



# Widespread occurrence of glyphosate in urine from pet dogs and cats in New York State, USA



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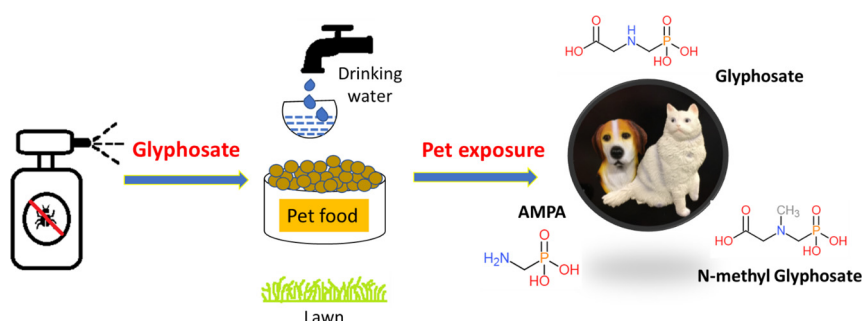
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## HIGHLIGHTS

- Glyphosate and its derivatives were measured in dog and cat urine for the first time.
- Glyphosate was found to be widespread in the urine of dogs and cats.
- Glyphosate concentration in cat urine was 2-fold higher than that in dogs.
- Exposure levels of glyphosate in dogs and cats were 2–4 orders of magnitude below the current ADI.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

### Article history:

Received 19 November 2018

Received in revised form 29 December 2018

Accepted 29 December 2018

Available online 31 December 2018

Editor: Jay Gan

### Keywords:

Glyphosate exposure

Pet animals

Dog urine

Cat

Herbicide

AMPA

## ABSTRACT

Glyphosate is one of the most widely used herbicides in the United States, which has led to its ubiquitous occurrence in food and water and regular detection in human urine at concentrations of 1–10 µg/L. Data pertaining to health risks arising from the ingestion of glyphosate are limited and are the subject of much debate, which demands the need for more exposure information for this herbicide. Very little is known about glyphosate exposure in pets. In this study, we determined concentrations of glyphosate (Glyp) and its derivatives, methyl glyphosate (Me-Glyp) and aminomethylphosphonic acid (AMPA), in urine collected from 30 dogs and 30 cats from New York State, USA. Glyp was the most predominant compound found in pet urine followed by AMPA and Me-Glyp. The mean urinary concentration of  $\sum$  Glyp (sum of Glyp + Me-Glyp + AMPA) in cats (mean: 33.8 ± 46.7 ng/mL) was 2-fold higher than that in dogs (mean: 16.8 ± 24.4 ng/mL). Cumulative daily intakes (CDI) of Glyp in dogs and cats estimated from the urinary concentrations were, on average, 0.57 and 1.37 µg/kg bw/d, respectively. The exposure doses were two to four orders of magnitude below the current acceptable daily intake (ADI) suggested by several international health organizations for humans.

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## 1. Introduction

Glyphosate (Glyp) is a non-selective herbicide (Curwin et al., 2005; Niemann et al., 2015) used heavily in agriculture, lawns, parks and home gardens in the United States (USA) and across the globe (Centner et al., 2019; Vandenberg et al., 2017). In 2014, the total global usage of Glyp was 8.9 million tons, with the USA accounting for 19% of

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the total global usage (Benbrook, 2016). Furthermore, Glyph accounts for 59% of the total pesticide usage in the USA. Glyph residues were reported to occur in foods from the United Kingdom (U.K.) and the USA and in fruits, vegetables, drinking water, surface water, wastewater, sediments, beer, tea, grains, human milk and indoor dust from several countries (Bai and Ogbourne, 2016; Chen et al., 2013; Curwin et al., 2005; Goen et al., 2017; Kolpin et al., 2006; Koskinen et al., 2016; Myers et al., 2016; Nagatomi et al., 2013; Osten and Dzul-Caamal, 2017; Schrubbers et al., 2016; Steinborn et al., 2016; Van Bruggen et al., 2018). However, few studies have reported exposure of humans to Glyph and its major metabolite, aminomethylphosphonic acid (AMPA), in urine (Conrad et al., 2017; Goen et al., 2017; Mills et al., 2017). Conrad et al. (2017) reported gradual increase in Glyph exposure in German adults between 2001 and 2015, and a 13-fold increase in Glyph exposure was reported in American adults from 1993 to 2016 (Mills et al., 2017). These studies suggest that there has been an increase in the usage and concomitant human exposure to Glyph from the environment.

