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

Zhong-Min Li\textsuperscript{a,b}, Morgan Robinson\textsuperscript{a,b}, Kurunthachalam Kannan\textsuperscript{a,b,}\textsuperscript{*}

\textsuperscript{a}Department of Pediatrics, New York University Grossman School of Medicine, New York, NY 10016, United States

\textsuperscript{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.

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

\textsuperscript{*}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-pyrimidinol (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, 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, neonicos, 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 neonicos, 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-dimethylphosphate, DEP-diethylphosphate, DMTP-dimethylthiophosphate, DETP-diethylthiophosphate, DMDTP-dimethylthiodiphosphate, and DEDTP-diethylthiodiphosphate) 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), flocambicide (FLO), N-desmethyl thiamethoxam (N-DMT), thiacloprid-amide (TA), imidaclothiz (IMZ), and N-desmethyl acetamiprid (N-DMTA) 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 neonic 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 neonic 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, neonic (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, neonic 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):

\[
DI = C \times \frac{V}{BW} \times \frac{MW_1}{MW_2} \times \frac{1}{F_{ue}}
\]

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.

**Acknowledgements**

The research reported here was supported, in part, by the US National Institute of Environmental Health Sciences (NIEHS) under award number U2CES026542 (KK). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIEHS.

**Data availability**

Data will be made available on request.

**References**

Ali N, Malik RN, Mehdi T, Eqani SAMAS, Javeed A, Neels H, Covaci A. 2013. Organohalogenated contaminants (OHCs) in the serum and hair of pet cats and dogs: biosentinels of indoor pollution. Sci. Total Environ 449, 29–36. [PubMed: 23403100]

Alvavanja MCR, 2009. Introduction: Pesticides use and exposure extensive worldwide. Rev. Environ. Health 24, 303–309. [PubMed: 20384038]

Aylward LL, Morgan MK, Arbuckle TE, Barr DB, Burns CJ, Alexander BH, Hays SM, 2010. Biomonitoring data for 2,4-dichlorophenoxyacetic acid in the United States and Canada: interpretation in a public health risk assessment context using biomonitoring equivalents. Environ. Health Perspect 118 (2), 177–181. [PubMed: 20123603]

Bertone ER, Snyder LA, Moore AS, 2003. Environmental and lifestyle risk factors for oral squamous cell carcinoma in domestic cats. J. Vet. Intern. Med 17 (4), 557–562. [PubMed: 12892308]

Brits M, Gorst-Allman P, Rohwer ER, De Vos J, de Boer J, Weiss JM, 2018. Comprehensive two-dimensional gas chromatography coupled to high resolution time-of-flight mass spectrometry for screening of organohalogenated compounds in cat hair. J. Chromatogr. A 1536, 151–162. [PubMed: 28866251]

Brits M, Brandsma SH, Rohwer ER, De Vos J, Weiss JM, de Boer J, 2019. Brominated and organophosphorus flame retardants in South African indoor dust and cat hair. Environ. Pollut 253, 120–129. [PubMed: 31302398]

Burns CJ, Swaen GMH, 2012. Review of 2,4-dichlorophenoxyacetic acid (2,4-D) biomonitoring and epidemiology. Crit. Rev. Toxicol 42 (9), 768–786. [PubMed: 22876750]

Calaf GM, 2021. Role of organophosphorous pesticides and acetylcholine in breast carcinogenesis. Semin. Cancer Biol 76, 206–217. [PubMed: 33766648]

CDC, 2018. Fourth national report on human exposure to environmental chemicals updated tables, March 2018, Volume One, NCEH, CDC. Available at: https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Mar2018.pdf. (Accessed May 2022).

CDC, 2021. Fourth national report on human exposure to environmental chemicals, updated tables, volume two. March 2021, DLS, NCEH, CDC. Available at: https://www.cdc.gov/exposurereport/ (Accessed March 2022).

Chinthakindi S, Kannan K, 2022. Urinary and fecal excretion of aromatic amines in pet dogs and cats from the United States. Environ. Int 163.

