Environ. Sci. Technol. **2006**, *40,* 6282−6289

Hydroxy-PCBs, PBDEs, and HBCDDs in Serum from an Elderly Population of Swedish Fishermen's Wives and Associations with Bone Density

JANA WEISS,[†] EWA WALLIN,[‡] ANNA AXMON,[‡] BO A. G. JÖNSSON,[‡] HELENE ÅKESSON,[‡] KAREL JANÁK,[§] LARS HAGMAR,[‡,||] AND ÅKE BERGMAN*[,†]

Department of Environmental Chemistry, Stockholm University, SE-10691 Stockholm, Sweden, Division of Occupational and Environmental Medicine and Psychiatric Epidemiology, University Hospital Lund, SE-221 85 Lund, Sweden, and Division of Environmental Medicine, Norwegian Institute of Public Health, NO-0403 Oslo, Norway

Lack of human exposure data is frequently reported as a critical gap in risk assessments of environmental pollutants, especially regarding "new" pollutants. The objectives of this study were to assess serum levels of the persistent 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153), hydroxylated polychlorinated biphenyl metabolites (OH-PCBs), polybrominated diphenyl ethers (PBDEs), and hexabromocyclododecanes (HBCDDs) in a group of Swedish middle-aged and elderly women expected to be relatively highly exposed, and to evaluate the impact of potential determinants (e.g., fish intake, age) for the inter-individual variation, as well as to investigate the association between these pollutants and bone density. No associations were found between bone mineral density or biochemical markers of bone metabolism and the analyzed environmental pollutants. Relatively high levels of CB-153 (median 260 ng/g fat) and Σ_3-OH-PCBs (median 1.7 ng/mL serum), and low concentrations of Σ_6PBDEs (median 3.6 ng/g fat) were determined. Total level of HBCDDs in serum was quantified by gas chromatography with mass spectrometric detection (median 0.5 ng/g fat). HBCDD diastereomeric and enantiomeric patterns were determined by liquid chromatography with mass spectrometric detection. The dominating stereoisomer was (−)α-HBCDD, but 1−3% of γ-HBCDD was also detected in the serum samples.

Introduction

While it is established that endocrine disruption occurs in wildlife for a range of persistent organic pollutants (POPs), it has not yet been possible to confirm such effects in humans (*1*). Still, there are numerous indications that such effects can play a role in human health. For example, several polychlorinated biphenyls (PCBs) have experimentally shown the ability to act as endocrine disrupting compounds (EDCs). PCBs and their hydroxylated metabolites (OH-PCBs) induce decreased peripheral vitamin A and thyroxin (T_4) levels in rodents and induce microsomal enzyme activities (*2*). PCBs have also been suggested to influence sperm quality (*3*, *4*) and possibly induce type 2 diabetes mellitus (*5*, *6*). More recently polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDDs) have come into focus due to increasing exposure levels (*7*−*9*) and concern about their potential health effects (*10*−*12*). Endocrine disruption, e.g., reproductive effects and developmental neurotoxicity in rodents, is the principle effect causing concern (*2*,*10*−*13*). So far PBDEs have been the most studied group of brominated flame retardants, whereas data on HBCDDs are still scarce (*10*, *14*).

A potential effect of human endocrine disruption may be osteoporosis as indicated in seals from the Baltic Sea (*15*). Fractures have become more common among elderly people all over the world, especially in northern Europe (*16*). Many factors influence the risk of fractures, such as age, menopause, heredity (*17*), and lifestyle habits (physical activity, smoking, alcohol intake, and diet) (*18*). Animal data show that dioxin-like POPs may impair normal bone metabolism and mineralization, as well as reduce collagen content and serum osteocalcin levels, which result in increased bone fragility (*19*−*22*).

Fatty fish from the Baltic Sea is a major source of POP exposure for the population in Sweden and other countries around the Baltic Sea (*23*, *24*). In a register-based study there was a significantly increased risk for vertebral fractures among fishermen's wives from the Swedish east coast as compared with fishermen's wives from the west coast. Fatty fish from the Baltic Sea is more contaminated with POPs compared to fish caught off the Swedish west coast (*24*). In a questionnaire study there were no differences in fracture incidence between east and west coast fishermen or fishermen's wives (*25*). However, for east coast fishermen's wives incidence of osteoporotic fractures correlated with consumption of fatty fish from the Baltic Sea (*25*). A clinical examination of 196 east coast fishermen and 184 east coast fishermen's wives showed no association between serum levels of 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153), a biomarker for PCB, or *p*,*p*′-DDE and bone mineral density (BMD) or biochemical markers of bone metabolism (*26*).

The objectives of the present study were as follows: (1) to assess serum levels of some selected environmental pollutants (CB-153, OH-PCBs, PBDEs, and HBCDDs) in a group of Swedish middle-aged and elderly women; (2) to evaluate the impact of potential determinants for the inter-individual variations in serum concentrations of the selected environmental pollutants; and (3) to investigate the associations between these pollutants and bone density and biochemical markers of bone metabolism.

Materials and Methods

Study Population and Interview. A previously established cohort of wives and ex-wives of professional fishermen from the Swedish east coast (*27*, *28*) was approached for this study. In 2000, postal questionnaires were sent to 1291 Swedish subjects born 1920−1954. There were 779 women (77%) who responded to the questionnaire. Out of these, 596 women were positive to participate in future studies. A subset of 184 women was invited and participated in a study mainly focused on the association between CB-153 and *p*,*p*′-DDE in serum and bone mineral density (*26*), but also comprised by other potential health effects of POP exposure. Details of the recruitment process and non-participant analysis have been given elsewhere (*26*). A subset of 53 women was selected for

* Corresponding author phone: +46-8-163995; fax: +46-8-163979; e-mail: ake.bergman@mk.su.se.
† Stockholm University.
‡ University Hospital Lund.
§ Norwegian Institute of Public Health.
|| Deceased.

10.1021/es0610941 CCC: $33.50
Published on Web 09/09/2006

TABLE 1. Background Characteristics of 53 Fishermen's Wives from the Swedish East Coast

	n	%	median	5%	95%
potential determinants for persistent organic pollutant concentration					
age (yr)			62	52	81
current body mass index (kg/m^2)			27	21	42
change in body mass index since age 25 (% increase)			18	0	73
current consumption of fatty fish from the Baltic Sea (meals/month)			2	0	12
outcome variables					
bone mineral density (g/cm^2)			0.44	0.24	0.61
S-Osteocalcin (μg/L)			28	12	58
S-CTX (ng/L)			386	114	877
potential confounders for the association between persistent organic pollutants and bone mineral density, osteocalcin and CTX					
body height (cm)			163	156	173
current body weight (kg)			71	55	112
body weight at age 25 (kg)			63	46	75
body mass index at age 25 (kg/m^2)			22	18	27
age at menopause			50	44	56
length of fertile period (years)			36	29	43
current hormone replacement therapy	10	19			
history of fracture[a]	2	4			
heredity for fracture[b]	10	19			
low physical activity at work at 25 and 35 yrs of age[c]	3	6			
currently low physical activity[d]	7	13			
current smoking (cigarettes/day)			0	0	15
cumulative cigarette smoking over life (pack-years)			0	0	33
current alcohol consumption (g/month)			37	0	362

[a] Osteoporotic fracture after 50 years of age. [b] Parents or siblings. [c] Defined as mostly sitting or standing with low muscle activity. [d] Defined as mostly sitting or standing with low muscle activity at work and participating in other physical activities (such as cycling, walking, gardening, etc.) less than 7 h per week.

the present study. The selection criterion was to include those with highest and lowest BMD measurement, to ensure contrast in outcome measure. The overlap between the subjects analyzed for CB-153, *p*,*p*′-DDE, and OH-PCBs and those analyzed for PBDEs and HBCDDs was almost complete, as serum from 50 of these women was analyzed for each compound. The participants were asked about individual factors that potentially might affect the concentrations of pollutants in serum and bone metabolism (Table 1). Data for calculation of the present body mass index (BMI) and as it was at the age of 25 were collected for each participant. All women had reached menopause. The study was performed in accordance with the Declaration of Helsinki and approved by The Lund University Ethic's Committee. All participants provided written informed consents.

