Taylor & Francis
Taylor & Francis Group

Inhalation Toxicology

International Forum for Respiratory Research

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/iiht20

Pilot study evaluating inhalation and dermal glyphosate exposure resulting from simulated heavy residential consumer application of Roundup®

Jennifer S. Pierce , Benjamin Roberts , Daniel G. Kougias , Chris E. Comerford , Alexander S. Riordan , Kara A. Keeton , Heidi A. Reamer , Neva F. B. Jacobs & Jason T. Lotter

To cite this article: Jennifer S. Pierce , Benjamin Roberts , Daniel G. Kougias , Chris E. Comerford , Alexander S. Riordan , Kara A. Keeton , Heidi A. Reamer , Neva F. B. Jacobs & Jason T. Lotter (2020): Pilot study evaluating inhalation and dermal glyphosate exposure resulting from simulated heavy residential consumer application of Roundup® , Inhalation Toxicology, DOI: 10.1080/08958378.2020.1814457

To link to this article: https://doi.org/10.1080/08958378.2020.1814457

Published online: 06 Sep 2020.

INHALATION TOXICOLOGY
https://doi.org/10.1080/08958378.2020.1814457

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Pilot study evaluating inhalation and dermal glyphosate exposure resulting from simulated heavy residential consumer application of Roundup®

Jennifer S. Pierce[a], Benjamin Roberts[a], Daniel G. Kougias[a], Chris E. Comerford[a], Alexander S. Riordan[a], Kara A. Keeton[a], Heidi A. Reamer[a], Neva F. B. Jacobs[b] and Jason T. Lotter[a]

[a]Cardno ChemRisk, Chicago, IL, USA; [b]Cardno ChemRisk, Arlington, VA, USA

ABSTRACT

Objectives: The purpose of this study was to evaluate the individual contributions of inhalation and dermal exposures to urinary glyphosate levels following the heavy residential consumer application of a glyphosate-containing herbicide.

Methods: A pilot study was conducted in which each participant mixed and continuously spray-applied 16.3 gallons of a 0.96% glyphosate-containing solution for 100 min using a backpack sprayer. Twelve participants were divided evenly into two exposure groups, one equipped to assess dermal exposure and the other, inhalation exposure. Personal air samples ($n = 12$) and dermal patch samples ($n = 24$) were collected on the inhalation group participants and analyzed for glyphosate using HPLC-UV. Serial urine samples collected 30-min prior to application and 3-, 6-, 12-, 24-hr (inhalation and dermal groups) and 36-hr (dermal group only) post-application were analyzed for glyphosate and glyphosate's primary metabolite (AMPA) using HPLC-MS/MS.

Results: The mean airborne glyphosate concentration was 0.0047 mg/m^3, and the mean concentrations of glyphosate for each applicator's four patch samples ranged from 0.04 µg/mm^2 to 0.25 µg/mm^2. In general, urinary glyphosate, AMPA, and total effective glyphosate levels were higher in the dermal exposure group than the inhalation exposure group, peaked within 6-hr following application, and were statistically indistinguishable from background at 24-hr post-application.

Conclusions: This is the first study to characterize the absorption and biological fate of glyphosate in residential consumer applicators following heavy application. The results of this pilot study are consistent with previous studies that have shown that glyphosate is rapidly eliminated from the body, typically within 24 hr following application.

ARTICLE HISTORY
Received 24 January 2020
Accepted 14 August 2020

KEYWORDS
Glyphosate; Roundup; toxicokinetics; exposure

Introduction

According to usage estimates through 2012, glyphosate was the most common active ingredient in herbicides used in the agricultural industry and the second most common active ingredient in herbicides used in the home and garden sector of the United States (U.S. EPA 2017). In 2015, the International Agency for Research on Cancer (IARC) classified glyphosate as '*probably carcinogenic to humans*' (i.e. a Group 2A carcinogen), based on '*limited evidence*' of cancer (e.g. non-Hodgkin lymphoma) in humans and '*sufficient evidence*' of cancer in experimental animals. However, based on its own review, the U.S. Environmental Protection Agency (U.S. EPA) classified glyphosate as a Group D chemical (i.e. not classifiable as to human carcinogenicity) and concluded that there are '*no risks to the public health from the current registered uses of glyphosate*' (U.S. EPA 1987, 2019a). The U.S. EPA's conclusion is consistent with the conclusions from other domestic and international agencies, including the U.S. Department of Agriculture (USDA), United Nations (UN) agencies [e.g. the World Health Organization (WHO), of which IARC is a part of, and the Food and Agriculture Organization (FAO)], European Union (EU) agencies [e.g. the European Food Safety Authority (EFSA) and the European Chemicals Agency (ECHA)], Health Canada, German Federal Institute for Occupational Safety and Health, Food Safety Commission of Japan, Australian Pesticides and Veterinary Medicines Authority, and the New Zealand Environmental Protection Agency (NZEPA) (Williams et al. 2000; SERA 2011; APVMA 2016; Tarazona et al. 2017; U.S. EPA 2017, 2019a, 2019b). While the use of glyphosate-containing herbicides is permitted in a majority of countries globally, albeit to some capacity, full bans of glyphosate-containing products have been enacted in Vietnam and member nations of the Gulf Cooperation Council. Luxembourg and Germany announced their intention to ban all products containing glyphosate beginning in 2020 and 2023, respectively. Glyphosate use has also been curtailed in multiple countries, including in Belgium, Bermuda, Czech Republic, Colombia, France, Italy, Mexico, the Netherlands, Portugal, and Sri Lanka. While

CONTACT Jennifer S. Pierce jennifer.pierce@cardno.com Cardno ChemRisk, Chicago, IL, USA

Thailand and Austria previously announced bans on glyphosate-containing products, both bans were later reversed.

To date, no health-based occupational exposure standard exists for glyphosate in the United States (ATSDR 2019). Specifically, the U.S. Occupational Safety and Health Administration (OSHA) has not established a permissible exposure limit (PEL) for glyphosate, and the American Conference of Governmental Industrial Hygienists (ACGIH) has yet to determine a threshold limit value (TLV) (although glyphosate is currently listed as a Tier 1 chemical '*Under Study*' by the ACGIH). The U.S. EPA has established an oral reference dose (RfD) of 2 mg/kg/day for glyphosate based on the maternal no observed effect level of 175 mg/kg/day from a rabbit developmental toxicity study and an uncertainty factor of 100 (U.S. EPA 1993; ATSDR 2019). However, to date the U.S. EPA has not determined a reference concentration (RfC) for the inhalation of glyphosate (ATSDR 2019).

Limited information regarding the toxicokinetics of glyphosate following inhalation, oral, or dermal exposures exists (ATSDR 2019). Multiple studies reported dermal absorption of glyphosate as the primary exposure route during application (Acquavella et al. 2004; Connolly, Coggins, et al. 2019), despite evidence indicating low rates of skin penetration (< 5%) when administered dermally as a diluted aqueous solution (on monkeys or using an *in vitro* human skin model) (Wester et al. 1991; Lavy et al. 1992; Wester et al. 1996). Exposure via inhalation is thought to be low in humans (Jauhiainen et al. 1991). Following absorption, glyphosate does not accumulate in the organs or tissues of humans or rats and is rapidly excreted in urine and feces, principally as the parent compound (ATSDR 2019). While glyphosate does not undergo significant metabolism in humans (IARC 2015), a small amount (< 1%) is metabolized possibly by gut microbiota in mammals, including humans, to aminomethylphosphonic acid (AMPA), which is similarly excreted in urine and feces (U.S. EPA 1993; Williams et al. 2000; ATSDR 2019, 2020). The arithmetic mean biological half-life of glyphosate has been estimated to be 5.5, 10, and 7.25 h, respectively, based on unadjusted, creatinine-adjusted, and urinary excretion rate-adjusted urine samples collected from seven horticultural workers who applied glyphosate-containing products (Connolly, Jones, et al. 2019). Connolly and colleagues cautioned that '*[a]lthough elimination kinetics from different uptake routes should be comparable, it is important to also consider the absorption kinetics*', including a possible delay in absorption via dermal exposure (Connolly, Jones, et al. 2019, p. 209).

No published studies to date have assessed non-occupational inhalation and dermal exposures to glyphosate from application of glyphosate-containing herbicides. Therefore, to understand glyphosate exposure in residential use settings, this exploratory study evaluated the individual contributions of inhalation and dermal exposures on urinary glyphosate and AMPA levels from the heavy application of a commercially available formulation of a glyphosate-containing herbicide. Inhalation and dermal exposures were further characterized in this study by analyzing air samples collected in the applicators' breathing zones and dermal patch samples from high exposure areas on applicators' bodies.

