Human Reproduction Vol.17, No.8 pp. 1973–1979, 2002

Decreased human semen quality and organochlorine compounds in blood

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BACKGROUND: Various studies have been performed in which potential effects of xenoestrogens on fertility or sperm parameters were investigated by comparing groups of subjects exposed to different levels of these chemicals. METHODS: In our study we used an alternative approach, as we selected one group of men with very poor semen quality and another group with normal semen quality and determined the blood organochlorine contents in order to determine whether a difference in these levels could be established. Organochlorine compounds, including polychlorinated biphenyls (PCB) and PCB metabolites, were detected using gas chromatography. The concentrations were compared between both groups, and related to semen parameters. RESULTS: A comparison of both groups did not reveal significant differences in organochlorine levels. Linear relationships were found when PCB and metabolite concentrations were related to the age of the volunteers. Focusing on the subgroup of men with normal semen quality showed that sperm count and sperm progressive motility were inversely related to the concentrations of PCB metabolites within this group. CONCLUSIONS: The finding of a significantly decreased sperm count in relation to an elevated PCB metabolite level within the subgroup of men with normal semen quality is important. This is the first time that a correlation between exposure to environmental pollutants with endocrine-disrupting capacity and human sperm quality has been observed.

Key words: endocrine disruptor/hydroxy-PCB/organochlorine/semen quality/subfertility

Introduction

A possible decline in semen quality (particularly in sperm count) has been observed and reported for many years (Nelson and Bunge, 1974; Leto and Frensilli, 1981; Bostofte *et al*., 1983; Osser *et al*., 1984; Bendvold, 1989; Bendvold *et al*., 1991). However, since the publication of a meta-analysis of 61 studies concerning human sperm count (Carlsen *et al*., 1992), the decline in male fertility has been extensively examined and disputed (Bromwich *et al*., 1994; Auger *et al*., 1995; Olsen *et al*., 1995; Bujan *et al*., 1996; Fisch *et al*., 1996; Irvine *et al*., 1996; Paulsen *et al*., 1996; Becker and Berhane, 1997; Swan *et al*., 1997, 2000; Emanuel *et al*., 1998; Saidi *et al*., 1999). Possible causes of this alleged decline, including the estrogenic activity of several environmental pollutants, have been the subject of extensive discussions (Sharpe and Skakkebaek, 1993; Jensen *et al*., 1995; Safe, 1995; Toppari *et al*., 1996; Cooper and Kavlock, 1997; Daston *et al*., 1997; Irvine, 1997). A striking fact in this context is that the estrogenic property of several of these compounds has been known for over half a century (Dodds and Lawson, 1936). The environmental estrogens might affect the development of the male reproductive system during fetal or childhood life (Sharpe and Skakkebaek, 1993; Jensen *et al*., 1995; Cooper and Kavlock, 1997; Irvine, 1997), but from studies on occupational exposure to high amounts of chemicals it is known that spermatogenesis can also be corrupted by exposure of the adult man to various organic chemicals (Whorton *et al*., 1977; Glass *et al*., 1979; Emmett *et al*., 1988; Tas *et al*., 1996; De Celis *et al*., 2000). Suspect agents of endocrine disrupting activity, possibly interfering with male reproductive capacity, are chlorinated hydrocarbons, for instance polychlorinated biphenyls (PCB) and pesticides. In-vivo formed PCB metabolites, especially hydroxy-PCB, might play an even more important role, since in in-vitro studies such metabolites have been shown to exhibit a much stronger estrogenic activity than the unmetabolized compounds (Korach *et al*., 1988; Waller *et al*., 1995; Fielden *et al*., 1997; Garner *et al*., 1999). Furthermore, anti-androgen activity (Kelce *et al*., 1995) and anti-estrogen activity might also be involved in the disturbance of the delicate hormonal balance that regulates spermatogenesis (Connor *et al*., 1997; Fielden *et al*., 1997; Moore *et al*., 1997; Golden *et al*., 1998; Navas and Segner, 1998).

The cause of involuntary childlessness in couples can be

found in either the male or the female counterpart, or in both. From couples visiting the Maastricht University Hospital, males were selected to be included in either a group of subfertile men, based on extremely poor semen quality [male factor subfertility (MFS) group] or in a group of men with normal (good) semen quality [female factor subfertility (FFS) group]. The classification was based on semen analyses performed on three previous occasions, and progressively motile sperm concentration (PMSC) was used as the parameter to make the classification.

