

Glyphosate Monitoring in Water, Foods, and Urine Reveals an Association between Urinary Glyphosate and Tea Drinking: A Pilot Study

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Abstract

Glyphosate is the most widely used agricultural herbicide in the world and in the United States. In this study, we measured glyphosate levels in water, foods, and human urine samples. We also specifically examined possible correlations between urinary glyphosate levels and physiological, dietary, or behavioral factors. The results show that water samples obtained from both Lake Erie and tap water contained nondetectable or very low levels of glyphosate (≤ 0.08 ppb). On the other hand, food samples showed various levels of glyphosate contamination. In particular, tea products (40–728 ppb in five samples), coffee powder (11 and 26 ppb in two samples), and honey (20 ppb) had higher glyphosate concentrations in comparison to other foods. Glyphosate was also detected in human urine samples, in particular at elevated levels (~2 fold) from participants who consumed tea in the past 24 h.

Keywords: Food, glyphosate, Lake Erie, tea, urine

INTRODUCTION

Glyphosate is the most widely applied herbicide in the world to eliminate broadleaf weeds and grasses that compete with crops. A recent estimate by the US Geological Survey projected that glyphosate would account for 53.5% of total agricultural herbicide used in the United States in 2009.^[1] The US Environmental Protection Agency classified glyphosate as “practically nontoxic and not an irritant” and established the maximum contaminant level goals (MCLGs) at 700 parts per billion (ppb). However, the effect of glyphosate on human health still remains very controversial. For example, in 2015, the International Agency for Research on Cancer classified glyphosate as “probably carcinogenic to humans,”^[2] whereas a review in 2012 examined 21 studies and “found no consistent pattern of positive associations indicating a causal relationship between total cancer (in adults or children) or any site-specific cancer and exposure to glyphosate.”^[3] Many other studies suggested the association between glyphosate and various adverse health effects seen in liver, kidney, reproductive system, endocrine system, nervous system, and embryonic development,^[4-14] even when

glyphosate concentrations were as low as 0.1 ppb.^[15] Some recent reports have also linked glyphosate with neurological conditions such as autism and attention-deficit/hyperactivity disorder.^[16,17]

In this study, glyphosate levels were measured using the enzyme-linked immunoabsorbent assay (ELISA) method in water samples collected from Lake Erie, at Erie, Pennsylvania, USA, as well as in foods and urine samples. The correlations between the urinary glyphosate concentration and factors such as gender, diet, body mass index (BMI), sleep duration, and exercise were examined. Our results show the presence of glyphosate at nondetectable or very low concentrations in water samples including tap water and water collected from Lake Erie. On the other hand, beverages and food samples contained various concentrations of glyphosate. In particular, tea products, coffee powder, and honey showed elevated

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glyphosate concentrations in comparison to the other food samples. Glyphosate was also detected in almost all urine samples, in particular at elevated levels from participants who consumed tea in the past 24 h.

MATERIALS AND METHODS

Sample collection

Water samples were collected from multiple sources. Lake water was collected from the beach at Lake Erie at Erie, PA. Bay water was collected from Presque Isle, Erie, PA. Tap water was collected from Gannon University, Erie, PA. Laboratory water was purified by Thermo Scientific ion exchange column. Food samples were collected from local grocery stores at Erie, PA. Solid samples were converted to liquids by soaking or homogenization. Supernatants were taken after liquid samples were centrifuged at 13,000x for 3 min. Details of sample preparation are listed in Table 1.

Urine samples were collected from participants recruited from an undergraduate course at Gannon University. The average age of participants was 20.3 years with an age range of 19–24 years. Out of 36 participants, 20 were male and 16 were female. The participants came from mixed races but were primarily Caucasian. Samples were collected at ~2 pm. There was no intake restriction of food or drink before sample collection. Participants were asked to fill out a questionnaire at the time of sample collection. The use of humans was approved by the Institutional Review Board of Gannon University.