Methods

Study protocol

The study was conducted outdoors on a single day in July 2019 in Monee, Illinois, during which temperatures ranged from 77.4 °F to 89.4 °F (25.2 °C to 31.9 °C) and relative humidity ranged from 40% to 62%. Sampling occurred while Roundup® Weed & Grass Killer Super Concentrate (EPA Reg. No. 71995-25) was mixed and applied using commercially available backpack sprayers made by the same manufacturer (Roundup® Commercial backpack sprayers), in a manner consistent with product instructions.

Participants were divided into two exposure groups, one equipped to only assess dermal exposure, and the other, only inhalation exposure. Six (three female and three male) study participants (i.e. 'applicators') comprised each exposure group, for a total of 12 subjects. For the dermal exposure group, applicators wore their own shorts, t-shirts, socks, and athletic shoes, which was believed to be consistent with the typical apparel worn by a residential consumer applicator on a warm day. The composition of the apparel worn by applicators in the dermal exposure group varied by applicator, but generally was composed of cotton, nylon, polyester, and elastane. Dermal group participants also wore half-face respirators equipped with OV/AG/P100 cartridges (3M 60921; 3M Company, St. Paul, MN). For the inhalation exposure group, applicators wore hooded Tyvek coveralls and chemical resistant gloves but no respirators. All applicators were explicitly instructed to abstain from applying lotion, make-up, or any other skin care product on the day of the study.

The duration of each exposure simulation was 100 min; this duration was exclusively selected to conform to the minimum air sampling duration specified within the OSHA Method PV2067, and was not based on typical residential consumer use durations, which are expected to be less than the duration of application in this study. During each exposure simulation, each applicator mixed and subsequently sprayed in a continuous fashion the Roundup® product. Throughout the application period, each applicator walked forward while using the built-in hand-operated pump and sprayer wand to continuously spray the product from side-to-side across their walking path. When the backpack was empty, it was refilled and mixed, and the process was repeated for a total of four mixing and spraying events per applicator in the 100 min sampling period. Following the exposure simulation, applicators washed their hands with soap and water per instructions on the product's container.

To mix the product, per the manufacturer's specifications, each applicator added ten fluid ounces (295.7 mL) of Roundup® concentrate containing 50.2% glyphosate to their backpack sprayer followed by four gallons (15.1 L) of water, which created a 0.96% glyphosate solution. The Roundup® product used in this evaluation was selected because (1) it

required mixing (and therefore there was a potential fordermal exposure from the concentrated product), and (2) after mixing, the resulting solution contained a comparable concentration of glyphosate found in ready-to-use Roundup® products available for noncommercial residential use (i.e. 1–2% glyphosate). Each applicator sprayed the 677-foot perimeter of a gravel and asphalt yard at an approximate pace of one foot per second (to maintain adequate distance between applicators), covering a total distance of more than 5000 ft (1524 m) over the sampling duration. This is consistent with the spraying around the perimeter of one square acre of land six times consecutively, and thus, clearly exceeds typical residential consumer application.

Sampling was conducted over the course of three sampling events. Two applicators in the dermal exposure group and two applicators in the inhalation exposure group participated in each event. During each event, a helper was assigned to each applicator to ensure that the applicators remained evenly spaced and were spraying and walking at approximately the same rate.

Study participants

All participants were employees of Cardno ChemRisk at the time the study was conducted. Before sampling began, all participants and investigators involved in the implementation of the study received training regarding the possible hazards of working with glyphosate, as well as relevant environmental safety training. Participants in the dermal exposure group were medically cleared and fit-tested to wear respirators prior to the initiation of the study.

Institutional review board (IRB) approval was obtained from a medical institutional review board that was in compliance with U.S. federal regulations (including, but not limited to 21 CFR Parts 50 and 56, and 45 CFR Part 46), various guidelines as applicable (both domestic and international, including but not limited to OHRP, FDA, U.S. EPA, ICH GCP as specific to IRB review, Canadian Food and Drug Regulations, the Tri-Council Policy Statement 2, and CIOMS), and the ethical principles underlying the involvement of human subjects in research (including The Belmont Report, Nuremberg Code, Declaration of Helsinki) (Protocol number: Pro00036892; Advarra Institutional Review Board, Inc., Columbia, MD).

Sampling and analytical methods

Urinary sampling and analysis for glyphosate and AMPA

Urinary testing kits for glyphosate and AMPA were obtained from the Health Research Institute (Fairfield, IA). Each kit consisted of a sealed sterile leak-tight polypropylene cup and a polyethylene cap. The methods for testing urinary glyphosate and AMPA levels were accredited to the ISO 17025:2005 standard for the competence of testing and calibration laboratories. The limit of detection (LOD) and limit of quantification (LOQ) for glyphosate in urine were 0.02 ng/mL (1 ng/mL = 1 ppb) and 0.05 ng/mL, respectively, whereas, for AMPA, the LOD and LOQ were 0.013 ng/mL and 0.05 ng/mL, respectively.

Urine samples were collected from all applicators 30min prior to application and 3-, 6-, 12-, and 24-hr after the completion of application (herein referred to as post-application). An additional urine sample was collected 36-hr post-application for the dermal exposure group due to the possible delay in absorption. The urine samples were stored at room temperature and were analyzed for glyphosate and AMPA by the Health Research Institute using high-performance liquid chromatography (HPLC) with triple quadrupole mass spectrometry (HPLC-MS/MS). Levels were adjusted for dilution effects using urine specific gravity.

Air sampling and analysis

Air samples were collected in the breathing zone of each inhalation group participant during mixing and spraying. Air sampling for glyphosate was conducted in accordance with OSHA Method PV2067 (OSHA 1989). This is a stopgap method partially validated for a specific set of sample collection parameters. Air samples were collected on glass fiber filters (37 mm, 0.45 µm pore size; Zefon International, St. Petersburg, FL) using GilAir3 Personal Air Samplers (Gilian, St. Petersburg, FL). All sampling pumps were calibrated with a Bios DryCal DCLite primary flow calibrator (Bios International Corporation, Butler, NJ) before and after each sampling event.

Two air samples were collected on each of the six inhalation group participants on their left and right lapels, for a total of 12 personal samples. All air samples were collected for 100 min (± 2 min) at a flow rate of 1.0 L/min (± 6%). An additional two blank samples were collected and sent to the laboratory for quality control.

All air samples were stored at room temperature and were analyzed by an American Industrial Hygiene Association (AIHA) accredited laboratory (Bureau Veritas Laboratories, Novi, MI) according to OSHA Method PV2067 using HPLC with an ultraviolet detector (HPLC-UV) (OSHA 1989). The mass-based reporting limit (RL) for each sample was 0.1 µg, which resulted in concentration-based RLs of approximately 0.001 mg/m^3.

Dermal patch sampling

Prior to the initiation of the study, but on the same day as the study, a pilot study was conducted during which a participant wearing Tyvek coveralls used the backpack sprayer to apply water containing a blue spray pattern indicator (Liquid Harvest Lazer™, Sanco Industries Inc., Fort Wayne, IN). Based on this assessment, it was visually determined that areas in which most of the solution deposited were the right shin (i.e. the front of the right leg below the knee), the left shin (i.e. the front of the left leg below the knee), the dorsal side of the forearm of the applicator's spraying arm, and the proximal portion of the anterior thigh (i.e. the upper front portion of the thigh) contralateral to the applicator's spraying arm. Using this information, four 142-mm borosilicate glass fiber patches were positioned and taped in

these locations on each applicator within the inhalation exposure group in order to assess dermal deposition in these higher loading zones. Patches were not used on the dermal exposure group because they would effectively reduce the surface area of the applicators' exposed skin, thereby resulting in an underestimation of dermal uptake.

Upon completion of each exposure event, patch samples were placed in sterile glass jars and sent at room temperature to an AIHA accredited laboratory for analysis (Bureau Veritas Laboratories, Novi, MI). Two additional blank patch samples were sent to the laboratory for quality control. All patch samples were analyzed by a modified OSHA PV2067 method with a RL of 1.0 µg.

Data analysis

Urinary analyses

A linear mixed model was used to characterize the differences in urinary glyphosate, AMPA, and 'total effective glyphosate' levels between the two exposure groups across each urine sample time point. Total effective glyphosate was calculated by the laboratory by summing the measured urinary glyphosate concentration plus 1.5 times the urinary AMPA concentration (FAO and WHO 2005). Urinary glyphosate or AMPA levels below the LOQ were assigned a value of one-half the LOQ (i.e. 0.025 ng/mL) for purposes of statistical analysis. A linear mixed model is appropriate for assessing repeated measures for each subject over time, as well as unbalanced data, which arose from the dermal exposure group providing an additional urine sample 36-hr after spraying (Cnaan et al. 1997; West et al. 2007). A p value less than 0.05 was considered statistically significant. To avoid violation of the normality of residuals, glyphosate, AMPA and total effective glyphosate levels were log-transformed, which is consistent with previous studies (Acquavella et al. 2004; Curwin et al. 2006; McGuire et al. 2016; Connolly et al. 2017; Connolly et al. 2018; Connolly, Coggins, et al. 2019; Connolly, Jones, et al. 2019).

