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Detection of herbicides in the urine of pet dogs following home lawn chemical application

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HIGHLIGHTS

- Lawn chemicals were commonly detected on treated and "untreated" lawns.
- The detection of lawn chemicals in the urine of pet dogs was widespread.
- Lawn chemicals persisted on the grass for at least 48h after application.
- Chemicals persisted longer on grass under certain environmental conditions.
- Dogs may serve as sentinels for human expsoures, and further study is justified.

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ABSTRACT

Exposure to herbicide-treated lawns has been associated with significantly higher bladder cancer risk in dogs. This work was performed to further characterize lawn chemical exposures in dogs, and to determine environmental factors associated with chemical residence time on grass. In addition to concern for canine health, a strong justification for the work was that dogs may serve as sentinels for potentially harmful environmental exposures in humans. Experimentally, herbicides [2,4-dichlorophenoxyacetic acid (2,4-D), 4-chloro-2-methylphenoxypropionic acid (MCPP), dicamba] were applied to grass plots under different conditions (e.g., green, dry brown, wet, and recently mowed grass). Chemicals in dislodgeable residues were measured by LC-MS at 0.17, 1, 24, 48, 72 h post treatment. In a separate study, 2,4-D, MCPP, and dithiopyr concentrations were measured in the urine of dogs and in dislodgeable grass residues in households that applied or did not apply chemicals in the preceding 48 h. Chemicals were measured at 0, 24, and 48 h post application in treated households and at time 0 in untreated control households. Residence times of 2,4-D, MCPP, and dicamba were significantly prolonged ($P < 0.05$) on dry brown grass compared to green grass. Chemicals were detected in the urine of dogs in 14 of 25 households before lawn treatment, in 19 of 25 households after lawn treatment, and in 4 of 8 untreated households. Chemicals were commonly detected in grass residues from treated lawns, and from untreated lawns suggesting chemical drift from nearby treated areas. Thus dogs could be exposed to chemicals through contact with their own lawn (treated or contaminated through drift) or through contact with other grassy areas if they travel. The length of time to restrict a dog's access to treated lawns following treatment remains to be defined. Further study is indicated to assess the risks of herbicide exposure in humans and dogs.

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; creat, creatinine; dithiopyr, 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl)pyridine-3,5-dicarbothioate; MCPP, 4-chloro-2-methylphenoxypropionic acid; UC, urothelial carcinoma.

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1. Introduction

Urinary bladder cancer (urothelial carcinoma, UC) causes >14,000 deaths yearly in the United States (Tanaka and Sonpavde, 2011). Approximately half of UC cases are thought to be due to exposure to cigarette smoke and chemicals in the workplace (Dietrich and Golka, 2012; Droller, 2006; Felknor and Delclos, 2006). Exposure to herbicides, pesticides, and contaminants in agricultural chemical mixtures could increase UC risk, although not all studies support this role (Alavanja and Bonner, 2012; Boers et al., 2010; Koutros et al., 2009; Singh et al., 2010). Genetic factors have been associated with increased UC risk, especially in relation to chemical exposures (Franekova et al., 2008; Murta-Nascimento et al., 2007). More than half of UC patients, however, have no known risk factors for the cancer. Studies are needed to further identify environmental and genetic factors, and gene–environment interactions that increase UC risk.

Dogs offer a highly relevant naturally-occurring animal model to identify environmental chemical exposures and gene–environment interactions that increase UC risk. Naturally-occurring UC in dogs closely mimics human invasive UC in physiologic age of onset, molecular features, biologic behavior, and treatment response (Knapp, 2006, 2007). Dogs offer the opportunity to study heritable risk factors and chemical–gene interactions leading to UC as specific breeds of dogs are much more likely to develop this cancer. This breed-associated, i.e. heritable, risk for UC includes an 18–20-fold increased risk in Scottish Terriers and a 3–5-fold increased risk in the genetically-related West Highland White Terriers, and a 3–5-fold increased risk in Shetland Sheepdogs and beagles (Knapp, 2006). With the tremendous genetic diversity in humans, groups of humans with this level of heritable risk for UC have not been identified. In addition, since dogs do not smoke, they can be a more specific sentinel for other causes of UC in humans.

Another intriguing aspect of UC in dogs is the latency period between chemical exposure and UC development. In dogs this can be as short as a year (range 1–10 yr) (Okajima et al., 1981), whereas latency periods in humans can extend to several decades (Dietrich and Golka, 2012; Droller, 2006; Felknor and Delclos, 2006). This indicates that dogs could serve as sentinels to environmental exposures that could be harmful to humans, as well as dogs, if not addressed in a timely fashion. Dogs have already been identified as sentinels for environmental exposures related to the risk of other types of cancer (Reif, 2011), with a notable example being the risk of mesothelioma from exposure to asbestos (Glickman et al., 1983; Kelsey et al., 1998). In a case control study, owners of dogs with mesothelioma were more likely than owners of control dogs to have been exposed to asbestos at work or through a hobby (Glickman et al., 1983). It is considered appropriate to follow the diagnosis of mesothelioma in a dog with a careful search for asbestos in the environment in order to prevent new or continued exposure to humans in the same area.

