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Profiles of urinary neonicotinoids and dialkylphosphates in populations in nine countries

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Abstract

The application of neonicotinoid insecticides (neonics) has increased dramatically as a replacement for organophosphate pesticides (OPs) in recent years. Nevertheless, little is known about human exposure to these pesticides in various countries. In this study, concentrations of 14 neonics and six dialkylphosphate metabolites (DAPs) were determined simultaneously in 566 urine samples collected from nine countries during 2010–2014. The highest sum concentration of 14 neonics was found in urine from Vietnam (median: 12.2 ng/mL) whereas that of six DAPs was from China (18.4 ng/mL). The median concentrations of Σ6 DAPs were twice higher than those of Σ14 neonics across the nine countries, which suggested a greater exposure to OPs than neonics. The overall pattern of urinary pesticide concentrations was similar among the nine countries with dimethylphosphate (DMP) and dimethylthiophosphate (DMTP) accounting for 51–89% of the total pesticide concentrations. Differences in urinary pesticide concentrations between genders (female and male), age groups (≤20, 21–49, and ≥50 years), and regions (cities of Shanghai, Guangzhou and Qiqihar) were examined. Total daily exposure doses to OPs were highest in China (515 μg/day) with 15% of the samples exceeding the U.S. Environmental Protection Agency's reference dose for chlorpyrifos (18 μg/day). This is the first study to establish baseline levels of OP and neonics exposure in general populations across nine countries.

Graphical Abstract

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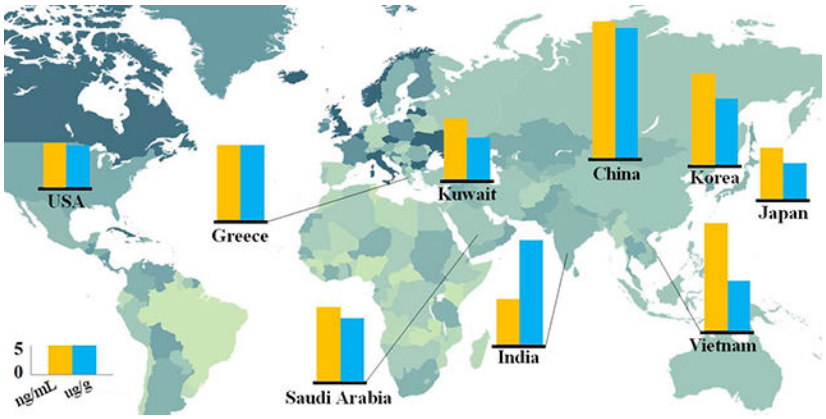
Declaration of interests

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.

Appendix A. Supplementary data

Supplementary data to this article can be found in a separate file.



Keywords

Neonicotinoid; Organophosphate; Metabolite; Chlorpyrifos; Urine; Biomonitoring

1. Introduction

Neonicotinoid insecticides (hereafter “neonics”) have emerged as alternatives to organophosphate (OP) pesticides, and their usage in agriculture has increased due to their broad-spectrum insecticidal activity, unique mode of neurotoxic action and presumed low mammalian toxicity (Casida and Durkin, 2013; Rundlöf et al., 2015). The application of neonics has also expanded to non-agricultural fields, such as homes, lawns, gardens and animal welfare (Morrissey et al., 2015; Zhang et al., 2018). The market share of neonics grew rapidly from the launch in the 1990s to >25% of the pesticide use in 2014 (Bass et al., 2015; Jeschke et al., 2011). The annual global production of neonic active ingredients was estimated at 20 thousand tonnes in 2010 (Wang et al., 2018). Thiamethoxam (THX, 37.6% of the total market share of $3.2 billion in 2012), imidacloprid (IMI, 33.5%), clothianidin (CLO, 14.7%), acetamiprid (ACE, 7.2%), thiacloprid (THI, 3.8%), dinotefuran (DIN, 2.9%) and nitenpyram (NIT, 0.3%) are the most commonly used neonics in over 140 crops in 120 countries, especially in Asia, North and South America (75% of total neonics use) and Europe (11%) (Bass et al., 2015; Sadaria et al., 2016).

Given their widespread use and physicochemical properties (systemic action and persistence in soil and water), neonics are ubiquitous in soil (Morrissey et al., 2015; Stewart et al., 2014), water (Klarich et al., 2017; Sadaria et al., 2016), house dust (Bennett et al., 2019), air (Tapparo et al., 2012; Ikenaka et al., 2019) and food (Mitchell et al., 2017; Chen et al., 2020). In spite of the original notion that neonics have low mammalian toxicity, evidences suggest adverse effects on non-target organisms such as honey bees (Rundlöf et al., 2015; Mitchell et al., 2017), insectivorous birds (Hallmann et al., 2014) and aquatic invertebrates (Morrissey et al., 2015), and further raising concerns about human health. Epidemiologic studies have linked human exposure of neonics with adverse developmental and neurological outcomes (e.g., congenital heart defects, anencephaly, autism spectrum disorders and memory loss) (Cimino et al., 2017; Zhang et al., 2018).

Human exposure to pesticides arises primarily via the ingestion of contaminated food and water (Bennett et al., 2019; Jusko et al., 2019; Klarich et al., 2017; Mitchell et al., 2017). Following enteric absorption, both neonics and OPs are metabolized by phase I enzymes, and some of the phase I metabolites undergo phase II conjugation to facilitate excretion in urine (Li et al., 2020a; Li and Kannan, 2018). Some neonics are excreted in urine as unchanged compounds due to their high water solubility whereas 70–75% of OPs are metabolized to dialkylphosphates (DAPs) (Ueyama et al., 2015) (Table S1). Very few studies have reported urinary concentrations of neonics and DAPs in general populations (Table S2; Table S3). An understanding of exposure patterns of neonics and DAPs in populations from various countries is lacking.