Airborne exposure analysis

A Kruskal-Wallis test was used to determine if the concentrations measured by the left and right lapel air samplers were significantly different from each other and whether there was a significant difference in measured airborne glyphosate levels between participants.

Dermal exposure analysis

The total mass of glyphosate collected on dermal patches was tabulated by filter location. The concentration of glyphosate was calculated by dividing the collected mass of glyphosate on the filter by the surface area of the filter. The Kruskal-Wallis test was used to determine if the concentration of glyphosate collected on the filters varied significantly by the filter location or by applicator.

Results

Urinary concentrations

Urinary glyphosate levels measured over the sampling period are reported in Table 1 and are displayed graphically in Figure 1(a,b) for the inhalation and dermal exposure groups, respectively. Data for urinary AMPA and total effective glyphosate concentrations are included in Appendix A (Table A1, Figure A1(a,b)) and Appendix B (Table B1, Figure B1(a,b)), respectively. Trends similar to that of glyphosate were observed for both AMPA and total effective glyphosate.

Baseline (pre-application) urinary glyphosate levels were not significantly different between the exposure groups and ranged from 0.26 ng/mL to 1.90 ng/mL in the inhalation exposure group and 0.30 ng/mL to 1.98 ng/mL in the dermal exposure group. In general, urinary glyphosate levels were the highest in the samples collected 3-hr post-application, except in two subjects with peak urinary levels at 6-hr post-application (one in the inhalation and one in the dermal exposure group), and in one subject in the dermal exposure group who had relatively low urinary glyphosate levels that peaked at 24-hr post-application. Peak urinary glyphosate concentrations ranged from 3.79 ng/mL to 17.23 ng/mL for the inhalation exposure group and 5.55 ng/mL to 310.91 ng/mL for the dermal exposure group.

As observed in Figure 1(b), the measured urinary glyphosate level at 3-hr post-application in one of the participants within the dermal exposure group (310.91 ng/mL) was a statistical outlier, as the value surpassed 1.5 times the interquartile range added to the 75th percentile of the distribution of the measurements (Hubert and Vandervieren 2008). When this outlier was excluded from the analysis, the highest urinary glyphosate level measured in the dermal exposure group was 57.36 ng/mL.

The results of the linear mixed model for the log-transformed urinary glyphosate data are presented in Table 2; similar analyses for AMPA and total effective glyphosate are presented in Appendices A and B, respectively. The log-transformed urinary glyphosate levels at baseline in the inhalation exposure group served as the reference group in this model. The regression coefficients were exponentiated to calculate the geometric mean (in ng/mL) for each variable. For both exposure groups combined, urinary glyphosate levels were significantly elevated relative to baseline until 24-hr post-application (i.e. at 3-, 6-, and 12-hr post-application). This held true when the model was run separately for the dermal group; however, for the inhalation group, urinary glyphosate levels were only significantly elevated relative to baseline until 12-hr post-application. Overall, the geometric mean urinary glyphosate levels were higher in the dermal exposure group than the inhalation exposure group; however, these differences were not statistically significant. Although gender was excluded to avoid overfitting the model, females appeared to have higher mean urinary glyphosate levels following application compared to males in both exposure groups, but these differences were not statistically significant and not of the similar magnitude as the difference between exposure groups (data not shown).

Table 1. Urinary glyphosate concentrations (ng/mL) for applicators by exposure group and urine collection time point.

Exposure group	Minimum	Median	Arithmetic mean	Standard deviation	Maximum
Inhalation (n = 6)					
30-min Pre-Application (Baseline*)	0.26	0.84	0.94	0.60	1.90
3-hr Post-Application	3.79	13.03	11.48	5.55	17.23
6-hr Post-Application[b]	2.91	4.04	6.09	3.77	12.17
12-hr Post-Application	1.10	1.66	2.11	1.08	3.55
24-hr Post-Application	0.25	0.90	0.91	0.59	1.83
Dermal (n = 6)					
30-min Pre-Application (Baseline*)	0.30	0.88	0.94	0.63	1.98
3-hr Post-Application	3.12	13.30 *(9.73)*	63.87 *(14.46)*	121.64 *(13.66)*	310.91 *(37.04)*
6-hr Post-Application[b]	3.80	17.77	24.46	22.86	57.36
12-hr Post-Application	3.50	6.54	11.94	11.01	31.69
24-hr Post-Application	0.68	1.54	3.08	2.96	6.96
36-hr Post-Application[b]	0.62	1.60	2.68	2.70	7.33

[a]Values in parentheses exclude the outlier measurement.
[b]$n = 5$; three samples were not analyzed (a 6-hr post-application sample from an inhalation group applicator, a 6-hr post-application sample from a dermal group applicator, and a 36-hr post-application sample from a separate dermal group applicator spilled during transit)
*Baseline urinary glyphosate concentrations were not statistically significantly different between the inhalation and dermal exposure groups.

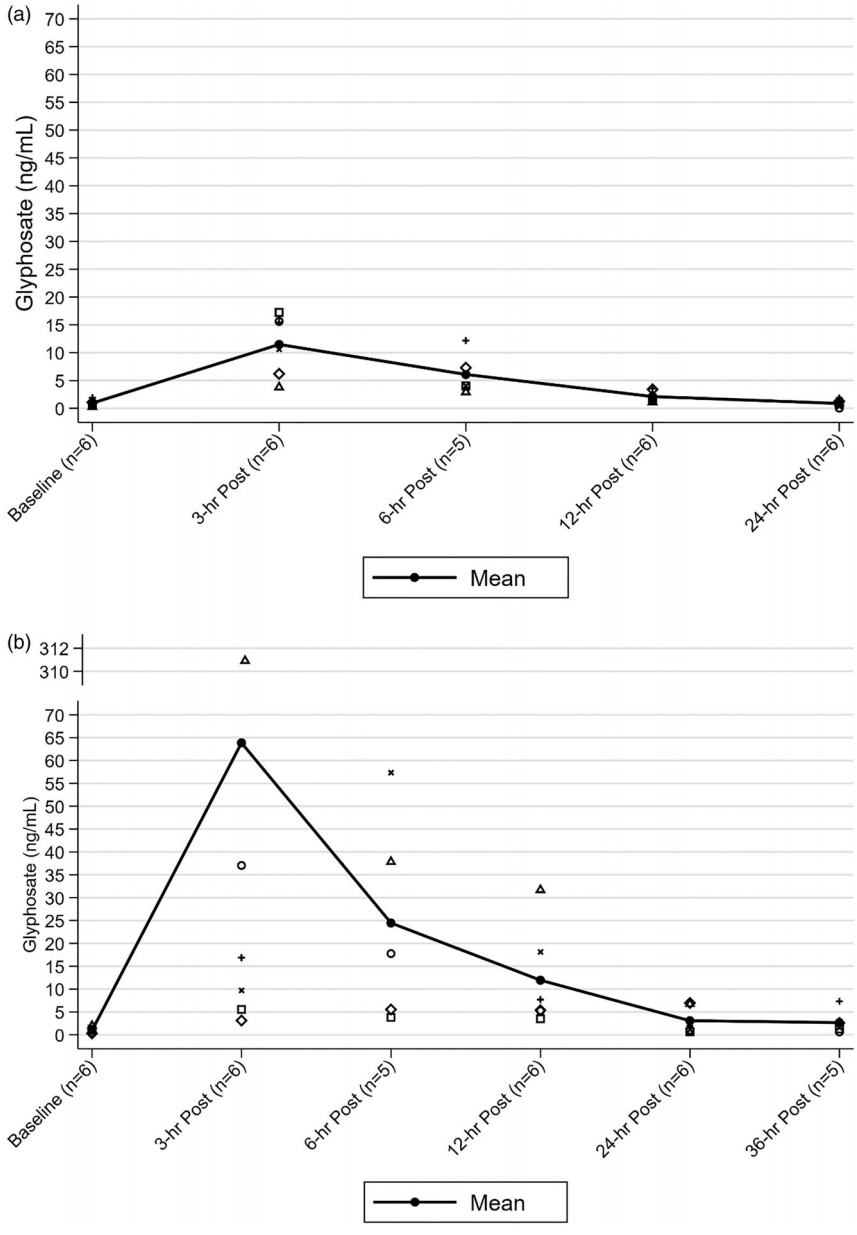

Figure 1. (a) Urinary Glyphosate Concentrations for Applicators ($n = 6$) in the Inhalation Exposure Group at Baseline, and 3-hr, 6-hr, 12-hr, and 24-hr After Application of Roundup®. (b) Urinary Glyphosate Concentrations for Applicators ($n = 6$) in the Dermal Exposure Group at Baseline, and 3-hr, 6-hr, 12-hr, 24-hr and 36-hr After Application of Roundup®.

