Estimations of past male and female serum concentrations of biomarkers of persistent organochlorine pollutants (POPs) and their impact on fecundability estimates

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ABSTRACT

Persistent organochlorine pollutants (POPs) have been suggested to have negative effects on a number of hormonal systems. Several studies performed retrospectively have reported a possible association between POP exposure and fertility, measured as time to pregnancy (TTP). However, these studies are lacking biomarkers of exposure at the time when the women tried to conceive. It has previously been found that past *female* serum concentrations of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) can be estimated using a complex decay model, assuming that the biological half-life is 5 years, the yearly environmental reduction of the compound has been 3 % since 1976, and that reduction of body burden due to lactation is 20 % for periods up to 6 months and 30 % for periods exceeding 6 months. In the present study it is established that the model is valid also for estimations of past *male* serum concentrations of CB-153. Furthermore, the complex decay model was found to be useful also for estimating past serum concentrations of 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p´-DDE), assuming that the biological half-life of the compound is 8 years, that the yearly reduction between 1971 and 1981 was 20 % and after that 9 %, and that the reduction of body burden due to lactation is the same as for CB-153. However, even though the estimated past serum concentrations of CB-153 and p,p'-DDE were found to be better proxy measures of actual past concentrations than current serum concentrations, there was little change in the rank order of the population investigated. Thus, the effect estimate for TTP was similar for both proxy measures when using categorized measures of exposure.

Key words: Biomarkers, polychlorinated biphenyls, DDT, fertility, past exposure

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INTRODUCTION

Persistent organochlorine pollutants (POPs), such as polychlorinated biphenyls (PCBs) and dichlorodiphenyl trichloroethane (DDT), have been released into the environment, mainly since the Second World War. PCB and DDT were restricted or totally banned in most countries during the 1970s and 1980s, but *e.g*. DDT is still used in some areas for malaria vector control. The lipophilic POPs are highly resistant to both abiotic and biotic degradation. They are transported through both atmosphere and watercourses, and trace amounts are found all over the world, even in places were PCBs never have been produced or used (AMAP 1998). These substances are stable and resistant to metabolism and have long biological half-lives. Due to persistence, biomagnification and bioaccumulation, several populations have increased body burdens of POPs. In Sweden, studies have shown that the body burdens of PCBs and the major DDT-metabolite 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p´-DDE) are still considerable in fishermen (Sjödin et al. 2000) and fishermen's wives.

In Sweden, a major source of exposure to POPs is through the consumption of fatty fish from the Baltic Sea, off the east coast of Sweden. Cohorts of professional Swedish fishermen (Svensson et al. 1995) and their wives (Rylander and Hagmar 1995) have been established. Both the east and west coast fishermen's families eat more locally caught fish than the general population (Hagmar et al. 1992, Rylander et al. 1995, 1996, Svensson et al. 1991). Since the fish caught of the west coast is less contaminated than Baltic Sea fish (Bergqvist et al. 1989), it is fair to assume that the east coast fishermen's families are more dietary exposed to POPs than both the west coast families and the general population. Indeed, higher serum levels of POPs in east than in west coast fishermen have been observed (Rignell-Hydbom et al. 2004, Svensson et al. 1995).

One of the most common PCB congener, 2,2´,4,4´,5,5´-hexachlorobiphenyl (CB-153) has been found to correlate very well with the total PCB concentration (Glynn et al. 2000, Grimvall et al. 1997, Richthoff et al. 2003), as well as with the TCDD (2,3,7,8-tetrachloro-dibenzo-*p*-dioxin)-equivalent (TEQ) and the total PCB derived-TEQ (Gladen et al. 1999). Thus, CB-153 may be considered a useful biomarker of body burden of POPs whereas p,p'-DDE is considered to be a good indicator of non-recent exposure to DDT.

