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Associations between neonicotinoids and liver function measures in US adults

National Health and Nutrition Examination Survey 2015–2016

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Background: Toxicological studies indicate that neonicotinoids may be associated with disruptions in liver function due to an increase in oxidative stress. There are scant epidemiological studies investigating the chronic hepatotoxic effects of neonicotinoids.
Objective: To examine the association between detectable concentrations of parent neonicotinoids and neonicotinoid metabolites with liver function markers among US adults, and whether sex modifies this association.
Methods: National Health and Nutrition Examination Survey 2015–2016 data were used to estimate associations between detectable neonicotinoids and serum alkaline phosphatase (ALP), alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transaminase (GGT), albumin, total bilirubin, total protein, and Hepatic Steatosis Index (HSI) using multiple linear regression.
Results: Detectable levels of *N*-desmethyl-acetamiprid were associated with a decrease in GGT (β = –3.54 unit/l; 95% confidence interval [CI] = –6.48, –0.61) and detectable levels of 5-hydroxy-imidacloprid were associated with a decrease in HSI (β = –1.11; 95% CI = –2.14, –0.07). Sex modified the association between any parent neonicotinoid and ALP (P_{int} = 0.064) and the association between clothianidin and ALP (P_{int} = 0.019), with a pattern of positive associations in males and inverse associations in females, though stratified associations did not reach statistical significance. Sex also modified the association between 5-hydroxy-imidacloprid and total protein (P_{int} = 0.062), with a significant positive association in females (β = 0.14 g/dl; 95% CI = 0.03, 0.25) and a null association in males.
Conclusion: Detectable concentrations of neonicotinoid metabolites were inversely associated with GGT and HSI in US adults. Evidence suggests neonicotinoids may influence liver function differently depending on sex. Future research is recommended to replicate the findings as the study was limited in its cross-sectional nature and inability to examine continuous neonicotinoid concentrations with liver function.

Keywords: 5-Hydroxy-imidacloprid; Bilirubin; Liver enzymes; Liver function; *N*-desmethyl-acetamiprid; Neonicotinoids

Introduction

Neonicotinoids are a type of pesticide, which are commonly used to protect agricultural crops from insects and other pests. Since these chemicals are more harmful to target organisms than humans, they have rapidly become popular in the global market since the introduction of imidacloprid in 1991.[1] Neonicotinoids persist in the environment due to their long half-life in soil and high water solubility, which can increase their concentrations in food and water sources.[2] Some of the common neonicotinoids include acetamiprid, clothianidin, imidacloprid, nitenpyram, thiacloprid, and thiamethoxam.[3] Several studies have found high levels of neonicotinoids in soil samples and water sources around the world.[4–13] Fruits and vegetables sampled in the United States (US) were found to have more than 90% detectable levels of neonicotinoids.[14,15] High levels of neonicotinoids have also been found in dust samples in China and the US,[16–18] air around farmland in Canada,[19] and among Italian household air samples.[20] This leads to concerns regarding unsafe exposure concentrations and potential long-term effects on human health.

Multiple studies have measured concentrations of neonicotinoids in human breast milk, blood, saliva, and urine samples.[21–24] A representative sample from the National Health and Nutrition Examination Survey (NHANES) showed

What this study adds:
Our research examines novel environmental contaminants, neonicotinoids, which are replacement insecticides for organophosphates, methyl carbamates, and pyrethroids. While toxicological studies provide evidence to support that neonicotinoids may be potential hepatotoxins, limited research has been published examining the relationship between neonicotinoids and a comprehensive set of liver function measures using a large, representative sample of adults in the United States.

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National Health and Nutritional Examination Survey 2015–2016. This is a publicly available database and can be accessed at https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2015.

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moderate detection frequencies of urinary neonicotinoids, with nearly 50% of the participants having a detectable concentration of at least one neonicotinoid biomarker tested.[25] High detection frequencies (70%) of urinary metabolites of neonicotinoids were also reported among samples collected from rural Iowa, US.[26] However, it is difficult to estimate neonicotinoid exposure over time in urine samples, since the intraclass correlation coefficient values for each neonicotinoid are relatively low.[27]

Several studies have shown the adverse effects of neonicotinoids in mammals through their harmful effects on the digestive, endocrine, hepatic, neurological, and reproductive systems.[28–31] Toxicological studies have reported that neonicotinoids are associated with changes in liver function as a result of inflammation precipitated by an increase in reactive oxygen species (ROS).[32–36] Hepatic cells may have increased susceptibility to neonicotinoids since they are highly soluble in bile, contributing to higher concentrations in the liver and potential induction of toxicity.[37] A study in rabbits showed that thiamethoxam was associated with a reduction in the activities of liver enzymes and an increase in levels of bilirubin.[30] Another study in pregnant mice showed a dose-dependent decrease in liver enzymes associated with nitenpyram among female offspring.[38] Neonicotinoids have been shown to precipitate inflammatory changes in the liver, leading to hepatomegaly and subsequent damage to normal liver function.[30] This was apparent in an epidemiological study, which showed that neonicotinoid concentrations in blood were positively associated with greater odds of being diagnosed with hepatic cancer.[39]