At baseline and 24-hr post-application, urinary AMPA concentrations were on average 55.7% and 33.5% of urinary glyphosate levels, respectively, in the dermal exposure group (see Figure 2). For the dermal exposure group, the time points at which urinary glyphosate concentrations were significantly elevated relative to baseline (i.e. at 3-, 6-, and 12-hr post-application), urinary AMPA concentrations were on average between 7.4% and 10.5% of urinary glyphosate. Additionally, the ratio of urinary AMPA to urinary glyphosate concentrations was significantly different from baseline at 36-hr post-application.

At baseline as well as at 12- and 24-hr post-application, urinary AMPA concentrations were on average between 20.7% and 49.5% of urinary glyphosate in the inhalation exposure group. At the time points in which the urinary glyphosate concentrations were significantly elevated relative to baseline in the inhalation group (i.e. at 3- and 6-hr post-application), urinary AMPA concentrations were on average 11.9% and 17.9% of urinary glyphosate concentrations, respectively.

Table 2. Fixed and random effects for the log-transformed urinary glyphosate data ($n = 63$)[a].

	Coefficient (GM)	SE	p Value	95% CI	
Intercept	−0.62 (0.54)	0.34	0.068	−1.29	0.05
Group					
Inhalation	Reference				
Dermal	0.72 (2.05)	0.40	0.072	−0.06	1.51
Time Point					
Baseline	Reference				
3-hr Post-Application	2.86 (17.46)	0.26	<0.001*	2.30	3.42
6-hr Post-Application	2.46 (11.71)	0.33	<0.001*	1.74	3.17
12-hr Post-Application	1.66 (5.26)	0.34	<0.001*	0.91	2.41
24-hr Post-Application	0.27 (1.31)	0.35	0.507	−0.52	1.06
36-hr Post-Application	0.48 (1.62)	0.45	0.359	−0.55	1.51
Random effects (variance)	Estimate	SE			
Subject: random intercept	0.01	0.02			
Subject: random slope	5.04×10^{-18}				
Rho	0.46	0.16			
Residual	0.90	0.28			

aThree samples were not analyzed (a 6-hr post-application sample from an inhalation group applicator, a 6-hr post-application sample from a dermal group applicator, and a 36-hr post-application sample from a separate dermal group applicator spilled during transit).

*Across both exposure groups, urinary glyphosate levels were significantly elevated relative to baseline until 24-hr post-application (i.e. at 3-, 6-, and 12-hr post-application). This held true for the dermal group when the model was run separately, but the urinary glyphosate levels were only significantly elevated for the inhalation exposure group until 12-hr post-application (data not shown).

Airborne concentrations

A total of 12 personal air samples were collected from the inhalation group participants and analyzed for glyphosate (Table 3). The airborne glyphosate concentrations ranged from 0.0030 mg/m^3 to 0.0075 mg/m^3, with an overall arithmetic mean of 0.0047 mg/m^3. The difference between the left and right lapel samples was not found to be statistically significant (data not shown). Similarly, the difference

Table 3. Airborne concentrations of glyphosate (mg/m^3) in the breathing zone of applicators.

Parameter*	Concentration ($n = 12$ samples)
Minimum	0.0030
Median	0.0046
Mean	0.0047
Standard Deviation	0.0014
Maximum	0.0075

*No statistically significant differences were found between the left and right samplers across the 6 inhalation group participants or between participants.

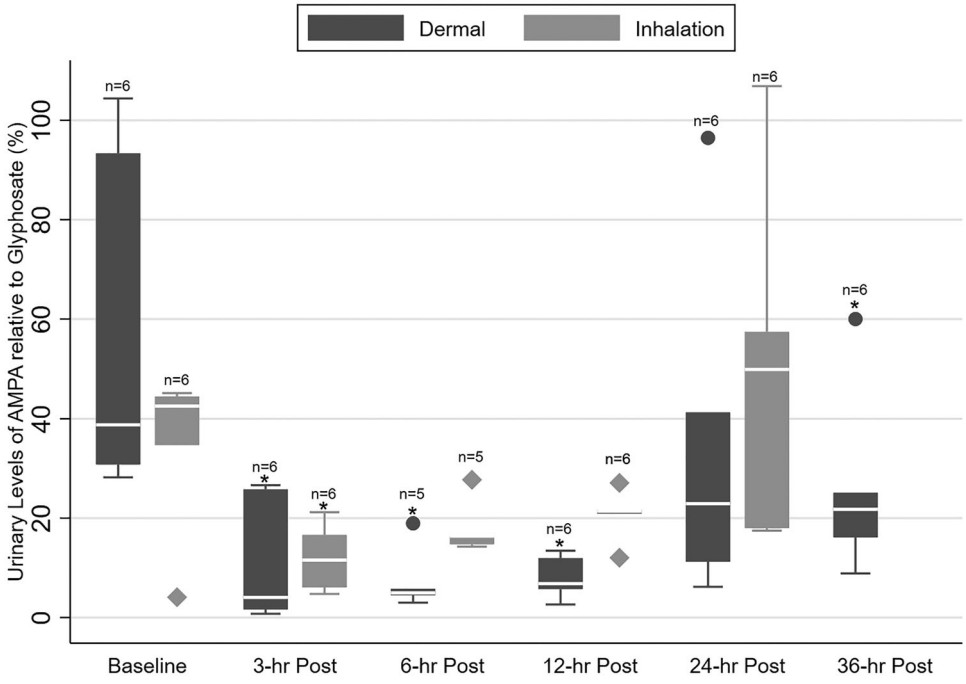

Figure 2. Comparison of Urinary Glyphosate and Urinary AMPA Levels for Applicators the Dermal Exposure Group ($n = 6$) and Inhalation Exposure Group ($n = 6$) After Application of Roundup®. The box represents the interquartile range. The whiskers represent the lowest and highest non-outlier measurements. *The asterisk indicates that the ratio was significantly different ($p < 0.05$) from baseline.

Table 4. Results of dermal sampling for glyphosate by patch location.

Patch location*	Min	Median	Mean	SD	Max
Total Mass Collected (μg) ($n = 24$)					
Right Shin	380	1005	2437	2698	6000
Left Shin	150	935	2285	2724	6800
Dorsal Forearm of Spraying Arm	9	660	1343	1685	4100
Thigh Opposite Spraying Arm	90	265	1653	2389	5700
Concentration (μg/mm^2) ($n = 24$)					
Right Shin	0.0240	0.0635	0.1539	0.1703	0.3789
Left Shin	0.0095	0.0590	0.1443	0.1719	0.4294
Dorsal Forearm of Spraying Arm	0.0005	0.0417	0.0848	0.1064	0.2589
Thigh Opposite Spraying Arm	0.0057	0.0167	0.1044	0.1508	0.3599

*No statistically significant differences were found between the four patch locations across the 6 inhalation group participants, nor between individual participants.

between participants was not found to be statistically significant (data not shown).

Dermal patch sample concentrations

A total of 24 patch samples were collected on the inhalation group participants. The total mass collected and average concentrations of glyphosate by patch sample location is presented in Table 4. Overall, the arithmetic mean concentrations of glyphosate for each applicator's four patch samples ranged from 0.04 μg/mm^2 to 0.25 μg/mm^2 (data not shown). In general, the highest concentrations of glyphosate were measured on the right shin followed by the left shin, which had arithmetic mean concentrations of 0.15 μg/mm^2 and 0.14 μg/mm^2, respectively. No statistically significant differences were found between patch location or applicator.

Discussion

Overview of the urinary results and comparison to published literature

In summary, within this exploratory study, measurable concentrations of glyphosate and AMPA were detected in the urine of both exposure groups following heavy residential consumer application of glyphosate. These levels generally peaked within 6-hr post-application with the greatest frequency at 3-hr post-application, which is consistent with the only other study that reported a peak urinary glyphosate concentration (Mesnage et al. 2012). Statistically, urinary glyphosate levels rapidly returned to baseline within 24 hr of application. This suggests that the use of glyphosate-containing products produces only a transient effect on urinary glyphosate levels, as glyphosate is rapidly excreted from the body (ATSDR 2019). In addition, as can be seen in Figure (1a,b), our results generally support that the biological half-life for glyphosate is roughly between 3 and 6 hr. This is consistent with what has previously been reported by Roberts et al. (2010) in adults following acute self-poisoning (3.1 hr; 95% CI: 2.7–3.6 hr), and less than the elimination half-lives [5.51 hr (95% CI: 3.56–7.46 hr) for unadjusted samples, 10.00 hr (95% CI: 5.47–14.53 hr) for creatinine-corrected concentrations, and 7.25 hr (95% CI: 5.38–9.12 hr) for adjustments by urinary excretion rates] reported by Connolly, Jones et al. (2019). Furthermore, as can be seen in Figure 2, the urinary concentration of AMPA relative to glyphosate is highest at baseline, and at 24- and 36-hr post-application, when exposures are presumed to be predominantly determined by diet. Although urinary glyphosate levels were higher in the dermal exposure group than the inhalation exposure group, these differences were not statistically significant, which was likely at least in part due to the small sample size.