A significant association between chemical exposure and UC risk has been described in dogs across many breeds (Glickman et al., 1989). A stronger association between lawn chemical exposure and UC risk has been reported in a genetically susceptible dog breed, Scottish Terriers (Glickman et al., 2004). In a case control study, Scottish Terriers exposed to lawn herbicides had a 3.6-fold increased risk for UC (OR 3.62, 95% CI 1.17–11.19, P = 0.03) compared to unexposed dogs. Dogs exposed to lawn herbicides and pesticides had a 7.2-fold increased UC risk (OR 7.19, 95% CI, P = 0.001) (Glickman et al., 2004). It was suggested that chemical carcinogens (or pre-carcinogens) on the lawn were internalized by the dogs and excreted in urine, thereby exposing the urothelium to harmful chemicals.

Herbicides can readily be measured in urine of dogs following experimental administration (Dickow et al., 2001). Similarly, in a community study, 2,4-D was detected in urine of dogs exposed to household lawns treated with herbicides within the previous 42 days (Reynolds et al., 1994). Among 44 dogs potentially exposed to 2,4-D treated lawns, 2,4-D concentrations of at least 10 μg/L were found in 75% of dogs, and concentrations ≥ 50 μg/L were found in 39% of dogs on average 11 days post lawn treatment (Reynolds et al., 1994). The study reported here was performed to confirm and extend these findings in a contemporary setting. The relationship between the herbicide concentration on the grass and the likelihood of exposure of dogs to lawns has not been previously reported. The primary goal of the current study was to prospectively measure herbicide concentrations on treated lawns and in the urine of dogs living in these homes, before and after lawn application, with comparison to concentrations on lawns and in urine of dogs from untreated households. A secondary goal was to identify host and environmental factors that could increase exposure potential.

For the household study, the concentrations of 3 chemicals commonly used in commercial lawn care products were measured including 2,4-dichlorophenoxyacetic acid (2,4-D), 4-chloro-2methylphenoxypropionic acid (MCPP), and dimethyl 2-(difluoromethyl)-4-(2-methylpropyl)-6-(trifluoromethyl) pyridine-3,5-dicarbothioate (dithiopyr). The chemical, 2,4-D, was selected for study because it is commonly included in lawn treatment mixtures and because it has been incriminated as a potential carcinogen in other studies. An association between 2,4-D exposure and risk of cancer, especially non-Hodgkins lymphoma, in humans has been described (Hardell et al., 1981; Hoar et al., 1986; Hoar-Zahm et al., 1990; Landgren et al., 2009; Miligi et al., 2006; Mills et al., 2005; Wigle et al., 1990). A positive association has also been reported between the risk of non-Hodgkins lymphoma in dogs and exposure to lawn chemicals (Takashima-Uebelhoer et al., 2012), and specifically 2,4-D on lawns (Hayes et al., 1991). Not all studies, however, support a link between this chemical exposure and lymphoma in dogs (Carlo et al., 1992; Kaneene and Miller, 1999) or humans (Bond and Rossbacher, 1993; Burns et al., 2011; Garabrant and Philbert, 2002; Hartge et al., 2005). Considerable concern has also been expressed regarding the risk of non-Hodgkins lymphoma and other diseases in Vietnam veterans exposed to a herbicide mixture containing 2,4-D (Agent Orange), although contaminating dioxins could be the most harmful component of this mixture (Hites, 2011). The other 2 chemicals measured in the household study (MCPP, dithiopyr) have not been studied as rigorously as 2,4-D. These chemicals were included in the study because they are commonly used in lawn treatment products, can be accurately measured, and could serve as markers of lawn chemical uptake in dogs.

2. Methods

2.1. Study overview

This study was approved by the Purdue Animal Care and Use Committee and the Purdue Institutional Review Board for human subject use. The two components of the study were: (1) an experimental grass plot study to confirm analytic methods used to measure chemicals on the grass and their residence time under different environmental conditions, and (2) a household study to determine concentrations of lawn chemicals on grass and in the urine of dogs exposed to treated lawns or control (untreated) lawns.

2.2. Experimental grass plot study

2.2.1. Sample grass plots

Sample grass plots were selected from established lawn areas at Purdue University that had been planted with mixed 50% bluegrass/50% fine fescue and which were restricted from any lawn treatment for at least 4 months before beginning the experiments. A 25 meter (m) boundary area surrounding the plots was sequestered from treatments for the same period. The 4 m^2 plots were located in open areas ≥ 8 m from buildings, trees, or shrubs. Two plots were used for each treatment/week. Three samples were collected at each time point from each plot. Within each condition type, assignment of treatment was randomized (random block design). Prior to herbicide

application, dislodgeable residue tests were performed on each plot to confirm the absence of detectable residues of any of the chemicals to be applied. Experimental plots were treated and analyzed in April. The same plots were used for initial application sampling (15 min post application) and sampling after treatments had dried, but different portions of the plot were sampled. Separate areas of the plots were sampled at 0.17, 1, 24, 48, and 72 h. The effects of different lawn conditions on chemical residence time were studied. Mist treatments (simulating watering or rain) were applied to separate plots. The effects of rain (amount of rain recorded), mowing the grass, or allowing the grass to become dry and brown were studied. Grass plots were identified in the selected test area that matched these conditions.