Glyphosate measurements

Glyphosate concentrations were measured by the ELISA method using a Glyphosate ELISA Microtiter Plate kit (Abraxis, Warminster, PA). The absorbance was measured at 450 nm using a microplate reader (Promega, Madison, WI). A series of standard samples provided by the kit (0.075–4.0 ppb) were used to construct a standard curve with a linear regression between log (absorbance) and log (concentration). In three measurements, the values of the correlation coefficient R^2 were 0.92, 0.96 and 0.99.

Data analysis

Statistical analyses (two-tailed independent Student's *t*-test) were performed using SPSS Statistics version 22.0 (IBM, New York, USA). The group average values are reported in the form of mean \pm standard error of the mean.

RESULTS

Glyphosate concentrations were measured in multiple samples of water. The glyphosate concentrations are listed in Table 2. Any value below the limit of detection (LOD) of the assay (0.075 ppb) in Table 2 was regarded as “not detected.” Both tap water and the water samples collected from Lake Erie had glyphosate concentrations below the LOD. Similarly, very low levels of glyphosate were recorded in the water samples collected from the Presque Isle Bay at Erie, PA (0.08 ppb).

Compared to water samples, food and beverages showed higher glyphosate concentrations. Among these samples, the highest levels of glyphosate were detected in tea bags, tea leaves, coffee powder, and honey [Table 3].

Glyphosate concentrations were also measured in human urine samples collected from 36 participants. One urine sample had a glyphosate concentration lower than the LOD. The remainder of the 35 human urine samples had a mean glyphosate concentration of 0.368 ± 0.055 ppb, with a median value of was 0.235 ppb and a range from 0.096 to 1.033 ppb. The majority of samples ($n = 26$) had glyphosate measurement of <0.4 ppb. A smaller group of samples ($n = 10$) had relatively higher glyphosate measurements ranging between 0.5 and 1.05 ppb [Figure 1a].

Next, a comparison of the urinary glyphosate levels according to gender and BMI was conducted. No significant difference was seen between females (0.375 ± 0.069 ppb, $n = 19$) and males [0.347 ± 0.069 ppb, $n = 16$, independent *t*-test $P = 0.78$, Figure 1b]. Participants with a lower BMI (BMI <24 , 0.384 ± 0.071 ppb, $n = 19$) also did not show a significant difference (independent *t*-test $P = 0.63$) compared to participants with a higher BMI [BMI >24 , 0.336 ± 0.066 ppb, $n = 17$, Figure 1c].

Table 1: Sample preparation methods

Sample type	Sample	Preparation method
Water samples	Tap water	Collected from university campus
	Lake water, bay water	Collected from the Lake Erie and Presque Isle Bay
	Laboratory water	Distilled water further was purified using the Thermo Scientific ion exchange column
Liquid samples	Soda, beer	Shaken for 10 min, centrifuged, then supernatant was collected
	Sports drink, fruit juice, popsicle	Centrifuged then supernatant was collected
	Nonorganic milk, organic milk, soy milk	Centrifuged twice then supernatant was collected
Solid samples	Corn, corn starch, cucumber, fish, beef	Sliced if necessary, homogenized in laboratory water, centrifuged, then supernatant was collected
	Tea leaves, tea bag, coffee powder	Soaked in 10 \times weight laboratory water for 8 h, liquid part was centrifuged then supernatant was collected and centrifuge-filtered with Durapore membrane (pore size 5 μ L, Fisher Scientific)
	Tofu, chicken, beef	Liquid component from retail package was collected, diluted if necessary, centrifuged then supernatant was collected
	Honey	1:40 diluted in laboratory water

We then examined whether dietary and behavioral factors were correlated with the urinary glyphosate levels. Participants who had consumed tea in the past 24 h showed higher urinary

Table 2: Glyphosate concentrations in water samples

Sample	Measured glyphosate (ppb)
Lake water	ND
Bay water	0.08
Tap water	ND
Laboratory water	ND

