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Seasonal Urinary Levels of Glyphosate in Children From Agricultural Communities

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Abstract

Background: Glyphosate is one of the most commonly used pesticides in agricultural activities worldwide. For the last 20 years, its use has increased rapidly becoming a public health concern. The IARC classified glyphosate as probably carcinogenic; however, the reported evidence is not enough to establish a statement.

Objective: This work aimed to measure glyphosate levels in the urine of children from a single rural community in an endemic region of chronic kidney disease and malnutrition.

Methods: A cross-sectional study was carried out in a rural community in western Mexico. The study included 95 children between the ages of 6 and 16. A urine sample (first-morning spot) was obtained from children and processed to measure glyphosate levels using high-performance liquid chromatography coupled with tandem mass spectrometry.

Results: All samples tested positive for glyphosate levels. Urine glyphosate levels were related to the season and the age of the children.

Conclusion: Glyphosate is present in children of all ages in the community even if they were not in direct contact with it. No toxicity cases were reported, nor were there other health problems related to glyphosate. However, more long-term studies should be done that provide a statement regarding the harmful effects that glyphosate has on public health.

Keywords

glyphosate, children, health effects, high-performance liquid chromatography

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Introduction

Glyphosate is a pesticide widely used in agriculture.[1] Glyphosate ($HOOCCH_2NHCH_2PO(OH)_2$) (CAS 1071-83-6) kills weeds by inhibiting enzyme 5-enolpyruvyl-3-shikimate phosphate synthase that reduces the production of aromatic amino acids vital for growth of plants.[2] The use of this herbicide has rapidly accelerated since 2000, and this increase has been associated in particular with the development of transgenic crops.[3]

In 2015, the International Agency for Research on Cancer (IARC), the specialized cancer agency from the World Health Organization (WHO), classified this herbicide as probably carcinogenic to humans (Group 2A). The IARC recognized strong evidence for genotoxicity for technical glyphosate and formulations based on 1000 studies.[4] However, the term "probably carcinogenic" is still under discussion. Several studies have reported that glyphosate exposure is not associated with multiple myeloma or solid tumors.[5,6] In 2012, Mink et al reviewed seven cohort studies and fourteen case-control studies on glyphosate. The authors found no positive association or causal relationship between cancer and exposure to glyphosate in adults or children.[7] While its carcinogenicity continues to be debated, a recent meta-analysis identified a 41% increase in non-Hodgkin lymphoma among exposed workers.[8]

Widespread use of glyphosate has been documented in Mexico, especially among rural communities with heavy agricultural activity. A total of 192 children included in a previous cross-sectional study, 73% of whom reported residues of glyphosate (urine first-morning spot), living near the largest lake in Mexico, Lake Chapala.[9]

In Mexico, glyphosate is widely used in farms. This herbicide is highly soluble in water and, due to spills, runoff, and leaching, has been identified in surface and groundwater sources. A clear example is Hopelchén and Mújica in Campeche (southern Mexico), an area of intensive agricultural activity. Glyphosate was found in the urine samples of farmers and in water wells.[10] A similar situation was also found in northern Mexico (the Mayo Valley, Sonora), where glyphosate was detected in urine samples from people residing in the region.[11] The former study showed a statistical correlation ($R^2 = 0.994$) between contaminated water intake and the presence of diabetes ($P \leq .03$) and hypertension ($P \leq .004$).

In 2005, the Food and Agriculture Organization of the United Nations (FAO) reported on the potential toxicological effects of glyphosate as a result of presence of glyphosate in food.[12] The health effects have been studied, but many of them have concluded the need for more epidemiological studies, prevention, and biomonitoring.[13,14]

This report aims to measure the frequency of detection and concentrations of glyphosate in urine of children from a single rural community in Chapala Lakeshore.

Table 1. Demographic Characteristics of the Complete Sample.

Total Sample (n = 95)				
Variable	Average mean	SD	Range	95% CI
Age (in years)	10	3	6–6	9.3–10.6
Weight (kg)	31.8	9.9	15–64	29.7–33.8
Height (meters)	1.35	0.13	1.07–1.63	1.32–1.37
BMI	16.9	2.2	12.6–25.8	16.4–17.34
SBP	97.9	10.9	51–121	95.6–100.1
DBP	58.1	10	41–84	56.0–60.1

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Methods

A cross-sectional study was performed in a rural community in the Chapala Lakeshore in Jalisco State (western Mexico). The compiled research related to human use that utilized all the relevant national regulations and institutional policies, and was performed under the principles of the Helsinki Declaration. As such, it has been approved by the ethics committee of the PhD in Public Health Sciences of the University of Guadalajara (DCSP/CEI/2016/260618/038) and with the permission of the parents of the children, the children themselves, as well as the local and municipal authorities. Informed consent: Informed consent has been obtained from all individuals included in this study.

