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An assessment of exposure to several classes of pesticides in pet dogs and cats from New York, United States

Zhong-Min Li[a,b], **Morgan Robinson**[a,b], **Kurunthachalam Kannan**[a,b,*]

[a]Department of Pediatrics, New York University Grossman School of Medicine, New York, NY 10016, United States

[b]Department of Environmental Medicine, New York University Grossman School of Medicine, New York, NY 10016, United States

Abstract

Exposure of pet dogs and cats to pesticides used in and around homes (e.g., lawns and gardens) is a significant health concern. Furthermore, some pesticides are directly used on dogs and cats for flea, lice, and tick control. Despite this, little is known regarding the extent of pesticide exposure in pets. In this study, we determined the concentrations of 30 biomarkers of pesticide exposure in urine collected from dogs and cats in New York State, USA: 6 dialkylphosphate (DAP) metabolites of organophosphates (OPs); 14 neonicotinoids (neonics); 3 specific metabolites of OPs; 5 pyrethroids (PYRs); and 2 phenoxy acids (PAs). The sum median concentrations of these 30 pesticide biomarkers (ΣPesticides) in dog and cat urine were 35.2 and 38.1 ng/mL, respectively. Neonics were the most prevalent in dogs (accounting for 43% of the total concentrations), followed by DAPs (17%), PYRs (16%), OPs (13%), and PAs (~10%). In cat urine, neonics alone accounted for 83% of the total concentrations. Elevated concentrations of imidacloprid were found in the urine of certain dogs (max: 115 ng/mL) and cats (max: 1090 ng/mL). Some pesticides showed gender- and sampling location- related differences in urinary concentrations. We calculated daily exposure doses of pesticides from the measured urinary concentrations through a reverse dosimetry approach. The estimated daily intakes (DIs) of chlorpyrifos, diazinon, and cypermethrin were above the chronic reference doses (cRfDs) in 22, 76, and 5%, respectively, of dogs. The DIs of chlorpyrifos, parathion, diazinon, and imidacloprid were above the cRfDs in 33, 14, 100, and 29%, respectively, of cats. This study thus provides evidence that pet dogs and cats are exposed to certain pesticides at levels that warrant immediate attention.

*Corresponding author at: MSB 6-698, 550 First Avenue, New York, NY 10016, United States. kurunthachalam.kannan@nyulangone.org (K. Kannan).

CRediT authorship contribution statement

Zhong-Min Li: Methodology, Data curation, Formal analysis, Writing – original draft. **Morgan Robinson:** Methodology, Writing – review & editing. **Kurunthachalam Kannan:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107526.

Keywords

Organophosphate; Neonicotinoid; Pyrethroid pesticides; Dog; Cat; Urine

1. Introduction

Pesticides are used extensively in agriculture and in disease vector control in and around homes (Alvavanja, 2009; Md Meftaul et al., 2020; Stanneck et al., 2012). Global annual pesticide consumption in 2019 was ~ 4.2 million tons, with the United States accounting for ~ 20% of usage (EPA, 2017; FAO, 2019). Neonicotinoids ("neonics"), pyrethroids (PYRs), and organophosphates (OPs) account for 24%, 15%, and 8%, respectively, of the global pesticide market (Sparks et al., 2020). The phenoxy acid (PA) herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) has been used for decades on lawns, turfs, and agricultural fields (Burns and Swaen, 2012).

There is a growing concern about health effects from chronic exposure to pesticides in humans and pet animals. Humans and pet animals can be exposed to pesticides through air, water, soil, and diet (Kim et al., 2017), as well as through veterinary medication for pets (Wise et al., 2022). Following ingestion, OPs are primarily metabolized to common dialkylphosphate (DAP) (~70–75%) metabolites, as well as specific metabolites such as 3,5,6-trichloro-2-pyridinol (TCPY, metabolite of chlorpyrifos), 4-nitrophenol (PNP, metabolite of parathion), and 2-iso-propyl-6-methyl-4-pyrimidiol (IMPY, metabolite of diazinon) (Gari et al., 2018; Ueyama et al., 2015). Several PYRs are generally metabolized to compounds such as 3-phenoxybenzoic acid (3-PBA), 4-fluoro-3-phenoxybenoic acid (4-F-3-PBA), *cis*- and *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid (*cis*-/*trans*-DCCA), and *cis*-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropane-1-carboxylic acid (*cis*-DBCA) (Gari et al., 2018). Neonics and PAs are mostly excreted unchanged in urine due to their high water solubility (Aylward et al., 2010; Ueyama et al., 2015). The biological half-lives of OPs, PYRs, and neonics in mammals range from a few hours to a few days (Harada et al., 2016; Li and Kannan, 2018; Li et al., 2020).

Human biomonitoring studies have reported widespread exposure to pesticides and their metabolites in the general population (CDC, 2018; 2021). Toxicological and epidemiological studies have reported associations between pesticide exposure and neurological, respiratory, dermatological, digestive, carcinogenic, reproductive, and developmental effects (Gonzalez-Alzaga et al., 2014; Kim et al., 2017; Saillenfait et al., 2015). In addition, neonic exposure is implicated in population-level effects on non-target organisms such as bees (Rundlof et al., 2015), aquatic invertebrates (Morrissey et al., 2015), and insectivorous birds (Hallmann et al., 2014). The International Agency for Research on Cancer (IARC) classified malathion and diazinon as probable carcinogens (Group 2A) and parathion, 2,4-D, and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) as possible carcinogens (Group 2B) (Guyton et al., 2015; IARC, 2015, 1987).

Pet dogs and cats share a common living environment with humans and can serve as sentinels of human exposure to environmental contaminants (https://factor.niehs.nih.gov/2022/1/feature/3-feature-sentinels/index.htm). Exposure of pet dogs and cats to various

environmental chemicals has been reported through the analysis of urine, feces, blood, hair, and silicone tags (Ali et al., 2013; Brits et al., 2019; Brits et al., 2018; Chinthakindi and Kannan, 2022; Gonzalez-Gomez et al., 2018; Karthikraj and Kannan, 2019; Mizukawa et al., 2016; Poutasse et al., 2019; Wise et al., 2020; Wise et al., 2022; Zhang et al., 2019). Positive correlations were found between exposure levels in humans and dogs from the same homes to several classes of environmental chemicals (Wise et al., 2020; Wise et al., 2022). Dogs and cats develop chronic diseases similar to those of humans but with a shorter latency period (Knapp et al., 2013). Pesticide exposure in dogs and cats has been linked to mammary cancer (Gautam et al., 2020), lymphoma (Takashima-Uebelhoer et al., 2012), bladder cancer (Glickman et al., 2004), and oral squamous cell carcinoma (Bertone et al., 2003), reflecting effects similar to those reported in human studies (Calaf, 2021; Fritschi et al., 2005; Koutros et al., 2016). Nevertheless, studies reporting the occurrence of pesticides in dog urine are limited (Forster et al., 2014; Karthikraj and Kannan, 2019; Knapp et al., 2013; Reynolds et al., 1994; Wise et al., 2022), and no previous studies have determined the exposure of cats to OPs, neonics, PYRs, or PAs.

In this study, we determined the concentrations of 30 pesticide biomarkers in dog and cat urine collected from New York State, USA, to elucidate profiles, exposure doses, and health risks. Six DAPs, 14 neonics, 3 OPs, 5 PYR metabolites, and 2 PAs (Fig. S1-S5, Supplementary material) were analyzed.

2. Materials and methods

2.1. Reagents, standards, and sample collection

Reagents and analytical standards used in this study were described previously (Li and Kannan, 2018; Li and Kannan, 2020) (Table S1). Dog and cat urine samples were collected from a veterinary hospital, an animal shelter, and individual pet owners from the Albany area of New York State, USA, during March–July 2017. Majority of the samples were collected at the veterinary hospital and the animal shelter, and an aliquot of urine was used in this study. Canine urine was collected directly in polypropylene (PP) containers, whereas feline urine samples were collected by cystocentesis or directly in PP containers. Details of breed, age, gender, and sampling location of pets are given in Table S2 (Karthikraj et al., 2018a; Karthikraj and Kannan, 2019). The numbers of urine specimens analyzed were 39–47 for dogs and 15–28 for cats (Table S3). The number of samples analyzed for each class of pesticides varied depending on the available sample volume. The samples were stored at −20 °C until analysis.