Baseline urinary glyphosate concentrations in the present analysis (0.26–1.98 ng/mL) were generally similar to or lower than what has been reported by other researchers. For example, Acquavella et al. (2004) reported that baseline urinary glyphosate concentrations among 48 farmers in South Carolina and Minnesota ranged from <1 to 15 ppb (<1–15 ng/mL) with a mean of 3.2 ppb (3.2 ng/mL). The authors suggested that glyphosate was not detected in the baseline urine of up to 40% of their study population (Acquavella et al. 2004). Connolly et al. (2018) reported that baseline urinary glyphosate concentrations among 20 applicators in Ireland ranged from 0.14 to 5.44 μg/L (0.14–5.44 ng/mL) with an arithmetic mean of 1.08 μg/L (1.08 ng/mL). The authors noted that 48% of their pre-task samples were potentially influenced by work tasks performed in the days prior to the study and by starting the work task before providing the pre-task (baseline) urinary sample (Connolly et al. 2018). Regarding the general population, Curwin et al. (2006) reported that urinary glyphosate concentration in 25 non-farm households in Iowa ranged from 0.13 to 5.4 ng/mL (mean: 1.4 ng/mL) for men and 0.062 to 5.0 ng/mL (mean: 1.2 ng/mL) for women (Curwin et al. 2006). McGuire et al. (2016) determined that the mean baseline urinary glyphosate concentration among 40 healthy, lactating women in the U.S. Pacific Northwest was 0.28 ng/mL (standard deviation: 0.38 ng/mL). The authors reported no significant differences in urinary glyphosate concentrations between those who consumed an organic compared to a conventional diet, or those who lived on or near a farm compared to those living in an urban or suburban region (McGuire et al. 2016). In addition, Parvez et al. (2018) recently reported that the urinary glyphosate concentration of 71 pregnant women in Indiana ranged from 0.50 to 7.20 ng/mL (mean: 3.40 ng/mL, standard deviation: 1.24 ng/mL). In contrast to the findings of McGuire et al. (2016), Parvez and colleagues reported that urinary glyphosate concentrations were higher among women who lived in rural areas. Overall, reported baseline urinary glyphosate concentrations tend to be higher for farmers and applicators than

for the general population. The baseline concentrations reported in the present analysis were within the ranges reported by other researchers and comparable to those of the general population.

Although other researchers have assessed urinary glyphosate concentrations following application of glyphosate-containing herbicides, the precise durations between application and urine sampling are often unclear, making it difficult to draw meaningful comparisons with the present study. One of the urinary glyphosate measurements (310.9 µg/L) from an applicator within the dermal group of the present study is the highest single urine sample reported following application in the literature. It is important to recognize that the conditions evaluated in this study likely resulted in worst-case residential consumer exposures (e.g. heavy application, coupled with wearing short-sleeved shirts, shorts, and permeable athletic shoes). Had dermal applicators worn chemical-resistant gloves, chemical-resistant clothing, and impermeable shoe covers, such as what is suggested on the Safety Data Sheet for the product used in our evaluation in instances when there is '*significant potential for contact*', the dermal exposures would undoubtedly have been much lower (Monsanto 2015). Furthermore, Acquavella et al. (2004) reported a urinary glyphosate concentration of 233 µg/L from a 24-hr composite urine sample. This is more than two-fold greater than the urinary glyphosate concentration of 96.8 µg/L from a 24-hr composite urine sample extrapolated from the dermal applicator with the highest glyphosate concentration in a spot urine sample (assuming equal urine volume at each time point) in the present study.

Overview of air and dermal sampling results and comparison to published literature

The majority of glyphosate exposure studies to date have evaluated agricultural workers, as it is believed that these workers have the highest exposure potential. However, a limited number of studies have evaluated airborne and dermal concentrations of glyphosate associated with lesser-exposed workers that may be more relevant to evaluations of residential consumer exposure levels. The airborne concentrations of glyphosate reported in the present study (air: $0.0030 - 0.0075\ mg/m^3$) are generally toward the lower end of the range of air concentrations previously reported for nonagricultural applications, as summarized from the following studies.

NIOSH (1985) conducted a Health Hazard Evaluation (HHE) to evaluate the U.S. Department of the Interior Bureau of Reclamation's pesticide application program. One of the pesticides evaluated was Roundup®, which was diluted to a concentration 0.5%; however, it was unclear if this was the concentration of Roundup® or glyphosate in the solution. Application reportedly involved walking alongside a vehicle with a hand-held hose; backpack sprayers were not used. Single breathing zone air samples were collected from both the applicator and vehicle driver over the duration of their workday (sample durations = 430 min and 425 min, respectively) encompassing both mixing and spraying operations, and resulted in glyphosate concentrations less than $0.028\ mg/m^3$ for both samples. Although the amount of glyphosate applied, as well as the duration of application, were not provided, mixing reportedly took place for less than 15 min.

Jauhiainen et al. (1991) conducted field studies in Finland in which they collected breathing zone air samples from forest workers who were engaged in spraying Roundup® using brush saws equipped with pressurized sprayers. Air samples were collected for durations of 1–6 hr, during which workers mixed the solution to a concentration of 8% Roundup®, periodically filled the tanks on the saws, and conducted spraying; the workers used an average of 9.8 liters of glyphosate-containing solution per day. Airborne concentrations of glyphosate ranged from $<0.00125\ mg/m^3$ to $0.0157\ mg/m^3$, which exceeds the highest concentration measured in our investigation ($0.0075\ mg/m^3$) by two-fold.

Johnson et al. (2005) conducted surveys to assess the potential inhalation and dermal exposure to applicators of glyphosate-based 'amenity herbicides' in the United Kingdom in 1998 and 1999. In the first of the two surveys, ATVs fitted with tanks and spray bars were used to apply Roundup® Pro Bioactive diluted to concentrations of 11–55 g/L (~1.1–5.5%) glyphosate (while amenity spraying was considered by the authors to be 'non-agricultural', ATVs or trucks are commonly used during agricultural application of herbicides). Short-term (30 min) breathing zone samples were collected from the ATV drivers as they operated the sprayers. Airborne glyphosate concentrations ranged from 0 to $36.5\ mg/m^3$ with an arithmetic mean of $15.0\ mg/m^3$, which is 2000 times the highest air concentration measured in the present study. Further, six sampling pads were affixed to the clothing of each ATV driver, including on the top of the head, chest, right upper forearm, mid-thigh of left leg, above the ankle of left leg, and on the upper back. Based on their results, the authors estimated full body dermal exposures ranged from 0.7 to 4.2 mL/h; the actual concentrations measured on each of the sampling pads were not reported.

During the second survey, individuals used backpack sprayers equipped with controlled droplet applicators to apply Roundup® Pro Bioactive diluted to concentrations of 72–167 g/L (~7.2–16.7%) glyphosate. The airborne glyphosate concentrations ranged from 0 to $0.616\ mg/m^3$, with an arithmetic mean of $0.074\ mg/m^3$. Dermal sampling pads were also placed on the applicators in the same positions as in the first survey and resulted in estimated total body dermal exposures for glyphosate ranging from 0.003 to 0.666 mL/h; similar to the first survey, the actual concentrations measured on each of the patches were not reported. For both surveys, the highest amount of glyphosate was found on the lower legs.

Connolly, Coggins et al. (2019) conducted dermal and inadvertent ingestion exposure assessments of horticultural workers in Ireland who applied glyphosate-based pesticides. Workers were grouped based on their method of application: backpack with manual lance, motorized backpack with pressurized lance, and controlled droplet applicator. The

controlled droplet applicator was noted by the authors to be similar to the manual backpack, but contained a premixed solution, thus eliminating the loading and mixing steps. A total of 20 workers applied the glyphosate-based pesticide products for between 30 min and 6 hr. Several different glyphosate-based products were used, with concentrations of the sprayed solutions ranging from approximately 2–4% glyphosate (Connolly, Coggins, et al. 2019). Workers wore gloves during application and wipe samples were collected from their hands after the removal of the gloves. The geometric mean glyphosate concentrations were 0.04 μg/cm^2 (range: 4.7×10^{-4} to 2.56 μg/cm^2) and 0.05 μg/cm^2 (range: 2.8×10^{-4} to 2.04 μg/cm^2) for the left and right hands, respectively (Connolly, Coggins, et al. 2019). Although not directly comparable due in part to differences in sampling locations, sampling methods, and the use of gloves, these dermal concentrations were generally orders of magnitude lower than those measured in the present study.