Several PCB congeners and DDT, as well as p,p'-DDE, have been suggested to have weak agonistic- or antagonistic effects on a number of hormonal systems (Bonefeld-Jorgensen et al. 2001, Kelce et al. 1998, Kelce et al. 1995). Thus they may be classified as "endocrine disruptors". An increasing body of evidence indicates that endocrine disruptors may cause developmental and reproductive abnormalities in animals: Due to a chemical spill, Lake Apopka, Florida, was extensively polluted with DDT. A series of reproductive abnormalities have been reported in alligators living in the lake, including reduced clutch size, altered gonadal morphology, smaller penis size and altered penis size (Guillette et al. 1999). In laboratory studies male rats exposed *in utero* to a single dose of 0.005, 0.20, or 0.80 µg/kg TCDD on gestation day 15 displayed reduced fertility, delayed puberty and altered reproductive organ weight (Gray et al. 1997). A single postnatal (day 21) dose of non-dioxin-like PCBs resulted in a decrease in sperm motility and sperm capability to penetrate hamsteroocytes in adult rats (Hsu et al. 2003b). Humans studies have shown that accidental high dietary exposure to PCBs and polychlorinated dibenzofurans (Yu-Cheng) had a negative effect on male reproductive function (Guo et al. 2000, Hsu et al. 2003a). Other studies with lower serum concentrations of PCB have shown adverse effects on especially sperm motility (Bush et al. 1986, Dallinga et al. 2002, Hauser et al. 2003, Richthoff et al. 2003, Rignell-Hydbom et al. 2004). Furthermore, small associations (both positive and negative) between

measured serum levels of POPs or reported fish consumption rates, and changes in menstrual cycle length have been reported in background-exposed populations (Axmon et al. 2004a, Cooper et al. 2005, Mendola et al. 1997, Windham et al. 2005).

The time that elapses between the cessation of contraceptive use and clinical recognition of a pregnancy (time to pregnancy; TTP) has been shown to be a useful tool for the assessment of reproductive effects of exposures in general (Joffe 1989), as well as for environmental exposures (Baird et al. 1986).

Several studies on TTP as an outcome after dietary exposure to POPs have been carried out on populations which are exposed to POPs through the consumption of contaminated fatty fish. The results of these studies are ambiguous in that some have found a possible association between fish consumption and TTP (Buck et al. 2000, Courval et al. 1999), whereas others have not (Axmon et al. 2000b, 2002, Buck et al. 1997). Since all previous studies have been performed retrospectively, they share the drawback of lacking biomarkers of exposure at the time when the woman tried to conceive. However, a method of estimating past exposure to PCBs, using a complex decay model has been developed previously (Rylander et al. 1998). Validation using biobanked pregnancy screening serum samples have shown that the backward estimation enhanced the fit with actual serum levels at time of pregnancy as compared with current serum levels (Axmon et al. 2001, Rylander et al. 1998). However, the method has not been validated for other biomarkers than CB-153, or for male exposure.

The aims of the present study were I) to validate the complex decay model for male exposure to CB-153 and p,p´-DDE, and II) to investigate the possible impact on fecundability estimates from using estimated past exposure measures instead of current exposure. The latter aim was evaluated for men and women separately.

MATERIALS AND METHODS

Study population

The recruitment of the male part of the study population is described in detail elsewhere (Rignell-Hydbom et al. 2004). Briefly, 648 fishermen from the Swedish east and west coasts were invited to participate in a study investigating a possible effect of POP exposure on semen quality. Although 266 men agreed to participate, during the field study 71 men were excluded due to change of mind (n=15), logistical reasons (n=22), sickness or recent vasectomy (n=10), or they were impossible to get in contact with (n=24). Moreover, five men failed to supply blood samples. In the present study we included 67 men whose partner supplied information on TTP (see also below).

Of the 67 men included in the present study, 39 had also participated in a study conducted in 1991 (Sjödin et al. 2000). In this study, blood was drawn and serum concentrations of CB-153 and p,p'-DDE were determined. These serum concentrations were used in the present study to validate the choice of parameters in the complex decay model.

