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Exposure to PCB and *p,p'*-DDE in European and Inuit populations: impact on human sperm chromatin integrity

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BACKGROUND: Persistent organochlorine pollutants (POP), such as polychlorinated biphenyls (PCB) and dichlorodiphenyldichloroethylene (*p,p'*-DDE), are widely found in the environment and considered potential endocrine-disrupting compounds (EDC). Their impact on male fertility is still unknown. METHODS: To explore the hypothesis that POP is associated with altered sperm chromatin integrity, a cross-sectional study involving 707 adult males (193 Inuits from Greenland, 178 Swedish fishermen, 141 men from Warsaw, Poland, and 195 men from Kharkiv, Ukraine) was carried out. Serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153), as a proxy of the total PCB burden, and of *p,p'*-DDE were determined. Sperm chromatin structure assay (SCSA) was used to assess sperm DNA/chromatin integrity. RESULTS: We found a strong and monotonically increasing DNA fragmentation index with increasing serum levels of CB-153 among European but not Inuit men, reaching a 60% higher average level in the highest exposure group. No significant associations were found between SCSA-derived parameters and *p,p'*-DDE serum concentrations. CONCLUSION: These results suggest that human dietary PCB exposure might have a negative impact on the sperm chromatin integrity of adult males but additional issues, including differences in the genetic background and lifestyle habits, still need to be elucidated.

Key words: DDT/DNA damage/polychlorinated biphenyls (PCB)/SCSA/sperm chromatin

Introduction

Polychlorinated biphenyls (PCB) and dichlorodiphenyldichloroethylene (*p,p'*-DDE, a metabolite of dichlorodiphenyltrichloroethane, DDT), are lipophilic persistent organochlorine pollutants (POP) of anthropogenic origin which are ubiquitous in the environment (Breivik *et al.*, 2002; Turusov *et al.*, 2002; Lintelmann *et al.*, 2003). These compounds bioaccumulate and are concentrated by up to a thousand times compared with background levels, particularly in high rank predators of the aquatic food chain, man included (Longnecker *et al.*, 1997; Smith, 1999). Even where their use has been restricted or banned some decades ago, POP are still detected in human blood, fat tissue and breast milk worldwide. The problem seems particularly serious in the Arctic regions, posing a potential health risk for the local populations (Hansen, 2000; Bonefeld-Jørgensen, 2004; Deutch *et al.*, 2004; Johansen *et al.*, 2004; Van Oostdam *et al.*, 2004).

Theoretically, 209 PCB congeners, varying in their stability and toxicity, can be formed, but considerably fewer are detected in biological samples. The most common PCB congener, 2,2′,4,4′,5,5′-hexachlorobiphenyl (CB-153), is considered a useful marker of body burden of PCB (Ayotte *et al.*, 2003; Hauser *et al.*, 2003a,b; Richthoff *et al.*, 2003; Axmon *et al.*, 2004; Rignell-Hydbom *et al.*, 2004, 2005), because it correlates very well with the overall total PCB concentration (Grimvall *et al.*, 1997; Glynn *et al.*, 2000). Moreover, among 44 American Vietnam veterans, the CB-153 concentration has been shown to be well correlated with the 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) equivalent (TEQ) and the total PCB-derived TEQ (Gladen *et al.*, 1999). The major DDT metabolite *p,p'*-DDE is likewise a good indicator of the exposure to DDT (Hauser *et al.*, 2003b).

Several PCB congeners and DDT (together with *p,p'*-DDE) have weak agonistic- or antagonistic effects on several hormonal systems (Kelce *et al.*, 1998; Bonefeld-Jorgensen *et al.*, 2001; Portigal *et al.*, 2002; Schrader and Cooke, 2003; Scippo *et al.*, 2004; Pocar *et al.*, 2005). Studies on wildlife and laboratory animals show that exposure to PCB and *p,p'*-DDE can adversely affect reproductive and endocrine functions (reviewed by Rosselli *et al.*, 2000; Faroon *et al.*, 2001; Guillette and Gunderson, 2001; Gray *et al.*, 2001; Norgil Damgaard *et al.*, 2002; Skaare *et al.*, 2002; Teilmann *et al.*, 2002; Toppari,

2002; Safe, 2004; Sharpe and Irvine, 2004; Toft *et al.*, 2004). Human epidemiological data are limited and major gaps in knowledge continue to preclude evidence-based risk assessment. Studies have shown that accidentally high simultaneous exposures to PCB and polychlorinated dibenzofurans have a negative effect on male reproductive function (Guo *et al.*, 2000; Hsu *et al.*, 2003). Lower serum concentrations of PCB, in the range of current levels for most European populations, have been associated with effects on semen characteristics, mainly sperm motility (Bush *et al.*, 1986; Dallinga *et al.*, 2002; Hauser *et al.*, 2003a, 2005; Richthoff *et al.*, 2003, Rignell-Hydbom *et al.*, 2004).

There are limited and contradictory epidemiological data on whether PCB, and possibly DDT/DDE, can damage human sperm DNA (Rozati *et al.*, 2002; Hauser *et al.*, 2003b; Rignell-Hydbom *et al.*, 2005). Reasons for this inconsistency might be different methodologies used to detect sperm DNA damage, varying exposure ranges to POP and their mixtures, and different inclusion criteria for the studies. These results are nevertheless of concern as sperm DNA integrity is essential for the accurate transmission of genetic information (reviewed in Evenson *et al.*, 2002; Agarwal and Said, 2003; Perreault *et al.*, 2003; Sakkas *et al.*, 2003a). Therefore, there is a need to investigate possible adverse effects of PCB and *p,p'*-DDE on human sperm genetic integrity in a sufficiently large study population with an adequate exposure contrast.

Sperm DNA integrity can effectively be evaluated by the sperm chromatin structure assay (SCSA) (Evenson *et al.*, 2002; Perreault *et al.*, 2003), which measures, by flow cytometry, the susceptibility of sperm nuclear DNA to denaturation *in situ* followed by Acridine Orange staining (Evenson *et al.*, 1980). It is an indirect indicator of DNA damage because it measures the amount of single-stranded DNA after treatments that normally do not denature sperm DNA. SCSA can detect two independent abnormal traits of the mature male gamete, i.e. sperm with altered chromatin condensation, which is indicated by high DNA stainability (HDS), and sperm with increased DNA damage, evidenced by the DNA fragmentation index (DFI). Increased fractions of sperm with abnormal chromatin, expressed in terms of DFI and HDS, have been associated with decreased natural fecundability (Evenson *et al.*, 1999; Spanò *et al.*, 2000) and reduced assisted pregnancy rates (Bungum *et al.*, 2004; Virro *et al.*, 2004).

In this study, cohorts were established including three European populations from Sweden, Poland and Ukraine, with regionally different levels of exposure to both PCB and *p,p'*-DDE, together with an Inuit population from Greenland. Characteristics and preliminary results for the Swedish cohort have been described elsewhere (Rignell-Hydbom *et al.*, 2004, 2005). The aim of this study was to investigate whether serum levels of CB-153 and *p,p'*-DDE were associated with sperm chromatin damage assessed by SCSA.