2.2.2. Treatment

Treatments were applied with a calibrated sprayer according to manufacturer's instructions. A standard broadleaf herbicide mixture (Triplet SF, Nufarm, Burr Ridge, IL; 0.169% 2,4-D, 0.045% MCPP, 0.015% dicamba) was applied at a rate of 117 μL/m^2 according to manufacturer's specifications.

Several dislodgeable residue sampling methods were assessed for their effectiveness. An EPA-approved drag block method (Nishioka et al., 1996, 1999) using 3 wipe passes over 3 adjacent strips defined by a 0.17 m^2 sampling frame was selected for sample collection in the study. Briefly, a single thickness control application pad of 100% soft cotton (5 × 20 cm strip) was mounted on a square block. The block was dragged across the area to be sampled, and the dislodgeable residue from the grass picked up by the cloth on the block. Collected dislodgeable residues were compared to herbicide deposition on cloth traps. For these traps, cloth identical to that on the drag blocks, was attached to a metal plate and mounted on a vertical stake 5 cm above the grass surface. The plate was positioned downwind from the site of application at an angle of 35° from horizontal facing the application site. The cloth traps were placed in the center of the sampling frame to determine the maximum amount of chemical that could have been deposited on the grass and to ascertain the effective period during which quantitative analysis of herbicide availability was feasible. Additional cloth traps were placed outside of the sprayed area for the detection of overspray/drift. Samples were stored at 4 °C in dim light (22 μE m^{-2} s^{-1}) until analyzed. All samples collected included a cloth-only (unexposed) blank that was used as a baseline negative control.

2.2.3. Herbicide analysis

Grass dislodgeable residues were extracted from sampling cloths with 100 mL methanol using vacuum filtration, reduced to 0.5 mL by rotary evaporation with rinsing, then diluted (1:1, v/v) with deionized water. Aliquots (300 μL) were transferred into glass vials for analysis. Herbicides were extracted from urine samples in methanol/chloroform (1:4, v/v) added 1:1, v/v to refrigerated samples. The chloroform phase of each sample was reduced under nitrogen gas and resuspended in 50% methanol, and 300 μL of each sample was transferred to individual glass vials. As indicated, multiple samples were collected at each site. Each sample was split into 3 aliquots to determine the variation within the sample preparation and analysis. No significant variation was observed. Quantitative recovery within the range of detection by liquid or gas chromatography mass spectroscopy (LC-MS or GC–MS) was evident within a 48 h window after application (Table 1).

In initial analyses, concentrations of dicamba, 2,4-D, and MCPP were determined by LC-MS using a Micromass LCT Premier (Beverly, MA) in ion monitoring mode using a C18 column and 0–55% acetonitrile gradient with 0.1% formic acid and genuine standards with indole-3-proprionic acid as an internal standard. These analyses were used in the experimental grass plot studies and in additional pilot experiments conducted on lawn sites that had been treated by commercial applicators and homeowners. These assays were subsequently expanded to include LC/MS-MS to detect MCPP and dithiopyr, and GC/MS to detect 2,4-D. For the LC/MS-MS detection of MCPP and dithiopyr, 5 μL samples were submitted to an Agilent 6460 HPLC-triple quadrupole MS (Agilent Technologies, Santa Clara, CA) fitted with a Zorbax SB-C18 column (2.1 × 50 mm, 3.5 μm), flow 0.30 mL/min, solvent A of water and solvent B acetonitrile (both 0.1% formic acid), utilizing an elution gradient 0 min at 50% B; 0–3 min gradient to 100% B; 3–6 min hold at 100% B; 6–7 min gradient to 50% B; 7–10 min hold at 50% B. The Jetstream ESI source was operated in positive ion mode for dithiopyr and negative ion mode for MCPP [nozzle voltage 1000 V, capillary 3500 V, nebulizer pressure 35 psi, drying gas (nitrogen) 325 °C with flow rate 8 L/min, sheath gas 250 °C with a flow rate of 7 L/min, and fragmentor voltage 80 V]. Multiple reaction monitoring (MRM) was used for selective detection. The mass transitions were 402.0 to 353.9 for dithiopyr and 213.0 to 140.9 for MCPP. Collision energies were 15 eV for dithiopyr and 10 eV for MCPP. A 100 ms dwell time was used for each transition. Compound retention times were 5.3 min for dithiopyr and 1.6 min for MCPP. Quantitation standards were prepared in the range of 0.1–100 pg/mL in methanol. Standard curves had a $r^2 > 0.99$.