Strengths and limitations

While exploratory in nature and relatively limited in sample size, this pilot study provides the most descriptive temporal profile of urinary glyphosate levels following the application of a glyphosate-containing herbicide in the published literature. Although other researchers, notably Connolly et al. (2018) and Connolly, Jones, et al. (2019), have collected multiple spot urine samples in order to estimate the peak urinary concentrations of glyphosate following its application, the present study had a well-defined mixing and application protocol that simplifies interpretation of the toxicokinetics of glyphosate. In particular, the current study standardized the mixing and application procedure with continuous application over a fixed duration, during which a fixed volume of a single type of glyphosate-containing herbicide with a known concentration was used. No other studies have controlled for these variables, and many lack even basic information on factors such as the concentration of glyphosate in the solution applied, the duration of application, and the time between application and urine collection.

This study was designed to evaluate a heavy residential consumer exposure scenario. Rather than being selected based on the typical duration of residential consumer use, the combined duration of mixing and application was selected to meet the minimum air sampling duration, as specified by the OSHA Method PV2067. The product used in our evaluation is intended to only be applied by residential consumers around flower beds, trees, driveways and walkways, and along fences. However, in our simulation, each applicator applied the product to approximately 5000 linear ft (or approximately 1.14 miles) of land. Therefore, it is likely that the amount of herbicide used in our investigation (approximately 16 gallons of solution per applicator) exceeds a typical residential consumer's use (and may be more indicative of an agricultural worker's use). As such, it is probable that the urinary concentrations reported herein represent upper-bound levels experienced by residential consumers.

Furthermore, while applicators in the inhalation group wore hooded Tyvek coveralls and chemical resistant gloves, their shoes (athletic sneakers) were not covered. Many of the applicators in both exposure groups reported that their shoes were wet following application. Therefore, individuals in the inhalation group may have also been incidentally exposed dermally through their feet. This is potentially why differences in urinary glyphosate concentrations between the dermal and inhalation exposure groups were not more pronounced. As described previously, the urinary measurements for one female applicator in the dermal exposure group were far higher than those of the remainder of the applicators in the dermal group. While the precise reasons for this were unclear, it may be due to the fact that this applicator shaved her legs in the morning prior to application, and compared to the other two female applicators in the dermal group (who also shaved their legs in the morning prior to application), this applicator had a longer lag time between application and the washing of her legs (9 hr, versus 15 min for one applicator, and 6.75 hr for the other).

In addition, since multiple study participants sprayed simultaneously during each run, there was potential for exposure to spray drift in addition to the glyphosate directly applied by the participant. Exposure resulting from drift would not be expected in a typical residential consumer setting. In addition, given that the applicators were specifically instructed to forego the use of lotions, makeups, and other skin care products on the day of the study, it is unclear how these items may affect dermal uptake. Lastly, future research and large-scale studies are warranted to corroborate these findings, to further characterize any potentially significant differences between the inhalation and dermal exposure routes among applicators, and to quantify the potential role of glyphosate elimination through the feces and by exhalation.

Conclusions

This is the first study to characterize the absorption and biological fate of glyphosate in residential consumer applicators following simulated heavy exposure conditions. The results of this study are consistent with previous studies, showing that glyphosate is quickly eliminated from the body, typically within 24 hr following application.

Disclosure statement

No potential conflict of interest is reported by the author(s).

ORCID

Jennifer S. Pierce http://orcid.org/0000-0001-7722-9901
Benjamin Roberts http://orcid.org/0000-0001-7451-925X
Daniel G. Kougias http://orcid.org/0000-0003-4902-8250
Chris E. Comerford http://orcid.org/0000-0001-8168-7462
Alexander S. Riordan http://orcid.org/0000-0002-4409-7508
Kara A. Keeton http://orcid.org/0000-0002-5157-5627
Heidi A. Reamer http://orcid.org/0000-0002-8415-3607
Neva F. B. Jacobs http://orcid.org/0000-0002-3757-0467
Jason T. Lotter http://orcid.org/0000-0003-0427-153X

References

Acquavella JF, Alexander BH, Mandel JS, Gustin C, Baker B, Chapman P, Bleeke M. 2004. Glyphosate biomonitoring for farmers and their families: results from the Farm Family Exposure Study. Environ Health Perspect. 112(3):321–326.

APVMA 2016. Regulatory position: consideration of the evidence for a formal recosideration of glyphosate. Australian Pesticides and Veterinary Medicines Authority (APVMA). https://apvma.gov.au/sites/default/files/publication/20701-glyphosate-regulatory-position-report-final.pdf.

ATSDR 2019. Toxicological profile for glyphosate. Draft for public comment. Atlanta (GA): Agency for Toxic Substances and Disease Registry (ATSDR), Division of Toxicology and Human Health Sciences, Environental Toxicology Branch.

ATSDR 2020. Toxicological profile for glyphosate. Atlanta (GA): Agency for Toxic Substances and Disease Registry (ATSDR), U.S. Department of Health and Human Services, Public Health Service.

Cnaan A, Laird NM, Slasor P. 1997. Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data. Statist Med. 16(20):2349–2380.

Connolly A, Basinas I, Jones K, Galea KS, Kenny L, McGowan P, Coggins MA. 2018. Characterising glyphosate exposures among amenity horticulturists using multiple spot urine samples. Int J Hyg Environ Health. 221(7):1012–1022.

Connolly A, Coggins MA, Galea KS, Jones K, Kenny L, McGowan P, Basinas I. 2019. Evaluating glyphosate exposure routes and their contribution to total body burden: a study among amenity horticulturalists. Ann Work Expo Health. 63(2):133–147.

Connolly A, Jones K, Basinas I, Galea KS, Kenny L, McGowan P, Coggins MA. 2019. Exploring the half-life of glyphosate in human urine samples. Int J Hyg Environ Health. 222(2):205–210.

Connolly A, Jones K, Galea KS, Basinas I, Kenny L, McGowan P, Coggins M. 2017. Exposure assessment using human biomonitoring for glyphosate and fluroxypyr users in amenity horticulture. Int J Hyg Environ Health. 220(6):1064–1073.

Curwin BD, Hein MJ, Sanderson WT, Striley C, Heederik D, Kromhout H, Reynolds SJ, Alavanja MC. 2006. Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in iowa. Ann Occup Hyg. 51(1):53–65.

FAO and WHO 2005. Pesticide Residues in Food. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues. Geneva, Switzerland. 20–29 September 2005: Food and Agriculture Organization of the United Nations (FAO), World Health Organization (WHO).

Hubert M, Vandervieren E. 2008. An adjusted boxplot for skewed distributions. Comput Stat Data Anal. 52(12):5186–5201.

IARC 2015. Glyphosate. International Agency for Research on Cancer (IARC).Glyphosate. In: Some organophosphate insecticides and herbicides: diazinon, glyphosate, malathion, parathion, tetrachlorvinphos. IARC Working Group, March 3–10, 2015, Lyon (France). Lyon (France): World Health Organization (WHO), International Agency for Research on Cancer (IARC) (IARC Monographs on the Evaluation of Carcinogen Risks to Humans, Vol. 112), p. 1–92. http://mono graphs.iarc.fr/ENG/Monographs/vol112/index.php

Jauhiainen A, Rasanen K, Sarantila R, Nuutinen J, Kangas J. 1991. Occupational exposure of forest workers to glyphosate during brush saw spraying work. Am Ind Hyg Assoc J. 52(2):61–64.

Johnson PD, Rimmer DA, Garrod AN, Helps JE, Mawdsley C. 2005. Operator exposure when applying amenity herbicides by all-terrain vehicles and controlled droplet applicators. Ann Occup Hyg. 49(1):25–32.

Lavy TL, Cowell JE, Steinmetz JR, Massey JH. 1992. Conifer seedling nursery worker exposure to glyphosate. Arch Environ Contam Toxicol. 22(1):6–13.

McGuire MK, McGuire MA, Price WJ, Shafii B, Carrothers JM, Lackey KA, Goldstein DA, Jensen PK, Vicini JL. 2016. Glyphosate and aminomethylphosphonic acid are not detectable in human milk. Am J Clin Nutr. 103(5):1285–1290.

Mesnage R, Moesch C, Le Grand R, Lauthier G, de Vendomois JS, Gress S, Seralini GE. 2012. Glyphosate exposure in a farmer's family. JEP. 03(09):1001–1003.

Monsanto 2015. Safety data sheet for roundup weed & grass killer super concentrate. Marysville (OH): Monsanto Company, Lawn & Garden Products.

NIOSH 1985. Health hazard evaluation report. HETA 83-341-1558. Denver (CO): Bureau of Reclamation, U.S. Department of the Interior, National Institute for Occupational Safety and Health (NIOSH).

OSHA 1989. Glyphosate Method PV2067. Salt Lake City (UT): Carcinogen and Pesticide Branch, OSHA Analytical Laboratory, Occupational Safety and Health Administration (OSHA). https://www.osha.gov/dts/sltc/methods/partial/t-pv2067-01-8911-ch/t-pv2067-01-8911-ch.pdf.

