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Association between serum levels of organochlorine pesticides and sex hormones in adults living in a heavily contaminated area in Brazil

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ABSTRACT

Background: Several studies have investigated the effects of organochlorine (OC) pesticides on adverse reproductive outcomes. However, few previous studies explored their effects on sex hormones.
Objective: To examine the association between serum concentrations of OC pesticides and levels of sex hormones in adult population in a rural area in Brazil heavily contaminated with these pesticides.
Methods: A cross-sectional study with 304 men and 300 women was undertaken. Wet weight serum concentrations of 19 OC pesticides (dichloro-diphenyl-trichloroethane [DDT] and hexachlorocyclohexane [HCH], among others) were determined in all participants. Testosterone levels were obtained for men and estradiol, progesterone, prolactin, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) for women. Associations between OC pesticides and sex hormones were evaluated using linear regression models.
Results: Prevalence of women with non-physiological hyperprolactinemia was 4%. After adjusting for serum lipids and confounders, heptachlor and *o,p′*-DDT concentrations in men were associated with lower testosterone levels, while peri- and postmenopausal women ($N = 77$) showed inverse associations between LH and hexachlorobenzene (HCB), *p,p′*-DDT, *p,p′*-DDD (dichloro-diphenyl-dichloroethane), endosulfan 1 and 2, aldrin and mirex, as well as between FSH and *p,p′*-DDD, endosulfan 1 and aldrin. Premenopausal women ($N = 210$) did not show statistically significant associations between OC pesticides and sex hormones.
Conclusions: Inverse associations between OC pesticide concentrations and testosterone in men and LH and FSH in peri-/postmenopausal women, together with the high proportion of women with elevated prolactin, suggest that these OC compounds may have triggered anti-androgenic effects in men and estrogenic effects in women in this population.

Introduction

Sex hormones are steroids that interact with androgen or estrogen receptors (Guerreiro, 2009). Among them, testosterone is the major reproductive hormone in human males, playing an important role in puberty development and secondary sex characteristics, normal sexual functioning, and overall physical and psychological well-being. 17β-estradiol is the predominant estrogen during female reproductive life and has a critical impact on reproductive and sexual functioning, also affecting other organs and tissues, including the bones. Another critical steroid hormone in humans is progesterone, which is involved in female fertility, pregnancy, and embryogenesis. Additional non-steroid hormones constitute the reproductive axis: the gonadotropins LH (luteinizing hormone) and FSH (follicle-stimulating hormone) regulate ovarian hormone production and ovulation, and prolactin stimulates the mammary glands to produce milk during the postpartum period.

Organochlorine (OC) pesticides are ubiquitous and highly persistent pollutants. Although the use of OC pesticides has been banned in many countries, they still remain in the environment and in human tissues (Needham et al., 2005). It has been reported that a number of OC pesticides are able to interfere with estrogenic and androgenic activity (Andersen et al., 2002; Briz et al., 2011; Danzo, 1997; Kelce et al., 1995; Ralph et al., 2003; Walsh and Stocco, 2000), with the strongest evidence for metabolites

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of DDT (dichloro-diphenyl-trichloroethane), which exhibit potent estrogen agonism (*o*,*p*′-isomers) and androgen antagonism (*p*,*p*′-isomers) *in vitro* and *in vivo* (ATSDR, 2002; Kelce et al., 1995; Sohoni and Sumpter, 1998).

Since OC pesticides may interfere with sex hormones activity, human exposure to these compounds might affect male and female reproductive function (Crain et al., 2008; Sharpe, 1995). In fact, most epidemiological studies on health effects of endocrine-disrupting chemicals explored outcomes such as decreased sperm quality, male reproductive tract abnormalities, puberty onset, altered menstrual cyclicity, early menopause, infertility, and reproductive cancers (Crain et al., 2008; Toft et al., 2004). However, studies evaluating the effect of exposure to OC pesticides on reproductive hormone levels are scarce, particularly in women (Perry et al., 2006; Windham et al., 2005). Studies in men are more numerous but are mostly focused on DDT exposure and have shown inconsistent results (Asawasinsopon et al., 2006; Blanco-Muñoz et al., 2012; Cocco et al., 2004; Dalvie et al., 2004; Ferguson et al., 2012; Giwercman et al., 2006; Goncharov et al., 2009; Hagmar et al., 2001; Martin et al., 2002; Rylander et al., 2006).

The present study aimed to examine the association between serum concentrations of several OC pesticides and levels of sex hormones in adult men and women in a rural village in Southeast Brazil heavily contaminated with these pesticides over the past decades.

Materials and methods

Study population

Cidade dos Meninos is a rural village located in the county of Duque de Caxias, state of Rio de Janeiro, Brazil. In the late 1940s, a factory was set up in this village for the production of hexachlorocyclohexane (HCH) and other OC pesticides, such as DDT and hexachlorobenzene (HCB), for vector control programs. The factory was closed in 1961 and the remaining pesticide products were abandoned outdoors in the vicinity of the plant (Ministério da Saúde, 2003). Since then, local residents have been exposed to these chemicals through contaminated soil, water, and local food (Asmus et al., 2008).

In 2003–2004, a population-based survey was conducted in Cidade dos Meninos to investigate residents' serum levels of OC pesticides. Population participating in the survey has been previously described (Freire et al., 2012). Briefly, 359 out of 381 contacted families agreed to participate in the study. Participation rate was 96%. Serum samples from 995 subjects, of which 787 (397 men, 390 women) were adults (>14 years old), were analyzed for OC pesticides and several biochemical and hematological parameters. An electricity blackout resulted in loss of 23% of the serum samples, limiting the study to 609 adults (306 men and 303 women) with completed information on OC pesticides and sex hormones levels. For the present analysis, three women >70 years old presenting abnormally low gonadotropins levels and two men with missing information on covariates were excluded. Thus, this study consists of a cross-sectional analysis of 304 men and 300 women with available data on serum levels of OC pesticides and sex hormones, which completed information on covariates. The study was approved by the Ethics Committee of the National School of Public Health/FIOCRUZ and a signed informed consent was obtained from all recruited participants.

Interviews

A questionnaire on information about sociodemographic characteristics and lifestyle was completed by study subjects through face-to-face interviews. Questionnaires were administered by trained staff after blood sample collection and without previous knowledge on participants' exposure status. The following variables have been used in this study: age (years), self-declared ethnicity (white or non-white), years residing in Cidade dos Meninos, period of life in Cidade dos Meninos (residents whose pregnancy occurred in Cidade dos Meninos, residents that moved to the study area at age 1–14 years; or residents moving at age >14 years), current alcohol use (any or none), smoking (never, ex-smoker, or current smoker), weight (kg), and height (cm). Women also provided information on age at menarche (years), breastfeeding history (any or none), and parity (number of liveborn infants).

Laboratory analysis

Fasting blood samples were collected before 10:00 a.m. at the local health unit. Concentrations of OC pesticides were determined by gas chromatography with electron-capture detection at the laboratory of the National School of Public Health/FIOCRUZ. The following analytes were determined: HCH (alpha, beta and gamma isomers), HCB, chlordane (alpha and gamma isomers), *trans*-nonachlor, heptachlor, DDT metabolites (*p*,*p*′-DDE, *o*,*p*′-DDT, *p*,*p*′-DDT, and *p*,*p*′-DDD), endosulfan 1 and 2, aldrin, endrin, dieldrin, methoxychlor, and mirex. Further information about sample collection, analytical methodology and detection limits (LD) have been previously reported (Freire et al., 2012; Sarcinelli et al., 2003).

