Canines on the Couch: Using Silicone Passive Samplers to Evaluate Pesticide Concurrent Exposures in People and Their Pet Dogs

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ABSTRACT

People are chronically exposed to various pesticides through the diet, but also through herbicide applications in lawns and pesticide treatments around the home. Chronic household exposure to pesticides affects people and their pets, and some studies suggest pesticide exposure in dogs may be associated with cancer. Companion animals are recognized for their value in comparative health studies, and their shared daily environment with people suggests they are valuable in supporting environmental health research. In this study, we used wearable silicone passive samplers to support a comparative exposure assessment. We recruited 30 people and their pet dogs (living in the same household) to participate in a study to determine how well silicone wristbands (for human) and dog tags (worn on dog collars) can predict urinary pesticide biomarkers of exposure. Participants wore the silicone samplers for 5 days. They collected urine from themselves and their dogs on Days 1, 3 and 5 of the study. Urine samples were pooled for analysis of pesticide biomarkers. Using targeted GC-MS analyses, we quantified 8 pesticides in silicone samplers. Using a suspect screening approach, we additionally identified N,N-diethyl-m-toluamide (DEET), promecarb, flamprop-methyl and fipronil on the silicone samplers with high detection frequencies. DEET and fipronil were confirmed with authentic analytic standards and had statistically significant correlations between wristbands and dog tags (r_s=0.86 and 0.67, respectively, p<0.01). Pooled urine samples were quantified for 15 pesticide metabolite biomarkers. Several pesticides, including permethrin, DEET and chlorpyrifos, were detected with high frequency (>70%) in wristbands and urine of both humans and dogs, as corresponding biomarkers. Compared to adults evaluated in the U.S. general population, these dog-owners had higher urinary pesticide metabolite concentrations. Significant and positive correlations were observed between silicone sampler levels of permethrin and DEET with their corresponding urinary metabolites (r_s= 0.50-0.96, p<0.05) in both humans and dogs. Dogs had significantly higher urinary concentrations of 2,4-D and para-nitrophenol compared to humans in our study. Owners that reported using flea and tick products containing fipronil on their dog had significantly higher levels of fipronil in wristbands (~10X) and dog tags (~100X) compared to those who did not (p<0.01). This study demonstrates that pet dogs can act as proxies for human pesticide exposures in the home environment, potentially providing a new way to study relationships between environmental exposures and disease etiology.

1. Are pesticide exposures correlated in people and their pet dogs?

2. Do pesticide exposures captured by wristbands reflect internal dose?

INTRODUCTION

- Homeowners can use up to 10x more pesticide per acre than a typical agricultural application[1]
- Pesticide exposures have been linked to many chronic diseases in people including, cancers, diabetes, reproductive and neurological disorders, and birth defects[2-4]
- Pesticide exposures have been linked to bladder cancer, lymphoma, and mammary carcinoma in dogs[5-7]

This study builds on our seminal work evaluating environmental exposures in people and their pet dogs living in the same household[8]. We previously demonstrated high correlations between species for exposures measured on silicone monitoring devices (Table 2).

Compound	Human DF	Human GM	Dog DF	Dog GM	r_s
Lindane	27	N/A	50	N/A	N/A
Chlorpyrifos	83	1.0	70	0.6	0.90*
trans-Chlordane	93	4.2	97	5.8	0.83*
cis-Chlordane	70	1.1	93	1.8	0.85*
trans-Permethrin	100	347.0	100	334.7	0.83*
cis-Permethrin	100	254.2	100	261.1	0.81*
cypermethrin	77	5.6	27	N/A	N/A
Azoxystrobin	80	1.3	50	N/A	0.15
Chlorfenapyr	10	N/A	13	N/A	N/A

Table 1. Descriptive statistics of pesticides measured on wristbands and dog tags
Detection frequency (DF; %); Geometric mean (GM) r_s = the spearman correlation coefficient between species; *p<0.0001. Analytes with a DF <50% were not analyzed and entered as not applicable (N/A).

