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Pyrethroid insecticide metabolites are associated with serum hormone levels in adult men

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ABSTRACT

Experimental studies have reported that pyrethroid insecticides affect male endocrine and reproductive function, but human data are limited. We recruited 161 men from an infertility clinic between years 2000–2003 and measured serum reproductive and thyroid hormone levels, as well as the pyrethroid metabolites 3-phenoxybenzoic acid (3PBA) and *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*cis*-DCCA and *trans*-DCCA) in spot urine samples. When adjusting for potential confounders, categories for all three metabolites, as well as their summed values, were positively associated with FSH (all *p*-values for trend <0.05). Statistically significant or suggestive positive relationships with LH were also found. In addition, *cis*-DCCA and *trans*-DCCA were inversely associated with inhibin B (*p* for trend = 0.03 and 0.02, respectively). Finally, there was evidence that *trans*-DCCA was inversely associated with testosterone and free androgen index (the ratio of testosterone to sex hormone binding globulin; *p* for trend = 0.09 and 0.05, respectively). The observed relationships were consistent with previous findings, but further research is needed for a better understanding of the potential association between pyrethroid insecticides and male reproduction.

1. Introduction

There is concern for adverse human health risks resulting from exposure to environmental endocrine-disrupting compounds (EDCs), which in men may be associated with or lead to declined reproductive capacity or possibly increased risk of testicular, prostate or thyroid cancer [1–3]. Recent evidence in men suggests the occurrence of secular declining trends in testosterone levels, and perhaps semen quality, and some have hypothesized that this may be related to human exposure to EDCs [4–6]. A number of environmental chemicals may cause altered hormone levels through various biological mechanisms and target sites, ranging from effects on hormone receptors to effects on hormone synthesis, secretion or metabolism. While the health impacts of sub-clinical alterations in circulating hormone levels remain unclear, there is a limited but growing body of evidence that environmental and occupational exposure to some commonly used chemicals is associated with hormone level alterations. In addition, because so many individuals are exposed to commonly used chemicals, such as certain pesticides, even seemingly subtle epidemiologic associations may result in large increases in reproductive and other endocrine-related disease among populations and thus should be of great public health concern.

Synthetic pyrethroid insecticides are among the most commonly available to consumers today due to increased use in recent years because of the need to replace common organophosphorus insecticides following use restrictions in the United States and other countries. A consequence of the increased availability, use, and broad-spectrum applicability of pyrethroid insecticides is widespread exposure among the general population. Urinary metabolites of pyrethroid insecticides have been measured in a substantial proportion of the general population in the United States and in Germany [7,8], and it is reasonable to expect these proportions to increase as organophosphorus insecticides continue to be phased out. Diet is a primary route of exposure to pyrethroids among non-occupationally exposed individuals [9], but permethrin and other pyrethroid insecticides have also been measured in a high proportion of household dust samples suggesting that the home environment may also comprise a major exposure source [10–13]. Thus, exposure to pyrethroid insecticides is likely to be multimedia and multi-route, making the use of exposure biomarkers

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to estimate internal dose advantageous in human epidemiological studies.

Data on altered reproductive or endocrine function resulting from pyrethroid insecticide exposure are limited, but animal and *in vitro* studies suggest that some pyrethroid insecticides or their metabolites may possess endocrine disrupting properties [14–20] and adversely affect semen quality [15,20–24]. In humans, several recent non-occupational studies have reported significant or suggestive associations of urinary pyrethroid insecticide metabolite concentrations with reduced sperm concentration, motility and morphology, and increased DNA damage [25–27]. Another recent study reported statistically significant relationships between pyrethroid insecticide metabolite concentrations and circulating hormone levels in non-occupationally exposed Chinese men [28]. Experimental studies have also implicated pyrethroid insecticides in altered thyroid function [29–32], although these associations remain untested in human studies.

The present study was conducted to assess the relationships between environmental pyrethroid insecticide exposure, as assessed by the measurement of several urinary metabolites, and altered circulating reproductive and thyroid hormone levels in men.