*Environ Int.* Author manuscript; available in PMC 2022 November 01.
Dymond NL, Swift IM. 2008. Permethrin toxicity in cats: a retrospective study of 20 cases. Aust. Vet. J 86 (6), 219–223. [PubMed: 18498556]

EPA, 2020. Imidacloprid proposed interim registration review decision case number 7605. Available at: https://www.epa.gov/sites/default/files/2020-01/documents/imidacloprid_pid_signed_1.22.2020.pdf. Accessed March 2022.

EPA, 2017. Pesticide industry sales and usage 2008–2012 market estimates; U.S. Environmental Protection Agency; Washington, DC, USA, 2017. Available at: https://www.epa.gov/sites/default/files/2017-01/documents/pesticides-industry-sales-usage-2016_0.pdf (Accessed May 2022).

FAO, 2019. Database collection of the food and agriculture organization of the United Nations. https://www.fao.org/faostat/en/#data/RP/visualize. Accessed May 2022.

Forster GM, Brown DG, Dooley GP, Page RL, Ryan EP. 2014. Multiresidue analysis of pesticides in urine of healthy adult companion dogs. Environ. Sci. Technol 48, 14677–14685. [PubMed: 25365583]

Fritschi L, Benke G, Hughes AM, Kricker A, Turner J, Vajdic CM, Grulich A, Milliken S, Kaldor J, Armstrong BK. 2005. Occupational exposure to pesticides and risk of non-Hodgkin’s lymphoma. Am. J. Epidemiol 162, 849–857. [PubMed: 16177143]

Gari M, Gonzalez-Quinteiro Y, Bravo N, Grimalt JO. 2018. Analysis of metabolites of organophosphate and pyrethroid pesticides in human urine from urban and agricultural populations (Catalonia and Galicia). Sci. Total Environ 622–623, 526–533.

Gautam S, Sood NK, Gupta K, Joshi C, Gill KK, Kaur R, Chauhan I. 2020. Bioaccumulation of pesticide contaminants in tissue matrices of dogs suffering from malignant canine mammary tumors in Punjab, India. Heliyon 6, e05274. [PubMed: 33163644]

Glickman LT, Raghavan M, Knapp DW, Bonney PL, Dawson MH. 2004. Herbicide exposure and the risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. J. Am. Vet. Med. Assoc 224, 1290–1297. [PubMed: 15112777]

Gomez SA, Picado A. 2017. Systemic insecticides used in dogs: potential candidates for phlebotomine vector control? Trop. Med. Int. Health 22, 755–764. [PubMed: 28326655]

Gonzalez-Alzaga B, Lacasana M, Aguilar-Garduno C, Rodriguez-Barranco M, Ballester F, Rebagliato M, Hernandez AF. 2014. A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure. Toxicol. Lett 230, 104–121. [PubMed: 24291036]

Gonzalez-Gomez X, Cambeiro-Perez N, Martinez-Carballo E, Simal-Gandara J. 2018. Screening of organic pollutants in pet hair samples and the significance of environmental factors. Sci. Total Environ 625, 311–319. [PubMed: 29289779]

Guo Y, Alomirah H, Cho H-S, Minh TB, Mohd MA, Nakata H, Kannan K. 2011. Occurrence of phthalate metabolites in human urine from several Asian countries. Environ. Sci. Technol 45, 3138–3144. [PubMed: 21395215]

Guyton KZ, Loomis D, Grosse Y, El Ghissassi F, Benbrahim-Tallaa L, Guha N, Scoccianti C, Mattock H, Straif K. 2015. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncol. 16, 490–491. [PubMed: 25801782]

Hallmann CA, Poppen RP, van Turnhout CA, de Kroon H, Jongejans E. 2014. Declines in insectivorous birds are associated with high neonicotinoid concentrations. Nature 511, 341–343. [PubMed: 25030173]

Harada KH, 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. [PubMed: 26731104]

Hoffman JM, Creevy KE, Franks A, O’Neill DG, Promislow DEL. 2018. The companion dog as a model for human aging and mortality. Aging Cell 17, e12737. [PubMed: 29457329]

IARC, 2015. IARC monographs volume 112: evaluation of five organophosphate insecticides and herbicides. 2015. Available at: https://www.iarc.who.int/wp-content/uploads/2018/07/ MonographVolume112-1.pdf (Accessed May 2022).