In those cases where the fisherman was married or cohabiting (n=87), his partner was invited to participate in the study. Of these women 67 (77%) participated. Furthermore, women who had previously answered a questionnaire regarding the association between fish consumption and skeletal fractures (Wallin et al. 2004), and who had stated that they would be willing to supply blood for a future study were also invited (n=1074), as were women who had previously participated in a study on TTP and fish consumption (Axmon et al. 2000b),

but who were not included in the study on skeletal fractures (n=1509). Of the 2650 women invited, 559 (21 %) were interviewed and provided blood samples. However, among these, TTP was missing for 40 (7%). The reasons for this was either that they had never been pregnant (n=5), had never had a planned pregnancy (n=7), did not remember their TTP (n=11), or had an assisted pregnancy (n=17).

Outcomes

The primary method of assessing TTP was simply asking the women how much time (weeks and/or months and/or years) that had passed from the time the couple stopped using birth control until the woman became pregnant. Using this information, a TTP in months was estimated assuming that each year was equal to 12 months, and that each week was a quarter of a month. The TTP in months was used as a proxy of TTP in cycles, which is the inherent unit of TTP. Of the women who provided information on TTP, 513 supplied information according to this method. The women were also asked about how many times they menstruated during the time period when they tried to become pregnant (categorized in the questionnaire as 0, 1, 2, 3 or more than 3 times). If the woman had not provided a TTP in weeks/months/year, this possibly censored measure was used when available (n=6). For neither of the methods was it required for the woman to state how the pregnancy was determined.

Exposure

Collection and analyses of blood samples drawn in 2001

Blood samples were drawn from a cubital vein into 10 ml vacuum tubes for serum collection without additives (Becton Dickinson, Maylan, France). After cooling to room temperature the tubes were centrifuged at 4000 *g* for 15 min. Serum was transferred with ethanol rinsed Pasteur pipettes to ethanol rinsed brown glass bottles (Termometerfabriken, Gothenburgh, Sweden). A piece of aluminium foil was placed on top of the bottles which were then sealed. Sera were stored at -80°C until analysis (< 1 year).

The levels of CB-153 and p,p'-DDE in serum were determined shortly after the blood was drawn, applying solid phase extraction using on-column degradation of lipids and analysis by gas chromatography mass spectometry (Jönsson et al. Submitted, Richthoff et al. 2003, Rignell-Hydbom et al. 2004). The relative standard deviations, calculated from samples analyzed in duplicate at different days, was 18% at 0.1 ng/mL, 10% and 0.5 ng/ml, and 10% at 2 ng/mL for CB-153 and 11% at 1 ng/mL, 8% at 3 ng/mL, and 7% at 8ng/mL for p,p'-DDE. The detection limits were 0.05 ng/mL for CB-153 and 0.1 ng/mL fpr p,p'-DDE.

Serum concentrations of triglycerides and cholesterol were determined by enzymatic methods as described elsewhere (Jönsson et al. Submitted). The total lipid concentration in serum was calculated by the following equations (Rylander et al. In press):

Men: Total (g/L) = 0.96 + 1.28*(triglycerides + cholesterol)

Women: Total (g/L) = 1.13 + 1.31*(triglycerides + cholesterol).

Backwards estimation of the POP biomarkers using a complex decay model

The estimations were done using a method which assumes a stable and relatively low background exposure, as well as a constant individual intake of fatty fish from the Baltic Sea,

but takes into account the reduction of the compounds in the environment, the biological half-life of the compounds, and reduction of body burden due to breast feeding (Axmon et al. 2001, Axmon et al. 2004b, Rylander et al. 1998).

We have previously found that for CB-153 it is reasonable to assume that the yearly reduction in the environment is 3% since 1976, that the biological half-life of CB-153 is 5 years in humans, and that women who breast feed for less than 6 months reduce their body burden by 20%, whereas women who breast feed for longer than 6 months reduce their body burden by 30% (Axmon et al. 2001).

The available literature on the reduction of DDT and its metabolites in the Baltic Sea presents results with respect to sDDT (DDT+DDE+DDD). However, in biological material from the Baltic Sea, p,p´-DDE represents about 95% of sDDT (Odsjö et al. 1997). Thus, it seems fair to use these parameter value for p,p´-DDE.

