



LUND UNIVERSITY

Serum Levels of 2,2,4,4,5,5 -Hexachlorobiphenyl (CB-153) in Relation to Markers of Reproductive Function in Young Males from the General Swedish Population.

Richthoff, Jonas; Rylander, Lars; Jönsson, Bo A; Åkesson, Heléne; Hagmar, Lars; Nilsson-Ehle, Peter; Stridsberg, Mats; Giwercman, Aleksander

Published in:
Environmental Health Perspectives

DOI:
[10.1289/ehp.5767](https://doi.org/10.1289/ehp.5767)

2003

[Link to publication](#)

Citation for published version (APA):

Richthoff, J., Rylander, L., Jönsson, B. A., Åkesson, H., Hagmar, L., Nilsson-Ehle, P., Stridsberg, M., & Giwercman, A. (2003). Serum Levels of 2,2,4,4,5,5 -Hexachlorobiphenyl (CB-153) in Relation to Markers of Reproductive Function in Young Males from the General Swedish Population. *Environmental Health Perspectives*, 111(4), 409-413. <https://doi.org/10.1289/ehp.5767>

Total number of authors:
8

General rights

Unless other specific re-use rights are stated the following general rights apply:
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Read more about Creative commons licenses: <https://creativecommons.org/licenses/>

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Serum Levels of 2,2',4,4',5,5'-Hexachlorobiphenyl (CB-153) in Relation to Markers of Reproductive Function in Young Males from the General Swedish Population

Jonas Richthoff,¹ Lars Rylander,² Bo A.G. Jönsson,² Heléne Åkesson,² Lars Hagmar,² Peter Nilsson-Ehle,³ Mats Stridsberg,⁴ and Aleksander Giwercman¹

¹Fertility Centre, Malmö University Hospital, Malmö, Sweden; ²Department of Occupational and Environmental Medicine and ³Department of Clinical Chemistry, Lund University Hospital, Lund, Sweden; ⁴Department of Medical Sciences, Clinical Chemistry, University Hospital, Uppsala, Sweden

A time-related deterioration in male reproductive function caused by exposure to endocrine disruptors, including persistent organochlorines (POCs), has been hypothesized. In animal studies, POCs were found to have adverse effects on male reproductive function. However, little is known about the impact of POC exposure on reproductive parameters in men. In a study of 305 young Swedish men 18–21 years old from the general population, we correlated lipid-adjusted serum levels of 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153)—an index substance for POC exposure—to markers of male reproductive function: testis size assessed by ultrasound, sperm concentration, total sperm count, sperm motility assessed manually and with a computer-aided sperm analyzer (CASA), and serum levels of follicle-stimulating hormone, inhibin B, testosterone, sexual hormone-binding globulin (SHBG), luteinizing hormone, and estradiol. We found weak but statistically significant, negative correlations between CB-153 levels and both the testosterone:SHBG ratio ($r = -0.25$, $p < 0.001$)—a measure of the biologically active free testosterone fraction—and CASA sperm motility ($r = -0.13$, $p = 0.02$). No statistically significant association with other seminal, hormonal, or clinical markers of male reproductive function was found. In previous studies of more highly POC-exposed groups of adult men, the correlation between POC exposure, including CB-153, and free testosterone levels was not statistically significant. The present study gives some tentative support for weak negative effects of CB-153 exposure on sperm motility and free testosterone levels in young men, but further semen studies on more highly exposed groups may give more firm conclusions on the hazard for male reproductive function from dietary POC exposure. **Key words:** dioxin, male reproduction, PCB, reproductive hormones, semen quality, sperm concentration, sperm motility. *Environ Health Perspect* 111:409–413 (2003). doi:10.1289/ehp.5767 available via <http://dx.doi.org/> [Online 30 October 2002]

Persistent organochlorine compounds (POCs)—for example, polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs)—are ubiquitous environmental contaminants. These compounds are highly resistant to both abiotic and biotic degradation, and thus persistent, and also have high lipid solubility, which results in bioaccumulation and biomagnification in the food chain. The main human exposure source is the diet, especially animal fat (Svensson et al. 1991). Most POCs are highly toxic and have shown a multitude of health effects both in animal experiments and in epidemiologic studies. Animal studies show that the critical effects of dioxins and dioxin-like (coplanar) PCBs seem to be both impaired neurodevelopment and disruption of reproductive development and function. Male rats exposed *in utero* to low doses of dioxins showed decreased sperm counts (Faqi et al. 1998; Gray et al. 1997; Mably et al. 1992). Moreover, *in utero* exposure to coplanar PCBs leads to reduced reproductive capability in the offspring (Brouwer et al. 1995). Adult rats exposed to coplanar PCBs showed decreased sperm counts and increased percentages of abnormal spermatozoa (Faqi et

al. 1998). And a recent human study has shown that *in utero* exposure to PCBs and to PCDFs has a deleterious effect on sperm parameters (Guo et al. 2000).