2.2. Analysis of urinary pesticides

Urinary pesticides were determined using the methods described elsewhere (Li and Kannan, 2018; Li and Kannan, 2020). Details of sample preparation and instrumental methods are provided in the Supplementary material. Briefly, the urinary DAPs (DMP-dimethyl-phosphate, DEP-diethylphosphate, DMTP-dimethylthiophosphate, DETP-diethylthiophosphate, DMDTP-dimethyldithiophosphate, and DEDTP-diethyldithiophosphate) were extracted using a weak anion-exchange cartridge (Biotage WAX; Waters Corp, Milford, MA, USA) and determined by high-performance

liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) under positive-ion mode electro-spray ionization (ESI) (Table S4). Neonics were extracted from urine using a nonpolar divinylbenzene-based neutral polymeric cartridge (Bond Elut Plexa; Agilent, Santa Clara, CA, USA) and analyzed using HPLC-MS/MS under positive-ion mode ESI for nitenpyram (NIT), thiamethoxam (THX), imidacloprid (IMI), acetamiprid (ACE), thiacloprid (THI), clothianidin (CLO), dinoteruran (DIN), flonicamid (FLO), *N*-desmethyl thiamethoxam (*N*-DMT), thiacloprid-amide (TA), imidaclothiz (IMZ), and *N*-desmethyl acetamiprid (*N*-DMA) and under negative-ion mode ESI for 6-chloronicotinic acid (6-CN) and sulfoxaflor (SUF) (Tables S5 & S6). PYRs, PAs and OPs were extracted from urine using a hydrophilic-lipophilic balanced cartridge (Oasis HLB; Waters Corp) following enzymatic digestion, and determined using HPLC-MS/MS under ESI negative-ion (for PNP, TCPY, 2,4-D, 2,4,5-T, 3-PBA, 4-F-3-PBA, *trans*-DCCA, *cis*-DCCA, and *cis*-DBCA) and positive-ion (for IMPY) modes (Table S7).

2.3. Quality assurance and quality control

An isotope dilution method was used to quantify target analytes. An 11- to 16-point calibration curve was prepared by injecting standard solutions at concentrations ranging from 0.01 to 200 ng/mL, along with 10 ng/mL of isotopically labeled internal standards. Two procedural blanks (containing HPLC-grade water instead of urine), two matrix blanks (synthetic urine from Cerilliant, Round Rock, TX, USA), two matrix spikes (fortified synthetic urine with target analytes at 10 ng/mL), and proficiency test (PT) samples from the German External Quality Assurance Scheme (G-EQUAS) round 67/2021 (samples 9A, 9B, 14/15A, and 14/15B) were analyzed with every batch of 25 samples. The limit of detection (LOD) was determined as the concentration at a signal-to-noise ratio (S/N) of 3. Sample-to-sample carryover of target analytes was monitored by injecting a pure solvent after every 10 samples. A mid-point standard solution (10 ng/mL) was injected after every 20 samples as a check for the stability of the instrumental response to target analytes.

2.4. Method performance

Typical chromatograms of the targeted analytes in both solvent and urine matrix are presented in Fig. S6. The correlation coefficient (r) of the calibration curve was > 0.99 for all analytes. Trace levels of DMP (0.03 ng/mL), DEDTP (0.11 ng/mL), IMZ (0.02 ng/mL), PNP (1.11 ng/mL), 3-PBA (0.23 ng/mL), 2,4-D (0.005 ng/mL), and *cis*-DBCA (0.18 ng/mL) were found in procedural blanks, and these concentrations were subtracted from those measured in samples. The LODs of all target analytes were between 0.001 and 0.053 ng/mL. The recoveries of all target analytes were in the range of 75–121%, with a relative standard deviation of 4–24%. The concentrations of target analytes measured in PT samples were within the acceptable ranges (Table S8). In this study, OP refers to PNP, IMPY and TCPY.

2.5. National health and nutrition examination survey data

Urinary pesticide concentrations measured in dogs and cats were compared with those of the U.S. general population reported in the National Health and Nutrition Examination Survey (NHANES). The geometric mean (GM) and 95th percentile (P95) values of creatinine-adjusted concentrations of pesticides with detection frequencies (DFs) ≥ 80% were used

for comparison. NHANES data for the survey years 2009/2010 (for TCPY, 2,4,5-T and *cis*-DBCA), 2011/2012 (for DMP, DEP, DMTP, DETP, DMDTP, and DEDTP), 2013/2014 (for PNP, 2,4-D, *trans*-DCCA, and IMPY), and 2015/2016 (for IMI, ACE, CLO, and *N*-DMA) were used (CDC, 2018; 2021; Ospina et al., 2019). For comparison of urinary pesticide concentrations in pets with the NHANES data, dog ages were rescaled to approximate human ages (Hoffman et al., 2018).

2.6. Statistical analyses

Statistical analyses were conducted for pesticides with DFs ≥ 80%. The concentrations below the LOD were replaced with LOD divided by the square root of 2. Normality of the distribution of pesticide concentrations was tested using a Shapiro-Wilk test. The differences in pesticide concentrations between cats and dogs, as well as between gender and sampling sites, were tested using a non-parametric test. Spearman's rank correlation was applied to examine the relations among urinary pesticide biomarkers. Statistical significance was set at $p < 0.05$. All statistical analyses were conducted using R (version 4.1.2; R Foundation for Statistical Computing).

3. Results and discussion

3.1. Concentrations in dog and cat urine

The median concentrations of ΣPesticides in dog and cat urine were 35.2 ng/mL (31.4 μg/g creatinine) and 38.1 ng/mL (17.2 μg/g creatinine), respectively. In dog urine, all DAPs and OPs had DFs ≥ 80%, whereas 10 neonics, 2 PRYs and 2 PAs had DFs ≥ 80%. Similarly, in cat urine, all DAPs and OPs were found with DFs ≥ 80%, whereas 11 neonics, 3 PYRs, and 2,4,5-T were found in ≥ 80% samples (Table 1 & Fig. 1). Our results suggest widespread exposure to multiple pesticides in pet dogs and cats. The median urinary concentrations of 2,4-D (0.80 ng/mL), *trans*-DCCA (1.09 ng/mL), PNP (2.55 ng/mL), and TCPY (0.92 ng/mL) in dogs were similar to those reported in a recent study from the U.S. states of North Carolina and New Jersey (0.96, 1.47, 2.86, and 1.30 ng/mL for 2,4-D, *trans*-DCCA, PNP, and TCPY in dog urine, respectively) (Wise et al., 2022). Low DFs for ACE, *N*-DMA, and THI were found in dog urine in both our (21.4–50%) and previous (0%) studies (Wise et al., 2022).

IMI is one of the most widely used insecticides in veterinary medicine (Vo et al., 2010), which may explain its high urinary concentrations in dogs and cats in our study (P95: 76.3 and 1090 ng/mL in dog and cat urine, respectively) and a previous canine study (P95: 126 and 584 ng/mL for IMI and 5-OH-IMI in dog urine, respectively) (Wise et al., 2022). The DFs of IMI (93.8–95.2%) and IMPY (100%) in our study were higher than those reported earlier (DFs: 26–42% for IMI and 0% for IMPY) (Forster et al., 2014; Wise et al., 2022). The concentrations of DMP (GM: 1.04 and 0.47 μg/g creatinine in dog and cat urine, respectively), DEP (1.32 and 1.47 μg/g creatinine, respectively), and DMTP (0.36 and 0.39 μg/g creatinine, respectively) in dog and cat urine were 2-5-fold lower than those reported for the U.S. general population (GM: 2.45, 2.29 and 1.62 μg/g creatinine for DMP, DEP and DMTP, respectively) (Fig. 2). The P95 concentrations of DETP and DMDTP in pet dog and cat urine were 1.5-3.8-fold lower than those reported for humans. The higher concentrations

of DAPs in human urine indicate greater exposure to DAPs or their parent compounds. In both dogs and humans, the urinary concentrations of DMP, DEP and DMTP tend to be higher in younger age groups (Table S9).