Although environmental chemicals undergo rigorous testing for hepatotoxicity and other adverse reactions before being disseminated into the consumer market, traditional methods of estimating toxicity may not always effectively judge chronic adverse effects due to low exposure levels, differences in body burden, and correlations with other exposures. Exploratory studies have found that high levels of heavy metals (such as lead and mercury) and polychlorinated biphenyls (PCBs) are associated with a significant increase in alanine aminotransferase (ALT), an enzyme measuring liver function.[40] PCBs have been extensively used in electric equipment, pesticides, flame retardants, paints, and printer ink.[41] Similarly, mercury is used in thermometers, batteries, and chemical factories,[42] and lead was used in pipes and household paint for several years.[43] However, adverse effects of these chemicals were not observed until they were in use for several years, which leads to the concern that risk assessment evaluations may not always detect long-term effects of environmental chemicals. Therefore, we can hypothesize that neonicotinoids may have undetected chronic hepatotoxic effects.

The objectives of this study were to examine the association between detectable urinary neonicotinoids and serum liver function markers in a representative sample of US adults, and potential effect measure modification by sex. Since the 2015–2016 cycle is the only NHANES cycle measuring common urinary neonicotinoids, we used this cycle to achieve our study objectives.

Methods

Study participants

Study participants were selected from the NHANES 2015–2016 public access database. NHANES is a program developed by the National Center for Health Statistics in which a representative sample of American adults and children is enrolled to obtain information on various health and nutritional parameters through interviews, examinations, and laboratory tests.[44] This survey collects information from noninstitutionalized US residents while accounting for clustering, stratification, and oversampling. All the data are deidentified and kept confidential.

The 2015–2016 NHANES cycle had a total of 9971 participants. Participants with information for at least one urinary neonicotinoid and at least one liver function measurement were included in this study (n = 1,695). Among these participants, we excluded those who: (1) were less than 20 years old (n = 286); (2) self-reported as pregnant (n = 17); (3) had a laboratory-confirmed diagnosis of hepatitis B (n = 104) or hepatitis C (n = 15); or (4) self-reported a diagnosed liver condition (n = 36). The final number of participants included in this study was 1,253.

Neonicotinoid assessment

The NHANES data included urinary measures of four parent neonicotinoids (acetamiprid, clothianidin, imidacloprid, and thiacloprid) and two neonicotinoid metabolites (5-hydroxy-imidacloprid and *N*-desmethyl-acetamiprid) collected from approximately one-third of the total number of participants in the survey. To obtain these measurements, enzymatic hydrolysis of urinary conjugates was followed by online solid phase extraction, reversed-phase high-performance liquid chromatography separation, and isotope dilution-electrospray ionization tandem mass spectrometry.[45] Proper quality control guidelines were followed in accordance with the Clinical Laboratory Improvement Amendments regulations.[46] Limits of detection for each compound were as follows: acetamiprid (0.30 µg/l), clothianidin (0.20 µg/l), imidacloprid (0.40 µg/l), thiacloprid (0.03 µg/l), 5-hydroxy-imidacloprid (0.20 µg/l), and *N*-desmethyl-acetamiprid (0.40 µg/l).[44]

Liver function assessment

Liver function parameters analyzed in this study were collected as a part of the standard biochemistry profile in NHANES using blood samples. ALT and aspartate aminotransferase (AST) were measured using a kinetic rate method and enzymatic rate method, respectively, to measure the rate of change in absorbance at 340 nm for a fixed interval of time. Alkaline phosphatase (ALP) was measured by a kinetic rate method using 2-amino-2-methyl-1-propanol as a buffer, where the rate of change of absorbance at 410 nm was observed for a fixed period of time. Gamma-glutamyl transaminase (GGT) was measured using an enzymatic rate method with subsequent procedures similar to the way ALP was measured. Higher liver enzyme (ALT, AST, ALP, and GGT) levels in serum are usually indicative of greater hepatotoxicity.[47] Total bilirubin was estimated using a timed-endpoint Diazo method where change in absorbance was monitored at 520 nm for a fixed period of time, while total protein was estimated by using a timed rate biuret method to monitor change in absorbance at 545 nm for a fixed period of time.[48] Low levels of total protein and high levels of total bilirubin in serum usually indicate liver dysfunction.[49] To estimate fatty liver severity, the Hepatic Steatosis Index (HSI) was calculated by using the following formula: HSI = 8 × [ALT (unit/l)/AST (unit/l)] + body mass index (BMI) (kg/m^2) + 2 (if having type 2 diabetes) + 2 (if female). A higher HSI score is generally indicative of greater severity of fatty liver disease.[50]

Statistical methods

Descriptive statistics were calculated for neonicotinoids (using weighted percent detection and percentiles) and liver function parameters (using weighted means and standard deviations). In general, neonicotinoids had a low detection frequency in urine with weighted percent detections at 0.4%, 7.9%, 4.1%, 0.1%, 19.2%, and 32.6% for acetamiprid, clothianidin, imidacloprid, thiacloprid, 5-hydroxy-imidacloprid, and *N*-desmethyl-acetamiprid, respectively (Table S1; http://links.lww.com/EE/A275). Since detection frequencies were generally low, neonicotinoid concentrations were examined as a binary variable (detect vs. nondetect, based on each neonicotinoid's respective limit of

detection) in this study. We focused on two-parent neonicotinoids (clothianidin and imidacloprid) and two neonicotinoid metabolites (5-hydroxy-imidacloprid and *N*-desmethyl-acetamiprid). Acetamiprid and thiacloprid were not included in the analysis due to low detection frequencies (0.4% and 0.1%, respectively).