ND: Not detected

Table 3: Glyphosate concentrations in water beverage and food samples

Sample (dilution ratio)	Measured glyphosate (ppb)	Before dilution (ppb)
Soda	0.116	0.116
Soy milk	0.140	0.140
Tofu	0.275	0.275
Fruit drink	0.400	0.400
Sports drink	0.411	0.411
Organic milk	0.442	0.442
Corn starch (1:2)	0.231	0.462
Nonorganic milk	0.533	0.533
Beef (1:3)	0.184	0.553
Cucumber (1:2)	0.317	0.634
Chicken “juice”	1.233	1.233
Fish (1:3)	0.498	1.495
Corn (1:2)	0.809	1.618
Popsicle	1.983	1.983
Beer (1:10)	0.276	2.757
Coffee brand #1 (1:50)	0.223	11.16
Coffee brand #2 (1:50)	0.526	26.32
Honey (1:40)	0.552	22.06
Tea leaves (1:10)	4.043	40.43
Tea bag brand #1 (1:200)	0.444	88.76
Tea bag brand #2 (1:200)	0.489	97.76
Tea bag brand #3 (1:200)	1.400	280.0
Tea bag brand #4 (1:200)	3.641	728.2

glyphosate concentrations (0.646 ± 0.148 ppb, $n = 5$) than participants who had not consumed tea in the past 24 h (0.315 ± 0.047 ppb, $n = 31$). This difference was statistically significant [Figure 2a, independent t -test, $P = 0.01$], and this result is consistent with higher concentrations of glyphosate detected in tea leaves and tea bags [Table 3].

When factoring in the location of each participants’ meals in the past 24 h [Figure 2b], those who had 2 or more meals at home had about the same urinary glyphosate concentration (0.329 ± 0.067 ppb, $n = 14$) compared to those who had only eaten one or no meal at home and instead consuming their meals in the university cafeteria or nearby restaurants (0.384 ± 0.068 ppb, $n = 21$, independent t -test $P = 0.58$).

Moreover, sleep duration appeared to have little effect on the urinary glyphosate levels [Figure 2c]. Those who had 6–7 h of sleep in the previous night had slightly lower levels of glyphosate compared to those who had 7.5–11 h of sleep (0.307 ± 0.055 ppb vs. 0.418 ± 0.085 ppb). This difference was not statistically significant (independent t -test $P = 0.26$).

Next, since physical exercise elevates metabolic levels, we compared participants according to their reported hours of physical exercise on the day before sample collection. Those that reported low levels (0–1.5 h) of physical exercise and those that reported moderate levels of (2–4 h) of physical exercise in the 24 h before sample collection had similar levels of urine glyphosate (0.366 ± 0.074 ppb, $n = 13$ vs. 0.382 ± 0.075 ppb, $n = 16$, respectively; independent t -test $P = 0.88$, Figure 2d).

In contrast, the medium-term physical exercise, as defined as in the week before sample collection, appeared to have a greater effect [Figure 2e]. Those who had 10 or more hours of physical exercise in the past week had lower urinary glyphosate levels (0.289 ± 0.056 ppb, $n = 20$) compared to those who had <10 h of physical exercise in the past week (0.460 ± 0.085 ppb, $n = 15$), but the difference was not statistically significant (independent t -test $P = 0.08$).

DISCUSSION

Glyphosate is currently the most heavily used herbicide in the world and continuing to trend upward.^[1] The purpose of

