



## Variability in urinary neonicotinoid concentrations in single-spot and first-morning void and its association with oxidative stress markers



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### ABSTRACT

Human exposure to neonicotinoid insecticides (hereafter “neonics”) is a concern. Spot urine samples have been widely used in the assessment of exposure to neonics. Urinary concentrations, however, can vary greatly over time due to variable exposure, potentially leading to exposure misclassification. In this study, within- and between-individual variability of urinary concentrations of 13 neonics and their metabolites collected consecutively for up to 44 days from 19 individuals were examined. We also measured seven oxidative stress biomarkers (OSBs) in repeated urine samples to elucidate their relationship with neonic exposure by mixed regression models. Intraclass correlation coefficients (ICCs, a ratio of between-individual variance to total variance) were used to assess the reproducibility of neonic/metabolite concentrations. Sensitivity and specificity were used to evaluate how well spot urine samples determined an individual’s average exposure over 44 days. A fair to good reproducibility was observed for *N*-desmethyl-acetamiprid (ICC = 0.42), whereas thiamethoxam, imidacloprid, clothianidin, imidaclothiz, 6-chloronicotinic acid, and sulfoxaflor showed poor reproducibility (ICC = 0.02–0.37). Use of single-spot urine samples to classify high (top 33%) exposure showed higher specificities (0.68–0.92) than sensitivities (0.32–0.88). The minimum number of specimens (*k*) required to estimate participant-specific mean for neonic exposures within 20% of the “true” values ranged from 16 to 172. Significant positive correlations were found between some of neonic and OSB concentrations. The high variability found in the urinary concentrations of most neonics/metabolites suggests that a single measurement can result in exposure misclassification.

### 1. Introduction

Neonicotinoid insecticides (hereafter “neonics”; Table S1) were developed to replace organophosphate and carbamate insecticides and are systemic in design for their neurotoxic action on the nicotinic acetylcholine receptors (Lu et al., 2018). Since the early 1990s, the use of neonics has increased dramatically worldwide, accounting currently for > 25% of the global insecticide market (Bass et al., 2015; Cimino et al., 2017; Wang et al., 2018). The annual global production of neonic active ingredients was estimated at 40 million lbs in 2010 (Wang et al., 2018). In the United States, over 4 million lbs of neonics have been applied annually on 79% of the cropland (~200 million acres) (Cimino et al., 2017; Douglas and Tooker, 2015). In addition, neonics are used on lawns, home gardens, livestock, and pets (Jeschke et al., 2011). Neonic residues have been reported to occur in soil, house dust, aquatic environment, and foods (Anderson et al., 2015; Basley and Goulson,

2018; Bennett et al., 2019; Bishop et al., 2018; Hladik et al., 2018; Lu et al., 2018; Mitchell et al., 2017; Zhang et al., 2018a). Neonics have long biological half-lives in the environment (e.g., > 1000 days in soils) (Bonmatin et al., 2015). Exposure of humans to neonics has been associated with adverse developmental or neurological outcomes, including congenital heart defects, anencephaly, and autism spectrum disorders (Cimino et al., 2017; Zhang et al., 2018b).

Neonics are metabolized by human cytochrome P450 enzymes via two different pathways: hydroxylation and dehydrogenation, and nitroimine reduction and cleavage to yield nitrosoimine, guanidine, urea derivatives and hydroxylated metabolites, which are eliminated in urine (Wang et al., 2018). The half-lives of neonics and their metabolites in human bodies are in hours to days. For example, dinotefuran (DIN), clothianidin (CLO), imidacloprid (IMI), and *N*-desmethyl-acetamiprid (N-DMA) have reported half-lives of 0.17–1.45 days in humans (Harada et al., 2016). Single-spot or first-morning-void (FMV) urine

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specimens have been used in biomonitoring of human exposure to neonics (Table S2) (Kabata et al., 2016; Marfo et al., 2015; Nomura et al., 2013; Osaka et al., 2016; Taira et al., 2013; Ueyama et al., 2015; Wang et al., 2015). However, suitability of a single-spot or FMV urine to represent integrated exposure in individuals over time has not been investigated. Because exposure to neonics is expected to be episodic, and their toxicokinetics vary in humans, appropriate exposure classification is important for epidemiologic data analysis (Harada et al., 2016; Osaka et al., 2016). Our recent study that examined daily variability in urinary concentrations of organophosphate and pyrethroid insecticides, as well as phenoxy herbicides, found considerable within-individual variations, which suggested episodic nature of exposure to these pesticides (Li et al., 2019a). Studies that determine inter- and intra-individual variability in urinary neonic levels are needed to evaluate suitability of a single-spot or FMV urine sample in exposure assessments. To date, no studies have evaluated temporal variability of neonics and their metabolites in urine.

An increasing number of *in vivo* and *in vitro* studies indicated that oxidative stress plays a significant role in neurotoxicity, immunotoxicity, hepatotoxicity, nephrotoxicity, and reproductive toxicity of neonics (Ozsahin et al., 2014; Pandey and Mohanty, 2015; Sheets et al., 2016). Research on neonic toxic mechanisms also suggested the involvement of oxidative stress (Al-Sarar et al., 2015; Galdíková et al., 2015; Lopez-Antia et al., 2015; Wang et al., 2018; Yan et al., 2015). The generation of reactive oxygen/nitrogen species represents an important role in neonic-induced oxidative damage to lipids, proteins, and DNA (Wang et al., 2018). Earlier studies of oxidative stress induced by neonics were conducted using cell bioassays (Al-Sarar et al., 2015; Galdíková et al., 2015) or *in vivo* animal studies (Lopez-Antia et al., 2015; Yan et al., 2015). Nevertheless, associations between markers of oxidative stress and neonic exposure in humans have not been investigated thus far.

To address the data gaps noted above, we performed a study with repeated analysis of neonic exposure in 19 healthy individuals, with the following aims: (1) to assess inter- and intra-individual variability in urinary concentrations of 13 neonics/metabolites in single-spot and FMV urine samples collected consecutively for up to 44 days; (2) to evaluate sensitivity and specificity of urinary measures of neonics/metabolites in individuals by comparing the “true” and “predicted” values; and (3) to examine the associations between urinary concentrations of 13 neonics/metabolites and biomarkers of oxidative damage to lipids, proteins and DNA.

## 2. Materials and methods

### 2.1. Study population and sample collection

Thirteen neonics and their metabolites were analyzed in repeated urine samples from a study designed to examine the variability in urinary levels of oxidative stress biomarkers (OSBs) (Martinez-Moral and Kannan, 2019). From February to April 2018, 19 volunteers (58% men; 42% women; Table S3) who resided in Albany, New York, USA, were recruited by convenience. The study volunteers were healthy, non-smoking, with no history of diabetes or renal disease that may affect metabolism, and no documented occupational exposure to neonics. The mean age of participants was  $33.8 \pm 12.4$  years, and the average body mass index (BMI) was  $23.6 \text{ kg/m}^2$ . Majority of the participants were Asians (68%) followed by Caucasians (32%). None of the participants was a habitual alcohol consumer. The Institutional Review Board of the New York State Department of Health approved this study.

The urine samples from 19 participants included spot and FMV samples, which were collected in 50-mL polypropylene (PP) tubes, consecutively, for up to 44 days. The samples were devoid of personal identifiers and were stored at  $-20^\circ\text{C}$  until analysis. A total of 515 urine samples, with an average of 27 samples per person, were available for this study (Table S3). Among the 515 urine samples, 243 samples were

FMVs, and the rest were spot urine. FMV refers to those urine samples that were collected immediately after waking up in the morning. For the purpose of data analysis, all 515 samples were considered in the category of spot urine, whereas 243 samples that were exclusively identified as FMVs were grouped in that category.

