BONE MINERAL DENSITY CHANGES IN RELATION TO ENVIRONMENTAL PCB EXPOSURE

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Introduction

It is well known that hormones, vitamins, pharmaceuticals, and metals can have adverse effects on bone. Bone effects, mainly congenital, have also been linked to persistent organochlorine exposure following a few notable poisoning incidents[1-3]. Recent epidemiological studies in populations with environmental organochlorine exposure have found inconsistent results with respect to whether organochlorine exposure has an effect on bone properties; several studies have indicated a possible association between organochlorine exposure and bone quality[4-6], but other studies have not found such an association[7-9].

While the epidemiological evidence is inconsistent, an increasing number of experimental studies lend biological plausibility to organochlorine induced bone effects[10, 11]. Animal studies have also demonstrated that the timing of exposure can be critical, with the same compound modulating bone quality differently depending on the developmental stage at exposure[12, 13], and that the oestrogen status of the exposed individual can influence the toxicity of organochlorines on bone[14-16]. Because of these various influences, the effect of exposure to a number of different organochlorines is likely to be difficult to predict, nonetheless, these animal studies suggest a possible causal relationship between organochlorine exposure and adverse bone effects.

The purpose of this study was to investigate the relationship between organochlorine exposure and BMD in a cohort of individuals living close to the Baltic coast (where elevated dietary PCB exposure from contaminated fatty fish consumption might be anticipated), and to a contaminated river, polluted with PCB containing paper pulp from an up stream paper mill[17].

Materials and Methods

Full details of the OSCAR study population can be found elsewhere[18]. In brief, subjects aged 16 to 80 who had resided near a nickel-cadmium battery plant for at least 5 years between 1910 and 1992 were invited into the OSCAR study; in total, 1,021 individuals (60%) agreed to participate. The OSCAR participants provided information on employment, residence, smoking, diet and medical history. Specially trained nurses collected urine and blood samples and measured height and weight. Forearm BMD was measured with an ambulant instrument (Osteometer DTX-200; Meditech A/S, Rødovre, Denmark), using dual energy X-ray absorptiometry, which is commonly used to evaluate BMD[6, 9, 19]. The distal site in the non-dominant forearm was measured with the patient in a supine position.

Blood samples from a subset of the OSCAR cohort consisting of participants aged 60 years and over (n=325) were included in this study. The analytical method for the measurement of PCBs was initially developed in serum[20, 21], but has been extended to plasma and whole blood. Samples were analysed for five mono-*ortho* chlorine substituted congeners (CBs 105, 118, 156, 157 and 167), expressed in terms of toxic equivalency ($TEQ_{mono\text{-}ortho}$), and individual CB 118 levels, to assess the effect of the "dioxin-like" activity. The sum of three most abundant non-dioxin-like (or di-*ortho* chlorine substituted) CBs 138, 153 and 180 (Σ_{3PCB}) was analysed as an indicator of the content of total PCBs in human samples[22], and individual CB 153 levels were also assessed as

the concentration of this congener correlates well with total and dioxin-like PCB concentrations in plasma and serum[23]. Finally, p,p'-DDE, the persistent metabolite of DDT, was also analysed, as this organochlorine has been measured in other studies exploring the effects of organochlorines on BMD[5, 6, 9]. Ethical approval for this study was obtained from the Karolinska Institutet ethics committee, and all participants gave their written informed consent prior to the OSCAR study.

Given the probable hormonal action of organochlorines on bone, and given the very large difference in osteoporosis incidence in males and females after the age of 60, it seemed most appropriate to assess the relationships between organochlorines and BMD measures in males and females separately. Because the OSCAR cohort was originally set up to explore the effect of cadmium on BMD, blood cadmium was included as a potential confounding variable in the linear and logistic regression analyses presented.

Results and Discussion

The blood concentration levels ranged from 0.002 to 0.067 pg $TEQ_{mono\text{-}ortho}$/ml for the dioxin-like PCBs, from 438 to 8960 pg/ml for Σ_{3PCB} and from 16.4 to 17500 pg/ml for p,p'-DDE. Women had significantly higher levels of CB 118 and p,p'-DDE than men.

As anticipated, age was negatively correlated with BMD and BMI was positively correlated with BMD. In unadjusted analyses, significant negative correlations were seen between CB 118 and BMD (Pearson correlation co-efficient = -0.130 (p=0.020)) when males and females were investigated as one group. When males and females were investigated separately none of the individual CB congeners were significantly correlated with BMD. There were significant positive correlations between each of the five organochlorine markers of interest.