Reliable biomarkers of POC exposure are necessary to establish a dose-effect relationship. A PCB congener, 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153), is a relevant and feasible exposure biomarker for POC. CB-153 in plasma correlated strongly with total PCB concentration (Atuma et al. 1998; Grimvall et al. 1997; Hagmar et al. 1998; Wicklund-Glynn et al. 2000). Furthermore, CB-153 was well correlated with the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent (TEQ) in plasma from PCBs (Grimvall et al. 1997), as well as with the total POC-derived TEQ in plasma (Asplund et al. 1994).

There are indications, although not unchallenged, that male reproductive function has deteriorated during the past few decades (Giwercman et al. 1993). According to a recent hypothesis, this trend might derive from increasing exposure to endocrine disruptors, such as POCs, during fetal life (Toppari et al. 1996). It is a matter of grave concern that the dioxin doses causing decreased sperm counts in rats are less than

one order of magnitude higher than the average daily human dioxin intake from the diet. It is not known whether lower body burdens of POCs, corresponding to what occurs in the general population of Western societies, might pose a hazard to the male reproductive function. Moreover, it is not known whether POCs might impair semen function only after exposure during the fetal and perinatal period, or whether exposure during adult life might also cause harmful effects.

Our aim in this study was to assess the association between serum levels of CB-153 as an index substance for POC exposure, and reproductive function parameters in young males from the general Swedish population.

Subjects and Methods

Subjects and medical examination. Approximately 95% of all Swedish males undergo a medical health examination before military service. Only those with serious chronic diseases are *a priori* excluded. Therefore, the group of men that undergo the conscript examination closely reflects the general population of young Swedish males. A total of 2,255 men born during the period 1979–1982 and living within an area of 60 km from the city of Malmö in southern Sweden were asked to participate. Of these, 13.5% ($n = 305$) agreed to enter the study. Their median age at the time of examination was 18.1 years (range, 18–21 years).

All subjects underwent an andrologic examination, including measurement of testicular volume by use of ultrasound. The information on their height and weight—as assessed at the compulsory medical examination before the military service—was used for calculation of

Address correspondence to A. Giwercman, Fertility Centre, Malmö University Hospital, Entrance 71, Level 6, SE 205 02 Malmö, Sweden. Telephone: 46-40-337904. Fax: 46-40-337043. E-mail: aleksander.giwercman@kir.mas.lu.se

We thank E. Stridsberg, R. Hofvander, and G. Svensson for their help in recruiting the participants in the study; A. Bremer, C. Anderberg, and K. Jepson for their technical assistance; and J. Schönebeck for his enthusiastic support.

The study was supported by Swedish governmental funding for clinical research, Crafoordska Fund, Ove Tulefjords Fund, and Foundation for Urological Research.

Received 7 May 2002; accepted 20 September 2002.

the body mass index (BMI, in kilograms per square meter). Their present smoking habits were recorded. Immediately after the examination, the subjects delivered a semen sample as well as blood samples drawn from a cubital vein. All subjects received a sum corresponding to US\$50 for their participation. The local ethics committee approved the study, and all subjects signed an informed consent form.

Ultrasound scanning. Ultrasound scanning of the testes was performed in supine position by use of a 7.5-MHz transducer connected to an Aloka 900 SSE scanner (Aloka, Tokyo, Japan). Each testis was investigated in two projections, and the volume was calculated as length \times width \times depth \times 0.52.

Hormone analysis. Circulating levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), sexual hormone-binding globulin (SHBG), testosterone, and estradiol were measured on an automated fluorescence detection system (Autodelphia, Wallac Oy, Turku, Finland) at the routine clinical chemistry laboratory, Uppsala University Hospital. Intraassay and total assay variation were below the level of 4.0 and 7.5%, respectively. Inhibin B levels were assessed using a specific immunometric assay, as previously described (Groome et al. 1996), with a detection limit of 15 ng/L and intraassay and total assay variation coefficients below 7%. The reference levels for the hormone analyses are given in Table 1.

Semen analysis. Each subject was asked to provide in a room at the laboratory a semen sample by masturbation into a wide-mouthed plastic cup. Three of the 305 participants were unable to produce an ejaculate; therefore, a total of 302 semen samples were included in the study. The men were asked to remain abstinent for at least 48 hr, but the

length of the actual abstinence period was recorded (Table 1). The weight of the empty plastic cup was subtracted from the total weight of cup and semen to get the semen volume (in milliliters). The measurements were done by use of a Sartorius scale, and the results were expressed with two decimals.