For GC/MS analysis of 2,4-D, dried extracts (from 300 μL) were silylated with 25 μL N-methyl-trimethylsilyltrifluoroacetamide (MSTFA) in 25 μL of pyridine at 60 °C for 1 h. Derivatized 2,4-D (2 μL) was analyzed on an Agilent 6890/LECO Pegasus III time-of-flight mass spectrometer (LECO Corp. St. Joseph, MI), with split ratio of 20:1, HP-5MS capillary column (30 m length × 250 mm i.d. × 0.25 μm film thickness), helium carrier at 1 mL/min, temperature gradient from 100 °C (0.2 min hold) to 300 °C at 10 °C/min, transfer line to MS of 250 °C, ion source of 200 °C, EI source 70 eV, and the mass scan range 30–800 m/z. Retention time for derivatized 2,4-D was 10.3 min. Quantitation of an extracted ion chromatogram (sum of fragment ion masses 233, 257, and 292) was used to generate standard curves from 1 μg/mL to 1.2 mg/mL with an $r^2 > 0.99$.

Dislodgeable residues were normalized to sampling area. The limit of detection of the assay was 0.5 μg/mL in the final sample. Results of grass plot experiments were analyzed by ANOVA using Student's

Table 1
Analyses of herbicides in dislodgeable residues with comparison to cloth traps and clipped grass samples.

	12 h	24 h	48 h	72 h	1week
Percentage of herbicide recovered in dislodgeable residue compared to herbicide recovered from cloth traps					
2,4-D	96%	81%	24%	11%	ND
MCPP	98%	93%	22%	18%	ND
Dicamba	81%	68%	17%	ND	ND
Detection limit over time of herbicide extracted from clipped grass with chloroform:methanol:water vs. dislodgeable residue collected from grass surfaces					
2,4-D	E+, DR+	E+, DR+	E+, DR+	E+, DR+	ND, ND
MCPP	E+, DR+	E+, DR+	E+, DR+	E+, DR+	ND, ND
Dicamba	E+, DR+	E+, DR+	E+, DR+	ND, ND	ND, ND

E+, detectable in grass clipping extracts; DR+, detectable in dislodgeable residues; ND, not detectable.

t-test and a post-hoc Tukey's analysis. Overspray was assessed by examination of dicot ornamentals in adjoining beds after 5 days and by both dislodgeable residue and trap sampling in adjoining grasses. Overspray (offsite deposition) of up to 10 m was detected with winds of ~5 mph and dicot ornamental damage (leaf curling) was detected at 25 m in winds of ~10 mph.

2.3. Household grass and pet dog study

2.3.1. Eligibility and recruitment

Households were recruited through web site (www.vet.purdue.edu/pcop) and flyers distributed in the Purdue University community. Study households were located within a 50 mile radius of West Lafayette, Indiana, USA (latitude 40.42°N, longitude 86.88°W), and included those who had already decided to either use or not use lawn chemicals during the next growing season. Other eligibility requirements for households included: (1) the presence of a pet dog of either sex, and of any breed, over the age of 12 months, and (2) written informed consent to participate. Treatment households were those whose occupants had decided to either apply commercial lawn products themselves during the study period (March–August) or have them applied by a licensed company. Control households were those whose owners were not going to apply lawn chemicals (herbicides, "fertilizer mix", etc.) during the study period, and who had not used lawn chemicals for the past 6 months.

2.3.2. Household information

Information collected for each household included: names of commercial products applied by the lawn care company or household occupant; chemical contents of products if known; application dates; areas treated; lawn watering history; gender, breed, weight, and age of the dog (1 dog/household); amount of time the dog typically spent on the lawn each day (weekdays and weekends); amount of time the dog spent on grass areas away from the household each day; the extent to which the dog was observed to eat grass; and the length of time the dog was kept off the lawn following chemical application. The guidelines that the dog owners received regarding the length of time to restrict their dog from the lawn after treatment varied across applicators and products, and ranged from 0 to 24 h.

2.3.3. Lawn treatment

All lawn chemicals were applied during the months of March through August. Lawn chemicals were applied by pet owners for 18 households and by a commercial lawn service for 7 households. The contents of the chemical mixture applied to the grass were obtained either from the commercial applicator or from the product label when applied by the owner. The study participants were asked to follow recommendations of the lawn care company or product label in regards to restricting their dog's access to treated grass.

2.3.4. Samples collected from grass and urine

The samples of dislodgeable residue from the lawn and urine from the dog were collected prior to (time 0) and at ~24 and 48 h after lawn chemical application. The samples were collected from control households at least once during the study period. The drag sled method was used to obtain dislodgeable residue from the grass. The grass residue collection methods used in the household study were the same as those used for the experimental grass plot study. A midstream free-catch urine sample (≥10 ml) was collected from each dog. The samples were stored at 4 °C (dim light, 22 $\mu E\ m^{-2}\ s^{-1}$) after addition of 25% (v/v) methanol. Herbicide concentrations in urine were normalized to urine creatinine (creat) concentration in order to determine actual chemical levels excreted in the urine regardless of how dilute or concentrated the dog's urine was at that given time.