Parvez S, Gerona RR, Proctor C, Friesen M, Ashby JL, Reiter JL, Lui Z, Winchester PD. 2018. Glyphosate exposure in pregnancy and shortened gestational length: a prospective Indiana birth cohort study. Environ Health. 17(1):23.

Roberts DM, Buckley NA, Mohamed F, Eddleston M, Goldstein DA, Mehrsheikh A, Bleeke MS, Dawson AH. 2010. A prospective observational study of the clinical toxicology of glyphosate-containing herbicides in adults with acute self-poisoning. Clin Toxicol (Phila). 48(2):129–136.

SERA 2011. Glyphosate: human health and ecological risk assessment. Manlius (NY): Syracuse Environmental Research Associates, Inc. (SERA).

Tarazona JV, Court-Marques D, Tiramani M, Reich H, Pfeil R, Istace F, Crivellente F. 2017. Glyphosate toxicity and carcinogenicity: a review of the scientific basis of the European Union assessment and its differences with IARC. Arch Toxicol. 91(8):2723–2743.

U.S. EPA 1987. Glyphosate; CASRN 1071-83-6. Integrated Risk Information System (IRIS), Chemical Assessment Summary, National Center for Environmental Assessment, United States Environmental Protection Agency (U.S. EPA). https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0057_summary.pdf.

U.S. EPA 1993. Reregistration Eligibility Decision (RED). Glyphosate. Washington (DC): Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency (U.S. EPA).

U.S. EPA 2017. Pesticides industry sales and usage: 2008–2012. Washington (DC): Biological and Economic Analysis Division, Office of Pesticide Programs, Office of Chemical Safety and Pollution Prevention, United States Environmental Protection Agency (U.S. EPA).

U.S. EPA. 2019a. EPA Takes Next Step in Review Process for Herbicide Glyphosate, Reaffirms No Risk to Public Health. United States Environmental Protection Agency (U.S. EPA); [cited 2019 Oct 11]. https://www.epa.gov/newsreleases/epa-takes-next-step-review-process-herbicide-glyphosate-reaffirms-no-risk-public-health

U.S. EPA 2019b. Glyphosate. [cited 2019 Nov 18]. https://www.epa.gov/ingredients-used-pesticide-products/glyphosate#actions

West BT, Welch KB, Galecki AT. 2007. Linear mixed models: a practical guide using statistical software. Boca Raton (FL): Chapman & Hall.

Wester RC, Melendres J, Sarason R, McMaster J, Maibach HI. 1991. Glyphosate skin binding, absorption, residual tissue distribution, and skin decontamination. Fundam Appl Toxicol. 16(4):725–732.

Wester RC, Quan D, Maibach HI. 1996. In vitro percutaneous absorption of model compounds glyphosate and malathion from cotton fabric into and through human skin. Food Chem Toxicol. 34(8):731–735.

Williams GM, Kroes R, Munro IC. 2000. Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. Regul Toxicol Pharmacol. 31(2 Pt 1):117–165.

Appendix A

Appendix B

Table A1. Urinary AMPA concentrations (ng/mL) for applicators by exposure group and urine collection time point

Exposure group	Minimum	Median	Arithmetic mean	Standard deviation	Maximum
Inhalation (n = 6)					
30-min Pre-Application (Baseline*)	0.03	0.29	0.36	0.31	0.86
3-hr Post-Application	0.80	0.99	1.10	0.38	1.83
6-hr Post-Application[a]	0.48	1.12	1.02	0.51	1.73
12-hr Post-Application	0.23	0.36	0.41	0.18	0.76
24-hr Post-Application	0.01	0.35	0.31	0.17	0.49
Dermal (n = 6)					
30-min Pre-Application (Baseline*)	0.11	0.45	0.43	0.22	0.71
3-hr Post-Application	0.18	1.01	1.25	1.03	2.59
6-hr Post-Application[a]	0.27	0.72	1.31	1.25	3.32
12-hr Post-Application	0.30	0.47	0.77	0.71	2.17
24-hr Post-Application	0.22	0.38	0.54	0.36	1.09
36-hr Post Application[a]	0.65	0.65	0.53	0.34	0.96

[a]$n = 5$; three samples were not analyzed (a 6-h post-application sample from an inhalation group applicator, a 6-h post-application sample from a dermal group applicator, and a 36-h post-application sample from a separate dermal group applicator spilled during transit).
*Baseline urinary AMPA concentrations were not statistically significantly different between the inhalation and dermal exposure groups.

Table A2. Fixed and random effects for the Log-Transformed urinary AMPA data ($n = 63$)[a]

	Coefficient (GM)	SE	*p* Value	95% CI	
Intercept	−1.43 (0.23)	0.33	<0.001	−2.07	−0.79
Group					
Inhalation	*Reference*				
Dermal	0.35 (1.42)	0.40	0.389	−0.44	1.14
Time Point					
Baseline	*Reference*				
3-hr Post-Application	1.20 (3.32)	0.27	<0.001*	0.68	1.72
6-hr Post-Application	1.01 (2.75)	0.26	<0.001*	0.50	1.52
12-hr Post-Application	0.52 (1.68)	0.25	0.034	0.04	1.01
24-hr Post-Application	0.06 (1.06)	0.25	0.793	−0.42	0.55
36-hr Post-Application	0.32 (1.38)	0.33	0.340	−0.34	0.98
Random Effects (variance)	Estimate	SE			
Subject: random intercept	0.43	0.23			
Subject random slope	3.45×10^{-18}				
Rho	−0.15	0.37			
Residual	0.37	0.08			

[a]Three samples were not analyzed (a 6-hr post-application sample from an inhalation group applicator, a 6-hr post-application sample from a dermal group applicator, and a 36-hr post-application sample from a separate dermal group applicator spilled during transit).
*Across both exposure groups, urinary AMPA levels were significantly elevated relative to baseline until 12-hr post-application (i.e. at 3- and 6-hr post-application). This held true when the model was run separately for the two exposure groups (data not shown).

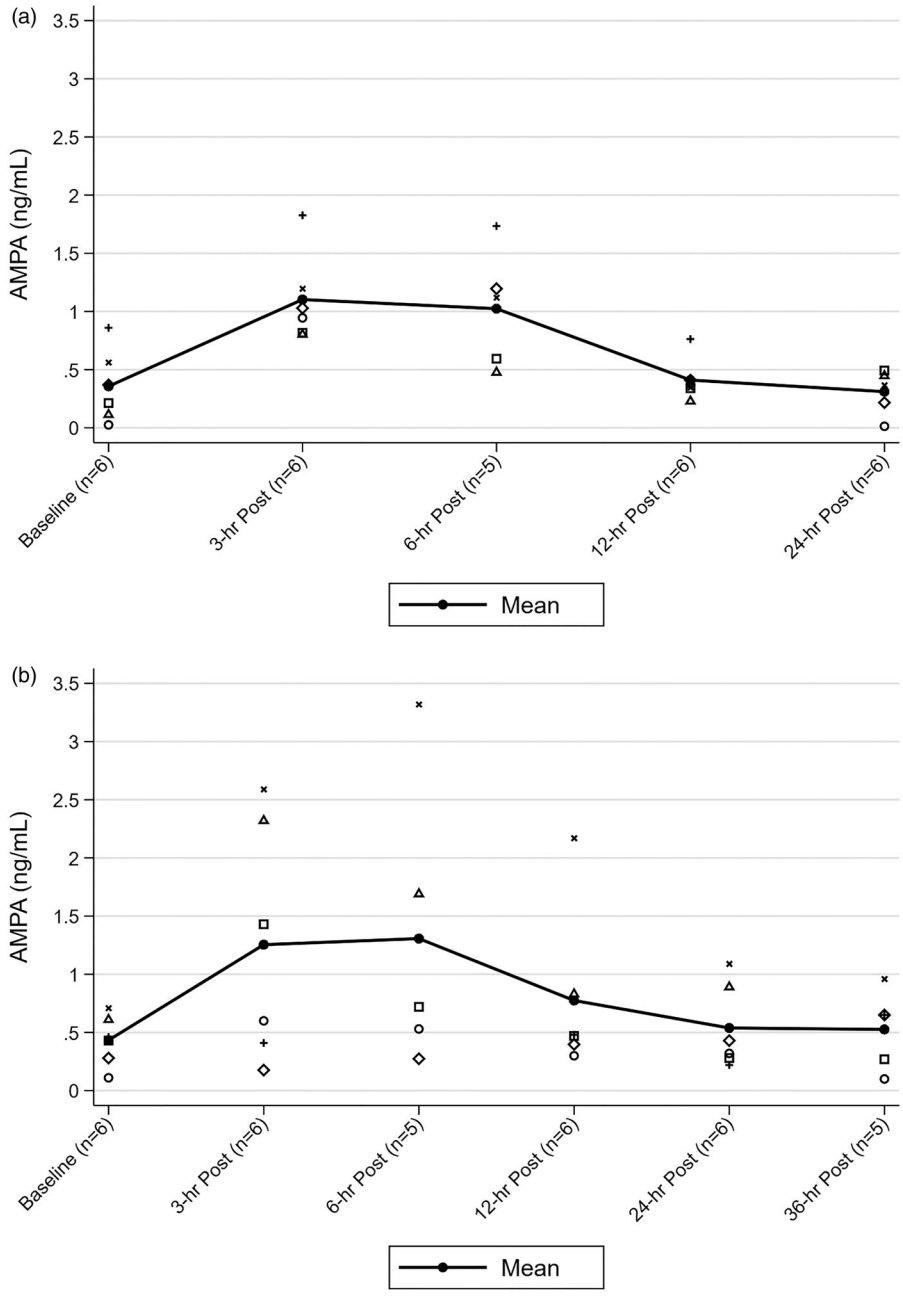

Figure A1. (a) Urinary AMPA Concentrations for Applicators ($n = 6$) in the Inhalation Exposure Group ($n = 6$) at Baseline, and 3-hr, 6-hr, 12-hr, and 24-hr After Application of Roundup®. (b) Urinary AMPA Concentrations for Applicators in the Dermal Exposure Group ($n = 6$) at Baseline, and 3-hr, 6-hr, 12-hr, 24-hr and 36-hr After Application of Roundup®.