The semen samples were analyzed according to the World Health Organization's (WHO) recommendations (WHO 1999). Sperm concentration was assessed by use of a modified Neubauer chamber (Developing Health Technology, Barton Mills, UK), and positive displacement pipettes were used for proper dilution of the ejaculate. Only three laboratory assistants performed the analyses of the ejaculates, and the interobserver coefficient of variation was found to be 8.5% for concentration assessment. According to the WHO recommendations, for determination of sperm motility, 200 spermatozoa were scored in categories A, B, C, and D, with A corresponding to rapid progressive motility, B to slow progressive motility, C to nonprogressive motility, and D to immotility. Because there is considerable interobserver variation in discriminating between A and B, these two scores were pooled together. Previous studies have indicated that the percentage of progressively motile spermatozoa (A + B) is the most significant of the WHO motility categories in relation to the fertility potential of a male (Jouannet et al. 1988). For that reason, and because the total sum of scores A, B, C, and D will always be 100, the category C was excluded from the analysis, which was restricted to the two types of motility, A + B and D.

Furthermore, the percentages of motile, locally motile, and immotile spermatozoa were assessed by use of CRISMAS computer-aided sperm motility analyzer (CASA), as previously

described by Larsen et al. (Larsen et al. 2000). Briefly, the analysis was performed in a 10- μ m Makler chamber at 37°C. The samples were diluted, if necessary, in a phosphate buffer. The motility assessment was based on capture sequences of 64 images (25 MHz), and for each sample at least 100 spermatozoa were analyzed. The designation of the motility status was based on the level of the curvilinear velocity, with $> 25 \mu\text{m}/\text{sec}$ for motile spermatozoa, $5\text{--}25 \mu\text{m}/\text{sec}$ for locally motile, and $> 5 \mu\text{m}/\text{sec}$ for immotile spermatozoa. Because of technical problems, ejaculates from only 285 of the 302 ejaculates were available for CASA analysis. However, the 17 subjects excluded from the CASA analysis did not differ significantly from the remaining 285 in sperm concentration or serum levels of CB-153.

Determination of CB-153. CB-153 was analyzed according to a modified method of Janak et al. (Janak et al. 1999). One milliliter of serum was added with 50 μL internal standard methanol solution containing 20 ng of $^{13}\text{C}_{12}$ -labeled CB-153/mL (Cambridge Isotope Laboratories, Andover, MA, USA). Preparation of external standards was performed by addition of different amounts of unlabeled CB-153 (Cambridge Isotope Laboratories) to 1.0 mL blank serum. The standard curve was corrected for the original contents of CB-153 in the blank serum. After the addition of the standards, the serum was stored at 4°C overnight.

The next morning, 2 mL formic acid:2-propanol (4:1) mixture was added to the serum samples. The sample was sonicated in an ultrasonic bath for 5 min. After about 30 min, 2 mL water:2-propanol (17:3) was added and the sample was sonicated again for 5 min. Isolute ENV+ (200 mg; 6 mL; International Sorbent Technology, Hengoed, UK) solid-phase extraction (SPE) columns

Table 1. Andrological parameters, and CB-153 and hormonal levels in young men from the general Swedish population.

Parameter	Reference interval	No.	Mean (SD)	Median	Range
CB-153 (ng/g lipid)	NA	305	68 (29)	65	23–250
BMI (kg/m ²)	18.5–24.9 ^a	305	22 (3.2)	22	15–42
Abstinence period (hr)	NA	302	85 (57)	67	12–504
Total testis volume (mL)	NA	305	29 (7.7)	29	13–53
Semen volume (mL)	$> 2.0^b$	302	3.2 (1.3)	3.2	0.3–8.4
Sperm concentration ($\times 10^6/\text{mL}$)	$> 20.0^b$	302	72 (66)	54	0.1–390
Total sperm count ($\times 10^6$)	$> 40^b$	302	210 (180)	167	0.5–1,200
Sperm motility (%)					
A + B	$> 50^b$	302	54 (17)	56	0–85
D	NA	302	31 (13)	29	6–99
CASA motile	NA	285	51 (22)	51	0–100
CASA immotile	NA	285	32 (23)	29	0–100
Inhibin B (ng/L)	100–240 ^c	305	210 (62)	200	54–420
FSH (IU/L)	1.0–10.51 ^c	305	3.5 (1.5)	3.0	0.5–12.5
LH (IU/L)	1.2–9.6 ^c	305	4.2 (1.8)	4.2	1.2–10.2
Testosterone (nM)	8.7–33 ^c	305	23 (5.3)	23	6.1–38
SHBG (nM)	13–50 ^c	305	28 (9.7)	28	7.2–67
Testosterone:SHBG	NA	305	1.0 (0.5)	1.2	0.1–2.1
Estradiol (pM)	60–150 ^c	305	80 (17)	77	43–144

NA, not available.