In order to determine whether presently occurring organochlorine levels play a role in the fertility defects of the MFS subgroup, various compounds, including several PCB and PCB metabolites, were analysed in blood samples of all volunteers and related to semen parameters. Moreover, the relationship between seminal plasma and blood levels of the unmetabolized organochlorine compounds was established by comparison of the samples of a small number of volunteers. Furthermore, to assess the potential role of genetic factors affecting PCB metabolism, polymorphic frequencies of the glutathione S-transferase (GST) GSTM1 and GSTT1 genes, encoding for these PCB-detoxifying enzymes, were determined in both the FFS and MFS groups.

Materials and methods

Study population

A total of 65 male counterparts of couples visiting the Maastricht University Hospital for fertility treatment were selected for this study, and donated blood and semen samples. One subgroup was composed of 31 men selected on the basis of normal (or better) sperm quality, where a PMSC of $\geqslant 10\times10^6$/ml was used as the criterion, measured at three different (previous) occasions. The mean of PMSC and sperm count ($\pm$ SD) of the three semen analyses in this group were 29.3 $\pm$ 15.3$\times10^6$ per ml and 94.5 $\pm$ 40.6$\times10^6$ per ml respectively, which is far above the best cut-off point for PMSC (4×10^6/ml) between normal and abnormal semen (Enginsu *et al*., 1992), as well as the WHO reference value for sperm density (20×10^6/ml) (World Health Organization, 1999). All men were of proven fertility, as demonstrated by successful IVF treatments. This group is referred to as the FFS group. The other subgroup consisted of 34 subjects, who were selected based on a PMSC of $\leqslant 1\times10^6$/ml, measured also on three different occasions. All these men were without marked clinical or pathological disorders. The mean of PMSC and sperm count of the three semen analyses in this group were 0.34 $\pm$ 0.48$\times10^6$ per ml and 6.8 $\pm$ 10.8$\times10^6$ per ml respectively. In this fashion, two groups were composed having maximal differences between them.

Questionnaires were used to obtain additional information on smoking behaviour, possible occupational exposure, etc. and the ages of the volunteers at the time of sampling were recorded. The study was approved by the Medical Ethical Commission of the University and the University Hospital and informed consent was obtained from all volunteers.

Semen quality

The semen samples, produced on the same occasion that the blood samples were taken, were investigated with regard to volume, sperm concentration (sperm count), overall and progressive motility and morphology. Conventional methods were used to evaluate the volume, sperm concentration and motility. A Makler counting chamber was used for concentration and motility evaluation. Two groups of motile sperm were recognized (World Health Organization, 1999): sperm with rapid and linear progressive movements (group A, equal to WHO grade 'a'), and sperm with all other types of movements: either slow, sluggish or hampered by a clearly visible morphological defect, non-progressive motility or immotility (group B, combining WHO grades 'b', 'c' and 'd'). The total number of sperm in group A was defined as the PMSC. The morphology was defined applying the morphology evaluation using strict criteria (Enginsu *et al*., 1992) and expressed as a percentage of the total number of sperm counted.

Isolation of organochlorine compounds from blood

The blood samples of all volunteers were investigated with regard to the organochlorine compounds hexachlorobenzene (HCB), *p,p*'-DDE:1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene *p,p*'-DDT:1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethylene, 2,3′,4,4′,5-pentachlorobiphenyl (PCB-118), 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB-153), 2,2′,3,4,4′,5-hexachlorobiphenyl (PCB-138) and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB-180). These four PCB were selected, based on their relatively high abundance in humans (Safe, 1994) and the potential endocrine-disrupting activity of their metabolites, due to the *ortho* substitution (McKinney and Waller, 1994). To this end, 3 ml samples were fortified with 50 µl of an internal standard solution of PCB-143 (100 ng/ml) and hydrolysed for 2 h at 60°C with 3 ml ethanolic sodium hydroxide (1 g NaOH in 25 ml water and 25 ml ethanol). The resulting solutions were extracted twice with 2 ml *n*-hexane, the combined hexane layers were dried over anhydrous sodium sulphate and further cleaned using deactivated silicon dioxide (SiO_2) columns. The solvent was removed by evaporation and the residue redissolved in 50 µl iso-octane.