2. Methods

2.1. Subject recruitment

Between April 2000 and April 2003, men between 18 and 54 years of age were recruited from the Vincent Memorial Andrology lab at Massachusetts General Hospital (MGH) and invited to participate in a study to assess the effects of environmental exposures on male reproductive health. Approximately 65% of eligible men agreed to participate. The primary reason cited by non-participants was lack of time. Exclusionary criteria included prior vasectomy or current use of exogenous hormones. A retrospective review of anonymized clinic records of non-participants, who met the same eligibility criteria as the study subjects, found that there were no differences between participants and non-participants in regards to age or semen parameters [33]. Height and weight were measured, and all men completed a brief nurse administered questionnaire at the time of recruitment, and provided health information. The Harvard School of Public Health (HSPH), MGH, and University of Michigan Human Subjects Committees approved the study and all subjects signed an informed consent.

2.2. Urine sample collection and analysis

A single spot urine sample was collected from each subject. Urine samples were frozen at −20 °C and sent to the U.S. Centers for Disease Control and Prevention (CDC) where the pyrethroid insecticide metabolites 3-phenoxybenzoic acid (3PBA), *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*cis*-DCCA), and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (*trans*-DCCA) were measured. 3PBA is a common metabolite of several pyrethroids including cyhalothrin, cypermethrin, deltamethrin, fenvalerate, and permethrin. *cis*-DCCA and *trans*-DCCA are metabolites of *cis* and *trans* isomers, respectively, of permethrin, cypermethrin and cyfluthrin. Analytical chemistry procedures used for measurement of pyrethroid metabolites in urine have been previously described [34]. Briefly, samples were fortified with stable isotope analogues of the target analytes, underwent solid-phase extraction, and were analyzed using high-performance liquid chromatography coupled with tandem mass spectrometry using turbo ion-spray atmospheric pressure ionization. The limit of detection (LOD) was 0.1 μg/l for all three metabolites. 4-Fluoro-3-phenoxybenzoic acid and *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid, specific metabolites of cyfluthrin and deltamethrin, respectively, were also measured but due to low detection rates were excluded from further analysis. Specific gravity (SG) was used to adjust urine samples for dilution. SG was measured using a handheld refractometer (National Instrument Company, Inc., Baltimore, MD, USA).

2.3. Serum hormones

One non-fasting blood sample was drawn between the hours of 9 a.m. and 4 p.m. on the same day that the urine sample was collected. Blood samples were centrifuged and serum stored at −80 °C until analysis. Testosterone was measured directly using the Coat-A-Count RIA kit (Diagnostics Products, Los Angeles, CA, USA), which has an interassay and intraassay coefficient of variation (CV) of 12% and 10%, respectively with a sensitivity of 4 ng/dl (0.139 nmol/l). The free androgen index (FAI) was calculated as the molar ratio of total testosterone to sex hormone binding globulin (SHBG). SHBG was measured using a fully automated system (Immulite: DPC, Inc., Los Angeles, CA, USA) which uses a solid-phase two-site chemiluminescent enzyme immunometric assay and has an interassay CV of less than 8%. Inhibin B was measured using a commercially available, double antibody, enzyme-linked immunosorbent assay (Oxford Bioinnovation, Oxford, UK) with interassay and intraassay CVs of 20% and 8%, respectively, limit of detection (LOD) of 15.6 pg/ml and a functional sensitivity (20% CV) of 50 pg/ml. Serum luteinizing hormone (LH), follicle stimulating hormone (FSH), estradiol, and prolactin concentrations were determined by microparticle enzyme immunoassay using an automated Abbott AxSYM system (Abbott Laboratories, Chicago, IL, USA). The Second International Reference Preparation (WHO 71/223) was used as the reference standard. The assay sensitivity for LH and FSH were 1.2 international units per liter (IU/l) and 1.1 IU/l, respectively. The intraassay CVs for LH and FSH are less than 5% and less than 3%, respectively, with interassay CVs for both hormones of less than 9%. The testosterone:LH ratio, a measure of Leydig cell function, was calculated by dividing testosterone (nmol/l) by LH (IU/l). The assay sensitivity for estradiol and prolactin were 20 pg/ml and 0.6 ng/ml, respectively. For estradiol the within-run coefficient of variation (CV) was between 3% and 11%, and the total CV was between 5% and 15%. For prolactin the within-run CV was ≤3% and the total CV was ≤6%.

