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Exposure to neonicotinoids and serum testosterone in men, women, and children

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Abstract

Neonicotinoids are the most used pesticides in the world and, despite being harmful to honeybees, they are considered safe for mammals. However, they have been associated with decreasing testosterone levels in several experimental animal models. In the present study, we aimed to determine the association of urinary neonicotinoids with serum testosterone in humans. We analyzed data on 2014 male and female participants to the National Health and Nutrition Examination Survey conducted between 2015 and 2016 aged 6 or older. In linear regression adjusted for age and potential confounders, serum total testosterone was 37.78% lower with 10-fold increase in urinary total neonicotinoids (95% CI: −58.82, −6.00), 20.81% lower with 10-fold increase in urinary 5-hydroxy-imidacloprid (95% CI: −34.94, −3.62) and 25.01% lower with 10-fold increase in urinary *n*-desmethyl-acetamiprid (95% CI: −39.80, −6.58) among males. Serum free androgen index (FAI) was also decreased with higher urinary *n*-desmethyl-acetamiprid. In females, serum total testosterone was 32.91% lower with 10-fold increase in urinary total neonicotinoids (95% CI: −54.93, −0.13), 21.32% lower with 10-fold increase in urinary 5-hydroxy-imidacloprid (95% CI: −29.31, −12.42) and 15.42% lower with urinary detection of 5-hydroxy-imidacloprid (95% CI: −22.80, −7.34). FAI was likewise reduced with higher urinary levels of 5-hydroxy-imidacloprid and *N*-desmethyl-acetamiprid. In conclusion, this study using a sample representative of the US population is the first to report that exposure to neonicotinoids is associated with decreased serum testosterone levels in humans. However, future prospective studies are warranted to confirm these findings.

KEYWORDS
endocrine disrupting chemicals, neonicotinoids, pesticides, testosterone

1 | INTRODUCTION

As the newest and most used pesticides in the world, neonicotinoids have been detected in the urine of half of the US population aged 3 years or older.[1,2] They account for 25% of the global insecticides market; about 50% of soybeans, 95% of cotton, and nearly 100% of corn are currently treated with neonicotinoids in the United States.[1] These pesticides are used not only in agriculture to protect crops from insects, but also in landscaping and to treat domestic animals against pests.[2] Neonicotinoids consist of seven pesticides classified into *N*-nitroguanidines (imidacloprid, thiamethoxam, clothianidin, and dinotefuran), nitromethylenes (nitenpyram) and *N*-cyanoamidines (acetamiprid and thiacloprid) that act as selective agonists to the nicotinic acetylcholine receptor (nAChR) of insects' central nervous system.[3] Due to their low molecular weight and high solubility in water, these compounds can infiltrate and stay in plant tissues for several

weeks after application.[1] They might also diffuse, accumulate, and persist in soil for up to 19 years and/or contaminate surface and ground water, fruits and vegetables, and bovine milk.[4] Human exposure to neonicotinoids occurs through ingestion of contaminated food and water, dermal absorption of the chemicals, and inhalation of contaminated particulates, vapors, or aerosols.[5]

Despite their harmful effects to honeybees, neonicotinoids have been considered safe in mammals and are being used as replacements to organophosphate pesticides, carbamates, and pyrethroids.[1] However, animal models suggest that chronic exposure to imidacloprid and acetamiprid, the two most common neonicotinoids, may significantly decrease testosterone levels by inhibiting serum cholesterol biosynthesis, inducing oxidative stress in Leydig cells, interacting with androgen receptor, and/or reducing the expression of genes involved in testosterone production.[6,7] Therefore, we tested the hypothesis that exposure to neonicotinoids is associated with reduced serum testosterone in humans, using a sample representative of the US male and female population aged 6 years or older. This study has important public health relevance, given the extensive use of neonicotinoids and the critical role of testosterone on reproductive, musculoskeletal, cardiovascular, respiratory, immune, neurological, and hematologic systems.[8]

2 | METHODS

2.1 | Data source

We used the 2015–2016 cycle of the National Health and Nutrition Examination Survey (NHANES) which included data on neonicotinoids. NHANES is a continuous survey of the US non-institutionalized civilian population conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). It collects data on participants' health through interviews, physical examinations, as well as laboratory tests and uses a complex multistage sampling design and sampling weights to derive a sample representative of the US population.[9] NHANES protocols were approved by the NCHS and the CDC's Institutional Review Boards and informed consent was obtained from all participants (details on IRB at http://www.cdc.gov/nchs/nhanes/irba98.htm).