Studies have shown that exposure to glyphosate instigates acute kidney injuries (AKI) in animals and humans (Seok et al., 2011; Wunnapuk et al., 2014). Exposure to Glyph in utero has been linked to fetal loss in mammals, chickens and frogs (Antonioni et al., 2012; Parvez et al., 2018). Nevertheless, information pertaining to health effects, including carcinogenic potential of Glyph, is controversial and debated (Centner et al., 2019). In 2017, the International Agency for Research on Cancer (IARC) classified Glyph as a “probable human carcinogen”. However, the US Environmental Protection Agency (EPA), the European Food Safety Authority (EFSA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) suggested the need for more biomonitoring and epidemiological studies to establish guidelines and to enable regulatory actions on Glyph (Centner et al., 2019).

Pet animals, particularly dogs and cats, have been used as sentinels of human exposure to emerging environmental chemicals (Reif, 2011). The USA has the largest number of pet animals globally (Karthikraj et al., 2018a; Statista, 2018). Earlier studies have linked chemical exposure in pets to major diseases such as hypothyroidism, obesity, kidney diseases and cancers (Cocchi et al., 2010; Peterson, 2012; Banfield Pet Hospital, 2016; Association for Pet Obesity Prevention, 2018). Few studies have reported the prevalence of pesticide exposure in pet dogs and cats (Caloni et al., 2016; Forster et al., 2014; Siqueira et al., 2015).

Glyph is used in home gardens and lawns, and exposure of pet animals from such use is plausible (Annett et al., 2014). Two recent studies reported the occurrence of Glyph in pet foods (Samsel and Seneff, 2015; Zhao et al., 2018), and a report from Italy indicated the prevalence of emergency calls related to glyphosate exposure in pet animals (Caloni et al., 2016). However, to our knowledge, no earlier studies have reported the occurrence of Glyph in pet urine. In this study, glyphosate and its derivatives were measured in urine of pet dogs and cats for the first time, to elucidate exposure levels and profiles.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Analytical standards of Glyph and AMPA were purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and Sigma-Aldrich (St. Louis, MO, USA), respectively. Methyl glyphosate or N-methyl-N-(phosphonomethyl)glycine (Me-Glyp) was purchased from Toronto Research Chemicals (North York, ON, Canada). The physicochemical properties of the target chemicals are listed in Table S1 (supporting information). The internal standards (IS) for Glyph (2-<sup>13</sup>C, 99%; <sup>15</sup>N, 98 + %) and AMPA (<sup>13</sup>C, 99%; <sup>15</sup>N, 98%; methylene D2) were purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and Cerilliant (Round Rock, TX, USA), respectively. Formic acid and ammonium hydroxide solution were purchased from Sigma-Aldrich (St. Louis, MO,

USA) and HPLC grade solvents (methanol, acetonitrile and water) were purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA).

### 2.2. Sample collection and preparation

Pet urine samples (n = 60) were collected in the Albany area of New York State, USA, during March to July 2017; further details of the samples are shown in Fig. 1. The urine samples were collected directly in polypropylene (PP) containers (for dogs) and/or by cystocentesis (for cats). Majority of the samples were originally collected for routine clinical diagnosis at the veterinary hospitals and animal shelters. We obtained an aliquot of those urine samples for our investigation. Information such as breed, specific gravity (SG), and creatinine concentration of urine has been reported elsewhere (Karthikraj et al., 2018b). Glyph and its derivatives were extracted from pet urine by a mixed-mode solid phase extraction method, with slight modifications of the methods reported by Connolly et al. (2017) and Karthikraj et al. (2018b). Briefly, 250 µL of urine, procedural blank and quality control samples were spiked with 20 ng each of the ISs. Samples were then vortexed, basified by the addition of 3 mL of 1% ammonium hydroxide in water, and kept at room temperature for 20 min. The basified urine sample was loaded onto an Oasis MAX cartridge (3 cc, 60 mg, 30 µm; Waters, Milford, MA, USA) at ~1 mL/min, which was pre-conditioned with 3 mL of methanol, 3 mL of water and 2 mL of 1% ammonium hydroxide, in that sequence. The cartridge was washed with 2 mL of 1% ammonium hydroxide and allowed to dry under vacuum. The target chemicals were eluted using 3 mL of 1% formic acid in methanol, and the eluent was evaporated to near dryness under a nitrogen stream. The residue was reconstituted in 250 µL of the HPLC mobile phase solution as described below, vortexed, centrifuged, and transferred into a polypropylene auto-sampler vial.

