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The relationship between urinary glyphosate and all-cause and specific-cause mortality: a prospective study

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Glyphosate (GLY) is a well-known herbicide with significant applications in both agriculture and non-agriculture. However, GLY overuse in recent years has resulted in detection of GLY residues in many crops, endangering human health and food safety. Our aim is to investigate the relationship between urinary GLY and mortality, as well as its influencing factors. The National Health and Nutrition Examination Survey (NHANES) data from 4740 American adults were examined. Fitted smooth curves, generalized summation models, and multiple logistic regression models were used to investigate the relationship between urinary GLY and mortality. To investigate potential regulatory elements between the two effects, perform subgroup analysis. During a median follow-up of 4.03 years, there were a total of 238 all-cause deaths, 75 cardiovascular disease (CVD) deaths and 52 cancer deaths. The urinary GLY is positively correlated with all-cause mortality. Each 1 ng/ml increase in urinary GLY was associated with a 40% increased risk of all-cause mortality (Hazard ratio (HR) 1.40, 95% confidence interval (CI) 1.09–1.80), and an 50% increased risk of all-cause mortality in High group compared with Low group (HR 1.50, 95% CI 1.05–2.14). In subgroup analysis, the association between urinary GLY and all-cause mortality was significantly modified by gender (P for interaction = 0.03), and the association between urinary GLY and cancer mortality was significantly modified by hypertension (P for interaction = 0.022). Higher urinary GLY seems to be associated with more all-cause death, and gender may affect this association. Furthermore, urine GLY may have a higher effect on cancer mortality in people without hypertension.

Keywords Glyphosate, All-cause mortality, Cardiovascular mortality, Cancer mortality, NHANES

Glyphosate (GLY) is the most widely used herbicide in the US agricultural sector[1]. It inhibits the 5-enolacetone shikimic acid-3-phosphate synthase (EPSPS), which interferes with the formation of aromatic amino acids and ultimately causes plant death in plants by acting on the shikimic acid pathway in plants[2,3]. Currently, farms, orchards, and gardens utilize it primarily for weeding[1,4]. However, because of the exponential increase in GLY use over the past century, its presence has been found in a wide range of foods[3,5–9]. As its use continues to rise, the public and scientific community are paying more and more attention to GLY's possible effects on human health. Several investigations have revealed that GLY is not as safe and innocuous as first thought. GLY can strongly irritate human skin, eyes, and respiratory tracts during acute exposure, resulting in discomfort sensations[10–12]. Even more concerning are the long-term health concerns associated with exposure, which can disrupt the endocrine system, alter hormone balance, and negatively impact vital physiological functions including development and reproduction[13,14]. GLY may be carcinogenic, as evidenced by some research that have connected it to an increased risk of cancer[15,16]. Furthermore, GLY may harm the cardiovascular system, immune system, the nervous system, the liver, kidneys, et[17–20]. In 2015, GLY was categorized as a chemical that was "potentially carcinogenic to humans" by the World Health Organization's International Agency for Research on Cancer (IARC)[1,21,22]. Furthermore, extensive and prolonged usage of GLY may have detrimental effects on aquatic and soil species[23–26]. Moreover, GLY usage over an extended period of time may cause weed resistance[27,28]. Higher doses of GLY or other herbicides are required to suppress weeds that have developed resistance to GLY, which increases agricultural production costs and strains the environment[29]. On the other hand, GLY is not

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expected to cause cancer, according to the findings of the Joint Meeting on Pesticide Residues between the Food and Agriculture Organization (FAO) and the World Health Organization (WHO)[30]. The U.S. EPA came to the conclusion that there was "inadequate information to assess carcinogenic potential," "carcinogenic to humans," or "likely to be carcinogenic to humans," based on the weight of the evidence and available data[31,32]. In a similar vein, the Glyphosate Assessment Group of the European Union declared that, when used in accordance with recommended usage, GLY is safe for all purposes and suggested declassifying it as carcinogenic[33]. Therefore, opinions on GLY's safety are currently divided. Nonetheless, it is easy to conclude from the first two NHANES cycles' data that GLY exposure is common among Americans and that most people's urine contains GLY[34,35].

Here, we examined the relationship between urine GLY and adult mortality in America by extracting data on urinary GLY and all-cause and cardiovascular mortality from the National Health and Nutrition Examination Surveys (NHANES) conducted from 2013 to 2018.