Concentrations of total cholesterol and triglycerides (in mg/dl) were determined by colorimetric enzymatic methods at the National Institute of Cancer (INCA), Rio de Janeiro. Serum concentrations of total testosterone, estradiol, progesterone, prolactin, LH, and FSH were measured by chemiluminiscence assay using ELISA kit (AlkaTecnologia®, São Paulo, Brazil) at the INCA. Laboratory reference values for sex hormones are presented in Supplemental Material (Table S1).

Information on women's reproductive status (*i.e.* premenopause, perimenopause and postmenopause), menstrual cycle phase, pregnancy status, and suspected use of oral contraceptives, as well as the presence of any abnormal hormonal profile, was obtained through the examination of individual hormone levels by an endocrinologist (R.C.). Peri- and postmenopausal status was defined as having high FSH, normal or low follicular-phase estradiol levels, respectively, and progesterone lower than 1.0 ng/ml (Harlow et al., 2012). Women of reproductive age presenting very high levels of estradiol, progesterone and prolactin were assumed to be pregnant. The menstrual cycle phase was considered to be luteal when progesterone levels were >1.2 ng/ml and was considered follicular when progesterone levels were $\leq$1.2 ng/ml, both together with estradiol and gonadotropin levels within the reference values. Use of oral contraceptives was suspected when gonadotropins, estradiol and progesterone levels were below the reference ranges (see Supplemental material).

Statistical analysis

Pesticide concentrations that were below the LD were substituted with the midpoint value between zero and the LD of each compound. Pesticide concentrations were treated as continuous variables since all of them but methoxychlor (which was categorized into values below and above the LD) were detected in more than 60% of the samples. Statistical analysis in women was stratified by menopausal status, dividing the sample into two groups: premenopausal women and peri-/postmenopausal women. Chi-square and Mann–Whitney tests were used to examine differences in characteristics of study population between genders, in sex hormone levels between age strata (men) or menopausal status (women), and in OC pesticide concentrations between genders and menopausal status.

Linear regression analyses were performed to examine crude and adjusted associations between characteristics of study population, OC pesticides and reproductive hormones, using natural-logarithm transformed hormone levels. Women with a hormonal profile compatible with pregnancy ($N=9$) of oral contraceptive use ($N=4$) were excluded from regression analyses. A separate model was built for each particular OC pesticides, including the wet-weight serum level of the pesticide and the serum lipid content (*i.e.* the sum of cholesterol and triglycerides levels) as independent variables. Associations between each OC pesticide and each sex hormone were adjusted by confounders that were chosen on the basis of bivariate associations ($p<0.10$) with OC pesticide concentrations and/or hormone levels.

In a second analysis, selected OC pesticides (beta-HCH and *p,p′*-DDE) were categorized into quartiles of concentrations to examine changes in sex hormone levels across exposure groups. Further multivariate analysis of data was conducted stratifying the study population according to three different windows of exposure, *i.e.* residents whose pregnancy occurred in Cidade dos Meninos and had lived there since birth; those moving to the study area during childhood (1–14 years); or residents that moved during adulthood (≥15 years).

A significance level of 0.05 was established. SPSS version 17.0 (SPSS Inc., Chicago, IL, US) was used for the analyses.

Results

Both men and women had a mean age of 39 years, had lived in Cidade dos Meninos for an average time of 23 years, had a BMI around 25 kg/m^2, and were mostly non-whites (Table 1). Almost 30% of participants were born in Cidade dos Meninos and half of them moved to the study area during adulthood. Most men and women were never smokers, although men were more likely to smoke than women ($p=0.005$). Half of the men and 24% of women reported drinking alcohol ($p<0.001$). Among females, mean age at menarche was 13 years, 36% of women had given birth to 3 or more children, 66% of them had breastfed anytime, and a peri-/postmenopausal profile was seen in 77 women (26%).

Testosterone in men decreased steadily from 20 years old on (p-trend = 0.001), except for individuals older than 80 years, who presented higher levels than younger men (Table 2). The prevalence of testosterone levels below normal values increased from 30 years old on, either using the lower limit of the testosterone kit used or a population-based reference value (Bhasin et al., 2011). Testosterone levels also decreased with number of years living in Cidade dos Meninos and with BMI (Table S2).

Hormonal profile of females showed that peri-/postmenopausal women had significantly lower levels of estradiol, progesterone and prolactin, and significantly higher LH and FSH, compared to premenopausal women (Table 3). Non-physiological hyperprolactinemia, characterized by high prolactin and very high estradiol and progesterone levels, was present in 12 women (4.0%) (data not shown). The highest prolactin levels (>50 ng/ml) were observed in women >30 years old, while minor elevations were seen in women <25 years old. In premenopausal women, those with higher parity had lower progesterone and higher LH, smokers had lower prolactin than never smokers, and current alcohol consumers had lower LH levels (Table S3). In addition, age at menarche was positively associated with prolactin, while years living in Cidade dos Meninos were positively associated with FSH and BMI. Regarding peri-/postmenopausal women, ex-smokers had higher levels of estradiol, while smokers had higher progesterone (Table S4). LH and FSH were inversely associated with BMI in these women, as well as they were higher among females who did not breastfeed. FSH was higher in peri-/postmenopausal women with > 2 children.

Table 1
Characteristics of study population.

	Men	Women
Sample size	304	300
Age (years), mean (SD, range)	39 (17, 15–94)	39 (17, 15–92)
Self-declared ethnicity, *n* (%)		
White	93 (30.6)	92 (30.7)
Non white	211 (69.4)	208 (69.3)
Years in Cidade dos Meninos, mean (SD, range)	23 (15, <1–61)	23 (14, <1–64)
Lifetime in Cidade dos Meninos, *n* (%)		
Born in Cidade dos Meninos	82 (27.0)	88 (29.3)
From 1 to 14 years	75 (24.7)	57 (19.0)
From ≥15 years	147 (48.3)	155 (51.7)
BMI (kg/m^2), mean (SD, range)	24.9 (4.3, 15.0–39.7)	25.2 (5.7, 14.5–50.8)
Alcohol consumption, *n* (%)		
No	142 (46.7)	228 (76.0)
Yes	162 (53.3)*	72 (24.0)
Smoking habit, *n* (%)		
Never	189 (62.2)	220 (73.3)
Ex-smoker	50 (16.4)	43 (14.3)
Current smoker	65 (21.4)*	37 (12.3)
Cholesterol (mg/dl), mean (SD, range)	175 (68, 37–399)	187 (49, 57–385)
Triglycerides (mg/dl), mean (SD, range)	127 (108, 29–468)	102 (58, 17–431)
Menarche age (years), mean (SD, range)	–	13 (9, 9–20)
Menopausal status, *n* (%)		
Premenopausal	–	223 (74.3)
Peri- and postmenopausal	–	77 (25.7)
Parity, *n* (%)		
None	–	84 (28.0)
1	–	45 (15.0)
2	–	64 (21.3)
≥3	–	107 (35.7)
Breastfeeding, *n* (%)		
Ever	–	197 (65.7)
Never	–	103 (34.3)

SD: standard deviation.
* $p<0.05$.

Additional information on sex hormone profiles is presented in Supplemental Material (Appendix 2).

Regarding serum concentrations of OC pesticides, *p,p′*-DDE and beta-HCH showed the highest prevalence both in men (>99%) and total women (>97%), followed by gamma-HCH, alpha-HCH, *p,p′*-DDT, and HCB (Table 4). Likewise, the highest concentrations in both sexes were observed for *p,p′*-DDE and beta-HCH, which were particularly high among peri-/postmenopausal women. In addition to *p,p′*-DDE and beta-HCH, significantly higher concentrations of HCB, *p,p′*-DDT, *p,p′*-DDD and methoxychlor were observed in peri-/postmenopausal women than premenopausal ones (Table 4). Except for HCB, aldrin, and methoxychlor, most correlations between pesticide concentrations were positive (Spearman correlation coefficients, $r>0.60$) and statistically significant ($p<0.05$). Correlation coefficient between beta-HCH and *p,p′*-DDE, which were amongst the compounds showing the highest prevalence and serum levels, was $r=0.68$ in males ($p<0.001$) and $r=0.72$ in females ($p<0.001$).