### 2.3. Instrumental analysis

An Agilent 1260 HPLC system coupled with an ABSciex 4500 Q-trap mass spectrometer (Applied Biosystems, Framingham, MA, USA) under the negative mode of electrospray ionization was used in the identification and quantification of target chemicals. A few studies have used an ion chromatographic (IC) column for the direct measurement (without

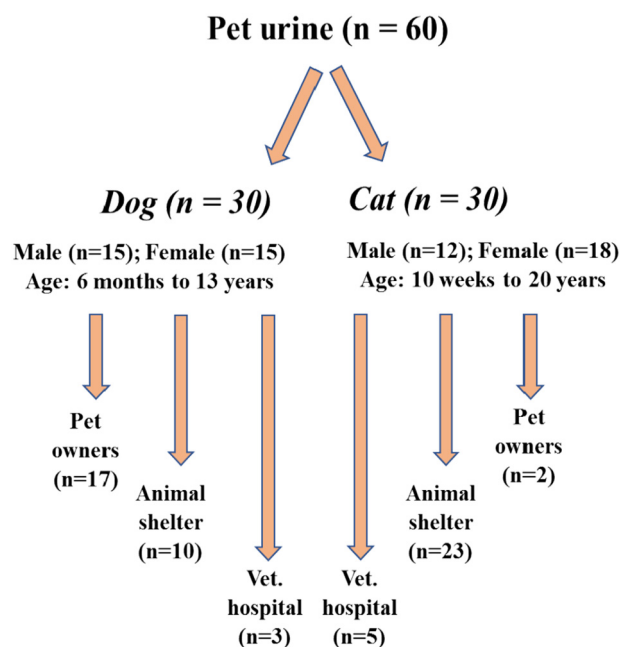


Fig. 1. Grouping of pet urine samples collected in this study for glyphosate analysis (sample size, gender, age, and source of pet urine collection).

**Table 1**  
Concentrations and detection rates (*dr* %) of glyphosate and its derivatives in urine of pet dogs and cats from the Albany area of New York State, United States. SG and creatinine values were previously reported (Karthikraj et al., 2018b).

	SG	Creatinine (mg/dL)	Glyphosate	AMPA	Methyl Glyphosate	∑Glyp
<b>Dogs (n = 30)</b>						
Mean (ng/mL)	1.035 ± 0.02	138 ± 90	13.0 ± 13.3	3.6 ± 10.3	0.4 ± 0.8	16.8 ± 24.4
Median (ng/mL)	1.037	119	7.0	<LOQ	<LOQ	7.0
Range (ng/mL)	1.002–1.089	25–355	0.4–49.1	<LOQ–55.8	<LOQ–3.5	1.2–94.8
Creatinine adjusted (µg/g)	–	–	10.6 ± 11.3	3.4 ± 6.6	0.5 ± 1.4	14.5 ± 19.3
SG adjusted (ng/mL)	–	–	13.4 ± 12.0	4.4 ± 9.2	0.7 ± 2.0	18.5 ± 23.1
<i>dr</i> (%)	–	–	100	43.3	26.7	–
<b>Cats (n = 30)</b>						
Mean (ng/mL)	1.054 ± 0.02	150 ± 85	20.8 ± 23.5	10.0 ± 5.0	3.1 ± 10.6	33.8 ± 46.7
Median (ng/mL)	1.061	140	17.8	5.00	<LOQ	22.8
Range (ng/mL)	1.011–1.080	25–312	<LOQ–111.0	<LOQ–48.1	<LOQ–52.1	<LOQ–193.2
Creatinine adjusted (µg/g)	–	–	16.3 ± 17.5	8.4 ± 12.9	2.2 ± 7.4	26.9 ± 37.8
SG adjusted (ng/mL)	–	–	19.7 ± 18.8	12.5 ± 20.6	2.3 ± 8.0	34.5 ± 47.3
<i>dr</i> (%)	–	–	86.7	63.3	13.3	–

