

Original Article

Urinary glyphosate levels and association with mortality in the 2013–16 National Health and Nutrition Examination Survey

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Abstract

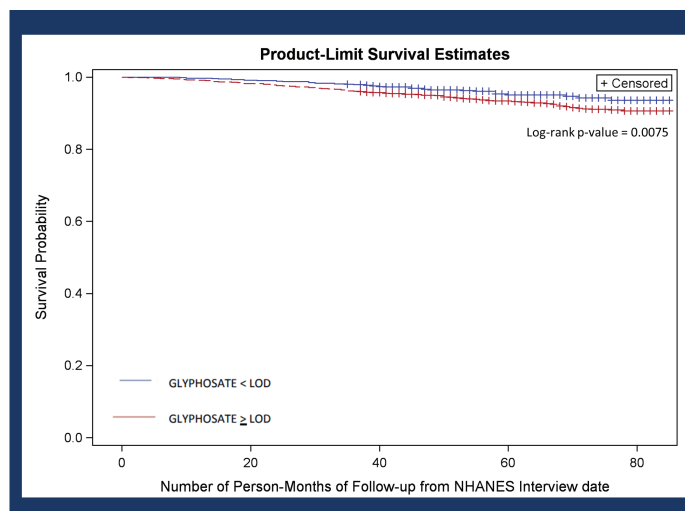
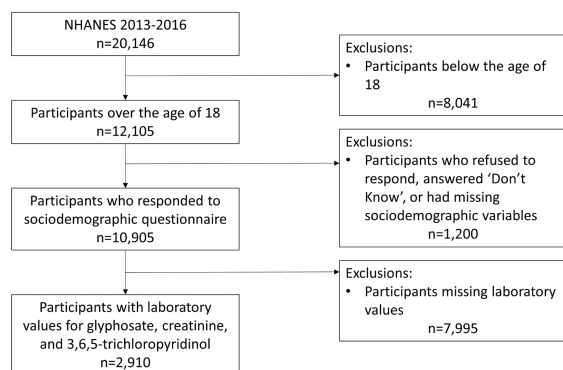
Objectives: Glyphosate is the most commonly used herbicide in the USA; however, its safety is still under debate. We assessed glyphosate levels and their association with overall mortality in a representative sample of the US adult population from the 2013 to 2016 National Health and Nutrition Examination Survey.

Methods: We extracted data on urinary glyphosate ($N = 2910$) measured by ion chromatography isotope-dilution tandem mass spectrometry. Associations between glyphosate concentrations and demographic, lifestyle and other exposures were analyzed. Data were linked to public-use Mortality Files for 2019.

Results: The mean (STD) glyphosate level was 0.53 (0.59) ng/ml, with 25.7% of the subjects having glyphosate levels at or below the detection limit. At multivariate analysis, age and creatinine were associated with glyphosate urinary levels (both $P < 0.0001$). There was a borderline association between glyphosate levels and mortality ($HR_{adj} 1.33$; 95% CI 0.99–1.77 $P = 0.06$). When 3,5,6-trichloropyridinol was excluded from the Cox model, glyphosate exhibits a significant association with mortality ($HR_{adj} 1.33$; 95% CI 1.00–1.77; $P = 0.0532$).

Conclusions: These nationally representative data suggest that recent exposure to glyphosate could be associated with increased mortality. More studies are necessary to understand population-level risk associated with the product, given its widespread use in agriculture.

Graphical Abstract



Abbreviations: BMI, body mass index; GBHs, glyphosate-based herbicides; NCHS, National Center for Health Statistics; NHANES, the National Health and Nutrition Examination Survey; NHL, non-Hodgkin's lymphoma; STD standard deviation.

Introduction

Glyphosate is the main component of a commonly used herbicide, Round-Up; however the exact composition of Round-Up and of the other glyphosate-based herbicides (GBHs) is unknown as of today. The use of GBHs has skyrocketed in the last 20 years. Their presence can now be measured in the environment in soil, water (1) and food (2) and is regularly found in human biofluids such as urine (3), serum (4) and breast milk (5).