Crude associations between beta-HCH and characteristics of study population are shown in Supplemental Material (Tables S2–S4). Beta-HCH was positively related with age and years living in Cidade dos Meninos in men (Table S2) and women (Tables S3 and S4), and with males' BMI (Table S2). Among premenopausal women, ex-smokers had higher concentrations of beta-HCH than never smokers, whereas non-white peri-/postmenopausal women had lower concentrations than white ones. Serum concentrations of most of the remaining pesticides were associated with age,

Table 2
Serum levels of testosterone (ng/dl) in men.

	N	Mean	SD	P25	P75	Range	Below 262 ng/dl[a]	Below Bhasin's P25[b]
All men	304	536	206	405	621	184–1600	14/304 (4.6%)	–
Age (years)								
15–19	36 (11.8%)	552	177	457	669	225–1076	1/36 (2.7%)	–
20–29	65 (21.4%)	612	229	484	704	284–1600	0/65	<295 = 1/65 (1.5%)
30–39	60 (19.7%)	526	194	413	615	221–1144	3/60 (5.0%)	<265 = 3/60 (5.0%)
40–49	64 (21.0%)	502	178	391	614	229–1248	3/64 (4.7%)	<267 = 4/64 (6.2%)
50–59	41 (13.5%)	456	184	324	533	188–1054	3/41 (7.3%)	<238 = 3/41 (7.3%)
60–69	22 (7.2%)	543	220	417	640	184–1157	2/22 (9.1%)	<243 = 2/22 (9.1%)
70–79	9 (3.0%)	501	235	296	689	229–916	2/9 (22.2%)	<203 = 2/9 (22.2%)
≥80	7 (2.3%)	636	314	369	776	348–1226	0/7	<196 = 0/7

SD: standard deviation; P25, P75: 25th and 75th percentiles.
[a] Laboratory reference for young men (<20 years).
[b] Value of the 2.5th percentile in a community-based sample of 3352 men older than 20 years (Bhasin et al., 2011).

Table 3
Serum levels of sex hormone in women.

	Mean	SD	P25	P75	Range
Estradiol (pg/ml)					
All women	138.8	370.8	20.0	88.7	20.0–2000[a]
Premenopausal	178.95	423.12	20.0	129.0	20.0–2000
Peri- and postmenopausal	22.95*	9.11	20.0	20.0	20.0–69.1
Progesterone (ng/ml)					
All women	3.52	7.55	0.20	1.51	0.20–40.00[a]
Premenopausal	4.65	8.47	0.22	6.36	0.20–40.00[a]
Peri- and postmenopausal	0.24*	0.09	0.20	0.26	0.20–0.75
Prolactin (ng/ml)					
All women	14.41	24.21	4.55	12.95	0.50–150.0
Premenopausal	17.35	27.40	5.03	15.60	0.50–150.0
Peri- and postmenopausal	5.88*	3.89	3.71	6.88	1.00–26.90
Luteinizing hormone (LH) (mU/ml)					
All women	13.52	15.35	2.62	20.20	0.10–81.40
Premenopausal	7.24	9.52	2.14	8.48	0.10–63.60[b]
Peri- and postmenopausal	31.70*	14.56	20.70	38.05	8.16–81.40
Follicle stimulating hormone (FSH) (mU/ml)					
All women	26.89	37.86	3.24	43.72	0.10–170.0
Premenopausal	7.66	12.14	2.19	7.71	0.10–121.0[b]
Peri- and postmenopausal	82.59*	31.31	56.95	100.0	32.30–170.0

P25, P75: 25th and 75th percentiles.
* $p < 0.001$.
[a] Including pregnant women.
[b] Ovulatory peak.

Table 4
Serum concentrations of OC pesticides (ng/ml).

	Men (*N* = 304)				Premenopausal women (*N* = 223)				Peri-/postmenopausal women (*N* = 77)			
	%>LD	Median	P25	P75	%>LD	Median	P25	P75	%>LD	Median	P25	P75
Alpha-HCH	95.5	2.52	0.97	0.65	95.1	2.77	1.00	6.09	94.8	2.43	1.14	6.08
Beta-HCH	99.8	6.00	2.08	15.4	97.1	6.32	2.45	14.43	98.7	11.72*	4.80	36.29
Gamma-HCH	96.0	0.95	0.44	2.21	91.9	0.89	0.36	2.28	96.1	1.07	0.57	2.04
HCB	88.0	0.33	0.14	0.63	94.2	0.36	0.14	0.61	97.4	0.42*	0.24	0.78
Alpha-chlordane	73.8	0.23	<LD	0.51	73.5	0.25	<LD	0.59	77.9	0.30	0.11	0.62
Gamma-chlordane	66.3	0.16	<LD	0.44	67.7	0.16	<LD	0.38	66.7	0.16	<LD	0.41
Trans-nonachlor	82.5	0.32	0.16	0.77	82.5	0.36	0.19	0.76	88.3	0.44	0.24	0.82
Heptachlor	71.2	0.31	<LD	0.89	74.3	0.36	<LD	0.91	68.4	0.33	<LD	0.66
p,*p*'-DDE	99.1	8.32	2.86	21.9	97.8	7.95	2.96	21.81	98.7	20.64*	6.21	65.60
o,*p*'-DDT	58.0	0.30	<LD	0.89	64.6	0.44	<LD	1.06	63.6	0.38	<LD	1.23
p,*p*'-DDT	93.7	3.09	0.94	6.96	91.5	3.00	1.02	7.30	97.4	4.72*	1.24	10.67
p,*p*'-DDD	80.9	0.61	0.19	1.34	78.5	0.59	0.19	1.19	93.5	0.87*	0.31	1.79
Endosulfan 1	63.0	0.22	<LD	0.46	60.5	0.22	<LD	0.42	70.1	0.23	<LD	0.51
Endosulfan 2	61.9	0.23	<LD	0.71	62.8	0.22	<LD	0.75	70.1	0.25	<LD	0.62
Aldrin	86.5	1.89	0.73	11.0	86.5	2.11	0.76	13.35	94.8	3.79	0.98	20.05
Endrin	87.4	0.63	0.24	1.48	87.9	0.59	0.27	1.57	87.0	0.54	0.23	1.38
Dieldrin	90.1	0.61	0.28	1.27	89.2	0.55	0.23	1.28	89.6	0.60	0.27	1.21
Mirex	68.3	0.47	<LD	0.97	70.0	0.39	<LD	1.04	77.9	0.48	0.21	0.95
Methoxychlor	32.5	<LD	<LD	0.27	38.6	<LD	<LD	0.31	51.9	<LD*	<LD	0.53

LD: limit of detection; P25, P75: 25th and 75th percentiles.
* $p < 0.05$ for differences in means between premenopausal and peri-/postmenopausal women.

Table 5
Adjusted regression coefficients (β) and 95% confidence intervals (CI) for the association between OC pesticides and log-transformed testosterone concentrations (ng/dl) in men.