Data on urinary nicotinoids was available in 3038 NHANES participants aged ≥3 years; of them, 2342 participants aged ≥6 years had data on serum sex hormones. After excluding women who were pregnant or had both ovaries removed, and participants with missing data on covariates ($N = 328$), the final sample size was arrived at 2014 participants.

2.2 | Urinary neonicotinoids

Spot urine collected mobile examination centers was frozen and shipped in dry ice to the CDC's National Center for Environmental Health (NCEH) where they were stored at −70°C until analysis. Urinary concentration of neonicotinoids was determined using enzymatic hydrolysis, online solid phase extraction, separation by reversed phase high-performance liquid chromatography, and detection by isotope dilution-electrospray ionization tandem mass spectrometry. The urine samples were tested for acetamiprid, clothianidin, imidacloprid, thiacloprid, 5-hydroxy-imidacloprid, and *N*-desmethyl-acetamiprid, with the following limits of detection (LOD): 0.2 μg/L for *N*-desmethyl-acetamiprid and clothianidin, 0.03 μg/L for thiacloprid and acetamiprid, and 0.4 μg/L for imidacloprid and 5-hydroxy-imidacloprid. Samples with neonicotinoids levels below the LOD were assigned the value $LOD/\sqrt{2}$. Detailed description of the laboratory methods and quality control procedures are published elsewhere.[2,10]

Our analysis of individual neonicotinoids was performed on 5-hydroxy-imidacloprid and *N*-desmethyl-acetamiprid; imiacloprid, thiacloprid, acetamiprid, and clothianidin were not studied as individual compounds due to their low urinary detection ranging from 0.7% to 7.9%.

2.3 | Serum sex hormones

Serum was sampled, processed, and stored under −20°C until shipping to the NCEH for testing. Serum total testosterone was measured using isotope dilution liquid chromatography tandem mass spectrometry (ID-LC–MS/MS). Serum sex hormone binding globulin (SHBG) levels were determined through reaction with immuno-antibodies and chemo-luminescence measurements of the reaction products. We calculated a free androgen index as the ratio of total testosterone (nmol/L) to SHBG (nmol/L) to indirectly approximate circulating free testosterone levels.[11] Details on the laboratory and quality control procedures are also described elsewhere.[12]

2.4 | Covariates

Data on age, sex, race/ethnicity, smoking, exposure to cigarette smoking, oral contraceptives or use of sex hormones, and family income were collected using questionnaires. Poverty to income ratio (PIR), used as a proxy for socioeconomic status, was estimated using guidelines and adjustment for family size, year, and state. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (9). BMI was categorized into <18 kg/m^2 (underweight), 18 to <25 kg/m^2 (normal), 25 to <30 kg/m^2 (overweight), and ≥30 kg/m^2 (obese) in adults aged 18 years or older. In children and adolescents younger than 18 years old, BMI was categorized into <5th percentile (underweight), ≥5th to <85th percentile (normal), ≥85th to <95th percentile (overweight), and ≥ 95th percentile (obese) as suggested by the CDC.[13,14] Smoking and exposure to environmental tobacco smoke (ETS) was defined as self-reported cigarette smoking or living with a household member who smoked inside the home. Furthermore, Serum cotinine was measured by isotope dilution-high performance liquid chromatography / atmospheric pressure chemical ionization tandem mass spectrometry. Diabetes was

defined as taking insulin or oral medications for diabetes or having hemoglobin A1c ≥6.5% or fasting plasma glucose ≥126 mg/dl.[15] Urinary creatinine served to account for urine dilution and was measured by quantitative enzymatic determination.[16]

2.5 | Statistical analysis

Descriptive analyses were performed to examine the prevalence of urinary neonicotinoids detection in the overall study population and by the characteristics of study participants and *p* values for differences were calculated using chi-square tests. Age-adjusted least-square means (SEs) of testosterone and free androgen index were computed compared urinary detection of the neonicotinoids. Multiple linear regressions were used to calculate the association of neonicotinoids with log-transformed concentrations of serum sex hormones. The models were adjusted for age, sex, race/ethnicity, PIR, BMI, serum cotinine levels, diabetes, and urinary creatinine to account for dilution[16]; in women, the models were additionally adjusted for use of oral contraceptives or sex hormones. For meaningful interpretations, the linear regression coefficients (β) were expressed as % change in serum hormone associated with urinary neonicotinoids, using the formula $(\exp^{\beta} - 1) \times 100$. We stratified age into the following groups: 6–11 years, 12–19 years, 20–39 years, and 40 years or older, and we tested whether the associations significantly differed by age through estimation of effect modification using the interaction term *exposure* × *age groups* included in the models. The analyses were performed in SAS (Version 9.4; SAS Institute, Cary, NC), accounting for NHANES sampling weights and the complex survey design to provide nationally representative estimates. *p* values <.10 for interactions and *p* values <.05 for all other analyses were considered significant.