Materials and methods

Subjects

Semen and blood samples were collected between May 2002 and February 2004 in Greenlandic Inuits, Swedish fishermen, and men from the general population in Warsaw, Poland and Kharkiv, Ukraine. In all regions, except Sweden, consecutive pregnant women were approached at the entry point for the study and their male spouses were enrolled in the male study. In all countries it was required that both the man and his partner should be >18 years of age and that they should both be born in the country of the study.

The eligible males were asked to fill in a questionnaire regarding lifestyle, occupational and reproductive history. All participants were encouraged to deliver a blood sample and the men were consecutively enrolled into the semen study with the objective of recruiting some 200 men in each region.

In Greenland, the eligible target population constituted 665 couples, of which 598 (90%) were enrolled in the study. In all, 256 male partners were asked to participate in the semen study. Thirty-five men did not want to participate and 20 dropped out (18 could not be reached and two did not attend after two reminders) giving a total of 201 semen samples collected. Due to limited amounts of semen, samples from only 193 of the 201 participants could be used for SCSA.

In all, 2614 Swedish fishermen were informed about the study and 266 gave their written informed consent to participate, but 71 subjects had to be excluded during the sampling period due to logistical reasons, change of mind, sickness or recent vasectomy during the field study period (Rignell-Hydbom *et al.*, 2004). Due to limited amounts of semen, samples from only 178 of the 195 participants could be used for SCSA (Rignell-Hydbom *et al.*, 2005). Eighty per cent of the remaining 178 fishermen had fathered at least one child.

In Poland, altogether 690 spouses of pregnant women, who either visited the obstetric outpatient clinic of the Gynecological and Obstetric Hospital of theWarsaw School of Medicine, Poland, or physicians at a collaborating hospital in the same city, were informed about the project and asked to participate. A total of 472 couples (68%) was enrolled. Of these, 141 men donated blood and semen samples and were interviewed from September 2002 to March 2003.

In Kharkiv, Ukraine, 2478 spouses of pregnant women, who visited one of eight antenatal clinics or three maternity hospitals in Kharkiv, were informed about the project and asked to participate in the semen study. From the eligible couples, 640 (26%) were enrolled in the study. Of those, 195 men donated both a semen sample and a blood sample and were interviewed from April 2003 to December 2004.

In total, 707 adult males (193 Inuits from Greenland, 178 from Sweden, 141 from Warsaw, Poland, and 195 from Kharkiv, Ukraine), with complete SCSA results and lipid-adjusted POP serum concentrations, were included in this study, representing participation rates of 79% for Greenland, 10% for Sweden, and 30% for Warsaw and Kharkiv. The overall participation rate in this project was 18%.

The study was approved by the local ethical committees representing all participating populations and all subjects signed an informed consent.

Collection of semen and blood samples

Semen samples were collected by masturbation either at the participant's residence (Sweden) or in privacy in a room at the hospital (other regions). The subjects were asked to abstain from sexual activities for ≥2 days before collecting the sample, if possible, and to note the actual abstinence time. The sample was kept close to the body to maintain a temperature close to 37°C when transported to the laboratory immediately after collection. Two Nunc cryotubes (VWR International, Roskilde, Denmark) with 0.2 ml aliquots of undiluted raw semen, collected 30 min after liquefaction, were prepared from each semen sample, coded and directly transferred into a −80°C freezer. In addition the samples were analysed for motility and concentration according to the World Health Organization

(1999). The results of the conventional semen analysis will be the subject of another paper.

Venous blood samples were collected within 1 week of the semen collection, except for a subgroup of 116 Greenlandic samples, which were collected up to 1 year in advance. The blood samples were centrifuged immediately after collection and sera were stored at −80°C for later analysis.

Sperm chromatin structure assay

The coded frozen semen samples were shipped on dry ice to the flow cytometry (FCM) facility of the Section of Toxicology and Biomedical Sciences (ENEA Casaccia, Rome, Italy), for SCSA analysis. On the day of analysis, the samples were quickly thawed in a 37°C water bath and analysed immediately. The SCSA was applied following the procedure described elsewhere (Spanò *et al.*, 2000; Evenson *et al.*, 2002). Briefly, 1–2 $\times 10^6$ cells were treated with an acid–detergent solution (0.08 N HCl, 0.15 mol/l NaCl, 0.1% Triton-X 100; pH 1.2) for 30 s and then stained with 6 mg/l of purified Acridine Orange (AO; Molecular Probes, Eugene, OR, USA) in a phosphate-citrate buffer, pH 6.0. Cells were analysed by a FACScan (Becton Dickinson, San José, USA) equipped with an air-cooled argon ion laser and standard optical filters to collect green and red fluorescence. Measurements began 3 min after AO staining and stopped when a total of 10 000 events had been accumulated for each sample, keeping the flow rate at ~200 cells/s. Off-line analysis of the flow cytometric data was carried out by using dedicated software (SCSASoft; SCSA Diagnostics, Brookings, SD, USA). The percentage of abnormal sperm with detectable DFI (%DFI) was calculated from the DFI frequency histogram obtained from the ratio between the red and total (red plus green) fluorescence intensity (Evenson *et al.*, 2002). High DNA stainability (%HDS) was calculated based on the percentage of sperm with high levels of green fluorescence (Evenson *et al.*, 2002).

For the flow cytometer set-up and calibrations, a reference semen sample retrieved from our laboratory repository was used. Calibration aliquots were thawed and measured at each start-up of the flow cytometer and after every 10 samples, to ensure standardization and stability of the instrument from sample to sample and from day to day. Inter-day SCSA variability, evaluated after 216 flow sessions and considering the coefficients of variation (CV) of the %DFI and %HDS, was 6.0 and 4.8% respectively. In addition, 358 randomly chosen samples (50.6% of the total) were measured twice in independent FCM sessions. Results from the two measurements were highly correlated (DFI, $r = 0.96$; HDS, $r = 0.97$).

Finally, the reliability and stability of SCSA measurements has also been challenged by an external quality control study that we carried out within the framework of this project, based on the blind analysis of three aliquots from seven different donors not selected among the participants in this study. The median inter-sample variability for %DFI, expressed as CV, was 1.5%.

Determination of CB-153 and p,p'-*DDE*

All samples were analysed at the department of Occupational and Environmental Medicine in Lund, Sweden. CB-153 and *p,p*'-DDE were extracted from serum by solid phase extraction using on-column degradation of the lipids and analysis by gas chromatography mass spectrometry as previously described (Richthoff *et al.*, 2003; Rignell-Hydbom *et al.*, 2004, 2005). The relative SD, calculated from samples analysed in duplicate on different days, was 18% at 0.1 ng/ml ($n = 990$), 10% at 0.5 ng/ml ($n = 990$) and 10% at 2 ng/ml ($n = 990$) for CB-153 and 11% at 1 ng/ml ($n = 1058$), 8% at 3 ng/ml ($n = 1058$) and 7% at 8 ng/ml ($n = 1058$) for *p,p*'-DDE. The detection 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 were part of the Round Robin inter-comparison program (Prof. Hans Drexler, Institute and Out-Patient Clinic for Occupational, Social and Environmental Medicine, University of Erlangen-Nuremberg, Germany) with analysis results within the tolerance limits.