Bignert et al (Bignert et al. 1998b) found a 10% yearly reduction of sDDT between 1967 and 1995. However, other reports suggests that the decrease started in the early 1970's and was most rapid during the first 6-10 years, where yearly decreases of as much as 20% were found (Bignert et al. 1998a, Odsjö et al. 1997). We chose to assume that the initial fast reduction occurred between 1971 and 1981, and that a slower yearly reduction was present after 1981. Since all our past serum concentrations of p,p'-DDE were measured in 1991, i.e. after the initial fast decrease, there was no possibility of validating this fast decrease. Based on the literature, we assumed this decrease to be 20% yearly. For the decrease after 1981, we ran four different models, assuming 1%, 3%, 6% and 9% yearly decrease, respectively (Table 1).

The half-life of p,p'-DDE in humans has in some studies been found to be more than 10 years (Longnecker et al. 1997), whereas others have found it to be less than seven years (Morrison and Newell 1999, WHO 1989). To determine a suitable parameter value for the complex decay model, we performed analyses assuming the half-life to be 4, 8 and 12 years, respectively (Table 1).

The reduction of the body burden of p,p'-DDE due to breast feeding is similar to the reduction of CB-153 (Rogan et al. 1986, Skaare and Polder 1990). Thus, the same parameter values for breast feeding was used for p,p'-DDE as for CB-153.

Past estimated concentrations of CB-153 and p,p'-DDE could be calculated for 536 women and 66 men. Furthermore, past actual exposure (blood samples taken in 1991) was available for 39 of the 66 men, and were used to validate the model for male exposure to CB-153 as well as to p,p'-DDE.

Statistics

To determine the best set of parameter values for estimation of past concentrations of p,p'-DDE, we determined the median difference between estimated and actual past concentrations for each combination of parameter values. To investigate the agreement between the estimated past male concentrations of CB-153 (parameter values determined previously) and p,p'-DDE (best set according to the procedure described above) on one hand and the actual concentrations measured in 1991 on the other, and to compare this to the agreement between concentrations measured in 1991 and in 2001 we used box-plots which were visually assessed.

The correlations between continuous variables were described using Spearman's coefficient of correlation (r_S).

To assess the possible impact on fecundability of different measures of CB-153 and p,p'-DDE, female data was trichotomized into three equally sized groups (approximately 170 women in each group). The number of men included was lower than the number of women, thus the male data was dichotomized (approximately 30 men in each group).

To estimate the association between exposure and TTP, logistic regression was applied to a database of months, estimating the per month relative odds of pregnancy. For each comparison, a fecundability ratio (FR) was calculated from the odds ratio (OR) for pregnancy in each month. In the analyses, TTP was censored at 12 months.

When comparing the FRs regarding past estimated exposure to those regarding current exposure, no potential confounders were considered. The rationale for this was that potential confounders would most likely have the same impact on FRs regardless of which measure of exposure was used.

RESULTS

Of the women who were invited to participate in the present study, some 80 % had been included in previous studies on fish consumption and reproductive outcomes (Axmon et al. 2000a, Axmon et al. 2000b). The information from the previous studies were used to assess possible differences between women who did and did not participate in the present study. The non-respondent analyses revealed that the respondents had somewhat higher level of education (29 % vs 17 % had studied at university or college) and were somewhat more likely to be non-smokers prior to their first pregnancy (61 % vs 55 %). However, there were only small differences with respect to ever having had a planned pregnancy (82 % vs 78 %), or having been pregnant more than once (95 % vs 91 %).

The median recall time for TTP was 19 years (range 0-38 years). Since the blood was drawn at the same time that TTP was determined, the length of time for which the biomarkers were estimated was the same.

The agreement between estimated past serum concentrations of p,p'-DDE and actual concentrations measured in 1991 improved as we increased the assumed yearly environmental decrease after 1981. However, based on prior knowledge concerning the decrease of POPs in fatty fish from the Baltic Sea, very high yearly decrease seemed implausible. Thus, a 9% yearly decrease was used in the final model.

With respect to the half-life of p,p'-DDE, the difference between estimated and actual past serum concentrations varied marginally when investigating half-lives of 4, 8 and 12 years, respectively. Thus, based on prior knowledge about the half-live in man, a half-life of 8 years seemed suitable as a parameter value.