BMD Measurement. BMD (g/cm^2) was measured in the distal section of the forearm (radius and ulna) using dual energy X-ray absorptiometry (DXA), as described elsewhere (*26*).

Blood Sampling. Blood was drawn between 8 and 10 a.m. after 12 h fasting, into sterile Vacutainer glass tubes (BD Vacutainer, Plymouth, UK). Sera were separated by centrifugation (4000 rpm, 10 min) and transferred to glass tubes before being stored at −80 °C until analysis.

Chemicals. CB-153 and $^{13}C_{12}$-labeled CB-153 were purchased from Riedel-deHaën (Seelze, Germany) and Cambridge Isotope Laboratories (CIL, Andover, MA), respectively. All OH-PCBs and PBDE congeners applied as internal and external standards for identification and quantification were synthesized in-house (*29*, *30*). The external standards for identification of α-, *β*-, and *γ*-HBCDD isomers were purchased from CIL. All chemicals were of the best available quality. Diazomethane was synthesized as described by Fieser et al. (*31*) from *N*-methyl-*N*-nitroso-*p*-toluene sulfone amide (Sigma-Aldrich, Stockholm, Sweden) as starting material, and kept in diethyl ether and stored in a freezer at −20 °C until use.

Chemical Analysis. Determination of CB-153, 4-hydroxy-2,3,3′,4′,5-pentachlorobiphenyl (4-OH-CB107), 4-hydroxy-2,2′,3,4′,5,5′-hexachlorobiphenyl (4-OH-CB146), and 4-hydroxy-2,2′,3,4′,5,5′,6-heptachlorobiphenyl (4-OH-CB187) in serum samples ($n = 53$) was performed at Lund University. Analyses of 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47), 2,2′,4,4′,6-pentabromodiphenyl ether (BDE-100), 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), 2,2,4,4′,5,6′-hexabromodiphenyl ether (BDE-154), 2,2′,4,4′,5,5′-hexabromodiphenyl ether (BDE-153), decabromodiphenyl ether (BDE-209), and of total 1,2,5,6,9,10-hexabromocyclododecane (sum of α-, *β*-, and *γ*-HBCDD), in serum samples ($n = 50$) were performed at Stockholm University.

The serum (1 mL) levels of CB-153 were determined as previously described (*3*, *4*). The method applied for the cleanup and analysis of OH-PCBs, PBDEs, and HBCDDs in serum (5 mL) is described by Hovander and co-workers (*32*), with some modification for the PBDE and HBCDD analysis, i.e., (1) no partitioning was performed with potassium hydroxide (*32*), due to the alkaline instability of HBCDD, and (2) to exclude the majority of the PCBs and remaining lipids in the PBDE and HBCDD extract, a fractionation was performed, applying a silica gel (0.7 g) column (*33*). The first fraction of hexane (3 mL) was discarded, and the next fraction, dichloromethane (5 mL), was collected.

The OH-PCBs were analyzed by GC−MS on a DB-1 (30 m) column with GC oven programmed from 100 °C to 210 °C (10 °C/min) and to 310 °C (30 °C/min). PBDE congeners and HBCDDs (sum of α-, *β*-, and *γ*-HBCDD) were analyzed by GC−MS on a DB-5 column (15 m). The injector was programmed from 60 °C (1 min) to 300 °C (150 °C/min, 22 min). The GC oven was programmed from 80 °C (1 min) to 300 °C (15 °C/min, 10 min). MS was run in electron capture negative ionization (ECNI) mode with selected ion monitoring (SIM) where CB-153 was monitored at m/z 360, $^{13}C_{12}$-labeled CB-153 was monitored at m/z 372, 4-OH-CB107, 4-OH-CB146, 4-OH-CB187, and 4-OH-CB193 were monitored at m/z 341.5, 375.5, 409.5, and 409.5, respectively, and the PBDE congeners and HBCDD were monitored for the bromide isotopes m/z 79 and 81.

The PBDE/HBCDD extracts were later pooled ($n = 25$, 125 mL/pool) for stereoisomer determination, by LC/MS−MS performed at the Norwegian Institute of Public Health, using a published method (*34*). Diastereomers were separated on a Symmetry C_{18} LC-column with a mobile phase composition programmed from water/methanol (60%:40%) at 250 μL/min in 5 min to methanol (100%), and held for 7 min. Enantiomers were separated on a chiral (NUCLEODEX beta-PM) LC-column using mobile phase programmed from water/methanol/acetonitrile (30%:20%:50%), 0.5 min, 500 μL/min in 10 min, to methanol/acetonitrile (45:55), and held for 8 min. MS was operated in electrospray ionization negative ion mode using multiple reaction monitoring (MRM).

Quality Control. The recovery of PBDE congeners and HBCDD was tested in a separate recovery study with low (1 ng) and high (10 ng) doses added. Mean recovery for low dose was 86−93% and for high dose was 75−98% depending on congener. The recovery of added surrogate standard to all samples was between 67 and 116%. The analysis of CB-153 was part of an inter-comparison program (*3*). The obtained results were within the tolerance limits (2*x* standard deviation).

The analytical limits of detections (LOD, $s/n = 3$) for CB-153, 4-OH-CB107, 4-OH-CB146, and 4-OH-CB187 were estimated to 7.3 ng/g fat, 0.02 ng/mL, 0.02 ng/mL, and 0.01 ng/mL respectively. The LOD ($s/n = 5$) was 0.012−0.024 ng/g fat for BDE-47, -99, -100, -153, and -154, and 0.12 pg/g fat for BDE-209 and HBCDD. LODs ($s/n = 3$) of α-, β-, and γ-HBCDD diastereomers were 0.03, 0.06, and 0.03 ng/g fat, respectively. For enantiomers, LODs were 1.5 ng/g fat for (+)α-HBCDD and (+)β-HBCDD and 0.7 ng/g fat for (+)γ-HBCDD. Limit of quantification (LOQ) was defined as 10 times the noise level or 3 times background blank levels. Background levels were detected for BDE-47 (0.12 ng/g fat), BDE-99 (0.06 ng/g fat), and BDE-209 (0.06 ng/g fat). In all analyses background levels found in blank samples were subtracted from measured values.

Quality control was performed by inter-laboratory calibration of OH-PCB analysis between three laboratories. Coefficient of variation (CV) between mean reported levels from each laboratory was 2−15%, and within laboratories was 3−13% (Stockholm), 1.5−17% (Lund), and 5−9% (Amsterdam). Intra-laboratory quality control of PBDE and HBCDD analysis was performed via repeated analysis of reference material (*35*). The interassays CV were 1−12% for BDE-47, -99, -100, -153, and -154 and 20−28% for BDE-209 and HBCDD.

Enzymatic Determination of Serum Lipids. Serum concentrations of triglycerides and cholesterol were determined enzymatically (*36*). Based on calculations reported by Rylander and co-workers (*37*) the total lipid concentration in serum (g/L) was calculated by the following equation: Total lipids = $0.9 + 1.3(C_{\text{triglycerides}} + C_{\text{cholesterol}})$.

Analyses of Biochemical Markers of Bone Metabolism. Crosslaps (CTX) and osteocalcin in serum were determined with immunoassays. Methods and imprecision of analyses have been described elsewhere (*26*).