Free T_4, Total T_3, and TSH concentrations were also determined in serum by microparticle enzyme immunoassay (AxSYM automated system, Abbott Diagnostics, Abbott Park, IL USA). The assay sensitivity for free T_4 and total T_3 were 0.01 ng/dl and 0.15 ng/ml, respectively. The interassay CVs for both hormones of less than 9%. For TSH, the ultrasensitive hTSH II assay (Abbott Diagnostics) was used with a functional sensitivity of 0.03 micro-international units per liter (μIU/l), and interassay CVs of less than 8%.

2.4. Statistical analysis

Data analysis was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA). Bivariate analyses were conducted between all hormone, pyrethroid insecticide metabolites, and demographic variables to investigate associations or differences between categories and the potential for confounding. Differences were tested statistically using Spearman correlations, Students *t*-tests, one-way ANOVA, or chi-square tests where appropriate.

Because of the high proportion of samples below the LOD for 3PBA (46%), *cis*-DCCA (47%) and *trans*-DCCA (49%), SG-adjusted pyrethroid insecticide metabolites were categorized into low, medium or high groups. The low group consisted of values below the median for each metabolite. The medium group was comprised of values greater than or equal to the median but less than the 75th percentile value, while the high groups consisted of values greater than or equal to the 75th percentile. Categories of the molar sum of *cis*-DCCA and *trans*-DCCA, as well as the sum of all three pyrethroid insecticide metabolites, were also modeled as exposure variables. For values below the LOD, zero was used in the calculation of these summed variables prior to categorizing into low, medium or high groups.

Multivariate linear regression was used to explore relationships between urinary pyrethroid metabolite groups and hormone levels. Serum concentrations of testosterone, estradiol, inhibin B, free T_4 and total T_3 closely approximated normality and were used in statistical models untransformed, while the distributions of FSH, LH, SHBG, FAI, prolactin and TSH concentrations were skewed left and transformed to the natural log (ln) for statistical analyses. Inclusion of covariates was based on statistical and biologic considerations [35]. Age and BMI were modeled as a continuous variable, smoking status was dichotomized by current smoker vs. never smoked or former smoker, and race/ethnicity was categorized into four groups: White, African American, Hispanic, and other. Timing of blood sample collection by season (winter vs. spring, summer or fall) and time of day (9:00 a.m.–12:59 p.m. vs. 1:00 p.m.–4:00 p.m.) were considered for inclusion in the models as dichotomous variables.

3. Results

Among the 161 men for which both urinary pyrethroid insecticide metabolite and serum hormone concentrations were determined, the majority were white (86%) and had never smoked (68%). The mean (SD) age and BMI were 36 (5.8) years and 28 (4.8), respectively. Distributions of SG-adjusted urinary pyrethroid insecticide metabolite concentrations and serum hormone levels are presented in Tables 1 and 2. In preliminary bivariate analyses, age, BMI, race and season were not significantly associated with pyrethroid insecticide metabolite groups (p-values > 0.05), but current smokers were less likely to be categorized in the high *cis*-DCCA group (p-value < 0.05). Among the hormones measured, inhibin B levels were lower in blood samples collected in the winter, prolactin and free T_4 levels were lower in blood samples collected in the morning, and current smokers had lower FAI and TSH compared to nonsmokers (p-values < 0.05). Age was inversely associated with FAI and total T_3 but positively associated with SHBG, while BMI was inversely associated with inhibin

Table 1
Distribution of SG-adjusted pyrethroid metabolite concentrations (ng/ml). *N* = 161.

