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Prevalence of neonicotinoid insecticides in paired private-well tap water and human urine samples in a region of intense agriculture overlying vulnerable aquifers in eastern Iowa

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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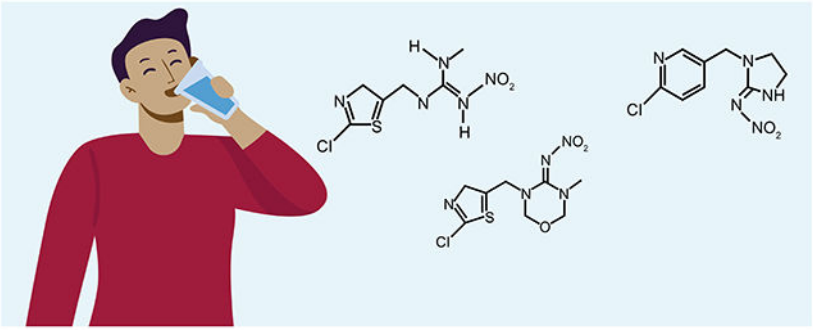
Abstract

A pilot study among farming households in eastern Iowa was conducted to assess human exposure to neonicotinoids (NEOs). The study was in a region with intense crop and livestock production and where groundwater is vulnerable to surface-applied contaminants. In addition to paired outdoor (hydrant) water and indoor (tap) water samples from private wells, urine samples were collected from 47 adult male pesticide applicators along with the completions of dietary and occupational surveys. Estimated Daily Intake (EDI) were then calculated to examine exposures for different aged family members. NEOs were detected in 53% of outdoor and 55% of indoor samples, with two or more NEOs in 13% of samples. Clothianidin was the most frequently detected NEO in water samples. Human exposure was ubiquitous in urine samples. A median of 10 different NEOs and/or metabolites were detected in urine, with clothianidin, nitenpyram, thiamethoxam, 6-chloronicotinic acid, and thiacloprid amide detected in every urine samples analyzed. Dinotefuran, imidaclothiz, acetamiprid-*N*-desmethyl, and *N*-desmethyl thiamethoxam were found in ≥ 70% of urine samples. Observed water intake for study participants and EDIs were below the chronic reference doses (CRfD) and acceptable daily intake (ADI) standards for all NEOs indicating minimal risk from ingestion of tap water. The study results indicate that while the consumption of private well tap water provides a human exposure pathway, the companion urine results provide evidence that diet and/or other exposure pathways (e.g., occupational, house dust) may contribute to exposure more than water contamination. Further biomonitoring research is needed to better understand the scale of human exposure from different sources.

Graphical Abstract



1. Introduction

Neonicotinoid insecticides (NEOs), first introduced in the 1990s, are the most widely used class of insecticides worldwide [1–4]. NEOs use has become prevalent in predominant corn and soybean regions such as Iowa and other cultivated areas in the United States [1, 5–9]. Use of treated seed has increased 3-fold in the last decade in the U.S. [10], and since 2000, use of acetamiprid in the US has increased more than seven-fold, imidacloprid use more than four-fold, and dinotefuran use more than five-fold,[5] the use of which is primarily concentrated in the Midwest. It's been estimated that most corn and soybean acreage in the U.S. is now planted with treated seeds using NEOs [10, 11]. Prior studies have shown that

NEOs have relatively long half-lives in soil, high water solubility, and low sorption in soil; these factors contribute to their persistence in water, soil and biota (Reviewed in [12]).

Several studies have reported that NEOs can adversely affect the health of mammals [13–15] with the potential for increased toxicity from select NEOs metabolites [12, 16, 17]. Despite the likelihood of widespread exposure, studies characterizing human health risks to NEOs are limited [12, 18, 19]. The few epidemiological studies that have been published highlighted concerns related to acute poisonings and possible chronic effects. This includes possible respiratory, cardiovascular, and neurological symptoms associated with acute poisonings as well as adverse pregnancy and birth outcomes with chronic exposure (Reviewed in [12]). Data from the 2015-2016 NHANES cycle found that nearly half of individuals sampled had NEOs and their metabolites in their urine [20]. Another study in Atlanta, GA, of donors with no documented exposure to NEOs, found that 90% of urine samples contained the metabolite N-desmethyl-acetamiprid [21].

Private wells are not regulated by the U.S. Environmental Protection Agency's Safe Drinking Water Act, and routine surveillance for contaminants in well water, including NEOs, is minimal [22]. Thompson et al suggest that due to the prevalence of NEOs in rural areas with high-intensity agriculture that exposure through well water might be particularly prevalent [23, 24]. In addition, they suggest the need to evaluate the relative contribution of NEOs exposure from multiple exposure pathways, the magnitude and variability of NEOs in dietary sources needs to be assessed [23, 24].

Prior work established the prevalence of NEOs in private well water sources throughout Iowa. Neonicotinoids are a common contaminant in Iowa groundwater [23, 24]. In a study of alluvial aquifers, at least one NEO was detected in 73% (n=37) of the wells sampled, with clothianidin (CLO), imidacloprid (IMI), and thiamethoxam (THX) being the most frequently detected [24]. Statistically significant inverse associations were observed between NEO concentrations and both well depth and water pH. A statewide follow-up study of groundwater in Iowa found that alluvial aquifers, wells with confining layers <15 m, and wells with depths shallower than 19.4 m had the greatest likelihood of NEO contamination [23]. Statewide, CLO was the most frequently detected NEO in groundwater (34%, max: 13.4 nanograms per liter [ng/L]), followed by THX (14.4%, max: 20.6 ng/L), and IMI (13%, max: 2.3 ng/L)[23]. The findings also suggest that NEO contamination may be present year-round in treated drinking water from vulnerable groundwater sources [23]. Although such research provides evidence that drinking water may represent a potential chronic source of human exposure [23], an important aspect of these previous studies is their focus on NEOs in ambient groundwater and not at the point of human exposure (i.e., tap water).

The primary objective of this pilot study was to assess human exposure to NEOs through private-well point-of-use tap water in an intense agricultural region overlying vulnerable aquifers of the midwestem United States. The study measured intake through direct measurements of NEOs from tap water, urine, and dietary surveys for adult study participants. The investigation also explored the potential relations to water treatment and farming/pesticide application practices to NEO concentrations in outdoor and indoor water samples. This study is important to understanding human exposure through different

pathways – drinking water, diet, and occupation. Biomonitoring research is necessary to better understand the scale of human exposure and potential risks to human health.

2. Methods

2.1. Participant Selection and Sample collection

A cross-sectional investigation was conducted from among a subset of participants enrolled in the Agricultural Health Study (AHS) Cohort's Biomarkers of Exposure and Effect in Agriculture (BEEA) study [25]. Among the Caucasian male pesticide applicators, aged 50-80 years old, enrolled in BEEA, we identified those living in an eight-county study area (Buchanan, Cedar, Delaware, Dubuque, Jackson, Johnson, Jones, or Linn) who met our study's inclusion criteria (Figure 1). The criteria for this study were selected to identify potential participants that had the highest risk of exposure through drinking water. This was done to assess the potential worst-case exposure. The inclusion criteria were:

1. participant's main source of drinking water over the past 12 months was from a private well,
2. participant's well depth was *EITHER* less than 45 m (150 ft) *OR* the predicted nitrate concentration at AHS enrollment (1993-1995) was > 5 mg/L as determined by a random forest model of private well nitrate concentrations developed for the Iowa participants of the AHS cohort [26],
3. participant personally performed farm work over the past 12 months; (4)
4. participant grew any major income-producing crop on over 0.20 km^2 (e.g., corn or soybeans) over the past 12 months,
5. participant used any type of pesticide on 75% of the farm's acreage over the past 12 months.