Over the years, the widespread application of glyphosate and GBHs to crops has created a large number of tolerant and resistant weeds and this has elicited more frequent applications at higher concentrations (6). The introduction of genetically modified seeds that are Round-Up resistant and the expansion of GBHs use in the early 2000s to speed up crop desiccation (6) have further contributed to a widespread GBHs utilization. Despite the increased exposure of the population to the various GBHs products through food, soil and water, little is known about their health effects in the general population.

The carcinogenic potential of glyphosate has been reviewed by several agencies with conflicting conclusions. In 2015, the International Agency for Research on Cancer classified glyphosate as a 'probable human carcinogen' (7), although in the same year the European Food Safety Agency declared that 'glyphosate is unlikely to pose a carcinogenic hazard to humans' (8) based on typical, expected exposures of the general public. The US Environmental Protection Agency's most recent review was in 2016 and concluded that glyphosate is 'not likely to be carcinogenic to humans' (9) based on typical, non-occupational exposures.

A recent systematic review of *in vivo*, *ex vivo* and *in vitro* mechanistic studies of human and experimental animals exposed to GBHs indicates strong evidence that these compounds possess 5 of the 10 key characteristics of carcinogenicity (10), as defined by International Agency for Research on Cancer (11).

In 2019, our group in collaboration with other scientists published a meta-analysis of occupational studies on exposure to glyphosate and non-Hodgkin's lymphoma (NHL) (12), and concluded that there is evidence of a link between exposures to GBHs and increased risk for NHL in occupationally exposed populations. However, we think that occupational exposure is only one fraction of the exposure that the general population experiences because of contaminated water, food and soil. To better assess the levels of exposure in the general population, we also conducted a review of the few studies on urinary levels of glyphosate (3) and found that the average urinary levels in occupationally exposed subjects varied from 0.26 to 73.5 µg/l; environmental exposure urinary levels ranged from 0.16 to 7.6 µg/l. Only two studies measured temporal trends in exposure, both of which show increasing proportions of individuals with detectable levels of glyphosate in their urine over time. We also focused our attention on the few studies including children (13), and found that all the studies confirm the presence of glyphosate in urine samples from children, with values exceeding those measured in adults, when the corresponding values were available. We therefore advocated in our publication for a national biomonitoring, to address changes of exposure over the years, seasonal changes, differences between adults and children, geographical differences and suggested

that the National Health and Nutrition Examination Survey (NHANES) should be the appropriate agency to conduct such measurements.

Recently, NHANES measured glyphosate in left over material from cycles 2013 to 2016, giving us the opportunity to address the following aims: (i) to assess average values of urinary glyphosate in the general US population, in a snapshot and over time; (ii) to assess the personal and behavioral characteristics associated with urinary glyphosate levels; (iii) to study if there is an association with urinary glyphosate levels and mortality in the US population.

Methods

Data/study population

NHANES is a National Center for Health Statistics (NCHS) program comprised of numerous surveys, laboratory tests and physical examinations designed to assess the health and nutritional status of American adults and children (14). The survey is conducted and released in biennial cycles, and the NHANES datasets are de-identified and available to the public. Additionally, NCHS provides public-use versions of linked mortality files, which include death certificate records from the National Death Index (15). NHANES data utilized in this study was linked to their respective mortality files. This study included participants who were 18 years or older from the 2013–14 to 2015–16 NHANES cycles ($n = 12\,105$ individuals). We restricted the sample to those who consented to future analyses, had urinary glyphosate levels measured in mid-late 2022, and had complete information on the other covariates (refused answering questions or missing responses on education status, $n_{\text{excl}} = 2$, smoking, $n_{\text{excl}} = 13$, country of birth, $n_{\text{excl}} = 5$, alcohol consumption, $n_{\text{excl}} = 11$, ratio of family income to poverty, $n_{\text{excl}} = 1150$ or death status, $n_{\text{excl}} = 19$), for a total of 2910 subjects (Supplementary Figure 1, available at *Carcinogenesis* Online).