3 | RESULTS

3.1 | Description of the study sample

Among the 2014 participants included in our analysis, 30.4% were aged 20–39 years old and 50.5% were aged 40 years or older. About 52.0% were men, and 62.4% were non-Hispanic White. The prevalence of overweight and obese BMI was 24.5% and 32.7% respectively; 38.1% of participants were exposed to ETS and 8.5% had diabetes. Approximately, 50.8% of the participants had a urinary detection of any neonicotinoid, 21.2% had urinary detection of 5-hydroxy-imidacloprid and 33.4% had urinary detection of *N*-desmethyl-acetamiprid (Table 1).

Participants with detected neonicotinoids in urine had lower diabetes prevalence and higher PIR than those with non-detected neonicotinoids. Participants with urinary 5-hydroxy-imidacloprid detection were mostly of "Other" race/ethnicity and had lower prevalence of diabetes and higher PIR compared to those without detection of the compound. Participants with urinary *N*-desmethyl-acetamiprid detection were younger (i.e., children and adolescents aged 6–19 years old), mostly of "Other" race/ethnicity, and had lower exposure to tobacco smoke compared to those without detection of the chemical (Table 1).

As shown in Table 2, age-adjusted least squared mean serum total testosterone was lower in males with urinary detection of 5-hydroxy-imidacloprid and in women with urinary detection of any neonicotinoid and *N*-desmethyl-acetamiprid than in those without detection of the compounds.

3.2 | Neonicotinoids and serum testosterone in males

In analysis adjusted for covariates and potential confounders, total testosterone was lower by 37.78% (95% CI: −58.82, −6.00) with 10-fold increase in Σ-neonicotinoids, lower by 20.81% (95% CI: −34.94, −3.62) with 10-fold increase in 5-hydroxy-imidacloprid and lower by 25.01% (−39.80, −6.58) with 10-fold increase in *N*-desmethyl-acetamiprid. Urinary detection of *N*-desmethyl-acetamiprid was associated with 20.78% lower (−35.96, −2.00) serum testosterone. FAI was also lower by 23.07% (−40.76, −0.11) with 10-fold increase in *N*-desmethyl-acetamiprid (Table 3). The association of urinary neonicotinoids and serum testosterone and FAI was not influenced by age (Table 4).

3.3 | Neonicotinoids and sex hormones in females

In adjusted analysis, serum total testosterone was lower by 32.91% (95% CI: −54.93, −0.13) with 10-fold increase in Σ-neonicotinoids, lower by 21.32% (95% CI: −29.31, −12.42) with 10-fold increase in 5-hydroxy-imidacloprid and lower by 15.42% (95% CI: −22.80, −7.34) with urinary detection of 5-hydroxy-imidacloprid. Likewise, FAI was decreased by 16.03% (95% CI: −28.90, −0.84) with 10-fold increase in 5-hydroxy-imidacloprid, decreased by 14.33% (95% CI: −24.58, −2.67) with *N*-desmethyl-acetamiprid urinary detection and by decreased 22.15% (95% CI: −38.61, −1.27) with 10-fold increase in *N*-desmethyl-acetamiprid (Table 3).

We observed significant effect modification by age on the association of *N*-desmethyl-acetamiprid urinary detection and log-transformed concentration with serum total testosterone ($p_{\text{-interaction}} = .01$) and of *N*-desmethyl-acetamiprid urinary detection with FAI ($p_{\text{-interaction}} = .03$) (Table 4). Urinary *N*-desmethyl-acetamiprid was associated with reduced serum testosterone and FAI only in women aged 40 years or older but not in other age groups. Urinary detection of *N*-desmethyl-acetamiprid was associated 18.55% lower (95% CI: −30.03, −5.20) total testosterone and 19.69% lower (95% CI: −31.13, −6.35) FAI while 10-fold increase in *N*-desmethyl-acetamiprid was associated with 26.19% lower (95% CI: −42.76, −4.83) total testosterone and 25.13% lower (95% CI: −43.20, −1.31) FAI (Table 5).