Initial recruitment of participants was performed by the National Cancer Institute's (NCI) contractor, Westat, Inc. The University of Iowa research team ran a second round of screening to confirm eligibility and to schedule site visits. Given the ubiquity of NEOs in modem agriculture planting crops like corn or soybeans were used as a proxy for NEO use since no information was available for their use from past BEEA studies.

Of the 95 potential participants whose contact information was provided by Westat, Inc, 34% (n=32) were determined to be ineligible because they had moved or had retired from farm work. Another 10% (n=9) of potential participants declined to participate and 4% (n=4) could not be reached by the investigators. Fifty participants that met all inclusion criteria were enrolled in the study with water samples ultimately collected from 47 of the 50 eligible participants. Two participants who originally provided consent withdrew from the study because the home visits were delayed, and one home could not be sampled due to hazardous winter road conditions at the scheduled time of sampling.

The research team obtained signed informed consent during home visits, collected outdoor and indoor water samples, and administered a questionnaire on tap water consumption, alcohol consumption, smoking history, farming practices and pesticide use, and diet during

the 24 hours prior to the home visit. During the home visit, a research team member reviewed the questionnaire and recall surveys for completeness with each participant. Daily water intake was recorded as part of this questionnaire and recall. These data were used to calculate drinking water exposure risks for the study participants. Human subject research approval was provided by Institutional Review Boards at the University of Iowa (#201809848) and the NCI prior to the study.

2.2. Analysis

Paired outdoor and indoor samples were collected at each household to compare the difference in NEO concentrations between the source of well water (ambient) and the tap (point of use). Samples were collected from December 2018 to February 2019. Water samples were collected from each households' hydrant (outdoor) as close to the wellhead as possible and kitchen cold water tap (indoor) to avoid going through the household water softener. Water samples were collected unfiltered as part of the collection procedures. Both outdoor and indoor samples were collected without prior flushing to represent/replicate homeowner use patterns when collecting tap water for consumption. Outdoor samples were untreated prior to collection. Conversely, about 27% (n=12) of indoor samples passed through some type of home water treatment system prior to collection. Unpreserved samples were collected directly into clean 1-Liter (L) amber glass bottles with Teflon-lined lids and immediately chilled. In the laboratory, samples were stored at <4 °C until they were prepared for analysis. Generally, samples were extracted within 48 days of collection and analyzed within one month of extraction. This time period is within the 64-day period for NEO stability results described previously [24]. A total of 94 water samples (47 indoor and 47 outdoor), 17 replicates, and 8 field blanks were collected. Ten replicate indoor samples and one field blank were analyzed at U.S. Geological Survey's (USGS) Organic Chemical Research Laboratory (Sacramento, California, USA) for independent validation of results [27]. Samples were analyzed by USGS using a previously published solid-phase extraction (SPE) method [28]. The 94 primary samples (47 outdoor and 47 indoor), 7 replicates, and 7 field blanks were analyzed at the State Hygienic Uaboratory at the University of Iowa (SHU) (Iowa City, Iowa, USA).

All water samples were analyzed using previously published methods [24, 28]. Briefly, 1 L water samples, blanks, and matrix spikes were filtered in the laboratory using a baked 0.7-mm nominal pore size GF/F-grade glass-fiber filters (Whatman, Piscataway, New Jersey, USA). Filtered water samples (1 L) were then spiked with a recovery surrogate, THX-d_3 (100 µL of a 1,000-ng/mF solution) and extracted using Oasis HUB SPE cartridges (6 cc, 500 mg; Waters Corporation, Milford, Massachusetts, USA) precleaned with methanol and organic-free water. Analytes were then eluted from the SPE cartridge into a clean glass concentrator tube using 10 mL of methanol. The eluant was evaporated using dry nitrogen in a nitrogen evaporator to 1 mL in a fume hood. The eluate was placed into a clean 10-mL volumetric flask, spiked with an internal standard, IMI-d_4 (C7D/N Isotopes, Pointe-Claire, Canada) (100 µL of a 1,000-ng/mF solution) and diluted to the final calibration mark of 10 mL with organic-free lab water.

Water samples, laboratory and field blanks, and matrix spikes were tested using a Agilent Zorbax Eclipse Plus C18, 1.8 μm, 2.1 x 50 mm column with electrospray ionization in positive mode (ESI+) using multiple-reaction-monitoring mode on an Agilent 1290 Infinity Liquid Chromatography System (Santa Clara, California, USA) and an AB Sciex Instruments Linear Ion Trap Quadrupole liquid chromatography with tandem mass spectrometer (LC/MS/MS) (Concord, Canada). Samples were tested for seven analytes: six NEOs (purity >99%) – acetamiprid (ACE), CLO, dinotefuran (DIN), IMI, thiacloprid (THL), THX, and a sulfoximine (purity >99%), sulfoxaflor (SUL), following methods described previously. The method detection limit for water samples was between 0.026 and 0.178 ng/L. Use of ACE, DIN, THL, and SUL has been intermittent over the past decade in Iowa [5]. These NEOs were included as analytes in this study to monitor their potential use. Replicates were then analyzed at two laboratories, the SHL (n=7) and a field blank (n=1) tested by the USGS were analyzed using a similar SPE method following a previously published method [28]. Additional analyses were run on tap water samples collected from these homes, including 437 unique organics, 32 inorganics, 5 *in vitro* bioassays, and 11 microbial assays.

The adult male pesticide applicators were provided three 90-mL collection containers for urine. Urine samples were collected before bedtime, as needed during the night, and first morning void on the day of the researchers' home visit. Samples were labeled with a unique study identifier assigned to each participant. These identifiers were used to associated urine samples by participant. All samples were refrigerated prior to the visit. Each sample was immediately placed into an individual bag, chilled, and transported on ice to the University of Iowa upon collection by researchers. All samples were stored at −80 °C until analyzed based upon guidance from the U.S. Center for Disease Control and Preventions recommendations for long-term storage of non-persistent pesticides [29]. The samples have been stored long-term for future analysis to examine additional chemical exposures.

Urine samples were analyzed for eight NEOs, including ACE, CLO, DIN, imidaclothiz (IMZ), IMI, nitenpyram (NIT), THL, THX, and four metabolites: 6-chloronicotinic acid (6-CN), acetamiprid-*N*-desmethyl (N-DMA), thiacloprid amide (TA), and *N*-desmethyl thiamethoxam (N-DMT). Samples were also analyzed for two NEO-like compounds: SUL, and flonicamid (FLO), a pyridine [12]. SUL is considered NEO-like because it has a similar mode of action to NEOs [30, 31]. FLO is often classified as an NEO but has a different mode of action [32, 33]. Use of IMZ, NIT, and FLO has not been documented in Iowa. The researchers tested urine samples for these additional NEOs to examine potential exposure to sources outside of agricultural use in Iowa.