### 2.2. Determination of neonics and their metabolites

Analytical standards (nitenpyram, NIT; thiamethoxam, THX; IMI; acetamiprid, ACE; thiacloprid, THI; CLO; sulfoxaflor, SUF; 6-chloronicotinic acid, 6-CN; *N*-desmethyl thiamethoxam, N-DMT; DIN; flonicamid, FLO; and thiacloprid-amide, TA) of purity 96.5–100% were purchased from AccuStandard (New Haven, CT, USA). Analytical standards of imidaclothiz (IMZ) and *N*-DMA of purity  $\geq 98\%$  were purchased from Toronto Research Chemicals (North York, Ontario, Canada) and Sigma-Aldrich (St. Louis, MO, USA), respectively. The isotopically labeled internal standards ( $\text{D}_5\text{-NIT}$ ,  $^{13}\text{C}_4\text{-}^{15}\text{N-THX}$ ,  $\text{D}_4\text{-IMI}$ ,  $^{13}\text{C}_6\text{-ACE}$ ,  $^{13}\text{C}_6\text{-THI}$ ,  $^{13}\text{C}_3\text{-}^{15}\text{N-CLO}$ ,  $\text{D}_3\text{-SUF}$ ,  $\text{D}_4\text{-IMZ}$ ,  $^{13}\text{C}_5\text{-DIN}$ , and  $^{13}\text{C}_6\text{-6-CN}$ ) of  $\geq 98\%$  purity were purchased from Cambridge Isotope Laboratories (Andover, MA, USA), Toronto Research Chemicals (North York, Ontario, Canada), C/D/N Isotopes (Pointe-Claire, Quebec, Canada) and Clearysynth (Mumbai, India). Formic acid (ACS reagent) and high-performance liquid chromatography (HPLC)-grade water were purchased from J.T. Baker (Center Valley, PA, USA). Methanol (LC/MS) was from Fisher Chemical (Fair Lawn, NJ, USA) and ammonium hydroxide was from Sigma-Aldrich (St. Louis, MO, USA).

The method for the extraction of neonics from urine by a solid phase extraction (SPE) method was described earlier (Honda et al., 2019). Briefly, urine samples (500  $\mu\text{L}$ ) were spiked with stable isotopically labeled internal standards and mixed with 2% formic acid (1.5 mL). The samples were then passed through an SPE cartridge (Bond Elut Plexa 3 cc, 60 mg, Agilent, MA, USA). The elutes (3 mL of methanol) were dried under a gentle stream of nitrogen and reconstituted with 250  $\mu\text{L}$  of methanol/water (1:1, v/v).

Neonics and their metabolites were chromatographically separated by a Waters Acquity UPLC I-Class system (Waters, Milford, MA, USA) connected with a Kinetex Phenyl/Hexyl (50  $\times$  2.1 mm, 2.6  $\mu\text{m}$ ; Phenomenex, CA, USA) or Betasil C18 column (100  $\times$  2.1 mm, 5  $\mu\text{m}$ ; Thermo Fisher Scientific, Waltham, MA, USA). The analytes were detected using an ABSCIEX 5500 electrospray triple quadrupole mass spectrometer (ESI-MS/MS; Applied Biosystems, Foster City, CA, USA) in either negative or positive ionization mode.

Quantification of neonics/metabolites was accomplished by use of native standards prepared in synthetic urine along with the addition of isotopically labeled internal standards. The recoveries of target analytes through the analytical method were examined by spiking a known amount of target neonics/metabolites (1.0, 10, and 20 ng/mL) into a synthetic urine sample. The recovery of DIN in the spiked urine sample was  $< 10\%$ , and, therefore, this compound was excluded from further analysis. The recoveries of the other 13 analytes ranged from 70% to 120%, with relative standard deviations (RSDs) of  $\leq 17\%$ . The method limits of detection (LODs) of the 13 analytes were in the range of 0.002–0.099 ng/mL. The concentrations of all target compounds in procedural blanks were below the LOD (Table S4). A total of 25 urine samples were analyzed in duplicate to evaluate method precision.

### 2.3. Determination of oxidative stress biomarkers

Seven OSBs, namely, *o,o'*-dityrosine (diY), 8-hydroxy-2'-deoxyguanosine (8-OHdG), malondialdehyde (MDA), 8-*iso*-15(R)-prostaglandin  $\text{F}_{2\alpha}$  (8,15-PGF $_{2\alpha}$ ), 8-*iso*-prostaglandin  $\text{F}_{2\alpha}$  (8-PGF $_{2\alpha}$ ), 11 $\beta$ -prostaglandin  $\text{F}_{2\alpha}$  (11-PGF $_{2\alpha}$ ), and 15(R)-prostaglandin  $\text{F}_{2\alpha}$  (15-PGF $_{2\alpha}$ ), were analyzed in urine samples by following the method described in detail elsewhere (Martinez-Moral and Kannan, 2019). Quantification of OSBs was based on an isotope-dilution method and the details of which are provided in the [Supplementary Data](#).

## 2.4. Determination of urinary creatinine

Urinary creatinine concentrations were determined by Acquity HPLC (Waters, Milford, MA, USA), coupled to Acquity TQD MS/MS (Waters, Milford, MA, USA) by following the method described earlier (Martinez-Moral and Kannan, 2019).

## 2.5. Data analysis

Data were analyzed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Concentrations below the LOD were replaced with a value of LOD divided by square root of 2. Data were log-transformed ( $\chi + 1$ ) to normalize their distributions for statistical analyses. Mixed regression models were developed to explore the associations between urinary concentrations of neonics/metabolites and OSBs.

Intraclass correlation coefficients (ICCs, a ratio of between-individual variance to total variance) were computed as a measure of the reproducibility of urinary concentrations of neonics/metabolites within individuals over time. The ICC values were categorized as excellent ( $ICC \geq 0.75$ ), fair to good ( $0.75 > ICC \geq 0.40$ ), and poor ( $ICC < 0.40$ ) for the assessment of reproducibility (Attfield et al., 2014; Wang et al., 2016). Between- and within-individual variances were calculated by a linear mixed-effects model, using maximum likelihood estimation. Akaike Information Criterion (AIC) values were used to evaluate the fitness of models of urinary concentrations reported as uncorrected values, creatinine-corrected values, and creatinine as a covariate. A lower AIC value indicates a better fitness of the model. By comparing the distribution of “true” and “predicted” levels, sensitivity (a true positive rate) and specificity (a true negative rate) were evaluated on one, two, or three randomly selected spot/FMV urine sample (s) from each participant as predictors of high (top 33%) exposure based on their 44-day average concentration (Wang et al., 2016). FMV urine samples ( $n = 243$ ) were analyzed separately to test whether they represent exposure better than spot urine samples. For the “true” levels, arithmetic means of log-transformed ( $\chi + 1$ ) concentrations of neonics/metabolites from each participant ( $n = 19$ ) were calculated for samples collected during the 44-day period. The values were scaled into tertiles, and the “true” top 33% exposure levels were identified. For the “predicted” levels, ten data sets, with each containing one randomly selected spot or FMV sample per participant, were produced (i.e., 19 observations per data set). Within each randomly selected data set, concentrations of the 19 observations were identified for the “predicted” top 33% exposure levels. The average sensitivity and specificity observed across the ten separately and randomly selected data against the “true” exposure were calculated for spot or FMV urine samples. To examine whether a collection of multiple urine samples would improve the sensitivity, we repeated the analysis, using arithmetic means of two or three urine samples that were randomly selected from each participant and collected on different days. The minimum number of spot or FMV urine samples ( $k$ ) required to predict the participant-specific mean concentration of neonics/metabolites, to be within 20% of the “true” values with a probability of 95%, was calculated after log-transformation ( $\chi + 1$ ) of measured concentrations, using the following equation:

$$k = (1.96 \times CV/20)^2$$

where CV is the within-individual coefficient of variation (Kim et al., 2014; Wang et al., 2016).

## 3. Results and discussion

### 3.1. Profiles of urinary neonics and their metabolites

Neonics and their metabolites were measured in 515 urine samples collected consecutively from 19 participants (13–40 samples per person) during February–April 2018. The available demographic information of the study participants is presented in Table S3. The

**Table 1**

Concentrations (ng/mL and creatinine-adjusted in  $\mu\text{g/g}$ ) of urinary neonicotinoid insecticides collected from 19 participants consecutively during a 44-day study period.