^aAs recommended by WHO. ^bAccording to WHO (1999). ^cClinical reference values for the laboratory performing the analyses.

Table 2. Pearson's *r* for CB-153 serum concentrations and reproductive parameters among young men from the general Swedish population.

Parameter	No.	CB-153 (ng/g lipid)	
		<i>r</i>	<i>p</i> -Value
Total testis volume (mL)	305	0.02	0.7
Semen volume (mL)	302	−0.01	0.4
Sperm concentration ($\times 10^6/\text{mL}$)	302	< 0.01	1.0
Total sperm count ($\times 10^6$)	302	< 0.01	1.0
Sperm motility (%)			
A + B	302	−0.09	0.14
D	302	0.11	0.05
CASA motile	285	−0.13	0.02
CASA immotile	285	0.11	0.06
Inhibin B (ng/L)	305	0.02	0.7
FSH (IU/L)	305	0.09	0.1
LH (IU/L)	305	−0.04	0.5
Testosterone (nM)	305	0.05	0.4
SHBG (nM)	305	0.25	< 0.001
Testosterone:SHBG	305	−0.25	< 0.001
Estradiol (pM)	305	−0.14	0.01

were conditioned by 1 mL methanol, 6 mL of dichloromethane (DCM), 5 mL methanol, and 3 mL water. The columns were mounted on a VacMaster-20 sample-processing station equipped with Teflon combined stopcock needles (International Sorbent Technology). Thirty minutes after the sonification of the serum samples, these were applied to the columns. The maximum flow rate of serum samples through the SPE column was 1 mL/min. The SPE columns were thereafter washed with 4 mL water:2-propanol (19:1) and with 2.5 mL water:methanol (9:1) at a flow rate up to 10 mL/min. The columns were dried with air suction for about 20 sec. One milliliter of concentrated sulfuric acid at a flow rate of 0.2 mL/min was then applied to the columns, after which these were washed with 2.5 mL water, 1.0 mL 1 M Na₂CO₃, 2.5 mL water, and 2.5 mL of water:methanol (9:1). Three drops of water:methanol (3:7, vol/vol) were added and the columns were dried with air suction for about 3 min. The columns were then centrifuged at 1,500g for 10 min and then again dried with air suction for 5 min. The CB-153 was eluted with 2 mL DCM, which was then evaporated and dissolved in 100 μ L of toluene.

The samples were analyzed by gas chromatography–mass spectrometry (GC-MS). The equipment consisted of a VG Trio 1000 quadrupole MS (Fisons, Manchester, UK) and a Carlo-Erba 8065 GC equipped with an A200S autosampler (Carlo-Erba, Milan, Italy). The column was a fused silica capillary column (30 m \times 0.25 mm inner diameter) with a DB-5 MS stationary phase and a film thickness of 0.25 μ m (J&W Scientific, Folsom, CA, USA). The samples were analyzed by a splitless injection technique. The injector temperature was kept at 300°C, and the injection volume was 2.0 μ L. The initial column temperature was 100°C for 1 min. The temperature was thereafter increased by

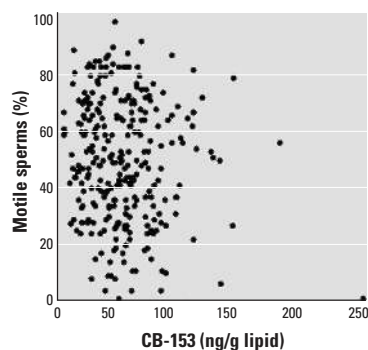


Figure 1. Correlation between the serum levels of CB-153 and percentage of motile spermatozoa, as assessed by CASA, in 285 young men from the general Swedish population. $r = -0.13$; $p = 0.02$.

15°C/min to 300°C. The MS interface was at 300°C, and the ion source was at 130°C. The MS was in the negative ion chemical ionization mode with ammonia as moderating gas. Selected ion monitoring of CB-153 was performed at m/z 326 and 360, whereas m/z 338 and 372 were chosen for the labeled internal standard.

The relative standard deviations, calculated from samples analyzed in duplicate at different days, were 7% at 0.6 ng/mL ($n = 76$) and 5% at 1.5 ng/mL ($n = 37$). The detection limit was < 0.05 ng/mL. The analysis of CB-153 is part of the Round Robin intercomparison program (H. Drexler, Institute and Out-patient Clinic for Occupational, Social and Environmental Medicine, University of Erlangen-Nuremberg) with analysis results within the reference limits.