Table 1 shows the results from multivariate linear regression analysis in males and females. In males, multivariate linear regression analysis indicated that age, BMI, blood cadmium and milk consumption explained 25% of the variability in BMD; none of the organochlorines were significantly associated with BMD when entered into the model individually. When the organochlorine variables were entered stepwise into the model, sequentially, in order of the smallest probability of F (if $F \leq 0.10$), until no more variables were eligible for inclusion or removal ($F \geq 0.15$), CB 118 was negatively associated (B=-0.00024; p=0.002) and Σ_{3PCB} positively associated (B=0.00002; p=0.003) with BMD in males, explaining an additional 6% of the variability in BMD. In females, multivariate linear regression analysis indicated that age, BMI, blood cadmium, age at menstruation and ever having being pregnant explained 39.5% of the variability in BMD. When organochlorines were entered individually into this model, CB 118 was significantly positively associated with BMD (B=0.00008; p=0.042), explaining a further 2% of the variability in BMD. No additional organochlorines contributed significantly to the model after stepwise entry.

Table 1: Linear regression analyses showing associations between BMD (g/cm^2) and explanatory variables (entered simultaneously) and organochlorines (pg/ml) (entered individually into the adjusted model).

	Males (n=150)			Females (n=134)		
Variable	Coefficient	SE	p	Coefficient	SE	p
Age	-0.004009	0.001123	<0.001	-0.005635	0.000953	<0.001
BMI	0.008255	0.002006	<0.001	0.006300	0.001209	<0.001
Ln B-Cd (nmol/l)	-0.013383	0.005946	0.026	-0.010184	0.008257	0.220
Milk (dl/week)	0.000544	0.000245	0.028	-	-	-
Age at menstruation	-	-	-	-0.009020	0.003598	0.013
Ever pregnant	-	-	-	0.037579	0.015894	0.020
CB 118	-0.000110	0.000062	0.079	0.000080	0.000039	0.045
$TEQ_{mono\text{-}ortho}$	-0.225387	1.156013	0.846	1.652927	0.861054	0.057
CB 153	0.000011	0.000011	0.331	0.000009	0.000010	0.385
Σ_{3PCB}	0.000008	0.000006	0.154	0.000007	0.000006	0.223
p,p'-DDE	-0.000003	0.000003	0.323	0.000003	0.000002	0.184

A dichotomous variable representing presence or absence of low BMD (defined as Z-score<-1[24]) was used in logistic regression analyses; 47 out of 154 (30.5%) of the males, and 31 out of 167 (18.6%) of the females had low BMD. Those organochlorines that were found to significantly explain variability in BMD after stepwise entry into the multivariate linear regression models (CB 118 and Σ_{3PCB} in males, and CB 118 in females) were included in the multivariate logistic regression as categorical variables (tertiles). Where a linear dose response relationship seemed apparent by tertiles, these variables were re-entered into the model as continuous variables to maximise power in assessing the dose response relationship. Table 2 shows the results from logistic regression analysis in males and females. In males, the risk of low BMD increased by tertile of CB 118, and when analysed as a continuous variable the odds ratio was 1.06 (95% confidence interval 1.01-1.12) for every 10 pg CB 118/ml. The risk of low BMD did not show a clear trend across tertiles of Σ_{3PCB}. In women, the risk of low BMD did not show a consistent trend with tertiles of CB 118.

Table 2: Logistic regression model for low BMD (Z-score < -1) in males and females.

	Odds Ratio	95% CI for OR		p
Variable		Lower	Upper	
Males (n=150)				
BMI	0.83	0.72	0.95	0.009
Ln Blood cadmium (nmol/l)	1.76	1.19	2.59	0.004
Milk consumption (dl/week)	0.97	0.95	0.99	0.004
CB 118 (<33rd percentile)	1.00	-	-	-
CB 118 (33rd-67th percentile)	1.48	0.55	3.97	0.437
CB 118 (>67th percentile)	2.11	0.63	7.12	0.227
CB 118 (continuous variable)	1.06	1.01	1.12	0.027
Σ_{3PCB} (<33rd percentile)	1.00	-	-	-
Σ_{3PCB} (33rd-67th percentile)	1.17	0.44	3.16	0.753
Σ_{3PCB} (>67th percentile)	0.91	0.27	3.02	0.879
Σ_{3PCB} (continuous variable)	1.00	0.99	1.00	0.101
Females (n=134)				
BMI	0.80	0.70	0.93	0.003
Ln Blood cadmium (nmol/l)	0.88	0.40	1.92	0.745
Age at menstruation	1.48	1.07	2.03	0.016
Ever pregnant	0.47	0.13	1.70	0.251
CB 118 (<33rd percentile)	1.00	-	-	-
CB 118 (33rd-67th percentile)	2.29	0.66	7.92	0.191
CB 118 (>67th percentile)	2.13	0.58	7.84	0.255

The present study is one of the largest to date to assess the relationship between organochlorine levels in blood and BMD in an environmentally exposed population. The blood concentration levels of these pollutants in this population are in the range of the values detected in human serum and/or blood samples for the general population[21, 25, 26]. Nonetheless, findings of this study indicate that exposure to some CB congeners, even at relatively low levels, may influence BMD, and that this effect may be sex specific.