Statistics. Age, current BMI, relative change in BMI from BMI at 25 years of age (% increase), and intake of fatty fish from the Baltic Sea were considered as possible predictors of serum concentrations of the POP biomarkers. In order to build multivariate models including these possible predictors, stepwise regression (backward) with p-to-include = 0.10 was used (SPSS for Windows version 12.0). Thus, in a first step, all possible predictors were included in the model. The variable that contributed the least to the total explained variance was excluded if the p-value for its contribution was >0.10. Residuals were checked using a normal probability plot. If the assumption of normality was not met, a model with log-transformed values was tried. The model based on log-transformed values was found to be appropriate for 4-OH-CB107, 4-OH-CB146, 4-OH-CB187, CB-153, BDE-47, BDE-99, BDE-100, BDE-154, BDE-209, and Σ_6PBDE but not for BDE-153 and HBCDD. When the outcome is log-transformed, interpretation of the slope of the regression line (β) is not straight forward. However, using the formula $100(e^{\beta} - 1)$ the percentage increase in the average value of the outcome per unit increase in the predictor was calculated.

To evaluate a possible effect of the POP biomarkers (all but CB-153, which has been analyzed previously (*26*) on BMD), and serum concentrations of osteocalcin and CTX, all biomarkers were dichotomized at their respective median (Table 2), resulting in a low- and a high-exposed group. Since none of the outcome variables fulfilled the normality criteria, and since the number of observations was small, differences between low and high exposure were evaluated using Mann−Whitney's U-test (StatXact-6). To evaluate possible confounding, pair-wise Spearman's correlations between the potential confounders in Table 1, and POP-biomarkers (continuous variables), BMD, osteocalcin, and CTX were calculated. If a variable was found to correlate ($r_S > 0.20$) with both the biomarker and the outcome, it was considered as a confounder, and its dichotomized version (cut at the median) was used to adjust the p-value. The analyses regarding 4-OH-CB107, 4-OH-CB146, and 4-OH-CB187 were performed using the wet weight concentrations, whereas all other analyses were based on lipid adjusted concentrations. Results below LOD were given a value of 0, and results below LOQ were given a value of 0.5 LOQ.

TABLE 2. Concentrations of CB-153 (n = 53), OH-PCBs (n = 50), PBDEs and HBCDDs (n = 50) in Human Serum

	median	5%	95%	minimum	maximum
fat content (%)	0.65	0.51	0.83	0.48	0.86
CB-153 (ng/g fat)	260	98	500	70	620
4-OH-CB107 (ng/mL)	0.54	0.17	1.9	0.12	3.3
4-OH-CB146 (ng/mL)	0.68	0.26	1.4	0.19	1.8
4-OH-CB187 (ng/mL)	0.47	0.21	1.0	0.14	1.4
BDE-47 (ng/g fat)	0.91	0.29	5.9	0.27	8.1
BDE-47 (pmol/g fat)	1.9	0.59	12	0.55	17
BDE-99 (ng/g fat)	0.20	<0.18[a]	1.9	<0.18[a]	3.1
BDE-99 (pmol/g fat)	0.35	<0.30[a]	3.4	<0.30[a]	5.5
BDE-100 (ng/g fat)	0.29	0.11	1.0	0.08	2.6
BDE-100 (pmol/g fat)	0.51	0.20	1.8	0.14	4.7
BDE-153 (ng/g fat)	1.1	0.46	2.5	0.29	4.7
BDE-153 (pmol/g fat)	1.7	0.71	3.9	0.44	7.2
BDE-154 (ng/g fat)	0.33	0.19	1.0	0.17	1.3
BDE-154 (pmol/g fat)	0.52	0.30	1.6	0.26	2.1
BDE-209 (ng/g fat)	0.46	<0.18[b]	3.0	<0.18[b]	3.3
BDE-209 (pmol/g fat)	0.48	<0.18[b]	3.2	<0.18[b]	3.5
Σ_6PBDE (ng/g fat)	3.6	1.5	12	1.1	20
Σ_6PBDE (pmol/g fat)	5.8	2.5	24	1.8	35
HBCDDs (ng/g fat)	0.46	<0.24[c]	1.2	<0.24[c]	3.4
HBCDDs (pmol/g fat)	0.71	<0.37[c]	1.9	<0.37[c]	5.4

[a] n < LOQ (LOD) = 14 (0). [b] n < LOQ (LOD) = 7 (10). [c] n < LOQ (LOD) = 6 (1).

The composition of enantiomeric HBCDD was expressed as enantiomer fractions (EFs) calculated from the peak areas (A) of enantiomers (±) in a pair by the following formula:

$$\text{ER} = \frac{(+)A}{(-)A + (+)A}$$

Results

OH-PCB fresh weight concentrations are given in Table 2. Lipid-adjusted concentrations of CB-153, PBDEs, and HBCDDs, expressed both in a molar scale (pmol/g fat) and on a weight basis (ng/g fat) are also given in Table 2. The median concentrations of the three OH-PCBs were rather similar (range 0.47−0.68 ng/mL). Among the PBDEs the highest median concentration was reported for BDE-153 (1.1 ng/g fat), followed by BDE-47 (0.9 ng/g fat), whereas on a molar basis BDE-47 level (1.9 pmol/g fat) was followed by BDE-153 (1.7 pmol/g fat) and then similar concentrations for BDE-154 (0.52 pmol/g fat), BDE-100 (0.51 pmol/g fat), BDE-209 (0.48 pmol/g fat), and BDE-99 (0.35 pmol/g fat).

The median concentration for HBCDDs was $^1/_{500}$ of CB-153, while only half of the BDE-47 level (cf. Table 2). Among the HBCDDs (Figure 1) α-HBCDD is the dominating diastereomer in the serum samples but γ-HBCDD was also detected (1−3%) in the samples. (−)α-HBCDD was identified as the dominating HBCDD enantiomer. The EF was estimated to be between 0.17 and 0.23 (Figure 2).

Age was positively associated with all three OH-PCBs, CB-153, and HBCDD, but only with BDE-154 among the PBDEs (Table 3). It cannot be excluded that the BDE-154 chromatography was influenced by coeluting 2,2′,4,4′,5,5′-hexabromobiphenyl. BDE-153 was negatively associated with current BMI, which means there were higher serum concentrations in relatively lean women. No significant association was observed between current BMI and any of the other compounds. Significant negative associations between per-