To determine whether liver function parameters, presence of any parent neonicotinoid, or presence of any neonicotinoid metabolite significantly differed by selected sociodemographic factors, health factors, or environmental factors, an analysis of variance was conducted with a significance level set at $P < 0.05$. All analyses were weighted to account for the NHANES complex survey design.

The associations between detectable concentrations of urinary neonicotinoids and liver function parameters were estimated using linear regression models, with statistical significance set at $P < 0.05$. All the models accounted for strata, primary sampling units, and weights as provided by NHANES, to make the findings more generalizable to the US population. Covariates considered for the final model were selected based on *a priori* knowledge of their association with neonicotinoids and/or liver function parameters as well as their availability within the NHANES database. These included sociodemographic characteristics such as sex, age, race/ethnicity, and family monthly poverty level index; health status factors such as smoking status (based on serum cotinine levels with a cutoff at 10 ng/ml),[51] current health status (self-reported), alcohol use (based on an average number of alcoholic drinks per day with a cutoff at 4 drinks per day demarcating low or high alcohol use),[52] BMI in kg/m^2 (with categories designated according to the Centers for Disease Control and Prevention guidelines),[53] physical activity (based on self-reported minutes of moderate and vigorous activity per week with cutoffs for being physically active at 150 minutes per week and 75 minutes per week, respectively, as recommended by the Centers for Disease Control and Prevention),[54] daily protein intake in grams (assessed as quartiles), and other environmental chemicals, such as bisphenol A (BPA) and di(2-ethylhexyl) phthalate (both assessed as quartiles and measured as µg/g creatinine). BPA and phthalates were considered for model inclusion since they have been reported to increase ALT and AST, both of which are significant markers of hepatotoxicity.[55,56]

Bivariate analysis was used as a tool to determine which covariates to include in the final model, with a significance level of $P < 0.20$ set as the criterion for inclusion. If a variable had a significant association with at least half of the liver function outcomes, it was included as a covariate in the final model. Based on the results of the bivariate analyses, the covariates included in the final model were: sex (male, female), age in years (20–29, 30–39, 40–49, 50–59, 60–69, >70), race/ethnicity (non-Hispanic Asian/other race/multiracial, non-Hispanic White, non-Hispanic Black, Mexican American/other Hispanic), current health status (excellent/very good, good, fair/poor), poverty level index (low [less than 1.30], middle [1.31–1.85], and high [greater than 1.85]), BMI (underweight [<18.5], normal [18.5–24.9], overweight [25–29.9], obese [≥30]), and daily protein consumption quartiles in grams (<56.6, 56.5–74, 74.1–95.8, >95.8).

Effect measure modification by sex was determined by including an interaction term between sex and detectable neonicotinoids in the regression models, with statistical significance of that term set at $P < 0.10$. We completed two sensitivity analyses: (1) inclusion of self-reported alcohol use in the models; and (2) additional adjustment for phthalates (di(2-ethylhexyl) phthalate). All statistical analyses were completed using STATA (STATACorp LLC, College Station, Texas).

Results

Study participants

This study had a higher percentage of participants between the ages of 20 and 29 years (19.3%) compared with the older age groups (Tables 1 and 2). There were more female participants (52.6%) than male participants (47.4%). A majority of the participants identified as non-Hispanic White (65.4%). Most of the participants reported their current health status to be excellent/very good (42.3%) or good (41.1%). Approximately two-thirds of the participants had a high monthly poverty level index (64.8%). A large number of participants (40.2%) had a BMI classifying them as obese.

Mean values for serum concentrations of ALP, ALT, AST, GGT, albumin, total bilirubin, and total protein were 66.7 ± 20.0 IU/l, 25.0 ± 16.1 IU/l, 25.3 ± 10.4 unit/l, 25.4 ± 24.0 unit/l, 4.4 ± 0.3 g/dl, 0.6 ± 0.3 mg/dl, and 7.1 ± 0.4 g/dl, respectively (Table S2; http://links.lww.com/EE/A275). Normal ranges for these values are also shown in Table S2; http://links.lww.com/EE/A275.[57] Mean values for the liver enzymes ALT, AST, and GGT were significantly higher in males (Table 1). ALP, ALT, and AST levels were significantly higher among Mexican Americans/other Hispanics. All four liver enzymes peaked around the age of 50–59 years, after which there was a slight decline. Significantly higher levels of liver enzymes ALP, ALT, and GGT were observed among obese participants and those reporting fair/poor current health status compared with those with normal weight and good health status. ALT levels were higher among individuals within the highest quartile of daily protein intake.