Main predictor

Urinary glyphosate level (ng/ml) was used as the main predictor of interest. A one-third subsample of participants aged 6 years and older from both NHANES cycles were randomly selected for participation along with all participants aged 3–5 years. Glyphosate levels were measured utilizing 200 µl of urine on 2D-on-line ion chromatography, tandem mass spectrometry and isotope-dilution quantification. Observations below the lower limit of detection of 0.2 ng/ml had an imputed fill value of the lower limit of detection divided by the square root of 2. Laboratory methodology and quality assurance were kept uniform across both survey cycles (16).

Outcomes

The primary outcome was mortality. Vital status and person months from date of initial interview to follow-up on 31 December 2019 as provided by the NCHS public use linked mortality files were used for analysis.

Covariates

The covariates of interest within the analysis were age in years at the time of participation, urinary creatinine (mg/dl), body mass index (BMI) [kg/m²], ratio of family income to poverty level, exposure to 3,5,6-trichloropyridinol (µg/l),

gender, ethnicity, country of birth, highest education attained, smoking status, alcohol consumption and physical activity level.

Age, creatinine, BMI, weight and ratio of family income to poverty were recorded as continuous variables. Creatinine is available for all participants aged 6 years and older by enzymatic method utilizing a Roche/Hitachi Cobas 6000 chemistry analyzer. Laboratory methodology and quality assurance were kept uniform among both survey cycles (17). Race/ethnicity included categories of Hispanic, Non-Hispanic White, Non-Hispanic Black, Non-Hispanic Asian and Other—including multi-racial participants. Highest education obtained was grouped into <9th grade, 9th–11th grade (including 12th grade with no diploma), high school graduate/GED or equivalent, some college or AA degree and college graduate or above. Individuals were grouped on whether they were born within the 50 US states or Washington, D.C., or if they were born elsewhere. Participants were classified as smokers if they smoked at least 100 cigarettes in their lifetime. Participants were categorized based on whether or not they had consumed 12 or more alcoholic beverages in the past year. Physical activity level was grouped into three categories: vigorous work, exercise or recreational activities for at least 1 day in a typical week; moderate physical activity, or low to no physical activity. Other compounds used in agricultural settings, such as fungicides (3,5,6-trichloropyridinol), pesticides (2,4,-D, 4-fluoro-3-phenoxy-benzoic acid, 3-phenoxybenzoic acid) and antimicrobial agents (Oxypyrimidine) were measured and available in this sample. Because some of them are known to be used together with glyphosate compounds, we assessed the correlation matrix in this sample population (Supplementary Table 1, available at *Carcinogenesis* Online), and used the compound with the strongest correlation with glyphosate (3,5,6-trichloropyridinol) as a covariate for adjustment in multivariate analyses. The final sample used in the analysis consisted of 2910 subjects.

Statistical analysis

Univariable and multivariable analyses were conducted to assess the association between glyphosate, demographics and mortality. Variables that had a significant association ($P < 0.05$) with mortality in a univariate Cox model were considered when running the adjusted model. Frequency tables and descriptive statistics of all covariates were generated for all adults within the two cycles. Distributions of urinary glyphosate for each individual cycle and the overall 4-year dataset were examined. Additionally, univariate linear regression models were used to determine covariates that had significant associations with urinary glyphosate levels. As NHANES utilizes a complex sampling design, weighted analyses were conducted using the survey procedures in SAS, in order to incorporate the design variables provided by NHANES to obtain national estimates (14).

All statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC).

Results

Within the 2013–14 and 2015–16 surveys, 2910 subjects had urinary glyphosate levels measured, and were included in the analyses. Population characteristics are reported in Table 1.