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. [PubMed: 20565065]

Environ Int. Author manuscript; available in PMC 2022 November 01.
Karthikraj R, Bollapragada R, Kannan K, 2018a. Melamine and its derivatives in dog and cat urine: An exposure assessment study. Environ. Pollut 238, 248–254. [PubMed: 29567446]

Karthikraj R, Borkar S, Lee S, Kannan K, 2018b. Parabens and their metabolites in pet food and urine from New York State, United States. Environ. Sci. Technol 52, 3727–3737. [PubMed: 29512377]

Karthikraj R, Kannan K, 2019. Widespread occurrence of glyphosate in urine from pet dogs and cats in New York State, USA. Sci. Total Environ 659, 790–795. [PubMed: 31096409]

Kassotis CD, Herkert NJ, Hammel SC, Hoffman K, Xia Q, Kullman SW, Sosa JA, Stapleton HM, 2020. Thyroid receptor antagonism of chemicals extracted from personal silicone wristbands within a papillary thyroid cancer pilot study. Environ. Sci. Technol 54, 15296–15312. [PubMed: 33185092]

Kim KH, Kabir E, Jahan SA, 2017. Exposure to pesticides and the associated human health effects. Sci. Total Environ 575, 525–535. [PubMed: 27614863]

Knapp DW, Peer WA, Conteh A, Diggs AR, Cooper BR, Glickman NW, Bonney PL, Stewart JC, Glickman LT, Murphy AS, 2013. Detection of herbicides in the urine of pet dogs following home lawn chemical application. Sci. Total Environ 456–457, 34–41.

Koutros S, Silverman DT, Alavanja MC, Andreotti G, Lerro CC, Heltshe S, Lynch CF, Sandler DP, Blair A, Beane Freeman LE, 2016. Occupational exposure to pesticides and bladder cancer risk. Int. J. Epidemiol 45, 792–805. [PubMed: 26411407]

Li AJ, Kannan K, 2018. Urinary concentrations and profiles of organophosphate and pyrethroid pesticide metabolites and phenoxyacid herbicides in populations in eight countries. Environ. Int 121, 1148–1154. [PubMed: 30808487]

Li AJ, Kannan K, 2020. Profiles of urinary neonicotinoids and dialkylphosphates in populations in nine countries. Environ. Int 145, 106120. [PubMed: 32949879]

Li AJ, Martinez-Moral MP, Kannan K, 2020. Variability in urinary neonicotinoid concentrations in single-spot and first-morning void and its association with oxidative stress markers. Environ. Int 135, 105415. [PubMed: 31869729]

Md Meftaul I, Venkateswarlu K, Dharmarajan R, Annamalai P, Megharaj M, 2020. Pesticides in the urban environment: A potential threat that knocks at the door. Sci. Total Environ 711, 134612. [PubMed: 31810707]

Mizukawa H, Nomiyama K, Nakatsu S, Iwata H, Yoo J, Kubota A, Yamamoto M, Ishizuka M, Ikenaka Y, Nakayama SM, Kunisue T, Tanabe S, 2016. Organohalogen compounds in pet dog and cat: Do pets biotransform natural brominated products in food to harmful hydroxlated substances? Environ. Sci. Technol 50, 444–452. [PubMed: 26630569]

Morrissey CA, Mineau P, Devries JH, Sanchez-Bayo F, Liess M, Cavallaro MC, Liber K, 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: a review. Environ. Int 74, 291–303. [PubMed: 25454246]

IARC, 1987. Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. IARC Monogr Eval Carcinog Risks Hum Suppl 7, 1987.

Available at: https://publications.iarc.fr/Book-And-Report-Series/Iarc-Monographs-Supplements/Overall-Evaluations-Of-Carcinogenicity-An-Updating-Of-IARC-Monographs-Volumes-1-42-1987 (Accessed May 2022).

