Exposure to CB-153 and p,p´-DDE and Bone Mineral Density and Bone Metabolism Markers in Middle-aged and Elderly Men and Women

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Abstract

The incidence of osteoporotic fractures is rising in western societies, partly due to unknown reasons. Persistent organochlorine compounds (POC) have in animal studies impaired the normal bone metabolism and resulted in increased bone fragility, which might have health implications for POC exposed human populations. The aim of the present study was to assess whether a high dietary intake of POC through fatty fish from the Baltic may result in decreased bone mineral density (BMD) or disturbances in biochemical markers of bone metabolism. From a study base of fishermen and fishermen's wives from the Swedish east coast that are considerably more POC exposed than the general Swedish population, 196 men (median age 59 years) and 184 women (median age 62 years) participated in an examination of their forearm BMD, using dual energy x-ray absorptiometry (DXA) dual energy. Further, POC exposure was assessed by analysis of lipid adjusted serum levels of 2,2´,4,4´,5,5´-hexachlorobiphenyl (CB-153) and 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene (p,p´-DDE). Cadmium in urine (U-Cd) was also analysed. Biochemical markers in serum of osteoblastic (osteocalcin) and osteoclastic (CrossLaps) functions were measured. Adjustment for potential confounders was made by employing multiple regression analyses. Univariate analyses showed significant negative associations between CB-153 concentrations and BMD, but after adjustment for age and body mass index these associations did not remain. None of the POC exposure variables were associated with CrossLaps or osteocalcin. There were no significant associations between U-Cd and BMD or any of the biochemical biomarkers. In conclusion, the results did not provide any support for the hypothesis that the current exposure levels to POC constitutes a hazard for impaired bone metabolism in the general Swedish population.

Keywords DDE; Bone Mineral Density; CrossLaps; fish consumption; osteocalcin; PCB,

Introduction

Persistent organochlorine compounds (POC), such as polychlorinated biphenyls (PCB), polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and pesticides such as dichlorodiphenyl trichloroethane (DDT), have been released into the environment, mainly since the Second World War. POC have been detected in human blood, adipose tissue and breast milk worldwide, but accumulate in particular in highly rank predators of the aquatic food chain.

It has been shown in animal studies that at least dioxin-like POC may impair normal bone metabolism and result in increased bone fragility (1-4). There are some sparse animal data indicating both impaired bone mineralization (1), and reduced collagen content and serum osteocalcin levels (2).

High accidental exposure to a POC, hexachlorobenzene, resulted in severe human osteoporosis (5), and infants exposed *in utero* to high concentrations of PCB and PCDF developed irregular calcification of their skull bones (6). Thus, high exposure levels to POC may impair normal bone metabolism in humans. The pertinent question is whether POC levels more relevant for different groups in the general population might be harmful for the human skeleton.

The mechanisms for POC related impairment of normal bone metabolism are not well known. Bone is a target tissue for estrogens and estrogen deficiency associated with menopause is the cause of the most rapid phase of bone loss in women (7). Moreover, estrogens seem to be involved in maintaining the male skeleton (8). Some POC compounds have estrogenic, some have anti-estrogenic and some have androgenic properties (9-11). Thus, the net effect *in vivo* on hormonal homeostasis of dietary exposure to a mixture of a large number of POCs can be difficult to predict (12). Moreover, a cross-talk between the Ah-receptor and the estrogen receptor pathways has also been postulated (13). Finally, some

PCBs are inhibitors of aromatase activity *in vitro* (14) and coplanar PCBs have been found to directly affect the expression of steroidogenic enzyme genes (15). Besides their mechanisms of action mediated by sex steroid hormones' receptors (16-18), PCBs were reported to inhibit estrogen sulfotransferase, the enzyme responsible for the inhibition of estrogen metabolism (19,20) and to alter GnRH gene expression, indicating also a hypothalamic level for endocrine disruption by these environmental toxicants (21). Thus, dioxins and other POC act as endocrine disruptors and modulate the homeostasis of estrogens and other steroid hormone systems, at either the receptor level, by altering metabolism or by affecting serum transport (cf 22), but the effect *in vivo* on bone metabolism is not easily predicted..

Recently it has been proposed that many of the toxic actions of dioxins and dioxin-like compounds are caused by their action to trigger severe stress responses by the affected cells, e.g. by stimulation of cellular production of cytokines (23). Whether this mechanism might be of relevance for POC effects on bone metabolism is presently not known.

There has been an increase in osteoporotic fractures in western societies during the last decades, even when accounting for age (24-26). Sweden is one of the countries with highest incidence of osteoporotic fractures (27). The reasons for this increase are not well known, but changes in environmental factors might be of importance (24,28). It is well known that high cadmium (Cd) exposure will damage the kidneys, followed by severe osteoporosis and osteomalacia as a secondary effect (29,30). Recent European studies have suggested that Cd can induce osteoporosis also at much lower exposure levels than previously reported (31-33). A high dietary intake of POC might act as another potential environmental risk factor for deteriorated bone quality in humans. Three small epidemiological studies show conflicting results with respect to whether dietary POC exposure decreases BMD (34-36).

In Sweden, consumption of fatty fish from the Baltic Sea, off the Swedish east coast, is the single major exposure source for POC (37,38), and cohorts of Swedish professional

fishermen and their families have been found to constitute excellent study bases for epidemiological evaluations of human health effects of POC. Swedish east coast fishermen had three times higher serum levels of POC than both fishermen living at the west coast of Sweden and the general Swedish population (38). Moreover, fishermen's wives had twice the fish consumption as control subjects (39) and fishermen's wives from the east coast had higher plasma levels of PCB than women from the general population (40).