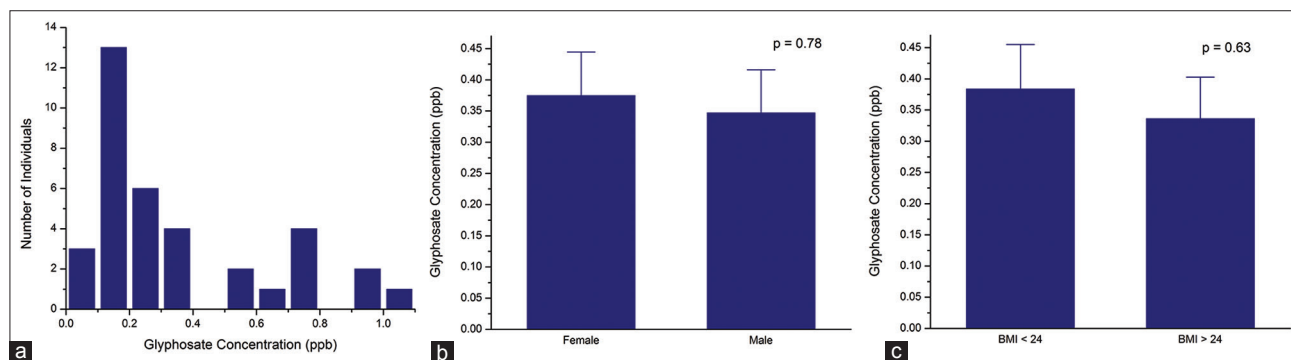


Figure 1: The presence of glyphosate in human urine samples. (a) The distribution of measurement results. (b) The comparison between genders. (c) The comparison between participants with lower and higher body mass index

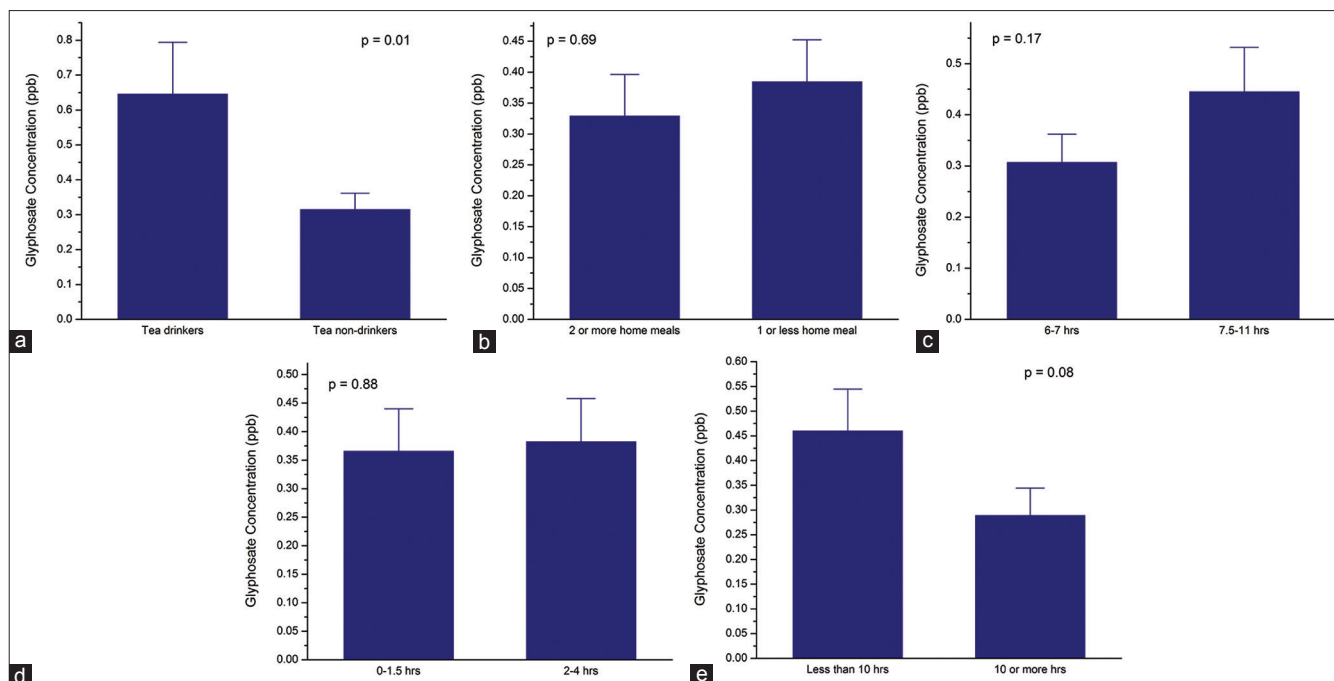


Figure 2: Comparison of urinary glyphosate by different factors. (a) Compares the participants who reported to have drunk tea to those who reported not to have drunk tea in the 24 h before sample collection. (b) Compares the participants who had 2 or more home meals to those who had 1 or zero home meals. (c) Compares those with sleep duration of 6–7 h in the previous night to those who had 7.5–11 h of sleep. (d) Compares those who had 2–4 h of physical exercise in the past day to those who had 0–1.5 h of physical exercise in the past day. (e) Compares those who had exercised 10 or more hours in the past week to those who had exercised <10 h

our study was to determine whether and how much water and foods had been contaminated and whether this contamination would be reflected in human urine samples.