Dog urine contained higher concentrations of neonics than those in humans of all age groups (rescaled ages: 3–5 y, 6–11 y, 12–19 y, and > 20 y) (Fig. 2 and Table S9). IMI (GM: 1.63 μg/g creatinine in dog urine vs < LOD in human urine) and CLO (GM: 0.40 μg/g creatinine in dog urine vs < LOD in human urine) were frequently found in dog urine. Both ACE and *N*-DMA had low DFs in dog and human urine. Nevertheless, IMI, ACE, CLO, and *N*-DMA concentrations in cat urine were higher than those in dog and human urine (GM: 0.02–23.5 ng/g creatinine; P95: 0.19–1830 ng/g creatinine), probably due to the use of neonics for the control of fleas, ticks, flies, and lice in dogs and cats (Vo et al., 2010). Concentrations in dog and cat urine were similar to those in humans, as reported in the NHANES for the U.S. general population, for PNP (GM: 2.03, 2.04, and 0.69 μg/g creatinine in dog, cat, and human urine, respectively), TCPY (0.68, 0.73, and 0.81 μg/g creatinine, respectively), and 2,4-D (0.56, 0.05, and 0.33 μg/g creatinine, respectively). However, the DFs of 2,4,5-T, *cis*-DBCA, *trans*-DCCA, and IMPY were higher in pet urine (DFs: 46.7–100%) than in human urine (DFs: < 50%) (CDC, 2018; 2021; Ospina et al., 2019), which suggested common exposure of dogs to certain PYRs (e.g., permethrin, cypermethrin) and OPs (e.g., diazinon) that humans are less likely to encounter. Flea and tick control and indoor application of pesticides are known to be sources of exposure in dogs and human children (Wise et al., 2020; Wise et al., 2022). In addition, the highest urinary IMI and *trans*-DCCA concentrations were found in younger dogs (rescaled ages 3–5 y); however, data for this age group in U.S. populations are not available for comparison (Table S9).

Spearman's rank correlations of pesticide biomarkers measured in dog and cat urine are shown in Fig. 3. Several pesticides measured in pet urine were positively correlated, despite belonging to five different classes of chemicals. For example, in dog urine, IMZ was significantly positively correlated with DMP, DEP, DMTP, DETP, DMDTP, THX, 6-CN, PNP, TCPY, 2,4,5-T, and IMPY (r_S: 0.33–0.46, $p < 0.05$). Similarly, the concentrations of several pesticides in cat urine were positively correlated: IMZ was significantly correlated with DEP, DMTP, DETP, DMDTP, NIT, TA, PNP, TCPY, and IMPY (r_S: 0.62–0.78, $p < 0.05$). These results suggest co-exposure to multiple pesticides as well as the existence of common precursors for some biomarkers measured (e.g., DETP and TCPY are both metabolites of chlorpyrifos). Our findings are consistent with those of previous studies, which also reported positive correlations among several classes of pesticides measured in human urine (Li and Kannan, 2018). These findings warrant attention from the view of cumulative and mixture toxicity.

3.2. Profiles in dog and cat urine

In dog urine samples, neonics (accounting for 43% of the total pesticide concentration) were the dominant class of pesticides, followed by DAPs (17%), PYRs (16%), OPs (13%), and PAs (~10%). In cat urine samples, neonics alone accounted for 83% of the total concentrations (Table 1 & Fig. 4). However, human studies have found higher concentrations

of DAPs than neonics in urine (Li and Kannan, 2020). This difference in profile might be explained by the direct usage of neonics on pet animals for flea and tick control (Vo et al., 2010).

The profiles of the six DAP metabolites tested in cat and dog urine were similar. DEP was the most abundant, accounting for, on average, 42% and 51% of the total DAP concentrations in dog and cat urine, respectively (Fig. 4). Among neonics, IMI and IMZ were abundant in dog urine, accounting for 54% and 30% of the total concentrations, respectively, whereas in cats, IMI alone accounted for 94% of the total concentrations. Pet collars containing a combination of 10% IMI (*w/w*) and 4.5% flumethrin (*w/w*) are reported to be effective in preventing tick and flea infestations and infection by some vector-borne pathogens in dogs (Stanneck et al., 2012). While IMI is effective against fleas and lice, PYRs such as permethrin and flumethrin are effective against ticks (acaricides). In addition to its use in pet products such as collars, soaps, and shampoos, IMI is also applied to pets topically as a treatment for fleas and ticks at 10–25 mg/kg body weight (BW), with dosing every 4–5 weeks (Gomez and Picado, 2017). Furthermore, application of pesticides in lawns and agricultural settings can also contribute to pet exposure. IMI is the most widely used neonic in the U.S. accounting for ~ 42% of the market (Jeschke et al., 2011) and has been registered in the U.S. for insect control in corn, lettuce, broccoli, apples, and potatoes (EPA, 2020). Use of neonics and PYRs in pet products (such as collars, shampoos) can also contribute to the exposure of humans in the indoor environment.

In both dog and cat urine samples, IMPY was the most abundant OP metabolite, followed by PNP and TCPY. However, parathion/methyl parathion (precursor of PNP) and diazinon (precursor of IMPY) are not permitted for use in veterinary medication in the U.S., indicating other exposure sources for diazinon. *Trans*-DCCA (metabolite of permethrin and other PYRs) was the predominant PYR found in dog urine, accounting for 81% of the total PYR concentrations. This finding is consistent with previous studies, which reported frequent detection of *trans*-DCCA in human and dog urine (DFs: 60–73%), as well as frequent detection of permethrin isomers on human wristbands and dog tags (DFs: 100%) (Kassotis et al., 2020; Wise et al., 2020; Wise et al., 2022). In cat urine, however, *cis*-DBCA (73%) and *trans*-DCCA (15%) were the dominant PYR compounds (Fig. 4). The relative distribution of 2,4-D and 2,4,5-T in PAs were similar in dogs and cats.

3.3. Differences in concentrations between dogs and cats

Cat urine contained higher concentrations of most pesticides than dog urine (Table 1). The concentrations of DETP (mean: 0.68 ng/mL in cat urine vs 0.47 ng/mL in dog urine; $p < 0.05$), DEDTP (1.82 vs 0.81 ng/mL; $p < 0.01$), IMI (211 vs 14.7 ng/mL; $p < 0.01$), DIN (2.37 vs 1.19 ng/mL; $p < 0.01$), *N*-DMT (0.41 vs 0.27 ng/mL; $p < 0.1$), TA (0.50 vs 0.13 ng/mL; $p < 0.01$), *N*-DMA (1.35 vs 0.19 ng/mL; $p < 0.01$), 6-CN (0.53 vs 0.42 ng/mL; $p < 0.05$), ΣNeonics (211 vs 26.2 ng/mL; $p < 0.01$), and IMPY (24.8 vs 3.44 ng/mL; $p < 0.01$) in cat urine were significantly higher than those in dog urine. In contrast, the concentrations of DMP (2.47 ng/mL in dog urine vs 0.92 ng/mL in cat urine; $p < 0.05$), 2,4-D (3.63 vs 0.18 ng/mL; $p < 0.01$), 2,4,5-T (2.48 vs 0.12 ng/mL; $p < 0.05$), and *trans*-DCCA (8.05 vs 0.56 ng/mL; $p < 0.01$) were significantly higher in dog urine than cat urine. These

differences remained significant even after the concentrations were adjusted for creatinine, except for IMI, 6-CN, ΣNeonics, and *trans*-DCCA. An earlier study reported a 2-fold higher concentration of glyphosate in cat urine than in dog urine (33.8 ± 46.7 ng/mL in cat urine vs 16.8 ± 24.4 ng/mL in dog urine) (Karthikraj and Kannan, 2019). Higher urinary concentrations of pesticides in cats than in dogs may be attributed to specific exposures and metabolic differences (van Beusekom et al., 2014). For instance, cats are sensitive to PYR (e.g., permethrin) toxicity due to their low glucuronidation capacity, and thus PYR-containing flea treatment products are intended only for dogs (Dymond and Swift, 2008; van Beusekom et al., 2014). This may explain the 14-fold lower *trans*-DCCA concentrations in cat urine than in dog urine (Table 1). In addition, elevated 2,4-D (herbicide) concentration in dog urine than in cat urine suggests frequenting of dogs in gardens and lawns where 2,4-D is commonly used.