In the final models, the difference between CB-153 measured in 1991 and 2001 was on average 100 ng/g lipid (median decrease), whereas the difference between CB-153 measured in 1991 and estimated CB-153 was 40 ng/g lipid. The corresponding numbers for p,p'-DDE were 400 ng/g lipid and 40 ng/g lipid (Table 1; Figure 1).

The correlations between current and estimated past concentrations of CB-153 (r_S=0.96 for men and r_S=0.97 for women) were higher than those between current and estimated past concentrations of p,p'-DDE (r_S=0.78 for both men and women). When assessing a possible relation between exposure and TTP, the FRs were similar irrespective of whether the estimated past concentrations or current concentrations of CB-153 and p,p'-DDE were used (Table 2).

DISCUSSION

The present study indicates that, in retrospective studies, estimations of past male concentrations of CB-153 and p,p'-DDE through a complex decay model provides better agreement with actual past concentrations than do concentrations measured at later time points. However, when investigating the relation between biomarkers for POPs and TTP, using categorized exposure measures, the risk estimates were similar whether using estimated past concentrations or current concentrations as proxy for actual past exposure.

The complex decay model used to estimate past concentrations of CB-153 and p,p'-DDE was derived in order to estimate the impact of female exposure to CB-153 on reproductive outcomes (Rylander et al. 1998). In a review, this model has been recommended to use when no repeated measures are available (Karmaus et al. 2004). The complex decay model is dependent on three parameter values: I) the reduction of the compound in the environment, II) the biological half-life of the compound, and III) the reduction of body burden due to breast feeding. Parameter values for female exposure have been previously determined and validated using CB-153 concentrations in serum samples from pregnancy screening programmes stored in a biobank (Axmon et al. 2001, Axmon et al. 2004b). However, the parameter values have not been previously validated for male concentrations of CB-153, neither have any parameter values for p,p'-DDE been determined.

In a previous validation of the complex decay model, we used the Kappa-values for categorized concentrations of female CB-153 (Axmon et al. 2001, Axmon et al. 2004b, Rylander et al. 1998). However, in the present study we have only two measures for each man, and all current and past concentrations are from the same time point, respectively (i.e. 1991 and 2001). Thus, there was no intra individual variation with respect to the time period for which the backwards estimation was performed. Therefore, we were forced to use the continuous, rather than categorized, concentrations of male CB-153 and p,p'-DDE in the validation process. A consequence of this was that we were not able compare the results from the present validation procedure to previous results.

There were some differences in the methods of analyses of lipid contents in 1991 and in 2001, in that the lipid content in 2001 was determined solely by triglyceride and cholesterol concentrations, whereas phospholipids were also analyzed in 1991. However, the formula used to determine lipid content based on triglyceride and cholesterol only have been previously validated, and it has been found that these two lipids explain as much as 97% of the variation in total lipid concentration (Rylander et al. In press). Thus, lipid adjusted serum concentrations in 1991 and in 2001 should be comparable.

The reduction of body burden of CB-153 due to lactation is considerable. Thus, the parameter value for this reduction has a major impact on the estimation of past exposure. Therefore, the complex decay model will be more sensitive to the choice of parameter values for reduction in the environment and the biological half-life when applied to men. Nevertheless, when comparing the estimated past concentrations with the actual past, there were small differences in absolute concentrations. Thus, there is a strong support in favour of the choice of parameter values made in previous studies.

Since POPs are stored in adipose tissue in the body, an individual with a drastic weight loss would get an equally drastic increased concentration of POPs in fatty tissue. However, it has been found that the average BMI in 1991 and in 2001 are similar for the

fishermen included in the present study (Lars Hagmar, personal communication). Thus, weight loss or weight gain should not pose a major problem in the present study.

The complex decay model has previously not been used to estimate past concentrations of p,p'-DDE, neither for men nor women. However, the two compounds have a large number of properties in common, and it is therefore reasonable to use the same model to estimate past p,p'-DDE as was used to estimate past CB-153.