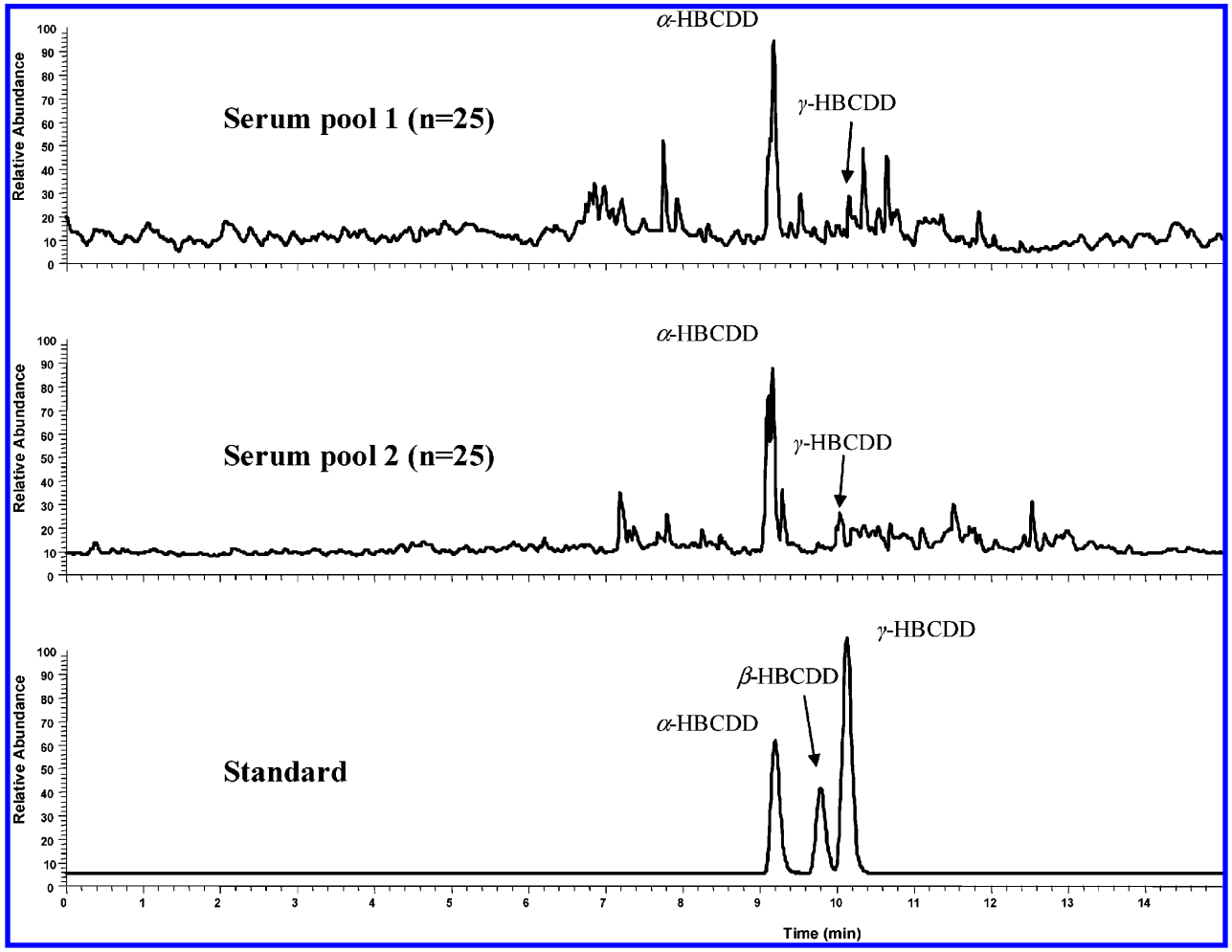

FIGURE 1. Separation of HBCDD diastereomers in two pooled samples ($n = 25$) of human serum as performed by LC/MS−MS (Symmetry C_{18} LC-colum).

cent increases in BMI from age 25 to the current situation were observed for all three OH-PCBs and for CB-153, but positive associations were seen for BDE-99 and BDE-100. Current consumption of fatty fish from the Baltic Sea was positively associated with CB-153 in serum, but not with any of the other POPs analyzed, indicating the fatty fish is not a major source of these compounds.

The linear regression models explained between 26 and 34% of the variance for the different OH-PCB congeners, which was lower than 41% for CB-153, but higher than for the PBDE congeners (8−24%) and HBCDDs (11%). None of the potential determinants explained any variation in BDE-209 concentrations.

There were negative associations between high serum concentrations of 4-OH-CB146 and 4-OH-CB187, respectively, and BMD, but when adjustments were made for age, weight, and BMI, no associations remained (Table 4). There were no other significant associations between the exposure and outcome variables.

Discussion

CB-153 is a well-established biomarker for PCB exposure and represents ~25% of total PCB (*38*, *39*), suggesting a total PCB concentration in serum of 280−2500 ng/g fat in the present cohort. OH-PCB levels have been reported to constitute between 5% and 40% of the total PCB on a fat basis (*23*, *35*, *40*, *41*). Σ_3OH-PCBs as presented in this study are on a level similar to that of CB-153, and within the previously mentioned range. Both CB-153 and Σ_3OH-PCB are considerably higher than reported serum levels in general populations in Europe at the same time period (*40*,*42*−*44*), but comparable with CB-153 concentrations in an elderly population of Swedish women (54−75 years old) (*45*). The PCB levels and hydroxylated metabolites are also comparable with a hot-spot exposure area in Slovakia (*46*), and in the Faeroe Islands (*47*), as well as with high and low fish consumers in Sweden, Latvia (*23*), and Finland (*48*). CB-153 levels correlate significantly with age of the individuals as reported earlier (*23*, *42*, *45*). It is thus expected that OH-PCB levels show similar trends (*23*).

In the present study, PBDEs did not correlate with age, which is in accordance with previous reports on human serum (*23*,*49*−*51*), but interestingly HBCDD does correlate with age. HBCDD total body half-life in humans has been estimated to 64 days, which is considerably lower than half-lives for tetraBDEs−hexaBDEs (1−10 years) (*52*), but longer than the apparent plasma half-life of BDE-209 (15 days) (*53*). Possibly, this difference can be explained by a continuous and ongoing use of HBCDD while the PBDE exposure has been disrupted (*7*, *54*). This reasoning is supported by the results of increasing HBCDD concentrations in human milk (*7*) and guillemot eggs (*54*).

PBDE congener levels in the fishermen's wives are comparable to or lower than those of other recently conducted studies on human serum in Europe (*44*, *49*, *55*). The Σ_6PBDE levels analyzed in human serum in this study were between 2 and 35 pmol/g fat. Studies on PBDE levels in human tissue have commonly reported BDE-47 as the dominating congener (*8*). In the current study BDE-153 concentrations were similar to BDE-47 which has been