TABLE 1 Characteristics of study participants ($N = 2014$)

Characteristics	All	Any neonicotinoid detected			5-hydroxy-imidacloprid detected			*N*-desmethyl-acetamiprid detected		
		Yes	No	*p*	Yes	No	*p*	Yes	No	*p*
Prevalence	100	50.8	49.2		21.2	78.8		33.4	66.6	
Age groups, *N* (%)				.10			.89			**.02**
6–11 years	7.8	9.4	6.3		7.0	8.2		**10.9**	**6.2**	
12–19 years	11.2	12.7	10.2		11.3	11.5		**13.2**	**10.3**	
20–39 years	30.4	29.9	30.7		32.1	30.4		**28.6**	**31.3**	
≥40 years	50.5	48.0	52.9		49.5	49.9		**47.3**	**52.3**	
Male (%)	52.0	49.5	53.8	.14	53.0	51.0	.61	47.0	54.3	.09
Race/ethnicity, *N* (%)				.07			**.04**			**.002**
Non-Hispanic Whites	62.4	60.8	64.1		**63.0**	**62.1**		**59.2**	**64.1**	
Non-Hispanic Blacks	11.6	11.7	11.1		**11.3**	**11.8**		**11.2**	**11.7**	
Hispanics	16.3	16.0	17.0		**13.9**	**17.5**		**17.1**	**16.0**	
Other	9.6	11.5	7.8		**11.9**	**8.6**		**12.4**	**8.2**	
BMI, *N* (%)				.71			.07			.44
Normal	38.8	39.9	38.4		34.2	40.0		41.3	37.5	
Underweight	4.0	3.9	4.1		2.4	4.7		4.6	3.6	
Overweight	24.5	25.6	23.7		29.0	23.1		24.2	24.6	
Obese	32.7	30.5	33.8		34.4	32.3		29.9	34.2	
Exposure to ETS	38.1	34.8	40.2	.19	39.1	37.2	.72	**28.2**	**42.9**	**< .001**
Diabetes	8.5	**5.9**	**11.0**	**.008**	**3.9**	**9.7**	**.001**	7.0	9.3	.23
PIR, mean (SE)	2.97 (0.10)	3.09 (0.12)	2.89 (0.11)	.07	**3.31 (0.15)**	**2.88 (0.10)**	**.002**	3.08 (0.13)	2.92 (0.11)	.20

Note: *p* value for differences in prevalence calculated using chi-square. *p* value for difference in PIR calculated using *t* test. Values in bold indicates significant results.
Abbreviations: BMI, body mass index; ETS, environmental tobacco smoke; PIR, poverty income ratio.

TABLE 2 Age-adjusted least square means of total testosterone and free androgen index in males and females, NHANES 2015–2016

	Total testosterone			Free androgen index		
	Least squared means (SE)			Least squared means (SE)		
Neonicotinoids	Neonic detected	Neonic non-detected	*p*	Neonic detected	Neonic non-detected	*p*
In males						
Any neonicotinoid	302.21 (10.30)	332.18 (10.65)	.08	30.69 (1.22)	31.73 (1.10)	.58
5-Hydroxy-imidacloprid	**290.35 (13.38)**	**326.42 (8.57)**	**.04**	32.18 (2.32)	31.01 (0.75)	.66
N-Desmethyl-acetamiprid	304.61 (13.87)	322.55 (9.22)	.35	31.40 (1.78)	31.07 (0.86)	.88
In females						
Any neonicotinoid	**19.25 (1.08)**	**27.84 (3.08)**	**.04**	**1.20 (0.09)**	**1.65 (0.11)**	**.03**
5-Hydroxy-imidacloprid	**16.63 (1.25)**	**24.79 (1.60)**	**.008**	**1.09 (0.10)**	**1.50 (0.07)**	**.01**
N-Desmethyl-acetamiprid	20.12 (1.39)	24.81 (2.15)	.18	1.24 (0.11)	1.49 (0.08)	.16

Note: Age-adjusted least-square means of testosterone and free androgen index compared urinary detection of the neonicotinoids using *t* test.