Determination of 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 CV for cholesterol and triglyceride determinations were 1.5–2.0%. The average molecular weight of triglycerides was 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. The total lipid concentration in serum (g/l) was calculated by the following equation (Rylander *et al.*, 2005):

$$\text{Total} = 0.96 + 1.28*(\text{triglycerides} + \text{cholesterols}).$$

Statistical analysis

We analysed sperm chromatin anomalies, mirrored by the SCSA variables %DFI and %HDS, as a function of current lipid-adjusted serum levels of CB-153 and *p,p*'-DDE respectively. In one set of analyses we used general linear models to analyse endpoints measured on a continuous scale (SAS GLM); in a second set of analyses the endpoints were dichotomized and analysed by logistic regression (SAS Logistic). Initially, all analyses were stratified by the four study groups (four regions corresponding to Greenland, Sweden, Warsaw and Kharkiv). If the risk estimates were not heterogeneous across regions, we estimated the strength of the associations based upon data aggregated from several regions. Statistical tests for heterogeneity of risk included Cochrane–Mantel–Haenzel test for 2×2 tables and significance test for interaction terms in multiple linear regression models.

For descriptive purposes the participants were divided into ten groups of roughly same size defined by the deciles of serum levels of CB-153 in each region—altogether 40 groups. Each of these groups was assigned the decile-specific median values of the endpoints, %DFI and %HDS. Scatter plots of the median values of endpoints by median values of CB-153 provided an overview of associations and supported the evaluation of heterogeneity of associations between regions. The corresponding linear regressions of the median values of %DFI on the median values of CB-153 weighted by the number of subjects in each decile were also performed. The CB-153 exposure measurement errors are of the 'classical type' leading to attenuation of regression coefficients, whereas measurement errors related to the aggregated exposure data are of the 'Berkson type' that are not associated with bias of the risk estimates (de Klerk *et al.*, 1989; Armstrong, 1998).

Subsequent analyses focused upon exposure–response relations and identification of possible thresholds of exposure below or above which effects were diminished. In order to accomplish this objective, participants were divided into five arbitrary groups by serum CB-153 levels that represented a reasonable trade-off between exposure contrast and group size (0–50, 51–100, 101–200, 201–400 and >401 ng/g lipid). As far as the *p,p*'-DDE analysis is concerned, participants were also divided into five arbitrary groups by serum *p,p*'-DDE levels (0–250, 251–500, 501–1000, 1001–1500 and >1501 ng/g lipid). Taking advantage of the properties of the logarithmic function, we used log transformation of the %DFI and %HDS to compute the percentage change in the endpoint at a given exposure level relative to the reference level (0–50 ng/g CB-153). The transformation of endpoints was also performed in order to equalize the variance of the skewed distributions

of the %DFI and %HDS. Test for equal group variances in a simplified one-way design model was performed with Levene's test for homogeneity of group variances (PROC GML SAS). After transforming the endpoints %DFI and %HDS by the natural logarithm, group variances were usually homogeneous.

We also regressed outcome variables on continuous exposure variables to examine exposure–response relations whenever the data indicated a trend in outcome variables across exposure categories. For analyses based upon continuous exposure variables, the logarithm of the measured CB-153 values was used because of the lognormal nature of the exposure distribution (Boleij *et al.*, 1995). The fit of the final multiple regression models was evaluated by plots of residuals by predicted values.

In addition to analyses of outcomes on the continuous scale, we dichotomized the %DFI values into high and low levels by a threshold value corresponding to the 75th percentile in the entire dataset (17.2%)—approximately the level above which %DFI is strongly related to reduced fecundability in the general population (Spanò *et al.*, 2000) and approximately the level when the male fertility potential shifts from excellent to good–fair, according to the SCSA categories proposed by Evenson *et al.* (2002). Using logistic regression, we modelled the risk for high level of %DFI according to the current serum concentration of CB-153 (groups and log-transformed continuous value). Prevalence odds ratios (OR) and 95% confidence intervals (95% CI) were calculated by standard methods (Hosmer and Lemeshow, 1989).

For the confounder analysis we applied a two-step analytic strategy. We first identified the few determinants of sperm chromatin anomalies that are well established in the literature, namely, period of sexual abstinence and age (Spanò *et al.*, 1998; Evenson *et al.*, 2000; Richthoff *et al.*, 2002; Giwercman *et al.*, 2003). The stratification variables (region, analyses of the aggregated data only), age and sexual continence were included in the models regardless of changes in risk estimates or significance levels [four regions corresponding to Greenland, Sweden, Warsaw and Kharkiv, age (as a continuous variable or categorized as <30, 30–45 and >45 years) and period of sexual abstinence prior to delivery of the semen sample (as logarithmically transformed continuous variable or categorized into 0–2, >2–4, >4–6, >6 days)]. Next, several suspected determinants of sperm chromatin anomalies were listed *a priori* and these variables were included in the final models according to the change-in-estimate method (Greenland, 1989; Maldonado and Greenland, 1993). Variables were included into the basic model one by one and were only kept for further analysis if the risk estimate was changed ≥10%. This subset of potential confounders was added to the model stepwise ordered according to effect on the risk estimate, but were only kept in the model if the risk estimate changed by ≥5%. The potential confounders included: season for sample collection (winter, spring or summer; yes/no); fever past 3 months (yes/no), spillage during semen sample collection (yes/no), current smoking status (yes/no), alcoholic beverage consumption (>21 drinks/week; yes/no), and self-reported urogenital infections (e.g. epididymitis, chlamydia, gonorrhoea, or mumps in adulthood (yes/no), and urogenital surgery (e.g. treatment for cryptorchidism, torsio testis, cancer testis, or varicose scrotal veins; yes/no).

Since the CB-153 and *p,p'*-DDE serum levels, especially in Inuits and Swedish fishermen, were highly correlated (r = 0.93 and 0.79 respectively), it proved unfeasible to keep both variables in the model in order to distinguish effects of these compounds. Lower correlation between CB-153 and *p,p'*-DDE among men from Warsaw (r = 0.26) and Kharkiv (r = 0.45) enabled models including both variables for these two study groups.

All analyses were carried out by SAS software (SAS, version 9.1; SAS Institute Inc., Cary, NC, USA).

Results

The characteristics of the 707 adult males with complete SCSA results and lipid-adjusted POP serum concentrations are shown in Table I. The median (range) concentrations of CB-153 and *p,p'*-DDE in the entire group were 88 ng/g lipid (3–5500) and 560 ng/g lipid (6–13 000) respectively.