OC pesticides (ng/ml)	Testosterone[a]	
	β	95% CI
Alpha-HCH	−0.002	−0.005, 0.00
Beta-HCH	−0.001	−0.002, 0.00
Gamma-HCH	−0.009	−0.02, 0.001
HCB	0.00	−0.005, 0.004
Alpha-chlordane	−0.02	−0.06, 0.02
Gamma-chlordane	−0.02	−0.07, 0.02
Trans-nonachlor	−0.03	−0.08, 0.02
Heptachlor	−0.03	−0.04, −0.009
p,p'-DDE	−0.001	−0.002, 0.00
o,p'-DDT	−0.02	−0.05, −0.003
p,p'-DDT	−0.001	−0.005, 0.003
p,p'-DDD	0.004	−0.02, 0.03
Endosulfan 1	−0.01	−0.07, 0.04
Endosulfan 2	0.003	−0.03, 0.03
Aldrin	0.002	−0.001, 0.003
Endrin	−0.01	−0.03, 0.007
Dieldrin	−0.01	−0.03, 0.01
Mirex	−0.003	−0.03, 0.02
Methoxychlor[b]	0.03	−0.05, 0.12

[a] Each line represents one separate model, adjusted for age, ethnicity, years in Cidade dos Meninos, BMI and smoking.
[b] Methoxychlor was treated as a dichotomous variable, categorized in levels below and above the LD.

ethnicity and years living in the study area in both sexes (data not shown).

In adjusted regression analysis, serum concentrations of OC pesticides in men were associated with decreasing testosterone, although associations were only statistically significantly for heptachlor and *o,p*′-DDT, and were of borderline significant for alpha-HCH, beta-HCH and *p,p*′-DDE (Table 5). In women, statistically significant associations between OC pesticide concentrations and sex hormones were not observed among premenopausal ones (Table 6). In contrast, peri-/postmenopausal women showed negative associations between OC pesticides and levels of gonadotropins, which were particularly consistent for LH (Table 7). Thus, statistically significant associations were found between decreased LH and concentrations of HCB, *p,p*′-DDT, *p,p*′-DDD, endosulfan 1 and 2, aldrin and mirex, and between decreased FSH and *p,p*′-DDD, endosulfan 1 and aldrin. In addition, estradiol was positively associated with aldrin in peri-/postmenopausal women, as well as borderline associations were observed between LH and beta-HCH, *p,p*′-DDE and *o,p*′-DDT, and between FSH and *p,p*′-DDT in these women.

A decrease in testosterone levels across beta-HCH quartiles (p-trend < 0.001) and *p,p*′-DDE (p-trend = 0.02) was observed (Fig. 1). Decreasing LH levels were also observed across quartiles of beta-HCH ($p = 0.008$) and *p,p*′-DDE ($p < 0.001$). Changes in levels of the remaining sex hormones in women associated with beta-HCH and *p,p*′-DDE exposure groups were not statistically significant.

Results from multivariate analysis stratifying by window of exposure (using the same potential confounders but "years of residence"), were not substantially different (data not shown). Thus, association between heptachlor and lower testosterone was only seen in the group of men that moved to the study area at age 1–14 years (regression coefficient, beta = −0.03; 95% confidence interval = −0.06, −0.01), while the association with *o,p*′-DDT did not remain in the stratified analysis. Instead, methoxychlor was associated with lower testosterone in men that were born in Cidade dos Meninos (beta = −0.18; 95% CI = −0.34, −0.02). Among premenopausal women, those moving to the study area at age 15 years or after ($N = 89$) showed significant associations between *trans*-nonachor and reductions in LH (beta = −0.38; 95% CI = −0.41, −0.02) and estradiol (beta = −0.38, 95% CI = −0.39, −0.22), and between heptachlor and lower estradiol (beta = −0.16, 95% CI = −0.24, −0.11). Regarding peri-/postmenopausal women, only 9 and 7 females, respectively, were born or moved to the study area during childhood, and thus, adjusted regression coefficients were only obtained for those moving to the study area during adulthood. Associations seen in the latter group of women were similar to those observed in the non-stratified analysis, except for the association between LH and HCB, which was not observed here.

Discussion

The sex hormone profile of this adult population residing in an area with a history of heavy OC pesticide contamination was, in general, consistent with physiological concepts. In men, it is widely known that increasing age is associated with a progressive decline in testosterone levels (Allan and McLachlan, 2004). Such a decrease with age leads to a significant increase in the prevalence of male hypogonadism from 60 years old on (Harman et al., 2001). Prevalence of testosterone levels below normal values in men in Cidade dos Meninos in creased from 30 years old on, in accordance with the literature (Bhasin et al., 2011). The findings of higher levels of testosterone observed in the oldest men in the present study may reflect the higher survival of men with higher testosterone, which is in agreement with studies showing that testosterone levels are inversely related to all causes of mortality during male aging (Haring et al., 2010). Obesity has also been shown to be associated with lower testosterone levels in men (Dhindsa et al., 2010), which is consistent with our finding of an inverse association with between testosterone and BMI. Regarding females, our findings are in accordance with the pronounced decrease in estradiol and progesterone and the increase in the pituitary gonadotropins LH and FSH commonly observed after menopause (Messinis, 2006; Touitou, 1995). Likewise, prolactin levels are usually lower after menopause than in reproductive years, as observed here.

However, prevalence of hyperprolactinemia in women in this study was higher than that reported elsewhere (Biller et al., 1999). Hyperprolactinemia causes menstrual disorders, anovulation, and female infertility, and known pathological causes of excess prolactin include the use of prolactin-increasing drugs, macroprolactinemia, hypothyroidism, renal failure and pituitary adenomas (Melmed et al., 2011; Vanderpump et al., 1998). It is also well known that estrogens stimulate pituitary prolactin release, which could explain our finding of higher hyperprolactinemia in older women who may have accumulated OC compounds with estrogenic activity. Nonetheless, levels of OC pesticides in those older women with high prolactin were not significantly higher than levels observed in the younger hyperprolactinemic women (data not shown).

Inverse associations were observed between testosterone levels and OC pesticide serum concentrations in men, while peri-/postmenopausal but not premenopausal women showed associations between OC pesticides and decreased levels of gonadotropins, particularly LH. These findings were observed after adjustment for potential confounders, suggesting that the potential effect of these compounds on sex hormones may be independent of the natural hormone decline with age and BMI. In addition, the effect of OC pesticides on sex hormone levels did not seem to depend on the window time of exposure, although women that moved to the study area after childhood seem to be more susceptible.

Previous studies examining the potential effect of OC pesticides on male total testosterone levels have reported, in general, none or inverse associations between exposure and hormone levels. Serum concentrations of *p,p*′-DDE were associated with lower testosterone in Mexican flower growers (Blanco-Muñoz et al., 2012), and

Table 6
Adjusted regression coefficients (β) and 95% confidence intervals (CI) for the association between OC pesticides and log-transformed concentrations of sex hormones in premenopausal women ($N = 210$).[a]