Ospina M, Wong LY, Baker SE, Serafim AB, Morales-Agudelo P, Calafat AM, 2019. Exposure to neonicotinoid insecticides in the U.S. general population: Data from the 2015-2016 national health and nutrition examination survey. Environ. Res 176,108555. [PubMed: 31288196]

Poutasse CM, Herbstman JB, Peterson ME, Gordon J, Soboroff PH, Holmes D, Gonzalez D, Tidwell LG, Anderson KA, 2019. Silicone pet tags associate tris(1,3-dichloro-2-isopropyl) phosphate exposures with feline hyperthyroidism. Environ. Sci. Technol 53, 9203–9213. [PubMed: 31290326]

Reynolds PM, Reif JS, Ramsdell HS, Tessari JD, 1994. Canine exposure to herbicide-treated lawns and urinary excretion of 2,4-dichlorophenoxyacetic acid. Cancer Epidemiol. Biomarkers Prev 3, 233–237. [PubMed: 8019373]

Rundlof M, Andersson GK, Bommarco R, Fries I, Hederstrom V, Herbertsson L, Jonsson O, Klatt BK, Pedersen TR, Yourstone J, Smith HG, 2015. Seed coating with a neonicotinoid insecticide negatively affects wild bees. Nature 521, 77–80. [PubMed: 25901681]
Saillenfait AM, Ndiaye D, Sabate JP, 2015. Pyrethroids: exposure and health effects—an update. Int. J. Hyg. Environ. Health 218, 281–292. [PubMed: 25648288]

Song S, Zhang T, Huang Y, Zhang B, Guo Y, He Y, Huang X, Bai X, Kannan K, 2020. Urinary metabolites of neonicotinoid insecticides: Levels and recommendations for future biomonitoring studies in China. Environ. Sci. Technol 54, 8210–8220. [PubMed: 32388996]

Sparks TC, Crossthwaite AJ, Nauen R, Banha S, Cordova D, Earley F, Ebbinghaus-Kintscher U, Fujioka S, Hirao A, Karmon D, Kennedy R, Nakao T, Popham HJR, Salgado V, Watson GB, Wedel BJ, Wessels FJ, 2020. Insecticides, biologics and nematicides: Updates to IRAC’s mode of action classification - a tool for resistance management. Pestic. Biochem. Physiol 167, 104587. [PubMed: 32527435]

Stanneck D, Krudedewagen EM, Fourie JJ, Horak IG, Davis W, Krieger KJ, 2012. Efficacy of an imidacloprid/flumethrin collar against fleas, ticks, mites and lice on dogs. Parasites Vectors 5, 102–119. [PubMed: 22647530]

Takashima-Uebelhoer BB, Barber LG, Zagarins SE, Procter-Gray E, Gollenberg AL, Moore AS, Bertone-Johnson ER, 2012. Household chemical exposures and the risk of canine malignant lymphoma, a model for human non-Hodgkin’s lymphoma. Environ. Res 112, 171–176. [PubMed: 22222006]

Ueyama J, Harada KH, Koizumi A, Sugitara 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. [PubMed: 26556224]

van Beusekom CD, Fink-Gremmels J, Schrickx JA, 2014. Comparing the glucuronidation capacity of the feline liver with substrate-specific glucuronidation in dogs. J. Vet. Pharmacol. Ther 37, 18–24. [PubMed: 2388985]

Vo DT, Hsu WH, Abu-Basha EA, Martin RJ, 2010. Insect nicotinic acetylcholine receptor agonists as flea adulticides in small animals. J. Vet. Pharmacol. Ther 33, 315–322. [PubMed: 20646191]

Wise CF, Hammel SC, Herkert N, Ma J, Motsinger-Reif A, Stapleton HM, Breen M, 2020. Comparative exposure assessment using silicone passive samplers indicates that domestic dogs are sentinels to support human health research. Environ. Sci. Technol 54, 7409–7419. [PubMed: 32401030]

Wise CF, Hammel SC, Herkert NJ, Ospina M, Calafat AM, Breen M, Stapleton HM, 2022. Comparative assessment of pesticide exposures in domestic dogs and their owners using silicone passive samplers and biomonitoring. Environ. Sci. Technol 56, 1149–1161. [PubMed: 34964617]

Yusa V, S FF, Dualde P, Lopez A, Lacomba I, Coscolla C, 2022. Exposure to non-persistent pesticides in the Spanish population using biomonitoring: A review. Environ. Res 205:112437. [PubMed: 34838757]