Analyte	Void	ng/mL		Creatinine-adjusted in $\mu\text{g/g}$	
		Median	GM <sup>a</sup>	Median	GM
NIT	Spot	< LOD <sup>b</sup>	0.004	< LOD	0.003
	FMV	< LOD	0.005	< LOD	0.003
THX	Spot	0.057	0.064	0.045	0.050
	FMV	0.075	0.075	0.037	0.045
IMI	Spot	0.091	0.095	0.071	0.074
	FMV	0.110	0.112	0.065	0.068
ACE	Spot	< LOD	< LOD	< LOD	< LOD
	FMV	< LOD	< LOD	< LOD	< LOD
THI	Spot	< LOD	0.003	< LOD	0.002
	FMV	< LOD	0.003	< LOD	0.002
CLO	Spot	0.193	0.205	0.161	0.159
	FMV	0.241	0.257	0.162	0.155
FLO	Spot	< LOD	0.016	< LOD	0.013
	FMV	0.011	0.021	0.009	0.013
N-DMT	Spot	< LOD	< LOD	< LOD	< LOD
	FMV	< LOD	< LOD	< LOD	< LOD
TA	Spot	< LOD	< LOD	< LOD	< LOD
	FMV	< LOD	< LOD	< LOD	< LOD
IMZ	Spot	0.177	0.209	0.166	0.163
	FMV	0.243	0.275	0.169	0.166
N-DMA	Spot	0.272	0.301	0.216	0.235
	FMV	0.272	0.297	0.172	0.179
6-CN	Spot	0.445	0.432	0.348	0.336
	FMV	0.507	0.524	0.333	0.317
SUF	Spot	0.019	0.019	0.014	0.015
	FMV	0.020	0.022	0.013	0.013

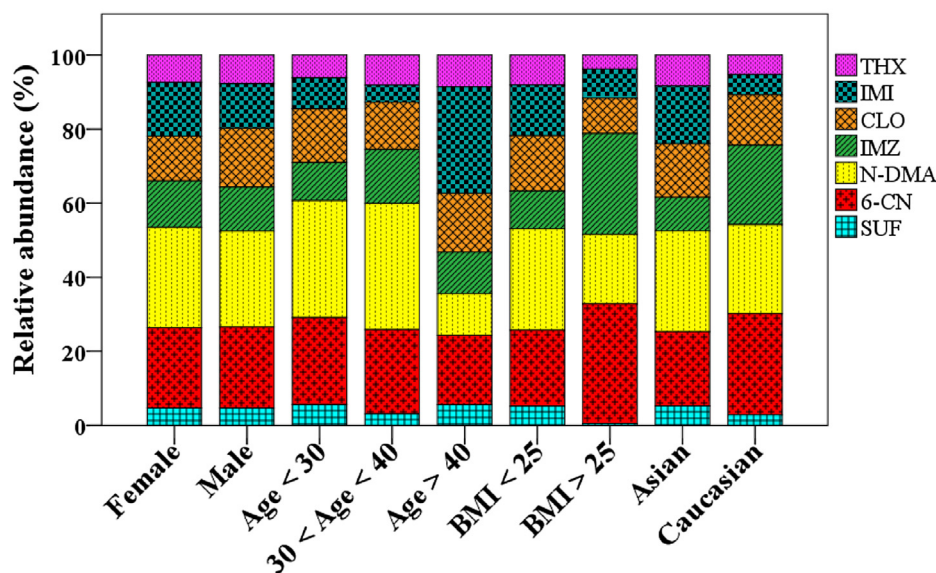
<sup>a</sup> Geometric mean.

<sup>b</sup> Limit of detection.

detection frequency (DF), distribution, and volume- and creatinine-adjusted concentrations of neonics/metabolites in spot and FMV urine are presented in Table 1, and Tables S5 and S6. The DF of neonics in urine varied widely among individuals. The most frequently detected neonics/metabolites in urine were in the decreasing order of: N-DMA > 6-CN > IMI  $\approx$  CLO > SUF > IMZ > THX (DFs, 75–100%). Other neonics/metabolites (FLO > NIT > N-DMT > ACE  $\approx$  THI > TA) were detected in 7–62% of the samples analyzed. Further analyses of the data were restricted to those analytes that had a DF of > 60% in all samples.

Creatinine-corrected concentrations of neonics and their metabolites were compared among gender, age (< 30, 30–40, and > 40 years), BMI ( $\leq 25$  and > 25  $\text{kg/m}^2$ ) and ethnic categories (Asians and Caucasians) (Fig. 1). The sum concentrations (ng/g creatinine) of the five neonics (i.e., THX, IMI, CLO, IMZ, and SUF) collectively accounted for 43–70% of the total (seven) neonic concentrations. Neonic metabolites, N-DMA (metabolite of ACE), and 6-CN (metabolite of IMI, ACE, THI, NIT and cycloxyprid) were the most abundant compounds, collectively accounting for 30–57% of the sum of seven neonics concentrations. It is worth noting that participants > 40 years of age had a high proportion of IMI (29%;  $p < 0.05$ ) and a low proportion of N-DMA (11%;  $p < 0.05$ ). IMZ concentrations were notable in individuals with BMI > 25 (27%,  $p < 0.05$ ) and who are Caucasians (21%,  $p < 0.05$ ). No significant gender-related difference was found in urinary neonic concentrations. A study in China reported no significant difference in neonic concentrations among age groups whereas higher concentrations were found in males than in females (Zhang et al., 2019). However, other studies reported no significant gender-related differences in urinary concentrations of neonics (Kabata et al., 2016; Osaka et al., 2016; Ueyama et al., 2014, 2015; Wang et al., 2015).

The creatinine-corrected concentrations of neonics/metabolites (micrograms per gram creatinine) in spot urine samples collected from the 19 participants over the 44-day period are shown in Fig. 2. There