Analytes were extracted from urine samples by SPE, and quantified using an isotope-dilution method as described earlier [34]. This method has also been previously used to assess exposure to the 14 analytes reported here [35]. All urine results were adjusted for creatinine by the National Center for Environmental Health, U.S. Centers for Disease Control and Prevention to normalize urinary concentrations of analytes and reported as μg/g. Briefly, urine samples (500 μL) were pipetted into 15-mL polypropylene (PP) tubes and fortified with 10 ng of an isotopically labeled internal standard mixture. The samples were acidified with 1.5 mL of 2% formic acid in water (v/v). The samples were then passed

through SPE cartridges (Bond Elut Plexa 60 mg, 3 mL; Agilent, Santa Clara, California, USA) that were conditioned with 3 mL of methanol containing 5% ammonia (v/v), followed by 3 mL of water. The urine samples were loaded onto the cartridges, and the sample tubes were rinsed with 3 mL of methanol. The cartridges were washed with 2 mL of 2% formic acid in a methanol/water (10:90, v/v) mixture and dried under a vacuum for 1 min. Target analytes were eluted with the rinsed solvent (3 mL of methanol) and concentrated to near-dryness under a gentle stream of nitrogen at room temperature. The analytes were reconstituted with 250 μL of 25% methanol in water (v/v) and transferred into liquid chromatography (LC) vials for instrumental analysis.

The chromatographic separation was carried out using ultra-performance liquid chromatography (Acquity I Class; Waters, Milford, Massachusetts, USA) coupled with electrospray triple quadrupole tandem mass spectrometry (API 5500; ABSciex, Framingham, Massachusetts, USA) ultra-performance liquid chromatography-electrospray-tandem mass spectrometry. For instrumental analysis, target analytes were separated into two groups. ACE, CLO, DIN, FLO, IMZ, IMI, NIT, THL, THX, N-DMA, TA, and N-DMT were analyzed using ESI+ mode. These compounds were separated chromatographically using a Betasil-C18 column (5 μm, 100 x 2.1 mm; Thermo Fisher Scientific, Waltham, Massachusetts, USA). SUL and 6-CN were analyzed using electrospray ionization in negative mode (ESI−), and these two compounds were separated chromatographically using a Kinetex phenyl/hexyl column (2.6 μm, 50 x 2.1 mm; Phenomenex, Torrance, California, USA). The method detection limit for urine samples was between about 0.015 and 0.024 ng/mL. further details of mass spectrometric conditions and liquid chromatography properties are described elsewhere [34, 35].

2.3. Statistics

Analyst and MultiQuant software (AB Sciex, Concord, Canada) was used to process LC/MS/MS data. Statistical analysis was done with SAS 9.4 (SAS Institute, Cary, North Carolina, USA). Non-detections were set at half the limit of detection [36]. This substitution was made based on the assumption that non-detects equal half the limit of detection would not significantly bias estimates and would provide a better estimate of the mean. Continuous variables were evaluated for normality using the Shapiro-Wilk test. Data that were not normally distributed are reported as median, maximum, and minimum to account for the skewed distribution. Non-parametric tests, Wilcoxon Rank-Sum Test (N=2) or Kruskal-Wallis Test (N=3), were used to compare differences in median NEO detections by water parameters and well depth. Spearman's correlation analyses were used to measure the strength and direction of association between continuous variables and NEO concentrations. Statistically significant variables ($p < 0.05$) were then categorized into discrete groups by quartiles and median values and univariate relationships were analyzed using chi-square tests and unadjusted univariate logistic regression. The unadjusted odds ratios (UORs) were calculated to estimate the potential association between NEO concentrations and covariables. Multivariate models were not considered for this pilot study.

2.4. Quality Control

Water results were validated using an external calibration curve, quality controls, and a recovery surrogate. An external calibration curve was run with each set of samples (range 10 to 2,500 ng/L). Both the linear and quadratic fits were evaluated and had mean coefficient of determination (*R*-squared) values >0.99. Concentrations detected above the highest calibration standard were diluted and retested to confirm the result. Quality controls including 15 blanks (8 field and 7 laboratory), 8 laboratory matrix spikes (3 at 25 ng/L and 5 at 250 ng/L), and replicate samples (n=17) were used to validate the results. Field and laboratory blanks used organic free or deionized water. No contamination was detected in any of the blanks tested. The recovery for the seven analytes in the matrix spikes ranged from 92.7 – 110.7% with a mean precision (relative standard deviation [RSD]) of 15% (standard deviation [SD], ± 5). The mean recoveries of the surrogate, THX-d_3. in indoor and outdoor samples were 102% (SD, ± 15) and 110.0% (SD, ± 10), respectively. Seventeen replicates, indoor (n=13) and outdoor (n=4), were also tested. Only CLO was detected in the samples and replicates tested. Replicates were then analyzed at two laboratories, the SHL (n=7) and at USGS's Organic Chemical Research Laboratory (n=10) [27]. Overall replicates varied by a mean of 2.6 ng/L (SD, ± 5) or 10.9%, with 100% agreement between detects (n=7) and non-detects (n=10). No recovery correction was made to the data reported.

The target analytes in urine were quantified by an isotopic-dilution method using a 14-point calibration curve (at concentrations ranging from 0.01 to 200 ng/mL) with the regression coefficient of ≥ 0.999. A pure solvent (methanol) and a mid-point calibration standard (10 ng/mL) were injected after every 15 samples to check for carry-over of target chemicals and instrumental drift in sensitivity. Several procedural blanks were analyzed to monitor for contamination that can arise from reagents and materials used in sample preparation steps. For each batch of 100 samples, five duplicates of the following were processed: method blank, matrix (urine) blank, and matrix (urine) spiked samples (at 10 ng/mL). Synthetic urine purchased from Cerilliant (Round Rock, Texas, USA) was used for the matrix blank and matrix spiked samples. High-performance liquid chromatography water was used for method/procedural blanks. Trace levels of IMI (1.03 – 2.53 ng/mL) and DIN (0.233 – 0.903 ng/mL) were found in method blanks. Mean method blank concentrations of IMI and DIN were subtracted from their respective concentrations in urine samples. Matrix spike samples for the 12 target analytes had mean recoveries of 86.0 – 112.8% with precision (RSD) of 5.0 – 19.8%. This method was applied to two external quality assessment scheme (G-EQUAS) samples, which have target values for 6-CN. The recoveries were 95.9 and 98.6% which are well within the acceptable error range of the analysis (±20 %).

2.5. Intake and Exposure assessment

Twenty-four-hour dietary recalls were collected from each participant and analyzed using the Automated Self-Administered 24-hour Dietary Assessment Tool (https://epi.grants.cancer.gov/asa24/), developed by the NCI, Bethesda, Maryland. In addition, a short survey was completed by participants detailing their consumption of water and alcohol, and smoking history.

Participants reported their daily water consumption. These values were used to calculate their actual exposure through drinking water. Estimated Daily Intakes were then calculated via water (EDI_w) to characterize risk for others. EDI_w were calculated for individual NEOs, and the sum of all NEOs intake (∑NNI). Water ingestion rates were used for different age groups, including infants (< 1 year old), toddlers (1-2 years old), children (3-12 years old), teenagers (13-18 years old), and adults (> 18 years old). The following formula was used:

$$EDI_W = \text{Concentration of NEOs in water(C)} * \text{Water Ingestion Rate}$$ [37]

Water ingestion rates for all analytes were calculated according to the U.S. Environmental Protection Agency exposure factors handbook, based upon “Consumer-Only Estimates of Direct and Indirect Water Ingestion (L / kg of body weight / day)” (Table S-1)[37].