2.3.5. Statistical analyses

Characteristics of dogs in treated households (N = 25) and control households (N = 8) were compared. For continuous variables (e.g., dog age, weight, and hours on lawn/week) a Wilcoxon Two-Sample test was used. For categorical variables (e.g., gender, neuter status, and whether or not specific herbicides were detected in urine at time 0) a Fisher's Exact test was used. Multivariable logistic regression models were used to calculate the odds of finding a detectable concentration of specific herbicide chemical in the urine of dogs for each hour the dog spent on its home lawn during a typical week when adjusted for dog's age, weight, gender, and neuter status. Ninety-five percent confidence limits were calculated for each odds ratio. The relationship between the finding of chemicals in the grass residue and in the urine of dogs from the same household was assessed by McNemar's test with calculation of the Kappa coefficient and P value. Statistical significance was defined as $P < 0.05$. Statistical analyses were performed using standard software (SAS version 9.3, SAS Institute Inc., Cary, NC).

3. Results and discussion

3.1. Experimental grass plot study

Conditions during the test plot experiments are summarized in Table 2. In dislodgeable residue analyses, 2,4-D was reproducibly detected from green lawns 48 h after application, whereas MCPP and dicamba residues were not significantly different from control measurements after 24 h (Fig. 1).

The grass plot studies demonstrated that lawn conditions can affect chemical residence time. Dislodgeable 2,4-D persisted significantly longer, i.e. had a significantly longer residence time, on dry brown grass at 24, 48 and 72 h after application ($P < 0.05$) compared to green grass (Fig. 1). A similar pattern was observed for MCPP at 48 and 72 h after application ($P < 0.05$). The significantly longer residence time of 2,4-D and MCPP on dry brown lawns is consistent with the mode of action for these herbicides which requires cellular uptake via hydrophobic diffusion across living cell membranes (Marchant et al., 1999; Swarup et al., 2001). In dry brown grass, cellular compartmentalization is unlikely to occur.

Dislodgeable 2,4-D, MCPP, and dicamba residues from lawns premisted before application to simulate wet conditions persisted at 48 h, slightly longer than those from dry green lawns (significantly for 2,4-D at 48 h, $P < 0.05$) (Fig. 1). This was most likely due to a delay in drying and absorption of the herbicide at the time of contact. Lawn mowing had little effect on persistence of dislodgeable residues, although 2,4-D concentrations from mowed lawns appeared slightly higher than those from dry green unmowed lawns at 72 h (Fig. 1). There was no significant difference in chemical residence times (concentrations detected at each time point) between green dry grass, green wet grass, or recently mowed grass.

The findings that lawn conditions can affect chemical residence time suggest that in future studies of chemical residence time, that

Table 2
Conditions during the course of the test plot experiments.

Date	Mean temp (°C)	High temp (°C)	Low temp (°C)	Precip (inches)
April 5	15	22	7	0
April 6	18	23	12	0
April 7	11	13	7	0
April 13	11	17	5	0.23
April 14	11	18	1	0
April 15	12	20	3	0
April 16	14	23	5	0
April 19	18	26	12	0
April 20	20	18	27	0.80
April 21	11	12	8	0.05