Our results revealed that glyphosate concentrations in the water samples collected were nondetectable or very low compared to the glyphosate MCLGs in drinking water (MLCG = 700 ppb). Tap water had a concentration below the LOD 0.075ppb. Very low levels were also seen in the water sample collected from Lake Erie (below the LOD) as well as in water samples from Presque Isle Bay, Erie, PA (0.08 ppb). This is probably due to the degradation of glyphosate into aminomethylphosphonic acid (AMPA) in soil by microbial activity, most notably *Pseudomonas* species, before the ultimate transport of limited amounts from glyphosate from land to water.^[18] A report showed that the half-time of glyphosate with elevated microbial activity was 47 days in the field.^[19] Our samples were collected in late fall, therefore may have been after the seasonal effect of glyphosate from nearby fields, if any.

The food and beverage samples we tested had detectable concentrations of glyphosate, but mostly under 3 ppb. Our result is consistent with several previous studies. A test in 2014 showed 3.3 mg/kg glyphosate and 5.7 mg/kg AMPA in genetically modified soybean samples from the US (1 mg/kg = 1 ppb).^[20] High levels (>20 mg/kg) of glyphosate were found in 7 out of 11 soybean samples from Argentina.^[21]

Among the food samples tested in this study, highest concentrations of glyphosate were seen in four specific sample

sources: tea bag, tea leaves, coffee powder, and honey. The high levels of glyphosate in the five tea products that were tested are probably the result of high levels of glyphosate applied in the fields where they were grown. The top tea-producing countries are in Asia, Africa, and South America according to the statistics of UN Food and Agriculture Organization.^[22] The source of the honey we sampled was also from South America.

We also examined the glyphosate levels in the urine samples collected from 36 participants. The average concentration was 0.368 ± 0.055 ppb in the range between 0.096 and 1.033 ppb. This is lower compared to the level seen in a nonagricultural population in Sri Lanka (mean value 3.3 ppb, range 1.2–5.5 ppb), but higher compared to the results (median 0.11–0.18 ppb in different years) of a multiyear study in Germans.^[23,24]

We also examined whether or not the urinary glyphosate concentrations correlated with physical, dietary, and behavioral factors including gender, BMI, drinking of tea, locations of meal preparation, physical exercise, and sleep durations. We observed elevated urinary glyphosate levels in participants who drank tea. This is consistent with the very high glyphosate levels we observed in tea products. Other comparisons did not generate statistically significant results, probably for two reasons. First, our sample size was relatively small ($n = 36$ in total and divided into two groups for comparisons), a much larger sample size would substantiate the comparison statistics in this pilot study. Second, for practical reasons, the participants did not have a controlled diet, a fixed amount of water intake,

or monitored behavioral conditions before sample collections. Based on this, there might be other factors that contribute to the urinary glyphosate levels that were not included in our survey. Nevertheless, the urinary glyphosate measurement results reflected our measurement results in tea samples. We also observed the lower urinary glyphosate levels, although not statistically significant, in the participants who had 10 or more hours of physical exercise in the past week compared to those with less physical exercise. A similar pattern related to mid-term exercise level was observed in another study on urinary atrazine levels using the same set of participants.^[25] In addition, higher atrazine levels in the tea products (28 and 49 ppb in tea bag brand #3 and tea leaves) were also observed. However, atrazine levels were not higher in the urine samples of tea drinkers compared to nontea drinkers. The difference in our observations of the two herbicides suggests tea drinks are the main contributor of glyphosate but not atrazine seen in urine.

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Conflicts of interest

There are no conflicts of interest.

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