Table 1. Descriptive statistics among adults included in 2013–16 NHANES

Variable	n = 2910	%	Mean	STD
Glyphosate (ng/ml)	2910		0.53	0.59
Age (years)	2910		47.88	18.19
BMI (kg/m ²)	2891		29.31	7.15
Family income to poverty ratio	2910		2.47	1.63
3,5,6-trichloropyridinol (µg/l)	2910		1.64	2.43
Creatinine (mg/dl)	2910		122.16	79.17
Sex				
Male	1410	48.45		
Female	1500	51.55		
Race/Ethnicity				
Hispanic	731	25.12		
Non-Hispanic White	1176	40.41		
Non-Hispanic Black	582	20.00		
Non-Hispanic Asian	307	10.55		
Other and multi-racial	114	3.92		
Education				
<9th grade	233	8.01		
9–11th grade (and 12th grade, no diploma)	377	12.96		
High school graduate/GED or equivalent	624	21.44		
Some college or AA degree	983	33.78		
≥College graduate	693	23.81		
Country of birth				
50 US states/Washington, DC	2102	72.23		
Other	808	27.77		
Smoked at least 100 Cigarettes in Life				
Yes	1260	43.30		
No	1650	56.70		
At least 12 alcoholic beverages in past year				
Yes	1896	69.73		
No	823	30.27		
Physical activity				
Low/None	890	30.58		
Moderate	883	30.34		
Vigorous	1137	39.07		
Mortality				
Assumed alive	2722	93.54		
Assumed deceased	188	6.46		
Underlying cause of death among deceased				
Diseases of heart	54	28.72		
Malignant neoplasms	44	23.40		
Cerebrovascular diseases	9	4.79		
All other causes	81	43.09		
Number of person-months of follow-up			59.83	15.71

The mean (STD) glyphosate level was 0.53 (0.59) ng/ml with a strong right skew both overall and within individual cycles, with 25.7% of the subjects having glyphosate levels at or below the detection limit. The mean values were not statistically significantly different between 2013–14 and 2015–16 cycles ($P = 0.1162$) (Supplementary Figure 2, available at *Carcinogenesis* Online).

At univariable analysis, factors associated with glyphosate urinary levels were age, 3,5,6-trichloropyridinol, race/ethnicity and creatinine. There was a non-statistically significant association with physical activity ($P = 0.08$). However, only age at screening and creatinine remained associated with glyphosate urinary levels at the multivariate analysis ($P < 0.0001$ for both; Table 2).

In univariable Cox models, all covariates demonstrated significant association with mortality except for BMI ($P = 0.92$), sex ($P = 0.34$), alcohol consumption ($P = 0.12$) and education ($P = 0.39$) (Table 3). The statistically significant covariates were considered for inclusion in the adjusted model. Mortality was positively associated with age ($HR_{adj} 1.08$; 95% CI 1.06–1.10 per year of age), as expected, and inversely

associated with the ratio of family income to poverty ($HR_{adj} 0.86$; 95% CI 0.76–0.97). There was a borderline association between glyphosate and mortality ($HR_{adj} 1.33$; 95% CI 0.99–1.77 $P = 0.06$) (Table 4). When 3,5,6-trichloropyridinol was excluded from the Cox model, glyphosate approaches a significant association with mortality ($HR_{adj} 1.33$; 95% CI 1.00–1.77; $P = 0.0532$) (data not shown).

Discussion

We present here the analysis of a subset of a representative cohort of the general US adult population, and we show that the average levels of urinary glyphosate were stable across the two temporal cycles. Roughly one quarter of the US population had urinary values of glyphosate at or below the detection limits in the 2013–16 cycles. A previous study on one NHANES cycle (2013–14) indicated that over four-fifths of the US general population ≥ 6 years experienced recent exposure to glyphosate, with variations according to dietary habits (18). We confirmed the overall results in a larger US sample, and we report here that glyphosate levels increase

Table 2. Univariate and multivariate linear model estimates of associations between covariates and urinary glyphosate levels (continuous)

