Risk Analysis, Vol. 0, No. 0, 2020 DOI: 10.1111/risa.13646

Risk Assessment of Glyphosate Exposures from Pilot Study with Simulated Heavy Residential Consumer Application of Roundup® using a Margin of Safety (MOS) Approach

Daniel G. Kougias,* Eric Miller, Abigail McEwen, Heidi Reamer, Michael Kovochich, and Jennifer Pierce

Due to the widespread application of glyphosate, a nonselective herbicide, to a variety of resistant food crops, the general population is exposed to glyphosate through dietary intake. Despite this, dietary exposures to glyphosate are considered low in comparison to application-related exposures. Although previous studies have evaluated exposure to horticultural and agricultural workers, to date only one study, which we recently conducted, has characterized exposure to glyphosate in consumers following heavy residential application of a glyphosate-containing herbicide in a residential yard and garden setting. In this previous study, we demonstrated that urinary glyphosate concentrations in these applicators were similar to or in some circumstances greater than those in occupational applicators, likely due to the nature of the simulation study, which ensured a heavy application protocol. However, it is unknown whether these urinary glyphosate concentrations in consumer applicators correspond to internal doses that may be of concern. Therefore, the purpose of this study is to provide a comprehensive risk assessment of glyphosate exposure in consumer applicators using a margin of safety approach. Here, we incorporated data collected from multiple spot urine samples across time from our previous study that assessed consumer exposure to glyphosate from Roundup® application. Estimated internal doses, even with the use of conservative assumptions across unique approaches, were below internal doses estimated from established health-based guidance values. Overall, this study demonstrates that glyphosate exposure from even heavy consumer application of a commercially available glyphosate-containing herbicide does not appear to be a health concern.

KEY WORDS: Biomonitoring; glyphosate; pesticide; risk assessment; urine

1. INTRODUCTION

Glyphosate, a nonselective herbicide, was first commercially available in 1974 and has since become the most ubiquitously used active ingredient in herbicides worldwide (Benbrook, 2016). Glyphosate-based products are applied to over a hundred glyphosate-resistant food crops, including but not limited to corn, soybean, cotton, canola, and sugar beets (U.S. Environmental Protection Agency [U.S. EPA], 2019a). Due to its widespread use in the environment, it has been presumed that the majority of the global population is exposed to glyphosate on a regular basis (Mills et al., 2017; Niemann, Sieke, Pfeil, & Solecki, 2015). Consequently, there has been a growing public, scientific, and regulatory interest in the potential health risks related to glyphosate exposure.

Cardno ChemRisk, Chicago, IL, USA.
*Address correspondence to Daniel G. Kougias, Cardno ChemRisk, 30 North LaSalle St Suite 3910, Chicago, Illinois, 60602–2590; Daniel.Kougias@cardno.com

Accordingly, several regulatory agencies and scientific organizations have provided comprehensive toxicological evaluations of glyphosate that assess the potential health effects related to glyphosate exposure. While the International Agency for Research on Cancer (IARC) classified glyphosate as having strong evidence of genotoxicity and as *probably carcinogenic to humans* in 2015 (International Agency for Research on Cancer (IARC), 2015), many other regulatory and expert scientific bodies, including the European Food Safety Authority (EFSA), the European Chemicals Agency (ECHA), the U.S. Environmental Protection Agency (EPA), the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Meeting on Pesticide Residues (JMPR), the Pest Management Regulatory Agency of Health Canada (PMRA), the Australian Pesticides and Veterinary Medicines Authority (APVMA), and the Food Safety Commission of Japan (FSCJ) have concluded that glyphosate is not mutagenic or genotoxic, at least at concentrations relevant to human exposure, and is not carcinogenic (ATSDR, 2019; APVMA, 2017; ECHA, 2017; EFSA, 2015; FSCJ, 2016; JMPR, 2016; PMRA, 2015; U.S. EPA, 2017). The difference of conclusions between these agencies and IARC is in part due to divergences in not only studies used and the corresponding interpretation of the results but also the weight assigned to human epidemiological studies (see Portier et al., 2016; Tarazona et al., 2017). Additionally, IARC opines on hazard with respect to the potential genotoxicity and carcinogenicity of glyphosate rather than on the account of a risk assessment (see IARC, 2019).

Based in part on these toxicological evaluations, several health-based guidance values specific to oral glyphosate exposure in the general population have been established and updated over the years. For instance, in 1987, the U.S. EPA Integrated Risk Information System (IRIS) program established a reference dose (RfD) of 0.1 mg/kg/day for glyphosate based on renal tubular dilation effects in a three-generation rat study (U.S. EPA, 1987). However, in their 1993 assessment, the U.S. EPA Office of Pesticide Programs (OPP) noted that the previously reported renal tubular dilation effects were not replicated at the highest dose tested in a two-generation rat study, such that the effects were considered "spurious rather than glyphosate-related" (U.S. EPA, 1993). Consequently, the U.S. EPA determined an oral RfD of 2 mg/kg/day for glyphosate based on the maternal no-observed-effect level of 175 mg/kg/day from a developmental toxicity study in rabbits (U.S. EPA, 1993). Currently, the U.S. EPA OPP is reevaluating glyphosate under the registration review program, and proposed a chronic population adjusted reference dose (cPAD) of 1 mg/kg/day, based on the maternal no-observed-adverse-effect level (NOAEL) of 100 mg/kg/day from a developmental toxicity study in rabbits (U.S. EPA, 2017). The lowest present-day health-based guidance value for glyphosate, separately established by PMRA in 2015 and APVMA in 2017, is an acceptable daily intake (ADI) of 0.3 mg/kg/day, which is believed to be the amount of a particular substance that can be ingested daily over a lifetime with no appreciable health risk (APVMA, 2017; PMRA, 2015). For the evaluation by PMRA, the ADI was based on a NOAEL of 32 mg/kg/day and 34 mg/kg/day (males and females, respectively) from a 26-month chronic toxicity and carcinogenicity study in rats that demonstrated reduced body-weights and increased incidences and severity of cellular alterations in the parotid and submandibular glands at a dose level of 100 mg/kg/day (i.e., the lowest-observed-adverse-effect level [LOAEL]); additionally, this selected point of departure was supported by a NOAEL of 30 mg/kg/day and a LOAEL of 100 mg/kg/day in three one-year studies in dogs (PMRA, 2015). For the evaluation by APVMA, the ADI was based on the highest tested dose of 30 mg/kg/day in a three-generation reproduction dietary study in rats (APVMA, 2017).

Although these health-based guidance values are specific to oral exposure, glyphosate can also be absorbed in humans via inhalation and dermal contact. These latter routes of exposure are considered minor for glyphosate alone partly due to its low vapor pressure and ionic nature; however, with the addition of surfactants in formulated products, glyphosate uptake is increased across lipid membranes. In fact, dermal absorption is considered the primary route of exposure for those involved in the application of glyphosate-containing herbicides while inhalation or inadvertent ingestion account for only small amounts of the total body burden (Connolly et al., 2019). Despite the limited information regarding the toxicokinetics of glyphosate following inhalation, dermal, or oral exposures (ATSDR, 2019), it has been reported that absorbed glyphosate undergoes little metabolism (less than 1%) to aminomethylphosphonic acid (AMPA), is confined primarily to the blood stream compartment and does not appear to accumulate within specific tissues, and is renally eliminated

from the blood with a half-life of approximately three to four hours (EFSA, 2015; JMPR, 2004; Roberts et al., 2010).

While the general population may be exposed to glyphosate in the air and in foods and drinking water, these environmental and dietary exposures are considered low in comparison to exposures that may occur during application of glyphosate-containing herbicides (ATSDR, 2019). To date only one pilot study, which we recently conducted, has characterized exposures to glyphosate following simulated heavy residential consumer application of a glyphosate-containing herbicide (see Pierce et al., 2020). In this previous study, we demonstrated that urinary glyphosate concentrations in these applicators generally peaked within six hours following application and were similar to or in some circumstances greater than those in occupational applicators, likely due to the nature of the simulation study, which ensured a heavy application protocol. However, it is unknown whether these urinary glyphosate concentrations in consumer applicators correspond to internal doses that may be of concern. Therefore, the purpose of this study is to provide a comprehensive risk assessment of glyphosate exposure in consumer applicators using a margin of safety (MOS) approach with unique methodologies to estimate the internal dose using data collected from multiple spot urine samples. In particular, risk is assessed by benchmarking the estimated internal doses in this study against the internal dose estimated from the most conservatively established health-based guidance value.

2. METHODS

2.1. Experimental Design

2.1.1. Study Protocol

An IRB-approved biomonitoring study involving the collection of individual urinary void spot samples was completed and previously reported (Pierce et al., 2020). In brief, the study was conducted during a single day in July of 2019 in a gravel and asphalt yard in Monee, IL. Participants were divided into two exposure groups: one designed to only assess dermal exposure and the other, inhalation exposure. There were six study participants (i.e., "applicators") per exposure group with equal representation of each gender, for a total of 12 applicators. For the dermal exposure group, applicators wore shorts, t-shirts, athletic shoes, and half-face respirators equipped with OV/AG/P100 cartridges (3M 60921, St. Paul, MN), whereas for the inhalation exposure group, applicators wore Tyvek suits and chemical resistant gloves but no respirator. Although farm workers as compared to residential sprayers have the potential to be exposed to larger amounts of glyphosate due to the application of greater volumes across more land, some farm workers can have reduced exposures with the use of personal protection and application equipment (e.g., respirators, Tyvek suits, chemical resistant gloves and boots, enclosed tractor cabins, etc.) that may not be the most accessible, or representative of, residential sprayers.