Zhang J, Wang L, Kannan K, 2019. Polyethylene terephthalate and polycarbonate microplastics in pet food and feces from the United States. Environ. Sci. Technol 53, 12035–12042. [PubMed: 31525038]
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. $\Sigma$DAP, sum concentration of dialkylphosphates; $\Sigma$Neonics, sum concentration of neonicotinoid insecticides; $\Sigma$OPs, sum concentration of organophosphate insecticides; $\Sigma$PYRs, sum concentration of pyrethroid insecticides; $\Sigma$PA, sum concentration of phenoxy acid herbicides; $\Sigma$Pesticides, 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.
Concentrations (ng/mL and μg/g creatinine in *bold italic*) of dialkylyphosphates (DAPs), neonicotinoid insecticides, specific metabolites of organophosphates (OPs), pyrethroid metabolites (PYRs), and phenoxyl 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    | Min       | Med          | N (DF%)   | Mean ± SD    | Min       | Med          |
| Creatinine (mg/dL) | 47 (100) | 157 ± 98    | 125       | 25          | 357       | 195 ± 134    | 154       | 25          |
| **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        | 3.89      | 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        | 0.26      | 16 (87.5)    | 0.04 ± 0.04| 0.02       | <LOD       | 0.02       | 0.13       |
|           | N (DF %) | Mean ± SD | GM | Min | Med | P95 |
|-----------|----------|-----------|----|-----|-----|-----|
| **Dog urine** |          |           |    |     |     |     |
| THI       | 42 (21.4)| 0.01 ± 0.01| <LOD| <LOD| 0.05| 16 (31.3)|
|           |          | 0.01 ± 0.01| <LOD| <LOD| 0.02| 0.02|<LOD|<LOD|0.04|
| CLO       | 42 (97.6)| 0.90 ± 1.07| 0.50| <LOD| 0.53| 4.13| 16 (100)|
|           |          | 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 ± 3.08| 0.81| <LOD| 0.84| 5.76| 16 (100)|
|           |          | 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 ± 0.03| 0.02| <LOD| <LOD| 0.13|0.02 ± 0.01|<LOD|0.07|
| N-DMT     | 42 (92.9)| 0.27 ± 0.39| 0.14| <LOD| 0.11| 1.08| 16 (75)|
|           |          | 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)|
|           |          | 0.12 ± 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)|
|           |          | 6.60 ± 3.99| 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)|
|           |          | 0.14 ± 0.28| 0.03| <LOD| <LOD| 1.08|1.94 ± 3.96**|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)|
|           |          | 0.29 ± 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)|
|           |          | 0.08 ± 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)|
|           |          | 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)|
|           |          | 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)|
|           |          | 1.33 ± 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 ± 25.7** | 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 |
|                |           | 1.33 ± 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 | 0.59 | 114  |           | 0.46 ± 0.53 | 0.17 | 0.03 | 0.32 | 1.88 |
| cis-DBCBA      | 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 |
|                |           | 2.42 ± 7.47 | 0.56 | 0.06 | 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 | 0.04 | 0.21 | 0.58 |
|                |           | 4.37 ± 8.18 | 1.40 | 0.07 | 1.17 | 16.3 |           | 0.19 ± 0.25** | 0.07 | 0.05 | 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
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) | Metabolite | 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 |
| Thiameifoxam     | 6                             | THX        | 0.08 | 0.10 | NC–0.52  | 0.18 | 0.19 | 0.03–0.57 |
| Clothianalin     | 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 |
| Dinofuran        | 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       | 50                            | DCCA        | 0.30 | 0.28 | 0.01–20.9 | 0.11 | 0.11 | 0.01–2.10 |
| Cypermethrin     | 10                            | DCCA        | 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.
Chronic reference dose (cRfD) for humans provided by the U.S. EPA were used for all pesticides except for nitenpyram and imidacloprid, for which the acceptable daily intake (ADI) values for humans proposed by the Chinese Ministry of Agriculture (NY/T 2874–2015) were used.

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

Indicates the sum of \textit{cis-} and \textit{trans-}DCCA.

Indicates the highest exposure estimate.