The objectives of this study were to establish baseline levels of exposure of general populations to neonics and OPs in nine countries (Table S1), and to elucidate the determinants of exposure and potential risks. An analytical method that was capable of measuring 14 neonics and 6 DAPs was developed and applied in the analysis of 566 urine samples collected from populations in nine countries (Table S4). By investigating geographic patterns in pesticide exposure profiles and demographic variables (i.e., gender and age), we established baseline levels needed for investigating future trends in exposures. On the basis of the concentrations measured in urine, daily exposure doses to target pesticides were calculated, which were then compared against available reference values.

2. Materials and methods

2.1. Study population

Spot urine samples (n = 566) were collected from nine countries during 2010–2014: the USA (n = 44; number of samples of males/females/unknown gender: 29/15/0), Greece (n = 118; 54/64/0), China (n = 84; 31/24/29), India (n = 41; 3/10/28), Saudi Arabia (n = 61; 28/33/0), Japan (n = 35; 27/8/0), Korea (n = 100; 47/53/0), Vietnam (n = 17; 8/9/0) and Kuwait (n = 66; 27/39/0) (Table S4). The samples originated from 12 cities in the nine countries, i.e., Albany (New York State), Athens, Guangzhou/Shanghai/Qiqihar, Mettupalayam (Tamil Nadu State), Jeddah, Ehime/Kumamoto, Seoul, Hanoi, and Kuwait. The age of the donors ranged from 1 to 87 years (mean ± standard deviation, 41.5 ± 19.7 years), with age groups of ≤20 (n = 66; 13%), 21–49 (n = 244; 48%) and ≥50 years (n = 198; 39%); the ratios of males to females were 0.9, 1.1 and 0.9, respectively, for the three age groups. These samples were collected as a part of our previous studies and all samples were void of personal identifiers. Institutional Review Board approvals were obtained from the New York State Department of Health for the analysis of urine samples. All samples were kept at −20 °C in polypropylene tubes until analysis.

2.2. Determination of neonicotinoids and dialkylphosphates in urine

Details pertaining to analytical standards, internal standards, and reagents used in this study are provided in the Supplementary Information and Table S5. The target neonics and DAPs were extracted from urine by a solid phase extraction (SPE) method, similar to that described earlier (Li et al., 2020a; 2020b). Briefly, urine samples (250 μL) were spiked with 2.5 ng each of stable isotopically labeled internal standard mixture (seven neonics and six

DAPs; Table S5) and mixed with 2% formic acid (1.5 mL). The samples were then passed through SPE cartridges (Bond Elut Plexa, 3 mL, 60 mg, Agilent; Lexington, MA, USA) that were conditioned with 5% ammonium hydroxide in methanol (3 mL) and water (3 mL). After loading samples, cartridges were washed with 2 mL of formic acid/methanol/water (2:10:88, *v/v/v*) and vacuum dried for 1 min. The eluates (3 mL of methanol) were dried under a gentle stream of nitrogen (Organomation Associates Inc.; West Berlin, MA, USA) and reconstituted with 250 μL of methanol/water (25:75, *v/v*).

Both neonics and DAPs were chromatographically separated using a Waters Acquity Class I HPLC system (Waters; Milford, MA, USA) connected with a Kinetex Phenyl/Hexyl column (50 × 2.1 mm, 2.6 μm, Phenomenex; Torrance, CA, USA) for the analysis of 6-CN and SUF, with a Betasil C18 column (100 × 2.1 mm, 5 μm, Thermo Fisher Scientific; Waltham, MA, USA) for the analysis of remaining 12 neonics, and with a Luna HILIC column (100 × 3 mm, 3 μm, Phenomenex; Torrance, CA, USA) for the analysis of six DAPs. The target analytes were detected and quantified 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. Further details of the instrumental analysis and compound specific parameters are provided in the Supplementary Information and Table S6.

2.3. Determination of creatinine

Urine samples (10 μL) were diluted 160-times using HPLC-grade water, and 1200 ng of D_3-creatinine was added. Creatinine concentrations were determined using a high performance liquid chromatography (Agilent 1100 series HPLC, Agilent Technologies; Santa Clara, CA, USA) coupled with an ABSCIEX 2000 ESI-MS/MS (Applied Biosystems; Foster City, CA, USA). The chromatographic separation was accomplished using a Betasil C18 column (100 × 2.1 mm, 5 μm, Thermo Fisher Scientific; Waltham, MA, USA). Further details of the instrumental analysis and compound specific mass spectrometric parameters are shown in the Supplementary Information and Table S6.

2.4. Method performance

Neonics and their metabolites were quantified using an isotope dilution method with a 10- to 11-point calibration curve prepared in synthetic urine at concentrations ranging from 0.02 to 50 ng/mL. The correlation coefficient (r) was >0.999 for all compounds. FLO (0.010 ng/mL) and N-DMA (0.005 ng/mL) were detected in procedural blanks and the concentrations found in blanks were subtracted from those measured for samples. The accuracy (% mean recovery) and precision (relative standard deviation; RSD) were measured by replicate analysis (n = 32) of a synthetic urine sample (Sigma-Aldrich; Round Rock, TX, USA) fortified at low (1 ng/mL), medium (10 ng/mL) and high concentrations (20 ng/mL) of target neionics. The recoveries of all target neonics were in the range of 85–110%, with RSDs of <14.6%. The limits of detection (LODs), defined as a signal-to-noise ratio of 3, ranged from 0.001 to 0.044 ng/mL (Table S7).

The six DAPs were quantified using an isotope dilution method with an 11 to 12-point calibration curve prepared at concentrations ranging from 0.02 to 100 ng/mL. The r was >0.999 for all compounds. Trace concentrations (ng/mL) of target compounds (0.043 for

DMP, 0.006 for DMTP, 0.002 for DMDTP and 0.016 for DETP) were detected in procedural blanks, and these concentrations were subtracted from those measured for samples. The accuracy and precision were determined by replicate analysis (n = 32) of a synthetic urine sample fortified at low (1 ng/mL), medium (10 ng/mL) and high concentrations (20 ng/mL) of DAPs. The recoveries of the six DAPs were in the range of 85–110%, with RSDs <12%. The LODs for DAPs ranged from 0.002 to 0.053 ng/mL (Table S7).