Metabolites were isolated separately, as follows. To 3 ml blood sample, 3 ml water and 3 ml methanol were added and the resulting solution was acidified with 0.5 mol/l sulphuric acid until pH <5. This solution was extracted with 3$\times$3 ml *n*-hexane/methyl-*t*-butylether (1:1); the combined organic layers were evaporated and the residue was redissolved in 2 ml *n*-hexane. From this solution, the polar metabolites were extracted with 1 ml 1.0 mol/l KOH solution (H_2O/CH_3OH 1:1), and, after acidification with sulphuric acid until pH <2 and extraction with 2$\times$1 ml *n*-hexane/methyl-*t*-butylether (1:1), the solvent was evaporated and the residue dissolved in 300 µl *n*-hexane. To this solution 200 µl of a diazomethane reagent was added and the resulting products were dissolved in *n*-hexane and cleaned over deactivated SiO_2 columns. After removal of the solvent, the residue was redissolved in 50 µl iso-octane, containing PCB-143 as an internal standard (Bergman *et al*., 1994).

Isolation of organochlorine compounds from seminal plasma

Semen samples were centrifuged to remove the sperm and 1 ml of the resulting plasma was fortified with 50 µl of the internal standard and extracted as described above for the blood samples (unmetabolized organochlorine compounds). The quantities of the samples in combination with the low concentration of metabolites did not allow for metabolite isolation and determination in the seminal plasma.

Determination of organochlorine compounds

Aliquots (1 µl) of the extracts were introduced into a gas chromatograph (HRGC Mega 2 Series, 8560 gas chromatograph; Interscience, Breda, The Netherlands), equipped with a cold on-column injection port. The samples were analysed using a capillary, 25 m length, 0.25 mm inside diameter, 0.25 µm film thickness fused silica CP Sil-8 CB column, at a programmed temperature of 80–270°C. Helium was used as the carrier gas and nitrogen as a make-up gas. An electron capture detector at 300°C was used to

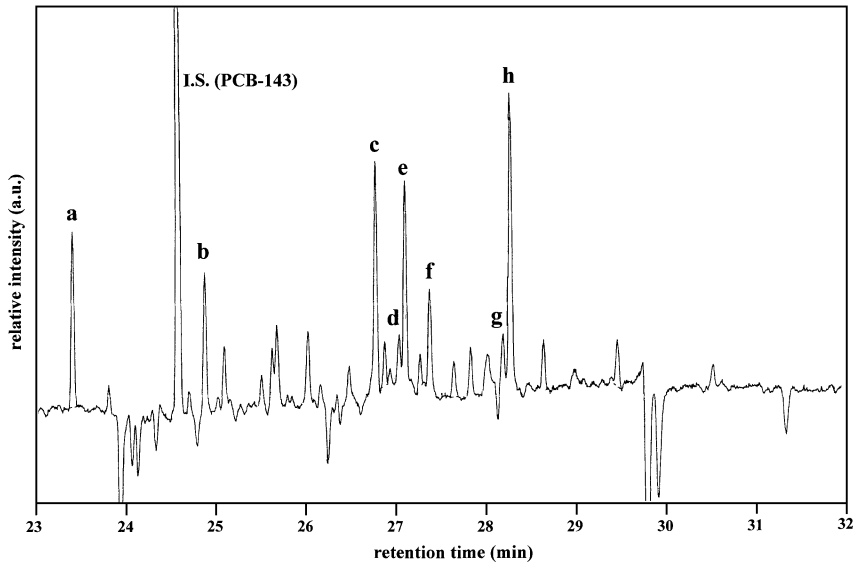

Figure 1. Gas chromatogram showing the organochlorine metabolites (labelled a–h). Metabolite c = 4-hydroxy-2,3,3′,4′, 5-pentachlorobiphenyl.

detect the organochlorine compounds in a sensitive and specific way Quantification was performed using the internal standard and based on peak area. Of the different metabolites, which were measured separately from the unmetabolized organochlorine compounds, one was identified by using the standard compound (metabolite c: 4-hydroxy-2,3,3′,4′,5-pentachloro biphenyl). The other seven metabolites were measured and compared using their relative retention times; these metabolites are bound to be hydroxy-PCB as well, because of the isolation and detection methods applied, analogous to other published methods (Bergman *et al*., 1994). The metabolites are labelled a–h in the example chromatogram presented in Figure 1.