Underweight participants had significantly higher levels of albumin, total bilirubin, and total protein levels compared with those in other BMI categories (Table 2). HSI was significantly higher in participants who were between 60 and 69 years old, Mexican American/other Hispanic, had a self-reported fair/poor current health status, and were obese. Participants with detectable concentrations of any parent neonicotinoid were significantly more likely to be non-Hispanic White (Table 3).

Association between neonicotinoids and liver function parameters

Neither of the parent neonicotinoids (clothianidin and imidacloprid) showed significant associations with any of the liver function parameters (Table 4). Similarly, no association was seen between the presence of any neonicotinoid metabolite and liver function. However, *N*-desmethyl-acetamiprid had a significant inverse association with GGT, where detectable levels of the metabolite in urine were associated with a decrease of 3.54 U/L of serum GGT (95% confidence interval [CI] = −6.48, −0.61) compared to nondetectable levels. Additionally, detectable urinary concentrations of 5-hydroxy-imidacloprid were inversely associated with HSI ($\beta = -1.11$, 95% CI = −2.14, −0.07).

Effect measure modification by sex

Sex significantly modified the association between the presence of any parent neonicotinoid ($P_{int} = 0.064$) and clothianidin ($P_{int} = 0.019$) with ALP (Figure 1 and Table S3; http://links.lww.com/EE/A275). Although sex-stratified findings were not statistically significant, we observed a pattern where detectable levels of any parent neonicotinoids and clothianidin were associated with higher ALP in males, while lower ALP levels were seen in females. Sex additionally modified the association between clothianidin and AST ($P_{int} = 0.090$), with a decrease in AST among males and increase in females, though sex-specific findings did not reach statistical significance (Table S3; http://links.lww.com/EE/A275). We did not observe any evidence of effect measure modification by sex for neonicotinoids with ALT, GGT, albumin, total bilirubin, or HSI. Sex also significantly modified the association between 5-hydroxy-imidacloprid and total protein ($P_{int} = 0.062$), with a

Table 1.

Study population characteristics for adults based on liver enzymes (20+ years), NHANES 2015–2016[a]

	n (%)	ALP (IU/l); mean (SD)	ALT (unit/l); mean (SD)	AST (unit/l); mean (SD)	GGT (unit/l); mean (SD)
Total	1253	66.7 (20.0)	25.0 (16.1)	25.3 (10.4)	25.4 (24.0)
Sex					
Male	580 (47.4)	66.8 (18.9)	29.4 (18.3)[b]	27.1 (11.1)[b]	30.8 (27.0)[b]
Female	673 (52.6)	66.6 (20.9)	21.0 (12.4)[b]	23.7 (9.4)[b]	20.5 (19.8)[b]
Age (years)					
20–29	222 (19.3)	63.5 (17.3)[b]	25.1 (19.6)[b]	24.7 (10.0)	20.4 (16.6)[b]
30–39	207 (17.5)	62.7 (18.3)[b]	25.5 (22.1)[b]	24.8 (12.7)	25.3 (27.7)[b]
40–49	219 (17.6)	65.6 (20.8)[b]	24.7 (11.6)[b]	24.8 (7.0)	26.5 (26.0)[b]
50–59	194 (17.7)	70.9 (18.8)[b]	28.8 (14.9)[b]	27.2 (12.8)	32.6 (27.1)[b]
60–69	219 (16.2)	70.2 (20.8)[b]	23.1 (10.4)[b]	24.9 (8.4)	23.2 (20.8)[b]
>70	192 (11.7)	68.3 (23.2)[b]	21.3 (10.4)[b]	25.4 (9.2)	24.3 (21.5)[b]
Race/ethnicity					
Non-Hispanic Asian/other race/multiracial	160 (9.1)	64.2 (18.3)[b]	25.3 (12.5)[b]	25.6 (7.4)[b]	28.5 (26.0)
Non-Hispanic White	449 (65.4)	65.9 (19.9)[b]	24.4 (13.1)[b]	25.3 (9.4)[b]	23.8 (19.7)
Non-Hispanic Black	248 (10.4)	66.5 (19.5)[b]	19.9 (8.3)[b]	23.0 (8.4)[b]	27.6 (33.3)
Mexican American/other Hispanic	396 (15.1)	71.8 (20.6)[b]	30.8 (27.7)[b]	26.7 (15.3)[b]	28.8 (30.6)
Current health status					
Excellent/very good	385 (42.3)	62.0 (17.3)[b]	23.1 (10.7)[b]	24.6 (7.0)	21.9 (16.9)[b]
Good	491 (41.1)	68.8 (19.5)[b]	26.1 (19.3)[b]	25.6 (12.6)	26.4 (24.3)[b]
Fair/poor	291 (16.7)	73.5 (24.7)[b]	26.6 (16.9)[b]	25.8 (10.8)	33.0 (34.3)[b]
Poverty level index					
Low	400 (22.2)	68.8 (18.9)	26.5 (21.7)	25.3 (13.0)	26.7 (27.6)
Middle	178 (13.0)	70.5 (21.1)	22.7 (14.0)	24.1 (8.8)	23.7 (23.0)
High	598 (64.8)	64.9 (19.7)	24.9 (14.4)	25.6 (9.7)	25.3 (23.1)
BMI					
Underweight	14 (1.8)	56.1 (13.7)[b]	22.1 (12.0)[b]	25.7 (11.0)	21.7 (28.2)[b]
Normal	310 (28.2)	62.0 (18.5)[b]	19.8 (8.4)[b]	24.3 (10.0)	19.0 (16.7)[b]
Overweight	382 (29.8)	68.6 (19.8)[b]	25.2 (13.8)[b]	25.4 (8.6)	25.8 (24.9)[b]
Obese	539 (40.2)	69.0 (20.7)[b]	28.6 (20.2)[b]	25.9 (11.6)	30.0 (26.4)[b]
Daily protein intake (g)					
<56.6	253 (22.0)	67.8 (19.9)	22.0 (12.4)[b]	24.4 (9.0)	23.8 (26.5)
56.6–74	252 (26.3)	66.9 (18.5)	25.1 (15.7)[b]	25.7 (11.9)	26.9 (27.6)
74.1–95.8	252 (26.9)	66.4 (21.3)	24.5 (15.0)[b]	24.3 (8.4)	23.9 (18.0)
>95.8	252 (24.8)	65.5 (19.4)	27.8 (20.2)[b]	26.8 (12.2)	27.1 (23.5)