Initially we analyzed the fracture incidence in the study base, which consisted of fishermen and fishermen's wives from the Swedish east and west coasts (41,42). As a complement we have now also performed a cross-sectional study of bone mineral density (BMD) and biochemical markers of bone metabolism within the east coast cohorts. We have used 2,2´,4,4´,5,5´-hexachlorobiphenyl (CB-153) as a biomarker for POC exposure, because it correlates very well with both total PCB concentration in plasma and serum ($r \geq 0.9$)(43,44), with the PCB derived dioxin-like effect ($r = 0.91$) (44) as well as the total POC derived dioxin-like effect ($r = 0.74$) (45). Another relevant exposure biomarker is the antiandrogenic compound 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene (p,p´-DDE), which is the major metabolite of DDT. p,p'-DDE is still present in relatively high serum concentrations in men consuming fatty fish from the Baltic Sea (46).

The aim of the study was to assess whether a high dietary intake of POC through fatty fish from the Baltic may result in decreased bone mineral density (BMD) or disturbances in biochemical markers of bone metabolism.

Subjects and methods

Study population and interview

Previously established cohorts of professional fishermen and their wives from the Swedish east coast (38,39) were linked to the National Cause-of-Death Register at Statistics Sweden,

and vital status was determined as of 31 December 1999 by linkage to the Swedish Population Register. A postal questionnaire, primarily aimed to assess the fracture incidence but also including a question about whether the subjects were interested to participate in a BMD study, was sent in year 2000 to 1500 fishermen and 1291 fishermen's wives that were born between 1920 and 1954, living in Sweden and still alive 31 December 1999 (42). There were 813 men (54 %) and 779 women (77 %) who responded to the questionnaire. Out of them 510 men and 596 women were positive to participate in the BMD study. We excluded 177 of the 596 women from participation because they had not yet reached their menopause. Another 5 women and 8 men were excluded as a linkage with the population register showed that they had deceased since responding to the questionnaire. The remaining 502 men and 414 women were invited by letter to participate in the study, and 351 men and 300 women accepted the invitation. We had a preset goal of examining 200 men and 200 women, and consecutively contacted subjects by phone for agreements until enough subjects were recruited. During the telephone recruitment 75 men and 45 women were found to have changed their mind about participation and another 12 men and 12 women did never show up at the agreed appointment. We examined, however, 208 men and 206 women.

Of these 414 examined subjects we had to exclude 5 men and 7 women due to incomplete blood samples or BMD investigations, 5 women that still not were in menopause, one woman with Cushing's syndrome and four women and two men that had been on long time treatment with oral steroids for rheumatoid arthritis. Moreover, we afterwards excluded 5 men and 5 women with high parathyroid hormone (PTH values), indicating hyperparathyroidism or vitamin D deficiency. Thus, the final data set comprised 196 men and 184 women.

The examinations were made at different locations, close to were the subjects were living, all along the Swedish east coast. The participants were interviewed, using a

standardized questionnaire. They were asked about factors that potentially might affect the BMD, such as at age at menarche and menopause, current hormone replacement therapy (HRT), history of osteoporotic fractures, heredity of fracture, physical activity at work (current and at 25 and 35 years of age), smoking habits, and current alcohol consumption. We measured current weight with an HL 120 Light Industrial Scale (Avery Berkel) and height using a standardized measuring rod. Descriptive data are given in Table 1.

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.

Non-participants

Five-hundred and two fishermen were invited to participate but 306 of them were not included in the final data set. Based on data from the original questionnaire, which was sent in year 2000, the non-participants had similar age distribution (median age in year 2000 was 58 years, range 45-80) as the participants (median 56, range 45-80). In addition, the BMI distributions were also very similar among the non-participants (median 26.5 kg/m^2, range17.1-39.9) and the participants (median 27.2, range 20.5-38.5). Of the male non-participants 1.3 % had a history of osteoporotic fracture after 50 years of age and 78 % were ever smokers. The corresponding figures among the participants were 1.5 % and 81 %, respectively.

Among the 230 female non-participants the median age in year 2000 was 61 years (range 45-80) and the median BMI was 26.2 kg/m^2 (range 19.7-38.2). The corresponding figures among the 184 participants were 59 years (range 45-79) and 26.0 kg/m^2 (range 18.7-39.6), respectively. Of the female non-participants 6.5 % had a history of osteoporotic fracture

after 50 years of age and 54 % were ever smokers. The corresponding figures among the participants were 7.6 % and 52 %, respectively.

Blood and urine sampling

Venous blood samples (125 ml) were drawn between 8.00 and 10.00 A.M, after 12 hr fasting, into sterile Vacutainer glass tubes (BD Vacutainer, Plymouth, UK). Serum was separated by centrifugation (4000 rpm, 10 minutes) and transferred to glass bottles and special tubes. Spot urine samples were collected in the morning in paper-cups and immediately transferred into two screw-capped 12ml polypropylene test tubes (Sartedt, Nümbrecht, Germany) that were initially kept in a refrigerator. Concentrated nitric acid was added to achieve a 2 % acid concentration. Paper-cups and test tubes were pre-tested for their Cd content and were found to be Cd free (i.e. below detection limit). All serum and urine samples were stored at –80^{o}C until analysis.

Bone Mineral Density measurement

A dual energy x-ray absorptiometry (DXA) was used. The Osteometer DTX-200 estimated the bone mineral content (BMC, g) and bone mineral density (BMD, g/cm^2) in the distal section of the forearm (radius and ulna). The participants were placed in an upright position with the dominant forearm placed in a container during the examination. The x-ray source was located on one side of the container and detector was located on the other side. The internal variation was checked by daily calibration using a phantom, with a known BMC.

Determination of CB-153 and p, p´-DDE in serum

The serum levels of CB-153 and p, p´-DDE were determined as previously described (47,48). Briefly, the CB-153 and p, p´-DDE were extracted from the serum by solid phase extraction

(Isolute ENV+; IST, Hengoed, UK) using on-column degradation of the lipids and analysis by gas chromatography mass spectrometry. $^{13}C_{12}$-labeled CB-153 and $^{13}C_{12}$-labeled p, p´-DDE were used as an internal standards. The relative standard deviations, calculated from samples analyzed in duplicate at different days, was 8 % at 0.8 ng/mL (n=76) and 7 % at 2.7 ng/mL for CB-153 and 7 % at 1.3 ng/mL and 9 % at 7.0 ng/mL for p, p´-DDE. The quantification limits were 0.05 ng/mL for CB-153 and 0.1 ng/mL for p, p´-DDE. The analyses of CB-153 and p, p´-DDE are part of the Round Robin inter-comparison program (Professor Dr. med. Hans Drexler, Institute and Out-Patient Clinic for Occupational, Social and Environmental Medicine, University of Erlangen-Nuremberg) with analysis results within the tolerance limits.