derivatization) of Glyp in beer, barley tea and foodstuffs (Nagatomi et al., 2013; Adams et al., 2017). We optimized the use of a Dionex IonPac AS21 IC column (250 mm × 2.0 mm, 7 µm; Thermo Fisher Scientific, Waltham, MA, USA) serially connected to a guard column (Dionex IonPac AS21, 2 × 50 mm) for the simultaneous measurement of Glyp, AMPA and Me-Glyp. An isocratic elution was used for the separation of target chemicals by employing 1% formic acid in a mixture of acetonitrile/water (5/95; v/v) as the mobile phase. The mobile phase flow rate and sample injection volume were 400 µL/min and 50 µL, respectively (total run time: 10 min). Quantification was achieved through an isotope dilution method. The optimized mass spectrometric parameters and MRM transitions for all target analytes are listed in Table S1. A typical extracted ion chromatogram (EIC; from matrix spike experiment) of the target chemicals is presented in Fig. S1.

#### 2.4. Quality assurance and quality control (QA/QC)

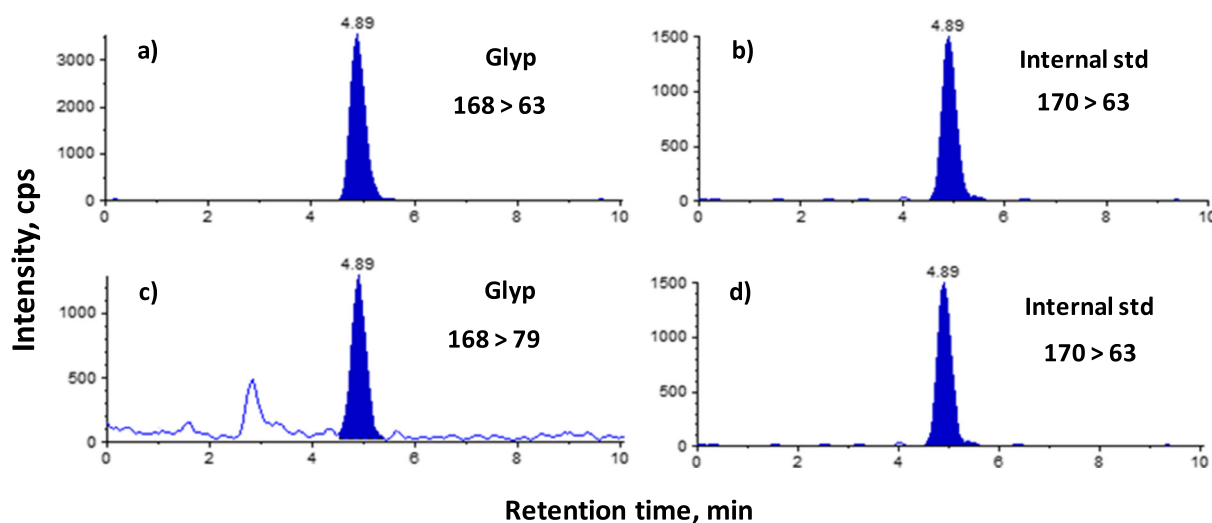
An 11-point calibration curve ranging in concentrations of target chemicals from 0.2 to 500 ng/mL showed correlation coefficients ( $r^2$ ) of >0.995. For every 10 samples, a blank solvent (methanol:water; 1:1 ratio by v/v) and a mid-point calibration standard (10 ng/mL) were injected to check for carryover of target chemicals between samples and drift in instrumental sensitivity. A procedural blank, matrix blank (pooled urine) and two levels of matrix spike (pooled urine spiked at 20 and 40 ng each of target chemicals) were analyzed with every 15

