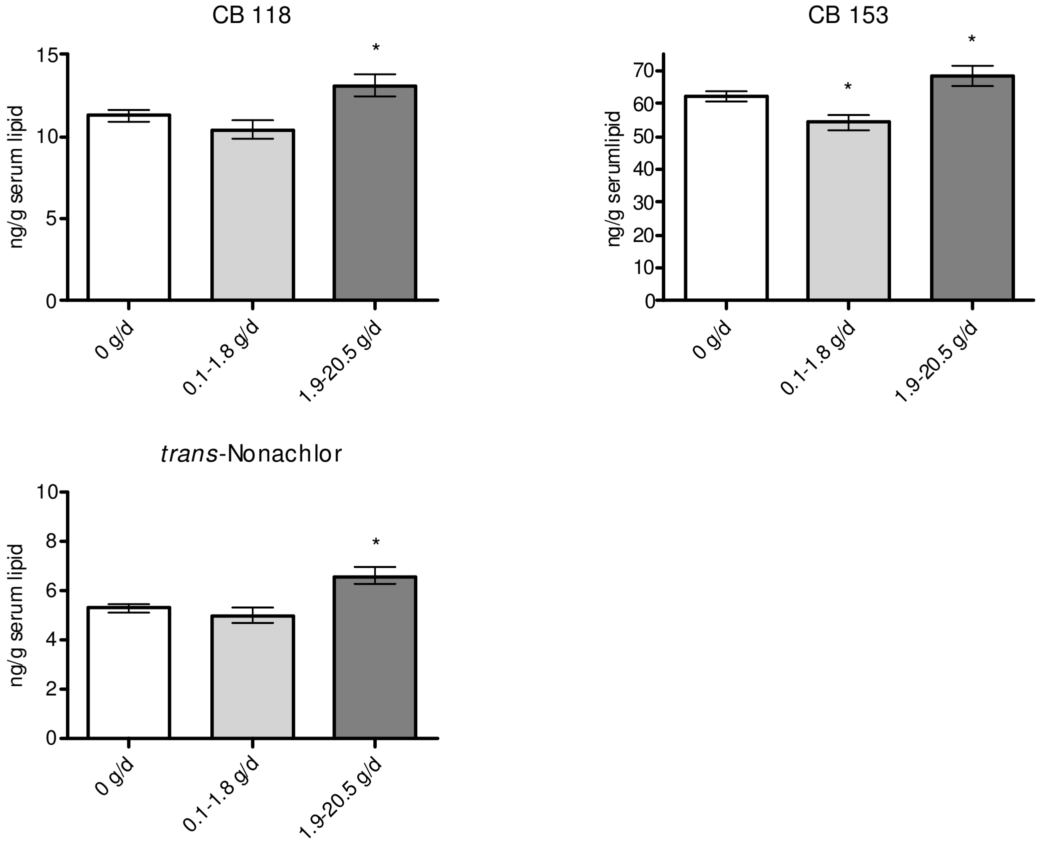
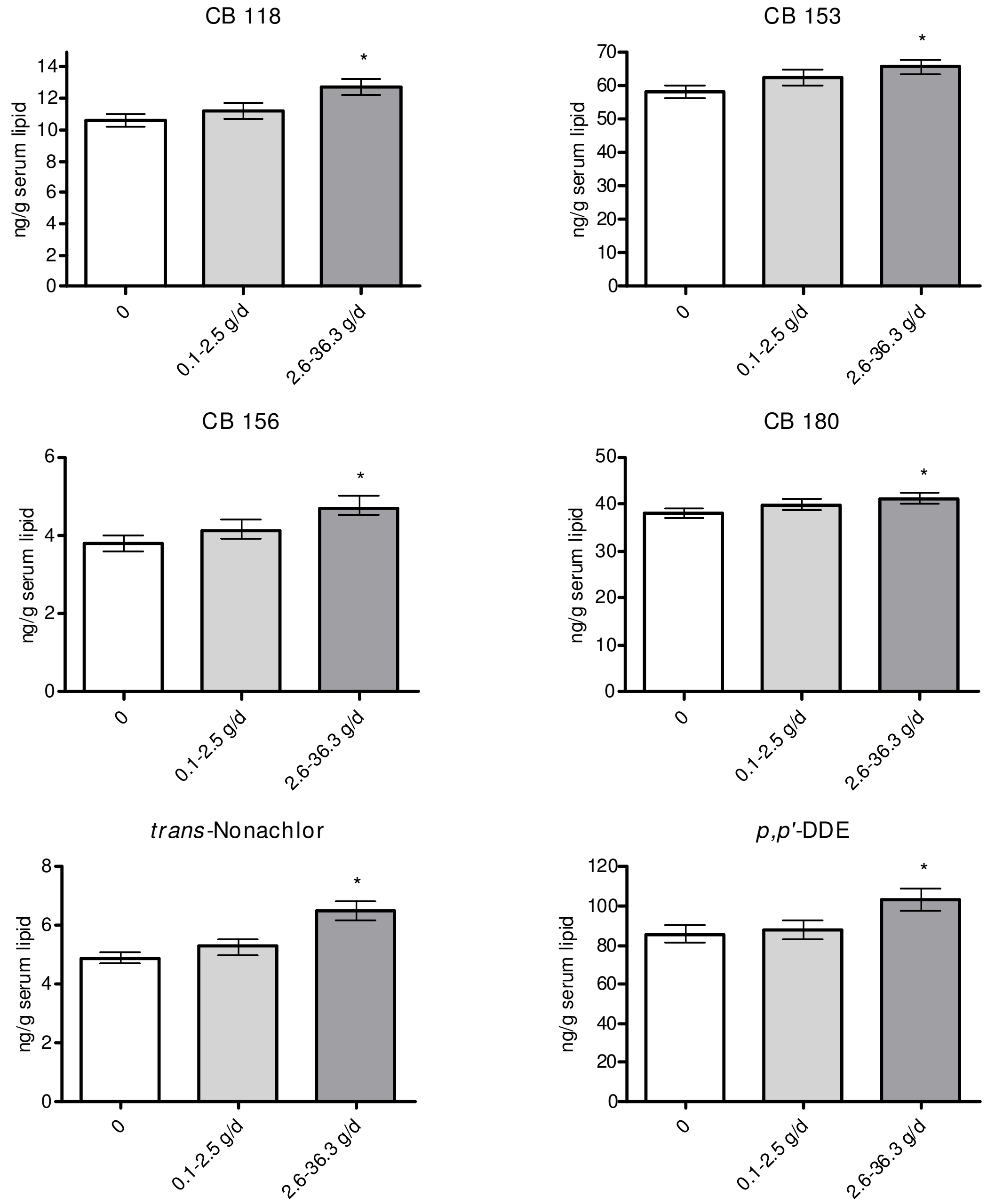