Because the primary predominant NEOs component was CLO, the CLO equivalent EDI_w of NEO intake was used to determine the relative potency factor (RPF).

$$RPF(i) = RfD \text{ (reference dose) } CLO/RfD(i)$$

Where i represents individual NEOs and RfD is chronic reference dose (Table S-2). Then the CLO equivalent of ΣNNI was calculated as follows:

$$\text{CLO equivalent(ng/L)} = CLO + ACE * 0.1 + DIN * 0.5 + IMI * 0.2 + SUL * 0.2 + THI * 2.5 + THX * 1.6$$

The hazard quotient (HQ) of individual NEOs was calculated as follows (Table S-3):

$$HQ = EDI_W \text{of individual NEOs/ RfD of NEOs}$$

The RfD values from U.S. Environmental Protection Agency were 71, 9.8, 20, 57, 50, 4, and 6 μg/kg body weight (bw)/day for ACE, CLO, DIN, IMI, SUL, THL, and THX respectively [38–43]. The hazard index was calculated by adding all individual HQ. A hazard index <1 indicates no significant health risk of a particular contaminant through an exposure pathway (i.e., ingestion).

3. Results

3.1. Water Exposure and Concentration

In the United States, the NEOs are not regulated under the Safe Drinking Water Act, which sets Maximum Contaminant Limits for contaminants in drinking water[44, 45]. Public water systems are therefore not required to test for them. Drinking water from private wells are also not regulated by the U.S. federal government [22]. Well water can be vulnerable to contamination from a variety of sources with treatment left to individual households [22, 46, 47]. In regions where groundwater is vulnerable to surface-applied contaminants, households that rely on private wells for drinking water may be place at heightened risk for exposure to NEOs. Several studies have observed increased risks for adverse health effects due to exposure to contaminants [48–52].

At least one NEO was found in 53% of outdoor hydrant samples and 55% of indoor tap water samples. In outdoor water samples, CLO was found in 53% (max: 570.6 ng/L) of samples, followed by IMI (13%, max: 3.0 ng/L), THX (6%, max: 16.1 ng/L), and DIN (2%, max: 3.9 ng/L) (Table 1). Two or more NEOs were found in 13% of outdoor water samples. Considering only those samples that contained any detectable NEO, CLO was detected in 92% and 100% of outdoor and indoor samples, respectively. Only two outdoor samples had a NEO detected without CLO also being found (DIN and IMI in one sample each). For indoor samples, the most frequently detected NEO was CLO (55%, max: 140.5 ng/L), followed by IMI (11%, max: 29.3 ng/L), and THX (6%, max: 13.1 ng/L) (Table 1). Spearman correlations showed that CLO, IMI, and THX concentrations in outdoor samples were positively correlated (coefficients ranged from 0.43 – 0.52, p-value < 0.05) with each other, indicating that the NEOs may have similar sources (i.e., agricultural land use) and subsurface pathways to groundwater. This finding is consistent with other studies that have similarly found correlations between NEO concentrations [53, 54]. In this study, the correlation may be limited, however, by the low detection frequencies of IMI and THX. CLO is also a major metabolite of THX in addition to being an NEO active ingredient [3]. Thus, in addition to chemical use, this degradation pathway may also partially explain the higher frequency and concentrations of CLO detected [3, 55]. The low detection frequency of THX may therefore be related to its degradation to CLO because its use is at the land surface. Previous research found mean concentrations for IMI and THX were twice as high during the summer months, whereas CLO concentrations were greater during winter [23]. This study only analyzed samples collected during winter. IMI and DIN are both active ingredients recommended in Iowa for treating the emerald ash borer, an exotic pest that colonizes ash trees and has been found in all of Iowa's 99 counties and across the upper midwestem United States [56]. ACE, SUL, and THL were not detected in any outdoor or indoor samples from this study.

3.2. Well and Hydrogeology Characteristics

This study sampled 47 participants from eight counties in eastern Iowa (Figure 1). By design, only wells from aquifers vulnerable to surface-derived contaminants were sampled, bedrock (81%) with karst topography and/or limited overburden and alluvial aquifers (19%). Hydrogeology data were obtained from Iowa Department of Natural Resources' Natural Resources Geographic Information Systems Library [57]. These data were previously used to model nitrate concentrations in groundwater to estimate drinking water exposure amongst the Iowa participants of the AHS cohort [26]. Based upon a Wilcoxon Rank-Sum analysis of the outdoor samples, only the median concentrations of CLO varied significantly between the two types of vulnerable aquifers sampled (p= 0.05). Detected concentrations of CLO were 4.8 times (UOR=4.8; 95% confidence interval (CI; 0.9-26.3), Chi-Square p=0.07) as likely to be above the median in the sampled bedrock aquifers compared to the sampled alluvial aquifers.

The median well depth in this study was 36.6 m, with a range of 8.5 to 187.5 m, with numerous wells (45%) being less than 30 m deep. Most of the wells were less than 45 m deep (62%, n=29) or had predicted nitrate concentration greater than 5 mg/L at time of enrollment (51%, n=24). Aquifer vulnerability was estimated based upon well depth and

dissolved oxygen (DO) concentrations, two factors that can provide a generalized estimate of ground water age (low: depth >50 m and DO <0.5 mg/L; intermediate: depth >50 m and DO >0.5 mg/L or depth ≤ 50 m and DO < 0.5 mg/L; and high: depth ≤ 50 m and DO ≥5 mg/L)[58]. Using these vulnerability criteria, only high (n=16) and intermediate (n=31) vulnerability wells were sampled by design (Figure 2). Median NEO concentrations had no statistical difference (Wilcoxon Rank-Sum, $p > 0.05$) among well depth or aquifer vulnerability class.

Median CLO concentrations were significantly different by the presence of karst topography at the well location (Wilcoxon Rank-Sum, p=0.01) and between alluvial and bedrock geology (Wilcoxon Rank-Sum, =0.04). Median total NEOs concentrations (ΣNEO) also differed by karst topography (Wilcoxon Rank-Sum, p=0.02), but not by geology (Wilcoxon Rank-Sum, p>0.05). Karst is a type of terrain with exposed soluble bedrock having an abundance of surface landforms, such as sinkholes, sinking streams, and springs, that reflect the presence of subsurface voids or caves [60]. The median concentration of CLO in wells located in areas of karst topography was 2.4 ng/L compared to a median of < 0.05 ng/L where karst topography was not present. Wells with karst topography (67%) had higher detection frequencies of CLO and any NEO detection. These findings are consistent with the researchers' prior study that suggested the potential for long-term contamination in vulnerable aquifers and chronic exposure risk [23]. Here the results indicate that groundwater from shallow wells in areas with porous geology may be at greater risk to contamination from NEO application on the surface.

3.3. Comparison of private and public-supply wells

In Iowa, a comparison of untreated private wells from this study and another[24] with public water samples from a prior study [23] indicates that some private well owners may consume higher concentrations of NEOs. The studied private wells consisted primarily of vulnerable wells from alluvial and bedrock aquifers, with a median depth of 37 m and ranging from 3.5 to 187.5 m. Alluvial aquifers are susceptible to surface-applied contaminants, such as pesticides and their degradates [58, 61]. In comparison, public supply well depths ranged from 6 to 850 m with median well depth of 56 m [23]. Samples collected from public supply wells were drawn from a mixture of six major aquifer groups: alluvial aquifers (30%), sand and gravel aquifers (9%), Cretaceous sandstone (Dakota Sandstone) (8%), Silurian-Devonian bedrock (26%), Mississippian bedrock (8%), and Cambrian-Ordovician bedrock (19%) [62]. Public supply wells with less than 15 m (50 ft) of confining bed thickness based on past water quality assessments are considered vulnerable by Iowa's Department of Natural Resources [63]. Wells with between 15 and 30 m (50-100 ft) and >30 m (>100 ft) of confining material are classified as intermediate and low vulnerability, respectively [63].