Urine samples (first-morning spot) were collected in 2 different periods. The first was in October, which is a season of low agricultural activity but of high consumption of the harvested products. The second, which was in May, is a high period of farming activity when pesticides are applied in the soil.

An extra sample in children from 3 to 6 years of age (kindergarten) was carried out in September when agricultural activities have not been entirely suspended (harvest starts). During this period, pesticides are not used, and the consumption of harvested products is low. The intention of this latter sample was to understand and compare urine glyphosate levels in younger children who are not in direct contact with glyphosate.

Urine samples were processed in order to determine pesticides associated with the HPLC/MS/MS (high-performance liquid chromatography coupled with the tandem mass spectrometry) method with Agilent Technologies® Model 1200 equipment for HPLC and Model 6430B for MS/MS spectrometry. The HPLC method used a column Zorbax Eclipse XDB C18, Rapid Resolution, 50 × 2.1 mm, 3.5 μm. Mobile phases: A, 0.1% formic acid in water; B, acetonitrile (ACN); gradient of 40% to 100% B; injection volume, 5 μL; flow, 0.5 ml/min; curve range for each pesticide, 0.01 to 1000 ng/ml.[9,15,16] The analytical detection of glyphosate in the mass spectrometer QQQ were as follows: precursor ion 168, product ions 149.9 and 124.2 using an 80-electron/volt fragmentor, a collision energy of 5 V and negative polarity. For development of this detection, a glyphosate standard Sigma Aldrich® was used. The internal standard was a deuterated glyphosate Sigma Aldrich®. The work curve was made with non-glyphosated urine, in which the external and internal standard were spiked.

The samples were kept inside a portable cooler for its transportation to the laboratory, followed by an immediate analysis by direct injection. The latter was performed at the Laboratory of Applied Pharmacokinetics of the University Center of Exact and Engineering Sciences of the University of Guadalajara.

For the statistical description, we utilized frequencies, percentages, means, standard deviations (SD), and 95% CI. Statistical significance was evaluated using the Mann–Whitney *U* test. Statistical significance was considered with a P of $\leq$.05. For data processing, Excel® (Microsoft, Redmond, WA, USA) and OpenEpi Info ver. 3.01 (Open Source Epidemiologic Statistics for Public Health, Bill and Melinda Gates Foundation, Emory University, Atlanta, GA, USA) statistical software was used.

Results

A total of 95 children were enrolled for the study (elementary and junior high local schools). A sampling schedule was programmed, and both parents as well as educational local authorities were informed of the sampling dates. Tables 1 and 2 show the demographic characteristics of the included children.

As previously mentioned, urine samples were processed to measure glyphosate levels using HPLC/MS/MS. The obtained results are shown in Table 3.

Results obtained from urine samples from kindergarten children (age = 3–6 years) are depicted in Table 4. These data were used in order to know if urinary glyphosate levels are related or not to agricultural activities and age.

The glyphosate means from this sample (0.03 ng/ml) was compared with the mean from the other two samples ($P < .00001$).

Discussion

Children from the study community are exposed to glyphosate year-round. The obtained results clearly show that glyphosate urine levels are higher in May which is the season for preparing the ground using pesticides, and glyphosate is widely used to kill weeds. As previously mentioned, children and women have a particular role in these sorts of activities. Therefore, urinary pesticide levels

Table 2. Demographic Data by Gender and Mean Differences.

Sample Divided by Gender (n) (male = 51) (Female = 44)					
Variable		Average mean	SD	95% CI[a]	*P* value[b]
Age (in years)	Male	13	3	12.1–13.8	$P < .05$
	Female	12	3	11.0–12.9	
Weight (kg)	Male	33.6	9.8	30.8–36.3	$P < .05$
	Female	29.7	9.8	26.7–32.6	
Height (meters)	Male	1.38	0.13	1.34–1.41	$P < .05$
	Female	1.32	0.12	1.28–1.35	
BMI	Male	17.2	2.1	16.6–17.7	$P > .05$
	Female	16.6	2.2	15.9–17.2	
SBP	Male	95.6	10.1	92.7–98.4	$P > .05$
	Female	91.6	11.5	88.1–95.0	
DBP	Male	59.1	10.6	56.9–62.88	$P > .05$
	Female	56.9	9.28	54.0–59.7	

[a]Confidence interval on the mean.
[b]*P* values correspond to differences between variable (by gender) groups estimated by the Mann–Whitney U test.

Table 3. Mean Differences of Glyphosate Levels by Periods.