TABLE 3 β (95% confidence interval) for the association of urinary neonicotinoids with serum testosterone and free androgen index in males and females, NHANES 2015–2016

	Testosterone	Free androgen index
In males		
Log_{10}-Σ neonicotinoid	**−37.78 (−58.82, −6.00)***	−33.05 (−60.25, 12.78)
5-Hydroxy-imidacloprid		
Detection	−7.94 (−21.58, 8.09)	2.01 (−19.87, 29.88)
Log_{10}-concentration	**−20.81 (−34.94, −3.62)***	−13.03 (−37.11, 20.25)
N-Desmethyl-acetamiprid		
Detection	**−20.78 (−35.96, −2.00)***	−21.45 (−38.38, 0.14)
Log_{10}-concentration	**−25.01 (−39.80, −6.58)***	**−23.07 (−40.76, −0.11)***
In Females		
Log_{10}-Σ neonicotinoid	**−32.91 (−54.93, −0.13)***	−34.57 (−61.09, 10.01)
5-Hydroxy-imidacloprid		
Detection	**−15.42 (−22.80, −7.34)****	−5.68 (−17.95, 8.42)
Log_{10}-concentration	**−21.32 (−29.31, −12.42)*****	**−16.03 (−28.90, −0.84)***
N-Desmethyl-acetamiprid		
Detection	−11.17 (−22.73, 2.11)	**−14.33 (−24.58, −2.67)***
Log_{10}-concentration	−17.71 (−34.06, 2.69)	**−22.15 (−38.61, −1.27)***

Note: Models adjusted for age, sex, race/ethnicity, poverty income ratio, body mass index, serum cotinine, diabetes, and urinary creatinine to account for dilution. In girls and women, models.
***$p < .001$. **$p < .01$. *$p < .05$.

4 | DISCUSSION

Our analysis of a nationally representative sample of the US population shows for the first time in humans that urinary detection and levels of neonicotinoids are associated with significant reductions of serum total testosterone and/or free androgen index in both males and females. The associations were observed for 5-hydroxy-imidacloprid and *N*-desmethyl-acetamiprid, the metabolites for imidacloprid and acetamiprid respectively.

Consistent with our results, reduced testosterone after exposure to acetamiprid or imidacloprid has been widely reported in animal studies. Acetamiprid administered by oral gavage at 12.5, 25, and 35 mg/kg for 90 days led to a dose dependent decrease in serum testosterone in Sprague Dawley rats.[6] Reduced serum testosterone was also observed in Sprague Dawley rats orally exposed to 30 mg/kg of acetamiprid for 35 days and in albino mice exposed to 0.16 and 0.22 mg/ml acetamiprid in drinking water for 4 weeks.[17,18] Similar results were found in adult Kunmin male mice which orally received 30 mg/kg/day for 35 days and in male Wistar rats exposed to 27 mg/kg acetamiprid by gavage for 45 days.[19,20] Although less reported, there is also evidence of lower testosterone related to imidacloprid exposure. This was observed in ICR male mice exposed to 3, 10, and 30 mg/L in drinking water for 10 weeks and in male Wistar rats orally exposed to 45 and 90 mg/kg or 0.5–8 mg/kg for 90 days.[7,21,22] Our results suggested that reduced testosterone associated with neonicotinoid exposure may occur not only in males, but also in

females and this was noted in female Nile tilapia exposed to acetamiprid mixed with a pyrethroids.[23] We found that the association of *N*-desmethyl-acetamiprid with lower serum total testosterone among females was dependent on age and was observed in women aged 40 years or older. Two animal studies also showed that the effects of acetamiprid and imidacloprid on the reproductive system may be more pronounced in mature than in immature rodents.[24,25] Interestingly, early life exposure to neonicotinoids did not cause testosterone reduction in C57BL/6J mice exposed to 1.0 or 10.0 mg/kg by gavage of acetamiprid from prenatal gestational day 6 to postnatal lactation day 21.[26]

There are several mechanisms by which neonicotinoids may affect sex hormones in both males and females. Acetamiprid has been reported to inhibit cholesterol, the precursor of steroid hormones and to prevent testosterone biosynthesis by blocking the conversion of cholesterol into testosterone as well as the entry of cholesterol in the mitochondria of Leydig cells.[18] In males, oxidative stress caused by acetamiprid damages Leydig cells' mitochondria, inhibits cyclic adenosine monophosphate (cAMP), a contributor to testosterone synthesis through cholesterol transportation into the steroidogenic pathway, and through stimulation of genes encoding for steroidogenic enzymes.[18] In females, imidacloprid may cause significant ovarian damage via oxidative stress to lower serum testosterone levels.[27] In both males and females, imidacloprid can be metabolized into desnitro-imidacloprid, a metabolite capable of crossing the blood brain barrier to affect the hypothalamus and suppress gonadotrophin-releasing hormone (GnRH), the regulator of sex hormones.[28] If the results of our analysis are confirmed, neonicotinoids could have serious systemic health effects mediated through reduced testosterone. In men, testosterone plays a crucial role in spermatogenesis and decreased testosterone leads to low sperm count with impaired fertility as well as erectile dysfunction.[18] In both men and women, decreased testosterone causes loss of libido, mood disturbance, reduced muscle mass, bone density, and cognitive function, as well as metabolic disturbances that significantly increase the risk of cardiovascular disease.[18,29]