Based on the literature and the results from models run with different parameter values, we chose three parameter values for estimation of past p,p'-DDE. Since we had no actual past concentrations for the women included in the present study, we had no possibility of validating the assumed reduction of body burden due to lactation. However, previous studies have indicated that the reduction of p,p'-DDE is similar to that of CB-153 (Rogan et al. 1986, Skaare and Polder 1990), which have been validated previously. Thus, we feel confident that the parameter value assumed for reduction of body burden due to lactation is appropriate.

To investigate the effect of using estimated past concentrations, instead of current concentrations, as proxy markers for actual past concentrations we trichotomized the female concentrations and dichotomized the male concentrations. The different categorizations techniques were applied since the number of men and women differed. The effect of male and female exposure on fecundability was assessed for both estimated past exposure and current exposure. We found that the results from the two different sets of analyses were quite similar. Thus, even if a persons body burden of POPs changes over time, she or he is likely to maintain hers or his rank order in a closed population such as the Swedish Fishermen's Families Cohort.

We can not rule out that the results would have been different, if we had chosen to use the biomarkers as continuous variables rather than categorized ones. However, there is no support, neither in this study, nor in the literature, that there is a dose-response relation between exposure to POPs and fecundability.

We have refrained from adjusting the FRs for potential confounders, such as age, BMI, smoking and alcohol. The reason for this is that the aim of the study was to compare risk estimates from two different measures of exposure: Current concentrations of POP and estimated past concentrations of POP. Since the outcome is the same in both analyses, adjusting for confounders would not change the relation of one FR to the other. In previous studies on TTP among fishermen and their wives (of which the persons included in the present study constitute a subgroup), efforts have been made to adjust for potential confounders (Axmon et al. 2000b, Axmon et al. Submitted). In these studies, it was found that the FRs did not change after inclusion of factors such as age and smoking. Thus, we suspect that including potential confounders in the present analyses would not affect the FRs. However, it should be acknowledged that the FRs presented in the present work are crude estimates, and should be used only to compare two measures of exposure.

Today, the use of PCBs and DDT has been banned or restricted in most parts of the world. Therefore, even though the compounds are still present in the environment, and can be found in almost all compartments of the biosphere, including animal and human tissues and body fluids (Brouwer et al. 1995), the current levels in the environment are lower than a few decades ago (Bignert et al. 1998a, Bignert et al. 1998b, Odsjö et al. 1997). From this follows, that in order to study men and women who were highly exposed to PCBs or DDT during their fertile years, retrospective studies are necessary. Several reproductive outcomes, such as birth weight and gestational length, can be collected through different registers. Furthermore, Joffe (Joffe et al. 1993) has found that it is quite possible to assess TTP retrospectively. However, a

drawback is obviously that biomarkers of POPs can not be measured retrospectively. Thus, to be able to estimate past concentrations of these biomarkers, is advantageous.

We make no claims of being able to estimate correctly past exposure of biomarkers of POP. To do this would indeed be difficult since the increase or decrease of a person's body burden of POP depends on factors which are difficult to take into consideration. These factors could include consumption of other food products than fatty fish, or weight loss. However, it must be concluded that in retrospective studies, using the complex decay model, with the parameter values suggested in the present study, will result in a more precise proxy than using current concentrations of the biomarker in question. Nevertheless, if the aim of a study is to determine the effect of exposure on a specific outcome, rather than focusing on the actual exposure levels, current concentrations of POPs may well be used as proxies for past concentrations.

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Table 1. Difference (median and quartiles) between serum concentrations of p,p'-DDE measured in 1991 and estimated past concentrations based on serum concentrations in 2001.

Yearly reduction after 1981	Half-life of p,p'-DDE		
	4 years	8 years	12 years
1%	-380 (-600, -140)	-370 (-600, -140)	-360 (-590, -130)
3%	-260 (-520, 120)	-250 (-510, -120)	-250 (-510, -110)
6%	-140 (-390, -50)	-140 (-360, -44)	-130 (-320, -24)
9%	-45 (-140, 71)	-43 (-130, 79)	-24 (-100, 100)

Table 2. Fecundability ratios (FRs) with 95% confidence intervals (CIs) for current and estimated past male and female serum concentration of 2,2'.4,4',5,5'-hexachlorbiphenyl (CB-153) and 1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethylene (p,p'-DDE).

