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.

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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

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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 testosteronetoSHBG 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 dose causing decreased sperm counts in rats is 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

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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 USS50 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 × width × depth × 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 (Autodelfia, 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 a sample 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-µm 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 µm/sec for motile spermatozoa, 5–25 µm/sec for locally motile, and < 5 µm/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 semen levels of CB-153.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference interval</th>
<th>No.</th>
<th>Mean (SD)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB-153 (ng/g lipid)</td>
<td>NA</td>
<td>305</td>
<td>68 (29)</td>
<td>65</td>
<td>23–250</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>18.5–24.9</td>
<td>305</td>
<td>22 (3.2)</td>
<td>22</td>
<td>15–42</td>
</tr>
<tr>
<td>Abstinence period (hr)</td>
<td>NA</td>
<td>302</td>
<td>85 (57)</td>
<td>67</td>
<td>12–504</td>
</tr>
<tr>
<td>Total testis volume (mL)</td>
<td>NA</td>
<td>305</td>
<td>29 (7.7)</td>
<td>29</td>
<td>13–53</td>
</tr>
<tr>
<td>Semen volume (mL)</td>
<td>&gt; 2.0</td>
<td>302</td>
<td>3.2 (1.3)</td>
<td>3.2</td>
<td>0.3–8.4</td>
</tr>
<tr>
<td>Sperm concentration (× 10⁹/mL)</td>
<td>&gt; 20.0</td>
<td>302</td>
<td>72 (66)</td>
<td>54</td>
<td>0.1–380</td>
</tr>
<tr>
<td>Total sperm count (× 10¹²)</td>
<td>&gt; 40.0</td>
<td>302</td>
<td>210 (180)</td>
<td>167</td>
<td>0.5–1,200</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A + B</td>
<td>&gt; 50</td>
<td>302</td>
<td>54 (17)</td>
<td>56</td>
<td>0–85</td>
</tr>
<tr>
<td>D</td>
<td>NA</td>
<td>302</td>
<td>31 (13)</td>
<td>29</td>
<td>6–99</td>
</tr>
<tr>
<td>CASA motile</td>
<td>NA</td>
<td>285</td>
<td>51 (22)</td>
<td>51</td>
<td>0–100</td>
</tr>
<tr>
<td>CASA immotile</td>
<td>NA</td>
<td>285</td>
<td>32 (23)</td>
<td>29</td>
<td>0–100</td>
</tr>
<tr>
<td>Inhibin B (ng/L)</td>
<td>100–200</td>
<td>305</td>
<td>210 (62)</td>
<td>200</td>
<td>54–420</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>1.0–10.5</td>
<td>305</td>
<td>3.5 (1.5)</td>
<td>3.0</td>
<td>0.5–12.5</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>1.2–9.6</td>
<td>305</td>
<td>4.2 (1.8)</td>
<td>4.2</td>
<td>1.2–10.2</td>
</tr>
<tr>
<td>Testosterone (nm/L)</td>
<td>8.7–33</td>
<td>305</td>
<td>23 (5.3)</td>
<td>23</td>
<td>6.1–38</td>
</tr>
<tr>
<td>SHBG (nm/L)</td>
<td>13–50</td>
<td>305</td>
<td>28 (9.7)</td>
<td>28</td>
<td>7.2–67</td>
</tr>
<tr>
<td>Testosterone:SHBG</td>
<td>NA</td>
<td>305</td>
<td>1.0 (0.5)</td>
<td>1.2</td>
<td>0.1–2.1</td>
</tr>
<tr>
<td>Estradiol (pm/L)</td>
<td>60–150</td>
<td>305</td>
<td>80 (17)</td>
<td>77</td>
<td>43–144</td>
</tr>
</tbody>
</table>