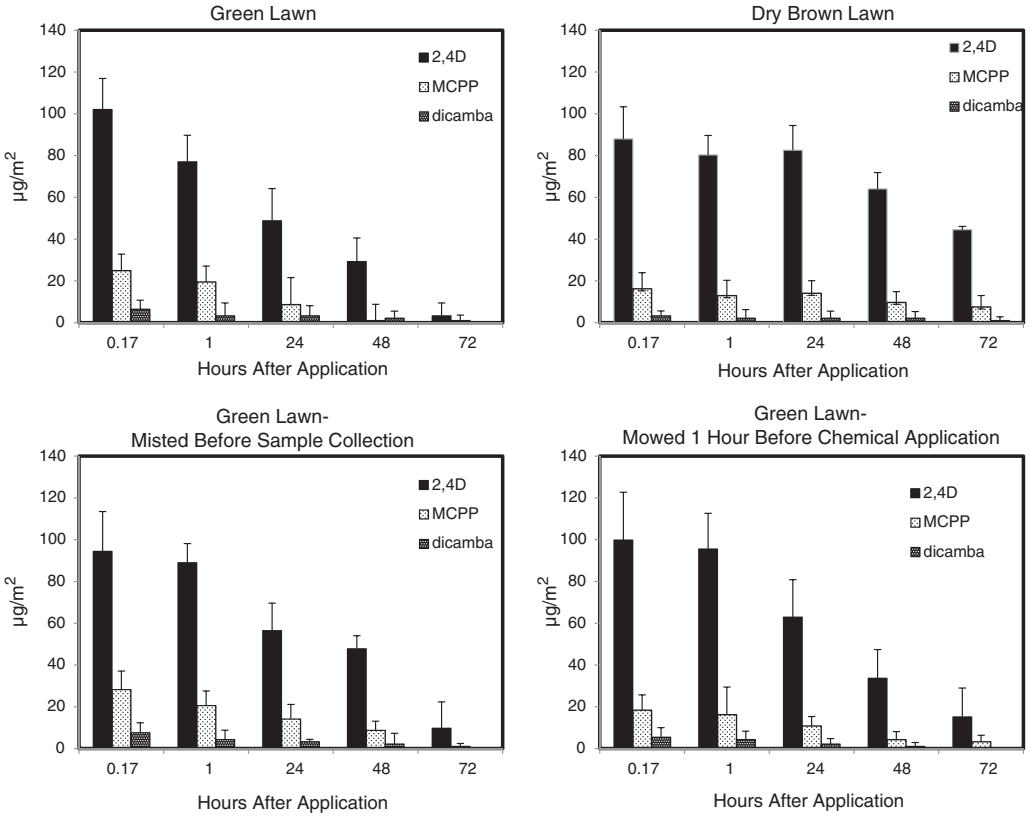

Fig. 1. Quantitative analysis of dislodgeable herbicide residue after application to 4 experimental grass plots. Conditions included: green lawn, dry brown lawn, green lawn that was misted with water prior to residue application, and green lawn that was mowed 1 h prior to herbicide residue collection. The herbicides that were quantitated were 2,4-D, MCPP and dicamba. Data are presented as mean ± standard deviation of 2 independent experiments, with 3 replicates each. Treatment conditions were compared to the green lawn control. Student's *t*- test, then followed by ANOVA with Tukey's pot-hoc analysis was performed. An asterisk (*) indicates that the chemical concentration detected in grass residue at that time point was significantly ($P < 0.05$) different between the green grass and the grass under the indicated experimental condition. For each case, control cloths were extracted by the same protocol, and no compounds were detected in these controls.

sampling be performed on dry green lawns wherever possible, and history of rain, watering, and mowing be recorded. Further studies are indicated which incorporate environment effects in defining the "period of risk" for chemical exposures to animals and humans following lawn chemical application.

3.2. Household study

The characteristics of the dogs participating in the study are summarized in Table 3. There were no significant differences between treated and control households in regards to the dogs' age, weight, gender, neuter status, or hours the dogs spent on the lawn in a typical week.

One of the most important findings from the study was the detection of herbicides in the urine of dogs that lived in untreated control households and in dogs living in treated households *before* chemicals had been applied. At least 1 of the 3 lawn chemicals studied was detected in urine of dogs from 4 (50%) of 8 control households. This included 2,4-D (292.42 µg/g creat) in 1 dog and MCPP (1.1, 1.1, and 197.3 ng/g creat) in dogs from 3 other control households. No 2,4-D was detected on the lawn of control households. The dog in the control household for which 2,4-D was detected in urine had been restricted to its home and yard; the source of the 2,4-D exposure for this dog was not identified. MCPP (90.8 ± 84.4 ng/m^2) was detected in the grass residue of 7 (87%) of the 8 control households including the 3 households in which MCPP was detected in the dogs' urine. No chemicals were detected in the blanks (assay controls).

In 14 (56%) of 25 treated households, at least 1 of the 3 study chemicals was detected in urine of dogs *before* the lawn was treated (time 0). This included 2,4-D in 6 dogs, MCPP in 7 dogs, and dithiopyr in 3 dogs (Table 4). For the 14 households in which chemicals were detected in dog urine before lawn treatment, the same chemicals were detected in the grass residue in 9 (64%) households before the

Table 3
Characteristics and baseline chemical exposure of dogs from treated (exposed) and control (not exposed) households.

	Exposed dogs (N = 25)		Not exposed dogs (N = 8)		P value [a]
	Mean	Std deviation	Mean	Std deviation	
Age	6.89	3.71	5.72	3.21	0.57
Weight (kg)	21.58	12.17	27.30	15.32	0.31
Hours on own lawn in typical week	4.68	6.72	3.43	2.69	0.75
	Number	Percent	Number	Percent	
Gender					
Female	11	44.0	3	37.5	0.30
Male	14	56.0	5	62.5	
Neuter status					
Neutered	23	92.0	7	87.5	0.44
Intact	2	8.0	1	12.5	
Detectable 2,4-D in urine at time 0					
Yes	6	24.0	1	12.5	0.65
No	19	76.0	7	87.5	
Detectable MCPP in urine at time 0					
Yes	7	28.0	3	37.5	0.67
No	18	72.0	5	62.5	

[a] Wilcoxon Two-Sample test for continuous variables; Fisher's Exact test for categorical variables.

lawn was treated. In another of the 14 (7%) households, the dog was reported to have had access to other grassy areas away from the home. The finding of chemicals in 4 (29%) of the 14 households where chemicals were detected in the dogs' urine before lawn treatment, could not be explained based on known exposures.

Multiple factors could contribute to the presence of herbicides in the urine of dogs on "untreated" lawns. In this observational study, dogs followed their routine activities which in some cases included visiting other grassy areas. Some dogs could have been exposed to chemicals away from their home. Ten of the 18 dogs on "untreated lawns" with positive urine tests, however, had been restricted to their home lawn. The role of previous chemical exposures and potential bioaccumulation of chemicals in fat tissues (Müllerová and Kopecký, 2007) in the dogs could not be determined. Although chemical concentrations in the dogs were lower than what would be likely to cause acute toxicity (Chen et al., 2010; Dickow et al., 2000; van Ravenzwaay et al., 2003), the health effects of long term exposure at the measured levels has not been determined.