samples. None of the target chemicals was detected in procedural blanks and matrix blanks. The respective mean ( $\pm$ SD) recoveries of target chemicals spiked into pooled urine at two levels were  $109 \pm 0.2$  and  $116 \pm 1.7\%$  for Glyp,  $101 \pm 2.1$  and  $117 \pm 0.6\%$  for AMPA, and  $119 \pm 1.4$  and  $107 \pm 6.5\%$  for Me-Glyp. The limits of detection (LODs) and limits of quantification (LOQs) were calculated as the signal-to-noise ratio of  $\geq 3$  and  $\geq 10$ , respectively. The LODs and LOQs were 0.15 and 0.5 ng/mL for both Glyp and AMPA, respectively, and 0.3 and 1 ng/mL for Me-Glyp, respectively. In this study,  $\sum$ Glyp represents sum concentrations of Glyp, AMPA and Me-Glyp. Data analyses were performed using Microsoft Excel 2016 and SPSS (version 22). Statistical significance was set at  $p \leq 0.05$ .

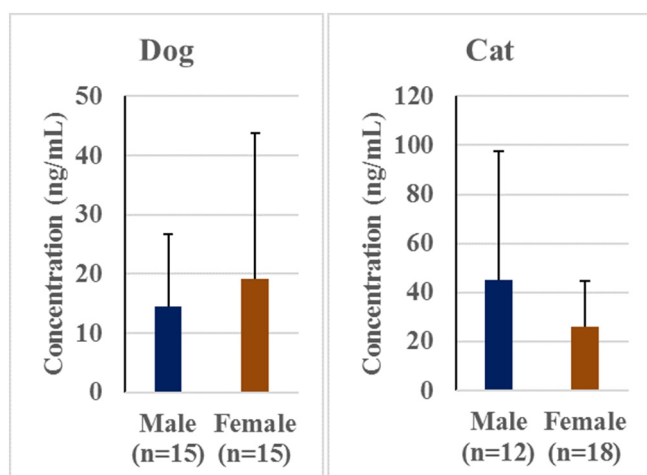
### 3. Results

#### 3.1. Concentrations and detection frequencies

Concentrations and detection frequencies (*dr* %) of target analytes found in pet urine are shown in Table 1. Although earlier studies have measured AMPA as a metabolite of Glyp in human urine, this is the first study that measures Me-Glyp in urine. Me-Glyp is a synthetic impurity and is thought to be present in Glyp mixture (Kwiatkowska et al., 2016). Glyp was the most abundant chemical found in the urine of dogs (*dr*: 100%) and cats (*dr*: 86.7%) at mean concentrations ( $\pm$ SD) of 13.0 ( $\pm$ 13.3) and 20.8 ( $\pm$ 23.5) ng/mL, respectively. Typical



**Fig. 2.** Typical LC-MS/MS chromatograms (a and c) of glyphosate (quantifier: 168 > 63 and qualifier: 168 > 79) and its corresponding labelled internal standards (b and d; 170 > 63) in a dog urine.



**Fig. 3.** Gender differences in  $\Sigma$ Glyp (sum of Glyp + AMPA + Me-Glyp) concentrations (mean: ng/mL) in urine of dogs and cats.

chromatograms (including both quantifier and qualifier ion transitions along with the IS) of Glyp in a dog urine (sample D10) are shown in Fig. 2. AMPA was the second most abundant chemical, found at a mean concentration of 3.6 ( $\pm 10.3$ ) ng/mL in pet dogs, which was 3.6 times lower than Glyp concentrations. The measured mean concentration of AMPA in cats was 10.0 ( $\pm 5.0$ ) ng/mL, which was 2 times lower than that of Glyp. No significant correlation existed between Glyp and AMPA concentrations in pet urine ( $p > 0.05$ ). This may be explained by a difference in metabolic rates between the two species of pets as well as existence of other sources of AMPA in pets (Grandcoin et al., 2017). Overall,  $\Sigma$ Glyp concentrations ranged from 1.2 to 94.8 ng/mL in dog urine and from <LOQ to 193 ng/mL in cat urine. The mean concentration of  $\Sigma$ Glyp in urine from cats was significantly higher than those in dogs, by 2-fold (two-tailed,  $p = 0.027$ ). The creatinine and SG normalized concentrations of  $\Sigma$ Glyp in cat urine were higher than those of dogs (Table 1). However, no significant correlation existed between creatinine/SG and Glyp concentrations in the urine of pet animals. Among the three age groups of cats and dogs, a notable pattern was found in the urinary Glyp concentrations. As creatinine level in urine increased,  $\Sigma$ Glyp concentration also increased, as we found earlier for melamine in the same set of samples (Karthikraj et al., 2018b).