[a]Percent, mean, and SD values presented are weighted to account for the NHANES complex survey design.

[b]Statistically different at $P < 0.05$.

SD indicates standard deviation.

significant positive association in females (β = 0.14 g/dl, 95% CI = 0.03, 0.25) and a null association in males (β = −0.01 g/dl, 95% CI = −0.14, 0.11) (Figure 1).

Sensitivity analyses

The overall findings did not change after adjusting for alcohol use (Table S4; http://links.lww.com/EE/A275). However, the magnitude of the association between *N*-desmethyl-acetamiprid and GGT increased (β = −5.66 unit/l, 95% CI = −8.90, −2.43) while the association between 5-hydroxy-imidacloprid and HSI was no longer significant. Additionally, adjusting for phthalate levels did not change the overall findings, with effect sizes being similar to those in the original model (Table S5; http://links.lww.com/EE/A275).

Discussion

This is the first epidemiological study to examine the associations between neonicotinoids and hepatotoxicity using a nationally representative population based in the US. The overall findings showed that 5-hydroxy-imidacloprid and *N*-desmethyl-acetamiprid were significantly associated with lower levels of serum GGT and lower HSI, respectively, which may lead to concerns regarding hepatotoxicity-induced liver cell damage.[30,38] Toxicological studies suggest that levels of liver enzymes higher or lower than normal could be associated with inflammatory changes and liver dysfunction. Therefore, significant inverse associations observed in this study could be linked to hepatotoxicity due to an increase in ROS and inflammation in liver tissue. However, these results should be interpreted with caution since this is a cross-sectional study and we were only able to estimate a potential association between detectable neonicotinoid levels and select liver function outcomes.

Similar to the findings of this study, a reduction in liver enzymes was seen after thiamethoxam exposure in rabbits[30] and nitenpyram exposure in mice.[38] Thiamethoxam is a broad-spectrum neonicotinoid, which has been linked to an increase in the incidence of hepatic tumors in mice;[58] while nitenpyram, a neonicotinoid comparable to imidacloprid, has been linked to the disruption of gastrointestinal microorganisms and a decrease in immune function.[59] A reduction in liver enzymes could be attributed to inflammatory pathological changes in the liver, such as infiltration of fatty acids, infiltration of inflammatory mediators, and fibrosis.[30] Furthermore, neonicotinoids are known to exacerbate oxidative stress by decreasing antioxidant enzymes, such as superoxide dismutase and catalase,[60,61] both of which are associated with an increase in ROS. These free radicals can lead to lipid peroxidation within cells hindering gene transcription and translation, which can be linked to direct cell injury and death.[62] An abundance of ROS may also lead to the overuse of glutathione with subsequent reduced levels of the antioxidant, further increasing ROS.[34,63] Another mechanism states that neonicotinoid exposure could be associated with a decrease in proliferation and diversity of gut microorganisms, which can hamper the normal metabolism of carbohydrates and proteins leading to inflammatory changes affecting biochemistry

Table 2.

Study population characteristics for adults based on other liver parameters (20+ years), NHANES 2015–2016[a]