Determination of serum lipids by enzymatic methods

Serum concentrations of triglycerides and cholesterol were determined by enzymatic methods using reagents from Roche Diagnostics (Mannheim, Germany). The inter-assay CVs for cholesterol and triglyceride determinations were 2-4 %. The average molecular weights of triglycerides were assumed to be 807. For cholesterol we used an average molecular weight of 571, assuming that the proportion of free and esterified cholesterol in plasma was 1:2. Based on a paper by Rylander et al the total lipid concentration in serum (g/l) was calculated by the following equations (49):

Men: Total = 0.96 + 1.28*(triglycerides + cholesterols)

Women: Total = 1.13 + 1.31*(triglycerides + cholesterols).

Determination of cadmium in urine

The concentration of Cd in urine was determined by inductively coupled plasma-mass spectrometry (Thermo X7, Thermo Elemental,Winsford, UK). Interference corrections were

made for ^{114}Cd for the spectral overlap of molybdenoxide and tin. The limit of detection, calculated as three times the standard deviation (SD) for the blank were 0.03 µg/L. All urine samples were prepared in duplicate and the method imprecision, calculated as the coefficient of variation for duplicate measurements, was 5.3 %. The analytical accuracy was checked against reference material. The results obtained were 4.4±0.02µg/L (mean±SD), n=20 vs. recommended 4.9 µg/L (Seronorm; batch FE1114, Nycomed, Oslo, Norway) and 0.75±0.03 µg/L (mean±SD), n=26 vs. certified 0.79 µg/L (batch D-03-01, Interlaboratory Comparison Programe, Centre de Toxicologie du Quebec; Canada). To correct for differences in urinary flow rate, the Cd concentration in the urine samples was adjusted for the creatinine content, determined enzymatically according to Mazzachi et al (50).

Analyses of biochemical markers of bone metabolism

CrossLaps (CTX) in serum was determined with the Elecsys β-CrossLaps immunoassay (Roche Diagnostics, Mannheim, Germany). This method specifically recognizes C-terminal fragments of type I collagen containing the β-isomerized octapeptide EKAHD- β-GGR. Total imprecision (CV) for this method is 6.6 and 4.6 % at 260 and 2600 ng/L, respectively. The upper normal limit of CTX in plasma for men is 710 ng/L (50-70 years) and 840 ng/L (> 70 years), whereas the upper normal limit for postmenopausal women is 730 ng/L.

Osteocalcin in serum was determined with the Elecsys N-MID Osteocalcin immunoassay (Roche Diagnostics), which measures the sum of intact osteocalcin (aa 1-49) and the large N-terminal fragment (aa 1-43). The method has a total imprecision of 2.3 and 2.4 % at 18 and 175 µg/L and a reference interval of 10-43 µg/L.

Parathyroid hormone (PTH) in serum was determined with the Elecsys PTH immunoassay (Roche Diagnostics), which employs two monoclonal antibodies, one reacting with the N-terminal part of the molecule (aa 1-37) and another directed at the C-terminal part

(aa 38-84). The total imprecision of this method is 4.4 and 4.1 % at 7.0 and 80 pmol/L, respectively. The reference interval is 1.6-6.9 pmol/L. PTH in serum was used to screen for possible hyperparathyroidism and vitamin D deficiency, but was not used as an outcome variable. The reason for that was that we had no a priori hypothesis that an effect of POC on bone metabolism would involve PTH.

Statistics

Separate analyses were performed for men and women, respectively. The effects of the exposure variables CB-153 and p,p'-DDE, respectively, on BMD, osteocalcin, and CTX were evaluated by linear regression models. The exposure variables were treated as continuous variables (untransformed and log transformed) as well as categorized into four equally sized groups. The categorized variables were entered as dummy variables in the models. Due to the high correlation between CB-153 and p,p'-DDE (women r=0.68; men r=0.64) these variables were not included in the models simultaneously. As potential confounders we considered the variables presented in Table 1. We also evaluated the effect of Cd in urine (continuous and categorized into three equally sized groups) on the outcome variables. If the potential confounders in univariate analyses showed any association ($p<0.15$) with the outcome variables, they were included in the models, one at a time, together with the exposure variable. The confounders were kept in the model if the effect estimate (i.e. the univariate effect of the exposure variable on the outcome variable) was changed more than 15 %. However, in the Tables the crude estimates are also shown. Model assumptions were checked by means of residual analysis.

Results

The distributions of the POC exposure variables and the outcome variables are given in Table 2. The univariate analyses showed in both men and women significant negative associations between CB-153 concentrations and BMD (Tables 3 and 4). An increase of the CB-153 concentrations in serum of 100 ng/g lipid corresponded to a decrease in BMD of 3.8 mg/cm^2 (95% CI -7.4, -0.1, Figure 1 and Table 3) among the men and a decrease of 10 mg/cm^2 (95% CI -18, -3.5, Figure 2 and Table 4) among the women. However, after adjustment for age and BMI none of these significant associations remained. Among the women the pattern was similar regarding the association between p,p'-DDE and BMD, whereas there were no such associations at all among the men. Neither log transformation nor categorization of the exposure variables changed these patterns.

None of the POC exposure variables were associated with CTX or osteocalcin.

There were for neither men nor women any significant associations between U-Cd and BMD or any of the biochemical markers (all $p>0.10$). Including age or smoking habits in multivariate models did not change these results.