OC pesticides (ng/ml)	Estradiol (pg/ml)[b]		Progesterone (ng/ml)[c]		Prolactin (ng/ml)[c]		LH (mU/ml)[c]		FSH (mU/ml)[c]	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Alpha-HCH	−0.002	−0.02, 0.01	0.00	−0.03, 0.03	0.003	−0.01, 0.02	−0.01	−0.03, 0.007	−0.01	−0.03, 0.004
Beta-HCH	−0.003	−0.01, 0.004	0.00	−0.01, 0.01	0.00	−0.006, 0.006	−0.005	−0.01, 0.003	0.00	−0.008, 0.008
Gamma-HCH	−0.02	−0.08, 0.04	0.00	−0.10, 0.10	−0.006	−0.06, 0.05	−0.03	−0.10, 0.03	−0.03	−0.10, 0.03
HCB	−0.04	−0.14, 0.06	0.005	−0.15, 0.16	−0.06	−0.14, 0.03	−0.06	−0.17, 0.05	−0.03	−0.13, 0.08
Alpha-chlordane	0.01	−0.19, 0.21	0.10	−0.21, 0.41	0.11	−0.06, 0.28	−0.18	−0.40, 0.03	−0.10	−0.31, 0.11
Gamma-chlordane	−0.10	−0.45, 0.25	−0.19	−0.74, 0.36	−0.11	−0.41, 0.18	−0.28	−0.66, 0.10	−0.25	−0.62, 0.12
Trans-nonachlor	−0.15	−0.38, 0.07	−0.02	−0.37, 0.36	−0.04	−0.24, 0.15	−0.14	−0.39, 0.11	0.04	−0.20, 0.28
Heptachlor	−0.02	−0.09, 0.05	−0.06	−0.17, 0.05	0.004	−0.06, 0.06	−0.03	−0.11, 0.05	0.00	−0.07, 0.08
p,p'-DDE	−0.004	−0.009, 0.002	−0.007	−0.02, 0.002	0.00	−0.006, 0.004	−0.003	−0.01, 0.003	0.00	−0.006, 0.006
o,p'-DDT	−0.08	−0.20, 0.05	−0.16	−0.35, 0.04	−0.04	−0.15, 0.07	−0.10	−0.24, 0.03	−0.04	−0.18, 0.09
p,p'-DDT	−0.01	−0.03, 0.006	−0.02	−0.05, 0.006	0.004	−0.01, 0.02	−0.01	−0.03, 0.008	0.004	−0.02, 0.02
p,p'-DDD	0.009	−0.09, 0.11	−0.02	−0.18, 0.13	0.04	−0.04, 0.12	−0.05	−0.16, 0.06	−0.005	−0.11, 0.10
Endosulfan 1	0.04	−0.27, 0.34	−0.009	−0.48, 0.47	0.07	−0.18, 0.33	−0.15	−0.48, 0.18	−0.20	−0.52, 0.12
Endosulfan 2	0.08	−0.06, 0.23	0.13	−0.10, 0.36	0.10	−0.02, 0.22	−0.07	−0.23, 0.09	−0.10	−0.26, 0.06
Aldrin	−0.001	−0.006, 0.004	0.00	−0.07, 0.07	0.004	0.00, 0.008	0.00	−0.006, 0.005	0.00	−0.005, 0.005
Endrin	0.007	−0.08, 0.09	−0.05	−0.19, 0.08	0.00	−0.07, 0.07	−0.04	−0.13, 0.06	−0.04	−0.13, 0.06
Dieldrin	0.03	−0.08, 0.14	0.005	−0.17, 0.18	0.04	−0.05, 0.13	−0.07	−0.19, 0.05	−0.08	−0.19, 0.04
Mirex	−0.02	−0.07, 0.03	−0.01	−0.10, 0.07	−0.01	−0.06, 0.04	0.02	−0.04, 0.09	0.06	0.00, 0.12
Methoxychlor[d]	0.25	−0.06, 0.57	0.24	−0.25, 0.73	0.25	−0.02, 0.52	0.14	−0.22, 0.49	−0.02	−0.36, 0.33

[a] Women with a hormone profile compatible with pregnancy or use of oral contraceptives were excluded from regression analyses.
[b] Each line represents one separate model, adjusted for age, ethnicity, years in Cidade dos Meninos, smoking, cholesterol and triglycerides serum content.
[c] Additionally adjusted for parity (progesterone), menarche age (prolactin), parity and alcohol consumption (LH) or BMI (FSH).
[d] Methoxychlor was treated as a dichotomous variable, categorized in levels below and above the LD.

Table 7
Adjusted regression coefficients (β) and 95% confidence intervals (CI) for the association between OC pesticides and log-transformed concentrations of sex hormones in peri- and postmenopausal women ($N = 77$).

OC pesticides (ng/ml)	Estradiol (pg/ml)[a]		Progesterone (ng/ml)[a]		Prolactin (ng/ml)[a]		LH (mU/ml)[b]		FSH (mU/ml)[b]	
	β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Alpha-HCH	0.002	−0.005, 0.01	0.002	−0.006, 0.009	0.002	−0.01, 0.02	−0.007	−0.02, 0.005	−0.003	−0.01, 0.007
Beta-HCH	0.001	0.00, 0.002	0.00	−0.002, 0.001	−0.002	−0.006, 0.001	−0.003	−0.006, 0.00	−0.002	−0.004, 0.001
Gamma-HCH	0.008	−0.02, 0.04	0.007	−0.02, 0.04	−0.005	−0.06, 0.05	−0.03	−0.07, 0.02	−0.02	−0.06, 0.02
HCB	0.06	−0.003, 0.13	0.06	−0.006, 0.13	−0.07	−0.22, 0.07	−0.13	−0.24, −0.02	−0.08	−0.17, 0.02
Alpha-chlordane	−0.02	−0.12, 0.09	−0.02	−0.13, 0.08	0.01	−0.21, 0.24	−0.12	−0.30, 0.05	−0.07	−0.22: 0.08
Gamma-chlordane	0.01	−0.13, 0.15	0.02	−0.12, 0.16	−0.04	−0.33, 0.25	−0.20	−0.42, 0.02	−0.11	−0.29, 0.08
Trans-nonachlor	0.10	−0.04, 0.25	0.01	−0.14, 0.16	−0.10	−0.41, 0.21	−0.17	−0.41, 0.07	−0.09	−0.29, 0.11
Heptachlor	−0.01	−0.08, 0.06	−0.003	−0.07, 0.07	−0.009	−0.15, 0.13	−0.10	−0.21, 0.01	−0.05	−0.14, 0.04
p,p'-DDE	0.00	0.00, 0.001	0.00	0.00, 0.001	0.00	−0.002, 0.001	−0.001	−0.002, 0.00	0.00	−0.001, 0.001
o,p'-DDT	−0.02	−0.06, 0.03	0.003	−0.04, 0.05	−0.003	−0.10, 0.09	−0.07	−0.15, 0.00	−0.04	−0.10, 0.02
p,p'-DDT	0.002	−0.003, 0.006	0.001	−0.003, 0.006	−0.002	−0.01, 0.007	−0.01	−0.02, −0.003	−0.006	−0.01, 0.00
p,p'-DDD	0.03	−0.01, 0.08	−0.004	−0.05, 0.04	0.02	−0.07, 0.12	−0.09	−0.17, −0.02	−0.09	−0.15, −0.03
Endosulfan 1	0.005	−0.13, 0.14	0.007	−0.13, 0.14	0.03	−0.25, 0.32	−0.24	−0.46, −0.03	−0.23	−0.41, −0.05
Endosulfan 2	0.02	−0.05, 0.09	0.006	−0.06, 0.08	0.04	−0.11, 0.19	−0.14	−0.25, −0.03	−0.08	−0.17, 0.01
Aldrin	0.006	0.001, 0.01	0.003	−0.002, 0.007	−0.002	−0.01, 0.008	−0.01	−0.02, −0.003	−0.007	−0.01, −0.001
Endrin	0.008	−0.05, 0.07	0.007	−0.05, 0.07	0.02	−0.10, 0.14	−0.09	−0.19, 0.01	−0.05	−0.13, 0.03
Dieldrin	0.01	−0.05, 0.07	0.01	−0.05, 0.07	−0.006	−0.14, 0.13	−0.07	−0.18, 0.03	−0.06	−0.15, 0.03
Mirex	0.01	−0.02, 0.05	0.01	−0.02, 0.04	0.001	−0.07, 0.07	−0.07	−0.12, −0.02	−0.04	−0.08, 0.006
Methoxychlor[c]	0.12	−0.004, 0.24	0.06	−0.07, 0.19	−0.06	−0.33, 0.20	0.05	−0.16, 0.26	−0.03	−0.06, 0.01

[a] Each line represents one separate model, adjusted for age, ethnicity, years in Cidade dos Meninos, smoking, cholesterol and triglycerides serum content.
[b] Additionally adjusted for BMI and breastfeeding.
[c] Methoxychlor was treated as a dichotomous variable, categorized in levels below and above the LD.

in adult Thai men (Asawasinsopon et al., 2006). A negative correlation between *p,p*′-DDE and the bioavailable/total testosterone ratio was found in young Mexican men (Ayotte et al., 2001). For their part, Martin et al. (2002) found a decrease in testosterone levels and in the free androgen index in male African-American farmers with the highest *p,p*′-DDE serum levels, compared to males with the lowest exposure levels. Null associations between testosterone and *p,p*′-DDE, *p,p*′-DDT, HCB or mirex were reported in other studies among adult populations in Europe and North America (Cocco et al., 2004; Ferguson et al., 2012; Goncharov et al., 2009; Hagmar et al., 2001). A unique positive relationship was observed between testosterone and DDT compounds in South-African malaria vector-control workers (Dalvie et al., 2004).