3.4. Sex-, sampling location- and breed-specific variations

The concentrations of DAPs, OPs, PYRs, and PAs were similar between males and females in both dogs (25 males and 22 females) and cats (9 males and 19 females) ($p > 0.05$) (Table S10). However, select neonics exhibited significant sex differences in concentrations in dogs. CLO (0.52 ng/mL in males vs 1.36 ng/mL in females; $p < 0.05$) and *N*-DMT (0.13 vs 0.40 ng/mL; $p < 0.05$) concentrations were significantly higher in female than in male dogs. The differences remained significant even after creatinine adjustment of urinary concentrations. NIT (0.12 μg/g creatinine in males vs 0.08 μg/g creatinine in females; $p < 0.05$) concentrations were significantly higher in male than in female dogs. In contrast, no sex-related differences in pesticide concentrations were found in cats (either volume- or creatinine-based concentrations), probably due to the limited statistical power of this analysis. Further studies with larger sample size are needed to confirm these findings. Furthermore, some animals in this study were spayed or neutered, which may have a significant impact on the metabolism and excretion of pesticides.

Pesticide concentrations were compared among dogs from individual owners (n = 16), animal shelter (n = 12) and veterinary hospital (n = 19) (Table S11). The urine of dogs from the veterinary hospital contained the highest concentrations of IMI (mean ± SD: 5.89 ± 15.6, 0.55 ± 0.44, and 25.4 ± 25.9 μg/g in dogs from individual owners, animal shelter and veterinary hospital, respectively; $p < 0.001$), ΣNeonics (mean ± SD: 16.2 ± 18.3, 8.87 ± 6.14, and 35.6 ± 29.9 μg/g, respectively; $p < 0.001$), *cis*-DCCA (mean ± SD: 1.60 ± 3.92, 0.05 ± 0.03, and 1.76 ± 5.97 μg/g, respectively; $p = 0.02$), and *trans*-DCCA (mean ± SD: 17.1 ± 40.4, 0.69 ± 0.48, and 19.4 ± 66.0 μg/g, respectively; $p = 0.03$). These findings indicate that dogs in veterinary hospitals have been treated with imidacloprid and cypermethrin (precursor compound of *cis*- and *trans*-DCCA), likely from veterinary medication.

The urinary pesticide concentrations were compared among dogs of different breed sizes (Table S12). The unadjusted urinary concentrations of DETP (mean ± SD: 0.61 ± 0.36 and 0.43 ± 0.78 ng/mL in medium/small and large breed dogs, respectively; $p = 0.02$) and 6-CN (mean ± SD: 0.65 ± 0.71 and 0.28 ± 0.55 ng/mL, respectively; $p = 0.03$) were significantly higher in small and medium breeds than those in large breeds. However, the differences were

not significant after creatinine adjustment. The creatinine-adjusted concentrations of CLO, IMZ, *cis*-DCCA, *trans*-DCCA, 2,4,5-T and ΣPA were higher in the urine of large dogs than medium and small dogs, whereas IMI concentrations were higher in the urine of medium and small dogs. However, these differences were not significant among unadjusted urinary concentrations.

3.5. Exposure assessment

We estimated the daily intakes (DIs) of parent pesticides from the concentrations of pesticides or its metabolites measured in urine with the DFs ≥ 80% (Table 2) using the following equation (Guo et al., 2011):

$$DI = \mathrm{C} \times \frac{V}{BW} \times \frac{MW_1}{MW_2} \times \frac{1}{F_{\mathrm{ue}}} \quad (1)$$

where DI is the daily intake of pesticides (μg/kg BW/day); C is the measured concentration of a pesticide or metabolite in pet urine (ng/mL); BW is the body weight (kg), which was estimated according to the breed and age of each pet; V is the 24-h average excretion volume of urine (mL/day), which was estimated according to the body size of dogs and age of cats (Karthikraj et al., 2018b). The estimated BW and V values are given in Table S2; MW_1 and MW_2 are the molecular weights (g/mol) of the parent pesticide and metabolite, respectively; and F_{ue} represents the fraction of the pesticide or its metabolite excreted in urine following exposure to parent molecule.

Cis- and *trans*-DCCA are the metabolites of cyfluthrin, cypermethrin, and permethrin, whereas 4-F-3-PBA is the metabolite of cyfluthrin and flumethrin. Due to the low DF of 4-F-3-PBA in both dog and cat urine samples, we assumed that the measured concentrations of *cis*- and *trans*-DCCA represent exposure to cypermethrin and permethrin. The DI of malathion was estimated from the urinary concentration of DMP, since its specific metabolite (malathion dicarboxylic acid) was not measured in this study, although DMP could arise from several parent OPs as well (Yusa et al., 2022). The F_{ue} values used in this study were based on those obtained from human or animal models (see Table S13 for details). Because no human or animal pharmacokinetic data were available for IMZ, the F_{ue} value of 0.127 was used (similar to that for IMI) (Harada et al., 2016). The estimated DI values of pesticides were then used for risk assessment through comparison with threshold/reference values. The suggested chronic reference dose (cRfD) values for pesticides, reported by the U.S. EPA, were used for comparison, except for NIT and IMZ, for which the acceptable daily intake (ADI) values proposed by the Chinese Ministry of Agriculture were used (as cRfD values are not available).

The estimated daily exposure doses to pesticides of dogs and cats are shown in Table 2 and Fig. 5. The DIs of the sum of all pesticides analyzed in dog and cat urine in this study were 0.43–87.3 (median: 9.55; GM: 9.14) and 0.13–1090 (median: 9.77; GM: 12.0) μg/kg BW/day, respectively. The median DIs for all pesticides in dogs were below the threshold/reference values by 3- (chlorpyrifos) to 54500-fold (nitenpyram) except for diazinon, for which the median intake was 1.9-fold higher than the cRfD. Furthermore, the DIs of chlorpyrifos, diazinon, and cypermethrin were above the respective cRfD values in

22, 76, and 5% of the dogs tested. The estimated DIs of pesticides in cats were below the reference values by 2- (chlorpyrifos) to 28900-fold (nitenpyram) except for diazinon, for which the median intake was 25-fold higher than the cRfD. Furthermore, the DIs of chlorpyrifos, parathion, diazinon, and imidacloprid were above the respective cRfD values in 33, 14, 100, and 29% of the cats tested (Table 2 and Fig. 5). Nevertheless, only 15 cat urine samples were included in the calculation of DIs, and therefore our results need to be interpreted with caution. Although the median DI values of all pesticides estimated for dogs and cats were similar to those reported for humans (DAPs and neonics: 3.72 μg/kg BW/day; OPs and PYRs: 0.44 μg/kg BW/day) (Li and Kannan 2018; Li and Kannan 2020), the DIs of diazinon in dogs (median: 0.37 μg/kg BW/day) and cats (5.03 μg/kg BW/day) were 22– and 296-fold higher than those estimated for the U.S. general population (0.017 μg/kg BW/day) (Li and Kannan 2018), indicating potential health risk from this OP insecticide.