Creatinine was quantified using an isotope dilution method with a nine-point calibration curve prepared at concentrations ranging from 100 to 2000 ng/mL (r = 0.9997). The concentration of creatinine in procedural blanks was below the LOD (19.4 ng/mL). The recovery of creatinine (fortified at 750 ng/mL in synthetic urine) through the analytical method was 99.6% (n = 10; RSD = 0.6%). Additionally, Standard Reference Materials (SRMs) 3672 and 3673 (creatinine in smokers' and non-smokers' urine, respectively), supplied by the National Institute of Standards and Technology (Gaithersburg, MD, USA), were analyzed for creatinine. The recoveries of creatinine from SRMs were from 97% to 102% (n = 8; RSDs <1.5%).

2.5. Data analysis

Data were analyzed using SPSS 19.0 (SPSS Inc.; Chicago, IL, USA). The concentrations below the LOD were assigned a value of LOD divided by the square root of 2. Prior to statistical analyses, data were log-transformed (χ + 1) to normalize their distributions. Creatinine-adjusted values were provided, as appropriate. Differences in urinary levels of neonics and DAPs among the nine countries and three age groups were examined by one-way ANOVA, if the data followed a normal distribution; otherwise, a non-parametric Kruskal-Wallis H test was applied. Differences in urinary levels of chemicals between genders were examined by a Student's t-test, if the data followed a normal distribution; otherwise, a Mann-Whitney U test was applied. Pearson's correlation analyses were conducted to explore associations between urinary concentrations of target pesticides. Values of $p < 0.05$ denote statistical significance.

3. Results and discussion

3.1. Urinary neonics and DAP concentrations in nine countries

The concentrations of Σ14 neonics and Σ6 DAPs were significantly different among the nine countries studied (Kruskal-Wallis H test, $p < 0.001$). The median urinary concentrations (ng/mL) of Σ14 neonics varied by an order of magnitude among the nine countries, with the highest concentration found in Vietnam (12.2), followed by Saudi Arabia (6.1), Greece (6.0), China (4.6), Japan (4.6), the USA (3.1), India (2.9), Kuwait (2.5), and Korea (1.9) (Fig. S4). The median concentrations (ng/mL) of Σ6 DAPs varied by a factor of five among the countries studied, and were the highest in China (18.4) followed in decreasing order by Korea (14.1), Kuwait (7.6), Saudi Arabia (7.3), Vietnam (6.9), Greece (6.2), Japan (4.0), the USA (3.8) and India (3.6) (Fig. S5). The differences in exposure patterns suggest varied usage patterns of neonics and OP pesticides among countries. We reported in our previous study that geographical differences existed in the distribution of urinary concentrations of 11

organophosphate and pyrethroid pesticides and phenoxy acid herbicides in eight countries, with Vietnam containing the highest concentrations (Li and Kannan, 2018).

The sum concentrations of Σ20 pesticides (sum of 14 neonics and 6 DAPs) differed significantly among the nine countries (Kruskal-Wallis H test, $p < 0.001$) in the decreasing order of China (median at ng/mL, 25.2), Vietnam (19.8), Korea (16.8), Greece (13.7), Saudi Arabia (13.6), Kuwait (11.3), Japan (9.6), India (8.5) and the USA (8.1) (Fig. 1). The median concentrations of Σ20 pesticides found in urine samples from China, Vietnam and Korea were 2–3 times higher than those found in Kuwait, Japan, India and the USA. The rate of annual pesticide use (kg/ha) during 2010–2014 was the greatest in Japan (18.9), followed by China (10.5), the USA (3.9) and India (0.3) (Zhang, 2018). The agricultural use of neonics was 23%, 22% and 11% of the total market share in 2012 in Asia, North America and Europe, respectively (Bass et al., 2015). Among all insecticides (94000 tonnes), OPs (30000 tonnes) were the most widely used in 2014 globally (Zhang, 2018). The rate of pesticide use among Chinese farmers reached three times the global average, leading to increased pollution (Zhang, 2018). In China, over 270000 tonnes of pesticides are used annually in agriculture, with OPs comprising approximately 70% of all pesticides used (Wang et al., 2017).

Urinary concentrations of neonics and DAPs were normalized for creatinine content. Pearson correlation analysis showed significant positive correlations between volume-based (ng/mL) and creatinine-adjusted (Σg/g creatinine) concentrations of pesticides for 191 out of 198 pairs (9 countries × (14 neonics + 6 DAPs + Σ14 neonics + Σ 6DAPs) (i.e., 9 × 22), regardless of individual or sum concentrations measured for the nine countries ($r = 0.240$–0.998, $p < 0.05$; Table S8). The volume-based and creatinine-adjusted concentrations of NIT, FLO, TA, SUF and DEDTP were not significantly correlated, which can be explained by their low detection frequencies in samples (2–37%) of these analytes. Significant positive correlations were found between volume-based and creatinine-adjusted concentrations of individual or total concentrations across the nine countries ($r = 0.682$–0.948, $p < 0.01$). These findings suggest that urine excretion volume at sampling (i.e., dilution factor) did not influence the measured concentrations of these pesticides (Fig. 1) (Li and Kannan, 2018). Further discussions on urinary pesticide concentrations were based on volumetric values (i.e., ng/mL), unless specified otherwise.