The applicators mixed and, using a backpack sprayer (Roundup® Commercial backpack sprayer), applied a commercially available glyphosate-containing herbicide (Roundup® Weed & Grass Killer Super Concentrate EPA Reg. No. 71995-25). It is no unreasonable to assume that residential consumers would use the same product and spray-application equipment, as both are readily available at home improvement and garden retailers and the backpack sprayer used in this study was made by the same manufacturer as the product. Ten fluid ounces of roundup concentrate containing 50.2% glyphosate was added to the backpack sprayer with four gallons of water to create a 0.96% glyphosate-containing solution. Each applicator sprayed the 677-foot perimeter of the yard at an approximate pace of 1 foot per second. Product was continuously sprayed until the backpack sprayer was empty, after which it was refilled, and the process was repeated for a total of four mixing and spraying events per applicator. This corresponded to a total of 100 minutes, with roughly 3–5 minutes and 20–22 minutes for each mixing and spraying event, respectively.

The study was conducted in three separate 100-minute runs at 0930, 1215, and 1415, with two applicators in the dermal exposure group and two applicators in the inhalation exposure group participating in a given run. Helpers were assigned to each applicator to ensure that the four applicators during a given run remained evenly spaced by walking and spraying at approximately the same rate in order to minimize additional exposure to spray drift among the four applicators during a run, as spray drift would not be expected in a residential consumer application setting.

2.1.2. *Urinary Sampling for Analysis of Glyphosate Residues*

Urine samples were collected from each applicator at baseline (30 minutes preapplication) and three, six, 12, and 24 hours postapplication. An additional urine sample was collected 36 hours postapplication only from applicators within the dermal exposure group due to the possible delay in absorption via dermal exposure.

Using testing kits obtained from the Health Research Institute (HRI) Laboratories (Fairfield, IA), urine samples were analyzed for glyphosate and AMPA with high-performance liquid chromatography-triple quadrupole mass spectrometry (HPLC-MS/MS). The limits of detection (LOD) and quantification (LOQ) for glyphosate in urine were 0.02 ppb and 0.05 ppb, respectively, whereas, for AMPA, they were 0.013 ppb and 0.05 ppb, respectively. Concentrations of glyphosate and AMPA were adjusted for dilution effects using urine specific gravity.

HRI Laboratories also calculated the "effective glyphosate" concentrations according to the FAO method to determine total glyphosate residues. This is achieved by summing the weights of measured urinary concentrations of glyphosate and 1.5 times AMPA.

2.1.3. *Applicator Fluid and Dietary Intake*

Each applicator recorded their dietary intake, including liquids, throughout the study period starting in the morning on the day of application and continuing to their last urinary void. This information was logged for each period between urinary void samples in order to potentially account for anomalies in the urinalysis data. Additionally, detailed information on fluid intake was documented to provide insight as a predictor of urinary output for internal dose calculation purposes.

2.2. Internal Dose Calculation

Internal dose serves as a biomarker of exposure that indicates the amount of glyphosate that is systemically available. With some minor adjustments, internal dose for glyphosate is generally calculated for a given individual by dividing the product of their daily glyphosate urinary concentration (C_{urine}; in mg/L) and daily urinary output (V_{urine}; in L) by their bodyweight (BW; in kg) (see Equation (1)) (see Acquavella et al., 2004; Niemann et al., 2015).

$$\text{Internal dose (mg/kg BW)} = \frac{C_{\text{urine}} \times V_{\text{urine}}}{\text{BW}} \quad (1)$$

A rudimentary internal dose calculation: Internal dose is generally calculated by dividing the product of glyphosate urinary concentration (C_{urine}; mg/L) and daily urinary output (V_{urine}) by bodyweight (kg).

However, given that our data provide urinary concentrations of several samples throughout a period of more than 24 hours without a direct measurement of total urinary output volume, additional considerations and assumptions were made and are discussed below. Bodyweights were self-reported values and were not complemented with direct measurements.

2.2.1. *Pharmacokinetic Recovery Adjustment*

Based on a pharmacokinetic study in monkeys, a recovery of approximately 94.9 ± 8.6 (mean ± *SD*) and 98.8 ± 3.8% were obtained in the urine across seven days following a single intravenous injection of glyphosate at a relatively high and low dose, respectively (Wester, Melendres, Sarason, McMaster, & Maibach, 1991). Hence, Acquavella et al. (2004) divided the amount of glyphosate excreted by 0.95 to estimate the amount of glyphosate that would have been recovered in urine with complete pharmacokinetic recovery. However, since the recovery of glyphosate limited to the first 24-hour period in the study by Wester et al. (1991) was approximately 82%, the present study used a value of 0.82 as an adjustment to account for pharmacokinetic recovery.

2.2.2. *Daily Amount of Glyphosate Residues Excreted*

Urinary "effective glyphosate" concentrations were used in calculations for internal dose estimations to conservatively account for any potential losses of glyphosate due to metabolism to AMPA. Moreover, certain health-based guidance values for glyphosate, like the ADI determined by the JMPR (JMPR, 2004), also consider AMPA concentrations.

The total amount of glyphosate residues excreted during a 24-hour period was calculated based on "effective glyphosate" concentrations in the three, six, 12, and 24 hours postapplication spot urine samples (samples collected 30 minutes preapplication and 36 hours postapplication were excluded), given

that these were consecutive samples that most closely represented a 24-hour period and corresponded to the highest average "effective glyphosate" concentrations. Due to the selection of these sampling times, a more conservative estimation of the daily amount of glyphosate residues excreted was calculated based off urinary output volume across 26.167 hours. In particular, the time between the preapplication urine sample and the subsequent urine sample totaled 310 min (i.e., 30 minutes prior to application, 100 minutes of application, and three hours postapplication) and no additional urinary voiding occurred between these two time-point samples.

2.2.3. Urine Production Considerations

Urinary production volume is known to vary as a function of fluid intake (Armstrong et al., 2012; Armstrong et al., 2010; Perrier et al., 2012). In particular, it has been demonstrated to rapidly respond to changes in fluid intake and stabilize within 24 hours following modified fluid intake (Perrier, Erica et al., 2013). Furthermore, two recent studies have demonstrated a strong relationship between daily fluid intake and 24-hour urine output volume (Athanasatou, Kandyliari, Malisova, & Kapsokefalou, 2019; Zhang et al., 2017).

In view of this, fluid intake data for each applicator was used to inform estimations of urinary output. According to the Centers for Disease Control and Prevention (CDC), normal urinary output is between 0.5 mL/kg BW/h and 1.5 mL/kg BW/h (CDC, n.d.). Consequently, each applicator was assigned a bodyweight-specific urine production rate dependent on their hydration status (i.e., fluid intake).

In conjunction with the selected bodyweight-specific urine production rate, two separate approaches were used for estimating the total urine volume between urinary void samples: (Approach #1) by assuming the time elapsed between each urine collection time-point was directly related to urine volume production and (Approach #2) by assuming equal urine volume between each time-point. In large part, both approaches are presented here to demonstrate two reasonable methods to address the uncertainty of this important variable.

2.2.4. Margin of Safety Calculation

The margin of safety (MOS) approach is a well-established risk assessment approach where a selected reference or benchmark dose is divided by the measured or expected exposure to quantify how many times lower the exposure is relative to the reference or benchmark dose (Equation (2)). Although MOS is not a probabilistic statement of risk, as the value increases, the concern regarding the exposure evaluated decreases. By using a health-based guidance value that already incorporates an uncertainty or safety factor of 100 for the benchmark dose, MOS values greater than 1 are interpreted to be acceptable, while values lower than 1 suggest that the chemical exposure risk is not likely to be acceptable.

$$\text{MOS} = \frac{\text{Reference or Benchmark Dose}}{\text{Calculated Internal Dose}} \quad (2)$$

Margin of safety (MOS) calculation. Margin of safety (MOS) is calculated by dividing the reference or benchmark dose by the calculated internal dose.

As outlined in Table I, several relevant health-based guidance values from various agencies are available to benchmark against internal doses calculated within this analysis. The ADI of 0.3 mg/kg/day separately established by APVMA and PMRA was chosen based on the fact that it was the lowest value, thus providing for the most conservative benchmark value. A correction factor of 20% to account for limited oral absorption was applied to convert the ADI to an internal dose of 0.06 mg/kg/day or 60 μg/kg/day (PMRA, 2015; APVMA, 2017).

3. RESULTS

3.1. Hydration Status

3.1.1. Fluid Intake

Individual fluid intake across the 26.167-hour study period ranged from 3.48 L to 9.32 L, with an average intake of 5.49 L (Table II). As expected, with each separate run occurring later in time from the previous run, preapplication fluid intake values, which represented the time between awakening and 30 minutes preapplication, increased across the three runs. Since a positive relationship is known to exist between fluid intake and urinary output volumes (Armstrong et al., 2012; Armstrong et al., 2010), the fluid intake values within the present study were used to inform estimations of urinary output volume. The approximate daily fluid intake values within this study were on the higher end of, or considerably above, daily fluid intake values from previous studies of comparable individuals based on age, gender, and body mass (Armstrong et al., 2013; Armstrong

Table I. Health-Based Guidance Values for Oral Exposure to Glyphosate

Organization	Year	Animal Model	NOAEL(underlying study)	Limit	Guidance Value (mg/kg/day)	Internal Dose (mg/kg/day)
U.S. EPA	1993	Rabbit	175 mg/kg/day for maternal toxicity[a]	RfD	2	0.4
JMPR	2004	Rat	100 mg/kg/day for salivary gland alterations	ADI	0–1	0.2
EFSA	2015	Rabbit	50 mg/kg/day for maternal and developmental toxicity	ADI	0.5	0.1
				ARfD	0.5	0.1
				AOEL	0.1	0.1
PMRA	2015	Rat	NOAEL of 32/34 mg/kg/day in a two-year study based on decreased bodyweight and increased incidences and severity of cellular alterations in the parotid and submandibular glands	ADI	0.3	0.06
		Dog	NOAEL of 30 mg/kg/day and LOAEL of 100 mg/kg/day from three one-year studies			
FSCJ	2016	Dog	100 mg/kg/day for reduced bodyweight gain, diarrhea, bloody feces, etc.	ADI	1	0.2
		Rabbit	100 mg/kg/day for maternal toxicity			
APVMA	2017	Rat	30 mg/kg/day was the highest tested dose in a three-generation reproduction dietary study	ADI	0.3	0.06
U.S. EPA	2017	Rabbit	100 mg/kg/day for maternal toxicity	cPAD	1[b]	0.2
ATSDR	2019	Rabbit	100 mg/kg/day for maternal toxicity	MRL	1[b]	0.2

Note: All oral health-based guidance values used an uncertainty or safety factor of 100 and were based on a no-observed-effect level (NOEL) or no-observed-adverse-effect level (NOAEL) from a particular animal study. Health-based guidance values, which vary by agency and review, included the reference dose (RfD), acceptable daily intake (ADI), acute reference dose (ARfD), acceptable operator exposure level (AOEL), chronic population adjusted reference dose (cPAD), and minimal risk level (MRL). Internal dose (mg/kg/day) assumes 20% oral absorption with respect to the health-based guidance value, except in the case of the AOEL, which is a health-based guidance value that already corresponds to an internal dose.