Discussion

The main result of the present study was that there were no remaining associations between the POC exposure markers (CB-153 and p,p'-DDE) and BMD when adjustments for age and BMI had been made. The univariate associations between the exposure markers and BMD were explained by that age strongly affected both exposure and BMD. Moreover, neither CTX, that reflects osteoclastic activity, nor osteocalcin that reflects osteoblastic activity, were associated with any of the POC exposure markers.

It is always important in cross-sectional studies to be aware of potential selection bias. A comparison between subjects who were invited but in the end not included, and the participants showed no obvious differences with risk factors for osteoporosis such as age, BMI, history of osteoporotic fractures or smoking habits. We do therefore not believe that selection bias is of major concern in the present study.

For the POC biomarkers CB-153 and p,p'-DDE, and for U-Cd, the analytic accuracies and precisions were good. The long biological half-lives of many years for CB-153 and p,p'-DDE means that current serum levels well reflect the POC exposure the years preceding the cross-sectional study. Thus, we have a good measure of exposure for the period when the bone metabolism could have been affected. Therefore, we don't consider misclassification of exposure to be a bias of importance in the present study.

We choose in the present field study to use DTX-200 for measuring distal forearm BMD partly because the equipment had to be portable and stable after transport. Moreover, distal forearm BMD is a relevant measure for risk for osteoporosis. In a recent meta-analysis it has been shown that BMD, irrespective of the measurement site, predicts the occurrence of osteoporotic fractures (51). The analytical accuracies and precisions of the biochemical bone markers were good.

We excluded subjects with known very strong risk factors for osteoporosis (high PTH values, Cushing's disease and long term treatment with oral steroids) to avoid that weak associations between the exposure variables and the outcome variables should be overshadowed by these factors.

A thorough and systematic analysis of the effect of including a number of potential confounders in the multivariate models showed that only age and BMI were of importance for assessing the impact of the POC exposure variables. Whether the association between POC and BMI should be expected to be positive or negative is dependent on the timing of blood sampling in relation to when the period of more substantial dietary POC exposure had taken place (52). If this exposure had been recent, subjects with high BMI, i.e. with large adipose distribution volumes, should be expected to have negative association between BMI and lipid adjusted POP levels in serum. On the other hand, if the blood samples, as in the present study, were drawn many years after end of a more substantial exposure than the current one, and the subjects were old enough to have reached a steady state of their body burdens of POC, considering the long biological half-lives of many of these compounds, a positive association between BMI and POC in serum could be expected. Thus, it was a reasonable finding that the association between CB-153 and BMD became slightly weaker when adjusting for BMI.

The main reason for analysing U-Cd in the present study was the need to exclude any potential confounding effect on associations between POC exposure and BMD and bone metabolism markers. However, when assessing the effect of U-Cd we did not observe significant associations with any of the outcome variables. High Cd exposure will damage the kidneys, followed by severe osteoporosis and osteomalacia as a secondary effect (29,30). A slightly alarming finding has been that recent European studies have suggested that Cd can induce osteoporosis also at much lower exposure levels than previously reported (31-33). We could, however, not corroborate their results. A reason for this discrepancy might be that in

the present study the average U-Cd level was lower and the exposure range was much narrower than in the previous ones, hampering evaluation of exposure-response associations.

POC have in animal studies impaired normal bone metabolism resulting in increased bone fragility (1-4). Dioxin inhibited tibial growth in rat in a dose dependent way (3). Moreover, a dioxin-like PCB-congener impaired the mineralization process of tibiae in rat (1), and also reduced the collagen content and serum osteocalcin levels, which resulted in impaired maximum torque and stiffness of the rat humerus (2). Further, the trabecular bone density in Baltic grey seal was lower during the period of maximum POC contamination in the Baltic Sea as compared with both a preceding period and the later period (53). Thus, data are accumulating supporting that POC may interfere with normal bone metabolism, but the mechanism(s) are not yet fully understood, nor whether these effects may occur also after exposure to non dioxin-like POC.

The results from previous epidemiology are rather ambiguous. In a Swedish study of 115 middle-aged and elderly men from the general population a number of different POC were analyzed in serum, but it was only p,p'-DDE that showed a slight negative association with BMD in the multivariate models (34). The exposure levels were slightly lower than for the men in the present study. Among 68 Australian middle-aged, sedentary women, there remained a weak negative association between p,p'-DDE and BMD, even after adjustment for age and HRT (35). The exposure levels for the Australian women were almost as high as for the women in the present study. Finally, in a study from the US of 103 middle-aged women, with repeated measurements of BMD performed in the 1980´s, no associations between BMD and serum levels of p,p'-DDE were observed after adjustment for confounders (36). These US women had averagely been about twice as highly exposed for p,p'-DDE as compared with the women in the present study.

It is noteworthy that the present study is the first epidemiological assessment of the impact of POC, not only on BMD, but also on biochemical markers of bone metabolism. Our findings were completely negative. However, these results ought to be evaluated in studies including even larger study samples and in longitudinal studies. Another aspect to consider is that even if we could not see any effect on radial BMD and the bone turnover markers appear, other mechanical properties of bone (i.e. bone size and quality) could be affected by POCs.

The results of the present study should also be considered in relation to the results from our studies on osteoporotic fracture incidence in Swedish fishermen's families, from which the present study group was recruited. In a register based study there was a significantly increased Incidence Rate Ratio (IRR 2.29, 95 % CI 1.23-4.28) for vertebral fractures among fishermen's wives from the Swedish east coast off the Baltic Sea where the fish was much more polluted with POC as compared with the Swedish west coast (41). Such cohort differences were not seen for any other fracture type. In a questionnaire study there were no differences in fracture incidence between fishermen or fishermen's wives from the east and west coasts, respectively (42). However, east coast fishermen's wives with more than one meal of fatty fish from the Baltic Sea per month had an increased incidence of osteoporotic fractures as compared with east coast wives that ate at most one such meal per month (age-adjusted IRR 1.68, 95% CI 1.00-2.84). No such exposure-response association was seen among the fishermen. Taken together, the fracture incidence studies were not very consistent with respect to an association between estimated POC exposure and enhanced risk for osteoporotic fractures. However, a caveat with this conclusion is that the fracture incidence studies performed so far have mainly ascertained symptomatic fractures, which constitute a small fraction only of vertebral fractures. An X-ray based study might provide more conclusive results.