### 3.2. Age and gender variations

We categorized dogs and cats into 3 different age groups (Karthikraj et al., 2018a and 2018b) for the comparison of concentrations. Dogs were grouped as (i) 0.5–2 years ( $n = 11$ ), (ii) >2–5 years ( $n = 8$ ),

and (iii) >5–13 years ( $n = 11$ ). Cats were grouped as (i) 0.2–2 years ( $n = 12$ ), (ii) >2–5 years ( $n = 7$ ), and (iii) >5–20 years ( $n = 11$ ). In both dogs and cats,  $\Sigma$ Glyp concentrations in urine decreased with increasing age, with young puppies and kittens exhibiting higher concentrations of Glyp than older animals. Regarding gender, clear differences existed in urinary Glyp concentrations of 15 male and 15 female dogs, as well as those of 12 male and 18 female cats. The  $\Sigma$ Glyp concentrations were 1.3 times higher in female dogs than in male dogs, whereas an opposite pattern was observed in cats, which showed 1.7 times higher concentrations in males than in females (Fig. 3). Nevertheless, these differences were not statistically significant.

### 3.3. Exposure assessment

We calculated cumulative daily intakes (CDI) of Glyp for cats and dogs based on the concentrations measured in their urine (supplementary information). Age, body weight, and daily urine excretion rates used in CDI calculations were reported in our previous publication (Karthikraj et al., 2018b).

A rodent exposure study showed that Glyp was excreted unchanged (>98%) in urine. Therefore, we assumed that Glyp is fully excreted unchanged in the urine of pets (Panzacchi et al., 2018). The calculated CDI values of Glyp in small dogs were higher (0.76  $\mu$ g/kg bw/d) than those of large dogs (0.55  $\mu$ g/kg bw/d) (Table 3). The highest CDI values in cats were found for medium-sized cats (1.69  $\mu$ g/kg bw/d), followed by those of small (1.51  $\mu$ g/kg bw/d) and large cats (0.92  $\mu$ g/kg bw/d) (Table 3). Overall, the average Glyp exposure in dogs was approximately 2.5-fold lower than that in cats (Table 3).

Several international health/environmental agencies have proposed acceptable daily intake (ADI) values for Glyp. We considered the ADI values proposed by the US EPA, Food Safety Commission of Japan (FSCJ), the European Food Safety Authority (EFSA), and the Australian Pesticides and Veterinary Medicines Authority (APVMA) for comparison against CDI values calculated for pets (FSCJ, 2016; Zhao et al., 2018) (Table 4). We calculated the hazard quotients (HQ) of Glyp by taking the ratio of CDI to ADI. Overall, the current exposure doses (both mean CDI and 95th percentile) of glyphosate in dogs and cats were two to four orders magnitude below the ADI recommended for humans (Table 4). Cats were at 3–4 times higher risk from Glyp exposure than dogs. It should be noted that all ADI values other than those provided by the APVMA are proposed for humans, and it is not known if these guideline values are protective of pets.