GSTM1 and GSTT1 polymorphisms

The GSTM1 and GSTT1 genotypes were determined using a PCR-based assay (Oude Ophuis *et al*., 1998). PCR was carried out in a total volume of 25 µl, containing 0.1 µg DNA, 0.2 mmol/l of each dNTP, 5 mmol/l Tris–HCl, 50 mmol/l KCl, 2 mmol/l $MgCl_2$, 1 IU of Taq polymerase (Pharmacia Biotech, Roosendaal, The Netherlands), 0.2 µmol/l of the CYP1A1 (control) primers and 0.4 µmol/l GSTM1 or GSTT1 primers. Exact information about the primers used for GSTM1 and GSTT1 can be found in the respective literature (Brockmöller *et al*., 1992; Pemble *et al*., 1994). After denaturation at 94°C for 4 min, the PCR was followed by 35 cycles of amplification. The PCR product was analysed on a 2% agarose gel for the presence of CYP1A1 bands at 204 bp (control) and 480 bp (GSTT1) or 650 bp (GSTM1).

Statistical methods

Comparison of the groups defined as FFS and MFS was performed using the non-parametric Mann–Whitney *U*-test. This test was also used to examine potential effects of polymorphisms and smoking on sperm parameters and organochlorine contents. Quantitative relationships with regard to various organochlorine levels, sperm parameters and age were investigated using simple linear regression.

Results

All data concerning age, polymorphisms and sperm quality of the subgroups of FFS and MFS volunteers have been collected in Table I. No significant differences in sperm parameters between smoking and non-smoking men were found (sperm count for smokers: 59.4 ± 59.1×10^6/ml, non-smokers 40.2 ± 53.8×10^6/ml; Mann–Whitney *U*-test, P = 0.22). The absence of an effect of smoking on sperm quality and the indifference for polymorphisms of both groups enabled us to elaborate the relationships between organochlorine data and sperm parameters without corrections for smoking or polymorphisms.

The concentrations of the various compounds and of the combined PCB, total organochlorine compounds (unmetabolized) and of the metabolites are summarized in Table II.

Comparison of PCB and PCB metabolite concentrations

A very strong positive correlation was found between the total PCB concentration and the total PCB metabolite concentration in blood (n = 65, R^2 = 0.36, P = 0.0001). This correlation was not affected by GSTM1 or GSTT1 polymorphisms. Similar strong correlations were found between individual PCB, between individual metabolites and between individual PCB on the one hand and metabolites on the other.

Correlation of age with other parameters

No relationship between the ages of the volunteers and any of the sperm parameters could be established, but the levels of organochlorines in blood appeared to be strongly positively related to the ages of the men. These relationships were found for most of the individual components, for all metabolites and for the combined PCB levels, combined metabolite levels and total organochlorine levels. As an example, the relationship between age and metabolite levels is shown in Figure 2.

Correlation of organochlorine contents and semen parameters

While examination of the data of the group of 65 volunteers showed no relationship between the organochlorine levels and

Table I. Overview of the composition of the subgroups of men in relation to sperm characteristics

Subgroup	Age (years)	Smoking	Ejaculate volume (ml)	Sperm count ($\times 10^6$/ml)	PMSC ($\times 10^6$/ml)	Overall motility (%)	Morphology (%)[a]	Polymorphism GSTM1 (+/–)	GSTT1 (+/–)
MFS group ($n = 34$)	34.5 ± 3.4	15/16[b]	3.4 ± 1.6	9.3 ± 15.7	1.6 ± 3.6	42.8 ± 24.5	1.6 ± 0.9	11/22[b]	24/7[b]
FFS group ($n = 31$)	36.7 ± 5.4	10/19[b]	3.6 ± 1.6	101.1 ± 51.6[c]	35.4 ± 23.8[c]	67.9 ± 14.2[c]	6.7 ± 3.6[c]	13/18	22/9

All values are mean ± SD.
[a]Morphology evaluation using strict criteria (Enginsu *et al*., 1992).
[b]Sum <34 or <31, due to missing data.
[c]Statistically significantly different ($P < 0.05$).
PMSC = progressively motile sperm concentration; MFS = male factor subfertility; FFS = female factor subfertility.