The fishermen and their wives comprising our study base were considerably higher exposed to POC than the general Swedish population (38-40). Thus, adding the results from the present cross-sectional study of BMD and bone metabolism with the results from the fracture incidence studies in the same study base (41,42) do not provide any support for the hypothesis that the current exposure levels to POC constitutes a hazard for impaired bone metabolism and fractures in the general Swedish population. There is, however, a need for complementary large-scale epidemiological studies, including longitudinal follow-up of BMD and X-ray studies of fractures in relation to POC exposure, before a more definite conclusion can be drawn.

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References

1. Lind PM, Eriksen EF, Sahlin L et al. (1999) Effects of the antiestrogenic environment pollutant 3,3',4,4',5-pentachlorobiphenyl (PCB#126) in rat bone and uterus: diverging effects in ovariectomized and intact animals. Toxicol Appl Pharmacol 154:236-244.
2. Lind PM, Larsson S, Oxlund H et al. (2000) Change of bone tissue composition and impaired bone strength in rats exposed to 3,3',4,4',5-pentachlorobiphenyl (PCB-126). Toxicology 150:41-51.
3. Jamsa T, Viluksela M, Tuomisto JT et al. (2001) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on bone in two rat strains with different aryl hydrocarbon receptor structures. J Bone Miner Res 16:1812-1820.
4. Miettinen HM, Pulkkinen P, Jämsä T et al. (2003) In utero and lactational TCDD exposure affects rat bone development. Organohalogen Compounds 64:274-277.
5. Cripps DJ, Peters HA, Gocmen A et al (1984) Porphyria turcica due to hexachlorobenzene: a 20 to 30 year follow-up study on 204 patients. Br J Dermatol 111:413-422.
6. Miller RW (1985) Congenital PCB Poisoning: A Reevaluation. Environ Health Perspect 60:211-214.
7. Turner RT, Riggs BL, Spelsberg TC (1994) Skeletal effects of estrogen. Endocrin Rev 15:275-296.

8. Slemenda CW, Longcope C, Zhou L et al. (1997) Sex steroids and bone mass in older men. J Clin Invest 100:1755-1759.

9. Bonefeld-Jorgensen EC, Andersen HR, Rasmussen TH et al. (2001) Effect of highly bioaccumulated polychlorinated biphenyl congeners on estrogen and androgen receptor activity. Toxicology 158:141-153.

10. Danzo BJ (1997) Environmental xenobiotics may disrupt normal endocrine function by interfering with the binding of physiological ligands to steroid receptors and binding proteins. Environ Health Perspect 105:294-301.

11. Kelce WR, Stone CR, Laws SC et al. (1995) Persistent DDT metabolite p,p'-DDE is a potent androgen receptor antagonist. 375:581-585.

12. Sohoni P, Sumpter JP (1998) Several environmental oestrogens are also anti-androgens. J Endocrinol 158:327-339.

13. Safe S, Wormke M (2003) Inhibitory aryl hydrocarbon receptor-estrogen receptor alpha cross-talk and mechanisms of action. Chem Res Toxicol 16: 807-816.

14. Woodhouse AJ, Cooke GM (2004) Suppression of aromatase activity in vitro by PCBs 28 and 105 and Aroclor 1221. Toxicol Lett 152: 91-100.

15. Fukuzawa NH, Ohsako S, Nagano R et al. (2003) Effects of 3,3',4,4',5-pentachlorobiphenyl, a coplanar polychlorinated biphenyl congener, on cultured neonatal mouse testis. Toxicol In Vitro 17: 259-269.

16. Sonnenschein C, Soto AM (1998) An updated review of environmental estrogen and androgen mimics and antagonists. J Steroid Biochem Mol Biol 65:143-150.

17. Gray LE (1998) Xenoendocrine disrupters: laboratory studies on male reproductive effects. Toxicol Lett 102-103: 331-335.

18. Daston GP, Cook JC, Kavlock RJ (2003) Uncertainties for endocrine disrupters: our view

on progress. Toxicol Sci 74:245-252.

19. Kester MH, Bulduk S, Tibboel D, et al. (2000) Potent inhibition of estrogen sulfotransferase by hydroxylated PCB metabolites: a novel pathway explaining the estrogenic activity of PCBs. Endocrinology 141:1897-1900.
20. Kester MH, Bulduk S, van Toor H, et al. (2002) Potent inhibition of estrogen sulfotransferase by hydroxylated metabolites of polyhalogenated aromatic hydrocarbons reveals alternative mechanism for estrogenic activity of endocrine disrupters. J Clin Endocrinol Metab 87:1142-1150.
21. Gore AC, Wu TJ, Oung T, et al. (2002) A novel mechanism for endocrine-disrupting effects of polychlorinated biphenyls: direct effects on gonadotropin-releasing hormone neurons. J Neuroend 14: 814–823.
22. Birnbaum LS, Cummings AM (2002) Dioxins and endometriosis: a plausible hypothesis. Environ Health Perspect 110:15-21.
23. Matsumura F (2003) On the significance of the role of cellular stress response reactions in the toxic actions of dioxin. Biochemical Pharmacology 66:527-540.
24. Boyce WJ, Vessey MP (1985) Rising incidence of fracture of the proximal femur. Lancet 1:150-151.
25. Gullberg B, Duppe H, Nilsson B et al. (1993) Incidence of hip fractures in Malmo, Sweden (1950–1991). Bone (Suppl):s23–s29
26. Gullberg B, Johnell O, Kanis JA (1997) World-wide projections for hip fracture. Osteoporos Int 7:407–413
27. Ismail AA, Pye SR, Cockerill WC et al. (2002) Incidence of Limb Fracture across Europe: Results from the European Prospective Osteoporosis Study (EPOS). Osteoporos Int 13:565–571

28. Lips P (1997) Epidemiology and predictors of fractures associated with osteoporosis. Am J Med 103:3-8.

29. Kido T, Nogawa K, Yamada Y et al. (1989) Osteopenia in inhabitants with renal dysfunction induced by exposure to environmental cadmium. Int Arch Occup Environ Health 61:271-276.

