ASSOCIATONS BETWEEN SERUM LEVELS OF 2,2´, 4,4´, 5,5´-HEXACHLORBIPHENYL (CB-153) AND MARKERS OF REPRODUCTIVE FUNCTION IN YOUNG SWEDISH MALES

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Introduction

A time-related deterioration in male reproductive function due to exposure to endocrine disrupters, including persistent organochlorines (POC), has been hypothesized[1,2]. There is evidence from animal studies that POC, even in low doses, can affect the male reproductive function[3-5]. A few small human studies concerning male reproduction have indicated an association between exposure to PCBs and abnormal sperm count and motility[6-8]. No previous study has, however, been performed on men randomly recruited from the general population.

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

Methods and Materials

302 young (median age 18, range 18-21) Swedish males that participated in the compulsory medical health examination prior to military service, accepted to participate. Their present smoking habits were recorded. Testicular volume was measured by ultrasound. Venous blood was sampled and analyzed for follicle stimulating hormone (FSH), luteinizing hormone (LH), sexual hormone binding globulin (SHBG), testosterone, inhibin B and oestradiol in serum, using routine techniques. We analysed 2,2´, 4,4´, 5,5´-hexachlorbiphenyl (CB-153) in serum as a proxy exposure biomarker. Briefly, the CB-153 was extracted from the serum by solid phase extraction (Isolute ENV+; IST, Hengoed, UK) using on-column degradation of the lipids and analysis by gas chromatography mass spectrometry. $^{13}C_{12}$-labeled CB-153 was used as an internal standard. The CB-153 concentrations were adjusted for total serum lipid concentration determined by enzymatic methods and expressed as ng/g lipids.

Each subject provided a semen sample by masturbation. They had been asked to keep an abstinence period of at least 48 hours, but the actual length of the actual abstinence period was recorded (Table 1). The semen samples were analyzed according to the WHO's recommendations[9]. Sperm concentration was assessed by use of a modified Neubauer chamber and positive displacement pipettes were used for proper dilution of the ejaculate. For conventional, manual determination of sperm motility 200 sperms were scored in categories A, B, C and D, with A corresponding to rapid progressive motility, B to slow progressive motility, C to non-progressive motility and spermatozoa given score D being immotile. Sperm motility was also assessed by use of CRISMAS® computer-aided sperm motility analyzer (CASA)[10].

Bivariate associations were evaluated by Pearson's correlation coefficient. The effects of CB-153 on the sperm and the hormone were evaluated by linear regression models, adjusting for potential confounders; BMI, length of abstinence period, and smoking habits (smokers [n=218] *vs.* non-smokers [n=87]). 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).

Results and Discussion

We found weak but statistically significant, negative correlations between CB-153 levels and both the free testosterone (testosterone/SHBG ratio) level ($r=-0.25$, $p<0.001$) and CASA sperm motility ($r= -0.13$, $p=0.02$) (Table 2). The same correlation, close to the level of statistical significance, was observed also for conventional motility parameters. These correlations were still evident after adjustments for potential confounders. An increase in CB-153 levels by 10 ng/g lipid was calculated to corresponded to a 1.0 % decrease in the percentage of CASA motile sperms (95% CI: -2.0, - 0.13) and was accompanied by an equal increase in the percentage of immotile cells. No statistically significant association with other markers of male reproductive function was found.

The present study gives some tentative support for weak negative associations between CB-153 in serum and sperm motility and free testosterone levels, respectively, 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.

Acknowledgments

The study was economically supported by the Swedish Environmental Protection Agency, the Swedish governmental founding for clinical research, Crafoordska Fund, Ove Tulefjords Fund and Foundation for Urological research.

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Table 1. Andrological parameters, CB-153 and hormonal levels in 302 young men from the general Swedish population.

	Reference interval	N	Mean (SD)	Median	Range
CB 153 (ng/g lipid)	-	305	68 (29)	65	23-250
BMI (kg/m^2)	18.5-24.9	305	22 (3.2)	22	15-42
Abstinence period (h)	-	302	85 (57)	67	12-504
Testis volume (mL)	-	305	29 (7.7)	29	13-53
Semen volume (mL)	>2.0	302	3.2 (1.3)	3.2	0.3-8.4
Sperm concentration ($x\ 10^6$/mL)	>20.0	302	72 (66)	54	0.1-390
Total sperm count ($x\ 10^6$)	>40	302	210 (180)	167	0.5-1200
Sperm motility (%) A+B	>50	302	54 (17)	56	0-85
D	-	302	31 (13)	29	6-99
CASA motile	-	285	51 (22)	51	0-100
CASA immotile	-	285	32 (23)	29	0-100
Inhibin B (ng/L)	100-240	305	210 (62)	200	54-420
FSH (IU/L)	1.0-10.5	305	3.5 (1.5)	3.0	0.5-12.5
LH (IU/L)	1.2-9.6	305	4.2 (1.8)	4.2	1.2-10.2
Testosterone (nM)	8.7-33	305	23 (5.3)	23	6.1-38
SHBG (nM)	13-50	305	28 (9.7)	28	7.2-67
Testosterone/SHBG	-	305	1.0 (0.5)	1.2	0.1-2.1
Estradiol (pM)	60-150[c]	305	80 (17)	77	43-144

Table 2. Correlation coefficients (Pearson's r) between serum concentrations of CB-153 and reproductive parameters in 302 young Swedish men.

	N	CB-153 (ng/g lipid)	
		r	p-value
Testis volume (mL)	305	0.02	0.7
Semen volume (mL)	302	-0.01	0.4
Sperm concentration (x 10^6/mL)	302	<0.01	1.0
Total sperm count (x 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