TABLE 4 Interaction *p* values for effect modification by age on neonicotinoids associations with testosterone and free androgen index in males and females, NHANES 2015–2016

	Testosterone	Free androgen index
In males		
Log_{10}-Σ neonicotinoid	0.22	0.89
5-Hydroxy-imidacloprid		
Detection	0.83	0.90
Log_{10}-concentration	0.52	0.92
N-Desmethyl-acetamiprid		
Detection	0.28	0.69
Log_{10}-concentration	0.14	0.80
In Females		
Log_{10}-Σ neonicotinoid	0.11	0.69
5-Hydroxy-imidacloprid		
Detection	0.21	0.28
Log_{10}-concentration	0.11	0.05
N-Desmethyl-acetamiprid		
Detection	**0.01**	0.09
Log_{10}-concentration	**0.01**	**0.03**

Note: Models adjusted for age, sex, race/ethnicity, poverty income ratio, body mass index, serum cotinine, diabetes, and urinary creatinine to account for dilution. In girls and women, models were additionally adjusted for oral contraceptives.

TABLE 5 β (95% confidence interval) for the association of urinary neonicotinoids with serum testosterone and free androgen index in females by age groups, NHANES 2015–2016

	Testosterone	Free androgen index
In females 6–11 years old		
Detection	−2.70 (−19.30, 17.31)	−2.93 (−27.07, 29.21)
Log_{10}-concentration	−13.81 (−34.54, 13.49)	−12.49 (−40.29, 28.25)
In females 12–19 years old		
Detection	21.06 (−4.50, 53.46)	11.16 (−18.35, 51.36)
Log_{10}-concentration	25.39 (−8.37, 71.60)	26.56 (−16.20, 91.12)
In females 20–39 years old		
Detection	13.14 (−6.77, 37.30)	−0.49 (−21.05, 25.43)
Log_{10}-concentration	14.14 (−5.78, 38.25)	−11.29 (−34.25, 19.70)
In females 40 years or older		
Detection	**−18.55 (−30.03, −5.20)***	**−19.69 (−31.13, −6.35)****
Log_{10}-concentration	**−26.19 (−42.76, −4.83)***	**−25.13 (−43.20, −1.31)***

Note: Models adjusted for age, sex, race/ethnicity, poverty income ratio, body mass index, serum cotinine, oral contraceptives, diabetes, and urinary creatinine to account for dilution.
**$p < .01$. *$p < .05$.

Our study had limitations. Due to its cross-sectional design, temporality and causality between exposure to neonicotinoids and reduced testosterone cannot be established. Neonicotinoids were only measured in a single spot urine sample. The accuracy of free androgen index as a proxy for free testosterone used in our analysis is still matter of debate.[30] Data on treatment with sex hormones was only available in females but not in male participants. Nonetheless, our analysis has major strengths. It was conducted on a large sample representative of the US population which increased the generalizability of the findings and allowed for stratified analyses by age groups. Urinary neonicotinoids were measured at NCEH of the CDC with rigorous quality control and quality assurance procedures. Our analysis adjusted for several relevant covariates, which reduced the likelihood of residual confounding. Importantly, our analysis is the first human study to confirm the previous results from animal studies that reported an association of neonicotinoids with reduced testosterone.

5 | CONCLUSIONS

In this US representative sample, the detection of neonicotinoids in urine was associated with significantly reduced levels of serum testosterones in both males and females. Future prospective studies with repeated measures of exposure to neonicotinoids are needed to confirm these findings and better understand the underlying mechanisms. This study has important public health relevance, as neonicotinoids are extensively used and detected in half of the US population and because of the importance of sex hormones in all stages of life for both males and females.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Angelico Mendy contributed to the study concept and design, analysis and interpretation of data, and writing of the manuscript. Susan M. Pinney contributed to the interpretation of data and writing of the manuscript. Both authors reviewed the manuscript for intellectual content. Angelico Mendy takes full responsibility for the integrity of the dataset and the analysis results.

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