	Selected percentiles						
	10th	25th	50th	75th	90th	95th	Max
3PBA	<0.10	<0.10	0.15	0.47	1.31	2.68	61.3
cis-DCCA	<0.10	<0.10	0.16	0.30	0.60	1.64	23.2
trans-DCCA	<0.10	<0.10	0.11	0.38	1.35	4.07	36.8
cis + *trans*-DCCA	<0.10	0.12	0.27	0.65	1.89	6.01	59.9
Sum pyrethroid (nmol/ml)	0.0001	0.0009	0.0022	0.0051	0.015	0.039	0.57

Abbreviations: 3PBA, 3-phenoxybenzoic acid; *cis*-DCCA, *cis*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid; *trans*-DCCA, *trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid.

B, testosterone and SHBG but positively associated with FAI (*p*-values < 0.05).

In multivariate linear regression (Table 3), serum hormone levels were regressed on categories of SG-adjusted pyrethroid insecticide metabolite concentrations (<50th, ≥50th–75th, and ≥75th percentile). Compared with men who had values below the median, men in the highest 3PBA category had significantly higher FSH levels ($p = 0.001$; p for trend among low, medium and high categories = 0.002) and suggestively higher levels of LH ($p = 0.054$). Men in the highest 3PBA category also had a suggestive decline in FAI ($p = 0.08$). For FSH, which was transformed by the natural logarithm, the regression coefficient of 0.38 represents a 3.49 (95% confidence interval [CI] 1.21–13.7) IU/l increase in adjusted geometric mean FSH level compared to men with 3PBA below the median. There was a significant inverse trend between 3PBA categories and prolactin in crude linear regression (not shown; p for trend = 0.04) that was no longer statistically significant when adjusting for age, BMI, smoking and time of day (Table 2; p for trend = 0.11).

Men in the highest *cis*-DCCA, *trans*-DCCA, or summed metabolite categories had elevated FSH and LH compared to men with concentrations of these metabolites below the median, with most of these relationships demonstrating a dose-dependent trend. There were also dose-dependent declines in inhibin B associated with increasing *cis*-DCCA and *trans*-DCCA categories (*p*-values for trend = 0.03 and 0.02, respectively). For *trans*-DCCA, the middle and high metabolite categories were associated with 4.8% (95% CI −23.9% to +14.2%) and 22.7% (95% CI −41.8% to −3.7%) declines in inhibin B, respectively, compared to the population median for inhibin B (153 pg/ml).

There were significant inverse relationships between *trans*-DCCA categories and testosterone and FAI in crude regression models (not shown; *p*-values for trend = 0.05 and 0.04, respectively) that remained suggestive when adjusting for important covariates. In multiple linear regression (Table 3) the high *trans*-DCCA category was associated with a 10% (95% CI −21.3% to +1.1%; *p*-value = 0.08) decline in testosterone relative to the population median for testosterone (373 ng/dl).

In adjusted regression models there were also significant declining trends for testosterone and FAI among increasing metabolite categories when all three pyrethroid metabolites were summed together (*p*-values for trend = 0.03 and 0.02, respectively). Finally, there was an inverse association between *cis*-DCCA categories and total T_3, but the relationship did not follow a monotonic dose-dependent pattern.

4. Discussion

In the present study we found relationships between urinary metabolites of pyrethroid insecticides and serum reproductive hormone levels in men. Specifically, we found evidence that urinary pyrethroid insecticide metabolites are positively associated with FSH and LH, and inversely associated with inhibin B, testosterone, and free androgen index. While we cannot rule out the possibility that some of our statistically significant or suggestive results were due to chance since multiple comparisons were made, these findings may be of concern due to the increased use of pyrethroid pesticides that results in widespread exposure among the general population.