From Table I, as far as the SCSA parameters are concerned, it can be seen that the arithmetic mean of %DFI for all the 707 men was 13.2% with a SE of 0.4%, and the median value was 10.0%. The unadjusted %DFI geometric mean values differed highly significantly between the four study groups. Following adjustment for age, period of sexual abstinence and serum CB-153 concentration, the three European groups of men did not differ mutually in their %DFI levels but all were significantly higher than the level found in Inuit men. The adjusted geometric mean values (SE) were 13.5% (1.06) in Swedish fishermen, 12.7% (1.06) in men from Kharkiv, 11.5% (1.07) in men from Warsaw and, finally, 8.1% (1.07) in Inuits. Contrary to %DFI, the adjusted %HDS did not differ between study groups with the lowest value for Warsaw men and the highest for Inuit men. The adjusted geometric mean %HDS values (SE) were 10.09% (1.05) in Inuit men, 9.1% (1.05) in Swedish fishermen, 9.3% (1.05) in men from Kharkiv, and 8.5% (1.06) in men from Warsaw.

There was no correlation between the SCSA variables %DFI and %HDS (analysis stratified by region: Spearman's rank order coefficient r = 0.047, P = 0.22).

Spearman non-parametric correlation was also used to determine associations between conventional semen characteristics and SCSA parameters in all samples. Consistent with our previous reports (Spanò *et al.*, 1998, 2000; Richthoff *et al.*, 2002; Giwercman *et al.*, 2003) and other studies carried out on the general population (Evenson *et al.*, 1991, 1999), the correlation analysis confirmed that the SCSA data are either unrelated or poorly associated with the parameters of the conventional semen quality assessment. Spearman correlation coefficients were 0.02 (P = 0.55), –0.17 (P < 0.001), and –0.23 (P < 0.001) for %DFI versus sperm concentration, percentage morphologically normal forms and percentage motile (A+B) sperm respectively. For %HDS, the corresponding correlation coefficients were –0.36 (P < 0.001), –0.46 (P < 0.001), and –0.32 (P < 0.001), versus sperm concentration, morphology and motility respectively.

A scatter plot of the median %DFI values by the corresponding median CB-153 values in each decile calculated for study group showed a strong heterogeneity of associations (Figure 1A). Whereas %DFI values were unrelated to CB-153 among the Inuit men (crude median based linear regression coefficient r = 0.008, P = 0.85) and men from Warsaw (crude linear regression coefficient r = 0.071, P = 0.44), increasing CB-153 serum levels were associated with increasing %DFI values in the Swedish cohort (crude linear regression coefficient r = 0.372, P = 0.001), among men from Kharkiv (crude linear regression coefficient r = 0.283, P = 0.027) and in the three groups of European men combined (linear regression coefficient r = 0.208, P < 0.0001, test for heterogeneity of slopes P = 0.43). Accordingly, all subsequent analyses of %DFI were stratified on Inuit and European men because of the

Table I. Characteristics of the study populations

	Inuit men (n = 193)	Swedish fishermen (n = 178)	Warsaw men (n = 141)	Kharkiv men (n = 195)	All (n = 707)
CB-153 (ng/g lipid)[a]	350, 220 (16–5500)	250, 190 (40–1500)	22, 19 (3–130)	56, 47 (6–560)	180, 88 (3–5500)
p,*p*'-DDE (ng/g lipid)[a]	890, 600 (6 –13 000)	340, 240 (40–2250)	570, 490 (200–2000)	1300, 990 (320 –12 000)	790, 560 (6–13 000)
Age (years)[a]	31.1, 30.6 (18.5–51.3)	47.1, 47.8 (23.8–67.5)	30.3, 29.5 (20.4–46.3)	26.6, 25.1 (18.0–45.3)	33.7, 31.0 (18.0–67.5)
Body mass index (kg/m^2)[a]	26.1, 25.6 (11.5–57.8)	26.8, 26.5 (20.8–37.2)	25.8, 25.3 (18.6–36.3)	24.2, 24.0 (17.7–32.1)	25.7, 25.3 (11.5–57.8)
Current smokers (%)	71.5	23.0	29.1	65.6	49.0
>20 alcoholic drinks a week (%)	10.9	NA	NA	2.8	3.5
Testicular disorders[b] (%)	0.5	1.7	3.1	2.1	1.9
Genital infection[c], %	82.9	19.7	5.1	6.3	30.2
Total sperm count ($\times 10^6$)[a]	245, 186 (9–1496)	191, 145 (7–904)	394, 227 (12–2071)	250, 174 (2–1442)	262, 181 (2–2071)
Sperm concentration ($\times 10^6$/ml)[a]	72, 53 (3–374)	59, 49 (4–215)	94, 65 (5–419)	74, 59 (1–320)	74, 54 (1–419)
Oligospermia rate ($<20\times 10^6$/ml) (%)	13.0	15.7	14.4	16.3	14.7
Period of abstinence (days)[a]	5.4, 3.0 (0.5–240)	3.8, 3.0 (0.5–21)	6.5, 4.0 (1–60)	3.9, 3.0 (1–11)	4.7, 3.0 (0.5–240)
Spillage during semen collection (%)	10.4	15.2	5.7	18.0	12.7
Fever in the last 3 months (%)	13.5	1.7	7.8	9.7	8.3
Season for semen sampling (%)[d]					
Winter	0	0	45.4	29.2	17.1
Spring	32.1	35.4	14.2	26.2	27.7
Summer	53.9	34.8	13.5	22.1	32.2
Autumn	14.0	29.8	27.0	22.6	22.9
SCSA DFI(%)[a]	9.0, 7.4 (1.3–37.8)	18.6, 14.9 (2.3–88.2)	12.2, 9.6 (2.9–49.4)	13.3, 10.5 (2.4–49.6)	13.2, 10.0 (1.3–88.2)
SCSA HDS(%)[a]	13.5, 11.3 (2.4–62.3)	10.3, 8.2 (1.6–42.8)	9.9, 8.3 (2.3–32.4)	12.0, 10.3 (2.3–46.8)	11.6, 9.4 (1.6–62.3)

[a]Values are mean, median (range).
[b]Testicular disorders: treatment for retracted testes, surgery for varicose veins, torsio testis or testis cancer.
[c]Genital infections: epididymitis, gonorrhoea, chlamydia, mumps in adulthood.
[d]Winter: December, January, February; Spring: March, April, May; Summer: June, July, August; Autumn: September, October, November.
SCSA = sperm chromatin structure assay; %DFI = percentage DNA fragmentation index; %HDS = percentage high DNA stainability; NA = not available.

heterogeneity between Inuits and Europeans and homogeneity within the three European cohorts with respect to levels of %DFI and the slopes of the regression of %DFI on CB-153. On the contrary, the corresponding scatter plot for %HDS did not reveal any association with CB-153 in any region (Figure 1B).