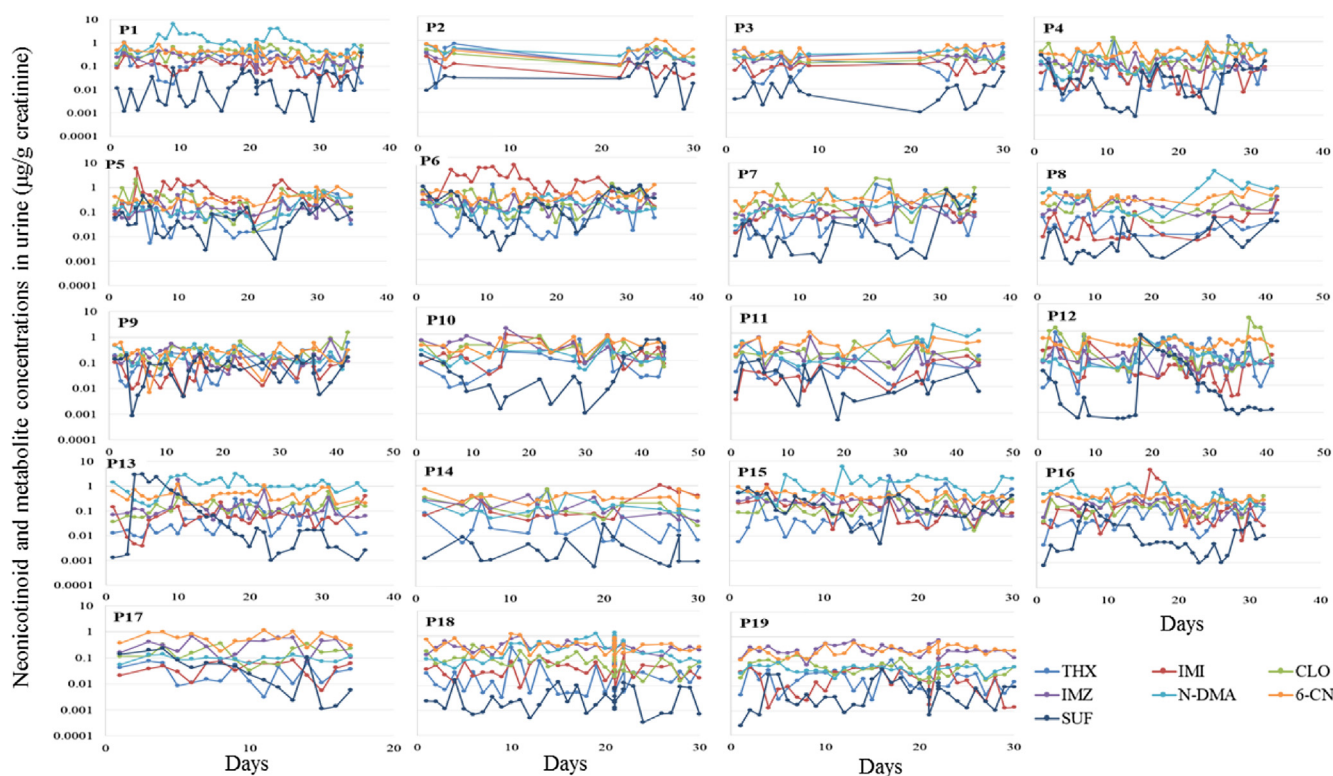


**Fig. 1.** Composition profile of urinary concentrations of neonicotinoid insecticides and their metabolites (to the sum of seven concentrations, ng/g creatinine) collected consecutively from 19 participants during a 44-day study period, stratified by gender, age, body mass index (BMI) and ethnicity. THX, thiamethoxam; IMI, imidacloprid; CLO, clothianidin; IMZ, imidaclothiz; N-DMA, *N*-desmethylacetamiprid; 6-CN, 6-chloronicotinic acid; SUF, sulfoxaflor.

were no consistent patterns in neonics/metabolites concentrations among the 19 participants. The urinary concentrations of THX and SUF varied by one to three orders of magnitude within individuals during the study period. The urinary concentrations of IMI, CLO, IMZ, N-DMA, and 6-CN varied by an order of magnitude for all participants, with the exception of IMI in three participants (P5, P13, and P16) and CLO in two participants (P5 and P12). All neonics/metabolites varied within an order of magnitude for two Asian Indian participants (P2 and P3) and a Caucasian (P8).

To the best of our knowledge, seven studies have reported urinary concentrations of neonics and their metabolites in general populations,

with four of the seven studies for a Japanese population (Table S2). The urinary concentrations of THX, IMI, CLO, 6-CN, IMZ, and N-DMA with DFs of  $\geq 55\%$  were also reported in a US population (Honda et al., 2019), and the urinary concentrations of these compounds (median, 0.07–0.43 ng/mL) were similar to those reported in our study. In comparison to the US population, Japanese adults had one to two orders of magnitude higher urinary concentrations of NIT, THX, IMI, ACE, THI, CLO, and N-DMA, which suggested the prevalence of neonic exposure in Japan (Marfo et al., 2015; Ueyama et al., 2014, 2015). In a study of Japanese children, urinary neonic levels were  $<$  LODs of 0.03–1.07 ng/mL and DFs were  $<$  25%, with the exception of DIN



**Fig. 2.** Creatinine-corrected concentrations of neonicotinoid insecticides and their metabolites ( $\mu\text{g/g}$  creatinine) in urine collected from 19 participants consecutively during a 44-day study period. Each figure represents an individual participant i.e., P1–P19. The dots in each figure denote concentrations in each spot urine sample (including first morning samples) collected during the sampling period.

**Table 2**

Apportionment<sup>a</sup> of variance in log-transformed ( $\chi + 1$ ) concentration of neonicotinoid insecticides and their metabolites in urine samples collected from 19 participants consecutively for up to 44 days ( $n = 515$ ).

	THX	IMI	CLO	IMZ	N-DMA	6-CN	SUF
<i>Uncorrected (ng/mL)</i>							
AIC <sup>b</sup>	-1030	-905	-941	-1099	-594	-1032	-1178
Between-individual $\sigma^2$ (%) <sup>c</sup>	0.001 (10)	0.005 (36)	0.001 (10)	0.001 (13)	0.006 (25)	0.002 (19)	0.001 (9)
Within-individual $\sigma^2$ (%) <sup>d</sup>	0.007 (90)	0.009 (64)	0.009 (90)	0.006 (87)	0.016 (75)	0.007 (81)	0.005 (91)
<i>Creatinine-corrected (<math>\mu\text{g/g creatinine}</math>)</i>							
AIC	-1313	-976	-1166	-1585	-814	-1451	-1428
Between-individual $\sigma^2$ (%)	0.0002 (4)	0.003 (30)	0.0003 (5)	0.0002 (6)	0.003 (25)	0.0002 (5)	0.0003 (9)
Within-individual $\sigma^2$ (%)	0.004 (96)	0.008 (70)	0.006 (95)	0.002 (94)	0.011 (75)	0.003 (95)	0.003 (91)
<i>Creatinine as a covariate</i>							
AIC	-1056	-910	-977	-1256	-651	-1191	-1189
Between-individual $\sigma^2$ (%)	0.001 (10)	0.005 (35)	0.001 (10)	0.0002 (4)	0.006 (31)	0.001 (11)	0.001 (9)
Within-individual $\sigma^2$ (%)	0.007 (90)	0.009 (65)	0.008 (90)	0.005 (96)	0.014 (69)	0.005 (89)	0.005 (91)

$\sigma^2$  = variance.

<sup>a</sup> Age, gender, body mass index, ethnicity, dietary supplement intake, exercise frequency, first morning void, alcohol and smoking consumption were included as covariates.

<sup>b</sup> AIC, Akaike Information Criterion values were used to assess the fitness of models.

<sup>c</sup> The proportion of between-individual variance to the total variance.

<sup>d</sup> The proportion of within-individual variance to the total variance.

(median, 0.44 ng/mL; DF, 58%) (Osaka et al., 2016). The median concentration of IMI measured in a general population in China (Wang et al., 2015) fell within the range reported in our study. A nationwide survey of urinary concentrations of neonics in China showed higher median concentrations (ng/mL) of THX (0.15), IMI (0.21), ACE (0.01) and CLO (0.24) than did our study (Zhang et al., 2019). All of these studies, however, used single-spot urine samples collected from a small sample size. None of the studies investigated temporal variability in urinary levels of neonics/metabolites within an individual.

### 3.2. Creatinine correction of urinary concentrations

Three different models were used to evaluate the influence of concentration corrections on the reproducibility of neonic measurements in spot urine samples. We examined between- and within-individual variance in urinary concentrations of neonics/metabolites by three different models, namely, uncorrected concentrations (i.e., volume-based), creatinine-corrected concentrations, and creatinine as a covariate (Table 2). The highest AIC values for the multilevel random-effects models were achieved with the uncorrected concentrations, which indicated the worst fit of the models. The AIC values decreased when urine dilution was taken into account. The lowest AIC values were found for creatinine-corrected concentrations of neonics. Urine dilution explained some of the observed variations in neonic concentrations, as the model fitness improved when urine dilution was accounted for (Li et al., 2019a; Wang et al., 2016). The apportionment of between- and within-individual variances in target chemical concentrations was similar for creatinine-corrected values and when creatinine was used as a covariate. Because creatinine-corrected concentrations of neonics presented the lowest AIC values, we used creatinine-corrected values in subsequent analyses.

### 3.3. Intraclass correlation coefficients and reproducibility

The within- and between-individual variances in creatinine-corrected concentrations of neonics/metabolites in spot and FMV samples from the 19 individuals are shown in Table 3. Fair to good reproducibility/reliability was observed for N-DMA (ICC = 0.42) during the 44-day period. Urinary concentrations of THX, IMI, CLO, IMZ, 6-CN, and SUF showed poor reproducibility (ICCs, 0.02–0.37) in both spot and FMV urine samples. The within-individual variance (58–98%) was much higher than the between-individual variance (2–42%). The FMV urine samples (ICCs, 0.02–0.37) did not show a better reproducibility in

comparison to the spot urine samples (ICCs, 0.06–0.42).