To our knowledge human studies of pyrethroid insecticide exposure and circulating hormone levels are limited to a single recent study [28]. Our findings of a suggestive positive association between 3PBA and LH are consistent with those reported by Han and colleagues among Chinese men [28]. In that study, which was similar in design to the present study, 199 men with no specific occupational exposure to pyrethroid insecticides were recruited through a Chinese infertility clinic and provided urine and blood samples. 3PBA concentrations were measured in urine, and FSH, LH, testosterone, estradiol, and prolactin were measured in serum. The authors reported a significant positive association between creatinine-adjusted 3PBA and LH in multiple linear regression

Table 2
Distribution of serum hormone concentrations. *N* = 161.

Hormone	Geometric mean	Selected percentiles						
		5th	10th	25th	50th	75th	90th	95th
FSH (IU/l)	7.84	3.67	4.21	5.12	7.16	10.4	15.6	24.9
LH (IU/l)	10.1	4.56	5.29	7.10	9.90	14.0	18.8	23.4
Inhibin B (pg/ml)	160[a]	44.1	68.1	108	153	200	273	301
Testosterone (ng/dl)	402[a]	228	247	306	373	479	588	663
SHBG (nmol/ml)	26.3	13.0	16.0	20.9	25.3	34.4	45.9	52.2
FAI	0.50	0.29	0.32	0.40	0.49	0.67	0.76	0.87
T:LH ratio	43.1[a]	15.9	21.3	30.4	39.4	54.8	68.5	79.8
Estradiol (pg/ml)	31.2[a]	<10	<10	25	31	38	47	51
Prolactin (ng/ml)	11.5	5.67	6.42	8.20	10.9	16.5	22.6	26.5
Free T_4 (ng/dl)	1.23[a]	0.96	0.99	1.08	1.18	1.37	1.56	1.73
Total T_3 (ng/ml)	0.96[a]	0.72	0.77	0.82	0.96	1.08	1.19	1.22
TSH (μIU/ml)	1.45	0.67	0.78	1.02	1.46	1.92	2.71	3.40

Abbreviations: FSH, follicle stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone binding globulin; FAI, free androgen index; TSH, thyroid stimulating hormone (thyrotropin).

[a] Arithmetic mean.

Table 3
Adjusted[a] regression coefficients (95% confidence intervals) for change in hormone levels associated with SG-adjusted pyrethroid metabolite groups. *N* = 161.