Variable	Univariate			Multivariate			
	Beta Coeff.	95% CI	P -value	Beta Coeff. ^a	95% CI	P -value	
Age (years)	0.003	0.002	0.004	<0.0001	0.004	0.002	0.006
BMI (kg/m ²)	0.004	0.0001	0.009	0.10	–0.0002	–0.004	0.004
Family income to poverty ratio	–0.02	–0.04	0.007	0.15	–0.01	0.04	0.008
3,5,6-trichloropyridinol (µg/l)	0.07	0.04	0.10	<0.0001	0.03	–0.002	0.06
Creatinine (mg/dl)	0.003	0.002	0.003	<0.0001	0.003	0.002	0.004
Sex				0.14			0.14
Female vs male	–0.04	–0.10	0.02		0.05	–0.02	0.12
Race/Ethnicity				0.007			0.17
Hispanic	REF	—	—		REF	—	—
Non-Hispanic White	0.07	–0.004	0.14		0.14	–0.01	0.28
Non-Hispanic Black	0.08	0.02	0.15		0.01	–0.06	0.08
Non-Hispanic Asian	–0.03	–0.11	0.05		–0.03	–0.11	0.05
Other and multi-racial	0.009	–0.06	0.08		0.01	–0.10	0.11
Education				0.72			0.60
<9th grade	REF	—	—		REF	—	—
9–11th grade (and 12th grade, no diploma)	–0.08	–0.21	0.70		–0.06	–0.19	0.06
High school graduate/GED or equivalent	–0.07	–0.21	0.07		–0.03	–0.18	0.13
Some college or AA degree	–0.08	–0.20	0.42		–0.06	–0.19	0.07
\geq College graduate	–0.09	–0.22	0.05		–0.04	–0.16	0.09
Country of birth				0.99			0.23
other vs 50 US states/Washington, DC	0.0003	–0.08	0.08		0.11	–0.07	0.29
Smoked at least 100 cigarettes in life				0.86			0.44
No vs Yes	0.007	–0.08	0.09		0.03	–0.05	0.11
At least 12 alcoholic beverages in past year				0.49			0.80
No vs Yes	0.05	–0.09	0.19		0.01	–0.10	0.13
Physical activity				0.08			0.17
Low/None	REF	—	—		REF	—	—
Moderate	–0.03	–0.15	0.09		0.002	–0.12	0.12
Vigorous	–0.08	–0.14	0.009		–0.05	–0.11	0.02

^aAdjusted for all the other covariates in the table.

Table 3. Univariate Cox model estimates of factors associated with mortality (in person-months).

Variable	Beta Coeff.	Hazard ratio	95% CI		P-value
Glyphosate (ng/ml)	0.43	1.54	1.22	1.93	0.0006
Age (years)	0.08	1.09	1.07	1.11	<0.0001
BMI (kg/m ²)	-0.02	1.00	0.96	1.03	0.92
Family income to poverty ratio	-0.17	0.85	0.77	0.94	0.002
3,5,6-trichloropyridinol (µg/l)	0.04	1.04	1.01	1.07	0.002
Creatinine (mg/dl)	-0.002	0.998	0.997	0.999	0.002
Sex					0.34
Female vs Male	-0.18	0.84	0.58	1.22	
Race/Ethnicity					0.0002
Hispanic	REF	REF	—	—	
Non-Hispanic White	0.78	2.17	1.31	3.60	
Non-Hispanic Black	0.77	2.16	1.29	3.61	
Non-Hispanic Asian	-0.38	0.68	0.25	1.89	
Other and multi-racial	1.45	4.27	2.11	8.62	
Education					0.39
<9th grade	REF	REF	—	—	
9–11th grade (and 12th grade, no diploma)	-0.58	0.56	0.22	1.43	
High school graduate/GED or equivalent	-0.27	0.76	0.37	0.58	
Some college or AA degree	-0.62	0.54	0.26	1.12	
≥College graduate	-0.60	0.55	0.26	1.18	
Country of birth					0.004
other vs 50 US states/Washington, DC	-0.59	0.55	0.37	0.82	
Smoked at least 100 cigarettes in life					0.003
No vs Yes	-0.61	0.54	0.37	0.80	
At least 12 alcoholic beverages in past year					0.12
No vs Yes	-0.30	0.74	0.51	1.09	
Physical activity					0.0002
Low/None	REF	REF	—	—	
Moderate	-0.57	0.57	0.35	0.92	
Vigorous	-1.54	0.22	0.11	0.42	

Table 4. Adjusted model estimates of factors independently associated with mortality in adults (2013–16 NHANES).