3.2. Profiles of urinary neonics and DAPs

The detection frequency (DF) and distribution of volume-based or creatinine-adjusted concentrations of neonics and DAPs in urine are presented (Tables 1 and S9). Among neonics, IMI, N-DMA (a metabolite of ACE) and 6-CN (a non-specific metabolite of IMI, ACE, THI, NIT and cycloxaprid) were found at a DF of >80% whereas other neonics were found at DFs in the range of 13–72%. Among six DAPs, DMP, DMTP, DMDTP and DETP were found in ≥92% of the urine samples while DEP and DEDTP were found in <70% of the samples analyzed. For further analysis, we selected those pesticides that were found at DFs of >80% in urine samples. Among the 20 pesticides analyzed, DMP was found at the highest concentration (median: 3.39 ng/mL), followed by DMTP (1.55), DETP (0.447), 6-CN (0.354), N-DMA (0.262), IMI (0.128) and DMDTP (0.112) (Table 1). Dimethyl

phosphates were detected more frequently and at higher concentrations than those of diethyl phosphates (Berman et al., 2013; Jusko et al., 2019; Oya et al., 2020; Sokoloff et al., 2016). This may be ascribed to greater exposure to dimethyl OP pesticides such as malathion and longer half-lives of dimethyl phosphates (Sokoloff et al., 2016). The distribution pattern of 6-CN, N-DMA and IMI found in our study was similar to that reported previously from the USA, China and Japan (Ikenaka et al., 2019; Li et al., 2020a; Wang et al., 2015). The highest urinary concentrations of IMI (median: 1.13–2.56 ng/mL) and 6-CN (median: 0.45–1.38 ng/mL) were reported among Chinese residents living near orchards (Tao et al., 2019). A significant correlation between the concentrations of neonics and DAPs in urine (Table S10) suggested a common exposure pathway for these two classes of pesticides (Osaka et al., 2016). Diet is thought to be the major source of exposure to both of these classes of pesticides (Chen et al., 2020; Lu et al., 2006).

The overall distribution pattern of urinary pesticide concentrations was similar among the nine countries, three age groups and between genders (Fig. 2). The sum concentrations of DMP and DMTP collectively accounted for 51–89% of the seven major urinary pesticide concentrations whereas neonics, IMI, N-DMA and 6-CN accounted for 4.9–42% of the total concentrations. It has been reported that, following absorption, 75% of the registered OP pesticides get metabolized to DAPs in the body (Ueyama et al., 2015). OP pesticides were banned for most residential uses in the USA and western European countries in the early 2000s. Nevertheless, the general populations are exposed to OPs through the ingestion of agricultural products (Furlong et al., 2014). Our previous study showed that the general populations in the studied countries were exposed to (methyl) parathion and chlorpyrifos extensively as implied by the abundance of corresponding urinary metabolites, *para*-nitrophenol and 3,5,6-trichloro-2-pyridinol (Li and Kannan, 2018). IMI currently accounts for ~42% of neonics market and the demand for other neonics, ACE, THI, NIT and DIN is increasing (Jeschke et al., 2011). The use of OPs has been constantly declining, from 70 to 20 million pounds during the period of 2000–2012 in the USA (EPA, 2017) and from 946 to 44 thousand pounds during the period of 2001–2011 in Japan (Ueyama et al., 2015). This change in usage pattern was attributed to the emergence of resistance towards OPs and concomitant development of neonics (Hladik et al., 2018; Ueyama et al., 2015). However, the median concentrations of Σ6 DAPs were twice higher than those of Σ14 neonics across the nine countries, suggesting greater exposure to OPs than that of neonics (Ueyama et al., 2015). It should be noted that several neonics are metabolized to hydroxylated as well as olefin derivatives and analytical standards are not commercially available to quantify those metabolites (Zhang et al., 2019; Song et al., 2020). Further studies on the measurement of neonics metabolites are needed to assess cumulative exposure doses to these insecticides.

3.3. Demographic factors and urinary pesticide concentrations

Country-specific differences in urinary concentrations were examined for individual and sum concentrations of seven major pesticides (DFs >80%) for the nine countries. The individual and sum concentrations of pesticides differed significantly among the nine countries (Kruskal-Wallis H test, $p < 0.01$) (Table 2). The highest urinary concentrations of 6-CN (median: 1.11 ng/mL), DMP (9.73) and DETP (1.54) were found in China; IMI (0.202) and N-DMA (1.22) in Vietnam; and DMTP (2.44) and DMDTP (0.243) in Korea.

Creatinine normalization of concentrations did not affect the geographic differences among countries except that India had the highest creatinine adjusted urinary concentration of IMI (median at μg/g creatinine, 0.232) and N-DMA (0.912) followed by Vietnam (IMI at 0.110; N-DMA at 0.667).

To examine location-specific differences in urinary concentrations of neonics and DAPs within a country, samples collected from Shanghai, Guangzhou and Qiqihar in China, were examined. Significant differences were found in N-DMA (Kruskal-Wallis H test, $p < 0.01$; Shanghai > Guangzhou > Qiqihar) and DMDTP (Kruskal-Wallis H test, $p < 0.01$; Qiqihar > Guangzhou > Shanghai) concentrations among the three cities in China. The creatinine adjusted concentrations did not alter our conclusions for N-DMA and DMDTP (Kruskal-Wallis H test, $p < 0.01$). Additionally, DMP (one-way ANOVA, $p < 0.05$; Guangzhou > Qiqihar > Shanghai) and DMTP (Kruskal-Wallis H test, $p < 0.05$; Qiqihar > Guangzhou > Shanghai) also displayed significant differences among the three cities. Location-specific variations in urinary concentrations of 3,5,6-trichloro-2-pyridinol, *trans/cis*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid, 2,4-dichlorophenoxyacetic acid and DAPs have been reported in China and Japan previously (Li and Kannan, 2018; Ueyama et al., 2015). Our results reiterate region-specific differences in pesticide exposures.

No significant differences were found for individual or sum concentrations of urinary neonics and DAPs between females and males (Mann-Whitney *U* test, $p > 0.05$) (Table 2). However, females had significantly higher creatinine adjusted urinary concentrations than males for both individual and sum concentrations (Mann-Whitney *U* test, $p < 0.01$) except for N-DMA (Mann-Whitney *U* test, $p > 0.05$). A similar gender-related difference (females > males) in urinary DAPs (Berman et al., 2013) and pyrethroid pesticide metabolites (Barr et al., 2010; Li and Kannan, 2018) was found for the general populations previously. Higher exposure levels of pesticides in females may be related to dietary preference for vegetables/fruits, and also an artifact of lower creatinine content in urine of females than males (Barr et al., 2005; Berman et al., 2013; Sokoloff et al., 2016; Tao et al., 2019). Gender-related difference was not found in urinary concentrations of neonics (i.e., ACE, IMI, THI, THX, CLO, DIN and NIT) among Japanese children (Osaka et al., 2016).