[a]Based on a reported NOEL for diarrhea, nasal discharge, and death (EPA, 1993).

[b]Draft or provisional value.

et al., 2012; Perrier, E. et al., 2013; Perrier et al., 2012); therefore, all applicators were considered to be well-hydrated and were assigned a bodyweight-specific urine production rate of 1.5 mL/kg BW/hr, the upper end of the normal range (CDC, n.d.).

3.1.2. Urine Specific Gravity

Urine specific gravity (i.e., the ratio of the density of urine to that of water) is a common urinalysis parameter used to evaluate kidney function with normal values ranging from 1.000 to 1.040 (Ridley, 2018). In view of this, all specific gravity measurements in this study were within the normal range, with an average urine specific gravity from all samples of 1.011 and a range of 1.003–1.030 (Table II). Urine specific gravity from three urinary void samples was not available due to a procedural error resulting in the loss of the sample.

Urine specific gravity is indicative of the concentration of excreted molecules and, therefore, is influenced by hydration status. However, first morning urine void samples provide the most uniform quantitative assessment of solutes, as it minimizes influences of diet, activity, and environmental and psychological stressors that affect urine specific gravity

Table II. Fluid Intake and Urine Specific Gravity by Individual. Total fluid Consumed (L) Prior to Each Urine Sample Time-Point (i.e., 30 Minutes Preapplication And Three, Six, 12, 24, and 36 Hours Postapplication) is Shown. Urine Specific Gravity is Provided for Each Available Urine Sample. "N/A" Indicated That the Value was Not Available Due to a Procedural Error Resulting in the Loss of the Sample

	Inhalation						Dermal					
	Run 1		Run 2		Run 3		Run 1		Run 2		Run 3	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Fluid Intake Prior to Sample Collection (L)												
Preapplication	0.70	0.83	1.48	1.83	2.87	2.45	1.21	1.06	1.68	0.97	2.48	2.40
3 hours postapplication	2.00	1.06	2.00	1.98	1.83	1.91	1.54	1.50	0.97	1.00	1.00	1.46
6 hours postapplication	1.50	0.71	2.10	1.77	1.77	0.50	2.60	1.00	1.10	0.71	0.50	0.75
12 hours postapplication	1.06	0.24	0.50	1.01	0.60	0.50	3.12	1.54	0.57	0.65	0.71	0.18
24 hours postapplication	0.60	1.95	1.95	2.07	3.08	1.50	2.06	1.18	2.48	1.95	1.27	1.86
36 hours postapplication							1.21	1.77	1.18	1.72	1.21	1.86
Approximate Daily (26.167 hours) Total	5.16	3.96	6.55	6.83	7.28	4.41	9.32	5.22	5.12	4.31	3.48	4.24
Specific Gravity												
Preapplication	1.021	1.011	1.014	1.003	1.007	1.005	1.006	1.007	1.006	1.011	1.009	1.004
3 hours postapplication	1.007	1.009	1.020	1.004	1.006	1.003	1.017	1.011	1.023	1.015	1.012	1.003
6 hours postapplication	1.009	1.010	1.023	N/A	1.003	1.017	1.020	1.024	1.023	1.016	1.015	N/A
12 hours postapplication	1.008	1.007	1.030	1.013	1.012	1.008	1.007	1.008	1.012	1.012	1.025	1.013
24 hours postapplication	1.018	1.003	1.008	1.003	1.011	1.004	1.005	1.010	1.006	1.007	1.012	1.006
36hours postapplication	–	–	–	–	–	–	1.004	N/A	1.010	1.013	1.021	1.023
Average daily (26.167 hours) specific gravity	1.011	1.007	1.020	1.007	1.008	1.008	1.012	1.013	1.016	1.013	1.016	1.007

independent of dehydration (Cheuvront, Kenefick, & Zambraski, 2015). Considering that this study did not collect a first morning urine sample, the urine specific gravity reported in the study was precluded from informing hydration status.

3.2. Additional Urinary Measurements

3.2.1. Glyphosate, AMPA, and Effective Glyphosate Concentrations

As had been previously reported, urinary glyphosate, AMPA, and "effective glyphosate" concentrations across the applicators followed similar temporal trajectories with concentrations generally peaking at three hours or six hours postapplication and returning to preapplication concentrations by 24 hours postapplication (see Pierce et al., 2020).

Urinary "effective glyphosate" concentrations are presented in Table III and are used in calculations for internal dose estimations. Like urine specific gravity, the urinary "effective glyphosate" concentration from three urinary void samples was not available due to a procedural error resulting in the loss of the sample. One of these samples was a 36-hours postapplication sample, and thus did not apply to internal dose calculations; however, two of the samples were six hours postapplication samples. To address this issue, the largest fold difference in an individual between the three hours and six hours postapplication samples (i.e., 4.577 from the male applicator in run 3 of the dermal group) was applied to the six hours postapplication samples that were lost.

3.2.2. Urine Volume

Urine production volume was estimated for all applicators within this study using the upper end of the normal urinary output range (1.5 mL/kg BW/hr). Consequently, estimated urine volume of the applicators averaged 3.039 L, with a range of 2.047 L to 4.184 L, during the 26.167-hour study period.

3.3. Dietary Intake

Individual dietary intake logs (not reported) were unremarkable and did not noticeably affect urinary glyphosate residue concentrations, at least relative to application exposures, as the range of urinary glyphosate residue concentrations at baseline (30 minutes preapplication) was much smaller than the range of concentrations at three, six, and 12 hours postapplication. Additionally, urinary glyphosate residue concentrations at baseline and at 24 hours and 36 hours postapplication were within the range of background urinary glyphosate concentrations observed in other studies (Gillezeau et al., 2019; Krüger et al., 2014; Niemann et al., 2015).

3.4. Bodyweights

Bodyweights are circuitously reported in Tables III and IV. Since it has been shown that both men and women overwhelmingly underestimate their bodyweight when self-reporting (Olfert et al., 2018), it is likely that the bodyweight values in this study are underestimated. Though with the use of a bodyweight-specific urine production rate, bodyweight *per se* has no impact on the calculated internal doses on a per kilogram bodyweight basis and no impact on margins of safety.

3.5. Internal Doses and Margins of Safety

3.5.1. Approach #1: Time-dependent urine volume between each time-point sample

In the first approach (see Table III), calculated total internal doses across the 26.167-hour study period for the applicators in the inhalation and dermal exposure groups averaged 19.982 μg (8.030–33.491 μg) and 97.280 μg (12.325-397.828 μg), respectively. Mean internal doses calculated on a per kilogram bodyweight basis for the applicators in the inhalation and dermal exposure groups were 0.290 μg/kg (0.118–0.615 μg/kg) and 1.045 μg/kg (0.170 –3.732 μg/kg), respectively. Calculated MOS values for applicators within the inhalation and dermal groups averaged 270 (98–508) and 140 (16–353), respectively.

Since these MOS values were based on internal doses from isolated inhalation or dermal exposures and it is likely that any real-world consumer use would lead to both inhalation and dermal exposures, a combined exposure MOS was calculated. To aid in representing a "worst-case" scenario, the highest bodyweight-adjusted internal dose (μg/kg) from each exposure group were summed, resulting in a maximum internal dose of 4.347 μg/kg. Accordingly, the lowest MOS calculated from the maximum internal dose for combined exposure routes was 14.