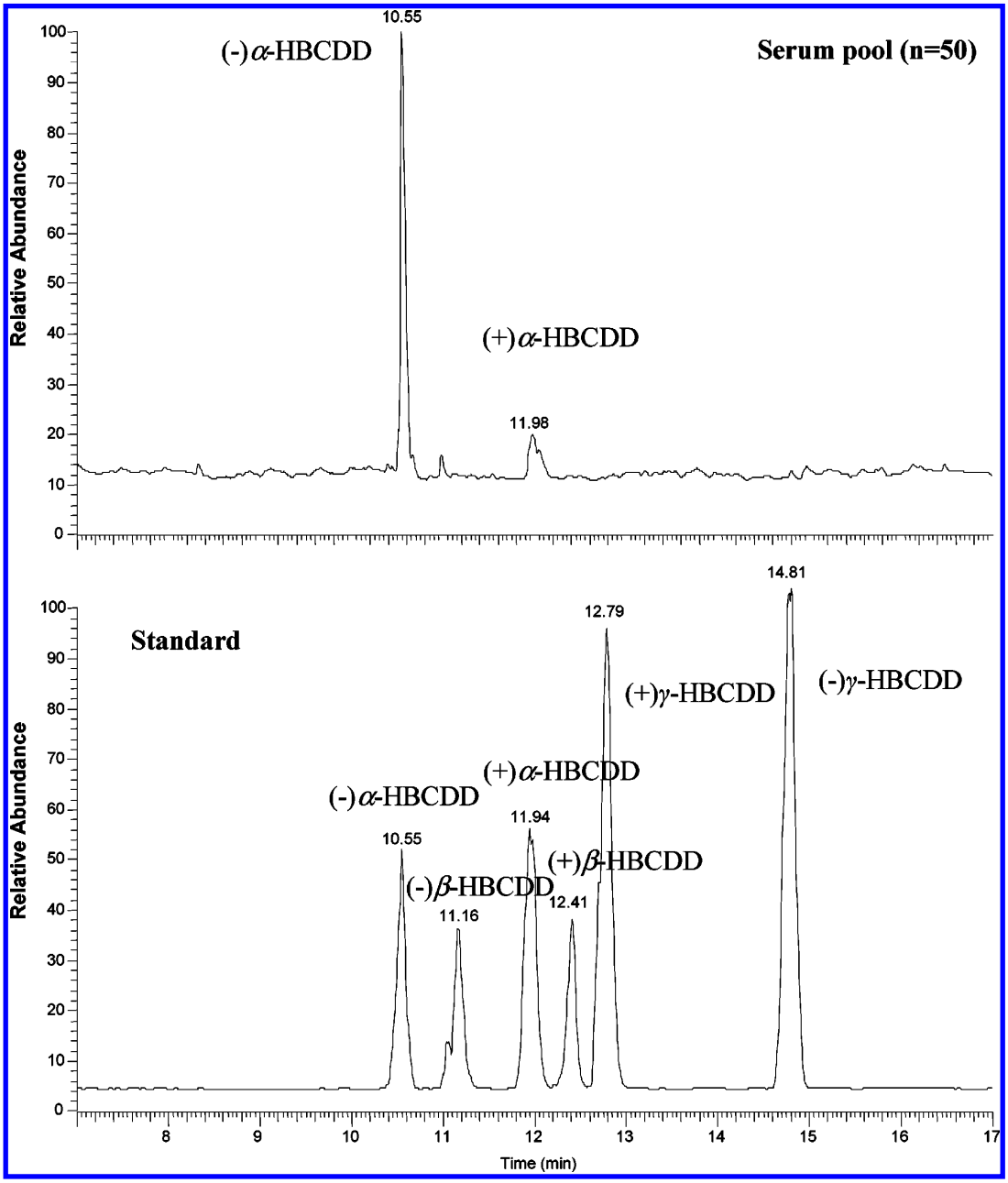

FIGURE 2. Separation of HBCDD enantiomers in one pool ($n = 50$) of human serum samples as performed by LC/MS−MS (chiral NUCLEODEX beta-PM LC-column).

reported in several other studies (*7*, *35*, *56*, *57*). This might be due to the suggested three times longer half-life of BDE-153 than of BDE-47 (*52*), possibly combined with a positive effect of the voluntary phase-out of PentaBDE in Sweden, as early as the beginning of the 1990s (*10*). PentaBDE is a major source of BDE-47 (30% of the mixture) whereas BDE-153 is a minor component in both PentaBDE and OctaBDE technical mixtures (*10*). Further, it cannot be excluded that abiotic decomposition or metabolism of BDE-209 to lower brominated PBDEs, such as BDE-153, can be a contributing factor to elevated BDE-153 concentrations (*58*−*60*).

There are few analyses conducted on HBCDDs in human tissue. In 2003 HBCDDs were for the first time identified and quantified in breast milk from a Swedish background population (*61*) and from a Norwegian population with high fish intake (*62*), and in 2004 in maternal and cord blood from The Netherlands (*57*) and in Mexican breast milk and blood (*63*). All HBCDD concentrations were in the same range as those reported here for the fishermen's wives (0.3−5.4 pmol/g fat). A time trend study on human milk levels of HBCDDs and PBDEs in Swedish mothers was recently finished, in which an increasing trend of HBCDDs was observed, starting from the mid 1980s, but leveling off over the last couple of years (*7*). The HBCDD concentrations in mothers' milk were below 1 pmol/g fat throughout the study period.

The technical mixture consist mainly of γ-HBCDD (~80%) while biological samples most commonly seem to contain α-HBCDD as the dominating isomer (*34*, *64*, *65*). It has been suggested that the exclusive presence of α-HBCDD is due to a significant metabolism of β-HBCDD and γ-HBCDD by cytochrome P450 (*64*). In the current survey (−)α-HBCDD was the dominating enantiomer in human serum with an EF of around 0.2 indicating enantioselective processes in the accumulation of (−)α-HBCDD. We are not yet able to explain this enantioselectivity. Between 1 and 3% γ-HBCDD was detected in these human samples which may reflect an active

TABLE 3. Linear Multiple Regression Coefficients (β with p-values) from Backward Stepwise Regression for Determinants for Human Serum Levels of CB-153 ($n = 53$), OH-PCBs ($n = 50$), and PBDEs and HBCDDs ($n = 50$)

	Age (yrs)			Current body mass index (kg/m^2)			Change in body mass index from age 25 (% increase)			Baltic Sea fatty fish (meals/month)			explained variance
	β	p	% inc[c]	β	p	% inc[c]	β	p	% inc[c]	β	p	% inc[c]	(%)
CB-153[a]	0.027	0.001	2.74	0.029	0.07	2.94	−0.011	0.01	−1.09	0.035	0.021	3.56	41
4-OH-CB107[a]	0.031	0.02	3.15	0.058	0.06	5.97	−0.018	0.02	−1.78	NI[b]			26
4-OH-CB146[a]	0.025	0.008	2.53	NI[b]			−0.009	0.004	−0.90	NI[b]			34
4-OH-CB187[a]	0.019	0.03	1.92	NI[b]			−0.008	0.01	−0.77	NI[b]			26
BDE-47[a]	NI[b]			NI[b]			0.011	0.07	1.11	NI[b]			8
BDE-99[a]	NI[b]			−0.184	0.08	−16.8	0.075	0.02	7.79	−0.150	0.09	−13.9	19
BDE-100[a]	NI[b]			−0.057	0.06	−5.54	0.023	0.006	2.33	NI[b]			18
BDE-153	NI[b]			−0.075	0.002		0.011	0.09	1.11	NI[b]			25
BDE-154[a]	0.020	0.02	2.02	NI[b]			NI[b]			NI[b]			12
BDE-209[a]	NI[b]			NI[b]			NI[b]			NI[b]			
Σ_6PBDE[a]	NI[b]			−0.057	0.03	−5.54	0.016	0.04	1.61	NI[b]			11
HBCDDs	0.023	0.03	2.33	NI[b]			NI[b]			NI[b]			11

[a] Based on log-transformed values. [b] NI = Not included. The variable did not fulfill the criteria to be included in the final model. [c] Percentage increase in the average value of the outcome per unit increase in the predictor. Calculated using the formula $100(e^{\beta} - 1)$ if the outcome is log-transformed.

TABLE 4. Effect of OH-PCBs, PBDEs, and HBCDDs in Human Serum, Respectively, on Bone Mineral Density and Serum Concentrations of Osteocalcin and Crosslaps