We categorized the samples into three age groups, namely, ≤20, 21–49, and ≤50 years, for the examination of age-related differences in urinary concentrations of neonics and DAPs. For both individual and sum concentrations of seven pesticides, no significant difference was found among the three age groups (Kruskal-Wallis H test, $p > 0.05$) (Table 2). After creatinine adjustment, we found significant differences in DMTP, DMDTP and DETP concentrations (Kruskal-Wallis H test, $p < 0.05$). The highest concentrations of the three compounds were all detected in the age group of ≤20 years (median: 0.122–1.97 μg/g creatinine), followed by ≥50 years (0.125–1.39) and 21–49 years (0.082–1.11). It was reported in an earlier study that children and elderly were at a greater risk of exposure to OP pesticides and the pyrethroid metabolite 3-phenoxybenzoic acid (Barr et al., 2010; 2011). Age related variations in urinary pesticide concentrations may be attributed to the differences in dietary patterns and metabolism (Barr et al., 2010; Li and Kannan, 2018).

3.4. Human exposure to pesticides

Urinary biomonitoring data can be used in the assessment of daily exposure doses of environmental chemicals. We estimated daily exposure doses of IMI, N-DMA, 6-CN, DMP, DMTP, DMDTP and DETP, which were detected in >80% of the samples analyzed, as shown in Eq. (1) for the nine countries (Guo et al., 2011; Li and Kannan, 2018):

$$DI = CV \times \frac{M1}{M2} \times \frac{1}{f} \quad (1)$$

where DI is the daily intake of pesticide (μg/day), C is the median urinary pesticide concentration (ng/mL), V is the daily excretion volume of urine (L/day; 24 h average urine excretion volume at 1.7 L/day for adults) (Guo et al., 2011; Li and Kannan, 2018), $M1$ and $M2$ are the respective molecular weights of parent pesticide and its metabolite (g/mol), and f is the ratio of compound/metabolite excreted in urine relative to the total exposure dose of the parent compound. The f values were obtained from published studies: 0.127 for IMI, 0.307 for N-DMA (Harada et al., 2016), 0.8 for 6-CN (Uroz et al., 2001), 0.1 for DMP, DMTP and DMDTP (Chen et al., 2013), and 0.93 for DETP (Griffin et al., 1999). For this assessment, we presumed that 6-CN was the metabolite of IMI; DMP, DMTP and DMDTP were only from malathion; and DETP only from chlorpyrifos. Malathion is a registered dimethyl OP pesticide used in agriculture and public health programs (e.g., mosquito control) whereas chlorpyrifos is one of the most commonly used diethyl OP pesticide; residues of both of these pesticides were frequently found in fruits and vegetables (Hernández et al., 2019; Sokoloff et al., 2016).

The estimated daily exposure doses of neonics and OPs by the populations in the nine countries are shown in Table 3. The average body weight of 60 kg was used for adults, in the estimation of chronic reference doses (cRfDs) for daily intakes (μg/day), as suggested by the U.S. EPA (57, 71, 70 and 0.3 μg/kg body weight/day for IMI, ACE, malathion and chlorpyrifos, respectively) (EPA, 1994; 2000; 2006; 2009). The estimated median daily intakes to neonics, IMI and ACE among the nine countries were thousand-fold below the cRfD values of the U.S. EPA. The highest daily intake of neonics was found for populations in Vietnam (12.7 μg/day). None of samples collected from the nine countries were above the cRfDs values of IMI and ACE. The estimated exposure doses of OPs, malathion and chlorpyrifos were approximately 10-fold below the cRfD values of the U.S. EPA. The median daily exposure doses estimated for OP pesticides for populations in China were the highest (515 μg/day) among the nine countries studied, and 13 out of 84 samples from China exceeded the cRfD value for chlorpyrifos of 18 μg/day.

4. Conclusions

This is the first study to describe exposure to 14 neonics and six DAPs in general populations from nine countries. An ultra-sensitive method was developed and applied in simultaneous analysis of 20 pesticides in urine. We found high exposure to OPs in China and Korea, and neonics in Vietnam. DMP and DMTP were the dominant urinary pesticide metabolites found in populations across the nine countries. Although this study is the first to report baseline levels of current use pesticides in populations in nine countries, our study has

several limitations. We employed a convenience sampling approach by recruiting individuals within a small community with a small sample size. Considering location specific differences, large number of samples covering several locations in a country is needed for elucidating country-specific exposure doses. In addition, our exposure estimates were based on a single spot urine sample, which may not represent exposures over time (Li et al., 2020a). Pesticide exposures are often sporadic (Li et al., 2020a) and therefore multiple samples need to be collected from individuals to draw conclusions on individual's exposure levels. Nevertheless, the data collected in this study can provide baseline information on exposures to neonics and OPs, which will lay the foundation for designing future studies to understand potential risks from exposure to pesticides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Neonicotinoids and dialkylphosphates were measured in urine from nine countries.
- The highest sum concentration of 14 neonicotinoids was found in Vietnam (12.2 ng/mL).
- The highest sum concentration of six dialkylphosphates was found in China (18.4 ng/mL).
- Dimethyl phosphates accounted for 51–89% of the total concentrations.
- Daily exposure dose to organophosphates was highest in China (515 μg/day).