Most of the variance in repeated measures of neonics was attributable to within-individual variability in both spot and FMV urine samples. The low ICC values of THX, IMI, CLO, IMZ, 6-CN, and SUF imply that measurements of these neonics/metabolites in a single-spot urine sample may not adequately represent exposure over a long period of time. High within-individual variability in urinary neonic concentrations is related to short half-lives of these pesticides in humans (Harada et al., 2016). Reproducibility of IMI and N-DMA concentrations (ICCs, 0.28–0.42) in urine was greater than that of the other target compounds (ICCs, 0.12–0.19), which could be related to the frequency of exposures and doses. IMI was the most frequently detected neonics in watersheds of both urban and agricultural areas in the USA (Hladik et al., 2018; Hladik and Kolpin, 2016). ACE, the parent compound of N-DMA, is one of the most widely used insecticides in the U.S. agriculture (Douglas and Tooker, 2015; Englert et al., 2017). The high within-individual variance in FMV urinary neonic concentrations suggests that FMV did not provide an accurate estimate of exposure for over a month. No earlier study has reported the reproducibility of neonics in urine. Our recent study on temporal variability in urinary concentrations of organophosphate and pyrethroid insecticides, and phenoxy herbicides also found considerable within-individual variability in both spot and FMV samples (Li et al., 2019a). This is probably due to the short half-lives of these pesticides, episodic nature of exposures, route(s) and frequency of exposure, and physiological characteristics of individuals (Aylward et al., 2014; Harada et al., 2016; Osaka et al., 2016).

### 3.4. Sensitivity and specificity analysis for predicting concentrations

Table 4 shows sensitivity and specificity results, to accurately categorize a high-exposure group (top 33% of 44-day average concentrations) based on one, two, or three randomly selected urine samples collected on different days. The proportion of participants who were correctly classified in the top 33% of average exposure from a single sample was 0.32–0.82 and 0.40–0.88 from spot and FMV samples, respectively. Analysis of two spot urine samples collected several days apart offered an increase in sensitivity for N-DMA (72%), whereas for other analytes, the increment was  $\leq 12\%$  in comparison to a single-spot urine sample. The analysis of two FMV urine samples increased the sensitivity by 6–40% for all compounds, except IMZ, in comparison to a single FMV sample. Analysis of three urine samples collected several days apart yielded moderate-to-high sensitivities in the ranges of 0.55–0.95 and 0.48–0.98 for spot and FMV samples, respectively. The

**Table 3**

The apportionment<sup>a</sup> of variance in log-transformed ( $\chi + 1$ ) creatinine-corrected concentration of neonicotinoid insecticides and their metabolites in spot and first morning void (FMV) urine samples collected from 19 participants consecutively during a 44-day study period.

	THX	IMI	CLO	IMZ	N-DMA	6-CN	SUF
<i>Spot urine sample (n = 515)</i>							
ICC <sup>b</sup>	0.06	0.36	0.09	0.10	0.42	0.08	0.13
Between-individual $\sigma^2$ (%) <sup>c</sup>	0.0003 (6)	0.004 (36)	0.0006 (9)	0.0003 (10)	0.008 (42)	0.0003 (8)	0.0005 (13)
(95% CI) <sup>d</sup>	(0.000, 0.0008)	(0.002, 0.009)	(0.000, 0.001)	(0.000, 0.0007)	(0.004, 0.015)	(0.000, 0.0007)	(0.000, 0.001)
Within-individual $\sigma^2$ (%) <sup>e</sup>	0.004 (94)	0.008 (64)	0.0057 (91)	0.0025 (90)	0.011 (58)	0.003 (92)	0.003 (87)
(95% CI)	(0.003, 0.005)	(0.007, 0.009)	(0.005, 0.006)	(0.002, 0.003)	(0.009, 0.012)	(0.002, 0.004)	(0.002, 0.004)
<i>First morning urine sample (n = 243)</i>							
ICC	0.04	0.37	0.04	0.02	0.28	0.02	0.19
Between-individual $\sigma^2$ (%)	0.0002 (4)	0.006 (37)	0.0003 (4)	0.00003 (2)	0.003 (28)	0.00007 (2)	0.0003 (19)
(95% CI)	(0.000, 0.001)	(0.003, 0.014)	(0.000, 0.002)	(0.000, 0.001)	(0.001, 0.008)	(0.000, 0.001)	(0.000, 0.001)
Within-individual $\sigma^2$ (%)	0.004 (96)	0.010 (63)	0.006 (96)	0.0019 (98)	0.008 (72)	0.0031 (98)	0.0013 (81)
(95% CI)	(0.003, 0.005)	(0.008, 0.012)	(0.005, 0.008)	(0.0016, 0.002)	(0.007, 0.010)	(0.003, 0.004)	(0.001, 0.002)

$\sigma^2$  = variance.

<sup>a</sup> Age, gender and body mass index were included as covariates.

<sup>b</sup> ICC, intraclass correlation coefficient to assess the reproducibility of repeated measurements.

<sup>c</sup> The proportion of between-individual variance to the total variance.

<sup>d</sup> Confidence interval for the variance components.

<sup>e</sup> The proportion of within-individual variance to the total variance.

specificity (0.68–0.99) was uniformly higher than the sensitivity (0.32–0.98) for both spot and FMV urine samples. This suggests that the ability to predict a false value (i.e., specificity) was higher than that of a true value (i.e., sensitivity). The minimum number of urine specimens ( $k$ ) required from a single person to estimate the participant-specific mean for the 13 neonics/metabolites within 20% of the “true” values was 16–172 and 17–127 for spot and FMV samples, respectively (Table 4).

In our sensitivity and specificity analyses, we observed trends similar to those found for ICCs. For example, use of single-spot urine samples to classify high (top 33%) exposure from the 44-day average concentrations showed high sensitivities for IMI (0.82) and N-DMA (0.73) but low sensitivities for other neonics (0.32–0.65). This finding implies that data from a single urine sample can result in exposure misclassification of neonics/metabolites. It is worth noting, however, that sensitivity and specificity values may be overestimated due to the contribution of “predicted” values in the calculation (Li et al., 2019a; Wang et al., 2016). A fraction of the increased sensitivity and specificity observed when selecting two or more samples (per participant) instead of a single sample may be due to the increased dependence between the errors of the “true” and “predicted” values (Hauser et al., 2004). The minimum number of urine samples required to predict neonic exposure was in the order of 10–100s, which is comparable to that reported in our previous study for other classes of pesticides (Li et al., 2019a). Our results suggest that adequate caution should be exercised

when extrapolating neonic exposures based on a single urine measurement.

### 3.5. Association between neonics and oxidative stress

Pearson’s correlation analysis was used to examine the relationships among the urinary concentrations (log-transformed) of seven neonics (Table S7) or seven OSBs (Table S8), in spot ( $n = 515$ ) and FMV ( $n = 243$ ) urine samples of the 19 participants. Significant positive correlations were found between the pairs of urinary CLO vs. THX and IMI, N-DMA vs. THX and CLO, and 6-CN vs. IMZ and N-DMA ( $r$ , 0.096–0.594,  $p < 0.05$ ) in both spot and FMV urine samples. Significant correlations were only found for SUF vs. THX, IMI and CLO ( $r$ , 0.205–0.266,  $p < 0.01$ ) in FMV samples. These results suggest that the sources of human exposure to THX, IMI, CLO, SUF and ACE are common (Douglas and Tooker, 2015; Englert et al., 2017). It is worth noting that the correlations between urinary concentrations of THX and CLO were the strongest ( $r$ , 0.537–0.594,  $p < 0.01$ ), which is in accordance with a recent report from China (Zhang et al., 2019). Besides direct exposure from food and water consumption (Chen et al., 2019; Klarich et al., 2017), CLO is formed from the metabolism of THX (Nauen et al., 2003; Zhang et al., 2019). Pearson’s correlations suggested significant positive relationships among most pairs of the seven OSBs with the correlation coefficients ranging from 0.086 to 0.394 (Table S8). Similar results have been reported previously (Li et al.,

**Table 4**

Sensitivity and specificity<sup>a</sup> analysis of urinary concentration<sup>b</sup> of neonicotinoid insecticides and their metabolites for classifying high exposures (top 33%) based on the analysis of one, two and three samples collected over a 44-day period.