Table III. Margin of Safety Approach #1: Time-Dependent Urine Volume Production

	Inhalation						Dermal					
	Run 1		Run 2		Run 3		Run 1		Run 2		Run 3	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Effective Glyphosate Level (ng/mL)												
Preapplication	1.619	0.433	0.822	0.743	2.110	3.194	0.720	2.900	1.880	0.540	1.750	1.760
3 hours postapplication	7.737	4.990	18.460	17.038	12.418	18.173	3.380	314.389	7.700	37.930	13.620	17.490
6 hours postapplication	9.091	3.620	4.918	N/A	5.720	14.769	5.947	40.380	4.880	18.570	62.340	N/A
12 hours postapplication	4.023	1.440	2.099	2.297	1.800	4.691	5.979	32.930	4.200	5.690	21.410	8.420
24 hours postapplication	1.565	1.603	1.596	Trace	0.895	2.330	7.610	8.090	1.098	1.460	2.770	2.300
36 hours postapplication	–	–	–	–	–	–	3.570	N/A	1.650	0.780	3.040	8.310
Urine Output (L)												
3 hours postapplication	0.721	0.527	0.756	0.422	0.650	0.404	0.721	0.826	0.563	0.450	0.721	0.439
6 hours postapplication	0.418	0.306	0.439	0.245	0.378	0.235	0.418	0.480	0.327	0.261	0.418	0.255
12 hours postapplication	0.837	0.612	0.878	0.490	0.755	0.469	0.837	0.959	0.653	0.523	0.837	0.510
24 hours postapplication	1.674	1.225	1.755	0.980	1.510	0.939	1.674	1.919	1.307	1.045	1.674	1.021
Total	**3.650**	**2.671**	**3.827**	**2.136**	**3.294**	**2.047**	**3.650**	**4.184**	**2.850**	**2.279**	**3.650**	**2.225**
Internal Dose (μg)												
3 hours postapplication	6.800	3.209	17.010	8.765	9.849	8.960	2.971	316.747	5.283	20.814	11.970	9.372
6 hours postapplication	4.639	1.352	2.631	23.294[b]	2.634	4.228	3.035	23.622	1.944	5.917	31.812	24.909[b]
12 hours postapplication	4.105	1.075	2.247	1.372	1.658	2.686	6.102	38.528	3.347	3.626	21.851	5.240
24 hours postapplication	3.195	2.395	3.416	0.060[a]	1.648	2.668	15.534	18.931	1.750	1.861	5.654	2.863
Total	**18.739**	**8.030**	**25.305**	**33.491**	**15.789**	**18.541**	**27.641**	**397.828**	**12.325**	**32.217**	**71.286**	**42.384**
26.167-hour dose (μg/kg/day)	0.202	0.118	0.260	0.615	0.188	0.355	0.297	3.732	0.170	0.555	0.767	0.748
Margin of safety	298	508	231	98	319	169	202	16	353	108	78	80

Note: Individual urinary "effective glyphosate" levels (ng/mL) are shown for each time-point (i.e., 30-minute preapplication and three, six, 12, 24, and 36 hours postapplication). "Effective glyphosate" level is the sum of urinary glyphosate levels and 1.5 times the urinary AMPA levels. Individual urine output volumes (L) were calculated using a conservative bodyweight-specific production rate of 1.5 cc/kg/h for the four time-points that correspond to the highest average concentrations and span 26.167 hours. Individual internal doses (μg) were estimated assuming 82% pharmacokinetic recovery in urine and were compared to internal doses estimated from the lowest health-based guidance value (i.e., APVMA's and PMRA's ADI of 0.3 μg/kg/d) to ascertain margins of safety. "Trace" indicated a glyphosate concentration between the LOD (0.02 ng/mL) and LOQ (0.05 ng/mL) and a nondetectable AMPA concentration (i.e., below the LOD of 0.013); therefore;

[a]the conservative value of 0.05 ng/mL for "effective glyphosate" concentration was used for subsequent calculations. "N/A" indicated that the value was not available due to a procedural error resulting in the loss of the sample. To address this;

[b]the largest fold difference in an individual between the three and six hours postapplication samples (i.e., 4.577 from the male applicator in run 3 of the dermal group) was applied to the 6 hours postapplication samples that were N/A.

Table IV. Margin of Safety Approach #2: Equal Urine Volume Production Between Each Time-Point Sample

	Inhalation						Dermal					
	Run 1		Run 2		Run 3		Run 1		Run 2		Run 3	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Urine Output (L)												
3 hours postapplication	0.912	0.668	0.957	0.534	0.823	0.512	0.912	1.046	0.712	0.570	0.912	0.556
6 hours postapplication	0.912	0.668	0.957	0.534	0.823	0.512	0.912	1.046	0.712	0.570	0.912	0.556
12 hours postapplication	0.912	0.668	0.957	0.534	0.823	0.512	0.912	1.046	0.712	0.570	0.912	0.556
24 hours postapplication	0.912	0.668	0.957	0.534	0.823	0.512	0.912	1.046	0.712	0.570	0.912	0.556
Total	**3.650**	**2.671**	**3.827**	**2.136**	**3.294**	**2.047**	**3.650**	**4.184**	**2.850**	**2.279**	**3.650**	**2.225**
Internal Dose (μg)												
3 hours postapplication	8.610	4.063	21.537	11.097	12.470	11.344	3.761	401.043	6.689	26.353	15.155	11.867
6 hours postapplication	10.116	2.947	5.738	50.794[b]	5.744	9.219	6.618	51.510	4.240	12.902	69.367	54.315[b]
12 hours postapplication	4.476	1.172	2.449	1.496	1.807	2.928	6.653	42.006	3.649	3.953	23.823	5.713
24 hours postapplication	1.741	1.305	1.862	0.033[a]	0.898	1.454	8.468	10.320	0.954	1.014	3.082	1.561
Total	**24.943**	**9.488**	**31.587**	**63.420**	**20.920**	**24.946**	**25.500**	**504.878**	**15.532**	**44.222**	**111.428**	**73.455**
26.167-hour dose (μg/kg/day)	0.268	0.139	0.324	1.165	0.249	0.478	0.274	4.736	0.214	0.762	1.198	1.296
Margin of Safety	224	430	185	51	241	125	219	13	280	79	50	46

Note: Individual urine output volumes (L) were calculated based on the conservative bodyweight-specific production rate of 1.5 cc/kg/h across 26.167 hours using the four time-points that correspond to the highest average "effective glyphosate" concentrations; however, unlike Approach #1, this approach assumed equal volume production between each time-point sample. Individual internal doses (μg) of "effective glyphosate" were estimated assuming 95% pharmacokinetic recovery in urine and were compared to internal doses estimated from the lowest health-based guidance value (i.e., APVMA's and PMRA's ADI of 0.3 μg/kg/d) to ascertain margins of safety.

3.5.2. Approach #2: Assuming equal urine volume production between each time-point sample

With the second approach (see Table IV), calculated total internal doses across the 26.167-hour study period averaged 29.217 μg (9.488–63.420 μg) and 129.169 μg (15.532–504.878 μg) for the inhalation and dermal exposure groups, respectively. Mean internal doses calculated on a per kilogram bodyweight basis for the applicators in the inhalation and dermal exposure groups were 0.437 μg/kg (0.139–1.165 μg/kg) and 1.413 μg/kg (0.214–4.736 μg/kg), respectively. MOS values for the inhalation and dermal exposure groups averaged 209 (51–430) and 115 (13–280), respectively.

For "worst-case" scenario purposes, using the sum of the highest bodyweight-adjusted internal doses from both the inhalation and dermal exposure groups (i.e., 5.901 μg/kg) resulted in a combined exposure MOS of 10.

4. DISCUSSION

This is the first study to perform a risk assessment of glyphosate exposures following consumer application. In this study, conservative assumptions were used across two unique approaches to estimate internal daily doses of glyphosate residues from multiple spot urine samples over time from 12 residential consumer applicators, with half of the applicators protected from dermal exposures and the other half from inhalation exposures. None of the individually calculated internal daily doses for an applicator, nor the sum of the two highest internal daily doses from each exposure group, approached the internal dose of 100 μg/kg/day estimated from the most conservative health-based guidance value established for glyphosate (i.e., an ADI of 0.3 mg/kg/day). Therefore, in using a margin of safety approach, this study demonstrates that daily glyphosate residue exposures from heavy residential consumer application of a commercially available glyphosate-containing herbicide results in internal doses that do not pose a health risk.

Considering that IARC is the only regulatory agency or scientific organization that has provided an opinion designating glyphosate as *probably carcinogenic to humans* and given that IARC opines on hazard rather than on risk (see IARC, 2019), their designation cannot be used to support a risk assessment. Until additional data enable the proposal of biological mechanisms at human-relevant exposures and/or provide a biomarker of cumulative exposure that is specific to glyphosate, no further considerations on the matter of potential carcinogenicity of glyphosate exposure in this context can be made.

4.1. A Comparison of the Simulated Heavy Residential Consumer Application Exposure Study and Occupational Exposure Studies

Given that the ADI used to calculate MOS values in this analysis incorporates a safety factor and is, by definition, an acceptable daily intake of glyphosate, any MOS with a value of 1 or greater can be interpreted as posing a negligible risk. Since the lowest MOS calculated by using the sum of the highest internal doses from each exposure group was 10, representing the highest achievable internal dose, our study suggests there should be little to no concern of any adverse health effects associated with daily residential consumer application. In fact, the sum of the highest internal dose from each exposure group is a gross overestimation of expected exposures in a residential setting, as the inhalation group likely also had incidental dermal exposures through their permeable athletic shoes worn that were not covered and because the dermal exposure group did not wear chemical-resistant gloves, chemical-resistant clothing, and impermeable shoe covers as recommended per the Safety Data Sheet of the product. Additionally, considering that four applicators sprayed simultaneously during each run in this study, there was potential for additional exposure to spray drift, though this was minimized by keeping applicators equidistantly spaced along the perimeter of the yard. Moreover, this risk assessment assumes daily residential use of a glyphosate-containing herbicide, even though realistic consumer application in a residential setting would occur on an infrequent basis. Lastly, the conditions of our simulated study likely overestimate use in any residential glyphosate application, as each applicator continuously applied the glyphosate-containing product totaling 16 gallons (60.6 L) sprayed to approximately 5,040 linear feet (i.e., approximately 0.955 miles) of land, which corresponds to 242.4 L being sprayed during each run of the study.