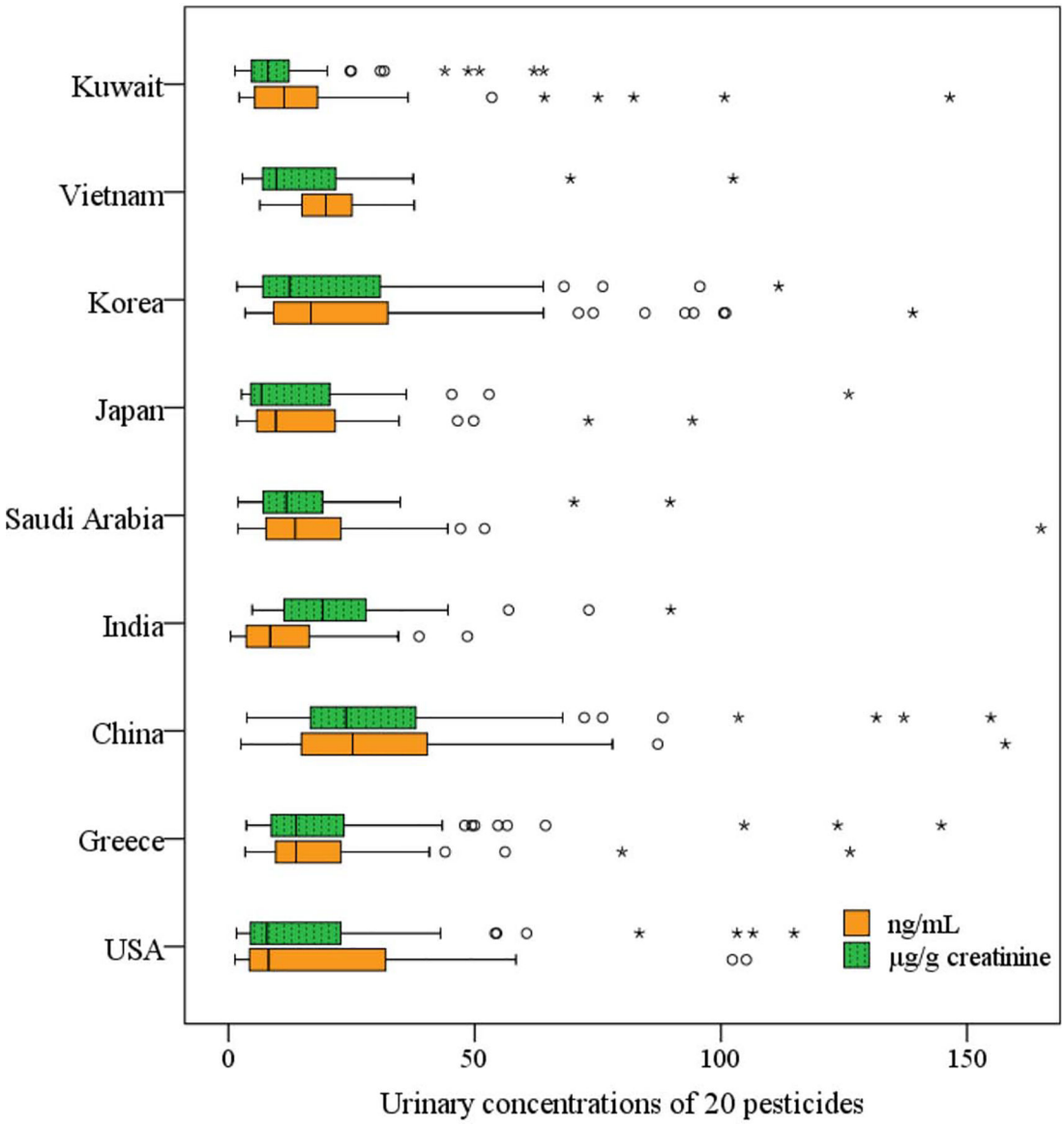

Fig. 1.

Urinary concentrations (ng/mL and µg/g creatinine) of pesticides (14 neonicotinoid insecticides and six dialkylphosphate metabolites) from nine countries. The vertical lines represent the minimum, 50th percentile, and maximum, and the boxes represent the 25th and 75th percentiles. Standard and extreme outliers are denoted by circles and stars, respectively, indicating values >1.5 and 3 times the interquartile range away from the 25th and 75th percentiles. Outliers' concentrations of sum of 20 pesticides over 160 ng/mL and µg/g creatinine are not shown in the figure.