Table II. Mean (± SD) concentration[a] of the organochlorine compounds in blood and seminal plasma

Component	Blood			Seminal plasma		
	Total ($n = 65$)	MFS group ($n = 34$)	FFS group ($n = 31$)	Total ($n = 10$)	MFS group ($n = 3$)	FFS group ($n = 7$)
HCB	0.11 ± 0.06	0.12 ± 0.07	0.11 ± 0.06	0.018 ± 0.011	0.013 ± 0.014	0.021 ± 0.010
p,p'-DDE	0.26 ± 0.33	0.22 ± 0.22	0.31 ± 0.42	0.015 ± 0.011	0.015 ± 0.014	0.015 ± 0.010
p,p'-DDT	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01			
PCB-118	0.05 ± 0.03	0.05 ± 0.03	0.06 ± 0.03			
PCB-153	0.41 ± 0.22	0.38 ± 0.22	0.44 ± 0.22	0.027 ± 0.016	0.011 ± 0.004	0.034 ± 0.014
PCB-138	0.37 ± 0.24	0.34 ± 0.26	0.41 ± 0.22	0.015 ± 0.017	0.003 ± 0.005	0.021 ± 0.017
PCB-180	0.33 ± 0.16	0.30 ± 0.15	0.37 ± 0.16	0.014 ± 0.012	0.008 ± 0.007	0.016 ± 0.013
Σ PCB	1.17 ± 0.60	1.07 ± 0.63	1.27 ± 0.56	0.056 ± 0.040	0.022 ± 0.013	0.071 ± 0.039
Σ Organochlorines	1.55 ± 0.85	1.41 ± 0.81	1.70 ± 0.88	0.089 ± 0.052	0.050 ± 0.036	0.106 ± 0.050
Metabolites[b]						
a	0.047 ± 0.042	0.040 ± 0.029	0.054 ± 0.052			
b	0.015 ± 0.010	0.014 ± 0.010	0.016 ± 0.011			
c	0.021 ± 0.013	0.020 ± 0.013	0.022 ± 0.013			
d	0.006 ± 0.004	0.005 ± 0.004	0.007 ± 0.005			
e	0.013 ± 0.008	0.012 ± 0.008	0.014 ± 0.008			
f	0.010 ± 0.008	0.010 ± 0.007	0.011 ± 0.010			
g	0.007 ± 0.006	0.007 ± 0.006	0.008 ± 0.006			
h	0.025 ± 0.014	0.023 ± 0.012	0.026 ± 0.015			
Σ metabolites	0.144 ± 0.079	0.132 ± 0.061	0.158 ± 0.095			

[a]Concentrations in ng/g blood, and in ng/ml seminal plasma.
[b]Metabolites a–h: see Figure 1.
HCB = hexachlorobenzene; *p,p*'-DDE = 1,1-dichloro-2-2-bis(p-chlorophenyl)ethane; *p,p*'-DDT = 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethylene; PCB-118 = 2,3′,4,4′,5-pentachlorobiphenyl; PCB-153 = 2,2′,4,4′,5,5′-hexachlorobiphenyl; PCB-138 = 2,2′,3,4,4′,5-hexachlorobiphenyl; PCB-180 = 2,2′,3,4,4′,5,5′-heptachlorobiphenyl.

the semen parameters, sperm count, progressive and overall motility, a significant relationship could be established between individual and combined PCB concentrations and sperm morphology (combined PCB: $n = 36$, $R^2 = 0.15$, $P = 0.02$). The seminal plasma levels of combined PCB also positively correlated with sperm count ($n = 10$, $R^2 = 0.79$, $P = 0.0005$), PMSC ($n = 10$, $R^2 = 0.86$, $P = 0.0001$) and sperm morphology ($n = 9$, $R^2 = 0.40$, $P = 0.05$).

Organochlorine concentrations in blood and in seminal plasma

A significant relationship was found between the combined PCB levels in blood and the corresponding levels in seminal plasma (simple regression, $n = 10$, $R^2 = 0.38$, $P = 0.05$), in which the blood levels appeared to be 20-fold higher than the seminal levels. When total organochlorine levels were compared, the relationship was similar, but not significant.