Determination of lipids by enzymatic methods. Plasma concentrations of triglycerides, cholesterol, and phospholipids were determined by enzymatic methods using reagents from Boehringer-Mannheim (triglycerides and cholesterol; Mannheim, Germany) and Waco Chemicals (phospholipids; Neuss, Germany). The total lipid concentration in plasma was calculated by summation of the amounts of triglycerides, cholesterol, and phospholipids. In these calculations, the average molecular weights of triglycerides and phospholipids were assumed to be 807 and 714, respectively. For cholesterol, we used an average molecular weight of 571, postulating that the proportion of free and esterified cholesterol in plasma was 1:2.

Statistical analysis. Bivariate associations between concentrations of lipid-adjusted CB-153 in serum and total testis volume, semen volume, sperm concentration, total sperm count, sperm motility, and inhibin B, FSH, LH, testosterone, SHBG, testosterone/SHBG, and estradiol levels, respectively, were evaluated by Pearson's correlation coefficient. To ensure that linear associations were reasonable (and accordingly the use of Pearson's r), scatter plots were assessed for all bivariate comparisons. The effects of CB-153 on the sperm and the hormone levels were evaluated by linear regression models, adjusting for potential confounders. Model assumption was checked by means of residual analysis. CB-153 was treated as a continuous variable as well as categorized into three equally sized groups (< 53.9, 53.9–74.6, > 74.6 ng/g lipid). As potential confounders, BMI,

length of abstinence period (treated as a continuous variable and alternatively categorized into four categories: < 49, 49–72, 73–96, and > 96 hr), and smoking habits [smokers ($n = 218$) vs. nonsmokers ($n = 87$)] were considered. If the adjusted estimate differed less than 15% from the crude estimate, we present only the crude results. When trends in the data were tested, the Jonckheere-Terpstra test was applied.

Results

The testicular volume was not correlated with the serum levels of CB-153.

A statistically significant negative, but weak, correlation was found between the CB-153 levels and the percentage of motile cells assessed by CASA (Table 2, Figure 1). The same pattern of correlations, close to the level of statistical significance, was observed also for conventional motility parameters. The estimated effects of changes in CB-153 levels on sperm motility parameters are shown in Table 3. Thus, an increase in CB-153 levels by 10 ng/g lipid corresponded to 1.0% decrease in the percentage of CASA motile spermatozoa [95% confidence interval (CI), –2.0 to –0.13; Table 3] and was accompanied by an equal increase in the percentage of immotile cells, a correlation that was almost statistically significant. No significant correlations were found between the CB-153 levels and seminal volume, sperm concentration, and total sperm count, respectively (Table 2). In an alternative analysis the CB-153 variable was categorized into three equal-sized groups. No other exposure–response associations than those seen in the linear analyses appeared.

There was a significant positive correlation between serum levels of CB-153 and SHBG, whereas the correlations were negative between CB-153 and the testosterone:SHBG ratio, and estradiol, respectively (Table 2). After adjustment for BMI, which was clearly associated with estradiol, testosterone, and the testosterone:SHBG ratio, the correlation between CB-153 and estradiol was no longer statistically significant (Table 4). CB-153 was not correlated with inhibin B, total testosterone, FSH, or LH.

Discussion

In the present study we found weak but significant negative correlations between CB-153 levels in serum and both CASA

Table 3. Estimated effects (β) of 10 ng/g lipid increase in CB-153 levels in serum on sperm motility parameters among young men from the general Swedish population, obtained from linear regression analyses.

Sperm parameter	No.	Intercept	β	95% CI
Sperm motility (%)				
A + B	302	57	–0.50	–1.2, 0.16
D	302	27	0.52	–0.0068, 1.0
CASA motile	285	58	–1.0	–2.0, –0.13
CASA immotile	285	26	0.89	–0.057, 1.8

sperm motility and the testosterone:SHBG ratio—a measure of the biologically active free testosterone fraction. The latter association was due to a positive correlation between the concentration of CB-153 and SHBG levels, whereas no association with total testosterone was seen. Furthermore, no statistically significant association with other seminal, hormonal, or clinical markers of male reproductive function was found, although the correlations with other motility parameters were almost significant. No congener other than CB-153 was analyzed in the present study.

It is known from animal experiments that relatively low doses of several POCs have negative impacts on male reproductive function (Toppari et al. 1996). However, the knowledge of adverse effects of POCs on the human male reproductive function is still limited. The present study is, to our knowledge, the first aiming to elucidate the impact of levels of exposure to POCs, corresponding to what occurs in general populations of Western societies, on a broad range of parameters of male reproductive function.