		Estimated past exposure	Current exposure
		FR (95% CI)	FR (95% CI)
Female exposure	CB-153[1]		
	Medium vs. low	1.12 (0.85-1.48)	1.08 (0.82-1.43)
	High vs. low	1.10 (0.83-1.45)	1.14 (0.86-1.51)
	p,p'-DDE[2]		
	Medium vs. low	1.02 (0.77-1.34)	1.16 (0.89-1.53)
	High vs. low	1.11 (0.84-1.47)	1.05 (0.79-1.39)
Male exposure	CB-153[3]		
	High vs. low	0.60 (0.30-1.21)	0.58 (0.29-1.16)
	p,p'-DDE[4]		
	High vs. low	0.60 (0.30-1.21)	0.44 (0.22-0.89)

[1] Estimated past: Cut-off low/medium=112 ng/g lipid, and medium/high=194 ng/g lipid. Current: Cut-off low/medium=67 ng/g lipid, and medium/high=106 ng/g lipid.

[2] Estimated past: Cut-off low/medium=497 ng/g lipid, and medium/high=1550 ng/g lipid. Current: Cut-off low/medium=107 ng/g lipid, and medium/high=203 ng/g lipid.

[3] Estimated past: Cut-off low/high=257 ng/g lipid. Current: Cut-off low/high=173 ng/g lipid.

[4] Estimated past: Cut-off low/high=633 ng/g lipid. Current: Cut-off low/high=207 ng/g lipid.

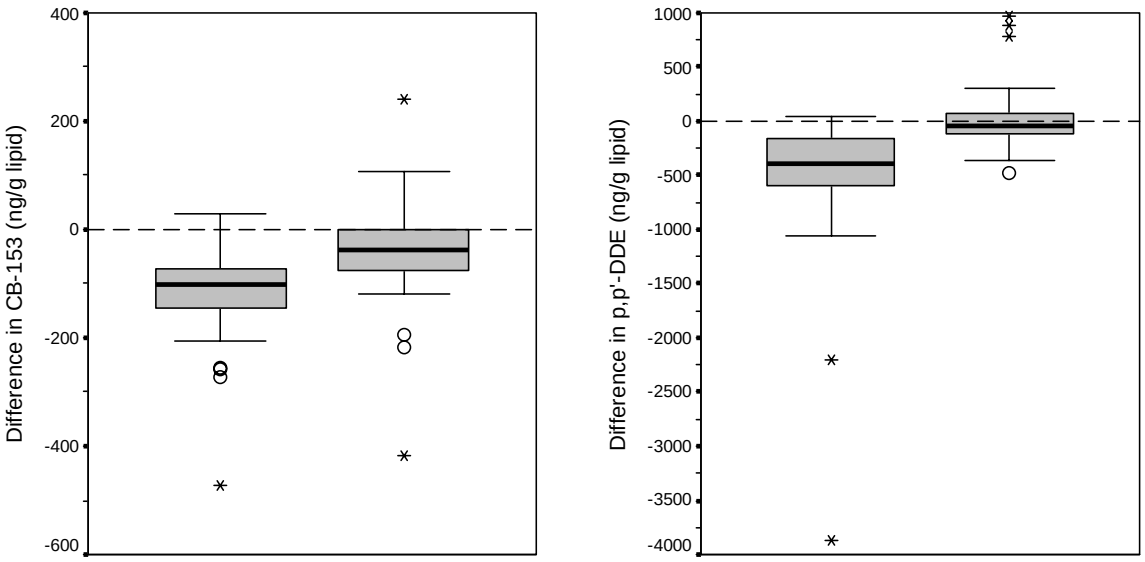

Figure 1. Difference between past (measured in 1991) concentrations of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153; left chart) and 1,1-dichloro-2,2-bis (p-chlorophenyl)-ethylene (p,p´-DDE; right chart) and current concentrations (measured in 2001; left box in each chart) and estimated past concentrations (right box in each chart). Circles denotes outliers (>1.5 times the inter-quartile difference), whereas stars denotes extremes (<3 times the inter-quartile difference).