	THX	IMI	CLO	IMZ	N-DMA	6-CN	SUF
<i>Spot urine sample (n = 515)</i>							
1 sample	0.57 (0.80)	0.82 (0.92)	0.60 (0.82)	0.55 (0.79)	0.73 (0.88)	0.32 (0.68)	0.65 (0.84)
2 samples	0.60 (0.82)	0.88 (0.95)	0.65 (0.84)	0.50 (0.77)	0.82 (0.92)	0.55 (0.79)	0.65 (0.84)
3 samples	0.62 (0.82)	0.95 (0.98)	0.70 (0.86)	0.58 (0.81)	0.87 (0.94)	0.55 (0.79)	0.62 (0.82)
$k^c$	137	71	48	37	36	16	172
<i>First morning urine sample (n = 243)</i>							
1 sample	0.40 (0.70)	0.70 (0.85)	0.50 (0.75)	0.65 (0.83)	0.60 (0.80)	0.50 (0.75)	0.88 (0.94)
2 samples	0.48 (0.74)	0.78 (0.89)	0.70 (0.85)	0.60 (0.80)	0.70 (0.85)	0.53 (0.76)	0.93 (0.96)
3 samples	0.48 (0.74)	0.75 (0.88)	0.80 (0.90)	0.68 (0.84)	0.78 (0.89)	0.55 (0.78)	0.98 (0.99)
$K$	127	54	50	28	38	17	114

<sup>a</sup> Mean sensitivity and specificity were computed based on ten data sets each containing one randomly selected urine sample per participant.

<sup>b</sup> Calculations used creatinine-corrected concentrations ( $\mu\text{g/g}$  creatinine) on the log ( $\chi + 1$ ) scale.

<sup>c</sup> The minimum number of urine samples required to estimate participant-specific mean within 20% of the “true” value.

**Table 5**

Mixed regression models<sup>a</sup> of log-transformed ( $\chi + 1$ ) creatinine-corrected concentration of urinary neonicotinoid insecticides and their metabolites and oxidative stress biomarkers.

Type of sample	Mixed regression models $\beta$ (95% CI) <sup>b</sup>							
	THX	IMI	CLO	IMZ	N-DMA	6-CN	SUF	$\Sigma$ 7 neonics
<i>Spot urine sample (n = 515)</i>								
diY	-0.001 (-0.04, 0.04)	0.05 (0.002, 0.10)*	-0.01 (-0.05, 0.04)	0.07 (0.04, 0.09)**	-0.05 (-0.11, 0.01)	0.03 (-0.01, 0.06)	-0.02 (-0.05, 0.02)	0.05 (-0.03, 0.12)
8-OHdG	-0.04 (-0.10, 0.01)	0.12 (0.03, 0.20)**	0.04 (-0.03, 0.11)	0.05 (0.004, 0.09)*	0.14 (0.04, 0.24)**	0.08 (0.02, 0.13)**	0.03 (-0.03, 0.08)	0.25 (0.13, 0.37)**
MDA	0.001 (-0.03, 0.03)	0.01 (-0.03, 0.05)	-0.01 (-0.04, 0.02)	0.01 (-0.01, 0.03)	0.03 (-0.01, 0.07)	0.03 (0.01, 0.06)**	-0.01 (-0.03, 0.02)	0.04 (-0.01, 0.09)
8-PGF <sub>2<math>\alpha</math></sub>	0.02 (-0.09, 0.13)	0.07 (-0.07, 0.22)	0.12 (-0.003, 0.25)	0.08 (-0.01, 0.16)	-0.002 (-0.18, 0.18)	0.14 (0.05, 0.24)**	0.05 (-0.05, 0.15)	0.26 (0.04, 0.48)*
11-PGF <sub>2<math>\alpha</math></sub>	-0.03 (-0.11, 0.05)	0.20 (0.10, 0.30)**	0.003 (-0.09, 0.09)	0.13 (0.07, 0.19)**	0.09 (-0.04, 0.21)	0.01 (-0.06, 0.08)	-0.05 (-0.12, 0.02)	0.19 (0.03, 0.34)*
15-PGF <sub>2<math>\alpha</math></sub>	-0.01 (-0.05, 0.03)	0.10 (0.05, 0.15)**	-0.01 (-0.05, 0.03)	0.09 (0.06, 0.12)**	0.03 (-0.03, 0.09)	0.08 (0.05, 0.11)**	-0.03 (-0.07, 0.001)	0.15 (0.07, 0.22)**
8,15-PGF <sub>2<math>\alpha</math></sub>	0.07 (0.004, 0.14)*	-0.01 (-0.10, 0.08)	-0.003 (-0.08, 0.08)	-0.02 (-0.08, 0.03)	0.07 (-0.04, 0.18)	0.05 (-0.01, 0.11)	-0.03 (-0.09, 0.03)	0.08 (-0.06, 0.21)
<i>First morning urine sample (n = 243)</i>								
diY	-0.003 (-0.06, 0.05)	0.04 (-0.04, 0.13)	-0.02 (-0.09, 0.04)	0.06 (0.02, 0.09)**	-0.04 (-0.12, 0.04)	0.06 (0.01, 0.10)**	-0.05 (-0.08, -0.02)**	-0.01 (-0.12, 0.10)
8-OHdG	-0.05 (-0.12, 0.02)	0.17 (0.02, 0.31)*	0.01 (-0.08, 0.10)	-0.01 (-0.06, 0.04)	0.08 (-0.05, 0.21)	0.04 (-0.03, 0.11)	-0.001 (-0.05, 0.05)	0.19 (0.04, 0.34)*
MDA	0.01 (-0.03, 0.05)	0.03 (-0.02, 0.09)	-0.01 (-0.05, 0.04)	0.01 (-0.02, 0.03)	0.05 (0.004, 0.11)*	0.03 (0.003, 0.06)*	-0.01 (-0.03, 0.02)	0.08 (0.00, 0.15)*
8-PGF <sub>2<math>\alpha</math></sub>	0.10 (-0.08, 0.28)	0.13 (-0.16, 0.41)	0.16 (-0.06, 0.38)	0.08 (-0.04, 0.20)	-0.05 (-0.31, 0.21)	0.18 (0.02, 0.33)*	0.00 (-0.10, 0.10)	0.26 (-0.11, 0.63)
11-PGF <sub>2<math>\alpha</math></sub>	-0.07 (-0.17, 0.03)	0.06 (-0.09, 0.22)	-0.03 (-0.16, 0.09)	0.07 (-0.001, 0.13)	-0.06 (-0.21, 0.08)	-0.02 (-0.11, 0.06)	-0.06 (-0.12, -0.01)*	-0.06 (-0.27, 0.14)
15-PGF <sub>2<math>\alpha</math></sub>	0.02 (-0.03, 0.08)	0.10 (0.02, 0.19)*	-0.004 (-0.07, 0.06)	0.09 (0.06, 0.13)**	0.04 (-0.04, 0.11)	0.11 (0.07, 0.16)**	-0.05 (-0.08, -0.02)**	0.16 (0.05, 0.26)**
8,15-PGF <sub>2<math>\alpha</math></sub>	0.07 (-0.02, 0.16)	-0.06 (-0.21, 0.09)	-0.04 (-0.16, 0.07)	-0.03 (-0.10, 0.03)	0.08 (-0.06, 0.21)	0.10 (0.02, 0.18)*	0.03 (-0.03, 0.08)	0.08 (-0.11, 0.27)

<sup>a</sup> Age, gender, body mass index, ethnicity, dietary supplement intake, exercise frequency and alcohol consumption were included as covariates.

<sup>b</sup> CI, confidence interval.

\* Correlation is significant at the 0.05 level.

\*\* Correlation is significant at the 0.01 level.

2019a, 2019b).