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References
1. Cripps, D.J., H.A. Peters, A. Gocmen, and I. Dogramici, *British Journal of Dermatology*, 1984. 111(4): p. 413-22.
2. Gocmen, A., H.A. Peters, D.J. Cripps, G.T. Bryan, and C.R. Morris, *Biomedical & Environmental Sciences*, 1989. 2(1): p. 36-43.
3. Miller, R.W., *Environmental Health Perspectives*, 1985. 60: p. 211-214.
4. Alveblom, A.K., L. Rylander, O. Johnell, and L. Hagmar, *International Archives of Occupational & Environmental Health*, 2003. 76(3): p. 246-248.
5. Beard, J., S. Marshall, K. Jong, R. Newton, T. Triplett-McBride, B. Humphries, and R. Bronks, *Archives of Environmental Health*, 2000. 55(3): p. 177-180.
6. Glynn, A.W., K. Michaelsson, P.M. Lind, A. Wolk, M. Aune, S. Atuma, P.O. Darnerud, and H. Mallmin, *Osteoporosis International*, 2000. 11(12): p. 1036-1042.
7. Bohannon, A.D., G.S. Cooper, M.S. Wolff, and D.E. Meier, *Archives of Environmental Health*, 2000. 55(6): p. 386-391.
8. Cote, S., P. Ayotte, S. Dodin, C. Blanchet, G. Mulvad, H.S. Petersen, S. Gingras, and E. Dewailly, *Environmental Health: A Global Access Science Source*, 2006. 5: p. 33.
9. Wallin, E., L. Rylander, B.A. Jonssson, T. Lundh, A. Isaksson, and L. Hagmar, *Osteoporosis International*, 2005. 16(12): p. 2085-2094.
10. Andrews, J.E., *Toxicology*, 1989. 57(1): p. 83-96.
11. Badraoui, R., N.B. Abdelmoula, Z. Sahnoun, Z. Fakhfakh, and T. Rebai, *Comptes Rendus Biologies*, 2007. 330(12): p. 897-904.
12. Jamsa, T., M. Viluksela, J.T. Tuomisto, J. Tuomisto, and J. Tuukkanen, *Journal of Bone & Mineral Research*, 2001. 16(10): p. 1812-20.
13. Miettinen, H.M., P. Pulkkinen, T. Jamsa, J. Koistinen, U. Simanainen, J. Tuomisto, J. Tuukkanen, and M. Viluksela, *Toxicological Sciences*, 2005. 85(2): p. 1003-12.
14. Lind, P.M., E.F. Eriksen, L. Lind, J. Orberg, and L. Sahlin, *Toxicology*, 2004. 199(2-3): p. 129-136.
15. Lind, P.M., E.F. Eriksen, L. Sahlin, M. Edlund, and J. Orberg, *Toxicology & Applied Pharmacology*, 1999. 154(3): p. 236-244.
16. Lind, P.M., S. Larsson, H. Oxlund, H. Hakansson, K. Nyberg, T. Eklund, and J. Orberg, *Toxicology*, 2000. 150(1-3): p. 41-51.
17. Bremle, G., P. Larson, T. Hammar, A. Helgee, and B. Troedsson, *Water Air and Soil Pollution* 1998. 107: p. 237-250.
18. Alfven, T., L. Jarup, and C.G. Elinder, *Environmental Health Perspectives*, 2002. 110(7): p. 699-702.
19. Alfven, T., C.G. Elinder, M.D. Carlsson, A. Grubb, L. Hellstrom, B. Persson, C. Pettersson, G. Spang, A. Schutz, and L. Jarup, *Journal of Bone & Mineral Research*, 2000. 15(8): p. 1579-1586.
20. Mariani, G., S. Carasi, E. Fattore, S. Nichetti, A. Guzzi, E. Benfenati, and R. Fanelli, *Organohalogen Compounds*, 2002. 55: p. 111-114.
21. Turci, R., G. Mariani, A. Marinaccio, C. Balducci, M. Bettinelli, R. Fanelli, S. Nichetti, and C. Minoia, *Rapid Communications in Mass Spectrometry*, 2004. 18(4): p. 421-34.
22. EFSA, *The European Food Safety Authority Journal* 2005. 284: p. 1-237.
23. Glynn, A.W., A. Wolk, M. Aune, S. Atuma, S. Zettermark, M. Maehle-Schmid, P.O. Darnerud, W. Becker, B. Vessby, and H.O. Adami, *Science of the Total Environment*, 2000. 263(1-3): p. 197-208.
24. Kanis, J.A., P. Delmas, P. Burckhardt, C. Cooper, and D. Torgerson, *Osteoporosis International*, 1997. 7(4): p. 390-406.
25. Botella, B., J. Crespo, A. Rivas, I. Cerrillo, M.F. Olea-Serrano, and N. Olea, *Environmental Research*, 2004. 96(1): p. 34-40.
26. Wilhelm, M., U. Ewers, and C. Schulz, *International Journal of Hygiene & Environmental Health*, 2003. 206(3): p. 223-9.