		Bone mineral density (g/cm^2)					Osteocalcin (μg/L)				CTX (ng/L)			
		median	2.5%	97.5%	p[a]	adj p[b]	median	2.5%	97.5%	p[a]	median	2.5%	97.5%	p[a]
4-OH-CB107	low (<0.54 ng/ml)	0.51	0.27	0.70			24	11	58		383	71	805	
	high (>0.54 ng/ml)	0.32	0.20	0.69	0.14		29	12	73	0.58	485	193	1351	0.24
4-OH-CB146	low (<0.68 ng/ml)	0.52	0.21	0.70			20	12	58		374	71	805	
	high (>0.68 ng/ml)	0.32	0.20	0.69	0.03	0.46	30	11	73	0.07	485	101	1351	0.12
4-OH-CB187	low (<0.47 ng/ml)	0.52	0.21	0.70			20	12	58		374	114	805	
	high (>0.47 ng/ml)	0.32	0.20	0.69	0.02	0.34	30	11	73	0.21	411	71	1351	0.46
BDE-47	low (<0.92 ng/g)	0.33	0.20	0.69			30	11	79		459	101	814	
	high (>0.92 ng/g)	0.52	0.21	0.70	0.07		22	13	73	0.36	323	71	1351	0.29
BDE-99	low (<0.20 ng/g)	0.32	0.20	0.69			30	11	79		425	101	1351	
	high (>0.20 ng/g)	0.51	0.21	0.70	0.24		22	13	73	0.19	374	71	1204	0.64
BDE-100	low (<0.287 ng/g)	0.33	0.20	0.69			29	11	79		425	101	814	
	high (>0.287 ng/g)	0.51	0.21	0.70	0.43		22	13	73	0.51	374	71	1351	0.57
BDE-153	low (<1.08 ng/g)	0.50	0.24	0.70			27	12	79		411	165	814	
	high (>1.08 ng/g)	0.44	0.20	0.61	0.35		29	11	73	0.81	383	71	1351	0.72
BDE-154	low (<0.335 ng/g)	0.50	0.20	0.69			29	12	79		425	163	814	
	high (>0.335 ng/g)	0.44	0.21	0.70	0.85		22	11	73	0.38	288	71	1351	0.19
BDE-209	low (<0.46 ng/g)	0.43	0.20	0.61			30	12	58		478	200	1351	
	high (>0.46 ng/g)	0.48	0.21	0.70	0.92		20	11	79	0.10	298	71	1204	0.12
Σ_6PBDE	low (<3.67 ng/g)	0.33	0.20	0.69			27	11	79		459	101	814	
	high (>3.67 ng/g)	0.51	0.21	0.70	0.30		28	13	73	0.79	323	71	1351	0.36
HBCDDs	low (<0.46 ng/g)	0.35	0.20	0.69			29	11	73		418	101	1204	
	high (>0.46 ng/g)	0.52	0.24	0.70	0.51		27	12	79	0.73	374	71	1351	0.46

[a] p-value for low vs high (Mann−Whitney's U-test). [b] p-values adjusted for age, weight, and BMI. Adjusted p-values are given only when unadjusted p-values were >0.05

or present source of exposure. To the best of our knowledge this is the first time HBCDD isomers and the enantiomer ratio have been reported in human serum.

No associations between BMD or the biochemical markers of bone metabolism and the analyzed environmental pollutants were found. One caveat with this conclusion is the limited number of samples, which hampers evaluation of weak or moderate associations. On the other hand, if there had been strong associations they would have been detected.

In this study a slight modification of a well-established analytical method was applied, owing to the special demands of both HBCDD and BDE-209, e.g., instability in alkaline solution and sufficient cleanup. A major problem for most brominated flame retardants, but in particular BDE-209, is background contamination of the samples. Solvent blank levels were low and highly sufficient for PBDE analysis, with instrumental LOQ between 0.008−0.12 pg/injection for BDE-47 to BDE-154. LOQ for HBCDDs and BDE-209 was 0.08 pg/injection.

Stereoisomers cannot be separated by GC and analysis must be performed by LC. However, higher LOD (1−2 pg/injection) together with 20 times larger injection volume demands a larger sample volume to detect trace levels of HBCDD. In this study, 125 mL of serum sample was used (end volume 0.5 mL) for diastereomer determination. Further, it has been reported that analyses of total HBCDD performed on GC and LC are in good agreement (*66*, *67*). Hence the present method is indeed suitable for analysis of low concentrations of HBCDDs and PBDEs in human blood.

Acknowledgments

Financial support was given by the European Commission RD Life Science Program to QLK4-CT-2000-0261, the Swedish Research Council for Medicine, the Swedish Research Council

for Environment, Agriculture Sciences and Spatial Planning, the Medical Faculty of Lund University, and Region Skåne funds. We thank Linda Linderholm, Ioannis Athanassiadis, and Ms. Berit Holmskov for their skilful technical assistance.

Literature Cited

(1) WHO. *Global Assessment of the State-of-the-Science of Endocrine Disruptors*; International Programme on Chemical Safety: Geneva, Switzerland, 2002.

(2) Hallgren, S.; Sinjari, T.; Håkansson, H.; Darnerud, P. O. Effects of polybrominated diphenyl ethers (PBDEs) and polychlornated biphenyls (PCBs) on thyroid hormone and vitamin A levels in rats and mice. *Arch. Toxicol.* **2001**, *75*, 200−208.

(3) Richthoff, J.; Rylander, L.; Jönsson, B. A. G.; Åkesson, H.; Hagmar, L.; Nilsson-Ehle, P.; Stridsberg, M.; Giwercman, A. Serum levels of 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) in relation to markers of reproductive function in young males from the general Swedish population. *Environ. Health Perspect.* **2003**, *111*, 409−413.

(4) Rignell-Hydbom, A.; Rylander, L.; Giwercman, A.; Jönsson, B. A. G.; Nilsson-Ehle, P.; Hagmar, L. Exposure to CB-153 and *p,p′*-DDE and male reproductive function. *Hum. Reprod.* **2004**, *19*, 2066−2075.

(5) Rylander, L.; Rignell-Hydbom, A.; Hagmar, L. A cross-sectional study of the association between persistent organochlorine pollutants and diabetes. *Environ. Health Glob. Access Sci. Source* **2005**, *4*.

(6) Longnecker, M. P.; Daniels, J. L. Environmental contaminants as etiologic factors for diabetes. *Environ. Health Perspect.* **2001**, *109*, 871−876.

(7) Fängström, B.; Strid, A.; Bergman, Å. *Temporal Trends of Brominated Flame Retardants in Milk from Stockholm Mothers, 1980−2004*; Dnr 721-2653-05Mm; The Swedish Environmental Protection Agency: Stockholm, 2005; *http://www.naturvardsverket.se/dokument/mo/modok/export/rapport2005_modersmjolk.pdf*.

(8) Hites, R. A. Polybrominated Diphenyl Ethers in the Environment and in People: A Meta-Analysis of Concentrations. *Environ. Sci. Technol.* **2004**, *38*, 945−956.

(9) Sjödin, A.; Patterson, D. G., Jr.; Bergman, Å. A review on human exposure to brominated flame retardants (BFRs) - particularly polybrominated diphenyl ethers (PBDEs). *Environ. Int.* **2003**, *29*, 829−839.

(10) Birnbaum, L. S.; Staskal, D. F. Brominated flame retardants: Cause for concern? *Environ. Health Perspect.* **2004**, *112*, 9−17.

(11) Legler, J.; Brouwer, A. Are brominated flame retardants endocrine disruptors? *Environ. Int.* **2003**, *29*, 879−885.

(12) Vos, J. G.; Becher, G.; van den Berg, M.; de Boer, J.; Leonards, P. E. G. Brominated flame retardants and endocrine disruption. *Pure Appl. Chem.* **2003**, *75*, 2039−2046.

(13) Eriksson, P.; Jakobsson, E.; Fredriksson, A. Brominated flame retardants: A novel class of developmental neurotoxicants in our environment? *Environ. Health Perspect.* **2001**, *109*, 903−908.

(14) Remberger, M.; Sternbeck, J.; Palm, A.; Kaj, L.; Strömberg, K.; Brorström-Lundén, E. The environmental occurrence of hexabromocyclododecane in Sweden. *Chemosphere* **2004**, *54*, 9−21.

(15) Lind, P. M.; Bergman, A.; Olsson, M.; Örberg, J. Bone mineral density in male baltic grey seal (*Halichoerus grypus*). *Ambio* **2003**, *32*, 385−388.

(16) Gullberg, B.; Johnell, O.; Kanis, J. A. World-wide projections for hip fracture. *Osteoporosis Int.* **1997**, *7*, 407−413.

(17) Pluijm, S. M. F.; Dik, M. G.; Jonker, C.; Deeg, D. J. H.; van Kamp, G. J.; Lips, P. Effects of gender and age on the association of apolipoprotein E ϵ4 with bone mineral density, bone turnover and the risk of fractures in older people. *Osteoporosis Int.* **2002**, *13*, 701−709.