We found significant positive associations between repeated measures of neonics/metabolites and OSBs, particularly 8-OHdG and 15-PGF<sub>2 $\alpha$</sub>  in spot and FMV samples collected during the 44-day study period (Table 5). Creatinine-corrected concentrations of diY, 8-OHdG, 11-PGF<sub>2 $\alpha$</sub> , and 15-PGF<sub>2 $\alpha$</sub>  were significantly correlated with IMI, IMZ, N-DMA, and 6-CN in spot samples, whereas diY, MDA, and 15-PGF<sub>2 $\alpha$</sub>  concentrations were significantly correlated with IMI, IMZ, N-DMA, and 6-CN in FMV urine samples. The significant positive association between repeated measures of neonics and OSBs support the previous finding of neonics-induced oxidative damage to lipids, proteins, and DNA (Wang et al., 2018), even at the low concentrations measured in this study. The low strength of the positive associations ( $\beta$ , 0.03–0.20), however, may be related to the low concentrations found in urine (median concentrations: < 0.5 ng/mL). A recent review showed oxidative stress induced by neonics in non-target organisms (Wang et al., 2018). Significant negative associations between creatinine-corrected concentrations of SUF and three OSBs (i.e., diY, 11-PGF<sub>2 $\alpha$</sub>  and 15-PGF<sub>2 $\alpha$</sub> ) were found in FMV urine samples. When the sum concentrations (log-transformed) of the seven neonics were considered for cumulative exposure assessment, a significant positive association was found with 8-OHdG, MDA, 8-PGF<sub>2 $\alpha$</sub> , 11-PGF<sub>2 $\alpha$</sub> , and 15-PGF<sub>2 $\alpha$</sub>  in either spot or FMV urine samples. This indicates cumulative effect of neonics on the oxidative damage to DNA (i.e., biomarker of 8-OHdG) and lipids (i.e., biomarkers of MDA, 8-PGF<sub>2 $\alpha$</sub> , 11-PGF<sub>2 $\alpha$</sub> , and 15-PGF<sub>2 $\alpha$</sub> ) in humans. For the first time, we report urinary levels of neonics in repeated samples and their associations with oxidative stress in humans.

#### 4. Conclusions

This study provides evidence that the US population is exposed to low levels of neonics, with the median urinary concentrations  $\leq$  0.5 ng/mL (including their metabolites). Repeated measurements of 13 neonics and their metabolites in urine displayed considerable within-individual variability in either spot or FMV samples. Low ICC values of urinary neonic concentrations indicated poor reproducibility of overall exposures from a single measurement. To better characterize average/integrated exposure over time, collection and analysis of repeated samples are recommended. Furthermore, we found positive associations between urinary concentrations of neonics and oxidative stress biomarkers of lipids, proteins and DNA damage.

The strengths of this study lie in its large sample size per individual and measurement of 13 neonics/metabolites in two types of urine samples collected over a 44-day period. In addition, for the first time, we report temporal variability of urinary levels of neonics in repeated samples and their associations with oxidative stress using mixed regression models. This study, however, was conducted on a convenience sample of 19 participants from a single geographic location. The participants for this study are limited to the age range of 11 to 56 years. In addition, our study did not exclude the influence of other environmental contaminants that may induce oxidative damage to lipids, proteins, and DNA. Therefore, caution is warranted in terms of the generalizability of neonic exposures to larger populations.

## Declaration of Competing Interest

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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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envint.2019.105415>.