The distribution of the crude median values of %DFI and %HDS according to grouped CB-153 serum levels and study group is displayed in Table II. We observed no association between serum CB-153 level and %HDS in any study group or in the aggregated data. On the other hand, consistent with the scatter plot in Figure 1, %DFI increased with increasing level of CB-153 in Swedish fishermen and men from Kharkiv but such relationships did not emerge among men from Warsaw or Greenland, the latter in spite of a wide and balanced span in exposure levels. The statistical analysis revealed a strong and monotonically increasing %DFI with increasing serum levels of CB-153 among European men reaching a 60% higher average level in the highest exposure group (Table III). The data revealed no indication of a threshold below which no effects could be revealed since even men with CB-153 belonging to the exposure range of 51–100 ng/g lipid had a significantly higher percentage of sperm cells with abnormal chromatin compared to the reference group (Table III). The outcome measures were adjusted for effects of study group, age and period of sexual abstinence but none of a variety of other potential confounders qualified for inclusion in the final models. Contrary to the consistent findings in European men, we found no association between CB-153 and %DFI among Inuit men (Table III and IV)—even though this cohort had the highest median serum concentrations and the widest exposure contrast for CB-153.

Results of analyses of risk of DFI levels above the 75th percentile of the DFI distribution (17.2%) according to CB-153 serum level are shown in Table IV. Consistent with the results of the multiple regression analyses, we observed an increasing risk with increasing level of CB-153 across all exposure ranges among European men, although only the risk in the highest exposed group reached statistical significance in comparison with the lowest exposed group. The proportion of Inuit men with %DFI ≥17% was also elevated, but the confidence intervals were broad and the results did not reach statistical significance.

We found no evidence that %DFI and %HDS was related to level of *p*,*p*'-DDE in serum in any of the study sites in spite of high exposure levels and wide contrasts among men, in particular in Kharkiv and Warsaw (Table V). This also applied to logistic regression of the risk of HDS levels below the 25 percentile (7%) according to *p*,*p*'-DDE serum concentration. In conclusion, no risk was related to levels of *p*,*p*'-DDE in any study group or in the entire dataset (data not shown).

Furthermore, for the two study groups with low to moderate correlation of serum CB-153 and *p*,*p*'-DDE, the inclusion of *p*,*p*'-DDE into the regression model did not weaken the significant effect of CB-153 on %DFI levels (regression coefficient without *p*,*p*'-DDE 0.156, $P = 0.003$ and with *p*,*p*'-DDE 0.163, $P = 0.001$).

Discussion

The main result of the study was the positive and significant associations between %DFI and CB-153 in the Swedish and Ukrainian cohorts, while no association emerged for the Polish and Greenlandic populations. The lack of association in men

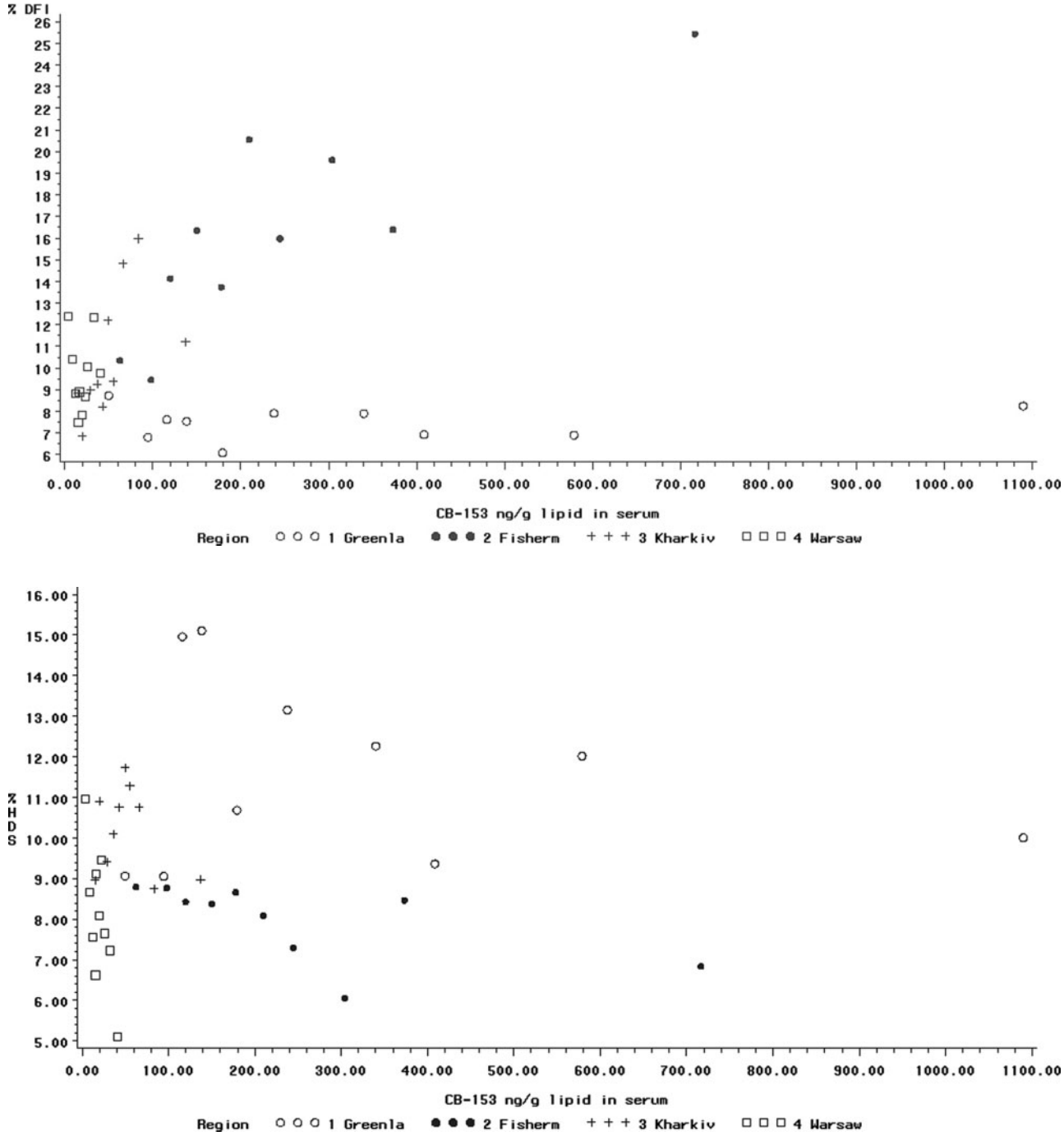

Figure 1. Scatter plot relating sperm chromatin structure assay (SCSA) characteristics and CB-153 serum concentration (ng/g lipid) stratified by region. (**A**) The relationship for the SCSA parameter percentage DNA fragmentation index (%DFI); (**B**) the relationship for the SCSA parameter percentage high DNA stainability (%HDS). For sake of clarity, instead of showing all the 707 data, men in each region were divided into 10 equally sized groups according to their CB-153 values. In each of these 10 groups, the median CB-153 value and the corresponding median %DFI and %HDS values were computed. The resulting 10 pairs of values from each region have been plotted in the scatter (10 points for each region). Therefore, the scatter plot reports the median values of SCSA parameters %DFI (**A**) and %HDS (**B**) as a function of the median CB-153 values, for each CB-153 decile obtained from the four study groups.

from Warsaw might be due to the low exposure levels and very limited exposure contrast within this population. However, by considering all 514 European men, an increasing risk with increasing level of CB-153 across all exposure ranges emerged, reaching statistical significance in the highest exposed group (> 401 ng/g lipid), a concentration range never explored before as far as its relationship with sperm genetic integrity is concerned.