A comparison of medians using the Wilcoxon Rank-Sum Test, showed that median concentrations of CLO and ΣNEO in private well water were significantly higher than median concentrations found in samples from public water systems ($p<0.05$). Median concentrations of IMI (Wilcoxon Rank-Sum Test, p=0.9) and THX (Wilcoxon Rank-Sum Test, p=0.3) were not significantly different. The maximum CLO concentration detected in private wells was 43 times greater than the maximum concentration found in samples

from public water systems. Maximum concentrations of IMI and ΣNEO were 3 and 26 times greater in the vulnerable private wells. No statistically significant difference in median concentrations was observed for IMI or THX when private well concentrations were compared to water from vulnerable aquifers, including untreated alluvial well samples (Wilcoxon Rank-Sum Test, IMI, p=0.3; THX, p=0.2) and treated public water supply (Wilcoxon Rank-Sum Test, IMI, p=0.7; THX, p=0.7). The difference in median ΣNEO when private wells were compared to public water from vulnerable aquifers was not statistically significant. These findings indicate that NEO exposures via drinking water may be higher in private well water, but also are taking place regardless of well type (i.e., public and private wells) when aquifers vulnerable to surface-applied contaminants are used as the drinking water source.

3.4. Comparison of Outdoor and Indoor Water Samples

The median number of detections and concentrations of individual NEOs and ΣNEO were not statistically different (Wilcoxon Rank-Sum tests, $p > 0.5$) between outdoor and indoor samples. One site, however, did have substantially lower outdoor NEO concentrations (CLO = 23.6 ng/L; THX = 3.1 ng/L; IMI = 2.8 ng/L) compared to indoor concentrations (CLO = 113.6 ng/L; THX = 13.11 ng/L; IMI = 29.3 ng/L). One possible explanation is during the household survey for this site it was reported that both flea/tick treatments for pets and insecticide granules on trees near the home took place over the 12 months prior to sampling. The granule ingredients were reported to include both CLO and IMI as active ingredients. However, THX is also a common active ingredient for many seed treatments. Although the ingredients of the flea/tick treatment used were not known, NEOs are common active ingredient in many household pet products for insect control [12]. More research would be helpful to understand if this phenomenon is specific to the households studied or whether applications within and around homes can affect indoor NEO concentrations in indoor water samples.

3.5. Tap Water Treatment

Only 27% (n=12) of the study's participants reported having any type of home water treatment system. The median concentration of CLO in any type of treated indoor samples (<0.05 ng/L) was statistically lower (Wilcoxon Rank-Sum tests, p=0.037) compared to untreated indoor samples (2.1 ng/L). This analysis was limited by a relatively small sample size, but samples collected by prior studies from public water systems in Iowa have also frequently detected NEOs in treated water [64, 67]. A 2017 study in Iowa City, Iowa, USA observed that conventional water treatment processes, such as rapid sand filtration, did not remove CLO or IMI; but THX concentrations were reduced by 40–60% following lime softening [65]. Granular activated carbon removed over 80% of all three NEOs [65]. In China, treatment such as aeration, coagulation, flocculation, sedimentation, sand filtration, and chlorination, was generally found to be ineffective, with <5% decrease in NEO concentrations [67]. Other studies have also found that treatment may contribute to the formation of metabolites. For example, desnitro-IMI and IMI-urea were detected in 100% and 56% of finished drinking water, respectively in Iowa City [66]. The investigators found that in the lime softening basin of a drinking water treatment plant, base-catalyzed hydrolysis occurred,[65] yielding two products from THX with one referred to as THX-H

237 found to be reactive toward chlorine [66]. The study also found CLO, IMI, desnitro-IMI and IMI-urea to be reactive during chlorination, leading to the potential to form novel disinfection by-products [66]. These results indicate that more research is needed to evaluate the efficacy of household treatment systems and to assess whether formation of transformation products during treatment are also possible.

3.6. Farming Practices

All participants reported to have farmed for at least the last 30 years, with many residing in the same location most of their lives. Generally, participants farmed 3 km^2 during the last growing season, of which 85% was cropland. All participants reported having applied pesticides and treated seeds. Thiamethoxam and clothianidin were the most common active ingredients in the commercial products participants reported using in the prior year. Only 26% of participants reported the trade name or active ingredient of products applied. During the prior growing season, most of the acreage was planted with corn (60%) or soybeans (28%). Most of the corn (92%) and soybeans (55%) were planted as treated seeds. Most participants reported using treated seeds for corn (79%) for over 10 years compared to only 25% for soybeans. Although 30% of participants reported never using treated soybean seeds, their use appears to be increasing as nearly 45% of participants reported significant or some increased use of treated soybean seeds compared to 33% who reported a change with treated corn seeds. The use of treated seeds has greatly increased NEO use in the United States [11].

A statistical analysis was conducted to determine if significant relations were observed between measured NEO concentrations and available ancillary factors related to farming practices. No significant relations, however, were observed between individual NEO concentrations or ΣNEO and percentage of cropland planted on the participants' farm with corn, soybean, or other crops. This is reasonable because all households were in an area of intense agricultural activity. The percentage of acreage planted with treated seeds was also not statistically significant associated with NEO concentrations in water. CLO, ΣNEO, and number of NEO detections were, however, inversely correlated with the percentage acreage used as cropland (Spearman, CLO, $p=0.05$; ΣNEO, $p=0.04$; total number of detects, $p=0.01$) and positively correlated with pasture acreage (CLO, $p=0.05$; ΣNEO, $p=0.05$; total number of detects, $p=0.01$). The latter observation is consistent with the results reported by a prior study [23]. A 2017-18 study investigating NEO contamination in 118 public water system wells across Iowa found significant positive correlations (Spearman, $p<0.05$) between CLO, IMI, and THX with percent grassland and pasture [23]. However, in that study, CLO concentrations were positively correlated with percent row crop, and NEOs were 3.8 times (UOR=3.8; 95% Cl (1.6-9.0), Chi-Square, $p=0.003$) more likely to be detected near row crops than any other dominant land cover [23]. Differences in the relation between acreage used as cropland and NEO concentrations in the previous study versus those in this study may be due to the intense agricultural activity surrounding all of the sampled sites in the current study causing the potentially spurious significant correlation observed. Participants reported a median of 90% (range: 50 – 100%) of their acreage was used for agriculture compared to a median of 10% (range: 0 – 50%) used for pasture. Median concentrations of CLO, IMI, and THX detected in outdoor samples were not statistically

different by any farming practices studied such as number of days planting, driving a combine, applying fertilizer, or other agricultural practices.

Median concentrations (Wilcoxon Rank-Sum tests) of CLO, IMI, and THX were not statistically significantly different by distance of well or home from pesticide mixing and storage areas. The observation is likely because NEOs are most likely used as seed treatments and in Iowa are not typically mixed on farm and applied through methods such as spraying. This could indicate that use of treated seeds minimizes the risk of well contamination compared to pesticides that are mixed on site. Median concentrations of CLO ($p=0.04$), ΣNEO ($p=0.04$), and number of detections ($p=0.02$) were significantly different by distance from the well to fields where treated seeds were planted. Concentrations of CLO ($p=0.02$), ΣNEO ($p=0.02$), and total number of detects ($p=0.01$) were positively correlated with well distance from fields where treated seeds were planted. This result is different than expected but we hypothesize this might be due to the study's sample size or specific geological features (i.e., sink holes) surrounding the households tested. In Iowa, prior studies have found herbicides and neonicotinoids are more frequently detected in alluvial and bedrock/karst region aquifers [23, 68]. In addition, only three wells were more than 182 m from where any pesticides or treated seeds were applied. This sample size was insufficient to test whether greater distance between application of pesticides and treated seeds were more protective of wells.