Altogether, these statements on overestimated exposures and exaggerated use indicate that this study may represent a "worst-case" residential consumer application scenario, which is supported by comparisons to studies with occupational use and exposure (see Table V). For example, in

Table V. A Comparison of the Simulated Heavy Residential Consumer Application Exposure Study and Occupational Exposure Studies

Reference Urine Sample Type, Number of Subjects, and Study Population Information on Study with an Emphasis on Glyphosate Use and Protection	*N*	Time of Urine Sample Collection	Urinary Glyphosate				Urinary AMPA			
				μg/L (ppb)				μg/L (ppb)		
			% ND or Below LOQ	Min	Max	Mean ± SD [median]	% ND or Below LOQ	Min	Max	Mean [or median]
Simulated Heavy Residential Consumer Application Exposure Study ($n = 1$)										
Pierce et al. (2020)										
Urine spot samples from 6 inhalation-exposed participants (3 males, 3 females) simulating heavy residential consumer application	29	Preapplication and 3, 6, 12, and 24 hour postapplication	3.45	> 0.02[†], < 0.05[‡]	17.2	[1.835]	7.41	< 0.013[†]	1.8	
Urine spot samples from 6 dermally exposed participants (3 males, 3 females) simulating heavy residential consumer application See Study Protocol section.	34	Spot samples: preapplication and 3, 6, 12, 24, and 36 hour postapplication	0	0.3	310.91	[4.5]	0	0.1	3.3	
Occupational Exposure Studies ($n = 12$)										
Jauhiainen et al. (1991)										
Urine spot samples from 5 male Finland forest workers Workers mixed and sprayed an 8% roundup-containing (360 g/L glyphosate as isopropylamine salt) solution with a brush saw each day during one workweek, using an average of 9.8 L/worker/6-hour day. There was no possibility to wash their hands in the field while filling the saw tanks (3.5 L) as needed or repairing the brush saws. The workers wore cotton overalls, cotton or rubber gloves, a hat or safety helmet, and rubber boots, and on two days that it rained, the workers wore rain clothes.	30	Postapplication of each workday during the study week and after the 3-week work period	100	All samples < 100[†]				All samples < 50[†]		

(*Continued*)

Table V (Continued)

Reference Urine Sample Type, Number of Subjects, and Study Population Information on Study with an Emphasis on Glyphosate Use and Protection	*N*	Time of Urine Sample Collection	Urinary Glyphosate				Urinary AMPA			
				μg/L (ppb)				μg/L (ppb)		
			% ND or Below LOQ	Min	Max	Mean ± SD [median]	% ND or Below LOQ	Min	Max	Mean [or median]
Lavy et al. (1992)							AMPA concentrations not tested			
24-hour urine samples collected over 1,138 worker-days from 14 conifer seedling nursery workers (in the United States near Brooklyn, MS, and Roseburg, OR) over at least eight weeks to calculate seasonal exposures The 3 applicators, 9 weeders, and 2 scouts monitored wore normal work clothing, which for applicators was a protective suit, rubber gloves, and boots. The 9 weeders wore rubber gloves and manually applied a glyphosate solution (Roundup®) as spot treatments within seedling beds up to 8.5 days during the season, corresponding to individual applications of approximately 2 gallons of a 1:40 dilution Roundup® (i.e., 0.49–0.60 kg of Roundup®), with application times of 56–68 hours and around 0.01 kg applied/hectare. One weeder handled the concentrate by diluting and filling the manual spray applicators for other weeders. The 3 applicators mixed and loaded spray bottles and drove tractors when applying glyphosate to a nearby field or between beds using conventional ground application spray equipment. The applicators individually applied 0.13–0.71 kg of Roundup® to 1.2–6.48 hectares, with application times of 2.5–5.0 hours and 0.11 kg applied/hectare. Scouts did not use or apply glyphosate, but they routinely reentered and worked in treated areas.	355	Before potential exposure and continuing throughout the application season	100	All samples < 10[a]						

(*Continued*)

Table V (Continued)

Reference Urine Sample Type, Number of Subjects, and Study Population Information on Study with an Emphasis on Glyphosate Use and Protection	*N*	Time of Urine Sample Collection	Urinary Glyphosate				Urinary AMPA			
				μg/L (ppb)				μg/L (ppb)		
			% ND or Below LOQ	Min	Max	Mean ± SD [median]	% ND or Below LOQ	Min	Max	Mean [or median]
Acquavella et al. (2004)							AMPA concentrations not tested			
Composite urine samples from 48 farmers (i.e., licensed pesticide applicators in South Carolina and Minnesota, USA) All the farmers used tractors and boom sprayers while farming at least 10 acres within 1 mile of their residence, and most applied the Roundup Ultra formulation. During the application, 60% of the farmers had enclosed tractor cabins, 29% did not wear rubber gloves, 31% had skin contact with the glyphosate formulation, 15% had spills during mixing and loading or application, and 27% repaired their equipment during application.	47	1 day preapplication	14.89	< 1†	15					
	48	Day of application	60.42	< 1†	233	3.2 ± 6.4				
	48	1 day postapplication	47.92	< 1†	126	1.7 ± 4.6				
	48	2 days postapplication	33.33	< 1†	81	1.1 ± 3.7				
	48	3 days postapplication	27.08	< 1†	68	1.0 ± 3.6				
Curwin et al. (2007)							AMPA concentrations not tested			
Spot samples from 24 fathers of farm households During May–August 2001, each household was visited on two occasions, with the first being 1–5 days after application of at least one of seven pesticides and the second, four weeks later. Thus, glyphosate was either not applied prior to a visit (i.e., in 27 of the 48 subject-visits), applied by a custom applicator (in 10 of the 48 subject-visits), or applied by the farm father (in 11 of the 48 subject-visits). Notably, glyphosate urinary levels did not differ by application status among the farm fathers.	92	Within 1–5 days postapplication, the next morning, and 4 weeks later in the evening and next morning	25	< 0.9†	18	1.9			

(*Continued*)

Table V (Continued)

Reference Urine Sample Type, Number of Subjects, and Study Population Information on Study with an Emphasis on Glyphosate Use and Protection	*N*	Time of Urine Sample Collection	Urinary Glyphosate				Urinary AMPA			
			% ND or Below LOQ	μg/L (ppb)			% ND or Below LOQ	μg/L (ppb)		
				Min	Max	Mean ± SD [median]		Min	Max	Mean [or median]
Mesnage et al. (2012)										
Urine spot samples from 1 farmer	NS	For 24 hours preapplication	100	All samples < 1†			All samples below unspecified LOD			
One farmer handled 55 L of an unspecified glyphosate-based herbicide at various concentrations for 4 hours, which entailed the mixing and application of 0.75 L with a hand sprayer and the remaining from his tractor across 3 fields, which were 1.5 km away from his residence. During the dilution of the formulation, he wore a mask and gloves but not while he was spraying from his tractor with the window open. When hand-spraying, he wore gloves but neither a mask nor protective suite. After 4 hours of handling pesticides, he went home to eat and carefully wash his hands and changed his cloths but did not shower.	4	Day of application: every 6 hours			9.5	4.35				
	4	1 day postapplication: every 6 hours				0.95				
	NS	2 days postapplication: unspecified				1.9				
Jayasumana et al. (2015)							AMPA concentrations not tested			
10 farmers, presumably paddy farmers, with endemic chronic kidney disease n Padavi-Sripura, Sri Lanka	10	One nonfasted spot urine sample in the morning per subject	0	28.2	> 80	[56.8]				
10 healthy farmers from same endemic area	10		0	40.2	> 80	[73.5]				
No information was provided in terms of the potential application of glyphosate from these farmers.										
Rendón-von Osten & Dzul-Caamal (2017)							AMPA concentrations not tested			
76 subsistence farmers in Hopelchén, Campeche, Mexico	76	Morning urine samples	31.58	< 0.05†	0.8754	0.26 ± 0.23[0.2814]				
No information was provided on the potential application of glyphosate.										

(*Continued*)

Table V (Continued)

Reference Urine Sample Type, Number of Subjects, and Study Population Information on Study with an Emphasis on Glyphosate Use and Protection	*N*	Time of Urine Sample Collection	Urinary Glyphosate				Urinary AMPA			
				μg/L (ppb)				μg/L (ppb)		
			% ND or Below LOQ	Min	Max	Mean ± SD [median]	% ND or Below LOQ	Min	Max	Mean [or median]
Connolly et al. (2017)							AMPA concentrations not tested			
Urine spot samples from 18 amenity horticulturists (17 males, 1 females) in the Republic of Ireland Between June and October of 2015, four similar exposure groups (SEGs) were characterized based on the pesticide used and the application task method; some workers participated in more than one SEG. For glyphosate-containing pesticide use, there were three SEGs based on application with a manual knapsack, a controlled droplet applicator, or a pressurized handheld lance (2–6 bar pressures). The average length of task time was 40 (range: 5–115), 81 (range: 33–195), and 133 (range: 24–375) minutes, respectively. The amenity horticulturists had an average history of 19.7 (range 0–40) years working with pesticides and an average of 5.2 (range 0–7) months of pesticide use within a year. 94% of the workers used gloves, 67% Tyvek suits, and 83% respiratory protective equipment.	31	Preapplication task	64.52	< 0.5†	3.43	0.71 ± 0.92				
	31	Within 1 hour postapplication task	45.16	< 0.5†	10.66	1.35 ± 2.18				

(*Continued*)

Table V (Continued)

Reference Urine Sample Type, Number of Subjects, and Study Population Information on Study with an Emphasis on Glyphosate Use and Protection	*N*	Time of Urine Sample Collection	Urinary Glyphosate				Urinary AMPA			
				μg/L (ppb)				μg/L (ppb)		
			% ND or Below LOQ	Min	Max	Mean ± SD [median]	% ND or Below LOQ	Min	Max	Mean [or median]
Connolly et al. (2018)							AMPA concentrations not tested			
Full void urine spot samples from 20 amenity horticulturists (18 males, 2 females) in the Republic of Ireland Between September 2016 and September 2017, three similar exposure groups (SEGs) using glyphosate-based herbicides were characterized based on the application method, that is, with a manual knapsack, a controlled droplet applicator, or a pressurized handheld lance. The average length of task time was 116 (range: 37–360), 158 (range: 38–286), and 256 (range: 60–380) minutes, respectively. The amenity horticulturists had an average history of 22 (range 10–35) years working with pesticides. 100% of the workers used gloves, 90% Tyvek suits, and 97% respiratory protective equipment.	29	Preapplication	37.93	< 0.5‡	5.44	1.08 ± 1.20				
	28	Within 1 hour postapplication	17.86	< 0.5‡	5.51	1.72 ± 1.53				
	29	Peak postapplication	6.90	< 0.5‡	7.36	2.53 ± 1.89				
	29	Morning after application	37.93	< 0.5‡	6.14	1.32 ± 1.32				
Perry et al. (2019)										
Spot urine samples from 18 Wisconsinite dairy farmers (self-reported glyphosate applicators) Between 1997 and 1998, individual urine spot samples were collected 8 hours after first of the season pesticide application on cropland from 18 dairy farmers who self-reportedly applied glyphosate-based herbicides (GBHs) and from 18 dairy farmers that did not apply glyphosate. Though glyphosate application and protection were not explicitly detailed in the study, it was noted that there was no association between urinary glyphosate concentrations and amount of land farmed, hours of application of non-GBHs, or number of acres to which non-GBHs were applied.	18	8 hours after first of the season application between 1997 and 1998	61.11	< 0.4†	12.0	4.04	94.44	< 1.0†	4.1	N/A