3.6. Strengths and limitations

This study has several strengths, including: (1) comprehensive evaluation of the occurrence of 30 biomarkers of five classes of the most widely used pesticides (DAPs, neonics, OPs, PYRs, and PAs) in dog and cat urine and (2) assessment of daily intakes of and risks from pesticides, including some that are directly used on dogs and cats. However, there are also reasons to interpret our results with some caution. One limitation is that, although prior studies have reported the occurrence of 5-hydroxy-imidacloprid (5-OH-IMI) and olefin-imidacloprid (Of-IMI) at concentrations higher than IMI (Ospina et al., 2019; Song et al., 2020), we did not measure 5-OH-IMI and Of-IMI in this study. Given the temporal variabilities in pesticide levels in urine samples (Li et al., 2020), measurement from a single spot urine sample may not accurately represent integrated exposure over time. Besides, information regarding health and exposure history of the pets and pesticide usage in and around homes were not available in this study. Furthermore, the toxico-kinetic parameters and threshold values used in assessing exposure and health risks were derived from human and rodent models, and have not been validated for dogs and cats. In general, the susceptibility of dogs and cats to pesticides is not well understood, and further studies are needed in this regard. Finally, the number of samples analyzed in this study is small. Nevertheless, our study provides critical baseline information on pesticide exposure and its potential health risks in pet dogs and cats.

4. Conclusions

This is a comprehensive survey of the occurrence of, and exposure to, organophosphates, pyrethroids, and neonicotinoids in pet dogs and cats. Neonicotinoids were the predominant pesticides found in both dog and cat urine samples, followed by organophosphates and pyrethroids. The pesticide concentrations measured were generally higher in cat urine than in dog urine. Age- and sampling site-related differences in urinary concentrations were found for certain pesticides. The daily intakes of chlorpyrifos, cypermethrin, and diazinon in dogs and chlorpyrifos, parathion, imidacloprid, and diazinon in cats were above the chronic reference doses, suggestive of possible health risks from exposure to those pesticides. The use of pesticides in flea and tick control products in pets may contribute to elevated exposure, including exposures above the current reference values in certain cases. Although

veterinary products are used specifically on pet animals, those applications also lead to human exposures in the indoor environment. It should be noted that humans and pet animals are also frequently exposed to pesticides through diet, water, air, and dust. Further studies are needed to investigate the health effects in pet dogs and cats following long-term exposure to such pesticides.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

Data will be made available on request.

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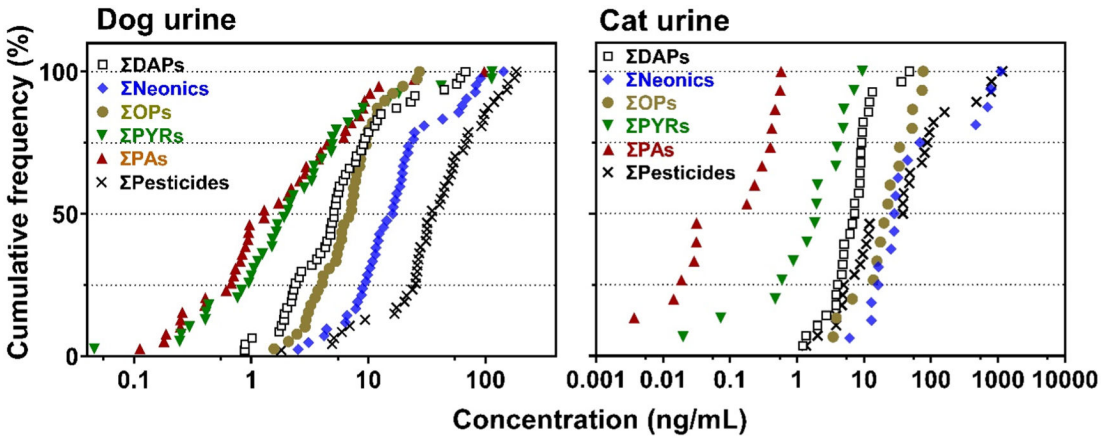

Fig. 1.

Frequency distributions of concentrations of pesticides (unadjusted) measured in dog and cat urine samples collected from New York State, USA. Pesticide concentrations were $\log_{10}$-transformed. ΣDAP, sum concentration of dialkylphosphates; ΣNeonics, sum concentration of neonicotinoid insecticides; ΣOPs, sum concentration of organophosphate insecticides; ΣPYRs, sum concentration of pyrethroid insecticides; ΣPA, sum concentration of phenoxy acid herbicides; ΣPesticides, sum concentration of all pesticides analyzed in this study.

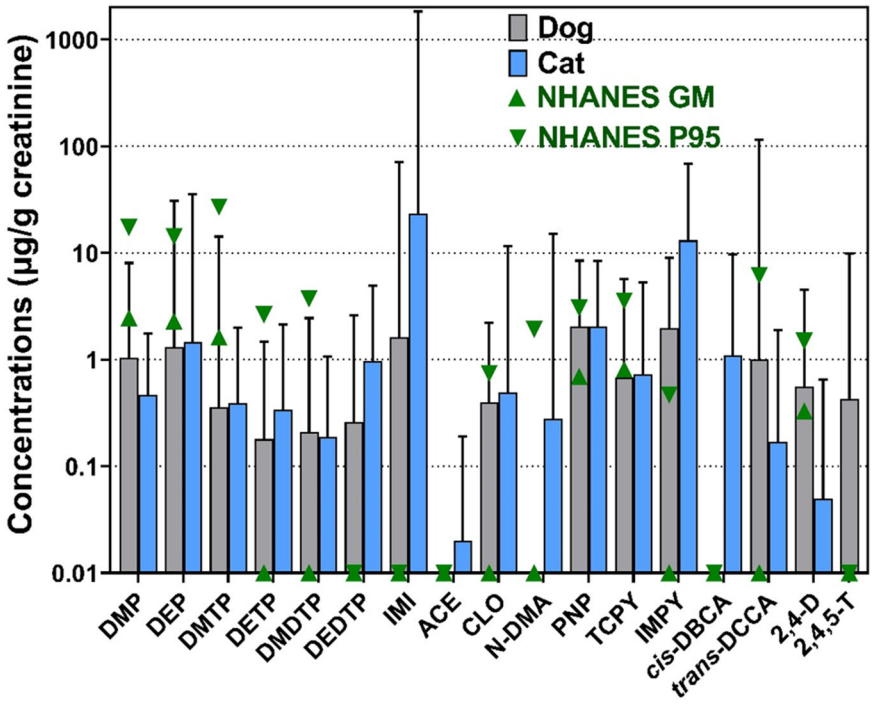

Fig. 2.

Comparison of urinary pesticide biomarker concentrations measured in pet dogs and cats in this study with NHANES data. The geometric mean (GM) and 95th percentile (P95) of the creatinine-adjusted concentrations in pet urine with DFs ≥ 80% are shown as bars and error bars. The corresponding most recent biomonitoring data (GM and P95) available in NHANES for the human general population are given for comparison (green triangles). NHANES values < LOD are plotted on the x-axis. NHANES data are from survey years 2009/2010 for TCPY, 2,4,5-T, and *cis*-DBCA; 2011/2012 for DMP, DEP, DMTP, DETP, DMDTP, and DEDTP; 2013/2014 for PNP, 2,4-D, *trans*-DCCA, and IMPY; and 2015/2016 for IMI, ACE, THI, CLO, and *N*-DMA.