Another important finding of the study was the widespread detection of chemicals in the urine of dogs following treatment of the lawn. Following treatment, at least 1 of the 3 chemicals was detected in urine of dogs in 19 (76%) of the 25 treated households (Table 4). Specifically, 2,4-D was detected in urine of 9 dogs (range 113.3–1508.2 μg/g creat), MCPP was detected in 15 dogs (range 0.6-505.7 ng/g creat), and dithiopyr was detected in 1 dog (187 ng/g creat) (Table 4). The mean time dogs in treated households were restricted from lawns post treatment was 5.8 ± 6.2 h (range 0–24 h). There was no significant association between the length of time the dog was restricted from the lawn after treatment or the dog's grass eating habits, and the concentration of chemicals in urine of the dogs. Similarly, there was no significant association between the time the dog spent on the lawn each week and the presence of chemicals in urine (Fig. 2). When the potential confounding variables namely age, weight, gender and neuter status, were removed from the logistic models, the results remained statistically non-significant.

The length of time dogs should be restricted from treated lawns remains to be defined. Recommended restrictions from lawn care companies ranged from, no recommendations, to no restrictions, to 24 h restriction, to "keeping pets and children off the lawn until the grass dries after treatment". The experimental grass plot data indicated that chemical exposures could occur up to 48 h following lawn treatment. The household lawn data, however, suggest that chemical exposures can occur at time points beyond 48 h, with the precise exposure window still to be determined. Urine samples were not collected at > 48 h following lawn treatment in the household study since the experimental grass plot study results suggested minimal chemical concentrations would be present after 48 h. Other investigators have reported 2,4-D in the urine of dogs with probable exposure to treated lawns >48 h post treatment, with detectable concentrations noted at 11 days post-treatment (Reynolds et al., 1994). With the long half-life of 2,4-D in dogs (99–134 h) (van Ravenzwaay et al., 2003), it is not known when the dogs in that study actually came in contact with the chemicals.

Along with the widespread detection of chemicals in the urine of dogs, chemicals were commonly detected in grass residue samples in control (7 of 8 households) and treated houseolds. Findings regarding chemicals in the grass residue in the 25 treated households are summarized in Table 4. At least 1 of the 3 chemicals studied was detected in lawn residue in 21(84%) households before treatment, in 24 (96%) households at 24 h after treatment, and in 21 (84%) of households at 48 h after treatment. Of 11 households in which 2,4-D was detected in grass residue samples post treatment at 24 or 48 h, 2,4-D was also detected in dogs' urine in 4 (36%) households. There was not a significant association between the presence of 2,4-D in the grass residue

Table 4
Presence of 2,4-D, MCPP, and dithiopyr in urine samples from dogs and in dislodgeable residue samples from grass from 25 treated households.

Urine samples			
	Pre treat	24 h post treat	48 h post treat
2,4-D present in dog urine			
Yes	6	6	7
No	19	19	18
2,4-D conc (mean ± sd, μg/g creat)			
Positive cases	329.7 ± 280.0	508.1 ± 517.3	398.1 ± 397.3
All dogs	79.1 ± 192.3	121.9 ± 323.7	111.5 ± 269.7
MCPP present in dog urine			
Yes	7	14	12
No	18	11	13
MCPP conc (mean ± sd, ng/g creat)			
Positive cases	7.5 ± 10.5	46.7 ± 135.1	8.1 ± 6.5
All dogs	2.1 ± 6.0	26.2 ± 102.2	3.9 ± 6.1
Dithiopyr present in dog urine			
Yes	3	1	1
No	22	14	14
Dithiopyr conc (ng/g creat)	0.05, 0.23, 2.28	1.87	1.52
Grass residue samples			
	Pre treat	24 h post treat	48 h post treat
2,4-D present in grass residue			
Yes	3	7	6
No	22	18	19
2,4-D conc (mean ± sd, μg/m^2)	387.2 ± 572.4	65.6 ± 26.9	72.1 ± 59.1
MCPP present in grass residue			
Yes	18	22	20
No	7	3	5
MCPP conc (mean ± sd, pg/ m^2)	649.9 ± 2,294.2	2088.8 ± 3715.3	1768.9 ± 3939.7
Dithiopyr present in grass residue			
Yes	11	12	6
No	14	13	19
Dithiopyr conc (mean ± sd, pg/ m^2)	5.3 ± 4.1	12.3 ± 17.6	9.8 ± 10.9