Comparison of the MFS and FFS groups

Comparison of the subgroups with regard to the levels of both metabolized and unmetabolized organochlorine compounds in blood using the Mann–Whitney *U*-test, revealed no significant differences in levels of individual components nor in combined PCB, metabolite or total organochlorine compounds. The PCB levels in seminal plasma were found to be higher in the samples of the FFS group than in the samples of the MFS-group, but these differences were not significant (Mann–Whitney *U*-test, $n = 10$, $P = 0.06$). The absence of significant differences in organochlorine levels for the two subgroups justifies the idea that the cause of the very poor sperm quality

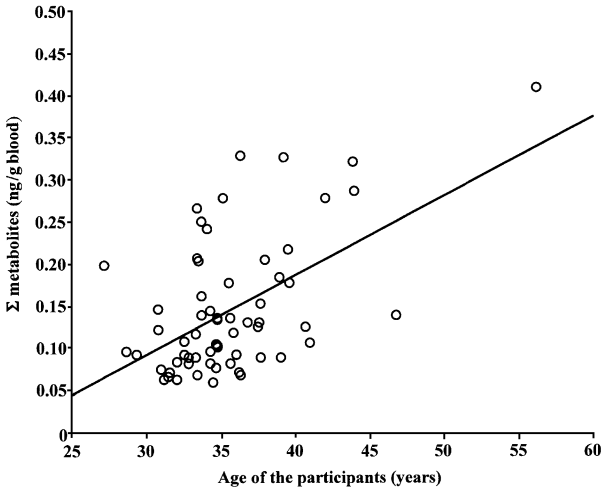

Figure 2. Relationship between organochlorine metabolite levels in blood and age of the participants. $n = 65$, $R^2 = 0.29$, $P = 0.0001$.

in the MFS subgroup has not resulted from exposure to organochlorine compounds, but from other, so far unknown, causes.

Sperm count and PCB metabolite concentrations in the FFS group

Within the FFS group, significant negative correlations between combined PCB metabolites concentration in blood and sperm count ($n = 31$, $R^2 = 0.14$, $P = 0.04$) and between PCB metabolites and PMSC ($n = 31$, $R^2 = 0.17$, $P = 0.02$) could be established (Figure 3). For the MFS group, corresponding (also negative) correlations were not significant ($n = 34$, $R^2 = 0.09$, $P = 0.08$ and $n = 33$, $R^2 = 0.07$, $P = 0.13$ respectively).

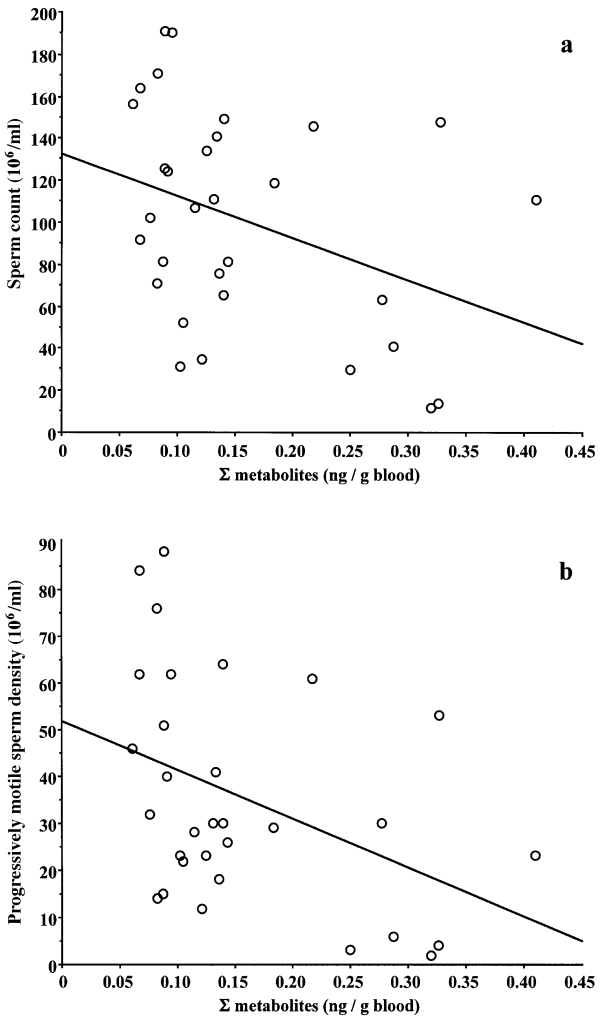

Figure 3. Relationship between sperm count and organochlorine metabolite concentration in blood (**a**) and between progressively motile sperm density and organochlorine metabolite concentration in blood (**b**) of the female factor subfertility subgroup ($n = 31$). $n = 31$, $R^2 = 0.14$, $P = 0.04$; $n = 31$, $R^2 = 0.17$, $P = 0.02$.