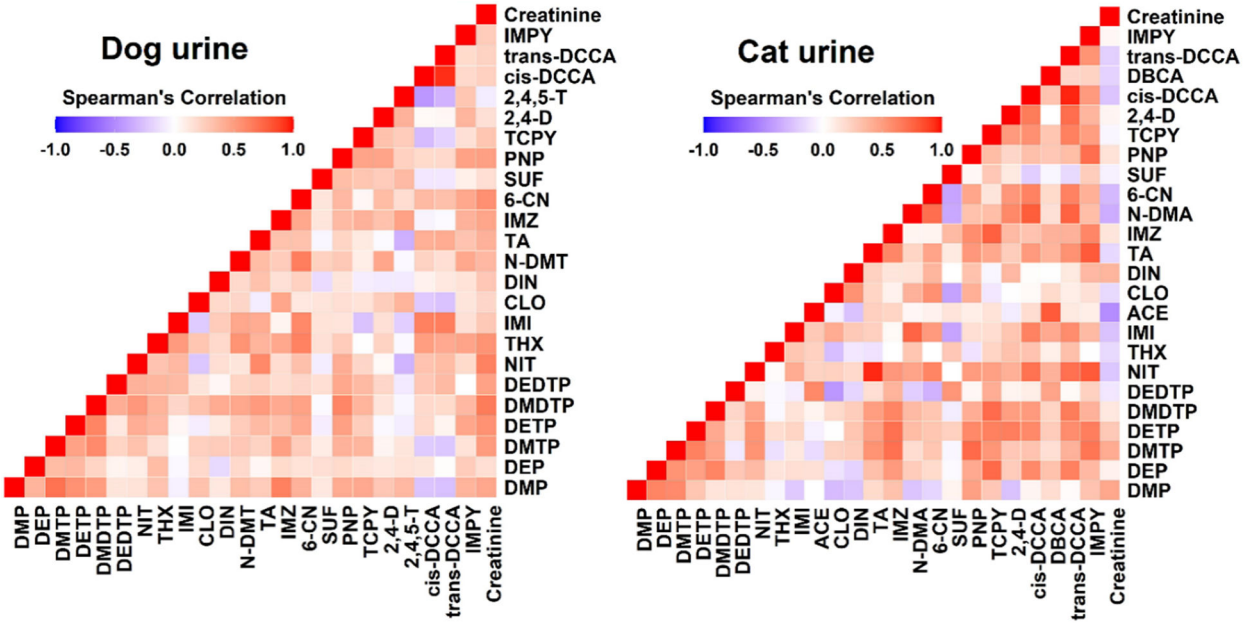

Fig. 3.

Heatmap of Spearman's rank correlation of pesticides measured in dog and cat urine. Only pesticides with DFs ≥ 80% were included in the analysis. Measures < LOD were replaced with LOD divided by square root of 2.

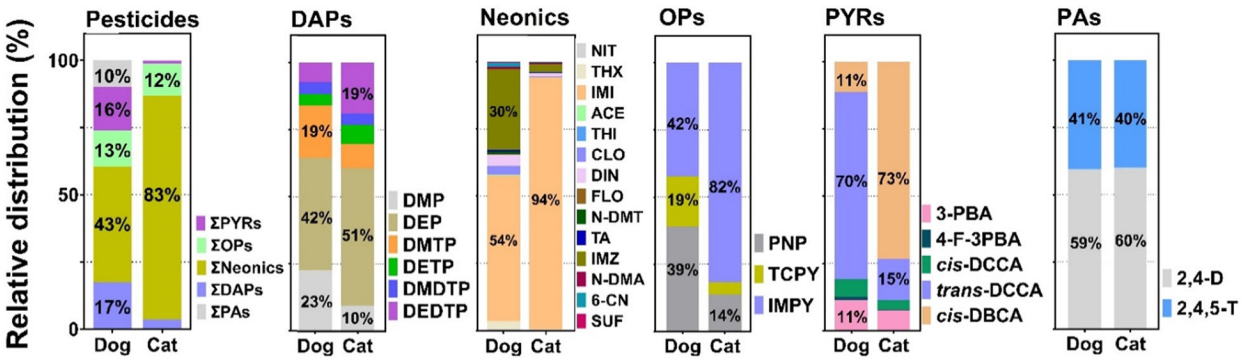

Fig. 4.

Relative distribution of all pesticides, dialkylphosphates (DAPs), neonicotinoids (Neonics), organophosphates (OPs), pyrethroids (PYRs), and phenoxy acids (PAs) in dog and cat urine collected from New York State, USA.

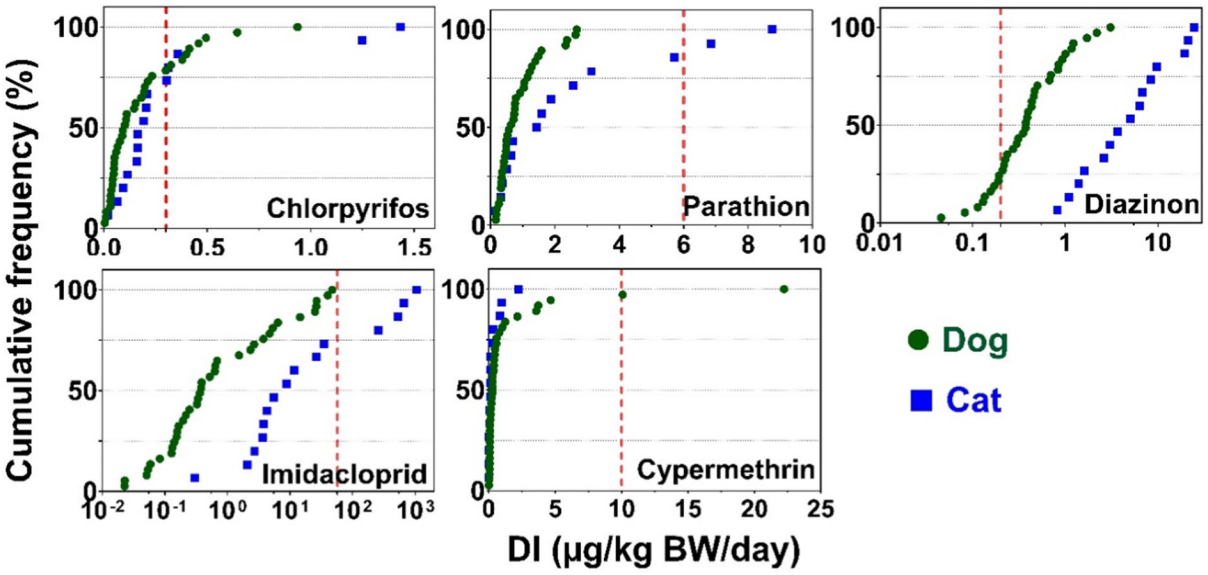

Fig. 5.

Frequency distribution of daily intake dose (μg/kg BW/day) of chlorpyrifos, parathion, diazinon, imidacloprid, and cypermethrin in dogs and cats estimated from measured urinary concentrations. The vertical lines indicate respective chronic reference dose (cRfD) values. The DI values of diazinon and imidacloprid were $\log_{10}$-transformed.

Table 1

Concentrations (ng/mL and μg/g creatinine in ***bold italic***) of dialkylphosphates (DAPs), neonicotinoid insecticides, specific metabolites of organophosphates (OPs), pyrethroid metabolites (PYRs), and phenoxy acid herbicides (PAs) measured in dog and cat urine collected from New York State, USA. The concentrations of pesticides in dog urine and cat urine were compared by Wilcoxon rank sum test.