3.7. Estimated Intake

Estimates (EDIs) of individual NEOs were calculated across different age groups considering NEO concentrations and water ingestion rate. Estimated were calculated to examine exposures for different aged family members. No direct quantification of intake was carried out for these groups. Estimates show infants would have increased exposure to NEOs as compared to other age groups. Mean EDI_w of ΣNNI was almost 4.5 times higher in infants (0.06 ng/kg body weight [bw]/day) and 1.6 times higher in children (0.023 ng/kg bw/day) as compared to adults (0.01 ng/kg bw/day) (Table 2). Similarly, maximum EDI_w of ΣNNI intake was highest among infants' group (13 ng/kg bw/day). Because the predominant NEO component was CLO in tap water sample, we also calculated CLO equivalent EDI_w of neonicotinoid intake using RPF. CLO intake contributed about 95% of total NEO intake across different age groups. However, EDIs of all individual NEOs were below the chronic reference dose (CRfD) and acceptable daily intake (ADI). The calculated HQ and hazard index (HI) were also <1 indicating no significant health risk of NEOs through ingestion of tap water.

The World Health Organization (WHO) ADI indicates 10 and 200 μg/kg·bw/day of various NEOs can be consumed daily for a lifetime without appreciable risk to health [69]. EDI_w was shown to be significantly less than 1% of these daily standards. The WHO and U.S. Environmental Protection Agency recommend that 12,000 – 350,000 ng/kg·bw/day of CLO, IMI, and THX can be consumed daily for a lifetime without appreciable risk to health [38–43, 69].

3.8. Biomonitoring

NEOs were detected in all 115 urine samples collected during evening (n=44), overnight (n=24), and first morning voids (n=47). Many human biomonitoring studies to date looked at single spot urine samples (Reviewed in [12]). Spot urine samples were collected over a 12-hour period to assess variation in excreted NEO concentrations based upon time of day. The median number of NEOs detected per urine sample was 10 with a range of 6-13 detects across all samples. Five analytes, CLO (max: 4.7 μg/g), NIT (max: 1.1 μg/g), THX (max: 3.4 μg/g), 6-CN (max: 7.0 μg/g), and TA (max: 1.5 μg/g) were ubiquitously detected in every sample collected (Table 3). The 6-CN acid is a common metabolite for four NEOs: IMI, NIT, THL, and ACE. In addition, another four analytes were detected in ≥ 70% of samples: DIN (max: 2.9 μg/g), IMZ (max: 15.3 μg/g), N-DMA (max: 16.9 μg/g), and N-DMT (max: 3.3 μg/g). SUL, IMI, ACE, THL, and FLO were detected in 9 to 53% of all urine samples.

Median urinary concentrations were different by the time of day of the sample for ACE (p=0.007), DIN (p=<0.001), IMI (p=<0.001), IMZ (p=0.007),SUL (p=0.011), and N-DMT (p=0.018) (Table 3). Concentrations of IMZ, N-DMT, and DIN were nearly twice as high during evening samples compared to concentrations detected in first morning void samples; however, IMI and SUL concentrations were highest among first morning void samples. The highest concentrations of ACE were detected during the overnight samples.

The occurrence of CLO and THX in urine is consistent with prior studies documenting their presence in Iowa's water resources. Along with IMI, both NEOs have been commonly detected in tap water in this study in addition to being frequently detected in surface and groundwater [23, 24, 28, 66, 70]. Both NEOs were detected in all of the tested urine samples. SUL, ACE, and THL have not been detected in any of the surface or groundwater studies published in the region to date [23, 24]. No published studies were found in the United States that assessed the occurrence of NIT, FLO, or IMZ in surface or groundwater. NIT is commonly used on sucking insect pests [71]. In the United States, it currently has no registered uses, and is regulated only by the U.S. Food and Drug Administration as an oral formulation for flea adulticide in dogs and cats [12]. Globally, it is commonly applied to rice paddies [71]. Only 10 participants in this study reported using any pet treatment over the prior 12 months. No applications had taken place prior to the study's home visit. Concentrations of NIT in urine samples did not differ by reported pet treatment use (Kruskal-Wallis, p>=0.05). FLO is primarily used against pests, like aphids and whiteflies [72]. Estimates of use show intermittent application from 2010 to 2019 in Iowa [5]. IMZ, a newer NEO, was detected in 99% of all urine samples. It is not registered for use in the United States [72, 74]. IMZ is a commercial pesticide developed in China to control sucking and biting insects, like aphids, whiteflies, beetles, and Lepidoptera species [75]. Little is known about IMZ because few studies characterizing its human and animal pharmacokinetic characteristics have been published [76, 77]. A recent study of 19 subjects repeatedly sampled over 44 days found IMZ concentrations were notable in Caucasians with body mass index > 25 [78]. The study did not find any significant difference between genders and NEO concentrations [78]. Because IMZ is not commonly used in the United States, imported foods may be the source. Craddock et al. (2019) notes that many imported commodities have the maximum concentrations of NEOs [64]. Bonmatin et al. (2021) similarly detected THL

in hair samples of Filipino farmers even though this chemical is not registered for use in the country [79]. The authors concluded that the most likely source of IMZ exposure was imported food containing this NEO [79].

NEOs and their metabolites have been shown to have short half-lives and are primarily excreted in the urine due to their low molecular weights and water solubility [16, 17, 80]. Pharmacokinetic models indicate that most of NEOs are excreted within three days of ingestion (Reviewed in [12]). This indicates that the observed exposure is likely recent and from a variety of sources including water and food.

Spearman correlations were conducted to assess associations between NEO concentrations in water and in first morning void urine samples with dietary intake. Most notably, the concentrations of most analytes detected in urine were not associated with concentrations of the same NEOs detected in tap water samples (Spearman, p=>0.05). Only urinary N-DMA was found to be positively correlated with the total number of NEOs detected in tap water (Spearman, p=0.044). These results indicate additional human exposure pathways (e.g., diet, occupational, or house dust) are contributing to NEO concentrations in urine. Increased urinary concentrations were not found to be associated with use of pesticides or treated seed on the farm. Increased detections were found to be correlated with increased tap water intake (Spearman, p=0.018) and inversely with increased bottled water intake (Spearman, p=0.015).

Neonicotinoids are commonly applied to foods including rice, fruits, vegetables, tea, and coffee, and other crops such as tobacco [2, 81, 82]. Between 1999 and 2015, data from the U.S. Department of Agriculture Pesticide Data Program's an increasing trend in use of acetamiprid, clothianidin, and thiamethoxam in domestic and imported commodities [64]. Previous studies have reported subacute intoxication from food and tea consumption [83]. Six patients that consumed greater than 500g/day of either domestic fruits/vegetables and/or tea reported symptoms including finger tremor, impaired short-term memory, fever, general fatigue, headache, palpitation/chest pain, abdominal pain, muscle pain/muscle weakness/ muscle spasm, and cough [83, 84].