Metabolite percentiles	Adjusted regression coefficients (95% CI)					
	FSH[b]	LH[b]	Inhibin B[c]	Testosterone[d]	SHBG[b]	FAI[b]
3PBA						
<50th	0	0	0	0	0	0
50th–75th	0.05 (−0.17, 0.28)	−0.05 (−0.25, 0.15)	−3.46 (−32.6, 25.7)	6.31 (−34.9, 47.5)	0.02 (−0.13, 0.16)	0.02 (−0.12, 0.15)
>75th	0.38 (0.15, 0.60)	0.20 (−0.004, 0.41)	−18.2 (−47.8, 11.4)	−27.4 (−68.3, 14.6)	0.08 (−0.07, 0.23)	−0.13 (−0.27, 0.02)
p for trend	0.002	0.10	0.25	0.27	0.30	0.12
cis-DCCA						
<50th	0	0	0	0	0	0
50th–75th	0.21 (−0.03, 0.44)	0.32 (0.12, 0.53)	−15.2 (−45.2, 14.8)	−6.67 (−49.7, 36.4)	0.05 (−0.10, 0.20)	−0.04 (−0.19, 0.10)
>75th	0.26 (0.02, 0.49)	0.22 (0.02, 0.43)	−32.0 (−61.8, −2.16)	−8.86 (−51.5, 33.8)	−0.03 (−0.18, 0.12)	0.002 (−0.14, 0.15)
p for trend	0.02	0.01	0.03	0.66	0.77	0.94
trans-DCCA						
<50th	0	0	0	0	0	0
50th–75th	0.08 (−0.14, 0.31)	0.05 (−0.15, 0.26)	−7.37 (−36.6, 21.8)	−7.84 (−49.1, 33.5)	−0.02 (−0.17, 0.13)	0.01 (−0.13, 0.14)
>75th	0.28 (0.05, 0.51)	0.20 (−0.004, 0.41)	−34.8 (−64.0, −5.64)	−37.7 (−79.7, 4.29)	0.08 (−0.07, 0.23)	−0.15 (−0.29, −0.01)
p for trend	0.02	0.06	0.02	0.09*	0.34	0.05
cis- + *trans*-DCCA						
<50th	0	0	0	0	0	0
50th–75th	0.09 (−0.14, 0.31)	0.05 (−0.15, 0.26)	0.43 (−28.6, 29.4)	−9.37 (−51.1, 32.3)	0.04 (−0.10, 0.19)	−0.03 (−0.17, 0.11)
>75th	0.29 (0.06, 0.52)	0.24 (0.03, 0.44)	−31.1 (−60.4, −1.70)	−26.9 (−68.9, 15.2)	0.04 (−0.11, 0.19)	−0.10 (−0.24, 0.04)
p for trend	0.03	0.03	0.06	0.21	0.57	0.16
Sum pyrethroids						
<50th	0	0	0	0	0	0
50th–75th	0.04 (−0.19, 0.26)	0.06 (−0.15 (0.26)	−8.70 (−37.8, 20.4)	−35.5 (−76.5, 5.56)	0.05 (−0.09, 0.20)	−0.09 (−0.22, 0.05)
>75th	0.32 (0.10, 0.55)	0.19 (−0.02, 0.39)	−23.7 (−53.3, 5.86)	−41.4 (−83.1, 0.36)	0.09 (−0.06, 0.24)	−0.16 (−0.30, −0.02)
p for trend	0.009	0.08	0.12	0.03	0.21	0.02

Metabolite percentiles	Adjusted regression coefficients (95% CI)					
	T:LH ratio	Estradiol[d]	Prolactin[b]	Free T4	Total T3	TSH[b]
3PBA						
<50th	0	0	0	0	0	0
50th–75th	1.33 (−6.60, 9.26)	0.49 (0.417, 5.15)	−0.09 (−0.27, 0.10)	0.02 (−0.07, 0.12)	0.04 (−0.02, 0.10)	−0.05 (−0.25, 0.15)
>75th	−5.08 (−13.1, 2.96)	−2.75 (−7.49, 2.00)	−0.15 (−0.34, 0.04)	−0.03 (−0.12, 0.07)	0.02 (−0.04, 0.09)	−0.11 (−0.32, 0.09)
p for trend	0.28	0.32	0.11*	0.67	0.33	0.27
cis-DCCA						
<50th	0	0	0	0	0	0
50th–75th	−8.99 (−17.1, −0.89)	−1.91 (−6.75, 2.93)	0.18 (−0.02, 0.37)	−0.001 (−0.10, 0.09)	−0.11 (−0.17, −0.05)	−0.01 (−0.22, 0.20)
>75th	−7.22 (−15.3, 0.82)	−2.54 (−7.34, 2.25)	0.05 (−0.15, 0.24)	−0.01 (−0.11, 0.08)	−0.05 (−0.11, 0.02)	−0.03 (−0.24, 0.17)
p for trend	0.04	0.27	0.44	0.82	0.05	0.74
trans-DCCA						
<50th	0	0	0	0	0	0
50th–75th	−3.51 (−11.4, 4.46)	1.34 (−332, 6.00)	−0.01 (−0.20, 0.18)	0.05 (−0.05, 0.14)	−0.02 (−0.08, 0.04)	−0.17 (−0.37, 0.03)
>75th	−5.52 (−13.6, 2.56)	−3.31 (−8.05, 1.43)	−0.002 (−0.20, 0.19)	0.01 (−0.08, 0.10)	−0.02 (−0.09, 0.04)	−0.07 (−0.27, 0.14)
p for trend	0.16	0.25	0.97	0.69	0.46	0.37
cis- + *trans*-DCCA						
<50th	0	0	0	0	0	0
50th–75th	−2.01 (−10.0, 5.98)	1.88 (−2.79, 6.55)	0.07 (−0.12, 0.26)	0.02 (−0.07, 0.11)	−0.06 (−0.12, 0.002)	−0.12 (−0.32, 0.09)
>75th	−5.91 (−14.0, 2.16)	−3.40 (−8.11, 1.31)	0.05 (−0.14, 0.25)	0.02 (−0.08, 0.11)	−0.03 (−0.10, 0.03)	0.004 (−0.20, 0.21)
p for trend	0.15	0.25	0.53	0.68	0.18	0.86
Sum pyrethroids						
<50th	0	0	0	0	0	0
50th–75th	−5.94 (−13.9, 1.99)	0.45 (−4.22, 5.11)	0.04 (−0.15, 0.23)	0.04 (−0.05, 0.13)	−0.03 (−0.09, 0.03)	−0.08 (−0.28, 0.12)
>75th	−5.30 (−13.3, 2.73)	−3.60 (−8.34, 1.15)	−0.02 (−0.21, 0.17)	−0.01 (−0.10, 0.08)	0.002 (−0.06, 0.07)	−0.05 (−0.25, 0.16)
p for trend	0.14	0.18	0.92	0.99	0.91	0.56