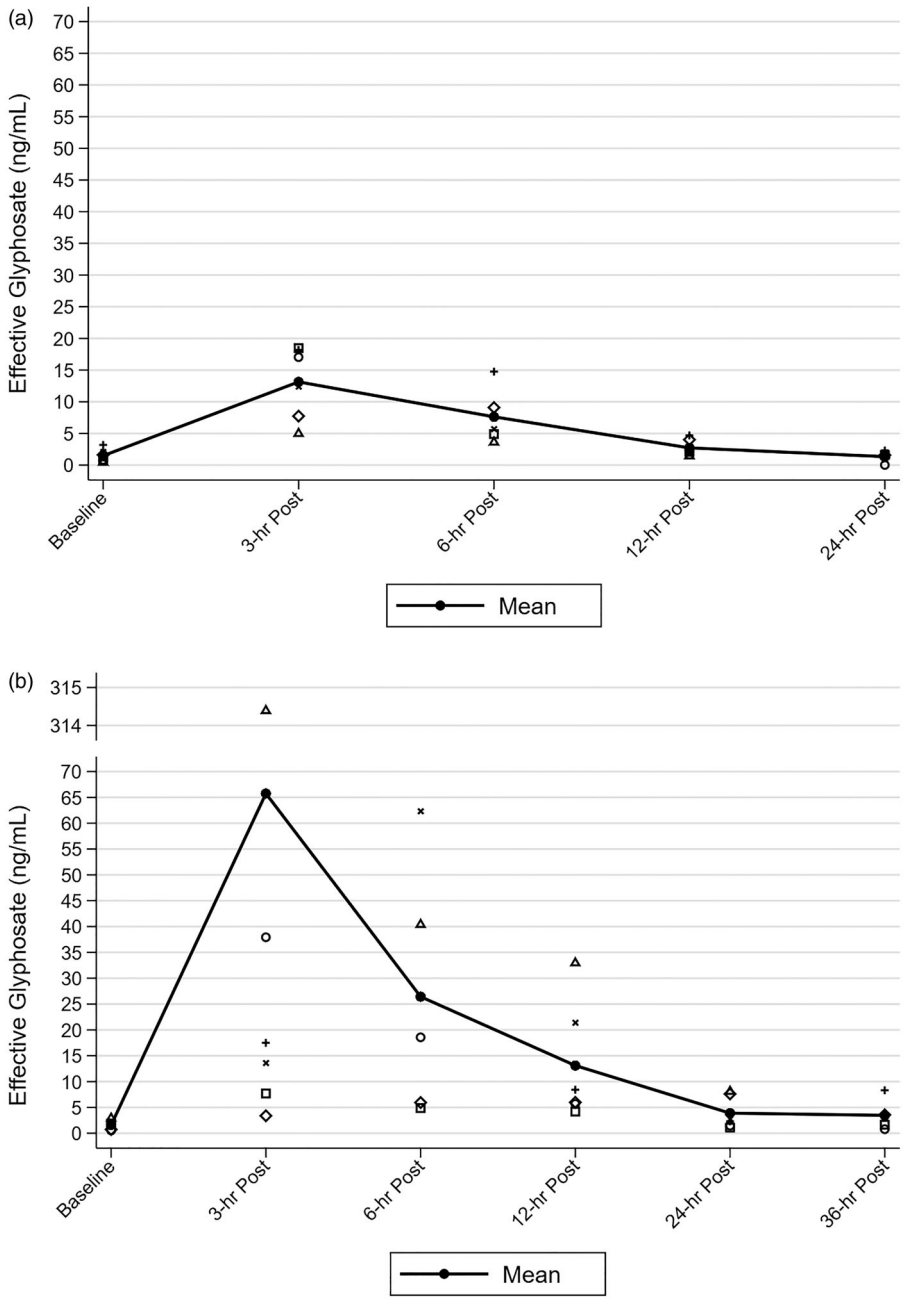

Figure B1. (a) Urinary Effective Glyphosate Concentrations for Applicators in the Inhalation Exposure Group ($n = 6$) at Baseline, and 3-hr, 6-hr, 12-hr, and 24-hr After Application of Roundup®. (b) Urinary Effective Glyphosate Concentrations for Applicators in the Dermal Exposure Group ($n = 6$) at Baseline, and 3-hr, 6-hr, 12-hr, 24-hr and 36-hr After Application of Roundup®.

Table B1. Urinary effective glyphosate concentrations (ng/mL) for applicators by exposure group and urine collection time point.

Exposure group	Minimum	Median	Arithmetic mean	Standard deviation	Maximum
Inhalation ($n = 6$)					
30-min Pre-Application (Baseline*)	0.43	1.22	1.49	1.04	3.19
3-hr Post-Application	4.99	14.73	13.14	5.74	18.46
6-hr Post-Application[b]	3.62	5.72	7.62	4.48	14.77
12-hr Post-Application	1.44	2.20	2.73	1.31	4.69
24-hr Post-Application	0.03	1.58	1.34	0.79	2.33
Dermal ($n = 6$)					
30-min Pre-Application (Baseline*)	0.54	1.75	1.59	0.86	2.90
3-hr Post-Application[a]	3.38	15.55 *(13.62)*	65.75 *(16.02)*	122.39 *(13.39)*	314.39 *(37.93)*
6-hr Post-Application[b]	4.88	18.57	26.42	24.65	62.34
12-hr Post-Application	4.20	7.20	13.10	11.57	32.93
24-hr Post-Application	1.10	2.54	3.89	3.13	8.09
36-hr Post-Application[b]	0.78	3.04	3.47	2.92	8.31

[a]Values in parentheses exclude the outlier measurement.
[b]$n = 5$; three samples were not analyzed (a 6-hr post-application sample from an inhalation group applicator, a 6-hr post-application sample from a dermal group applicator, and a 36-hr post-application sample from a separate dermal group applicator spilled during transit).
*Baseline urinary effective glyphosate concentrations were not statistically significantly different between the inhalation and dermal exposure groups.

Table B2. Fixed and random effects for the Log-Transformed urinary effective glyphosate data ($n = 63$)[a].

	Coefficient (GM)	SE	*p* Value	95% CI	
Intercept	−0.09 (0.91)	0.332	<0.760	−0.73	0.53
Group					
Inhalation	*Reference*				
Dermal	0.69 (1.99)	0.38	0.071	−0.06	1.43
Time Point					
Baseline	*Reference*				
3-hr Post-Application	2.50 (12.18)	0.28	<0.001*	1.94	3.06
6-hr Post-Application	2.11 (8.25)	0.36	<0.001*	1.41	2.81
12-hr Post-Application	1.35 (3.86)	0.37	<0.001*	0.62	2.08
24-hr Post-Application	0.16 (1.17)	0.39	0.680	−0.61	0.93
36-hr Post-Application	0.32 (1.38)	0.52	0.3541	−0.70	1.33
Random Effects (variance)	Estimate	SE			
Subject: random intercept	0.02	0.02			
Subject: random slope	5.88×10^{-12}				
Rho	0.40	0.17			
Residual	0.80	0.24			

[a]Three samples were not analyzed (a 6-hr post-application sample from an inhalation group applicator, a 6-hr post-application sample from a dermal group applicator, and a 36-hr post-application sample from a separate dermal group applicator spilled during transit).
*Across both exposure groups, urinary effective glyphosate levels were significantly elevated relative to baseline until 24-hr post-application (i.e. at 3-, 6-, and 12-hr post-application). This held true when the model was run separately for the dermal group, but the urinary glyphosate levels were only significantly elevated for the inhalation exposure group until 12-hr post-application (data not shown).