30. Kjellström T (1992) Mechanisms and epidemiology of bone effects of cadmium [review]. IARC Sci Publ 118:301-310.

31. Järup L, Alfvén T, Persson B et al. (1998) Cadmium may be a risk factor for osteoporosis. Occup Environ Med 55:435-439.

32. Staessen JA, Roels HA, Emlianov D et al. (1999) Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study. Lancet 353:1140-1144.

33. Alfvén T, Elinder C-G, Carlsson MD et al. (2000) Low-level cadmium exposure and osteoporosis. J Bone Miner Res 15:1579-1586.

34. Glynn AW, Michaelsson K, Lind PM et al. (2000) Organochlorines and bone mineral density in Swedish men from the general population. Osteoporosis Int 11:1036-1042.

35. Beard J, Marshall S, Jong K et al. (2000) 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane and reduced bone mineral density. Arch Environ Health 55:177-180.

36. Bohannaon AD, Cooper GS, Wolff MS et al. (2000) Exposure to 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDT) in relation to bone mineral density and rate of bone loss in menopausal women. Arch Environ Health 55:386-391.

37. Svensson BG, Nilsson A, Hansson M et al. (1991) Exposure to dioxins and dibenzofurans through the consumption of fish. N Engl J Med 324:8-12.

38. Svensson BG, Nilsson A, Jonsson E et al. (1995) Fish consumption and exposure to persistent organochlorine compounds, mercury, selenium and methylamines among Swedish fishermen. Scand J Work Environ Health 21:96-105.
39. Rylander L, Hagmar L (1995) Mortality and cancer incidence among women with a high consumption of fatty fish contaminated with persistent organochlorine compounds. Scand J Work Environ Health 21:419-426.
40. Rylander L, Dyremark E, Strömberg U et al. (1997) The impact of age, lactation and dietary habits on PCB in plasma in Swedish women. Sci Total Environ 207:55-61.
41. Alveblom A-K, Rylander L, Johnell O et al. (2003) Incidence of hospitalized osteoporotic fractures in cohorts with high dietary intake of persistent organochlorine compounds. Int Arch Occup Environ Health 76:246-248.
42. Wallin E, Rylander L, Hagmar L (2004) Dietary exposure to persistent organochlorine compounds through fish consumption and incidence of osteoporotic fractures. Scand J Work Environ Health 20:30-35.
43. Grimvall E, Rylander L, Nilsson-Ehle P et al. (1997) Monitoring of polychlorinated biphenyls in human blood plasma with respect to age, lactation and fish consumption; methodology developments. Arch Environ Contam Toxicol 32:329-336.
44. Glynn AW, Wolk A, Aune M et al. (2000) Serum concentrations of organochlorines in men: a search for markers of exposure. Sci Total Environ 263:197-208.
45. Gladen BC, Longnecker MP, Schecter AJ (1999) Correlations among polychlorinated biphenyls, dioxins, and furans in humans. Am J Ind Med 35:15-20.
46. Sjödin A, Hagmar L, Klasson-Wehler E et al. (2000) Influence of the consumption of fatty Baltic Sea fish on plasma levels of halogenated environmental contaminants in Latvian and Swedish men. Environ Health Perspect 108:1035-1041.

47. Richthoff J, Rylander L, Jonsson BA et al. (2003) 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 111:409-413.

48. Rignell-Hydbom A, Rylander R, Giwercman A et al. (2004) Exposure to CB-153 and p,p‘-DDE and male reproductive function. Hum Reprod 19:2066-2075.

49. Rylander L, Nilsson-Ehle P, Hagmar L. A simplified precise method for adjusting serum levels of persistent organohalogen pollutants to total serum lipids. Chemosphere (in press)

50. Mazzachi BC, Peake MJ, Ehrhardt V (2000) Reference range and method comparison studies for enzymatic and Jaffe creatinine assays in plasma and serum and early morning urine. Clin Lab 46:53-55

51. Marshall D, Johnell O, Wedel H (1996) Meta-analysis of how well measures of bone density predict occurrence of osteoporotic fractures. BMJ 312:1254-1259.

52. Wolff MS, Anderson HA (1999)Correspondence re: J. M. Schildkraut et al., Environmental contaminants and body fat distribution. Cancer Epidemiol Biomark Prev., 8:179-183. Cancer Epidemiol Biomark Prev 8:951-952.

53. Lind PM, Bergman A, Olsson M et al. (2003) BMD in male Baltic grey seal. Ambio 32:385-388.

Table 1. Distributions of risk factors for osteoporosis, which might act as potential confounders, among 196 fishermen and 184 fishermen's wives from the Swedish east coast off the Baltic Sea.

	Women			Men		
	n	%	Median (5^{th}, 95^{th} perc.)	n	%	Median (5^{th}, 95^{th} perc.)
Age (yr)			62 (51, 77)			59 (49, 75)
Body height (cm)			163 (153, 172)			176 (166, 187)
Body weight (kg)			72 (58-97)			88 (70-114)
BMI (kg/m^2)			27.7 (21.8-35.8)			28.3 (23.5-35.5)
Age at menopause			50 (44-55)			-
Length of fertile period			37 (30-43)			-
Current hormone replacement therapy	44	23		-	-	
History of fracture[a]	12	6.5		2	1.0	
Heredity for fracture[b]	38	21		23	12	
Low physical activity at work at 25 and 35 yrs of age[c]	21	11		3	1.5	
Currently low physical activity[d]	20	11		13	6.7	
Current smoking	31	17		39	20	
Cumulative cigarette smoking over life (pack-years)			0.7 (0, 33)			13 (0, 65)
Current alcohol consumption (g/month)			46 (0, 420)			250 (0, 1300
U-Cd (µmol/mol creatinine)			0.35 (0.13-0.91)			0.23 (0.09-2.90)

[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 hours per week.