Differences in results across studies in men could be due to a difference in exposure levels, as those observed in population in Cidade dos Meninos are quite higher than most previous studies (Asawasinsopon et al., 2006; Ferguson et al., 2012; Goncharov et al., 2009). Our results seem to be in accordance with previous findings of inverse relationships, although few associations between OC pesticides and testosterone were statistically significant. Such an hormone-lowering effect, although subtle, should be considered as relevant since decreased concentrations of circulating testosterone can result in adverse health effects, including infertility (Small et al., 2007), bone density loss (Schow et al., 1997), obesity (Kenny et al., 2001), depression (Barrett-Connor et al., 1999), increased risk of metabolic syndrome (Wang et al., 2011), heart attack (Zhao and

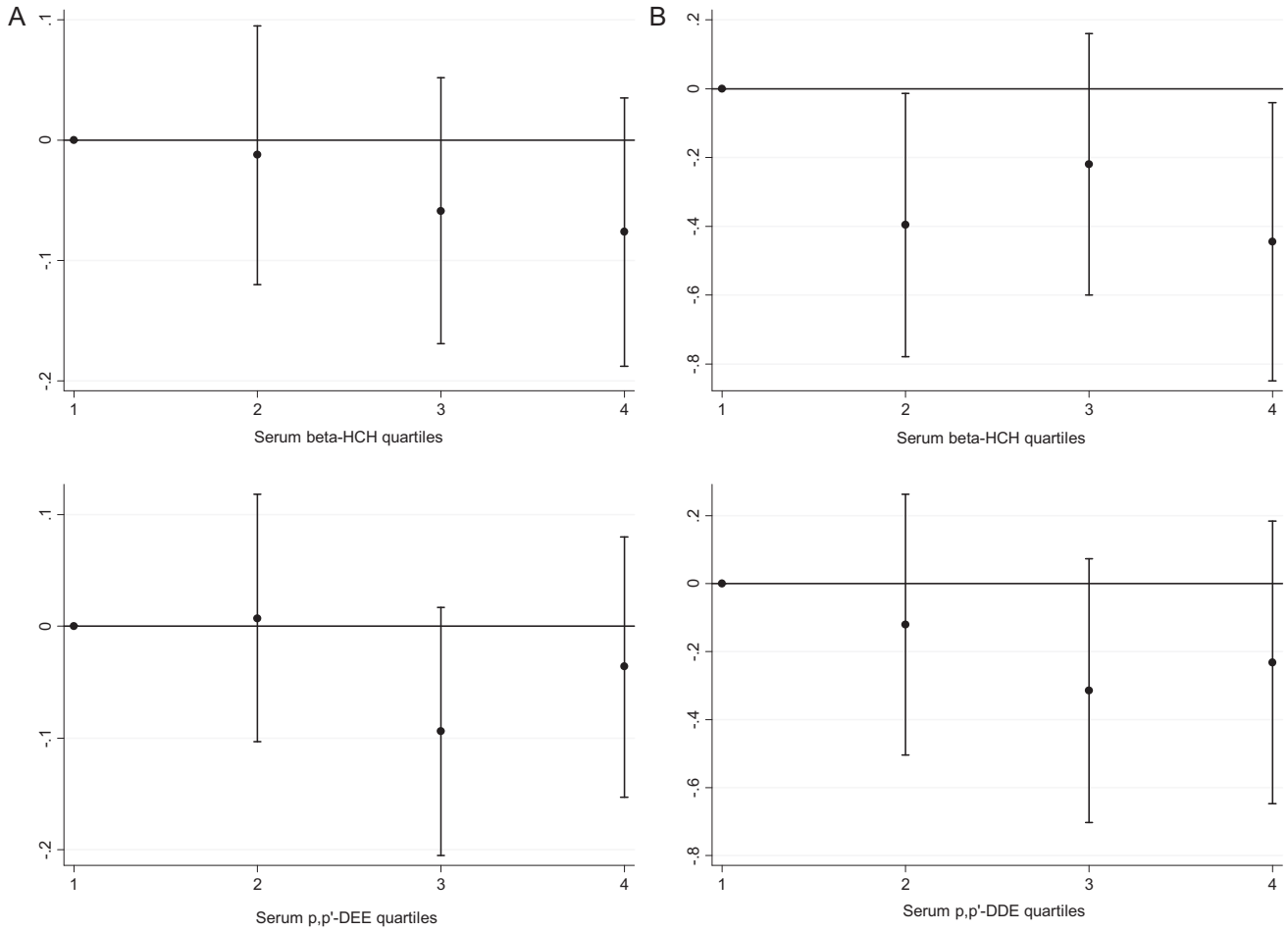

Fig. 1. Adjusted regression coefficients (dots) and 95% confidence intervals for changes in log-transformed testosterone levels (ng/dl) in men (panel A) and LH levels (mU/ml) in women (panel B) associated with quartiles of serum concentrations (ng/ml) of beta-HCH and *p,p′*-DDE.

Li, 1998) and elevated risk for Alzheimer's disease (Moffat et al., 2004). Evidence of effects different from androgen antagonism was not observed in men in the present study. For instance, experimental studies have reported that HCB has mixed agonist/antagonist androgenic actions (Ralph et al., 2003), while alpha-HCH, endosulfan, dieldrin, and lindane have shown estrogen-like effects (Briz et al., 2011; Pavlíková et al., 2012). However, because of colinearity among OC pesticides, it would be difficult to assess their possible independent effects with certainty.

In female population, one study showed inverse associations between DDT and urinary metabolites of estrogens during the periovulatory phase and with urine pregnanediol-3-glucuronide (the major metabolite of progesterone) during the luteal phase of the menstrual cycle among 287 Chinese women (Perry et al., 2006). Accordingly, a previous study of Southeast Asian immigrants in California found a significant decrease in progesterone (but not in estrogen) metabolite levels during the luteal phase with increasing *p,p′*-DDE serum levels (Windham et al., 2005). Another study in pregnant found a significant correlation between increasing DDT and HCH serum concentrations and lower LH (Liu et al., 2005). We did not find any significant reduction in estradiol or progesterone levels associated with OC pesticides, but did find significant associations with decreased LH and, to a lesser extent, FSH in peri-/postmenopause. Both LH and FSH are suppressed by endogenous or exogenous estrogens, giving support to the plausibility of the causal relation between OC pesticide exposure and decreased LH and FSH. In fact, it has been suggested that gonadotropins hormone receptors present steroidal-like binding sites (Rossi et al., 2009). In our study, LH and FSH decreases were significant in peri-/postmenopause, which may be explained by the fact that levels of endogenous estrogens are lower after menopause, thus making estrogen-binding sites more available to exogenous agonist estrogens in the menopause or the postmenopause than in non menopausal women. In addition, LH and FSH levels are elevated after the menopause, making any reduction in levels of these hormones more likely to be detected.