Table 2. Pearson’s r’s for CB-153 semen concentration and reproductive parameters among young men from the general Swedish population.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No.</th>
<th>r</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB-153 (ng/g lipid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total testis volume (mL)</td>
<td>305</td>
<td>0.02</td>
<td>0.7</td>
</tr>
<tr>
<td>Semen volume (mL)</td>
<td>302</td>
<td>−0.01</td>
<td>0.4</td>
</tr>
<tr>
<td>Sperm concentration (× 10⁹/mL)</td>
<td>302</td>
<td>&lt; 0.01</td>
<td>1.0</td>
</tr>
<tr>
<td>Total sperm count (× 10¹²)</td>
<td>302</td>
<td>&lt; 0.01</td>
<td>1.0</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A + B</td>
<td>302</td>
<td>−0.09</td>
<td>0.14</td>
</tr>
<tr>
<td>D</td>
<td>302</td>
<td>0.11</td>
<td>0.05</td>
</tr>
<tr>
<td>CASA motile</td>
<td>285</td>
<td>0.13</td>
<td>0.02</td>
</tr>
<tr>
<td>CASA immotile</td>
<td>285</td>
<td>0.11</td>
<td>0.06</td>
</tr>
<tr>
<td>Inhibin B (ng/L)</td>
<td>305</td>
<td>0.02</td>
<td>0.7</td>
</tr>
<tr>
<td>FSH (IU/L)</td>
<td>305</td>
<td>0.09</td>
<td>0.1</td>
</tr>
<tr>
<td>LH (IU/L)</td>
<td>305</td>
<td>−0.04</td>
<td>0.5</td>
</tr>
<tr>
<td>Testosterone (nm/L)</td>
<td>305</td>
<td>0.05</td>
<td>0.4</td>
</tr>
<tr>
<td>SHBG (nm/L)</td>
<td>305</td>
<td>0.25</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Testosterone:SHBG</td>
<td>305</td>
<td>−0.25</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Estradiol (pm/L)</td>
<td>305</td>
<td>−0.14</td>
<td>0.01</td>
</tr>
</tbody>
</table>
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 sonication 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 1,500 g 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 µL 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 × 0.25 mm inner diameter) with a DB-5 MS stationery phase and a film thickness of 0.25 µm (J&W Scientific, Italy). The column was a fused silica capillary and a Carlo-Erba 8065 GC equipped with an Environmental Health Perspectives – VOLUME 111 – NUMBER 4 – April 2003

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 × 0.25 mm inner diameter) with a DB-5 MS stationary phase and a film thickness of 0.25 µm (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 µL. The initial injector temperature was kept 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 = 70) 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 parameters.

**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.**

<table>
<thead>
<tr>
<th>Sperm parameter</th>
<th>No.</th>
<th>Intercept</th>
<th>β</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm motility (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A + B</td>
<td>302</td>
<td>57</td>
<td>−0.50</td>
<td>−1.2, 0.16</td>
</tr>
<tr>
<td>D</td>
<td>302</td>
<td>27</td>
<td>0.52</td>
<td>−0.008, 1.0</td>
</tr>
<tr>
<td>CASA motile</td>
<td>285</td>
<td>58</td>
<td>−1.0</td>
<td>−2.0, −0.13</td>
</tr>
<tr>
<td>CASA immotile</td>
<td>285</td>
<td>26</td>
<td>0.89</td>
<td>−0.007, 1.8</td>
</tr>
</tbody>
</table>

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.
a study based on Danish first pregnancy

decrease in the percentage of motile sperma-
tones with 10 ng/g lipid implied a 1%
which implies a lower free testosterone frac-
tion. Furthermore, the increase in SHBG concen-
tration of SHBG is known to be affected by the
(dioxins and dioxin-like PCB have more of
potential endocrine disrupters. Some PCB
meters 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
(Toppap et al. 1996). Other compounds,
such as the main p,p’-dichlorodiphenyl-
trichloroethane (DDT) metabolite 1,1-
dichloro-2,2-bis(p-chlorophenyl)ethene
(DDE), are anti-androgenic. Hepatic synthe-
sis of SHBG is known to be affected by the
esterogen–andro
gen 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 PCBs,
for which CB-153 serves as a proxy marker.
Furthermore, the increase in SHBG concen-
tration—by rising serum CB-153—was not
accompanied by rising testosterone levels,
which implies a lower free testosterone frac-
tion 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 sperma-
tooza 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 contami-
nant 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 concentra-
tion 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 evalua-
tion, 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 (Hauzer 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 popu-
lation, 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 con-
sumption 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 reproduc-
sive 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 nec-
essarily 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 represen-
tative 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SHBG (nmol/L)</th>
<th>Crude</th>
<th>95% CI</th>
<th>β Adjusted</th>
<th>95% CI</th>
<th>Crude</th>
<th>95% CI</th>
<th>Estradiol (pmol/L)</th>
<th>Crude</th>
<th>95% CI</th>
<th>β Adjusted</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB-153 (ng/g lipid)</td>
<td>0.08</td>
<td>0.47</td>
<td>1.21</td>
<td>0.56</td>
<td>0.18–0.94</td>
<td>-0.024</td>
<td>0.035–0.013</td>
<td>-0.82</td>
<td>1.5–0.17</td>
<td>-0.66</td>
<td>1.4–0.028</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.09</td>
<td>1.3</td>
<td>0.61</td>
<td>-0.77</td>
<td>1.1–0.42</td>
<td>0.016</td>
<td>0.0061–0.025</td>
<td>0.66</td>
<td>0.067–1.3</td>
<td>0.46</td>
<td>0.16–1.1</td>
<td></td>
</tr>
</tbody>
</table>

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

*Estimated 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 the semen in the 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. CB-153 had any effect on whether a subject managed to deliver semen in the presample period. Some tentative support for weak negative effects of CB-153 exposure on sperm motility and free testosterone levels in young men. CB-153 had any effect on whether a subject managed to deliver semen in the presample period.

References


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