[a] Adjusted for age, BMI, smoking and time of day at blood draw. *N* = 161.
[b] Variable transformed by the natural logarithm.
[c] Additionally adjusted for season.
[d] Additionally adjusted for ln-transformed SHBG.
* Statistically significant trend in unadjusted (crude) model (*p*-value for trend <0.05).

analysis. However, there were several discrepancies between the findings of that study compared to the present study, as the authors also reported significant inverse association between 3PBA and estradiol but no association between 3PBA and FSH. It is important to note that 3PBA concentrations among the Chinese men were higher, where the 50th and 75th percentile values for unadjusted 3PBA were 1.15 and 1.99 μg/l compared to 0.15 and 0.42 μg/l in the present study. In addition, *cis*-DCCA and *trans*-DCCA were not measured in the Chinese study which limits our ability to further compare results.

We recently reported inverse associations between urinary pyrethroid insecticide metabolites and human semen quality [25], as have others [26,27]. Insight into the biological mechanisms involved in the relationship between pyrethroid insecticide expo-

sure and declined semen quality may be provided by the present findings. Here we report that pyrethroid insecticide metabolites are positively associated with FSH and inversely associated with inhibin B. FSH and inhibin B are the two hormones most highly associated with semen quality, where elevated levels of FSH and/or low levels of inhibin B are highly predictive of poor semen quality [36–38]. FSH, a gonadotropin produced and secreted by the anterior pituitary, acts on Sertoli cells in the seminiferous tubules to initiate spermatogenesis. In addition to nurturing and protecting developing germ cells during spermatogenesis, Sertoli cells also produce and secrete inhibin B, a protein hormone, which then exerts negative feedback on the anterior pituitary to inhibit FSH secretion [39,40]. Although we do not have direct evidence of this, we hypothesize that exposure to pyrethroid insecticides or their metabolites may be associated with adverse effects on the Sertoli cells or their FSH receptors to alter spermatogenesis and inhibin B production. This may, in turn, alter negative inhibin B feedback and increase pituitary FSH production and secretion, ultimately resulting in poorer semen quality and possibly reduced male fertility. There is also limited evidence for altered Leydig cell function in association with urinary pyrethroid insecticide metabolite concentrations, particularly for *trans*-DCCA, where increasing metabolite categories were inversely associated with testosterone and FAI but positively associated with LH.