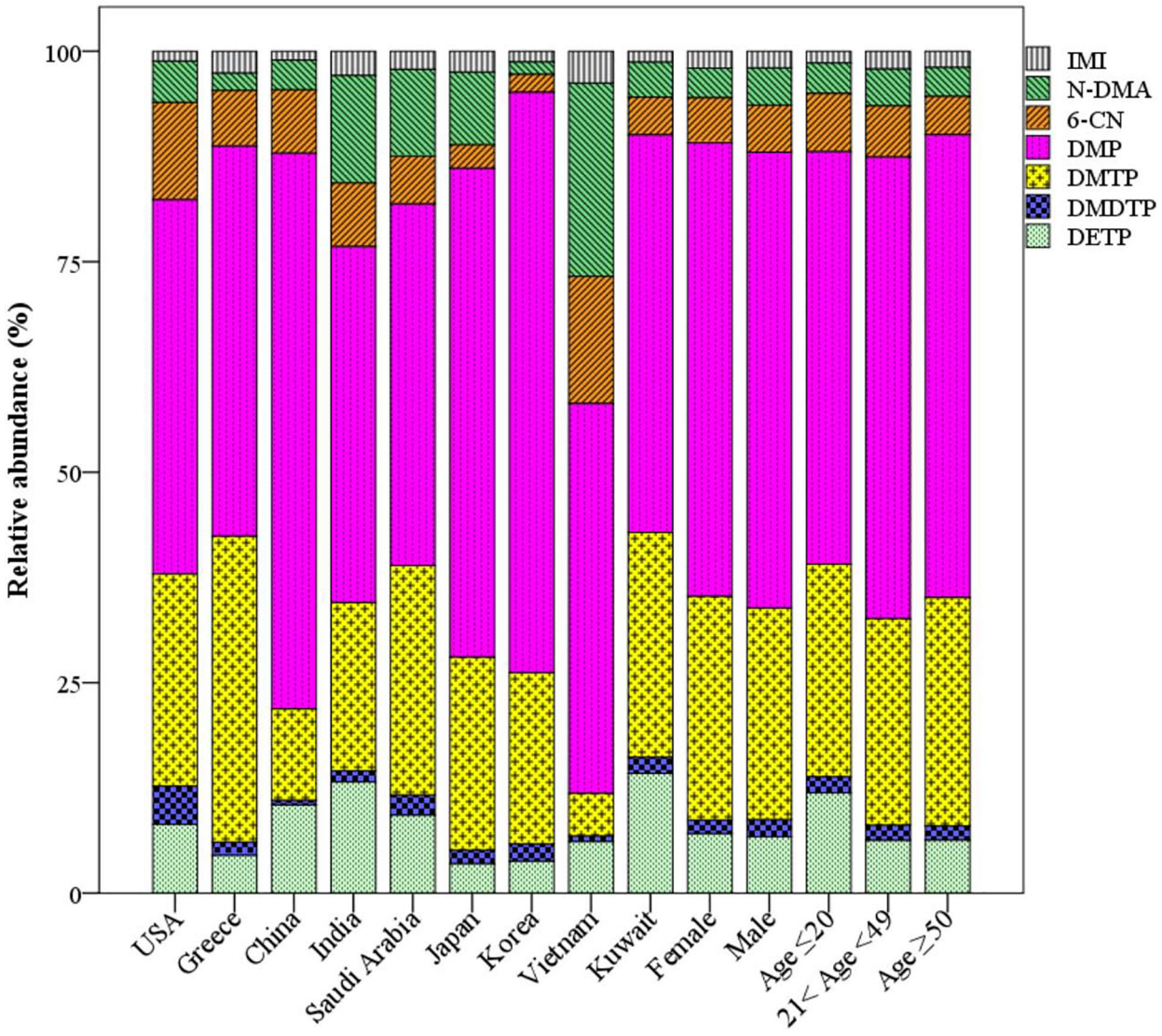

Fig. 2.

Composition profiles of urinary pesticides from nine countries (to sum of three neonicotinoid insecticides and four dialkylphosphate metabolites with detection frequencies >80%). IMI, imidacloprid; N-DMA, *N*-desmethyl acetamiprid; 6-CN, 6-chloronicotinic acid; DMP, dimethylphosphate; DMTP, dimethylthiophosphate; DMDTP, dimethyldithiophosphate; DETP, diethylthiophosphate.

Table 1

Descriptive statistics of concentrations (ng/mL) of urinary neonicotinoids and organophosphates and their metabolites from nine countries (n = 566).

Analyte	DF (%)	Mean (ng/mL)	Percentile (ng/mL)			
			25th	50th	75th	100th
NIT	53.7	0.079	<LOD	0.006	0.094	3.89
THX	72.3	0.282	<LOD	0.114	0.344	6.57
IMI	89.4	0.193	0.051	0.128	0.230	2.78
ACE	21.2	0.014	<LOD	<LOD	<LOD	2.16
THI	13.1	0.003	<LOD	<LOD	<LOD	0.412
CLO	72.1	0.262	<LOD	0.105	0.237	24.3
DIN	66.8	3.05	<LOD	0.681	2.85	187
FLO	47.7	0.070	<LOD	<LOD	0.069	9.42
N-DMT	57.1	0.282	<LOD	0.035	0.222	16.6
TA	41.9	0.066	<LOD	<LOD	0.096	0.712
IMZ	69.1	0.425	<LOD	0.165	0.509	8.76
N-DMA	97.7	0.750	0.103	0.262	0.672	13.7
6-CN	91.9	0.841	0.176	0.354	0.760	29.5
SUF	15.2	0.010	<LOD	<LOD	<LOD	1.38
∑14 neonics		**6.33**	**2.17**	**3.70**	**7.01**	**189**
DMP	98.6	9.21	1.41	3.39	8.77	216
DMTP	99.8	5.24	0.631	1.55	3.55	517
DMDTP	92.0	1.39	0.032	0.112	0.327	297
DEP	69.8	1.57	<LOD	0.244	1.48	47.3
DETP	97.7	1.52	0.186	0.447	1.34	132
DEDTP	8.5	0.068	<LOD	<LOD	<LOD	20.1
∑6 DAPs		**19.0**	**3.44**	**8.10**	**18.2**	**1035**

DF, detection frequency. LOD, limit of detection. ∑14 neonics, sum concentration of the 14 neonicotinoid insecticides. ∑6 DAPs, sum concentration of the six dialkylphosphate metabolites.

Table 2

Country-wise concentrations (ng/mL and μg/g creatinine in bold *italic*) of urinary neonicotinoid insecticides and dialkylphosphate metabolites among the nine countries studied.