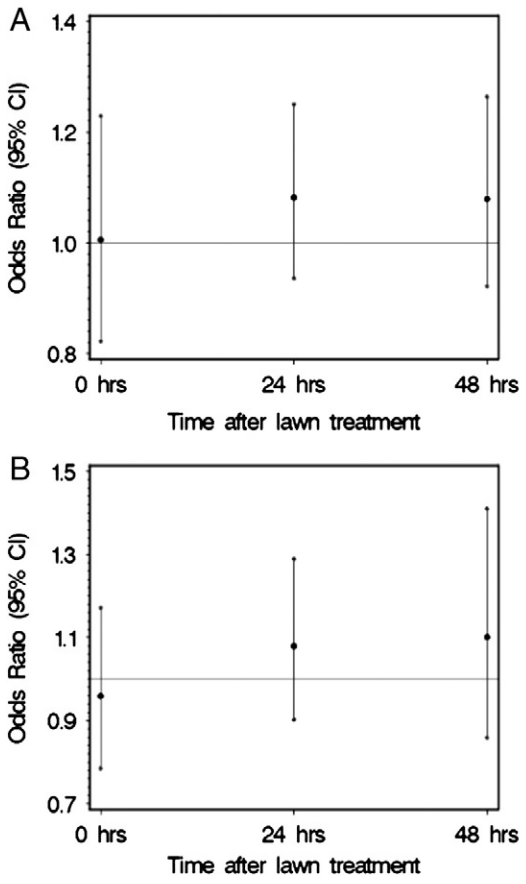

Fig. 2. Odds of finding a detectable concentration of 2,4-D (A) or MCPP (B) in the urine of dogs for each hour spent on the lawn during a typical week. Adjusted for age, weight, gender, and neuter status.

and in the urine of the dog in the same household ($P = 0.056$). Of 23 households in which MCPP was detected in the grass residue after treatment, MCPP was also detected in dog urine in 13 (56%) households. There was a significant association between finding MCPP in the grass residue and MCPP in the urine of the dog in the same household ($P < 0.02$). Of 13 households in which dithiopyr was detected in grass residue after treatment, the chemical was present in the urine of 1 (7%) dog.

A likely explanation for the presence of chemicals on untreated lawns is airborne drift from surrounding treated areas, especially from herbicides applied on windy days. Although lawn chemical drift has not been well characterized, agricultural herbicide drift has been widely recognized (Egan and Mortensen, 2012; Ward et al., 2006). In addition, in the grass plot study, overspary was detected up to 25 m from the treated areas. It is expected that under windy conditions, drift would be even more widespread. In the household study, the chemical applicators did not report any restrictions on spraying related to wind conditions.

Taken together, the results of this study indicate that dogs can be exposed to lawn chemicals in at least 3 ways: (1) through contact with chemicals intentionally applied to their household lawn, especially if the dog is not kept off of the lawn appropriately, (2) through contact with chemicals on their "untreated" household lawn which has been contaiminated by airborne chemical drift from adjancent treated areas, and (3) through the dog traveling to other treated areas. Furthermore, in a scenario when neither the herbicides nor the pets are restricted to a particular area, it would be difficult to prevent chemical exposure to the dog.

In addition to affecting the health of pet dogs, a serious concern from the results of this study is that herbicide exposures detected in dogs could translate into human exposures as well. Herbicide exposure has been well documented in farm workers (Arcury et al., 2010) and professional chemical applicators (Harris et al., 2010), and the potential chemical exposure to humans from sports fields has been raised (Gilden et al., 2012). Studies of herbicide exposure levels to adults and children participating in sports and other activities that involve close and frequent contact with treated grass, however, have not been reported. An additional concern regarding lawn chemical exposures is that chemicals (e.g. 2,4-D, diazinon) can be tracked into the house from the yard by pets and people (Morgan et al., 2008; Nishioka et al., 2001) leading to further opportunties for chemical exposure.

The 3 chemicals measured in the household study were selected in large part because of their frequent inclusion in lawn treatment mixtures and not because of a potential role in the development of UC. The carcinogenic activity of specific chemicals and solvents used for chemical dilution and distribution requires further study. It is of note that the "active" weed killing chemicals comprise only a small percentage by volume of the total product applied. In the grass plot study, for example, the active ingredients comprised <0.2% of the total product applied. The possibility that "inert" organic solvents, other components of herbicide formulations, and potential contaminants (Hardell, 2008) may contribute to their toxicity cannot be ruled out. An additional factor to consider in potential carcinogenic effects of lawn chemicals is that enzyme systems exist in plants which can convert the herbicides to more toxic intermediates *in planta* (Murphy and Taiz, 1999).

4. Conclusions

In conclusion, the results of this study: (1) suggest widespread exposure of pet dogs to commonly used lawn chemicals and the presence of chemicals in urine where contact with urothelium would occur, (2) raise concern for potential human exposure from lawn chemicals, (3) show persistence of lawn chemicals for at least 48 h after application, with longer residence times under certain environmental conditions, (4) provide justification to restrict dogs' access to treated lawns, (5) confirm that substantial amounts of chemicals can be present on "untreated" lawns, and (6) justify continued investigation of the health effects, including cancer risk, associated with the chemicals found in lawn care products.

Conflict of Interest

None of the authors have any conflicts of interest regarding the study or financial support for the study.

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