The present study has several limitations. First, only one blood collection was performed. However, several studies have shown that for most reproductive hormone measurements there is little temporal variability (Bjornerem et al., 2006). On the other hand, because OC serum levels reflect stored concentrations in body tissues, individual levels of OC pesticides in serum are unlikely to vary greatly over time. Second, blood samples were not dated according to each woman's menstrual cycle day. However, there is no reason to think that distribution of exposure levels has been different among women in different phases of the menstrual cycle and that the effects have been misestimated. Third, information on hormonal contraception, replacement therapy (HRT) or use of medication that could interfere with hormone levels was not available in the study population, preventing us to better understand the sex hormone profile of the study participants. For instance, elevated prolactin can be present in women using certain drugs, while menopausal women under HRT could present elevated estradiol, prolactin or progesterone levels. Fourth, information on reproductive hormones other than testosterone, such as sex-hormone binding globulin (SHBG) and estradiol, was not obtained for men

since knowledge about the potential impact of OC compounds on other male reproductive hormones was poor at the time of data collection. Indeed, information on other sex hormones would have provided further hints about the interaction of OC pesticides with the hypothalamic-pituitary-testicular axis and about the interference with testosterone metabolism and activity. Also, testosterone levels were not available for women, but commercially available testosterone kits are not sensitive enough for low values.

Conversely, this study presents some strengths. No previous study in Latin America analyzed the relationship between human exposure to endocrine-disrupting OC pesticides and sex hormones. It is also the first epidemiological investigation, to our best knowledge, exploring the potential impact of certain OC pesticides such as *trans*-nonachlor, chlordane, endosulfan or methoxychlor, on reproductive hormone levels. The large sample size (304 men and 300 women), the relatively homogeneous study population, as well as the possibility of evaluating the differential effect according to temporal windows of exposure, represent additional strengths of our study.

Conclusion

Serum concentrations of beta-HCH and *p,p′*-DDE were associated with decreased levels of testosterone in men and LH and FSH in peri- and postmenopausal women. These finding seem to support the known capacity of OC pesticides to exert estrogenic and anti-androgenic activity, affecting sex hormone systems through mechanisms of action that may be different for each individual compound.

Competing financial interests

The authors declare that there are no competing financial conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijheh.2013.07.012.

References

Allan, C.A., McLachlan, R.I., 2004. Age-related changes in testosterone and the role of replacement therapy in older men. Clin. Endocrinol. (Oxf.) 60, 653–670.

Andersen, H.R., Vinggaard, A.M., Rasmussen, T.H., Gjermandsen, I.M., Bonefeld-Jorgensen, E.C., 2002. Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity in vitro. Toxicol. Appl. Pharmacol. 179, 1–12.

Asawasinsopon, R., Prapamontol, T., Prakobvitayakit, O., Vaneesorn, Y., Mangklabruks, A., Hock, B., 2006. Plasma levels of DDT and their association with reproductive hormones in adult men from northern Thailand. Sci. Total Environ. 355, 98–105.

Asmus, C.I., Alonzo, H.G., Palácios, M., da Silva, A., Filhote, M.I., Buosi, D., Câmara, V., 2008. Assessment of human health risk from organochlorine pesticide residues in Cidade dos Meninos, Duque de Caxias, Rio de Janeiro, Brazil. Cad. Saude Publica 24, 755–766.

ATSDR, 2002. Agency for toxic substances and disease registry. In: Syracuse Research Corporation (Ed.), Toxicological profile for DDT, DDE, and DDD: update. US Department of Health and Human Services, Atlanta, GA, pp. 2–10.

Ayotte, P., Giroux, S., Dewailly, E., Hernández Avila, M., Farias, P., Danis, R., et al., 2001. DDT spraying for malaria control and reproductive function in Mexican men. Epidemiology 12, 366–367.

Barrett-Connor, E., Von Muhlen, D.G., Kritz-Silverstein, D., 1999. Bioavailable testosterone and depressed mood in older men: the Rancho Bernardo Study. J. Clin. Endocrinol. Metab. 84, 573–577.

Bhasin, S., Pencina, M., Jasuja, G.K., Travison, T.G., Coviello, A., Orwoll, E., et al., 2011. Reference ranges for testosterone in men generated using liquid chromatography tandem mass spectrometry in a community-based sample of healthy nonobese young men in the Framingham Heart Study and applied to three geographically distinct cohorts. J. Clin. Endocrinol. Metab. 96, 2430–2439.

Biller, B.M., Luciano, A., Crosignani, P.G., Molitch, M., Olive, D., Rebar, R., et al., 1999. Guidelines for the diagnosis and treatment of hyperprolactinemia. J. Reprod. Med. 44, 1075–1084.

Bjornerem, A., Straume, B., Oian, P., Berntsen, G.K., 2006. Seasonal variation of estradiol, follicle stimulating hormone, and dehydroepiandrosterone sulfate in women and men. J. Clin. Endocrinol. Metab. 91, 3798–3802.

Blanco-Muñoz, J., Lacasaña, M., Aguilar-Garduño, C., Rodríguez-Barranco, M., Bassol, S., Cebrián, M.E., et al., 2012. Effect of exposure to p,p'-DDE on male hormone profile in Mexican flower growers. Occup. Environ. Med. 69, 5–11.

Briz, V., Molina-Molina, J.M., Sánchez-Redondo, S., Fernández, M.F., Grimalt, J.O., Olea, N., et al., 2011. Differential estrogenic effects of the persistent organochlorine pesticides dieldrin, endosulfan, and lindane in primary neuronal cultures. Toxicol. Sci. 120, 413–427.

Cocco, P., Loviselli, A., Fadda, D., Ibba, A., Melis, M., Oppo, A., et al., 2004. Serum sex hormones in men occupationally exposed to dichloro-diphenyl-trichloro ethane (DDT) as young adults. J. Endocrinol. 182, 391–397.

Crain, D.A., Janssen, S.J., Edwards, T.M., Heindel, J., Ho, S.M., Hunt, P., et al., 2008. Female reproductive disorders: the roles of endocrine-disrupting compounds and developmental timing. Fertil. Steril. 90, 911–940.

Dalvie, M.A., Myers, J.E., Lou, T.M., Dyer, S., Robins, T.G., Omar, S., et al., 2004. The hormonal effects of long-term DDT exposure on malaria vector-control workers in Limpopo Province South Africa. Environ. Res. 96, 9–19.

Danzo, B.J., 1997. Environmental xenobiotics may disrupt normal endocrine function by interfering with the binding of physiological ligands to steroid receptors and binding proteins. Environ. Health Perspect. 105, 294–301.

Dhindsa, S., Miller, M.G., McWhirter, C.L., Mager, D.E., Ghanim, H., Chaudhuri, A., et al., 2010. Testosterone concentrations in diabetic and nondiabetic obese men. Diabetes Care 33, 1186–1192.

Ferguson, K.K., Hauser, R., Altshul, L., Meeker, J.D., 2012. Serum concentrations of p,p'-DDE, HCB, PCBs and reproductive hormones among men of reproductive age. Reprod. Toxicol. 34, 429–435.

Freire, C., Koifman, R.J., Sarcinelli, P., Rosa, A.C., Clapauch, R., Koifman, S., 2012. Long term exposure to organochlorine pesticides and thyroid function in children from Cidade dos Meninos, Rio de Janeiro, Brazil. Environ. Res. 117, 68–74.

Giwercman, A.H., Rignell-Hydbom, A., Toft, G., Rylander, L., Hagmar, L., Lindh, C., 2006. Reproductive hormone levels in men exposed to persistent organohalogen pollutants: a study of inuit and three European cohorts. Environ. Health Perspect. 114, 1348–1353.