## References

- Al-Sarar, A.S., Abobakr, Y., Bayoumi, A.E., Hussein, H.I., 2015. Cytotoxic and genotoxic effects of abamectin, chlorfenapyr, and imidacloprid on CHO<sub>K1</sub> cells. *Environ. Sci. Pollut. Res.* 22, 17041–17052.
- Anderson, J.C., Dubetz, C., Palace, V.P., 2015. Neonicotinoids in the Canadian aquatic environment: a literature review on current use products with a focus on fate, exposure, and biological effects. *Sci. Total Environ.* 505, 409–422.
- Attfield, K.R., Hughes, M.D., Spengler, J.D., Lu, C., 2014. Within- and between-child variation in repeated urinary pesticide metabolite measurements over a 1-year period. *Environ. Health Perspect.* 122, 201–206.
- Aylward, L.L., Hays, S.M., Smolders, R., Koch, H.M., Cocker, J., Jones, K., Warren, N., Levy, L., Bevan, R., 2014. Sources of variability in biomarker concentrations. *J. Toxicol. Environ. Health B* 17, 45–61.
- Basley, K., Goulson, D., 2018. Effects of field-relevant concentrations of clothianidin on larval development of the butterfly *Polyommatus icarus* (Lepidoptera, Lycaenidae). *Environ. Sci. Technol.* 52, 3990–3996.
- Bass, C., Denholm, I., Williamson, M.S., Nauen, R., 2015. The global status of insect resistance to neonicotinoid insecticides. *Pestic. Biochem. Physiol.* 121, 78–87.
- Bennett, B., Workman, T., Smith, M.N., Griffith, W.C., Thompson, B., Faustman, E.M., 2019. Longitudinal, seasonal, and occupational trends of multiple pesticides in house dust. *Environ. Health Perspect.* 127, 17003. <https://doi.org/10.1289/EHP3644>.
- Bishop, C.A., Moran, A.J., Toshack, M.C., Elle, E., Maisonneuve, F., Elliott, J.E., 2018. Hummingbirds and bumble bees exposed to neonicotinoid and organophosphate insecticides in the Fraser Valley, British Columbia, Canada. *Environ. Toxicol. Chem.* 37, 2143–2152.
- Bonmatin, J.-M., Giorio, C., Girolami, V., Goulson, D., Kreuzweiser, D.P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E.A.D., Noome, D.A., Simon-Delso, N., Tapparo, A., 2015. Environmental fate and exposure; neonicotinoids and fipronil. *Environ. Sci. Pollut. Res.* 22, 35–67.
- Chen, Y., Zang, L., Shen, G., Liu, M., Du, W., Fei, J., Yang, L., Chen, L., Wang, X., Liu, W., Zhao, M., 2019. Resolution of the ongoing challenge of estimating nonpoint source neonicotinoid pollution in the Yangtze River Basin using a modified mass balance approach. *Environ. Sci. Technol.* 93, 2539–2548.
- Cimino, A.M., Boyles, A.L., Thayer, K.A., Perry, M.J., 2017. Effects of neonicotinoid pesticide exposure on human health: a systematic review. *Environ. Health Perspect.* 125, 155–162.
- Douglas, M.R., Tooker, J.F., 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in U.S. field crops. *Environ. Sci. Technol.* 49, 5088–5097.
- Englert, D., Bakanov, N., Zubrod, J.P., Schulz, R., Bundschuh, M., 2017. Modeling remobilization of neonicotinoid residues from tree foliage in streams—a relevant exposure pathway in risk assessment? *Environ. Sci. Technol.* 51, 1785–1794.
- Galdíková, M., Šíviková, K., Holečková, B., Dianovský, J., Drážovská, M., Schwarzbacherová, V., 2015. The effect of thiacloprid formulation on DNA/chromosome damage and changes in GST activity in bovine peripheral lymphocytes. *J. Environ. Sci. Health B* 50, 698–707.
- Harada, K.H., Tanaka, K., Sakamoto, H., Imanaka, M., Niisoe, T., Hitomi, T., Kobayashi, H., Okuda, H., Inoue, S., Kusakawa, K., Oshima, M., Watanabe, K., Yasojima, M., Takasuga, T., Koizumi, A., 2016. Biological monitoring of human exposure to neonicotinoids using urine samples, and neonicotinoid excretion kinetics. *PLoS ONE* 11, e0146335. <https://doi.org/10.1371/journal.pone.0146335>.
- Hauser, R., Meeker, J.D., Park, S., Silva, M.J., Calafat, A.M., 2004. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. *Environ. Health Perspect.* 112, 1734–1740.
- Hladik, M.L., Corsi, S.R., Kolpin, D.W., Baldwin, A.K., Blackwell, B.R., Cavallin, J.E., 2018. Year-round presence of neonicotinoid insecticides in tributaries to the Great Lakes, USA. *Environ. Pollut.* 235, 1022–1029.
- Hladik, M.L., Kolpin, D.W., 2016. First national-scale reconnaissance of neonicotinoid insecticides in streams across the USA. *Environ. Chem.* 13, 12–20.
- Honda, M., Robinson, M., Kannan, K., 2019. A simple method for the analysis of neonicotinoids and their metabolites in human urine. *Environ. Chem.* <https://doi.org/10.1071/EN18240>.
- Jeschke, P., Nauen, R., Schindler, M., Elbert, A., 2011. Overview of the status and global strategy for neonicotinoids. *J. Agric. Food Chem.* 59, 2897–2908.
- Kabata, R., Nanayakkara, S., Senevirathna, S., Harada, K.H., Chandrajith, R., Hitomi, T., Abeyssekera, T., Takasuga, T., Koizumi, A., 2016. Neonicotinoid concentrations in urine from chronic kidney disease patients in the North Central Region of Sri Lanka. *J. Occup. Health* 58, 128–133.
- Kim, K., Bloom, M.S., Fujimoto, V.Y., Browne, R.W., 2014. Number of specimens required to estimate average follicular fluid high-density lipoprotein particle components in women undergoing in vitro fertilization. e44. *Fertil. Steril.* 101. <https://doi.org/10.1016/j.fertnstert.2014.04.019>.
- Klarich, K.L., Pflug, N.C., DeWald, E.M., Hladik, M.L., Kolpin, D.W., Cwiertny, D.M., LeFevre, G.H., 2017. Occurrence of neonicotinoid insecticides in finished drinking water and fate during drinking water treatment. *Environ. Sci. Technol. Lett.* 4, 168–173.
- Li, A.J., Martinez-Moral, M.P., Al-Malki, A.L., Al-Ghamdi, M.A., Al-Bazi, M.M., Kumosani, T.A., Kannan, K., 2019b. Mediation analysis for the relationship between urinary phthalate metabolites and type 2 diabetes via oxidative stress in a population in Jeddah, Saudi Arabia. *Environ. Int.* 126, 153–161.
- Li, A.J., Martinez-Moral, M.P., Kannan, K., 2019a. Temporal variability in urinary pesticide concentrations in repeated-spot and first-morning-void samples and its association with oxidative stress in healthy individual. *Environ. Int.* 130. <https://doi.org/10.1016/j.envint.2019.104904>.
- Lopez-Antia, A., Ortiz-Santaliestra, M.E., Mougeot, F., Mateo, R., 2015. Imidacloprid-treated seed ingestion has lethal effect on adult partridges and reduces both breeding investment and offspring immunity. *Environ. Res.* 136, 97–107.
- Lu, C., Chang, C.H., Palmer, C., Zhao, M., Zhang, Q., 2018. Neonicotinoid residues in fruits and vegetables: an integrated dietary exposure assessment approach. *Environ. Sci. Technol.* 52, 3175–3184.
- Marfo, J.T., Fujioka, K., Ikenaka, Y., Nakayama, S.M.M., Mizukawa, H., Aoyama, Y., Ishizuka, M., Taira, K., 2015. Relationship between urinary N-desmethyl-acetamidiprid and typical symptoms including neurological findings: a prevalence case-control study. *PLoS ONE* 10, e0142172. <https://doi.org/10.1371/journal.pone.0142172>.
- Martinez-Moral, M.P., Kannan, K., 2019. How stable is oxidative stress level? An observational study of intra- and inter-individual variability in urinary oxidative stress biomarkers of DNA, proteins, and lipids in healthy individuals. *Environ. Int.* 123, 382–389.
- Mitchell, E.A.D., Mulhauser, B., Mulot, M., Mutabazi, A., Glauser, G., Aebi, A., 2017. A worldwide survey of neonicotinoids in honey. *Science* 358, 109–111.
- Nauen, R., Ebbinghaus-Kintscher, U., Salgado, V.L., Kaussmann, M., 2003. Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. *Pestic. Biochem. Physiol.* 76, 55–69.
- Nomura, H., Ueyama, J., Kondo, T., Saito, I., Murata, K., Iwata, T., Wakusawa, S., Kamijima, M., 2013. Quantitation of neonicotinoid metabolites in human urine using GC-MS. *J. Chromatogr. B* 941, 109–115.
- Osaka, A., Ueyama, J., Kondo, T., Nomura, H., Sugiura, Y., Saito, I., Nakane, K., Takaishi, A., Ogi, H., Wakusawa, S., Ito, Y., Kamijima, M., 2016. Exposure characterization of three major insecticide lines in urine of young children in Japan—neonicotinoids, organophosphates, and pyrethroids. *Environ. Res.* 147, 89–96.
- Ozsahin, A.D., Bal, R., Yilmaz, O., 2014. Biochemical alterations in kidneys of infant and adult male rats due to exposure to the neonicotinoid insecticides imidacloprid and clothianidin. *Toxicol. Res.* 3. <https://doi.org/10.1039/c4tx00006d>.
- Pandey, S.P., Mohanty, B., 2015. The neonicotinoid pesticide imidacloprid and the dithiocarbamate fungicide mancozeb disrupt the pituitary-thyroid axis of a wildlife bird. *Chemosphere* 122, 227–234.
- Sheets, L.P., Li, A.A., Minnema, D.J., Collier, R.H., Creek, M.R., Peffer, R.C., 2016. A critical review of neonicotinoid insecticides for developmental neurotoxicity. *Crit. Rev. Toxicol.* 46, 153–190.
- Taira, K., Fujioka, K., Aoyama, Y., 2013. Qualitative profiling and quantification of neonicotinoid metabolites in human urine by liquid chromatography coupled with mass spectrometry. *PLoS ONE* 8, e80332. <https://doi.org/10.1371/journal.pone.0080332>.
- Ueyama, J., Harada, K.H., Koizumi, A., Sugiura, Y., Kondo, T., Saito, I., Kamijima, M., 2015. Temporal levels of urinary neonicotinoid and dialkylphosphate concentrations in Japanese women between 1994 and 2011. *Environ. Sci. Technol.* 49, 14522–14528.
- Ueyama, J., Nomura, H., Kondo, T., Saito, I., Ito, Y., Osaka, A., Kamijima, M., 2014. Biological monitoring method for urinary neonicotinoid insecticides using LC-MS/MS and its application to Japanese adults. *J. Occup. Health* 56, 461–468.
- Wang, L., Liu, T., Liu, F., Zhang, J., Wu, Y., Sun, H., 2015. Occurrence and profile characteristics of the pesticide imidacloprid, preservative parabens, and their metabolites in human urine from rural and urban China. *Environ. Sci. Technol.* 49, 14633–14640.
- Wang, X., Anadón, A., Wu, Q., Qiao, F., Ares, I., Martínez-Larrañaga, M.-R., Yuan, Z., Martínez, M.-A., 2018. Mechanism of neonicotinoid toxicity: impact on oxidative stress and metabolism. *Annu. Rev. Pharmacol. Toxicol.* 58, 471–507.
- Wang, Y.-X., Feng, W., Zeng, Q., Sun, Y., Wang, P., You, L., Yang, P., Huang, Z., Yu, S.-L., Lu, W.-Q., 2016. Variability of metal levels in spot, first morning, and 24-hour urine samples over a 3-month period in healthy adult Chinese men. *Environ. Health Perspect.* 124, 468–476.



- Yan, S., Wang, J., Zhu, L., Chen, A., Wang, J., 2015. Toxic effects of nitenpyram on antioxidant enzyme system and DNA in zebrafish (*Danio rerio*) livers. *Ecotoxicol. Environ. Saf.* 122, 54–60.
- Zhang, P., Ren, C., Sun, H., Min, L., 2018a. Sorption, desorption and degradation of neonicotinoids in four agricultural soils and their effects on soil microorganisms. *Sci. Total Environ.* 615, 59–69.
- Zhang, Q., Li, Z., Chang, C.H., Lou, J.L., Zhao, M.R., Lu, C., 2018b. Potential human exposures to neonicotinoid insecticides: a review. *Environ. Pollut.* 236, 71–81.
- Zhang, T., Song, S., Bai, X., He, Y., Zhang, B., Gui, M., Kannan, K., Lu, S., Huang, Y., Sun, H., 2019. A nationwide survey of urinary concentrations of neonicotinoid insecticides in China. *Environ. Int.* 132. <https://doi.org/10.1016/j.envint.2019.105114>.