Several POCs interact with hormonal pathways and can therefore be considered potential endocrine disrupters. Some PCB congeners have estrogenic properties, whereas dioxins and dioxin-like PCB have more of antagonistic effects on the estrogen receptors (Toppari et al. 1996). Other compounds, such as the main *p,p'*-dichlorodiphenyl-trichloroethane (DDT) metabolite 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE), are anti-androgenic. Hepatic synthesis of SHBG is known to be affected by the estrogen-androgen balance, and finding of a positive correlation between CB-153 levels and SHBG might be due to the estrogenic or anti-androgenic properties of the POCs, for which CB-153 serves as a proxy marker. Furthermore, the increase in SHBG concentration—by rising serum CB-153—was not accompanied by rising testosterone levels, which implies a lower free testosterone fraction and thereby reduced androgenic activity.

We found that an increase in CB-153 levels with 10 ng/g lipid implied a 1% decrease in the percentage of motile spermatozoa as assessed by CASA. Interestingly, in a study based on Danish first pregnancy

planners, percentage of motile spermatozoa as assessed by CRISMAS CASA, but not manually, was found to be an independent predictor of the fecundity of the couple (Bonde et al. 1998; Larsen et al. 2000). CASA analysis of sperm motility is subject to a lesser degree of intralaboratory variation than is the manual analysis (Auger et al. 2000; Boone et al. 2000; Jørgensen et al. 1997) and may therefore be a more sensitive measure of male fertility.

We did not find any correlation between CB-153 levels and the other important seminal predictor of fertility (Bonde et al. 1998)—sperm concentration. However, in a recent study, an impairment of sperm motility—without changes in daily sperm production or morphology—was found in diethylstilbestrol-treated adult rats (Goyal et al. 2001). In another report (van Birgelen et al. 1999), 13 weeks of exposure of 4-week-old rats to 3,3',4,4'-tetrachloroazoxybenzene, a contaminant of 3,4-dichloroaniline and the anilide herbicide Diuron, which acts through the aryl hydrocarbon receptor in a dioxin-like manner, decreased the motility but not the concentration of the epididymal spermatozoa. The opposite effect was found in mice, and neither species reached a no-observed-adverse-effect level. A small semen-quality study of teenagers who had been substantially exposed *in utero*, because of ingestion of POCs by their mothers via contaminated cooking oil, did show increased percentages of morphologically abnormal cells as well as reduced motility and ability to penetrate hamster oocytes compared with spermatozoa from control subjects (Guo et al. 2000). In another study, levels of some PCB congeners have been shown to be inversely correlated with sperm motility in semen samples in which sperm concentration was below 20 million/mL (Bush et al. 1986). In a small pilot study of 29 men presenting to a U.S. andrology laboratory for semen evaluation, there were indications that those subjects with normal sperm concentrations, motility, and morphology had somewhat lower serum concentrations of total PCB and *p,p'*-DDE, compared with the others (Hauser et al. 2002). The serum concentrations of CB-153 were very similar to those observed in the present study. Thus, previous studies have already

indicated that POCs can induce negative effects on male reproductive health, including deterioration of semen characteristics, also in humans. However, the key questions are what doses are needed for causing such effects, and whether the body burdens in the general population, which almost exclusively derive from the diet, are sufficiently high for impairing the male reproductive function.

A positive correlation between POC exposure and SHBG levels was also found in 110 Swedish and Latvian adult men, but after age adjustment, the correlation was no longer significant (Hagmar et al. 2001). It should be noticed that the CB-153 levels in serum from the men in the present study were low, with a median value of 65 ng/g lipids (range, 23–250). This can be compared with median levels, in samples collected in 1991, of 450 ng/g lipids for adult Swedish men with a high consumption of fatty fish from the Baltic Sea, 410 for those with more moderate fish consumption habits, and 220 for those with no such consumption (Sjodin et al. 2000). However, the lack of a significant association between CB-153 and SHBG levels in the more highly exposed men might at least be due to a lower statistical power compared with the present study of 305 men, in which age correction was not necessary because of a similar age of all the participants. Furthermore, given that the male reproductive system is most sensitive to the effect of endocrine disrupters during early fetal or perinatal life (Sharpe et al. 1993), the CB-153 values obtained at adult age may not necessarily express the level of exposure at that critical time window of development.

However, the lack of coherence among the studies calls for caution. A possibility that cannot be excluded is that weak associations observed in the present study were chance findings. It is therefore important to try to assess these associations in other populations as well. A study among subjects with higher and more varying body burdens of POCs may clarify this matter.

It can be assumed that the cohort of 305 young men included in this study is representative for this age group of men in southern Sweden, despite the fact that only 13.5% of eligible subjects agreed to deliver a semen

Table 4. Estimated effects (β) on hormone levels by 10 ng/g lipid increase in CB-153 concentrations in serum and BMI in 305 young men from the general Swedish population, obtained from linear regression analyses.