It is more difficult to understand why no association was found in Greenland where high exposure levels and high exposure contrasts were obtained. Actually, the %DFI levels were quite low in Inuit men compared to the European men. Perhaps

Table II. Crude SCSA %DFI (upper panel) and %HDS (lower panel) according to serum CB-153 concentration and study groups

CB-153 (ng/g lipid) in serum	Inuit men			European men[1]			Swedish fishermen			Warsaw men			Kharkiv men		
Quintiles	N	%DFI median	Range[2]	N	%DFI median	Range	N	%DFI median	Range	N	%DFI median	Range	N	%DFI median	Range
0–50	9	8.7	9	251	9.1	47	5	11.1	10	137	9.5	47	109	8.9	47
51–100	25	8.4	8	92	11.4	41	22	9.1	38	3	9.7	12	67	13.4	67
101–200	56	7.1	7	85	14.4	59	66	14.6	59	1	10.8	0	18	12.4	18
201–400	53	7.0	7	64	16.7	84	64	16.7	84	0	–	–	0	–	–
>401	50	7.3	7	22	21.7	55	21	22.6	55	0	–	–	1	11.0	0
CB-153 (ng/g lipid) in serum	Inuit men			All men			Swedish fishermen			Warsaw men			Kharkiv men		
Quintiles	N	%HDS median	Range	N	%HDS median	Range	N	%HDS median	Range	N	%HDS median	Range	N	%HDS median	Range
0–50	9	8.2	17	260	9.1	42	5	8.4	14	137	8.3	30	109	10.5	41
51–100	25	14.1	42	117	10.5	45	22	9.8	28	3	12.0	22	67	10.4	42
101–200	56	13.3	29	141	10.3	32	66	8.1	28	1	3.2	0	18	9.0	24
201–400	53	10.5	60	117	8.4	61	64	7.5	41	0	–	–	0	–	–
>401	50	10.6	29	72	10.4	29	21	6.8	24	0	–	–	1	11.3	0

[1]Inuit men were not included in the pooled %DFI analyses (upper panel) because of heterogeneity of associations.
[2]Range is the arithmetical difference between the highest and the lowest value.

Inuit men are less susceptible to toxic effects of PCB because of genetic differences compared to other European populations, but little is known about possible differences in gene polymorphisms between Caucasians (Europeans) and populations of Asian origin (Inuits) for genes involved in metabolism of POP (Miyoshi and Noguchi, 2003). This issue needs to be investigated and experiments are in progress to study also whether the androgen receptor (AR) gene polymorphism is involved in such differences (Y.Giwercman, personal communication). We have measured the serum concentration of CB-153 as a proxy of the whole burden of PCB and differences in the actual composition of the PCB mixture between the populations cannot be excluded. Different PCB congeners and other POP can be grouped as potentially estrogenic, anti-estrogenic, androgenic, anti-androgenic, and dioxin-like, varying in their biological and toxicological properties. Characterization of the xenobiotic hormone-like activity in serum cleared for endogenous steroid hormones and the integrated serum dioxin activity is in progress in a subgroup of these samples (E.C.Bonefeld-Jørgensen, personal communication), which can provide a deeper understanding of country-specific variations in exposure. For example, a significantly higher ratio of CB-153 (non-coplanar, non-dioxin-like PCB) to co-planar PCB (dioxin-like) was observed for Canadian Inuits compared to Canadian Caucasians (Dewailly *et al.*, 1994) indicating that some caution must be taken for use of CB-153 as a marker globally. Among other factors to be investigated remain possible protective effects of sperm chromatin integrity from substances other than POP associated with Inuit diet and lifestyle. For example, it is known that Inuits' intake of selenium and unsaturated fatty acids (*n*-3 UFA), including decosahexaenoic acid (DHA) is much higher (up to 10 times) than that of Caucasians (Deutch, 2003; Van Oostdam and Tremblay, 2003; Deutch *et al.*, 2004; Hansen *et al.*, 2001). It has also been shown that a diet rich in *n*-3 UFA can improve the motion characteristics of cool-stored stallion semen (Brinsko *et al.*, 2005). Although contradictory information is also available for humans (Hansen and Deguchi, 1996), selenium (and vitamin E) supplementation has been shown to improve human sperm motility and morphology (Wong *et al.*, 2000). Selenium deficiency can lead to low sperm production and quality in animals (Beckett and Arthur, 2005), whereas patients with low selenium intake showed sperm quality and fertility improved after selenium supplementation (Beckett and Arthur, 2005). Therefore, we can speculate that POP-associated diet factors found in Inuits' food, such as selenium and *n*-3 UFA, might have a neutralizing, protective effect on sperm chromatin damage. Whether the higher intake of selenium and *n*-3 UFA, or other unknown factors, might also contribute to explain the relatively low level of chromatin-defective sperm in Inuits clearly deserves further research.

The participation rate differed between the study groups and was exceptionally high in Greenland. Although the participation rates were lower in the three European regions, though at levels typical of many other semen studies (Bonde *et al.*, 1996), selection bias is an unlikely explanation of the findings since delivery of a semen sample was not related to fertility measured as time taken to conceive (G.Toft, personal communication). Moreover, the clear exposure response patterns in Swedish fishermen and in men from Kharkiv are unlikely to be produced by selection of men during the recruitment phase of the study.