	Dog urine						Cat urine					
	***N* (DF%)**	**Mean ± SD**	**GM**	**Min**	**Med**	**P95**	***N* (DF%)**	**Mean ± SD**	**GM**	**Min**	**Med**	**P95**
Creatinine (mg/dL)	47 (100)	157 ± 98	125	25	133	357	28 (100)	195 ± 134	154	25	183	549
DAPs												
DMP	47 (100)	2.47 ± 3.84	1.29	0.08	1.30	12.9	28 (100)	0.92 ± 0.71*	0.73	0.13	0.73	2.93
		1.75 ± 2.20	***1.04***	***0.06***	***0.97***	***8.04***		***0.66 ± 0.47****	***0.47***	***0.04***	***0.59***	***1.75***
DEP	47 (95.7)	4.57 ± 9.36	1.61	<LOD	1.21	34.4	28 (92.9)	4.91 ± 8.62	2.36	<LOD	2.39	34.5
		4.49 ± 9.88	***1.32***	***<LOD***	***0.90***	***30.6***		***4.32 ± 8.95***	***1.47***	***<LOD***	***1.64***	***35.6***
DMTP	47 (100)	*2.13 ± 5.98*	0.45	0.03	0.36	17.0	28 (100)	0.87 ± 0.77	0.59	0.05	0.69	2.86
		1.48 ± 4.19	***0.36***	***0.02***	***0.34***	***14.2***		***0.59 ± 0.53***	***0.39***	***0.05***	***0.53***	***2.00***
DETP	47 (87.2)	*0.47 ± 0.66*	0.25	<LOD	0.29	1.20	28 (100)	0.68 ± 0.63*	0.52	0.07	0.54	2.50
		0.33 ± 0.51	***0.18***	***<LOD***	***0.17***	***1.48***		***0.53 ± 0.56****	***0.34***	*0.03*	*0.37*	***2.12***
DMDTP	47 (100)	*0.48 ± 0.90*	0.26	0.02	0.26	1.97	28 (96.4)	0.41 ± 0.31	0.29	<LOD	0.35	1.16
		0.37 ± 0.69	***0.21***	***0.04***	***0.20***	***2.44***		***0.33 ± 0.29***	***0.19***	***<LOD***	***0.25***	***1.07***
DEDTP	47 (87.2)	*0.81 ± 1.42*	0.35	<LOD	0.26	3.35	28 (100)	1.82 ± 1.19**	1.49	0.38	1.73	4.88
		0.52 ± 0.69	***0.26***	***<LOD***	***0.18***	***2.60***		***1.50 ± 1.38****	***0.97***	***0.08***	***1.07***	***4.89***
ΣDAPs	47 (100)	*10.6 ± 15.6*	5.62	0.88	5.08	59.6	28 (100)	9.25 ± 9.88	6.61	1.22	7.23	42.5
		8.65 ± 14.8	***4.50***	***0.67***	***3.80***	***39.5***		***7.61 ± 10.3***	***4.30***	***0.53***	***5.15***	***42.3***
Neonics												
NIT	42 (97.6)	0.14 ± 0.12	0.10	<LOD	0.10	0.42	16 (93.8)	0.23 ± 0.30	0.11	<LOD	0.08	1.12
		0.10 ± 0.09	***0.08***	***<LOD***	***0.07***	***0.31***		***0.25 ± 0.43***	***0.09***	***<LOD***	***0.06***	***1.59***
THX	42 (97.6)	0.78 ± 0.79	0.47	<LOD	0.52	2.92	16 (100)	0.76 ± 0.48	0.62	0.23	0.74	1.75
		0.56 ± 0.50	***0.38***	***<LOD***	***0.33***	***1.75***		***0.68 ± 0.58***	***0.48***	***0.09***	***0.46***	***1.97***
IMI	42 (95.2)	14.7 ± 27.0	2.15	<LOD	1.06	76.3	16 (93.8)	211 ± 357**	28.9	<LOD	15.1	1085
		11.1 ± 21.4	***1.63***	***<LOD***	***0.82***	***71.1***		***309 ± 609***	***23.5***	***<LOD***	***11.9***	***1826***
ACE	42 (42.9)	0.04 ± 0.06	0.02	<LOD	<LOD	0.26	16 (87.5)	0.04 ± 0.04	0.02	<LOD	0.02	0.13

	Dog urine						Cat urine					
	N **(DF%)**	**Mean ± SD**	**GM**	**Min**	**Med**	**P95**	***N*** **(DF%)**	**Mean ± SD**	**GM**	**Min**	**Med**	**P95**
		0.03 ± 0.03	***0.01***	***<LOD***	***<LOD***	***0.12***		***0.04 ± 0.05***	***0.02***	***<LOD***	***0.01***	***0.19***
THI	42 (21.4)	0.01 ± 0.01	0.01	<LOD	<LOD	0.05	16 (31.3)	0.02 ± 0.02	0.01	<LOD	<LOD	0.04
		0.01 ± 0.01	***0.004***	***<LOD***	***<LOD***	***0.02***		***0.02 ± 0.02***	***0.01***	***<LOD***	***<LOD***	***0.04***
CLO	42 (97.6)	0.90 ± 1.07	0.50	<LOD	0.53	4.13	16 (100)	0.89 ± 0.75	0.63	0.08	0.60	2.90
		0.70 ± 0.71	***0.40***	***<LOD***	***0.55***	***2.22***		***1.25 ± 2.79***	***0.49***	***0.04***	***0.51***	***11.6***
DIN	42 (97.6)	1.19 ± 1.31	0.81	<LOD	0.84	5.76	16 (100)	2.37 ± 1.64**	1.85	0.20	1.74	6.60
		1.30 ± 3.08	***0.62***	***<LOD***	***0.54***	***4.04***		***1.87 ± 1.42****	***1.44***	***0.27***	***1.51***	***6.02***
FLO	42 (35.7)	0.04 ± 0.04	0.03	<LOD	<LOD	0.18	16 (37.5)	0.03 ± 0.02	0.02	<LOD	<LOD	0.07
		0.03 ± 0.03	***0.02***	***<LOD***	***<LOD***	***0.13***		***0.02 ± 0.01***	***0.01***	***<LOD***	***<LOD***	***0.03***
N-DMT	42 (92.9)	0.27 ± 0.39	0.14	<LOD	0.11	1.08	16 (75)	0.41 ± 0.47#	0.27	<LOD	0.18	1.81
		0.22 ± 0.40	***0.11***	***<LOD***	***0.07***	***1.27***		***0.41 ± 0.55****	***0.23***	***<LOD***	***0.13***	***2.03***
TA	42 (97.6)	0.13 ± 0.18	0.07	<LOD	0.07	0.62	16 (93.8)	0.50 ± 0.89**	0.21	<LOD	0.19	3.57
		0.12 ± 0.22	***0.05***	***<LOD***	***0.05***	***0.82***		***0.58 ± 1.29****	***0.15***	***<LOD***	***0.10***	***5.09***
IMZ	42 (100)	8.19 ± 5.38	6.25	0.26	6.81	18.6	16 (100)	6.78 ± 4.34	5.50	1.81	5.52	15.1
		6.60 ± 5.39	***4.94***	***0.66***	***4.83***	***19.6***		***6.00 ± 5.63***	***4.28***	***1.36***	***3.57***	***19.9***
N-DMA	42 (50)	0.19 ± 0.34	0.05	<LOD	<LOD	1.38	16 (93.8)	1.35 ± 2.01**	0.34	<LOD	0.26	6.40
		0.14 ± 0.28	***0.03***	***<LOD***	***<LOD***	***1.08***		***1.94 ± 3.98****	***0.28***	***<LOD***	***0.20***	***15.0***
6-CN	42 (97.6)	0.42 ± 0.64	0.21	<LOD	0.16	2.13	16 (93.8)	0.53 ± 0.51*	0.36	<LOD	0.34	1.75
		0.29 ± 0.40	***0.16***	***<LOD***	***0.15***	***1.04***		***0.60 ± 0.77****	***0.29***	***<LOD***	***0.26***	***2.54***
SUF	42 (83.3)	0.10 ± 0.18	0.06	<LOD	0.05	0.43	16 (100)	0.07 ± 0.08	0.03	0.002	0.07	0.30
		0.08 ± 0.13	***0.05***	***<LOD***	***0.04***	***0.33***		***0.05 ± 0.05***	***0.02***	***0.001***	***0.03***	***0.17***
ΣNeonics	42 (100)	26.2 ± 29.5	16.7	2.52	16.3	90.0	16 (100)	211 ± 350**	53.5	6.09	29.0	1099
		20.6 ± 23.3	***13.2***	***3.04***	***10.7***	***75.6***		***303 ± 601****	***41.7***	***5.10***	***25.5***	***1870***
OPs												
PNP	39 (100)	3.17 ± 2.40	2.48	0.64	2.55	10.3	15 (93.3)	4.16 ± 3.60	2.75	<LOD	2.20	12.2
		2.73 ± 2.29	***2.03***	***0.40***	***1.98***	***8.48***		***3.15 ± 2.53***	***2.04***	***<LOD***	***2.89***	***8.40***
TCPY	39 (100)	1.52 ± 1.71	0.83	0.04	0.92	5.80	15 (100)	1.42 ± 1.51	0.93	0.06	1.08	5.85
		1.33 ± 1.8	***0.68***	***0.08***	***0.62***	***5.71***		***1.30 ± 1.55***	***0.73***	***0.11***	***0.65***	***5.27***