## 4. Discussion

The high detection rates of Glyp in pet urine suggests occurrence of this herbicide in pet foods, drinking water and dermal/inhalation exposure from the use of this compound in lawns, public parks and home

**Table 2**  
Summary of urinary concentrations of glyphosate reported for human populations.

Country	Sample size (n)	Glyphosate	Mean (ng/mL)	Range (ng/mL)	Detection rate (%)	Reference
Ireland	50 adults	Glyp	Median (Glyp): 0.87	0.80–1.35	Glyp: 20	Connolly et al. (2018a)
European countries	182 people from 18 European countries	Glyp and AMPA	Mean (Glyp): 0.21; Mean (AMPA): 0.18	Glyp: <0.15–1.56; AMPA: <0.15–2.63	Glyp: 80 AMPA: 65	Hoppe (2013)
Germany	199 adults (considered population only from 2011 to 2015)	Glyp and AMPA	Median (Glyp): 0.13; Median (AMPA): <0.11	Glyp: <0.10–0.16; AMPA: <0.1–0.18	Glyp: 40 AMPA: 42.5	Conrad et al. (2017)
USA	100 older adults (considered population only from 2014 to 2016)	Glyp and AMPA	Mean (Glyp): 0.314; Mean (AMPA): 0.285	Not available	Glyp: 70 AMPA: 71	Mills et al. (2017)
USA	71 pregnant women	Glyp	Mean (Glyp): 3.4	Glyp: <0.5–7.20	Glyp: 93	Parvez et al. (2018)

**Table 3**  
Cumulative daily intakes (CDI) of glyphosate at average and worst-case scenarios (95th percentile) for dogs and cats ( $\mu\text{g}/\text{kg}$  bw/d) with respect to body size or age<sup>a</sup>.

Dog Size group	CDI ( $\mu\text{g}/\text{kg}$ bw/d)		Cat Age group	CDI ( $\mu\text{g}/\text{kg}$ bw/d)	
	Glyph			Glyph	
	Mean	95th		Mean	95th
Small	0.76	1.70	10–12 weeks	1.51	4.55
Medium	0.40	1.00	3–6 months	1.69	2.75
Large	0.55	1.70	$\geq 1$ year	0.92	2.62
Average	0.57	1.45	Average	1.37	3.30

<sup>a</sup> Size groupings: For dogs, small: 5.9–7.7 kg; medium: 17.2–25 kg; and large: 34 kg and above. For cats, small: 10–12 weeks (0.8–1 kg); medium: 3–6 months (1.4–2 kg); and large:  $\geq 1$  year (2–6.8 kg) (Karthikraj et al., 2018b).

gardens. Several studies have documented the occurrence of Glyph in human food products in the USA (FDA, 2016; Vandenberg et al., 2017). The majority (>90%) of corn, soybean, and canola grown in the USA are Glyph-resistant, suggesting that the production of these food crops entail use of glyphosate (USDA, 2014). Samsel and Seneff (2015) reported the occurrence of Glyph and AMPA in 9 cat and dog foods. Recently, Zhao et al. (2018) reported widespread occurrence of Glyph in commercially available pet foods (18 foods from 8 different manufacturers) sold in the USA. Notably, Glyph was detected in all pet food samples (78.3 to 2140  $\mu\text{g}/\text{kg}$  dry weight) at a median concentration of 198  $\mu\text{g}/\text{kg}$  dry weight, which was higher than the Glyph concentration reported in human foods. Although plant-based ingredients were suggested as a source for Glyph in pet foods, meat and fish-based pet foods also contained notable concentrations of Glyph. The findings of measurable concentrations of Glyph in pet urine are supported by sources originating from pet foods. Similarly, several studies have shown that drinking water is a major source of exposure to Glyph (Centner et al., 2019; Bai and Ogbourne, 2016; Myers et al., 2016; Osten and Dzul-Caamal, 2017). Exposure of pets to Glyph from its use in domestic lawns also cannot be ruled out. Glyph is strongly retained in soils, and therefore lawn soils can contain Glyph residues (Myers et al., 2016). Although dogs may spend longer time in lawns and other outdoor activities, we found higher concentrations of Glyph in cats than in dogs. These results suggest pet food as the major source of Glyph. Pesticides such as DDT were reported to be present at higher concentrations in cats than dogs (Ali et al., 2013; Kunisue et al., 2005).