Parameter	SHBG (nmol/L)				Crude testosterone/SHBG		Estradiol (pmol/L)			
	Crude		Adjusted ^a		β	95% CI	Crude		Adjusted ^a	
	β	95% CI	β	95% CI			β	95% CI	β	95% CI
Intercept	23		42		1.0		85		74	
CB-153 (ng/g lipid)	0.84	(0.47–1.21)	0.56	(0.18–0.94)	–0.024	(–0.035–0.013)	–0.82	(–1.5–0.17)	–0.66	(–1.4–0.028)
Intercept	50		—		0.51		65		—	
BMI (kg/m ²)	–0.93	(–1.3–0.61)	–0.77	(–1.1–0.42)	0.016	(0.0061–0.026)	0.66	(0.067–1.3)	0.46	(–0.16–1.1)

If the adjusted estimate differed < 15% from the crude estimate, we present only the crude results.

^aEstimated when CB-153 and BMI simultaneously were included in the model.

sample. It cannot be expected that men 18–21 years old have any knowledge about their reproductive capability. The low participation rate should therefore not imply any selection bias with respect to fertility. Accordingly, in a recent Danish conscript study, with a correspondingly low participation rate, it was concluded that the participants seemed to be representative for the whole group of conscripts, based on the levels of reproductive hormones in participants and nonparticipants in the semen study (Andersen et al. 2000). We found the same mean BMI for the group of men delivering semen samples in the present study as was found for the whole group of Swedish young men who had taken part in the conscript examination. This is also an indication that the sampled group was representative for the total group. Furthermore, it seems unlikely that the level of exposure to CB-153 had any effect on whether a subject agreed to participate in the study. No associations were found between the length of abstinence period and the motility and hormone variables, respectively. Therefore, length of abstinence period was not a confounder for these outcome measures.

In conclusion, the present study gives some tentative support for weak negative effects of CB-153 exposure on sperm motility and free testosterone levels in young men. Previous findings of the lack of correlation between POC exposure and free testosterone levels in more highly exposed adult men supports the possibility that the present results might be chance findings. Future studies of reproductive function on more highly POC-exposed groups are needed in order to draw more firm conclusions.