Table 2. Distributions of exposure variables and outcome variables, among 196 fishermen and 184 fishermen's wives from the Swedish east coast off the Baltic Sea.

	Women	Men
	Median (5^{th}, 95^{th} perc)	Median (5^{th}, 95^{th} perc)
Exposure variables		
S-CB-153 (ng/g lipid)	240 (98, 620)	370 (110, 1010)
S-p,p'-DDE (ng/g lipid)	600 (110, 2310)	580 (110, 2140)
Outcome variables		
BMD [a] (mg/cm^2)	430 (290, 580)	580 (450, 700)
S-Osteocalcin (μg/L)	25 (12, 48)	20 (11, 36)
S-Crosslaps (ng/L)	360 (130, 760)	340 (150, 650)

[a] 28 of 184 women (15.2 %) and 26 of 196 (13.3 %) men had age and gender standardized T-scores <-2.5, indicating osteoporosis, and 90 of 184 women (48.9 %) and 65 of 196 men (33.7 %) had T-scores -2.5, -1.0, indicating osteopenia.

Table 3 The effect of CB-153 and p,p'-DDE, respectively, on bone mineral density (BMD) among 196 fishermen from the Swedish east coast obtained from linear regression models. The exposure variables were treated as continuous (estimated effects (*ß*) on BMD by 100 ng/g lipids increase of exposure concentrations) as well as categorized variables. Crude and adjusted effects are showed with 95 % confidence intervals (CI). In addition, the p-values are shown.

	Bone Mineral Density (mg/cm^2)			
	Crude		Adjusted[a]	
Exposure variable	ß 95% CI	p	ß 95% CI	p
CB-153 (ng/g lipid)				
Continuous				
↑ 100	-3.8 (-7.4, -0.1)	0.04	-3.0 (-6.6, 0.6)	0.10
Categorical				
-218[b]	ref		ref	
>218 – 365	5.2 (-26, 37)	0.75	21 (-8.4, 51)	0.16
>365 – 556	-4.1 (-36, 27)	0.80	8.2 (-22, 38)	0.59
>556	-19 (-50, 13)	0.25	-5.6 (-36, 25)	0.72
p,p'-DDE (ng/g lipid)				
Continuous				
↑ 100	-0.3 (-1.8, 1.2)	0.72	-0.4 (-2.0, 1.1)	0.59
Categorical				
-345[b]	ref		ref	
>345 – 573	-3.4 (-35, 28)	0.83	-3.6 (-33, 26)	0.81
>573 – 1050	12 (-20, 43)	0.47	8.0 (-22, 38)	0.60
>1050	-9.4 (-41, 22)	0.56	-18 (-50, 15)	0.28

[a] Adjusted for age and BMI.
[b] Reference category.

Table 4 The effect of CB-153 and p,p'-DDE, respectively, on bone mineral density (BMD) among 184 fishermen's wives from the Swedish east coast obtained from linear regression models. The exposure variables were treated as continuous (estimated effects (ß) on BMD by 100 ng/g lipids increase of exposure concentrations) as well as categorized variables. Crude and adjusted effects are showed with 95 % confidence intervals (CI). In addition, the p-values are shown.

	Bone Mineral Density (mg/cm^2)					
	Crude			Adjusted[a]		
Exposure variable	ß	95% CI	p	ß	95% CI	p
CB-153 (ng/g lipid)						
Continuous						
↑ 100	-10	(-18, -3.5)	0.004	1.7	(-4.6, 8.0)	0.59
Categorical						
-159[b]	ref			ref		
>159 – 237	-16	(-50, 18)	0.36	12	(-17, 40)	0.42
>237 – 344	-11	(-46, 23)	0.51	19	(-10, 48)	0.20
>344	-56	(-89, -22)	0.001	4.6	(-26, 35)	0.42
p,p'-DDE (ng/g lipid)						
Continuous						
↑ 100	-1.4	(-2.8, -0.1)	0.04	-0.1	(-1.2, 1.1)	0.91
Categorical						
-300[b]	ref			ref		
>300 – 597	-23	(-58, 12)	0.19	-17	(-45, 10)	0.22
>597 – 1051	-38	(-72, -3.6)	0.03	-24	(-52, 4.6)	0.10
>1051	-48	(-83, -13)	0.007	-22	(-52, 7.2)	0.14

[a] Adjusted for age and BMI.
[b] Reference category.

LEGENDS

Figure 1. The association between the serum concentrations of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and bone mineral density among 196 fishermen from the Swedish east coast off the Baltic Sea.

Figure 2 The association between the serum concentrations of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153) and bone mineral density among 184 fishermen's wives from the Swedish east coast off the Baltic Sea.