	Albumin (g/dl); mean (SD)	Total bilirubin (mg/dl); mean (SD)	Total protein (g/dl); mean (SD)	HSI, mean (SD)
Total	4.4 (0.3)	0.6 (0.3)	7.1 (0.4)	38.1 (8.1)
Sex				
Male	4.5 (0.3)[b]	0.6 (0.3)[b]	7.2 (0.4)[b]	38.2 (8.0)
Female	4.3 (0.3)[b]	0.5 (0.2)[b]	7.0 (0.4)[b]	38.1 (8.1)
Age (years)				
20–29	4.5 (0.4)[b]	0.6 (0.3)	7.3 (0.4)[b]	36.3 (9.4)[b]
30–39	4.4 (0.3)[b]	0.6 (0.3)	7.2 (0.5)[b]	38.6 (8.3)[b]
40–49	4.4 (0.3)[b]	0.6 (0.3)	7.1 (0.4)[b]	38.7 (7.4)[b]
50–59	4.4 (0.3)[b]	0.6 (0.3)	7.1 (0.4)[b]	39.0 (7.2)[b]
60–69	4.3 (0.3)[b]	0.6 (0.3)	7.0 (0.4)[b]	39.6 (8.3)[b]
>70	4.2 (0.3)[b]	0.6 (0.3)	6.9 (0.4)[b]	36.6 (6.1)[b]
Race/ethnicity				
Non-Hispanic Asian/other race/multiracial	4.4 (0.3)[b]	0.5 (0.3)	7.2 (0.4)[b]	37.5 (7.7)[b]
Non-Hispanic White	4.4 (0.3)[b]	0.6 (0.3)	7.1 (0.4)[b]	37.4 (7.7)[b]
Non-Hispanic Black	4.2 (0.4)[b]	0.5 (0.3)	7.3 (0.5)[b]	39.6 (8.9)[b]
Mexican American/other Hispanic	4.3 (0.3)[b]	0.5 (0.3)	7.2 (0.4)[b]	40.6 (8.4)[b]
Mexican American/other Hispanic	4.3 (0.3)[b]	0.5 (0.3)	7.2 (0.4)[b]	40.6 (8.4)[b]
Current health status				
Excellent/very good	4.4 (0.3)[b]	0.6 (0.3)[b]	7.1 (0.4)	35.8 (7.1)[b]
Good	4.4 (0.3)[b]	0.6 (0.3)[b]	7.1 (0.4)	39.3 (8.1)[b]
Fair/poor	4.3 (0.4)[b]	0.5 (0.3)[b]	7.1 (0.5)	41.4 (8.9)[b]
Poverty level index				
Low	4.3 (0.3)[b]	0.5 (0.3)[b]	7.2 (0.5)[b]	39.9 (9.0)
Middle	4.3 (0.3)[b]	0.5 (0.3)[b]	7.1 (0.5)[b]	38.0 (8.0)
High	4.4 (0.3)[b]	0.6 (0.3)[b]	7.1 (0.4)[b]	37.6 (7.8)
BMI				
Underweight	4.7 (0.3)[b]	0.7 (0.5)[b]	7.4 (0.3)[b]	25.2 (3.0)[b]
Normal	4.5 (0.3)[b]	0.6 (0.3)[b]	7.2 (0.5)[b]	30.5 (2.6)[b]
Overweight	4.4 (0.3)[b]	0.6 (0.3)[b]	7.1 (0.4)[b]	36.3 (2.7)[b]
Obese	4.3 (0.3)[b]	0.5 (0.3)[b]	7.1 (0.4)[b]	45.9 (6.5)[b]
Daily protein intake (g)				
<56.6	4.3 (0.3)[b]	0.5 (0.2)	7.1 (0.5)	36.9 (8.0)
56.6–74	4.3 (0.3)[b]	0.6 (0.3)	7.1 (0.4)	38.6 (8.0)
74.1–95.8	4.4 (0.3)[b]	0.6 (0.3)	7.1 (0.5)	38.2 (8.0)
>95.8	4.4 (0.3)[b]	0.6 (0.3)	7.2 (0.4)	39.2 (8.4)

[a]Percent, mean, and SD values presented are weighted to account for the NHANES complex survey design.

[b]Statistically different at $P < 0.05$.

SD indicates standard deviation.

findings.[38] Although no other studies have reported an inverse association with hepatic biomarkers, findings pertaining to thiamethoxam and nitenpyram could be extrapolated to other neonicotinoid compounds since these compounds may share similar mechanisms of action. In addition, NHANES only has information pertaining to a small subset of neonicotinoid compounds, which may not describe the full extent of the body burden of neonicotinoids in participants.

However, there are some toxicological studies that contradict the findings of the current study.[36,64,65] An experimental study in male Wistar rats treated with 45 mg/kg body weight of imidacloprid for 28 days showed significant increases in liver enzymes compared with controls.[64] Another study showed similar findings for rats dosed with 10 mg/kg body weight of acetamiprid for 5 weeks.[65] Female mice who were given acetamiprid in their diet for 28 days showed a dose-dependent increase in liver enzymes.[36] These findings could be related to a neonicotinoid-mediated increase in membrane permeability of hepatocytes, leading to a leakage of liver enzymes into the bloodstream.[32,33,63] Additionally, an increase in free radicals due to inflammatory changes in the liver can be associated with the destruction of cell membranes and a reduction of mitochondrial membrane potential.[66] A hospital-based epidemiological study in China supported the hepatotoxic findings of toxicological studies by reporting that higher concentrations of acetamiprid and imidacloprid in blood were associated with approximately 3.5 times and 9 times the odds of having a liver cancer diagnosis, respectively.[39] Since ROS-mediated oxidative stress is the primary influence of degenerative inflammatory changes in cells, we can hypothesize that neonicotinoids may be potential mediators of oxidative stress.