Variable	Beta estimate	Hazard ratio _{adj} ^a	95% CI		P-value
Glyphosate (ng/ml)	0.28	1.33	0.99	1.77	0.06
3,5,6-trichloropyridinol (µg/l)	0.01	1.01	0.96	1.06	0.64
Age (years)	0.08	1.08	1.06	1.10	<0.0001
Ratio of family income to poverty	-0.15	0.86	0.76	0.97	0.02
Race/Ethnicity					
Hispanic	REF	REF	—	—	
Non-Hispanic White	0.33	1.40	0.83	2.35	0.20
Non-Hispanic Black	0.40	1.49	0.87	2.55	0.14
Non-Hispanic Asian	-0.51	0.60	0.24	1.53	0.28
Other race—including multi-racial	1.09	2.97	1.46	6.02	0.004
Country of birth					
Others vs Born in 50 US states or Washington, DC	-0.03	0.97	0.60	1.57	0.89
Smoked at least 100 cigarettes in life					
No vs Yes	-0.35	0.70	0.46	1.08	0.11
Physical activity					
Low/none	REF	REF			
Moderate	-0.39	0.68	0.41	1.13	0.13
Vigorous	-0.67	0.51	0.25	1.04	0.06
Creatinine (mg/dl)	-0.0003	1.00	1.000	1.002	0.75

^aAdjusted for all the other variables in the table.

with age and are associated with the presence of other fungicides and pesticides, probably suggesting that subjects were exposed occupationally, or use these chemicals for gardening or other leisure activities around the house. Unfortunately, the 2013–16 data set did not have complete information on occupation; therefore, we were not able to assess the association between glyphosate urinary levels and type of occupation or able to distinguish the association with occupation rather than with environmental exposure.

After linkage with publicly available mortality data, we observed an increase in overall mortality with urinary glyphosate levels, after adjustment for confounders. This is the first attempt, to our knowledge, at linking mortality with glyphosate exposure in the general US population. While previous studies on the same dataset suggested that urinary glyphosate levels were associated with other endpoints such as endocrine disruption (19), neurological health outcomes such as depression and decline in cognitive function (20), as well as red blood cell damage (21), we now show that there could be more systemic, persistent damage that translates into increased mortality associated with glyphosate exposure. We can only speculate that the increased mortality may be due to some specific cancer types, given the previously reported association with NHL (12), or to cardiovascular diseases, given the reported effect on red blood cells (21), or to serious neurological conditions (20).

The present analysis has several strengths, including providing the first preliminary evidence of an association between glyphosate exposure and overall mortality in a representative sample of American adults extracted from NHANES, a reliable and comprehensive dataset. The presence of several personal and behavioral characteristics, such as smoking status, alcohol, physical activity and BMI, allows for statistical adjustments that make the inference less probably to be due to confounders. This study is also the first comprehensive measure of urinary levels of glyphosate in a large random sample of US adult residents, with measurements relative to a recent period, between 2013 and 2016.

However, the study has some limitations as well. Because of the relatively small number of deaths and the lack of granularity in the NHANES public dataset, we were not able to identify the specific cause of death that is driving this small increase in mortality. The cross-sectional design of this investigation does not allow for causal inferences, as the association is based on one single urinary glyphosate measure. It is not clear if one single measure is representative of chronic exposure, and one measure does not reflect any temporal or seasonal variations. Glyphosate's half-life in humans is relatively short, and there could be biochemical factors that affect urinary glyphosate levels irrespective of overall glyphosate exposure. Furthermore, the measure of glyphosate metabolites is not available in NHANES.

The results are still preliminary because they refer to a relatively young population (mean age at enrollment 48 years) and a short follow-up (59 months average), but it calls for further studies on a larger sample size, with repeated urinary measures that include glyphosate metabolites, accompanied by the collection of specific causes of death. Because of the widespread exposure to glyphosate in the USA, even a small increase in mortality associated with exposure may have a large public health impact. Therefore, mechanistic studies along with epidemiologic studies are urgently necessary to address this issue.

Supplementary material

Supplementary data are available at *Carcinogenesis* online.

Conflict of interest statement

E.T. is retained as expert on behalf of plaintiffs in litigation pertaining to the development of cancer following glyphosate exposure. However, no one except the authors contributed to or reviewed this manuscript in advance of submission to the journal, nor was E.T. compensated by any law firm for her work on this article. All other authors have no conflicts to declare.

Data availability

This manuscript makes use of publicly available data from published studies; therefore, no original data are available for sharing.

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