There are only three previous studies regarding the association between PCB exposure and sperm genetic damage in humans. In an Indian study (Rozati *et al.*, 2002), there was a significant positive correlation between seminal total PCB level and the percentage of single-stranded DNA in sperm (r = 0.56). In a US study (Hauser *et al.*, 2003b), using the neutral single cell microgel electrophoresis assay (Comet assay), 212 male partners of subfertile couples (mean age 36 years) were

Table III. Adjusted percentage differences (Δ) in DNA fractionation index (%DFI) as a function of the adjusted lipid serum concentration of CB-153 (ng/g lipid) (stratified by study group)

Quintiles	Inuit men			European men											
				All European men			Swedish fishermen			Warsaw men			Kharkiv men		
	N	Δ (%)	95% CI	*N*	Δ (%)	95% CI	*N*	Δ (%)	95% CI	*N*	Δ (%)	95% CI	*N*	Δ (%)	95% CI
0–50	9	8.0[a]	5.5–11.6	251	9.7[a]	8.9–10.6	5	10.9[a]	8.5–14.0	137	10.0[a]	9.1–11.0	109	9.6[a]	9.6–8.6
51–100	25	+2.6	−41–46	92	+22.6*	6–39	22			3	−6.0	−74–64	67	+30.9*	+13–49
101–200	56	−9.6	−50–36	85	+37.6*	15–60	66	+36.3	–8–64	1	−21.1	–94–136	18	+13.6	−17–45
201– 400	53	−15.6	−56–25	64	+48.6*	21–76	64	+39.0*	+9–70	0	–	–	0	–	–
>401	50	−5.3	−36–47	22	+61.7*	27–96	21	+52.4*	+14–94	0	–	–	1	+43.4	−74–161
Linear regression coefficient[b]	193	−1.8	−11–7	514	14.3*	6–23	178	+18.0*	3–33	141	−2.9	−18–13	196	16.2*	4–28

[a]Adjusted geometric mean value, the reference level for the indicated percentage changes of %DFI at higher exposure levels. Adjustment for study group (European men only), period of sexual abstinence and age.
[b]Multiple linear regression of %DFI on serum CB-153 concentration as continuous variable (transformed by the natural logarithm). Adjustment for period of abstinence and age—the only determinants that qualified for inclusion into the final model among a number of potential confounders according to the change-in-estimate method.
*$P < 0.05$.
CI = confidence interval.

Table IV. Adjusted risk odds ratio (OR_{adj}) for increased DNA fragmentation index (%DFI ≥17.2) as a function of CB-153 serum concentrations (ng/g lipid) among Inuit and European men

Quintiles	Inuit men					European men																			
						All European men					Swedish fishermen					Warsaw men					Kharkiv men				
	n	*N*	%	OR_{adj}[a]	95% CI	*n*	*N*	%	OR_{adj}[a]	95% CI	*n*	*N*	%	OR_{adj}[a]	95% CI	*n*	*N*	%	OR_{adj}[a]	95% CI	*n*	*N*	%	OR_{adj}[a]	95% CI
0–50	2	32	6.3	1.00	–	54	251	21.5	1.00	3/4	0	5	0	1.00	3/4	31	137	22.6	1.00	3/4	23	109	21.1	1.00	3/4
51–100						31	92	33.7	*2.13*	0.8–3.7	5	22	22.7			1	3	33.3	2.37	0.2–32.3	25	67	37.3	2.24	1.1–4.5
101–200	5	56	8.9	2.53	0.3–23.2	32	85	37.7	2.44	0.7–4.9	26	66	39.4	2.23	0.7–7.4	0	1	0	-		6	18	33.3	1.42	0.4–4.6
201– 400	5	53	9.4	2.53	0.3–23.7	29	64	45.3	3.32	0.6–6.2	29	64	45.3	2.47	0.7–9.0	0	0	0	-		0	0	0	-	
>401	8	50	16.0	4.74	0.5–42.3	12	22	54.6	4.87	1.2–17.4	12	21	57.1	3.76	0.8 –18.0	0	0	0	-		0	1	0	-	
Continuous variable[b]	20	193	10.4	1.34	0.8–2.4	158	514	30.7	1.51	1.1– 2.1	72	178	40.5	1.44	0.8 –2.6	32	141	32.7	0.83	0.4–1.7	32	196	22.7	1.63	1.0–2.7

[a]Adjusted for study group (European men only), age (natural logarithm of years) and period of abstinence (natural logarithm of days), no other potential risk factors changed the risk estimate with >10%.
[b]Logistic regression of risk for a %DFI ≥17.2 on the natural logarithm of the serum concentration of CB-153 with adjustment for study group (European men only), age and period of abstinence. The OR estimates the risk related to one unit increase of CB-153 on the natural logarithm scale. CI = confidence interval. Note that the %DFI value of 17.2% is the threshold value corresponding to the 75th percentile in the entire dataset; *n* is the number of men in the specified exposure range with a %DFI ≥17.2; *N* is the number of men in the specified exposure range; '%' is *n*/*N*.

Table V. Crude %DFI (upper panel) and %HDS (lower panel) according to serum p,p'-DDE concentration and study groups

p,p'-DDE (ng/g lipid) in serum	Inuit men			All European men[#]			Swedish fishermen			Warsaw men			Kharkiv men		
Quintiles	N	%DFI median	Range[1]	N	%DFI median	Range	N	%DFI median	Range	N	%DFI median	Range	N	%DFI median	Range
0–250	35	8.5	17.3	141	11.5	41.2	95	13.4	41.2	11	12.7	27.0			
251–500	47	7.1	23.1	176	10.5	86.9	50	16.7	82.9	62	9.7	46.4	17	12.6	46.3
501–1000	49	7.1	27.4	211	9.1	43.5	24	21.0	41.6	57	8.9	35.0	81	9.4	36.9
1001– 1500	29	6.8	24.2	89	8.9	59.4	7	25.5	54.5	7	10.6	36.4	46	11.1	41.6
>1501	33	8.3	34.4	90	9.3	38.5	2	9.3	3.1	4	10.2	8.3	51	10.3	38.5
p,p'-DDE (ng/g lipid) in serum	Inuit men			All men			Swedish fishermen			Warsaw men			Kharkiv men		
Quintiles	N	%HDS median	Range	N	%HDS median	Range	N	%HDS median	Range	N	%HDS median	Range	N	%HDS median	Range
0–250	35	11.3	45.0	141	8.8	45.0	9	8.4	33.8	11	8.3	23.0	.	.	.
251–500	47	12.7	29.0	176	9.1	42.2	50	7.9	40.7	62	8.2	23.3	17	9.9	40.1
501–1000	49	12.4	57.0	211	9.5	60.7	24	8.1	26.0	57	8.8	30.1	81	9.6	43.3
1001–1500	29	10.1	29.7	89	10.1	29.7	7	13.3	13.6	7	6.9	10.1	46	10.7	20.2
>1501	33	10.0	29.2	90	10.2	33.6	2	5.5	0.6	4	10.2	18.6	51	10.7	33.6

[#]Inuit men were not included in the %DFI pooled analyses (upper panel) because of heterogeneity of associations.
[1]Range is the arithmetical difference between the highest and the lowest value.

enrolled. The mean total level of PCB in serum was 226 ng/g lipid (range 56–1590) whereas the mean level for CB-153 was 44 ng/g lipid (range 9–421). Even though all the Comet parameters evaluated (Comet extent, %DNA in tail, tail distributed moment, and number of long cells) showed a clear trend to increasing values (deterioration of semen quality) with increasing concentrations of PCB, the authors did not find any statistically significant associations between sperm DNA damage and serum levels of any individual PCB congeners (CB-153 included), or sum of PCB. The third study refers to a Swedish cohort (Rignell-Hydbom *et al.*, 2005) also included in this dataset. A threshold-like effect was noted when categorizing CB-153 serum levels into equally sized quintiles with a significantly lower fraction of sperm with DNA damage (%DFI) found in the lowest CB-153 quintile (<113 ng/g lipid) compared to the other quintiles. In the present study, the mean CB-153 serum level was 88 ng/g lipid with an exposure range of 3–5500 ng/g lipid, thus offering a much wider exposure interval and a much larger number of participants than previous studies. A monotonically increasing trend of %DFI with increasing CB-153 levels emerged when considering the European men (but not the Inuit cohort), without any obvious threshold below which no effect could be demonstrated. These results reinforce the limited earlier findings that low-level serum concentration of PCB may also elicit weak effects on sperm genomic integrity.