Goncharov, A., Rej, R., Negoita, S., Schymura, M., Santiago-Rivera, A., Morse, G., Akwesasne Task Force on the Environment, Carpenter, D.O., 2009. Lower serum testosterone associated with elevated polychlorinated biphenyl concentrations in Native American men. Environ. Health Perspect. 117, 1454–1460.

Guerreiro, G., 2009. Vertebrate sex steroid receptors: evolution, ligands, and neurodistribution. Ann. N.Y. Acad. Sci. 1163, 154–168.

Hagmar, L., Björk, J., Sjödin, A., Bergman, A., Erfurth, E.M., 2001. Plasma levels of persistent organohalogens and hormone levels in adult male humans. Arch. Environ. Health 56, 138–143.

Haring, R., Völzke, H., Steveling, A., Krebs, A., Felix, S.B., Schöfl, C., et al., 2010. Low serum testosterone levels are associated with increased risk of mortality in a population-based cohort of men aged 20–79. Eur. Heart J. 31, 1494–1501.

Harlow, S.D., Gass, M., Hall, J.E., Lobo, R., Maki, P., Rebar, R.W., et al., 2012. Consensus statement: executive summary of the stages of reproductive aging workshop + 10: addressing the unfinished agenda of staging reproductive aging. J. Clin. Endocrinol. Metab. 97, 1159–1168.

Harman, S.M., Metter, E.J., Tobin, J.D., Pearson, J., Blackman, M.R., Baltimore Longitudinal Study of Aging, 2001. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. J. Clin. Endocrinol. Metab. 86, 724–731.

Kelce, W.R., Stone, C.R., Laws, S.C., Gray, L.E., Kemppainen, J.A., Wilson, E.M., 1995. Persistent DDT metabolite p,p′-DDE is a potent androgen receptor antagonist. Nature 375, 581–585.

Kenny, A.M., Prestwood, K.M., Gruman, C.A., Marcello, K.M., Raisz, L.G., 2001. Effects of transdermal testosterone on bone and muscle in older men with low bioavailable testosterone levels. J. Gerontol. A. Biol. Sci. Med. Sci. 56, M266–M272.

Liu, G.H., Yang, K.D., Liu, X.P., Qin, Q.F., 2005. Effects of residues of organochlorine pesticides on reproductive endocrine in human. Wei. Sheng. Yan. Jiu. 34, 524–528.

Martin Jr., S.A., Harlow, S.D., Sowers, M.F., Longnecker, M.P., Garabrant, D., Shore, D.L., et al., 2002. DDT metabolite and androgens in African-American farmers. Epidemiology 13, 454–458.

Melmed, S., Casanueva, F.F., Hoffman, A.R., Kleinberg, D.L., Montori, V.M., Schlechte, J.A., et al., 2011. Diagnosis and treatment of hyperprolactinemia: an endocrine society clinical practice guideline. J. Clin. Endocrinol. Metab. 96, 273–288.

Messinis, I.E., 2006. Ovarian feedback, mechanism of action and possible clinical implications. Hum. Reprod. 12, 557–571.

Ministério da Saúde, 2003. Secretaria de Políticas de Saúde, Departamento de Ciência e Tecnologia em Saúde, Exposição Humana a Resíduos Organoclorados na Cidade dos Meninos, Município de Duque de Caxias, Rio de Janeiro. Relatório de Trabalho da Comissão Técnica Assessora ao Ministério da Saúde, Brasília, DF.

Moffat, S.D., Zonderman, A.B., Metter, E.J., Kawas, C., Blackman, M.R., Harman, S.M., et al., 2004. Free testosterone and risk for Alzheimer disease in older men. Neurology 62, 188–193.

Needham, L.L., Ozkaynak, H., Whyatt, R.M., Barr, D.B., Wang, R.Y., Naeher, L., et al., 2005. Exposure assessment in the National Children's Study: introduction. Environ. Health Perspect. 113, 1076–1082.

Pavlíková, N., Bláhová, L., Klán, P., Bathula, S.R., Sklenář, V., Giesy, J.P., et al., 2012. Enantioselective effects of alpha-hexachlorocyclohexane (HCH) isomers on androgen receptor activity in vitro. Chemosphere 86, 65–69.

Perry, M.J., Ouyang, F., Korrick, S.A., Venners, S.A., Chen, C., Xu, X., et al., 2006. A prospective study of serum DDT and progesterone and estrogen levels across the menstrual cycle in nulliparous women of reproductive age. Am. J. Epidemiol. 164, 1056–1064.

Ralph, J.L., Orgebin-Crist, M.C., Lareyre, J.J., Nelson, C.C., 2003. Disruption of androgen regulation in the prostate by the environmental contaminant hexachlorobenzene. Environ. Health Perspect. 111, 461–466.

Rossi, M., Dimida, A., Ferrarini, E., Silvano, E., De Marco, G., Agretti, P., et al., 2009. Presence of a putative steroidal allosteric site on glycoprotein hormone receptors. Eur. J. Pharmacol. 623, 155–159.

Rylander, L., Wallin, E., Jönssson, B.A., Stridsberg, M., Erfurth, E.M., Hagmar, L., 2006. Associations between CB-153 and p,p′-DDE and hormone levels in serum in middle-aged and elderly men. Chemosphere 65, 375–381.

Sarcinelli, P.N., Pereira, A.C., Mesquita, S.A., Oliveira-Silva, J.J., Meyer, A., Menezes, M.A., et al., 2003. Dietary and reproductive determinants of plasma organochlorine levels in pregnant women in Rio de Janeiro. Environ. Res. 91, 143–150.

Schow, D.A., Redmon, B., Pryor, J.L., 1997. Male menopause – how to define it, how to treat it. Postgrad. Med. 101, 62–74.

Sharpe, R.M., 1995. On the importance of being earnest. Decline in semen quality among fertile men in Paris during the past 20 years. Hum. Exp. Toxicol. 14, 463–464.

Small, C.M., Cheslak-Postava, K., Terrell, M., Blanck, H.M., Tolbert, P., Rubin, C., et al., 2007. Risk of spontaneous abortion among women exposed to polychlorinated biphenyls. Environ. Res. 105, 247–255.

Sohoni, P., Sumpter, J.P., 1998. Several environmental oestrogens are also anti-androgens. J. Endocrinol. 158, 327–339.

Toft, G., Hagmar, L., Giwercman, A., Bonde, J.P., 2004. Epidemiological evidence on reproductive effects of persistent organochlorines in humans. Reprod. Toxicol. 19, 5–26.

Touitou, Y., 1995. Effects of ageing on endocrine and neuroendocrine rhythms in humans. Horm. Res. 43, 12–19.

Vanderpump, M.P., French, J.M., Appleton, D., Tunbridge, W.M., Kendall-Taylor, P., 1998. The prevalence of hyperprolactinaemia and association with markers of autoimmune thyroid disease in survivors of the Whickham Survey cohort. Clin. Endocrinol. (Oxf.) 48, 39–44.

Walsh, L.P., Stocco, D.M., 2000. Effects of lindane on steroidogenesis and steroidogenic acute regulatory protein expression. Biol. Reprod. 63, 1024–1033.

Wang, C., Jackson, G., Jones, T.H., Matsumoto, A.M., Nehra, A., Perelman, M.A., 2011. Low testosterone associated with obesity and the metabolic syndrome contributes to sexual dysfunction and cardiovascular disease risk in men with type 2 diabetes. Diabetes Care 34, 1669–1675.

Windham, G.C., Lee, D., Mitchell, P., Anderson, M., Petreas, M., Lasley, B., 2005. Exposure to organochlorine compounds and effects on ovarian function. Epidemiology 16, 182–190.

Zhao, S.P., Li, X.P., 1998. The association of low plasma testosterone level with coronary artery disease in Chinese men. Int. J. Cardiol. 63, 161–164.