(*Continued*)

Table V (Continued)

Reference Urine Sample Type, Number of Subjects, and Study Population Information on Study with an Emphasis on Glyphosate Use and Protection	*N*	Time of Urine Sample Collection	Urinary Glyphosate				Urinary AMPA			
			% ND or Below LOQ	μg/L (ppb)			% ND or Below LOQ	μg/L (ppb)		
				Min	Max	Mean ± SD [median]		Min	Max	Mean [or median]
Balderrama-Carmona et al. (2020)										
Agricultural workers in Sonora, Mexico 30 urine samples were obtained from agricultural workers in the Valle del Mayo that regularly apply herbicides 24 hours after the application of either a glyphosate or picloram-based herbicide. No further details were provided regarding the application or protective equipment used.	30	24 hour postapplication	100	All samples below < 5†			93.33	Only two samples had detectable levels§		
Zhang et al. (2020)										
Spot urine samples from 134 workers exposed to pure glyphosate during production at distinct posts: crystallization ($n = 28$), centrifugation ($n = 32$), filtration (n = 17), drying ($n = 22$), packaging ($n = 27$), and glyphosate feeding ($n = 8$) Other workers were excluded from the study based on the following criteria: (1) working life <24 months, (2) exposure frequency within the last 2 years to other pesticides >3 times/year with exposures >8 hours, (3) urine specific gravity <1.010 or >1.030, and (4) liver or kidney dysfunction or use of hepatotoxic or nephrotoxic drugs within 3 months. The 134 selected workers had 16.1 ± 10.4 years of employment and 8.2 ± 3.7 years of glyphosate exposure. The workers did not have personal respiratory protective equipment, even though the air concentrations of glyphosate were found to be relatively very high.	134	Within 1 hour of the end of each of the monitored shifts	13.43	< 20†	17202	Geometric mean: 262 [292]	18.66	< 10†	2730	Geometric mean: 72 [68]

Abbreviations: AMPA = aminomethylphosphonic acid; h = hour(s); LOD = limit of detection; LOQ = limit of quantification; N = number of urine samples; n = number of studies; N/A = not applicable; ND = not detectable; NS = not specified. Key: † = limit of detection (LOD). ‡ = limit of quantification (LOQ). « = lower limit of method validation (LLOMV). = arithmetic mean, unless otherwise noted. § = two urine samples reportedly had detectable levels of AMPA, specifically each at 0.42 and 2.23 μg/L; however, the limit of detection for AMPA was reportedly 15 μg/L. Several other concerns with this study have been documented (see Kougias, 2020).

comparison to occupational use, workers in one study used an average of 9.8 L of an 8% Roundup® (360 g/L glyphosate) solution across an approximate six-hour workday (Jauhiainen, Rasanen, Sarantila, Nuutinen, & Kangas, 1991) while, in another study, a farmer handled 55 L of a glyphosate-based herbicide at various concentrations for four hours, which entailed the mixing and application of 0.75 L with a hand sprayer and the remaining from his tractor across three fields (Mesnage et al., 2012). It is important to mention that the personal protective gear worn, and equipment used across and even within the occupational application studies differs greatly. It is likely due to these protective measures that, in one study, some farmers did not have detectable glyphosate in their urine samples (<1 μg/L [i.e., the LOD]), even though applications exceeded 100 acres (Acquavella et al., 2004).

In comparison to the 11 identified studies with occupational application-related exposures, though exposure duration and sampling time after application varies across the studies, the current study includes urinary glyphosate data from applicators simulating heavy residential consumer application in Pierce et al. (2020) that is similar to or in most circumstances greater than those in occupational applicators. In particular, while this study has the highest reported urinary glyphosate concentration of 310.9 μg/L following the application of a glyphosate-based herbicide (Pierce et al., 2020), only one of the 11 studies on occupational application-related exposures has relatively higher urinary glyphosate concentrations: Acquavella et al. (2004) reported a 24-hour composite urinary glyphosate concentration of 233 μg/L that is nearly twofold greater than the current study's highest 26.167-hour composite of 120.67 μg/L (Approach #2). It is important to mention that, despite the current study having comparable exposures to those observed following occupational application, the exposures endured during either occupational or residential consumer application are magnitudes lower than the occupational exposures obtained during production of glyphosate, with a maximum urinary glyphosate concentration of 17,202 μg/L reported by Zhang et al. (2020).

4.2. Approach-Specific Considerations of the Current Risk Assessment

In this study, three dermal applicators had a 36 hours postapplication sample with higher glyphosate residue concentrations than the 24 hours postapplication sample, and if these higher values were used instead, only trivial differences would have appeared in those respective individual MOS values. Nonetheless, when using the higher of the two urinary concentration values to calculate the internal dose of each dermal applicator, the lowest MOS value for that exposure group remained the same. Therefore, regardless of whether the glyphosate residue concentrations from the 24 hours or 36 hours postapplication are used, the lowest combined exposure MOS value would not have changed.

The use of a pharmacokinetic recovery value of 0.82 was a uniquely conservative assumption in this study. Previous studies either have used a less conservative value of 0.95 (Acquavella et al., 2004) or have just not accounted for pharmacokinetic recovery (Niemann et al., 2015), as it was assumed that there was no metabolism, no accumulation, and complete excretion via urine of the absorbed amount of glyphosate. In view of this, the present study also distinctively accounted for metabolism with the use of "effective glyphosate" concentrations, which is the sum of glyphosate and 1.5 times AMPA, rather than glyphosate concentrations alone. Additionally, in this study, the "effective glyphosate" concentrations at the 24 and 36 hour postapplication samples were not statistically distinguishable from background levels (see Pierce et al., 2020) such that the amount of glyphosate that is theoretically recovered after 24 hours does not significantly increase glyphosate concentrations above background.

In our study, we chose to adopt a bodyweight-specific urine production rate of 1.5 mL/kg BW/hr to calculate the total urine output volume across the 26.167-hour study period for all applicators. Considering this was the upper end of the normal urinary output range (CDC, n.d.), the selection of this rate, as opposed to lower rates, resulted in the largest individual urine production volumes and internal dose estimations. This selection was marginally warranted seeing as the approximate daily fluid intake values within this study were on the higher end of, or considerably above, daily fluid intake values from previous studies of comparable individuals based on age, gender, and body mass (Armstrong et al., 2013; Armstrong et al., 2012; Perrier, E. et al., 2013; Perrier et al., 2012). Furthermore, by selecting this bodyweight-specific urine production rate, we calculated total urine output volumes that were also generally on the higher end of, or considerably above, daily urine output volumes of similar subpopulations

(Armstrong et al., 2013; Armstrong et al., 2012; Perrier, E. et al., 2013; Perrier et al., 2012).

Although other studies, notably Niemann et al. (2015), have conservatively assumed individual daily urine output volumes of 2 L based on the recognized mean daily urine excretion of 1.5–2 L, our study provided estimated daily urine output volumes for applicators that were individualized and all above 2 L, with an average of 3.039 L and a range of 2.047–4.184 L. Though the estimated daily urine output volumes within the current study provide for an even more conservative estimate than the uniform assumption of 2 L, we may be cautiously overestimating these values, as we do not account for fluid losses by other routes. In fact, sweating was apparent in all applicators, especially those in Tyvek suits (i.e., the inhalation exposure group), due to the laborious nature of our consumer application protocol and the summer heat. Additionally, the daily fluid intake values reported in our study are actually within the range for individuals in hot climates or with high activity levels (U.S. EPA, 2019b).

Urinary production volume is influenced by diurnal fluctuations as evidenced by one study's demonstration of lower production overnight, throughout the morning, and in the evening before sleep, with a significant increase in the afternoon (Perrier, Erica et al., 2013). In fact, based off the data in Perrier et al. (2013), nearly 50% of the daily urine production occurs between 12:00 and 20:00 hours. However, this somewhat conflicts with a study by Athanasatou et al. (2019), which demonstrated that the urinary output within the morning's first six-hour period encompasses 76% of the total urine excretion over a day. Given the inconsistency across studies in diurnal fluctuations with respect to urine output, which is heavily influenced by diurnal activities, diet, and environmental conditions (see Cheuvront et al., 2015), there was no appropriate method to incorporate this into our analysis. Nonetheless, even if the assumption that 100% of the total estimated urine output volume were voided during the period corresponding to the sample with the highest peak concentration of glyphosate residues, all calculated internal daily doses, including the sum of the two highest from each exposure group, would still be well below the internal dose of 100 μg/kg/day.

4.3. Urinary Concentrations as a Biomarker of Exposure

In rats, glyphosate in urine seems to be the most accurate biomarker of exposure to glyphosate-based herbicides because only a small portion of glyphosate is metabolized to AMPA such that urinary glyphosate concentrations are several times, and even up to 100-fold, greater than urinary concentrations of AMPA, which can be below or near the LOQ, ultimately affecting the reliability of the measurement (Panzacchi et al., 2018). Similarly, this appears to be the case for humans, as evidenced by the six studies in Table V that examine both glyphosate and AMPA concentrations in urine.