STUDY DESIGN

Wise et al., 2020
30 people and their pet dogs participated
- Silicone monitoring devices (left) were worn for 5 days
  - Extracted and cleaned up using column chromatography
  - Analyzed with mass spectrometry for targeted and suspect screening

- Pooled urine samples were made from 3 samples/individual
  - Sent to the CDC for pesticide biomarker analyses
  - Concentrations were specific gravity corrected

- Questionnaires were administered
- Spearman's correlations were used to determine associations between matrices and species

RESULTS

Biomarker	Abbreviation	LOD (µg/L)	DF	n	GM	95th	DF	n	GM	95th	r_s
Organophosphates											
3,5,6-Trichloro-2-pyridinol	TCPY	0.1	100	30	2.3	5.9	100	30	1.7	9.9	0.22
para-Nitrophenol	PNP	0.1	100	30	1.8	8.6	100	30	2.9	6.5	-0.14
2-isopropyl-4-methyl-6-hydroxypyrimidine	IMPY	0.1	30	30	N/A	0.7	0	30	N/A	N/A	N/A
Phenoxy acid											
2,4-Dichlorophenoxyacetic acid	2,4-D	0.15	93	30	0.5	1.9	97	30	0.9	3.9	-0.23
Pyrethroids											
3-phenoxybenzoic acid	3-PBA	0.1	100	30	3.4	21.2	20	30	N/A	1.5	N/A
4-fluoro-3-phenoxybenzoic acid	4-F-3-PBA	0.1	33	30	N/A	1.0	17	30	N/A	1.2	N/A
trans-3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid	*trans*-DCCA	0.6	60	30	2.4	22.2	73	30	2.8	368.9	0.38*
Neonicotinoids											
5-Hydroxy imidacloprid ‡	5-OH-IMI	0.2	38	16‡	0.8	38.4	50	26‡	1.8	583.9	N/A
Acetamiprid	ACET	0.2	3	30	N/A	N/A	0	N/A	N/A	N/A	N/A
Acetamiprid-N-desmethyl ‡	ACET-Ndes	0.4	48	29‡	0.8	5.6	0	N/A	N/A	N/A	N/A
Clothianidin	CLOTH	0.4	0	N/A	N/A	N/A	3	30	N/A	N/A	N/A
Imidacloprid ‡	IMI	0.3	46	24‡	0.6	1.6	42	24‡	0.9	126.2	N/A
Thiacloprid	THIAC	0.2	0	N/A	N/A	N/A	0	N/A	N/A	N/A	N/A
DEET											
3-(diethylcarbamoyl)benzoic acid	DCBA	0.03	100	30	51.1	21972.1	100	30	16.8	255.9	0.82**
3-(ethylcarbamoyl)benzoic acid ‡	ECBA	0.2	100	29‡	21.0	8389.6	83	30	4.7	98.3	0.87**

Table 2. Descriptive statistics of specific gravity corrected urinary concentrations (µg/L) of pesticides biomarkers measured in human and dog samples
Limit of detection (LOD) values are not specific gravity corrected. Detection frequency (DF; %); ‡ Some samples had mass spectrometry interferences that prevented accurate quantification of some biomarkers; those samples were excluded from statistical analyses. The 'n' column indicates the number of urine samples for which data was collected and which there was no mass spectrometry interference. Geometric mean (GM); 95[th] percentile (95[th]) r_s = the spearman correlation coefficient estimated from human and dog urinary biomarkers; * p<0.05; ** p<0.0001 Analytes with a DF <50% were not analyzed and entered as not applicable (N/A).

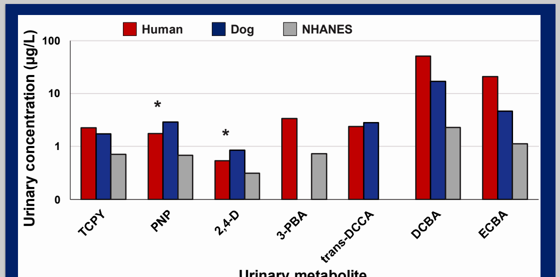

Figure 1. A comparison of pesticide biomarker concentrations in urine samples from humans and dogs in this study compared with NHANES adult data
Comparisons are shown for the geometric mean based on raw uncorrected concentrations of frequently detected metabolites measured in human and dog urine in our study compared to the corresponding and most recent NHANES biomonitoring data for the general population. NHANES data are from adults 20+ years in 2013/2014 except for DBCA and ECBA which had data available from 2015/2016, and TCPY which only had data from 2009/2010 limited to adults age 20-59. Comparisons were not included for analytes with a high proportion of samples with concentrations <LOD. * denotes statistically different between dogs and people in our data set.