		IMI	N-DMA	6-CN	DMP	DMTP	DMDTP	DETP	∑7
Country		**	**	**	**	**	**	**	**
USA (*n* = 44)	DF	79.5	97.7	93.2	100	100	93.2	95.5	
	Median	0.047	0.195	0.461	1.77	1.00	0.181	0.326	4.91
	Median	***0.052***	***0.181***	***0.442***	***1.22***	***1.11***	***0.140***	***0.258***	***4.99***
Greece (*n* = 118)	DF	95.8	99.2	94.9	99.2	100	93.2	99.2	
	Median	0.152	0.121	0.386	2.70	2.12	0.092	0.262	6.77
	Median	***0.144***	***0.098***	***0.340***	***2.23***	***1.53***	***0.077***	***0.265***	***5.70***
China (*n* = 84)	DF	89.3	100	97.6	100	100	94.0	100	
	Median	0.156	0.520	1.11	9.73	1.60	0.082	1.54	18.2
	Median	***0.131***	***0.498***	***1.19***	***9.80***	***1.39***	***0.090***	***1.86***	***19.6***
India (*n* = 41)	DF	85.4	100	90.2	100	97.6	82.9	87.8	
	Median	0.097	0.428	0.253	1.42	0.670	0.044	0.443	4.74
	Median	***0.232***	***0.912***	***0.577***	***3.08***	***1.11***	***0.083***	***0.870***	***9.08***
Saudi Arabia (*n* = 61)	DF	85.2	95.1	98.4	91.8	100	96.7	96.7	
	Median	0.138	0.659	0.363	2.75	1.74	0.153	0.591	8.78
	Median	***0.113***	***0.458***	***0.324***	***1.67***	***1.81***	***0.137***	***0.504***	***7.01***
Japan (*n* = 35)	DF	88.6	100	60.0	100	100	82.9	100	
	Median	0.080	0.279	0.090	1.87	0.740	0.052	0.112	3.59
	Median	***0.059***	***0.210***	***0.085***	***1.45***	***0.477***	***0.053***	***0.086***	***2.91***
Korea (*n* = 100)	DF	99.0	99.0	96.0	100	100	96.0	98.0	
	Median	0.152	0.178	0.253	8.27	2.44	0.243	0.454	14.3
	Median	***0.108***	***0.152***	***0.195***	***6.46***	***1.57***	***0.189***	***0.391***	***10.1***
Vietnam (*n* = 17)	DF	100	100	100	100	100	76.5	94.1	
	Median	0.202	1.22	0.803	2.46	0.270	0.035	0.326	6.19
	Median	***0.110***	***0.667***	***0.538***	***1.67***	***0.195***	***0.024***	***0.266***	***4.41***
Kuwait (*n* = 66)	DF	74.2	89.4	81.8	97.0	100	90.9	100	
	Median	0.080	0.258	0.276	2.92	1.65	0.118	0.879	7.94
	Median	***0.064***	***0.209***	***0.181***	***3.04***	***1.34***	***0.089***	***0.645***	***6.60***
Gender									
Female (*n* = 255)	DF	89.8	98.0	94.1	98.4	100	92.2	98.4	
	Median	0.133	0.234	0.355	3.58	1.77	0.107	0.470	9.00
	Median	***0.125***	***0.250***	***0.324***	***3.22***	***1.59***	***0.129***	***0.504***	***8.63***
Male (*n* = 254)	DF	90.6	96.9	89.4	98.4	100	93.3	98.4	
	Median	0.118	0.263	0.331	3.21	1.49	0.120	0.397	8.68
	Median	***0.092***	***0.192***	***0.238***	***2.55***	***1.05***	***0.078***	***0.324***	***6.25***
Age									
≤20 (*n* = 66)	DF	77.3	93.9	87.9	97.0	100	95.5	98.5	
	Median	0.081	0.211	0.402	2.85	1.47	0.111	0.693	7.38

		IMI	N-DMA	6-CN	DMP	DMTP	DMDTP	DETP	∑7
	Median	***0.076***	***0.187***	***0.322***	***3.11***	***1.97***	***0.122***	***0.621***	***7.26***
21–49 (*n* = 244)	DF	91.0	98.4	89.8	98.8	100	91.4	98.0	
	Median	0.133	0.274	0.379	3.43	1.54	0.112	0.392	8.11
	Median	***0.107***	***0.216***	***0.291***	***2.60***	***1.11***	***0.082***	***0.373***	***6.00***
≥50 (*n* = 198)	DF	93.4	97.5	95.5	98.5	100	93.4	99.0	
	Median	0.135	0.240	0.316	3.84	1.89	0.118	0.438	10.0
	Median	***0.111***	***0.242***	***0.268***	***3.39***	***1.39***	***0.125***	***0.390***	***8.41***

DF, detection frequency (%).

$^{**}p < 0.01$.

Table 3

Median daily intake (DI; μg/day) of pesticides estimated from urinary neonicotinoid insecticides and dialkylphosphate metabolites for the nine countries studied.

	Imidacloprid	Acetamiprid	∑neonics [a]	Malathion	Chlorpyrifos	∑OPs [b]	Total [c]
cRfD	57 [d]	71		70	0.3		
	3420 [e]	4260		4200	18		
Country							
USA	2.2	1.2	3.4	125 (1) [f]	1.2	127	130
Greece	3.4	0.7	4.1	208 (1)	1.0 (1)	209	213
China	5.9	3.1	9.0	500 (2)	5.8 (13)	506	515
India	2.2	2.5	4.7	91.4	1.7 (1)	93.1	97.8
Saudi Arabia	3.1	3.9	7.0	197	2.2 (5)	199	206
Japan	1.4	1.6	3.0	115	0.4	115	118
Korea	2.9	1.1	4.0	474 (4)	1.7 (6)	476	480
Vietnam	5.5	7.2	12.7	122	1.2 (1)	123	136
Kuwait	2.0	1.5	3.5	200 (1)	3.3 (3)	203	207
Gender							
Female	3.4	1.4	4.8	262 (4)	1.8 (13)	263	268
Male	3.5	1.6	5.0	246 (4)	1.5 (12)	247	253
Age							
≤20	3.0	1.2	4.3	212 (1)	2.6 (2)	214	219
21–49	3.6	1.6	5.2	250 (3)	1.5 (13)	252	257
≥50	3.4	1.4	4.8	280 (4)	1.7 (10)	282	287
All [g]	2.9	1.5	4.5	216 (9)	1.7 (30)	218	223

[a] ∑neonics refers to sum DIs of imidacloprid and acetamiprid.

[b] ∑OPs refers to sum DIs of malathion and chlorpyrifos.

[c] Total refers to sum DIs of imidacloprid, acetamiprid, malathion and chlorpyrifos.

[d] cRfD, chronic reference doses of U.S. EPA (μg/kg body weight/day).

[e] Estimated chronic reference doses from U.S. EPA (μg/day, the body weight assumed as 60 kg for adults).

[f] Number of samples exceeded the estimated reference doses.

[g] All refers to median DIs of pesticides estimated from urianry concentrations for the entire dataset from the nine countries.