	Dog urine						Cat urine					
	***N* (DF%)**	**Mean ± SD**	**GM**	**Min**	**Med**	**P95**	***N* (DF%)**	**Mean ± SD**	**GM**	**Min**	**Med**	**P95**
IMPY	39 (100)	3.44 ± 3.14	2.42	0.31	2.55	10.9	15 (100)	24.8 ± 20.7 **	16.7	2.83	16.6	66.0
		2.95* ± *2.59	***1.97***	***0.27***	***2.30***	***8.96***		***21.7* ± *22.5*** **	***13.1***	***3.18***	***7.97***	***68.5***
ΣOPs	39 (100)	8.13 ± 5.88	6.58	1.56	7.18	26.1	15 (100)	30.1 ± 23.7 **	21.0	3.47	22.8	76.4
		7.01* ± *5.52	***5.37***	***0.86***	***5.53***	***20.0***		***25.9* ± *25.1*** **	***16.5***	***4.32***	***11.4***	***75.8***
PYRs												
3-PBA	39 (23.1)	1.32 ± 2.00	0.39	<LOD	<LOD	5.34	15 (26.7)	0.28 ± 0.12	0.26	<LOD	<LOD	0.42
		2.20* ± *3.83	***0.36***	***<LOD***	***<LOD***	***10.9***		***0.16* ± *0.04***	***0.15***	***<LOD***	***<LOD***	***0.22***
4-F-3PBA	39 (48.7)	0.14 ± 0.19	0.05	<LOD	<LOD	0.62	15 (20)	0.01 ± 0.01	0.01	<LOD	<LOD	0.03
		0.08* ± *0.11	***0.03***	***<LOD***	***<LOD***	***0.36***		***0.01* ± *0.01*** #	***0.01***	***<LOD***	***<LOD***	***0.02***
cis-DCCA	39 (100)	0.76 ± 2.24	0.13	0.01	0.09	9.10	15 (80)	0.13 ± 0.15	0.07	<LOD	0.04	0.49
		1.23* ± *4.31	***0.10***	***0.01***	***0.07***	***12.8***		***0.11* ± *0.11***	***0.06***	***<LOD***	***0.07***	***0.35***
trans-DCCA	39 (100)	8.05 ± 22.4	1.24	0.04	1.09	97.0	15 (100)	0.56 ± 0.78 **	0.22	0.01	0.22	2.78
		13.4* ± *46.7	***1.01***	***0.10***	***0.89***	***114***		***0.46* ± *0.53***	***0.17***	***0.003***	***0.32***	***1.88***
cis-DBCA	39 (56.4)	1.30 ± 1.39	0.57	<LOD	0.06	4.65	15 (80)	2.70 ± 2.99	1.33	<LOD	1.02	9.46
		1.49* ± *2.34	***0.40***	***<LOD***	***0.04***	***8.82***		***2.40* ± *2.82***	***1.09***	***<LOD***	***1.09***	***9.72***
ΣPYRs	39 (100)	9.91 ± 25.5	2.30	0.05	2.06	113	15 (100)	2.90 ± 2.77	1.35	0.02	1.95	9.47
		16.0* ± *52.7	***1.88***	***0.18***	***1.56***	***133***		***2.51* ± *2.79***	***1.06***	***0.03***	***1.89***	***10.3***
PAs												
2,4-D	39 (100)	3.63 ± 15.3	0.69	0.01	0.80	8.35	15 (93.3)	0.18 ± 0.20 **	0.06	<LOD	0.03	0.58
		2.24* ± *7.47	***0.56***	***0.005***	***0.53***	***4.51***		***0.15* ± *0.22*** **	***0.05***	***<LOD***	***0.06***	***0.65***
2,4,5-T	39 (92.3)	2.48 ± 3.69	0.54	<LOD	0.37	10.2	15 (46.7)	0.12 ± 0.10 *	0.07	<LOD	<LOD	0.27
		2.30* ± *3.15	***0.43***	***<LOD***	***0.39***	***9.84***		***0.07* ± *0.06*** *	***0.05***	***<LOD***	***<LOD***	***0.17***
ΣPAs	39 (100)	5.92 ± 15.8	1.71	0.11	1.29	24.7	15 (100)	0.23 ± 0.22 **	0.10	0.004	0.21	0.58
		4.37* ± *8.18	***1.40***	***0.07***	***1.17***	***16.3***		***0.19* ± *0.25*** **	***0.07***	***0.005***	***0.10***	***0.80***
ΣPesticides	47 (100)	53.8 ± 46.4	36.2	1.81	35.2	169	28 (100)	148 ± 293	29.7	1.37	38.1	1002
		49.7* ± *65.9	***28.9***	***1.54***	***31.4***	***188***		***196* ± *486***	***19.3***	***0.53***	***17.2***	***1810***

DF, detection frequency; GM, geometric mean; Med, median; Min, minimum; P95, 95th percentile.

** $p < 0.01$

Environ Int. Author manuscript; available in PMC 2022 November 01.

* $p < 0.05$

# $p < 0.1$.

Table 2

Estimated daily intakes (DI) of pesticides in dogs and cats (μg/kg BW/day) calculated from urinary concentrations of pesticides and their metabolites. The DI values of both permethrin and cypermethrin were estimated from the sum of *cis*- and *trans*-DCCA, and therefore, our calculations represent maximum exposure for permethrin and cypermethrin.

Parent pesticide	Reference value (μg/kg BW/day)[a]	Metabolite[b]	Dog (μg/kg BW/day)			Cat (μg/kg BW/day)		
			GM	Med	Range	GM	Med	Range
Organophosphate insecticides								
Malathion	70	DMP	1.54	1.67	0.09–18.8	1.58	1.67	0.13–5.82
Chlorpyrifos	0.3	TCPY	0.10	0.10	0.005–0.94	0.20	0.19	0.02–1.44
Parathion	6	PNP	0.66	0.64	0.17–2.68	1.31	1.51	NC–8.76
Diazinon	0.2	IMPY	0.38	0.37	0.05–3.08	4.67	5.03	0.83–24.9
Neonicotinoid insecticides								
Imidacloprid	57	IMI	0.70	0.38	NC–47.0	17.62	8.75	NC–1055
Acetamiprid	71	ACE	NC	NC	NC	0.06	0.08	NC–0.63
Nitenpyram	530	NIT	0.01	0.01	NC–0.15	0.02	0.02	NC–0.17
Thiamethoxam	6	THX	0.08	0.10	NC–0.52	0.18	0.19	0.03–0.57
Clothianidin	9.8	CLO	0.04	0.04	NC–0.35	0.08	0.09	0.01–0.39
Thiacloprid	4	6-CN	0.11	0.09	NC–3.58	0.40	0.40	NC–2.89
Dinotefuran	20	DIN	0.04	0.04	NC–0.37	0.16	0.14	0.03–0.68
Imidaclothiz	25	IMZ	2.37	2.32	0.43–14.9	3.45	2.40	1.17–14.7
Sulfoxaflor	50	SUF	0.003	0.003	NC–0.06	0.003	0.01	NC–0.02
Pyrethroid insecticides								
Permethrin[d]	50	DCCA[c]	0.30	0.28	0.01–20.9	0.11	0.11	0.01–2.10
Cypermethrin[d]	10	DCCA[c]	0.32	0.30	0.01–22.3	0.12	0.12	0.01–2.23
Deltamethrin	10	*cis*-DBCA	NC	NC	NC	0.39	0.29	NC–4.26
Phenoxyacid herbicides								
2,4-D	10	2,4-D	0.04	0.04	NC–6.33	0.01	0.003	NC–0.06
2,4,5-T	10	2,4,5-T	0.03	0.02	NC–0.82	NC	NC	NC
AT	—	—	9.14	9.55	0.43–87.3	12.0	9.77	0.13–1090

BW, body weight; GM, geometric mean; Med, median; NC, not calculated.

[a] Chronic reference dose (cRfD) for humans provided by the U.S. EPA were used for all pesticides except for nitenpyram and imidaclothiz, for which the acceptable daily intake (ADI) values for humans proposed by the Chinese Ministry of Agriculture (NY/T 2874–2015) were used.

[b] The metabolites shown here indicate the metabolites selected for the calculation of DI value.

[c] Indicates the sum of *cis*- and *trans*-DCCA.

[d] Indicates the highest exposure estimate.