In human biomonitoring studies, Glyph was the major compound found in urine, in comparison to AMPA (Conrad et al., 2017; Mills et al., 2017). A similar pattern of higher concentrations and detection rates for Glyph than AMPA, was found in pet urine. AMPA is a metabolite of Glyph found in plants and soils (Bai and Ogbourne, 2016; Niemann et al., 2015), and can also be formed through the degradation of amino polyphosphonates (used as membrane anti-fouling agents) (Grandcoin et al., 2017). Considering that its toxicity is comparable to that of Glyph, it is necessary to include AMPA in future human and pet animal biomonitoring studies (Bai and Ogbourne, 2016). Anadon et al.

**Table 4**  
Hazard quotients (HQ) calculated from exposure to glyphosate at mean and 95th percentile concentrations in pet dogs and cats on the basis of acceptable daily intakes (ADI) proposed by the U.S. EPA, FSCJ-Japan, EU-EFSA and Australia-APVMA, for humans (Zhao et al., 2018).

International agency	ADI ( $\mu\text{g}/\text{kg}$ bw/d)	Glyph (mean <sup>a</sup> )		Glyph (95th percentile)	
		HQ (pet dogs)	HQ (pet cats)	HQ (pet dogs)	HQ (pet cats)
		U.S. EPA	1750	0.0003	0.0008
FSCJ-Japan	1000	0.0006	0.0014	0.0014	0.0033
EFSA-EU	500	0.0011	0.0027	0.0029	0.0066
Australia-APVMA	300	0.0019	0.0046	0.0048	0.0110

<sup>a</sup> Mean is the calculated CDI based on mean concentration (ng/mL).

(2009) reported that both Glyph and AMPA have short half-lives of between 3 and 15 h in rats, and are therefore excreted without any structural change in urine. A recent study by Connolly et al. (2018b) estimated that the average biological half-life of Glyph in humans ranged from 5½ to 10 h, which further suggests that urine is an ideal matrix for biomonitoring of Glyph exposure.

Human biomonitoring studies of Glyph have focused mostly on occupationally or incidentally exposed populations such as farmers (Connolly et al., 2017; Jayasumana et al., 2015). A few studies have reported urinary levels of Glyph in the general human population. The reported concentrations of Glyph in human urine were comparable or lower than those found in dogs and cats (Table 2). Another study reported that Glyph exposure in the United States population was 10 times higher than that of Europe (Honeycutt and Rowlands, 2014) (Table 2). Parvez et al. (2018) reported a mean Glyph concentration of 3.4 ng/mL in pregnant women from Central Indiana, USA, which was the highest among the studied populations (Table 2). Glyph was found only in 20% of urine samples from an Irish population with a median concentration of 0.87 ng/mL (Connolly et al., 2018a). Based on the mean concentrations reported for humans, it can be inferred that dogs and cats were 4–41 and 6–66-times, respectively, more highly exposed to Glyph than humans (comparison of mean values from Tables 1 and 2). For the Irish and German populations, only median urinary Glyph concentrations (Table 2) were reported and therefore we compared median values of dog and cat urine (Table 1) and found 8–54 and 20–137 fold, respectively, higher concentrations in pets. Kruger et al. (2014) reported the occurrence of Glyph residues in urine from cows and rabbits, and indicated that exposures in these animals were higher than those of humans. Zhao et al. (2018) also stated that exposure doses of Glyph in pet animals were higher than those in humans. These studies further support the high detection rates and concentrations of Glyph found in pet animals in our study.

## 5. Conclusions

For the first time, we demonstrate notable exposure of pets to glyphosate, through a biomonitoring approach. The measured urinary concentrations of glyphosate in cats were 2-fold higher than those in dogs. The high detection rate of glyphosate in pet urine suggests widespread exposure of pets through food, drinking water and outdoor activities. Age and gender-related differences existed in urinary concentrations of Glyph in pets. The current exposure doses of glyphosate in pets were two to four orders of magnitude below the acceptable daily intakes recommended for humans. Further studies are needed to assess the toxicity of Glyph in pet animals.

## Declarations

The authors declare no conflict of interest.

## Acknowledgements

We thank Ms. Sunmi Lee and other volunteers for help with pet urine collection. We also thank the Saratoga County Animal Shelter (Saratoga County, New York, USA) and the staff of Central Veterinary Hospital (Albany, New York, USA) for providing pet urine samples.

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2018.12.454>.

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