This study found that coffee, fruit, and smoking history were all found to be significantly positively correlated with urinary concentrations. Higher urinary concentrations of ACE (p=0.001), IMZ (p=<0.0001), NIT (p=0.024), total urinary NEO concentrations (p=0.004), and number of NEO detections (p=0.001) in urine were positively correlated with both self-reported coffee intake and estimated caffeine (mg) consumption. Participants that reported consuming 4 or more cups of coffee per day had up to 10 times higher median concentrations compared to individuals who did not drink coffee or had less. Spearman correlation found daily dietary caffeine (mg) concentrations were also associated with increased urinary concentrations of ACE (p=0.027), NIT (p=0.002), THL (p=0.002), 6-CN (p=0.029), and TA (p=0.006). Years of smoking history were found to be positively correlated with increased concentrations of FLO (p=0.036), IMZ (p=<0.0001), N-DMT (p=0.005), NIT (p=<0.0001), total urinary NEO concentrations (p=0.018), and number of NEO detections in urine (p= =<0.0001). Only 8 people reported ever smoking with only one a current smoker. Future research could assess the risk of smoking status on NEO exposure.

Total fruit intake (including juice), intake of citrus, melons, and other fruits had a significant positive association with increased urinary concentrations of SUL, THX, ACE, CLO, and N-DMA. These positive correlations are attributed to NEO application on the foods, tea, coffee, and tobacco consumed by the study's participants.

There may be other sources of exposure that have not been assessed as part of this study. This research was conducted during Iowa's winter months (i.e., December to February) to minimize the potential for occupational exposure from farming activities. In Iowa, NEOs are typically applied from April to May as seed treatments. Higher urinary concentrations may be observed during this planting period due to exposure to treated seeds. Study participants may also be exposed to other sources of NEOs in the environment, such as dust, that were not assessed in this study. NEOs have been frequently detected in soil and indoor dust in prior studies [85–88]. Other pesticides were also commonly reported in indoor dust samples collected from the BEEA cohort [25].

3.9. Limitations

As a pilot study the findings of this research may not be generalizable to the overall population. The study only enrolled adult males that were participants to the BEEA study following strict inclusion criteria. These criteria were set to assess a potential worst-case exposure. The subject of this study have a history of NEO use through agricultural practices and primarily consume water from sources vulnerable to contamination. More research is needed better understand the risk of exposure for the general population.

4. Conclusions

This study found NEOs, primarily CLO, to be prevalent in both outdoor and indoor samples collected from private wells in this region of eastern Iowa where intense agriculture (crop and livestock production) overlies aquifers that are vulnerable to surface applied contaminants. At least one NEO was detected in 53% of alluvial and vulnerable bedrock outdoor samples and 55% of indoor samples. CLO was the most frequently detected NEO in both outdoor and indoor samples, with maximum detected concentrations of 570.6 ng/L and 140.5 ng/L, respectively.

The ubiquitous presence of a suite of NEOs in the paired urine samples collected indicate that exposure to NEOs and NEO-like insecticides is commonplace among the participants (i.e. farmers) in the study region. The presence of NEOs in the companion tap water documents that the consumption of drinking water is contributing to human exposures of NEOs. However, the urine sample results show exposure to a wider variety of NEOs compared to those measured in both indoor and outdoor samples. This indicates that other sources, like dietary intake, may be important additional routes of human exposure. Other pathways (e.g., occupational exposure from application, or house dust) may also contribute to NEO concentrations in urine.

Our research supports the importance of biomonitoring of NEOs to understanding human exposure to the most heavily used class of insecticides globally. Such biomonitoring research is needed to determine current baseline human exposure levels and to provide

an understanding of the primary drivers to human exposure (i.e., drinking water, diet). Determining primary drivers of NEOs exposure will help elucidate areas where modifications would be best directed towards exposure reductions. Baseline data is critical to understanding if changes in NEO use are reflected in corresponding reductions in exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Neonicotinoids prevalent in aquifers vulnerable to surface-applied contaminants
- Neonicotinoids were ubiquitously detected (median = 10) in farmer's urine samples
- Consumption of well water only partially explains the urine neonicotinoid results
- Diet is likely an important factor contributing to the urine neonicotinoid results

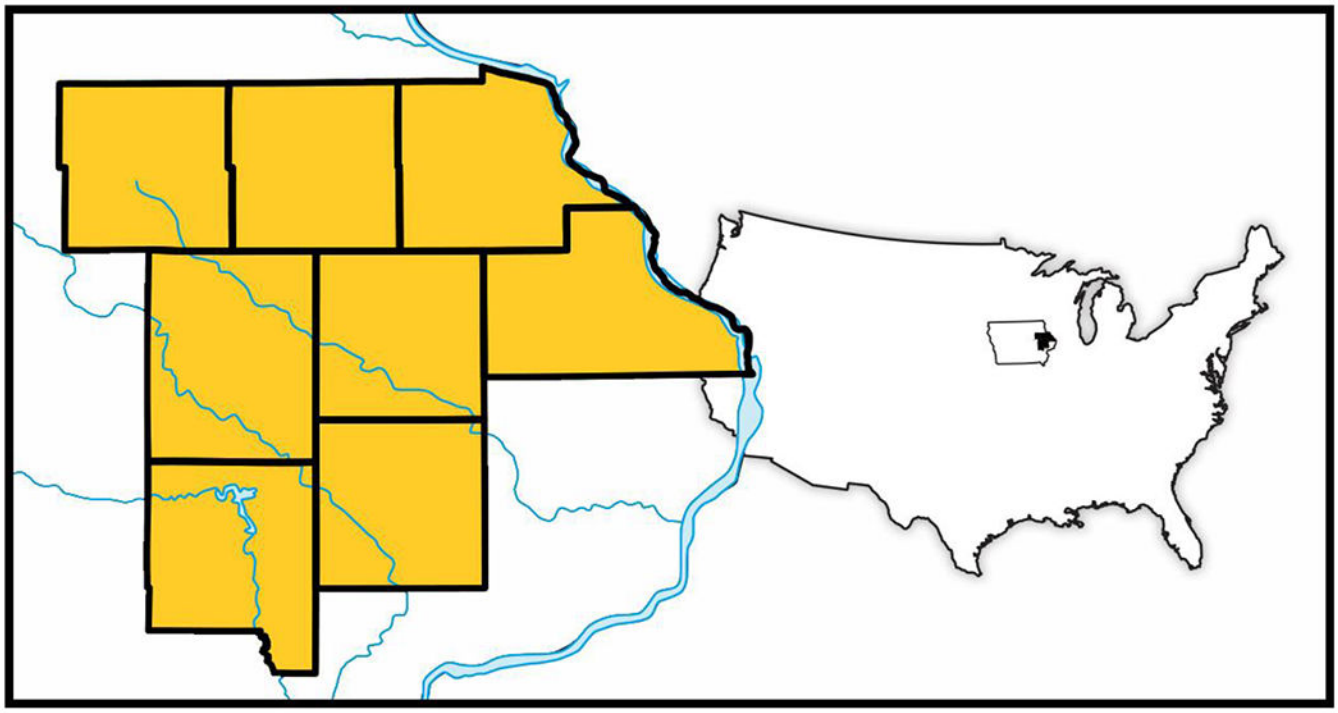

Figure 1:
Map showing the location of the eight counties in eastern Iowa where the study participants (n=47) reside.