Discussion

Comparison of the MFS and FFS groups, composed on the basis of large differences in PMSC, did not reveal significant differences in organochlorine concentrations between these groups. Obviously, the actual causes of the subfertility in the MFS subgroup could be manifold, thus masking a possible effect of the organochlorine compounds on any of the sperm parameters. However, more detailed investigation of the body burden of these compounds, in relation to individual sperm parameters, produced evidence for a significant (positive) correlation between PCB contents and sperm morphology and for a significant negative correlation between PCB metabolite concentration and sperm count and PMSC within the FFS subgroup. So far we do not understand the biological rationale for the observed relationship between PCB contents in blood and sperm morphology.

Perhaps not surprising—although previously unreported—is a positive correlation between organochlorine levels in blood and age. It appears that bioaccumulation in men has not reached an equilibrium at adulthood, but the levels of HCB, individual and combined PCB and metabolites continue to rise with age, whereas for *p,p*'-DDE and *p,p*'-DDT the relationships are less pronounced. Alternatively, the higher concentrations at an older age might be due to a greater exposure of the older persons at times that elevated levels of these compounds occurred. However, although the PCB concentrations in food are declining, this decline is not sharp; exposure to PCB from food is still the most important exposure route for man. From our data it is not possible to distinguish between the two explanations, but the effects would be the same for either of these two possibilities. The higher levels of organochlorine compounds in the FFS group, when compared with the MFS group, can be accounted for with this age–organochlorine relationship: the mean age of the fertile men is ~2 years higher than that of the subfertile men. After correction of PCB levels for age, no difference between the two subgroups remains. The relationships shown in Figure 3 are not influenced by the age–PCB concentration relationship; correction of the metabolite concentrations for age does not lead to different correlations.

The discussion concerning the potential relationship between environmental estrogens and decreasing semen quality is mainly focused on exposure *in utero* (Sharpe and Skakkebaek, 1993; Jensen *et al*., 1995). In our hypothesis, an impact of

environmental endocrine disruptors on spermatogenesis might also occur in adult males. However, in our study group, we found that semen quality parameters, such as sperm count, motility and morphology, appeared to be strongly positively correlated with the seminal plasma levels of organochlorine compounds. Enhanced organochlorine levels in semen without pathological findings when compared to sperm samples with various pathological findings were reported, but no relationship of these levels with sperm count, motility and morphology was found (Ensslen *et al*., 1990). In another study, an inverse correlation between seminal PCB levels and motility was found (Bush *et al*., 1986). Occupational exposure to 1,2-dibromo-3-chloropropane (a pesticide) was shown to be associated with a decreased sperm count (Whorton *et al*., 1977; Glass *et al*., 1979), chlordecone reversibly inhibited spermatogenesis (Guzelian, 1982). Exposure to PCB was related to a non-significantly decreased sperm count (Emmett *et al*., 1988). Recently, adverse effects of exposure to aromatic hydrocarbons on sperm count, motility and morphology were demonstrated (De Celis *et al*., 2000).

In the relatively homogeneous FFS subgroup, we found a strong negative correlation between organochlorine metabolite levels in blood and sperm count. This is the first example of a demonstrable correlation between an environmentally widespread pollutant at a background level and parameters of human semen quality. The fact that, despite strong correlations between the blood levels of organochlorine compounds and metabolites of these compounds, such correlations were not found between the unmetabolized component concentrations and sperm parameters strengthens the idea that the PCB metabolites are the biologically active and thus interesting compounds. It seems that the combination of intake (predominantly from food) and individual metabolism governs the effect of organochlorine compounds on the reproductive performance of men. We could not demonstrate an influence of the GSTT1 and GSTM1 polymorphisms, but polymorphisms for other PCB metabolizing enzymes, such as CYP1A1 and CYP1A2, may be involved. To elucidate the role of their and other rather low frequency polymorphisms for PCB bioactivation and/or biodetoxification, much larger population studies are required. From the data presented, it appears that sperm count and progressive motility are the sperm parameters most affected by the organochlorine metabolite levels, since effects on overall motility and morphology could not be detected in the present dataset (FFS group). Whether such effects on sperm count and PMSC of organochlorine metabolites may affect the occurrence of pregnancies by natural conception remains to be elucidated.

Acknowledgement

The authors wish to thank Prof. Åke Bergman from Stockholm University for his kind gift of 4-hydroxy-2,3,3′,4′,5-pentachloro biphenyl.

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Submitted on April 17, 2001; resubmitted on December 14, 2001; accepted on April 19, 2002