CB 118
ng/g serum lipid
15
10
5
0
*
0 g/d
0.1-1.8 g/d
1.9-20.5 g/d
CB 153
ng/g serumlipid
70
60
50
40
30
20
10
0
*
*
0 g/d
0.1-1.8 g/d
1.9-20.5 g/d
trans-Nonachlor
ng/g serum lipid
10
8
6
4
2
0
*
0 g/d
0.1-1.8 g/d
1.9-20.5 g/d

Figure 2


CB 118
ng/g serum lipid
0
2
4
6
8
10
12
14
*
0
0.1-2.5 g/d
2.6-36.3 g/d
CB 153
ng/g serum lipid
0
10
20
30
40
50
60
70
*
0
0.1-2.5 g/d
2.6-36.3 g/d
CB 156
ng/g serum lipid
0
2
4
6
*
0
0.1-2.5 g/d
2.6-36.3 g/d
CB 180
ng/g serum lipid
0
10
20
30
40
50
*
0
0.1-2.5 g/d
2.6-36.3 g/d
trans-Nonachlor
ng/g serum lipid
0
2
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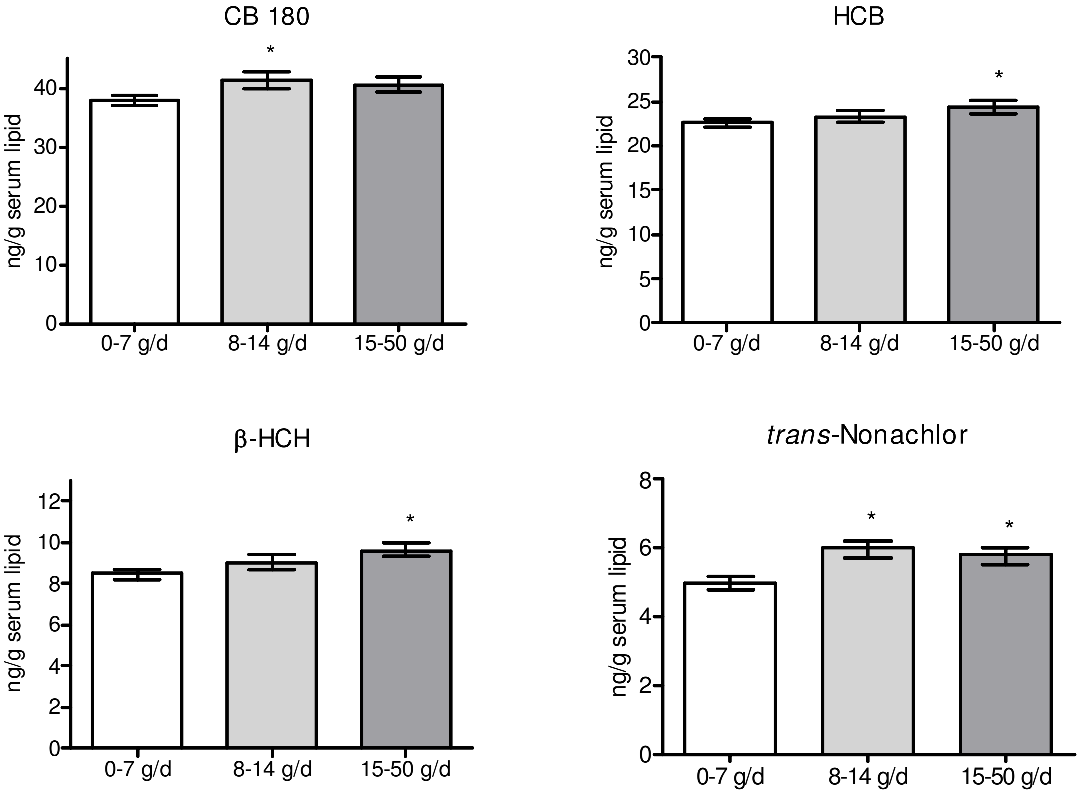
0
0.1-2.5 g/d
2.6-36.3 g/d
p,p'-DDE
ng/g serum lipid
0
20
40
60
80
100
120
*
0
0.1-2.5 g/d
2.6-36.3 g/d

Figure 3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10
20
30
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HCB
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0
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0-7 g/d
8-14 g/d
15-50 g/d
β-HCH
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trans-Nonachlor
ng/g serum lipid
0
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0-7 g/d
8-14 g/d
15-50 g/d

Figure 4