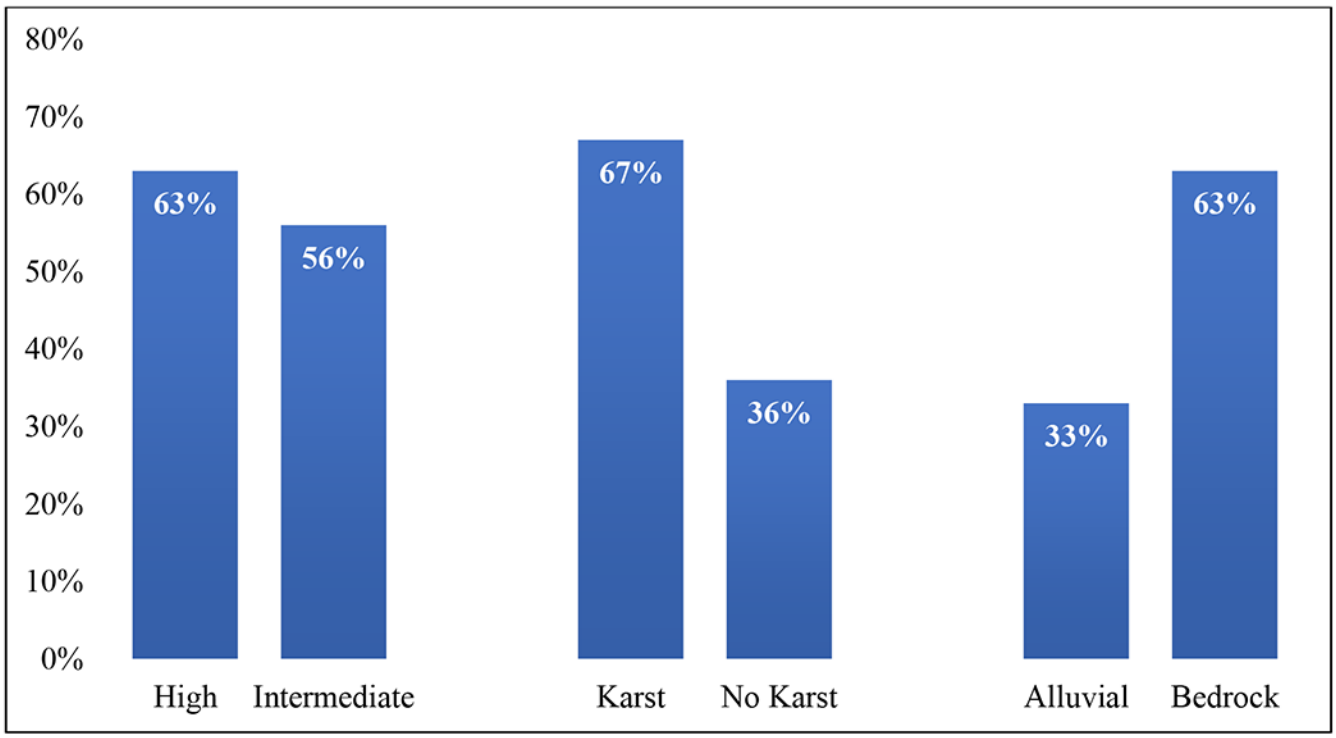

Figure 2:
Detection frequency of at least one NEO insecticide by vulnerability (high vs. intermediate), topography (karst vs. no karst), and geologic categories (alluvial vs. bedrock) in eastern Iowa (n=47).

Table 1.

Concentration (ng/L) of NEO insecticides detected in outdoor (n=47) and indoor (n=47) groundwater samples.

Analyte	Detects	Frequency	Median	Min	Max
Outdoor Hydrant Samples					
Clothianidin	25	53%	0.5	<0.05	570.6
Dinotefuran	1	2%	<0.18	<0.18	3.9
Imidacloprid	6	13%	<0.09	<0.09	3.0
Thiamethoxam	3	6%	<0.03	<0.03	16.1
Indoor Tap Samples					
Clothianidin	26	55%	0.5	<0.05	140.5
Imidacloprid	5	11%	<0.09	<0.09	29.3
Thiamethoxam	3	6%	<0.03	<0.03	13.1

< Below the limit of detection

Table 2.

Estimated daily intake via water (EDI_w, ng/kg body weight/day) of NEOs component, ΣNNI, and CLO equivalent (CLO equivalent residue of NNI based on ingestion of tap water across different age groups.

Analyte	Median EDI_w					Maximum EDI_w				
	Infants	Toddlers	Children	Teenagers	Adults	Infants	Toddlers	Children	Teenagers	Adults
Acetamiprid	0.004	0.001	0.001	0.001	0.001	0.004	0.001	0.001	0.001	0.001
Clothianidin	0.041	0.014	0.013	0.007	0.009	12.645	4.356	4.075	2.248	2.810
Dinotefuran	0.008	0.003	0.003	0.014	0.002	0.008	0.003	0.003	0.014	0.002
Imidacloprid	0.004	0.001	0.001	0.001	0.001	2.635	0.908	0.849	0.469	0.586
Sulfoxaflor	0.002	0.001	0.001	0.000	0.000	0.002	0.001	0.001	0.000	0.000
Tliiacloprid	0.003	0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.001	0.001
Thiamethoxam	0.001	0.000	0.000	0.000	0.000	1.180	0.407	0.380	0.210	0.262
Sum of NEO intake ΣNNI	0.063	0.021	0.02	0.024	0.014	16.477	5.677	5.309	2.943	3.662
Clothianidin equivalent intake (CLO equivalent residue of NNI)	0.058	0.020	0.019	0.010	0.013	12.715	4.380	4.097	2.261	2.826

[1]Based on water ingestion rate (L/kg bw/day) of 0.090, 0.031, 0.029, 0.016, 0.020 for infants, toddlers, children, teenagers, and adults, respectively.

Table 3.

Creatinine adjusted concentration (μg/g) of analytes detected in urine samples.

	AM (n=47)			PM (n=44)			Overnight (n=24)			
Analyte	**Freq**	**Median**	**Max**	**Freq**	**Median**	**Max**	**Freq**	**Median**	**Max**	**p-value**
Acetamiprid	9%	0.01	0.1	11%	0.01	0.38	17%	0.01	0.4	0.007
Clothianidin	100%	0.2	3.3	100%	0.3	3.9	100%	0.2	4.7	0.654
Dinotefuran	51%	0.04	2.9	93%	0.4	2.6	67%	0.1	1.4	<0.001
Flonicamid	36%	0.01	0.3	32%	0.01	0.4	29%	0.01	0.6	0.472
Imidacloprid	53%	0.1	7.4	18%	0.01	1.2	13%	0.01	0.4	<0.001
Imidaclothiz	98%	1.1	10.3	100%	1.6	15.3	100%	1.4	13.1	0.007
Nitenpyram	100%	0.2	0.7	100%	0.1	1.1	100%	0.2	0.8	0.693
Sulfoxaflor	23%	0.01	0.7	11%	0.01	0.1	21%	0.01	0.1	0.011
Thiacloprid	19%	0.01	0.4	34%	0.01	0.1	13%	0.01	0.0	0.124
Thiamethoxam	100%	0.3	3.4	100%	0.4	1.8	100%	0.3	1.3	0.132
6-Chloronicotinic acid	100%	0.9	5.2	100%	1.0	7.0	100%	0.9	2.0	0.138
Acetamiprid-N-Desmethyl	89%	0.1	3.4	89%	0.1	16.9	71%	0.1	4.6	0.937
Thiacloprid Amide	100%	0.2	1.5	100%	0.2	1.4	100%	0.3	0.5	0.860
(N-desmethyl thiamethoxam	91%	0.1	1.3	100%	0.2	3.3	96%	0.2	1.1	0.018
ΣNEO	100%	3.8	30.8	100%	5.4	33.2	100%	4.5	17.0	0.083

*Gray shading indicates significant difference in median concentration by time of collection.