(18) SBU. *Osteoporosis- Prevention, Diagnosis and Treatment*; 165; The Swedish Council on Technology Assessment in Health Care: Stockholm, Sweden, 2003.

(19) Jamsa, T.; Viluksela, M.; Tuomisto, J. T.; Tuomisto, J.; Tuukkanen, J. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on bone in two rat strains with different aryl hydrocarbon receptor structures. *JBMR* **2001**, *16*, 1812−1820.

(20) Lind, P. M.; Eriksen, E. F.; Sahlin, L.; Edlund, M.; Örberg, J. Effects of the antiestrogenic environment polluttant 3,3′,4,4′,5-pentachlorobiphenyl (PCB#126) in rat bone and uterus: diverging effects in ovariectomized and intact animals. *Toxicol. Appl. Pharmacol.* **1999**, *154*, 236−244.

(21) Lind, P. M.; Larsson, S.; Oxlund, H.; Håkansson, H.; Nyberg, K.; Eklund, T.; Örberg, J. Change of bone tissue composition and impaired bone strength in rats exposed to 3,3′,4,4′,5-pentachlorobiphenyl (PCB126). *Toxicology* **2000**, *150*, 41−51.

(22) Miettinen, H.; Pulkkinen, P.; Jämsä, T.; Tuomisto, J.; Tuukkanen, J.; Viluksela, M. In utero and lactational TCDD exposure affects rat bone development. *Organohalogen Compd.* **2003**, *64*, 274−277.

(23) Sjödin, A.; Hagmar, L.; Klasson Wehler, E.; Björk, J.; Bergman, Å. Influence of the consumption of fatty Baltic Sea fish on plasma levels of halogenated environmental contaminants in Latvian and Swedish men. *Environ. Health Perspect.* **2000**, *108*, 1035−1041.

(24) Alveblom, A.; Rylander, L.; Johnell, O.; Hagmar, L. Incidence of hospitalized osteoporotic fractures in cohorts with high dietary intake of persistent organochlorine compounds. *Int. Arch. Occup. Environ. Health* **2003**, *76*, 246−248.

(25) Wallin, E.; Rylander, L.; Hagmar, L. Exposure to persistent organochlorine compounds through fish consumption and the incidence of osteoporotic fractures. *Scand. J. Work Environ. Health* **2004**, *30*, 30−35.

(26) Wallin, E.; Rylander, L.; Jönsson, B. A. G.; Lundh, T.; Isaksson, A.; Hagmar, L. Exposure to CB-153 and *p,p′*-DDE and bone mineral density and bone metabolism markers in middle-aged and elderly men and women. *Osteoporosis Int.* **2005**, *16*, 2085−2094.

(27) Rylander, L.; Strömberg, U.; Hagmar, L. Decreased birthweight among infants born to women with a high dietary intake of fish contaminated with persistent organochlorine compounds. *Scand. J. Work Environ. Health* **1995**, *21*, 368−375.

(28) Svensson, B. G.; Nilsson, A.; Jonsson, E.; Schutz, A.; Akesson, B.; Hagmar, L. Fish consumption and exposure to persistent organochlorine compounds, mercury, selenium and methylamines among Swedish fishermen. *Scand. J. Work Environ. Health* **1995**, *21*, 96−105.

(29) Marsh, G.; Hu, J.; Jakobsson, E.; Rahm, S.; Bergman, Å. Synthesis and characterization of 32 polybrominated diphenyl ethers. *Environ. Sci. Technol.* **1999**, *33*, 3033−3037.

(30) Örn, U.; Eriksson, L.; Jakobsson, E.; Bergman, Å. Synthesis and characterization of polybrominated diphenyl ethers - unlabelled and radiolabelled tetra-, penta- and hexa-bromodiphenyl ethers. *Acta Chem. Scand.* **1996**, *50*, 802−807.

(31) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; John Wiley and Sons: New York, 1967; Ch. 1.

(32) Hovander, L.; Athanasiadou, M.; Asplund, L.; Jensen, S.; Klasson Wehler, E. Extraction and cleanup methods for analysis of phenolic and neutral organohalogens in plasma. *J. Anal. Toxicol.* **2000**, *24*, 696−703.

(33) Meironyté Guvenius, D.; Hassanzadeh, P.; Bergman, Å.; Norén, K. Metabolites of polychlorinated biphenyls in human liver and adipose tissue. *Environ. Toxicol. Chem.* **2002**, *21*, 2264−2269.

(34) Janák, K.; Covaci, A.; Voorspoels, S.; Becher, G. Hexabromocyclododecane in Marine Species from the Western Scheldt Estuary: Diastereoisomer- and Enantiomer-Specific Accumulation. *Environ. Sci. Technol.* **2005**, *39*, 1987−1994.

(35) Fängström, B., Human exposure to organohalogen compounds at the Faroe Islands. Ph.D. Thesis, Department of Environmental Chemistry, Stockholm University, 2005.

(36) Jönsson, B. A. G.; Rylander, L.; Lindh, C.; Rignell-Hydbom, A.; Giwercman, A.; Toft, G.; Pedersen, H. S.; Ludwicki, J. K.; Góralczyk, K.; Zvyezday, V.; Spanò, M.; Bizzaro, D.; Bonefeld-Jörgensen, E. C.; Manicardi, G. C.; Bonde, J. P.; Hagmar, L. Inuendo Inter-population variations in concentrations, determinants of and correlations between 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (p,p′-DDE): a cross-sectional study of 3161 men and women from Inuit and European populations. *Environ. Health Glob. Access Sci. Source* **2005**, *4*.

(37) Rylander, L.; Nilsson-Ehle, P.; Hagmar, L. A simplified but accurate and precise method for adjusting serum levels of persistent organohalogen pollutants to total serum lipids. *Chemosphere* **2006**, *62*, 333−336.

(38) Grimvall, E.; Rylander, L.; Nilsson-Ehle, P.; Nilsson, U.; Strömberg, U.; Hagmar, L.; Östman, C. Monitoring of polychlorinated biphenyls in human blood plasma: Methodological developments and influence of age, lactation, and fish consumption. *Arch. Environ. Contam. Toxicol.* **1997**, *32*, 329−336.

(39) Wicklund Glynn, A.; Wernroth, L.; Atuma, S.; Linder, C.-E.; Aune, M.; Nilsson, I.; Darnerud, P. O. PCB and chlorinated pesticide concentrations in swine and bovine adipose tissue in Sweden 1991−1997: spatial and temporal trends. *Sci. Total Environ.* **2000**, *246*, 195−206.