Animal data on altered endocrine or reproductive function in relation to permethrin and other pyrethroid insecticides are also limited but provide some support for our results and hypothesized biological mechanisms. However, it must be noted that most studies to date have used dose levels that are much higher than what is encountered by non-occupationally exposed humans. Oral administration of permethrin for 10 days resulted in antiandrogen-like effects in 5-week-old male rats, measured as reductions in androgen-dependent sex accessory tissue weights [41]. Another recent study of mice orally administered *cis*-permethrin for 6 weeks reported significant dose-dependent declines in testicular and circulating levels of testosterone, along with a dose-dependent increase in circulating LH and declines in epididymal sperm count and sperm motility [15]. Testicular residue concentrations of *cis*-permethrin from the individual animals were also strongly inversely correlated with testicular testosterone levels. Exposure-related reductions in mRNA and protein expression levels of peripheral benzodiazepine receptor (PBR), steroidogenic acute regulatory protein (StAR), and cytochrome P450 side-chain cleavage (P450scc) were observed, as well as structural changes in Leydig cell mitochondria, suggesting these disruptions resulted in a reduction of cholesterol transport and conversion essential for testicular steroidogenesis. In a follow-up study in mice comparing the effects of exposure to either *cis*- or *trans*-permethrin isomers, Zhang et al. [42] reported similar effects in relation to *cis*-permethrin but not *trans*-permethrin. The authors also reported that *cis*-permethrin caused structural abnormalities in the seminiferous tubules, which may be consistent with our hypothesis of pyrethroid effects on Sertoli cells based on the observed associations with increased FSH and declined inhibin B levels in the present study.

There is experimental evidence for endocrine disruption in relation to pyrethroids other than permethrin that are metabolized to 3PBA, *cis*-DCCA and/or *trans*-DCCA. Several *in vitro* studies have demonstrated that cypermethrin and fenvalerate may exert estrogenic and/or anti-androgenic activity [15,43,44]. A study of cypermethrin exposure in rats found declined testosterone, FSH, and LH levels, along with histological abnormalities in the seminiferous tubules and reduced sperm counts, following ingestion of high doses of the pesticide in drinking water [23]. Conversely, another study that simultaneously dosed male rats with cypermethrin and the organophosphorus pesticide methyl parathion reported significant increases in serum FSH levels in relation to exposure [29]. Among fenvalerate-exposed rats increases in FSH and LH, and declines testosterone, have been reported [18,19]. Another finding which is potentially relevant to our results and proposed biological mechanisms is that fenvalerate inhibits FSH-stimulated progesterone production in human ovarian luteinizing-granulosa cells. Finally, 3PBA itself has also demonstrated endocrine disrupting properties *in vitro* [14,20,45]. Based on these findings and the results of the present human study, more reproductive toxicology research on pyrethroids is needed to provide a clearer understanding on the specific exposures and mechanisms potentially involved in the relationships reported here.

The associations we report here are among men with urinary pyrethroid insecticide metabolite concentrations similar to or slightly lower than those measured among the US general population in the most recent (Third) National Report on Human Exposure to Environmental Chemicals [7]. Median, 75th and 95th percentile concentrations for unadjusted 3PBA among males in the Third National Report were 0.29, 0.68 and 3.23 μg/l compared to 0.15, 0.42 and 2.24 μg/l in the present study. For *trans*-DCCA these percentiles were <LOD, 0.40 and 2.37 μg/l among males in the Third National Report compared to 0.10, 0.36 and 3.34 in the present study. Pyrethroid insecticide metabolite concentrations in the present study were also similar to those found in urine samples from adults and children in the German general population [46,47]. A recent cross-validation study showed that comparable pyrethroid insecticide metabolite data was obtained among unknown urine samples between the US and German laboratories using different extraction and analytical techniques [48].

In conclusion, we found evidence for increased gonadotropin levels, and decreased androgen and inhibin B levels, in relation to urinary metabolites of pyrethroid insecticides at concentrations representative of those found among the US general population. However, the specific pesticide(s) and biological mechanisms potentially involved are not yet clear. Because pyrethroid use is common and likely increasing worldwide, more research is needed on their potential to adversely impact the male reproductive system.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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