Figure 1

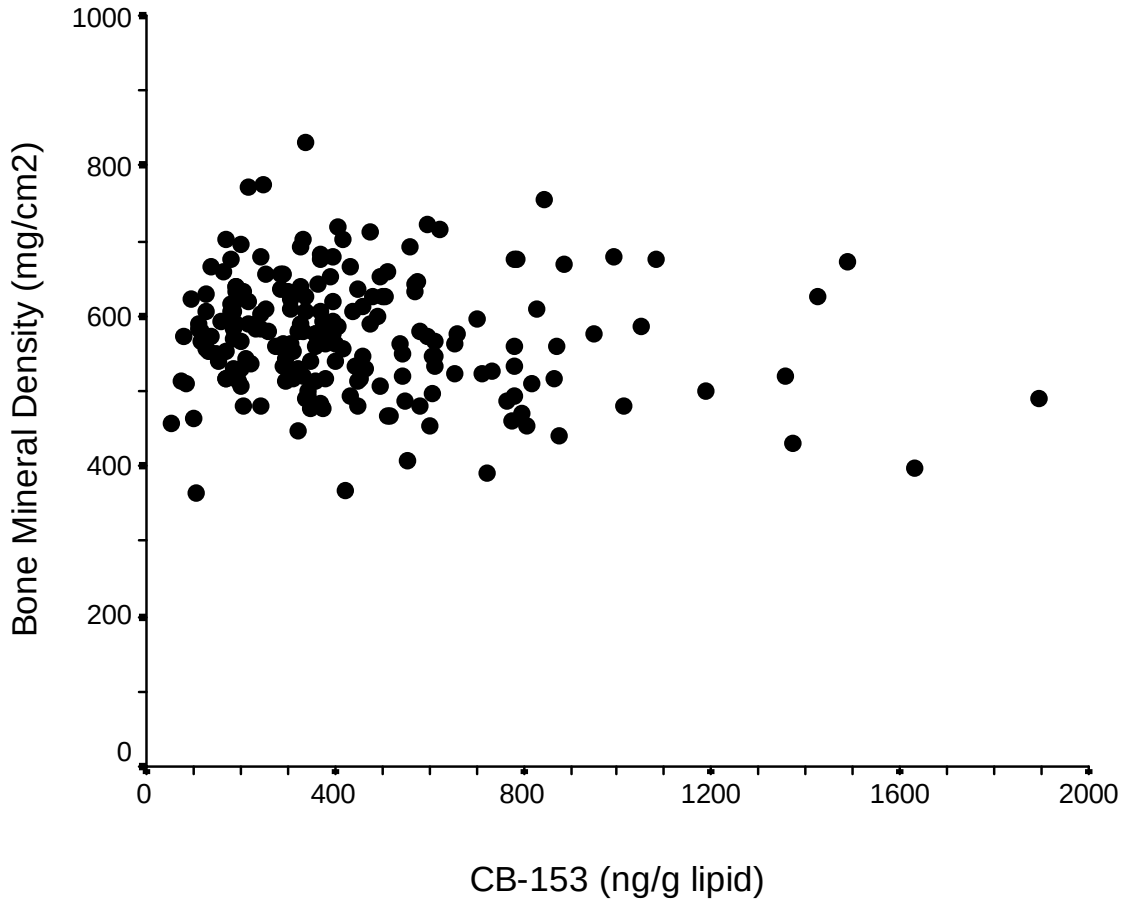
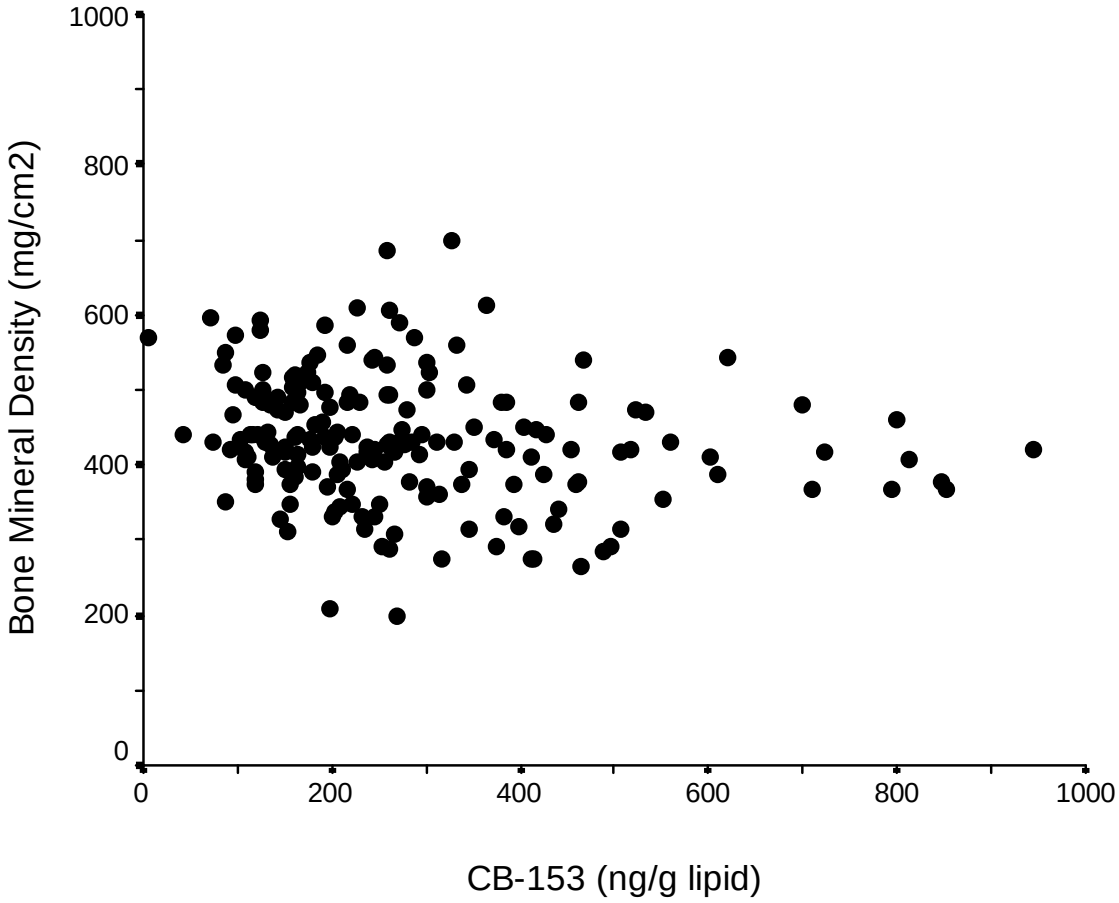

Bone Mineral Density (mg/cm2)
1000
800
600
400
200
0
0
400
800
1200
1600
2000
CB-153 (ng/g lipid)

Figure 2


Bone Mineral Density (mg/cm2)
1000
800
600
400
200
0
0
200
400
600
800
1000
CB-153 (ng/g lipid)