(40) Soechitram, S. D.; Athanasiadou, M.; Hovander, L.; Bergman, Å.; Sauer, P. J. J. Fetal exposure to PCB and their hydroxylated

metabolites in a Dutch cohort. *Environ. Health Perspect.* **2004**, *112*, 1208−1212.
(41) Fängström, B.; Athanasiadou, M.; Grandjean, P.; Weihe, P.; Bergman, Å. Hydroxylated PCB metabolites and PCBs in serum from pregnant Faroese women. *Environ. Health Perspect.* **2002**, *110*, 895−899.
(42) Gabrio, T.; Piechotowski, I.; Wallenhorst, T.; Klett, M.; Cott, L.; Friebel, P.; Link, B.; Schwenk, M. PCB-blood levels in teachers, working in PCB-contaminated schools. *Chemosphere* **2000**, *40*, 1055−1062.
(43) Pauwels, A.; Covaci, A.; Weyler, J.; Delbeke, L.; Dhont, M.; De Sutter, P.; D'Hooghe, T.; Scheepens, P. J. C. Comparison of persistent organic pollutant residues in serum and adipose tissue in a female population in Belgium, 1996−1998. *Environ. Contam. Toxicol.* **2000**, *39*, 265−270.
(44) Meironyté Guvenius, D.; Aronsson, A.; Ekman-Ordeberg, G.; Bergman, Å.; Norén, K. Human prenatal and postnatal exposure to polybrominated diphenyl ethers, polychlorinated biphenyls, polychlorobiphenylols and pentachlorophenol. *Environ. Health Perspect.* **2003**, *111*, 1235−1241.
(45) Wicklund Glynn, A.; Granath, F.; Aune, M.; Atuma, S.; Darnerud, P. O.; Bjerselius, R.; Vainio, H.; Weiderpass, E. Organochlorines in Swedish women: Determinants of serum concentration. *Environ. Med.* **2003**, *111*, 349−355.
(46) Hovander, L.; Linderholm, L.; Athanasiadou, M.; Athanassiadis, I.; Bignert, A.; Fängström, B.; Kocan, A.; Petrik, J.; Trnovec, T.; Bergman, Å. Levels of PCBs and their metabolites in the serum of residents of a highly contaminated area in eastern Slovakia. *Environ. Sci. Technol.* **2006**, *40*, 3696−3703.
(47) Weihe, P.; Hoppe, H.-W.; Grandjean, P. Sustained high concentrations of PCBs in Faroese pregnant women despite dietary intervention. *Organohalogen Compd.* **2003**, *63*, 389−392.
(48) Kiviranta, H.; Vartiainen, T.; Tuomisto, J. Polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls in fishermen in Finland. *Environ. Health Perspect.* **2002**, *110*, 355−361.
(49) Thomsen, C.; Lundanes, E.; Becher, G. Brominated flame retardants in archived serum samples from Norway: a study on temporal trends and the role of age. *Environ. Sci. Technol.* **2002**, *36*, 1414−1418.
(50) Schecter, A.; Pavuk, M.; Päpke, O.; Ryan, J. J.; Birnbaum, L.; Rosen, R. Polybrominated diphenyl ethers (PBDEs) in U.S. mothers' milk. *Environ. Health Perspect.* **2003**, *111*, 1723−1729.
(51) Petreas, M.; She, J.; Brown, F. R.; Winkler, J.; Windham, G.; Rogan, E.; Zhao, G.; Bhatia, R.; Charles, M. J. High body burdens of 2,2′,4,4′-tetrabromo diphenyl ether (BDE-47) in California Women. *Environ. Health Perspect.* **2003**, *111*, 1175−1179.
(52) Geyer, H. J.; Schramm, K.-W.; Darnerud, P. O.; Aune, M.; Feicht, A.; Fried, K. W.; Henkelmann; Lenoir, D.; Schmid, P.; McDonald, T. A. Terminal elimination half-lives of the brominated flame retardants TBBPA, HBCD, and lower brominated PBDEs in humans. *Organohalogen Compd.* **2004**, *66*, 3867−3872.
(53) Thuresson, K.; Höglund, P.; Hagmar, L.; Sjödin, A.; Bergman, Å.; Jakobsson, K. Apparent half-lives of hepta- to decabrominated diphenyl ethers in humans as determined in occupationally exposed workers. *Environ. Health Perspect.* **2006**, *114*, 176−181.
(54) Sellström, U.; Bignert, A.; Kierkegaard, A.; Häggberg, L.; de Wit, C. A.; Olsson, M.; Jansson, B. Temporal trend studies on tetra- and pentabrominated diphenyl ethers and hexabromocyclododecane in guillemot egg from the Baltic Sea. *Environ. Sci. Technol.* **2003**, *37*, 5496−5501.
(55) Schröter-Kermani, C.; Helm, D.; Herrmann, T.; Päpke, O. The German environmental specimen bank - application in trend monitoring of polybrominated diphenyl ethers in human blood. *Organohalogen Compd.* **2000**, *47*, 49−52.
(56) Fängström, B.; Athanasiadou, M.; Athanassiadis, I.; Bignert, A.; Grandjean, P.; Weihe, P.; Bergman, Å. Polybrominated diphenyl ethers and traditional organochlorine pollutants in fulmars (*Fulmarus glacialis*) from the Faroe Islands. *Chemosphere* **2005**, *60*, 836−843.
(57) Weiss, J.; Meijer, L.; Sauer, P.; Linderholm, L.; Athanassiadis, I.; Bergman, Å. PBDE and HBCDD levels in blood from Dutch mothers and infants - Analysis of a Dutch Groningen infant cohort. *Organohalogen Compd.* **2004**, *66*, 2677−2682.
(58) Kierkegaard, A.; Balk, L.; Tjärnlund, U.; de Wit, C. A.; Jansson, B. Dietary uptake and biological effects of decabromodiphenyl ether in Rainbow trout (*Oncorhynchus mykiss*). *Environ. Sci. Technol.* **1999**, *33*, 1612−1617.
(59) Stapleton, H. M.; Brazil, B.; Holbrook, D. R.; Mitchelmore, C. L.; Benedict, R.; Konstantinov, A.; Potter, D. *In vivo* and *in vitro* debromination of decabromodiphenyl ether (BDE 209) by juvenile rainbow trout and common carp. *Environ. Sci. Technol.* **2006**, *40*, 4653−4658.
(60) Söderström, G.; Sellström, U.; de Wit, C. A.; Tysklind, M. Photolytic debromination of decabromodiphenyl ether (BDE 209). *Environ. Sci. Technol.* **2004**, *38*, 127−132.
(61) Barregard, L.; Aune, M.; Claesson, A.; Darnerud, P. O. Persistent organic pollutants in breast milk - HBCD now detected in Swedish samples; International Society of Exposure Analysis, 13th annual conference; ISEA: Boston, MA, 2003; p 32.
(62) Thomsen, C.; Froshaug, M.; Leksnes, H.; Becher, G. Brominated flame retardants in breast milk from Norway. *Organohalogen Compd.* **2003**, *64*, 33−36.
(63) Lopez, D.; Athanasiadou, M.; Athanassiadis, I.; Estrade, L. Y.; Diaz-Barriga, F.; Bergman, Å. A preliminary study on PBDEs and HBCDD in blood and milk from Mexican women. The Third International Workshop on Brominated Flame Retardants; University of Toronto: Toronto, Ontario, Canada, 2004; pp 483−487.
(64) Zegers, B. N.; Mets, A.; Van Bommel, R.; Minkenberg, C.; Hamers, T.; Kamstra, J. H.; Pierce, G. J.; Boon, J. P. Levels of Hexabromocyclododecane in Harbor Porpoises and Common Dolphins from Western European Seas, with Evidence for Stereoisomer-Specific Biotransformation by Cytochrome P450. *Environ. Sci. Technol.* **2005**, *39*, 2095−2100.
(65) Janák, K.; Sellström, U.; Johansson, A. K.; Becher, G.; de Wit, C. A.; Lindberg, P.; Helander, B. Enantiomer-specific accumulation pattern of hexabromocyclododecane in eggs of predatory birds breeding in Sweeden. *Organohalogen Compd.* **2005**, *67*, 204−207.
(66) Petersen, M.; Hamm, S.; Schäfer, A.; Esser, U. Comperative GC/MS and LC/MS detection of hexabromocyclododecane (HBCD) in soil and water samples. *Organohalogen Compd.* **2004**, *66*, 226−233.
(67) Zaruk, D.; MacInnis, G.; Marvin, C.; Sverko, E. Comparison of hexabromocyclododecane determinations using GC/MS and LC/MS/MS. *Organohalogen Compd.* **2005**, 226−228.

Received for review May 8, 2006. Revised manuscript received August 4, 2006. Accepted August 7, 2006.

ES0610941