RESULTS

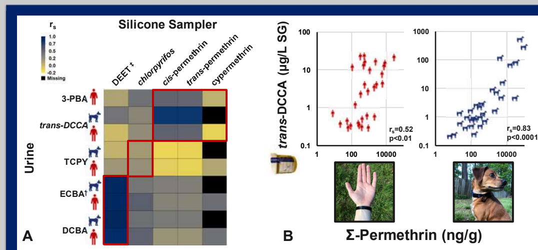

Figure 2. (A) Heatmap of Spearman's correlations for pesticides measured on wristbands/dog tags with urinary biomarker concentrations in each species (B) Example scatterplots for urinary biomarker *trans*-DCCA and total permethrin on silicone samplers
Analyses were restricted to chemicals with data available for known chemical/biomarker relationships. Direct relationships with parent chemicals (DEET, chlorpyrifos, isomers of permethrin, and cypermethrin) and their metabolites are highlighted in red boxes. Correlations were conducted using specific gravity corrected concentrations (µg/L SG). †Correlations coefficients for ECBA in human urine were done using n=29. Cypermethrin was detected on <50% of dog tags. 3-PBA was only detected in 20% of dog urine samples and therefore excluded. ‡DEET values are semi-quantitative and are based on area responses normalized to the nearest internal standard (retention time). Spearman's correlation coefficient (r_s)

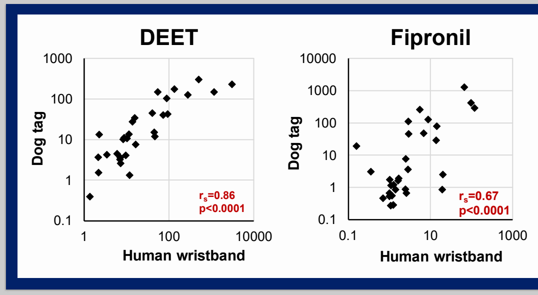

Figure 3. Scatterplots and Spearman's correlation coefficients (rs) for pesticides identified through suspect screening on human wristbands and dog tags; (a) DEET, (b) fipronil
Data are semi-quantitative and are based on area responses normalized to the nearest internal standard (by retention time).

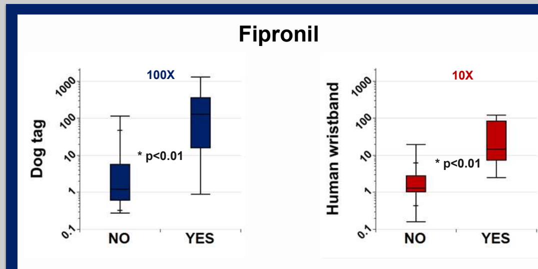

Figure 4. The relative amount of fipronil measured on (a) dog tags and (b) human wristbands based on reported use of a fipronil containing flea and tick medication
Groupings are based on whether people reported using a flea and tick product containing fipronil on their dog (YES, n=9) and those that did not (NO, n=21).

CONCLUSIONS

- Wearable samplers demonstrate that people and their pet dogs experience similar pesticide exposures in the home
- Urinary pesticide biomarker concentrations in human specimens were generally higher in our study compared to previous measurements from the U.S. adult general population
- Dogs had ~2x higher concentrations of 2,4-D and PNP (GM = 0.8 and 2.7 µg/L, respectively) compared to humans (GM = 0.4 and 1.2 µg/L, respectively).
  - These differences were statistically significant for both 2,4-D (p<0.05) and PNP (p<0.01)
- Significant and positive correlations were observed for some pesticides measured on silicone samplers and their corresponding biomarkers
- No significant correlation between chlorpyrifos measured on the silicone samplers and the urinary metabolite (TCPY) was observed likely due to dietary chlorpyrifos exposures
- A majority of the correlations between the pesticide levels in the silicone monitors and urinary concentrations pesticide metabolites were stronger in dogs than in humans, similar to what we previously observed with organophosphate esters[8]

FUTURE DIRECTIONS

A Canine Model for Human High-risk Non-muscle Invasive Human Bladder Cancer – Molecular and Environmental Considerations

We are using this study design to conduct a case-control study with pet dogs to investigate associations between environmental exposures and bladder cancer.

- 24 dogs enrolled with a positive urinary *BRAF* mutation detection
- 63 breed, age and sex matched control dogs enrolled with no urinary *BRAF* mutation detected

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