Another remarkable finding of the present study was that *p,p'*-DDE, in agreement with previous reports (Hauser *et al.*, 2003b; Rignell-Hydbom *et al.*, 2005) was not associated with any sperm chromatin/DNA damage. In the present study, the mean *p,p'*-DDE serum concentration was 550 ng/g lipid (range 6–13 000 ng/g lipid), which was somewhat higher than in the US study (mean 254 ng/g lipid, range 73–7776) (Hauser *et al.*, 2003b). The results from both studies are consistent and indicate that the antiandrogenic compound *p,p'*-DDE does not negatively affect sperm DNA integrity, at least not in the studied exposure ranges.

The SCSA %DFI and %HDS parameters, as expected also from other studies (Spanò *et al.*, 2000; Larson-Cook *et al.*, 2003, Rignell-Hydbom *et al.*, 2005), were not correlated. That means that they mirror two different aspects of sperm abnormalities, one linked to the occurrence of DNA damage, the other related to the maturation process of DNA packaging around the protamine core leading to chromatin condensation alteration. No associations were found between serum levels of CB-153 and the %HDS. We have also mentioned that the %DFI level and, at to lesser extent, the %HDS fraction can be related to the fertility potential (Evenson *et al.*, 1999; Spanò *et al.*, 2000): in the general population the probability of fathering a child (fecundability) starts to decrease when %DFI>20% and becomes negligible from 30–40% onwards. Thus, even though the large majority of the men included in this study were certainly fertile, PCB exposure might negatively impact reproductive capabilities especially for men who, for other reasons, already have a higher fraction of defective sperm. Studies of time-to-pregnancy on these men and their spouses are in progress to give some insight into this issue (G.Toft, personal communication).

At present, only speculative hypotheses can be proposed with respect to the mechanism of action. Human ejaculates always exhibit a certain fraction of sperm cells with abnormal DNA and reduced chromatin integrity (Evenson *et al.*, 1991; Spanò *et al.*, 1998; Sakkas *et al.*, 2003b). The origin of these abnormal cells is not fully understood but it is believed that they can arise intratesticularly as the results of defective apoptotic mechanisms and/or derailments during the cellular and nuclear remodelling occurring during the latest stages of spermiogenesis (unrepaired nicks operated by the DNA topoisomerase II and/or altered histone-to-protamine substitution) (reviewed in Sakkas *et al.*, 2002, 2003a; Agarwal and Said, 2003). Another possible source is an excessive load of reactive oxygen species (ROS) overwhelming the natural antioxidative defences present in the semen (reviewed in Agarwal and Saleh,

2004; Ford, 2004). We have evidence from animal models that, for example, decreased testosterone levels are associated with the induction of sperm chromatin abnormalities (Krishnamurthy *et al.*, 2000, Traina *et al.*, 2003). Despite the certainty that androgen action is essential for the regulation of spermatogenesis in adult mammals, little is known about the mechanisms via which this regulation is exerted. One of the three highly bioaccumulated PCB, CB-138, was shown to act as an AR antagonist (Bonefeld-Jørgensen *et al.*, 2001) and it makes us speculate that the binding of PCB congeners to the AR can affect testosterone levels. It has recently been demonstrated in rodents that testosterone acts as a positive regulator of DNA topoisomerase II gene expression, the key enzyme that nicks and ligates DNA molecules during the histone-to-protamine transition process (McPherson and Longo, 1993; Marcon and Boissonneault, 2004). Moreover, testosterone is required for the maintenance of topoisomerase II expression during spermatogenesis (Bakshi *et al.*, 2001). A Swedish study on young males reported a weak, negative association between PCB (as CB-153) and free testosterone levels (Richthoff *et al.*, 2003). We have previously observed that free testosterone levels can have a weak impact on the %DFI (Richthoff *et al.*, 2002). Also the minor non-dose-related changes in chromatin condensation (as assayed by the FCM SCSA) in mice treated with a mixture of POP including PCB (as Arochlor 1254) and *p,p*'-DDE (Wade *et al.*, 2002) is of interest. Another potential mechanism whereby PCB may produce DNA damage is through hydroxylated PCB metabolites, which are capable of multiple adverse effects, including gap junction inhibition, AhR-mediated activity, and antiestrogenicity (Machala *et al.*, 2004). Hydroxylated PCB metabolites, which have been found in human semen (Rozati *et al.*, 2002; Younglai *et al.*, 2002), are extremely potent inhibitors of human estrogen sulphotransferase and can indirectly induce estrogenic activity by increasing estradiol bioavailability (Kester *et al.*, 2000). These metabolites can be further oxidized to form quinones (Schlezinger *et al.*, 1999; McLean *et al.*, 2000) and superoxides with the formation of ROS leading to oxidative DNA damage and strand breaks (Oakley *et al.*, 1996; Srinivasan *et al.*, 2001). Moreover, PCB quinones can also inhibit topoisomerase II activity (Srinivasan *et al.*, 2002), the key enzyme for sperm nucleus remodelling.

As demonstrated from the low level of variability of the measurements using the reference sample and from the quality control exercise, SCSA was confirmed to be a sound and reliable technique to assay sperm chromatin integrity (Evenson *et al.*, 1991; Giwercman *et al.*, 1999). SCSA seems particularly fit for epidemiological surveys, because only a small (0.1 ml) amount of semen is needed for the analysis and it can be frozen, stored, shipped, and assayed at the end of the sampling period, thus minimizing inter-assay variation (Perreault *et al.*, 2003). The SCSA has already successfully been applied to medium- to large-scale epidemiological studies (Larsen *et al.*, 1998; Kolstad *et al.*, 1999; Perreault *et al.*, 2000; Selevan *et al.*, 2000; Bonde *et al.*, 2002; Sanchez-Peña *et al.*, 2004) but the present study represents by far the largest survey ever attempted in the field of molecular epidemiology, demonstrating the feasibility and robustness of the approach also in the case of samples collected in remote geographical areas.

In conclusion, our results suggest that human dietary PCB exposure might have a negative impact on the sperm chromatin integrity of European adult males. As Inuit men, confirmed to have high POP serum levels, do not follow this trend, additional issues (e.g. differences in the genetic background and lifestyle habits, characterization of actual POP mixture and their xenohormonal activities) need to be investigated. Finally, no associations were observed for *p,p*'-DDE.

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