Urinary concentrations of glyphosate are considered a good biomarker for exposure because glyphosate not only is poorly and exclusively metabolized to AMPA but also does not appear to bioaccumulate. Based on physicochemical properties, glyphosate is highly hydrophilic (ionized) at physiological pH and, therefore, is unlikely to bioaccumulate in fatty tissues or media, like breast milk (Bus, 2015). In fact, glyphosate was not detected in breast milk from lactating mothers with detectable glyphosate in their urine (McGuire et al., 2016). Additionally, as summarized by JMPR, there is no evidence of bioaccumulation based on the recovered amount of radioactivity after administration of radiolabeled glyphosate in numerous studies that differ in their use of species, doses, routes of administration, and exposure durations. In all of these toxicokinetic studies with radiolabeled glyphosate, nearly complete elimination of glyphosate was observed with less than 1% remaining in tissues after seven days, and, importantly, repeated dosing did not appear to alter absorption, distribution, or excretion of glyphosate (JMPR, 2004).

Altogether, given that glyphosate is rapidly excreted unchanged or as AMPA, glyphosate is not expected to bioaccumulate; however, recent data, with relatively lower doses and longer durations of exposure than the radiolabeled studies, challenge this conclusion. In particular, Panzacchi et al. (2018) demonstrated that there were increased urinary concentrations of glyphosate at the end of a longer exposure duration in rats administered glyphosate at a dose of 1.75 mg/kg/day as pure glyphosate or as the formulated Roundup Bioflow. It is important to mention that these doses are significantly above the projected doses that the applicators were exposed to in the current study, and the data used in the current study from Pierce et al. (2020) demonstrate that the urinary concentrations of glyphosate, AMPA, and "effective glyphosate" returned to baseline (preapplication) concentrations by 24 hours postapplication. Nonetheless, in the study by Zhang et al. (2020),

four workers that had urinary concentrations of glyphosate and AMPA analyzed at different time points had relatively high baseline levels, with one of the workers having urinary glyphosate concentrations around 500 μg/L at baseline (pretask) and over 1000 μg/L the following morning. Therefore, in view of these more recent findings, a biomarker of effect specific to glyphosate toxicity may be more accurate at determining the impact of cumulative exposure in scenarios with relatively high exposures; however, at this time, no such biomarkers are available.

4.4. Conclusions

In the present study, careful consideration was given to the impact of each selected assumption on the analysis, and in essentially all cases, modulating these assumptions would have coincided with an increase in the margins of safety. Thus, with the use of the most conservative assumptions across unique approaches, all internal doses calculated from our simulation study on heavy consumer application of a glyphosate-containing herbicide were below the internal doses estimated from established health-based guidance values. Overall, this study suggests that glyphosate exposure from consumer application of a commercially available glyphosate-containing herbicide does not appear to be a health concern; indeed, it would be helpful if larger-scale studies were able to corroborate these findings.

ACKNOWLEDGMENTS

At the time this study was conducted, all of the authors were employed by a consulting firm that provides scientific advice to the government, corporations, law firms, and various scientific/professional organizations. This firm has been engaged by companies involved in litigation; however, none of the authors have served as testifying experts in glyphosate litigation. The time invested by the authors to write this article was provided by their employer, and no client or defendant in litigation requested that this work be performed. No external funding was received for the research supporting the analysis, nor the time needed to prepare the article. Furthermore, the work product including the conclusions drawn are exclusively those of the authors.

REFERENCES

Acquavella, J. F., Alexander, B. H., Mandel, J. S., Gustin, C., Baker, B., Chapman, P., & Bleeke, M. (2004). Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study. *Environmental Health Perspectives*, *112*(3), 321–326. https://doi.org/10.1289/ehp.6667

Agency for Toxic Substances and Disease Registry (ATSDR). (2019). *Toxicological profile for Glyphosate. (Draft for public comment)*. Atlanta, GA: Retrieved from https://www.atsdr.cdc.gov/toxprofiles/tp214.pdf.

Armstrong, L. E., Johnson, E. C., McKenzie, A. L., & Munoz, C. X. (2013). Interpreting common hydration biomarkers on the basis of solute and water excretion. *European Journal of Clinical Nutrition*, *67*(3), 249–253. https://doi.org/10.1038/ejcn.2012.214

Armstrong, L. E., Johnson, E. C., Munoz, C. X., Swokla, B., Le Bellego, L., Jimenez, L., & Maresh, C. M. (2012). Hydration biomarkers and dietary fluid consumption of Women. *Journal of the Academy of Nutrition and Dietetics*, *112*(7), 1056–1061. https://doi.org/10.1016/j.jand.2012.03.036

Armstrong, L. E., Pumerantz, A. C., Fiala, K. A., Roti, M. W., Kavouras, S. A., Casa, D. J., & Maresh, C. M. (2010). Human hydration indices: acute and longitudinal reference values. *International Journal of Sport Nutrition and Exercise Metabolism*, *20*(2), 145–153. https://doi.org/10.1123/ijsnem.20.2.145

Athanasatou, A., Kandyliari, A., Malisova, O., & Kapsokefalou, M. (2019). Fluctuation of water intake and of hydration indices during the day in a sample of healthy Greek adults. *Nutrients*, *11*(4). https://doi.org/10.3390/nu11040793

Australian Pesticides and Veterinary Medicines Authority (APVMA). (2017). *Final regulatory position: Consideration of the evidence for a formal reconsideration of glyphosate*. KINGSTON ACT 2604 Australia: Retrieved from https://apvma.gov.au/node/26561.

Balderrama-Carmona, A. P., Valenzuela-Rincon, M., Zamora-Alvarez, L. A., Adan-Bante, N. P., Leyva-Soto, L. A., Silva-Beltran, N. P., & Moran-Palacio, E. F. (2020). Herbicide biomonitoring in agricultural workers in Valle del Mayo, Sonora Mexico. *Environmental Science and Pollution Research International*, *27*(23), 28480–28489. https://doi.org/10.1007/s11356-019-07087-6

Benbrook, C. M. (2016). Trends in glyphosate herbicide use in the United States and globally. *Environmental Science Europe*, *28*(1), 3. https://doi.org/10.1186/s12302-016-0070-0

Bus, J. S. (2015). Analysis of Moms Across America report suggesting bioaccumulation of glyphosate in U.S. mother's breast milk: Implausibility based on inconsistency with available body of glyphosate animal toxicokinetic, human biomonitoring, and physico-chemical data. *Regulatory Toxicology and Pharmacology*, *73*(3), 758–764. https://doi.org/10.1016/j.yrtph.2015.10.022

Centers for Disease Control and Prevention (CDC). (n.d.). *Urine output*. Retrieved from https://www.cdc.gov/dengue/training/cme/ccm/page57297.html.

Cheuvront, S. N., Kenefick, R. W., & Zambraski, E. J. (2015). Spot urine concentrations should not be used for hydration assessment: A methodology review. *International Journal of Sport Nutrition and Exercise Metabolism*, *25*(3), 293–297. https://doi.org/10.1123/ijsnem.2014-0138

Connolly, A., Basinas, I., Jones, K., Galea, K. S., Kenny, L., McGowan, P., & Coggins, M. A. (2018). Characterising glyphosate exposures among amenity horticulturists using multiple spot urine samples. *International Journal of Hygiene and Environmental Health*, *221*(7), 1012–1022. https://doi.org/10.1016/j.ijheh.2018.06.007

Connolly, A., Coggins, M. A., Galea, K. S., 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. *Annals of Work*

Exposures and Health, *63*(2), 133–147. https://doi.org/10.1093/annweh/wxy104

Connolly, A., Jones, K., Galea, K. S., Basinas, I., Kenny, L., McGowan, P., & Coggins, M. (2017). Exposure assessment using human biomonitoring for glyphosate and fluroxypyr users in amenity horticulture. *International Journal of Hygiene and Environmental Health*, *220*(6), 1064–1073. https://doi.org/10.1016/j.ijheh.2017.06.008

Curwin, B. D., Hein, M. J., Sanderson, W. T., Striley, C., Heederik, D., Kromhout, H., Alavanja, M. C. (2007). Urinary pesticide concentrations among children, mothers and fathers living in farm and non-farm households in iowa. *Annals of Occupational Hygiene*, *51*(1), 53–65. https://doi.org/10.1093/annhyg/mel062

European Chemicals Agency. (2017). *Glyphosate not classified as a carcinogen by ECHA*. Retrieved from https://echa.europa.eu/-/glyphosate-not-classified-as-a-carcinogen-by-echa.

European Food Safety Authority (EFSA). (2015). Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate. *EFSA Journal*, *13*(11). https://doi.org/10.2903/j.efsa.2015.4302

Food Safety Commission of Japan (FSCJ). (2016). Risk assessment report: Pesticides - glyphosate summary. *Food Safety*, *4*(3), 93–102. https://doi.org/10.14252/foodsafetyfscj.2016014s

Gillezeau, C., van Gerwen, M., Shaffer, R. M., Rana, I., Zhang, L., Sheppard, L., & Taioli, E. (2019). The evidence of human exposure to glyphosate: a review. *Environmental Health*, *18*(1). https://doi.org/10.1186/s12940-018-0435-5

International Agency for Research on Cancer (IARC). (2015). *IARC monographs 112: Glyphosate*. Lyon, France: IARC.

International Agency for Research on Cancer (IARC). (2019). *IARC monographs on the identification of carcinogenic hazards to humans: Preamble. Amended January 2019*. Lyon, France: IARC. Retrieved from https://monographs.iarc.fr/wp-content/uploads/2019/01/Preamble-2019.pdf.