Our findings indicated that the associations between specific neonicotinoid compounds and liver function may be sexually dimorphic. Males who had detectable concentrations of any parent neonicotinoid or clothianidin had higher levels of ALP. Higher than normal levels of serum liver enzymes can be considered to be an indicator of liver dysfunction, such as intra- and extrahepatic bile obstruction, hepatitis, and cirrhosis.[67] Females may also be susceptible to potential liver dysfunction as elevated AST was observed with detectable clothianidin concentrations, signifying potential liver damage. Results additionally support that 5-hydroxy-imidacloprid may play a role in the pathogenesis of liver disorders among males and females, though the cascade of liver function changes may differ by sex. Males with detectable concentrations of 5-hydroxy-imidacloprid had lower serum total protein levels, whereas higher total serum protein was observed among females. Lower total protein may suggest liver disorders that inhibit albumin and globulin production, while hepatitis has been linked to higher total serum protein.[68]

There are no toxicological studies directly examining sex differences in the relationship between neonicotinoids and liver function markers. An epidemiological study in China found no significant sex differences associated with the reporting of liver cancer diagnosis.[39] Although the current study shows insignificant results as well, sex-stratified findings displayed patterns where males had positive associations between neonicotinoids

Table 3.

Study population characteristics for adults based on detectable levels of neonicotinoids (20+ years), NHANES 2015–2016[a]

	n (%)	Detectable parent neonicotinoid, n (%)	Detectable neonicotinoid metabolite, n (%)
Total	1253	142 (11.8)	516 (42.2)
Sex			
Male	580 (47.4)	71 (51.0)	207 (43.1)
Female	673 (52.6)	71 (49.0)	309 (56.9)
Age (years)			
20–29	222 (19.3)	24 (15.5)	98 (20.1)
30–39	207 (17.5)	27 (21.4)	89 (17.8)
40–49	219 (17.6)	32 (25.0)	95 (17.3)
50–59	194 (17.7)	27 (22.2)	70 (17.9)
60–69	219 (16.2)	18 (8.8)	83 (14.9)
>70	192 (11.7)	14 (7.2)	81 (12.0)
Race/ethnicity			
Non-Hispanic Asian/other race/multiracial	160 (9.1)	32 (14.9)[b]	80 (10.5)
Non-Hispanic White	449 (65.4)	44 (61.5)[b]	168 (64.2)
Non-Hispanic Black	248 (10.4)	23 (8.8)[b]	103 (10.3)
Mexican American/other Hispanic	396 (15.1)	43 (14.8)[b]	165 (15.0)
Current health status			
Excellent/very good	385 (42.3)	46 (47.4)	167 (46.7)
Good	491 (41.1)	51 (38.8)	199 (39.8)
Fair/poor	291 (16.7)	30 (13.8)	112 (13.5)
Poverty level index			
Low	400 (22.2)	35 (20.8)	148 (19.9)
Middle	178 (13.0)	19 (13.1)	69 (12.1)
High	598 (64.8)	74 (66.1)	265 (68.0)
BMI			
Underweight	14 (1.8)	-	3 (1.5)
Normal	310 (28.2)	55 (36.9)	127 (27.6)
Overweight	382 (29.8)	43 (35.8)	157 (30.8)
Obese	539 (40.2)	44 (27.3)	226 (40.1)
Protein intake (g)			
<56.6	253 (22.0)	26 (21.7)	109 (21.0)
56.6–74	252 (26.3)	22 (21.4)	101 (25.6)
74.1–95.8	252 (26.9)	27 (23.7)	107 (25.0)
>95.8	252 (24.8)	35 (33.2)	114 (28.4)

[a]Percent and mean values presented are weighted to account for the NHANES complex survey design.
[b]Statistically different at $P < 0.05$.

and liver function, while females had primarily inverse associations. A possible explanation for these findings could be related to differences in the metabolism of neonicotinoids between males and females. Males may metabolize neonicotinoids faster than females, which could accelerate the onset of oxidative stress and release of ROS.[69] A study showed that male Sprague–Dawley rats had significant inflammatory changes in the liver after 24 hours of imidacloprid exposure, while female rats showed no inflammatory liver changes.[70] This could be attributed to estrogen playing a role as a protective factor against inflammation in females. Higher levels of estrogen have been linked to inhibition of nuclear factor κB (an important inflammation-inducing signaling factor), decreases in the expression of proinflammatory adhesion molecules, and a reduction in inflammatory mediators such as interleukins and tumor necrosis factor.[71] Mixed findings related to sex in this study could be attributed to low statistical power or previously unexplored hormonal differences between males and females. Future studies should further explore these sex differences in a longitudinal population with relatively higher detection frequencies of neonicotinoids.

This study had several strengths. The use of NHANES data provided a large, nationally representative sample for analysis, thereby increasing the generalizability of the study findings. Second, the analysis was adjusted for several important demographic, health, and environmental confounders. Additionally, we analyzed potential effect measure modification by sex for the association between neonicotinoids and liver function.