REFERENCES

- Andersen AG, Jensen TK, Carlsen E, Jørgensen N, Andersson A-M, Krarup T, et al. 2000. High frequency of sub-optimal semen quality among young men of the general Danish population. *Hum Reprod* 15:366–372.
- Asplund L, Svensson B-G, Nilsson A, Eriksson U, Jansson B, Jensen S, et al. 1994. Polychlorinated biphenyls, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDT) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)-ethylene (*p,p'*-DDE) in human plasma related to fish consumption. *Arch Environ Health* 49:477–486.
- Atuma S, Aune M, Bergh AS, Wicklund-Glynn A, Darnerud PO, Larsson M, et al. 1998. Polychlorinated biphenyls in salmon (*Salmo salar*) from the Swedish east coast. *Organohalogen Compounds* 39:153–156.
- Auger J, Eustache F, Ducot B, Blandin T, Daudin M, Diaz I, et al. 2000. Intra- and inter-individual variability in human sperm concentration, motility and vitality assessment during a workshop involving ten laboratories. *Hum Reprod* 15:2360–2368.
- Bonde JP, Ernst E, Jensen TK, Hjøllund NHI, Kolstad H, Henriksen TB, et al. 1998. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. *Lancet* 352:1172–1177.
- Boone WR, Shapiro SS, Shapiro SS. 2000. Using videotaped specimens to test quality control in a computer-assisted semen analysis system. *Fertil Steril* 73:636–640.
- Brouwer A, Ahlborg UG, Vand der Berg M, Birnbaum LS, Boersm Bosveld B, Denison MS, et al. 1995. Functional aspects of developmental toxicity of polyhalogen aromatic hydrocarbons in experimental animals and human infants. *Eur J Pharmacol* 293:1–40.
- Bush B, Bennett A, Snow J. 1986. Polychlorinated biphenyl congeners, *p,p'*-DDE, and sperm function in humans. *Arch Environ Contam Toxicol* 15:333–341.
- Faqi AS, Dalsenter PR, Merker HJ, Chahoud I. 1998. Effects on developmental landmarks and reproductive capability of 3,3',4,4'-tetrachlorobiphenyl and 3,3',4,4',5-pentachlorobiphenyl in offspring of rats exposed during pregnancy. *Hum Exp Toxicol* 17:365–372.
- Giwerzman A, Carlsen E, Keiding N, Skakkebaek NE. 1993. Evidence for increasing incidence of abnormalities of the human testis: a review. *Environ Health Perspect* 101(suppl):65–71.
- Goyal HO, Braden TD, Mansour M, Williams CS, Kamaledin A, Srivastava KK. 2001. Diethylstilbestrol-treated adult rats with altered epididymal sperm numbers and sperm motility parameters, but without alterations in sperm production and sperm morphology. *Biol Reprod* 64:927–934.
- Gray LE, Wolf C, Mann P, Ostby JS. 1997. *In utero* exposure to low doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin alters reproductive development of female Long Evans hooded rat offspring. *Toxicol Appl Pharmacol* 146:237–244.
- Grimvall E, Rylander L, Nilsson-Ehle P, Nilsson U, Stromberg U, Hagmar L, et al. 1997. Monitoring of polychlorinated biphenyls in human blood plasma: methodological developments and influence of age, lactation, and fish consumption. *Arch Environ Contam Toxicol* 32:329–336.
- Groome NP, Illingworth PJ, O'Brien M, Pai R, Rodger FE, Mather JP, et al. 1996. Measurement of dimeric inhibin B throughout the human menstrual cycle. *J Clin Endocrinol Metab* 81:1401–1405.
- Guo YL, Hsu PC, Hsu CC, Lambert GH. 2000. Semen quality after prenatal exposure to polychlorinated biphenyls and dibenzofurans. *Lancet* 356:1240–1241.
- Hagmar L, Becher G, Heikkilä JT, Frankman O, Schütz A, Ahlborg UG, et al. 1998. Influence of consumption of fatty fish from the Baltic Sea on specific PCB congeners in cord blood and venous blood from newly delivered mothers. *J Toxicol Environ Health* 53:581–591.
- Hagmar L, Björk J, Sjödin A, Bergman Å, Erfurth EM. 2001. Plasma levels of persistent organohalogen and hormone levels in male human adults. *Arch Environ Health* 56:138–143.
- Hauser R, Altshul L, Chen Z, Ryan L, Overstreet J, Schiff I, et al. 2002. Environmental organochlorines and semen quality: results of a pilot study. *Environ Health Perspect* 110:229–233.
- Janak K, Jensen E, Becher G. 1999. Determination of polychlorinated biphenyls in human blood by solid-phase extraction including lipid decomposition. *J Chromatogr B Biomed Sci Appl* 734:19–27.
- Jouannet P, Ducot B, Feneux, D, Spira A. 1988. Male factors and the likelihood of pregnancy in infertile couples. I. Study of sperm characteristics. *Int J Androl* 11:379–394.
- Jørgensen N, Auger J, Giwerzman A, Irvine DS, Jensen TK, Jouannet P, et al. 1997. Semen analysis performed by different laboratory teams: an intervariation study. *Int J Androl* 20:201–208.
- Larsen L, Scheike T, Jensen TK, Bonde JP, Ernst E, Hjøllund H, et al. 2000. Computer-assisted semen analysis parameters as predictors for fertility of the general population. *Hum Reprod* 15:1562–1567.
- Mably TA, Bjerke DL, Moore RW, Gendron-Fitzpatrick A, Peterson RE. 1992. In utero and lactational exposure to 2,3,7,8-tetrachloro-*p*-dioxin. III. Effects on spermatogenesis and reproductive capability. *Toxicol Appl Pharmacol* 114:118–126.
- Sharpe RM, Skakkebaek NE. 1993. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* 341:1392–1395.
- Sjödin A, Hagmar L, Klasson-Wehler E, Björk J, Bergman A. 2000. Influence of the consumption of fatty Baltic Sea fish on plasma levels of halogenated environmental contaminants in Latvian and Swedish men. *Environ Health Perspect* 108:1035–1041.
- Svensson BG, Nilsson A, Hansson M, Rappe C, Åkesson B, Skerfving S. 1991. Exposure to dioxins and dibenzofurans through the consumption of fish. *N Engl J Med* 324:8–12.
- Toppari J, Larsen JC, Christiansen P, Giwerzman A, Grandjean P, Guillelte LJ Jr, et al. 1996. Male reproductive health and environmental xenoestrogens. *Environ Health Perspect* 104:741–803.
- van Birgelen AP, Herbert CD, Wenk ML, Grimes LK, Chapin RE, Travlos GS, et al. 1999. Toxicity of 3,3',4,4'-tetrachloro-azoxybenzene in rats and mice. *Toxicol Appl Pharmacol* 156:206–221.
- Wicklund-Glynn A, Wolk A, Aune M, Atuma S, Zettermark S, Moehle-Schmid M, et al. 2000. Serum concentrations of organochlorines in men: a search for markers of exposure. *Sci Total Environ* 263:197–208.
- WHO. 1999. WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. Cambridge:Cambridge University Press.