Jauhiainen, A., Rasanen, K., Sarantila, R., Nuutinen, J., & Kangas, J. (1991). Occupational exposure of forest workers to glyphosate during brush saw spraying work. *American Industrial Hygiene Association Journal*, *52*(2), 61–64. https://doi.org/10.1080/15298669191364334

Jayasumana, C., Gunatilake, S., & Siribaddana, S. (2015). Simultaneous exposure to multiple heavy metals and glyphosate may contribute to Sri Lankan agricultural nephropathy. *BMC Nephrol*, *16*, 103. https://doi.org/10.1186/s12882-015-0109-2

Joint FAO/WHO Meeting on Pesticide Residues (JMPR). (2004). *Pesticide residues in food – Evaluations 2004: Part II – Toxicological*. Geneva, Switzerland: WHO. Retrieved from http://www.inchem.org/documents/jmpr/jmpmono/v2004pr01.pdf.

Joint FAO/WHO Meeting on Pesticide Residues (JMPR). (2016). *Summary report from the may 2016 joint FAO/WHO meeting on pesticide residues (JMPR) on diazinon, glyphosate, and malathion*. Geneva, Switzerland: WHO. Retrieved from http://www.who.int/foodsafety/jmprsummary2016.pdf?ua=1.

Kougias, D. G. (2020). Letter to the editor: Re: "Herbicide biomonitoring in agricultural workers in Valle del Mayo, Sonora Mexico" by Balderrama-Carmona et al. (2019) in Environ Sci Pollut Res Int. *Environmental Science and Pollution Research International*, *27*(14), 17429–17433. https://doi.org/10.1007/s11356-020-08388-x

Krüger, M., Schledorn, P., Schrödl, W., Hoppe, H.-W., Lutz, W., & Shehata, A. A. (2014). Detection of glyphosate residues in animals and humans. *Journal of Environmental & Analytical Toxicology*, *04*(02). https://doi.org/10.4172/2161-0525.1000210

Lavy, T. L., Cowell, J. E., Steinmetz, J. R., & Massey, J. H. (1992). Conifer seedling nursery worker exposure to glyphosate. *Archives of Environmental Contamination and Toxicology*, *22*(1), 6–13. https://doi.org/10.1007/BF00213295

McGuire, M. K., McGuire, M. A., Price, W. J., Shafii, B., Carrothers, J. M., Lackey, K. A., & Vicini, J. L. (2016). Glyphosate and aminomethylphosphonic acid are not detectable in human milk. *American Journal of Clinical Nutrition*, *103*(5), 1285–1290. https://doi.org/10.3945/ajcn.115.126854

Mesnage, R., Moesch, C., Grand, R. L. G., Lauthier, G., Vendômois, J. S. d., Gress, S., & Séralini, G.-E. (2012). Glyphosate exposure in a farmer's family. *Journal of Environmental Protection*, *3*(9), 3. https://doi.org/10.4236/jep.2012.39115

Mills, P. J., Kania-Korwel, I., Fagan, J., McEvoy, L. K., Laughlin, G. A., & Barrett-Connor, E. (2017). Excretion of the herbicide glyphosate in older adults between 1993 and 2016. *JAMA*, *318*(16), 1610–1611. https://doi.org/10.1001/jama.2017.11726

Niemann, L., Sieke, C., Pfeil, R., & Solecki, R. (2015). A critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, *10*(1), 3–12. https://doi.org/10.1007/s00003-014-0927-3

Olfert, M. D., Barr, M. L., Charlier, C. M., Famodu, O. A., Zhou, W., Mathews, A. E., & Colby, S. E. (2018). Self-reported vs. measured height, weight, and BMI in young adults. *International Journal of Environmental Research and Public Health*, *15*(10). https://doi.org/10.3390/ijerph15102216

Panzacchi, S., Mandrioli, D., Manservisi, F., Bua, L., Falcioni, L., Spinaci, M., & Belpoggi, F. (2018). The Ramazzini Institute 13-week study on glyphosate-based herbicides at human-equivalent dose in Sprague Dawley rats: study design and first in-life endpoints evaluation. *Environmental Health*, *17*(1), 52–52. https://doi.org/10.1186/s12940-018-0393-y

Perrier, E., Demazières, A., Girard, N., Pross, N., Osbild, D., Metzger, D., & Klein, A. (2013). Circadian variation and responsiveness of hydration biomarkers to changes in daily water intake. *European Journal of Applied Physiology*, *113*(8), 2143–2151. https://doi.org/10.1007/s00421-013-2649-0

Perrier, E., Rondeau, P., Poupin, M., Le Bellego, L., Armstrong, L. E., Lang, F., & Klein, A. (2013). Relation between urinary hydration biomarkers and total fluid intake in healthy adults. *European Journal of Clinical Nutrition*, *67*(9), 939–943. https://doi.org/10.1038/ejcn.2013.93

Perrier, E., Vergne, S., Klein, A., Poupin, M., Rondeau, P., Le Bellego, L., & Tack, I. (2012). Hydration biomarkers in free-living adults with different levels of habitual fluid consumption. *British Journal of Nutrition*, *109*(9), 1678–1687. https://doi.org/10.1017/s0007114512003601

Perry, M. J., Mandrioli, D., Belpoggi, F., Manservisi, F., Panzacchi, S., & Irwin, C. (2019). Historical evidence of glyphosate exposure from a US agricultural cohort. *Environmental Health*, *18*(1), 42. https://doi.org/10.1186/s12940-019-0474-6

Pest Management Regulatory Agency (PMRA). (2015). *Proposed re-evaluation decision: Glyphosate. health Canada report no. RVD2015-01. Issued April 13, 2015*. Ottawa, Ontario: Health Canada. Retrieved from https://static1.squarespace.com/static/568c9773d82d5e25a61fe201/t/56c1212759827ef22c06dd43/1455497515928/H113-27-2015-1-eng.pdf.

Pierce, J. S., Roberts, B., Kougias, D. G., Comerford, C. E., Riordan, A. S., Keeton, K. A., & Lotter, J. T. (2020). Pilot study evaluating inhalation and dermal glyphosate exposure resulting from simulated heavy residential consumer application of Roundup((R)). *Inhalation Toxicology*, *32*(8), 354–367. https://doi.org/10.1080/08958378.2020.1814457

Portier, C. J., Armstrong, B. K., Baguley, B. C., Baur, X., Belyaev, I., Bellé, R., Zhou, S.-F. (2016). Differences in the carcinogenic evaluation of glyphosate between the international agency for research on cancer (IARC) and the European food safety authority (EFSA). *Journal of Epidemiology and Community Health*, *70*(8), 741–745. https://doi.org/10.1136/jech-2015-207005

Rendon-von Osten, J., & Dzul-Caamal, R. (2017). Glyphosate residues in groundwater, drinking water and urine of subsistence farmers from intensive agriculture localities: A survey in Hopelchen, Campeche, Mexico. *International Journal of*

Environmental Research and Public Health, *14*(6). https://doi.org/10.3390/ijerph14060595

Ridley, J. W. (2018). *Procedures for complete urinalysis/confirmation testing fundamentals of the study of urine and body fluids* (pp. 203–249). Cham, Switzerland: Springer International Publishing.

Roberts, D. M., Buckley, N. A., Mohamed, F., Eddleston, M., Goldstein, D. A., Mehrsheikh, A., & Dawson, A. H. (2010). A prospective observational study of the clinical toxicology of glyphosate-containing herbicides in adults with acute self-poisoning. *Clinical Toxicology (Philadelphia)*, *48*(2), 129–136. https://doi.org/10.3109/15563650903476491

Tarazona, J. V., 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. *Archives of Toxicology*, *91*(8), 2723–2743. https://doi.org/10.1007/s00204-017-1962-5

U.S. Environmental Protection Agency. (1987). Integrated risk information system (IRIS): Chemical assessment summary of glyphosate (CASRN 1071-83-6). Integrated Risk Information System (IRIS) U.S. Environmental Protection Agency Chemical Assessment Summary.

U.S. Environmental Protection Agency. (1993). *Reregistration eligibility decision (RED): Glyphosate*. Retrieved from https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/red_PC-417300_1-Sep-93.pdf.

U.S. Environmental Protection Agency. (2017). *Revised glyphosate issue paper: Evaluation of carcinogenic potential*. Retrieved from https://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=534487&Lab=OPP.

U.S. Environmental Protection Agency. (2019a). *EPA takes next step in review process for herbicide glyphosate, reaffirms no risk to public health*. Retrieved from https://www.epa.gov/newsreleases/epa-takes-next-step-review-process-herbicide-glyphosate-reaffirms-no-risk-public-health.

U.S. Environmental Protection Agency. (2019b). *Exposure factors handbook chapter 3 (Update): Ingestion of water and other select liquids*. (EPA/600/R-18/259F). Washington, DC: EPA.

Wester, R. C., Melendres, J., Sarason, R., McMaster, J., & Maibach, H. I. (1991). Glyphosate skin binding, absorption, residual tissue distribution, and skin decontamination. *Fundamental and Applied Toxicology*, *16*(4), 725–732. https://doi.org/10.1016/0272-0590(91)90158-z

Zhang, F., Xu, Y., Liu, X., Pan, L., Ding, E., Dou, J., & Zhu, B. (2020). Concentration distribution and analysis of urinary glyphosate and its metabolites in occupationally exposed workers in Eastern China. *International Journal of Environmental Research and Public Health*, *17*(8), 2943. https://doi.org/10.3390/ijerph17082943

Zhang, N., Du, S., Tang, Z., Zheng, M., Yan, R., Zhu, Y., & Ma, G. (2017). Hydration, fluid intake, and related urine biomarkers among male college students in Cangzhou, China: A cross-sectional study-applications for assessing fluid intake and adequate water intake. *International Journal of Environmental Research and Public Health*, *14*(5). https://doi.org/10.3390/ijerph14050513