	Mean Glyphosate Levels in ng/ml (SD)		
	95% CI		
Number of Samples (%)	October 2017	May 2018	*P* value[a]
Males *n* = 51 (54)	0.4 (0.32) 0.3–0.5	3.05 (3.04) 2.1–3.9	$P < .0001$
Females *n* = 44 (46)	0.4 (0.33) 0.3–0.5	2.9 (2.83) 2.0–3.7	$P < .0001$
Total *n* = 95 (100)	0.37 (0.30) 0.3–0.4	3.2 (3.28) 2.5–3.8	$P < .0001$

[a]Mann–Whitney U test.

Table 4. Glyphosate Levels in Children From the Kindergarten Community.

Number of Samples September 2018	Age (mean)	Mean Urinary Glyphosate Levels in ng/mL (SD)	95% CI
42	3.7	0.03 (0.04)	0.01–0.04

obtained in October 2017 can be explained since in that season, agricultural activities are related to harvesting, and pesticide exposure is minimum.

The sample collected in September 2018 has the lowest urinary pesticide levels. The mean age corresponds to children that have indirect participation in agricultural activities. These children are frequently working in the field while their mothers and older brothers are working at harvesting or are engaged in other farming activities which require minimum use of pesticides. Mean differences, with regard to urinary pesticide levels, are remarkable ($P < 0.00001$). No data are available about intoxication or diseases in children related to pesticide exposure in the area. However, the harmful effects of pesticides could be present in the long-term after exposure.

Since the end of the last century, it has been widely documented that children from rural communities are exposed to pesticides, even if they are not related to agricultural activities. This exposure has been studied mainly in developed countries where there are strict regulations regarding the use of pesticides.[17,18] However, there is enough evidence to indicate that pesticides are brought into homes through work clothes, footwear, and on the skin from family members engaged in agriculture activities.[19-21]

Pesticides are capable of reaching homes through the air.[22,23] Volatile pesticide particles accumulate on furniture, floors, and playgrounds. This phenomenon does not happen in homes far from agricultural fields. Some researchers have reported that urinary pesticide levels vary depending on the season or the time of application of the pesticide.[24-26]

Ever since glyphosate entered the market in 1974, its effects on human health have been a controversial matter. In 2016, a systematic review reported an association with attention-deficit hyperactivity disorder among children born to glyphosate users (OR = 3.6, 1.3–9.6), but no other associations such as birth defects, that were minor for gestational age at birth, had significant associations.[27]

Recent studies have detected glyphosate in food and humans at environmentally relevant levels as observed in the laboratory[28] which induces oxidative stress, anti-androgen, and anti-estrogenic activity.[29-31] The role of glyphosate as an endocrine disruptor has been reported in several scientific papers. In 2013, Thongprakaisang et al reported the effect of glyphosate in human breast cancer cells. The study focused on estrogen receptor-mediated transcriptional activity and their expressions. The authors were aware of the risk of environmental exposure to the pesticide which is commonly used for soybean production.[32] Based on the volume of information, public health authorities should take strong measures since evidence concerning cancer that is related to glyphosate is everyday more evident, and although several scientific reports are based on animal studies,[33] the long-term effects in humans are not well known, and as such, the final results could be catastrophic.

The main concern in our research is the unknown effect in the health of children who are exposed to glyphosate. Previous studies have been carried out in rural communities in the same area that sought links between glyphosate exposure and chronic kidney disease, malnutrition, and birth defects, but to date, nothing has been evident.[34]

The fact is that until now our results have shown that children in this area are exposed to glyphosate, but there is no evidence of any harmful effects on children exposed to it. However, children who have been exposed should be of concern to public health authorities, especially if epidemiological tracing is absent, thus leaving the health of children vulnerable. But the problem is worse since children are exposed to more than one pesticide. Our previous research reported urine samples with 16 pesticides.[9] Long-term effects of those pesticide exposures should be traced and avoided if possible.

Conclusion

For around 50 years, glyphosate has been used in agricultural activities. While its carcinogenic and other harmful effects on health continue to be debated, several authors have reported increased incidences of some specific effects in exposed workers and their children. Although there is a lack of reports about the long-term effects of glyphosate exposure, cumulative scientific reports over the years have increased rapidly. However, with time, the consequences that are negative or neutral will become clearer, thereby making it possible to come to a decision, suggest regulatory framework for public health, and lastly, to put aside any assumptions.

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Research Data

The data that support the findings of this study are available from the corresponding author (Dr Erick Sierra-Diaz), but restrictions apply to the availability of these data, which were used under license for the current study, and as such, are not publicly available. Data are, however, available from the authors upon reasonable request and with permission from the Public Health Department of the University of Guadalajara.

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