Although this study shows significant associations between neonicotinoids and liver function, further research is needed to address the limitations. First, since this was a cross-sectional study, temporality of the association cannot be established. Second, since neonicotinoids were examined as a binary variable (detect vs. nondetect), a dose-dependent relationship between neonicotinoids and liver function parameters could not be analyzed. Third, specific dietary components such as the consumption of fruits and vegetables were not included in this study (since this was not measured by NHANES). These could act as potential confounders for measured concentrations of neonicotinoids. Finally, there could be exposure misclassification due to the short half-life of neonicotinoids. Half-lives for clothianidin, dinotefuran, and imidacloprid are approximately 14, 4, and 35 hours, respectively,[1] which may lead to concerns that the neonicotinoid concentrations measured at a single time point may not reflect the true body burden of the participants.

Conclusion

This is the first epidemiological study that assesses whether detectable levels of neonicotinoids in urine are associated with changes in liver function in a representative population from the US. We identified a significant inverse association between neonicotinoid metabolites and certain liver function indicators. There was evidence of effect measure modification by sex with varying directionality of the associations. Results indicate that both males and females may be susceptible to the potential hepatotoxic effects of neonicotinoids depending on the compound or metabolite, suggesting differing modes of action for liver dysfunction by sex. Overall findings provide evidence of potential hepatotoxicity induced by neonicotinoids, which may be primarily linked to inflammatory changes leading to liver cell damage

Table 4.

Estimated differences (*β*) and 95% confidence intervals (CIs) in liver function measures by detectable concentrations of urinary neonicotinoids in US adults, NHANES, 2015–2016[a]

	ALP (IU/l) *β* (95% CI)	ALT (unit/l) *β* (95% CI)	AST (unit/l) *β* (95% CI)	GGT (unit/l) *β* (95% CI)	Albumin (g/dl) *β* (95% CI)	Total bilirubin (mg/dl) *β* (95% CI)	Total protein (g/dl) *β* (95% CI)	HSI *β* (95% CI)
Any parent neonicotinoid								
Detect	−2.77 (−9.38, 3.85)	0.82 (−3.17, 1.52)	0.01 (−2.25, 2.27)	0.80 (−5.28, 6.89)	−0.06 (−0.15, 0.03)	−0.003 (−0.04, 0.04)	−0.07 (−0.16, 0.02)	0.25 (−0.70, 1.20)
Nondetect	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Clothianidin								
Detect	−3.54 (−11.34, 4.27)	−0.91 (−4.54, 2.73)	−0.59 (−3.96, 2.78)	1.44 (−7.44, 10.32)	−0.07 (−0.17, 0.04)	0.03 (−0.02, 0.08)	−0.06 (−0.18, 0.06)	0.76 (−0.20, 1.72)
Nondetect	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Imidacloprid								
Detect	−2.07 (−11.26, 7.12)	−1.20 (−4.34, 1.93)	1.12 (−2.74, 4.97)	−1.31 (−11.44, 8.81)	−0.03 (−0.13, 0.06)	−0.02 (−0.13, 0.08)	−0.10 (−0.22, 0.03)	−0.88 (−2.58, 0.82)
Nondetect	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Any neonicotinoid metabolite								
Detect	−3.26 (−7.02, 0.51)	0.11 (−2.67, 2.89)	−0.32 (−1.72, 1.08)	−2.19 (−5.17, 0.80)	−0.01 (−0.06, 0.04)	0.01 (−0.03, 0.05)	−0.01 (−0.08, 0.07)	−0.31 (−1.10, 0.48)
Nondetect	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
5-Hydroxy-imidacloprid								
Detect	−1.67 (−6.45, 3.10)	−0.96 (−4.18, 2.26)	−0.79 (−2.55, 0.97)	0.20 (−4.38, 4.77)	0.04 (−0.02, 0.11)	0.02 (−0.03, 0.07)	0.07 (−0.02, 0.15)	−1.11 (−2.14, −0.07)[b]
Nondetect	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
N-desmethyl-acetamiprid								
Detect	−3.13 (−6.75, 0.48)	0.71 (−1.92, 3.35)	0.31 (−0.88, 1.50)	−3.54 (−6.48, −0.61)[b]	−0.02 (−0.06, 0.03)	0.001 (−0.04, 0.05)	−0.05 (−0.11, 0.01)	0.06 (−0.78, 0.89)
Nondetect	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)

[a]Adjusted by age, race/ethnicity, sex, current health status, poverty level index, BMI, and daily protein consumption.
[b]Statistically significant at $P < 0.05$.
Ref indicates referent.

Figure 1. Estimated differences and 95% confidence intervals in liver function markers by detectable parent and metabolite urinary neonicotinoid compounds in US adults, stratified by sex, NHANES, 2015–2016. Adjusted by age, race/ethnicity, sex, current health status, poverty income index, BMI, and daily protein consumption.

and degeneration. However, these findings do not indicate causation and should be interpreted cautiously. Neonicotinoid measurements taken at a single time point may not accurately represent the overall body burden in the participants. Future epidemiological studies should use a prospective study design to further examine the association between neonicotinoids and liver function while considering neonicotinoid measurements across several time points, populations, and geographical locations. Future studies should also consider the use of more robust data to further investigate whether neonicotinoid exposure contributes to liver function impairment.

Conflicts of interest statement

The authors declare that they have no conflicts of interest with regard to the content of this report.

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