Methods

Study design and population

National Health and Nutrition Examination Survey (NHANES) is a cross-sectional study conducted by the U.S. Centers for Disease Control and Prevention (CDC). NHANES utilizes a complex, multistage sampling design designed to be representative of the nation's diverse population groups. The data collected include physical examinations, interviews, and laboratory tests that help researchers analyze health trends and inform public health policy. Ethical approval for the study was obtained from the National Center for Health Statistics (NCHS) Ethics Review Board, and all NHANES participants provided written informed consent. The data collected and related documents are publicly available. For more information, please visit the official NHANES website. In addition to the core survey, NHANES links participant data to the National Death Index (NDI) to track mortality outcomes. NDI mortality data are available at https://www.cdc.gov/nchs/data-linkage/mortality.htm.

We conducted a secondary analysis using data from three independent NHANES cycles between 2013 and 2018 to investigate the association between GLY exposure and all-cause and cardiovascular mortality. A total of 8,507 participants with available urinary GLY data were included for the study. Due to the vulnerability of children and adolescents, we excluded 3,109 participants under the age of 18. Next, we excluded 17 participants with missing mortality data and 641 participants with missing urinary GLY data. In the end, 4,740 eligible individuals were included in the final analysis (Fig. 1).

Urinary GLY

In the NHANES, urinary GLY measurements are part of the environmental exposure data collected to assess the levels of this widely used herbicide in the U.S. population. The eligible sample consisted of all examined participants aged 3 to 5 years and one-third of the examined participants aged 6 years and older. NHANES's official website provides information about urinary GLY's range and handling options. The median GLY level in the population's urine serves as the grouping concentration for the independent variables. Urinary GLY was measured by using 200 µl of urine and was based on 2D-on-line ion chromatography coupled with tandem mass spectrometry (IC-MS/MS) and isotope dilution quantification[36]. The analytical measurements were conducted following strict quality control/quality assurance CLIA guidelines. Along with the study samples, each analytical run included high- and low-concentration quality control materials (QCMs) and reagent blanks to assure the accuracy and reliability of the data. The concentrations of the high-concentration QCMs and the low-concentration QCMs, averaged to obtain one measurement of high-concentration QCM and low-concentration QCM for each run, were evaluated using standard statistical probability rules[37].

All-cause, cardiovascular mortality and cancer mortality

In this study, the outcome variables included all-cause mortality, cardiovascular mortality and cancer mortality. These mortality data were obtained through linkage with the NDI[38]. Specifically, participants without a recorded death were considered alive during the follow-up period, which extended from the time of their participation in the survey until December 31, 2019. All-cause mortality encompasses deaths from any cause. Cardiovascular mortality was defined using the International Classification of Diseases, 10th Revision (ICD-10) codes: I00-I09, I11, I13, I20-I51, and I60-I69, which represent a range of cardiovascular-related conditions, including heart disease, hypertensive heart disease, and cerebrovascular disease. Cancer mortality include any death brought on by cancer.

Potential covariates

The covariates in this study were pre-selected based on prior research identifying risk factors for all-cause mortality. After variable screening, the final multivariable logistic regression analysis included the following covariates: Continuous variables include age, poverty-to-income ratio (PIR), body mass index (BMI, kg/m^2), alanine aminotransferase (ALT, U/L), serum creatinine (SCR, µmol/L), blood urea nitrogen (BUN, mg/dL), estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2), uric acid (UA, µmol/L), fasting blood glucose (FBG, mmol/L), glycated hemoglobin (HbA1c, %), total cholesterol (TC, mmol/L), and triglycerides (TG, mmol/L); Categorical variables include sex, ethnicity, education level, smoking and drinking status, physical activity, presence of hypertension, and presence of diabetes. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation: $eGFR = 141 \times \min(SCR/\kappa, 1)^{\alpha} \times \max(SCR/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] $\times 1.159$ [if black], κ is 0.7 for females and 0.9 for males, α is -0.329 for females and −0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1[39]. Hypertension was defined as either a self-reported diagnosis from a healthcare professional, a systolic blood pressure ≥ 140 mmHg, and/or diastolic blood pressure ≥ 90 mmHg[40]. Diabetes was defined as a self-reported diagnosis, a fasting blood glucose (FBG) ≥ 7 mmol/L, or HbA1c > 6.5%[41].

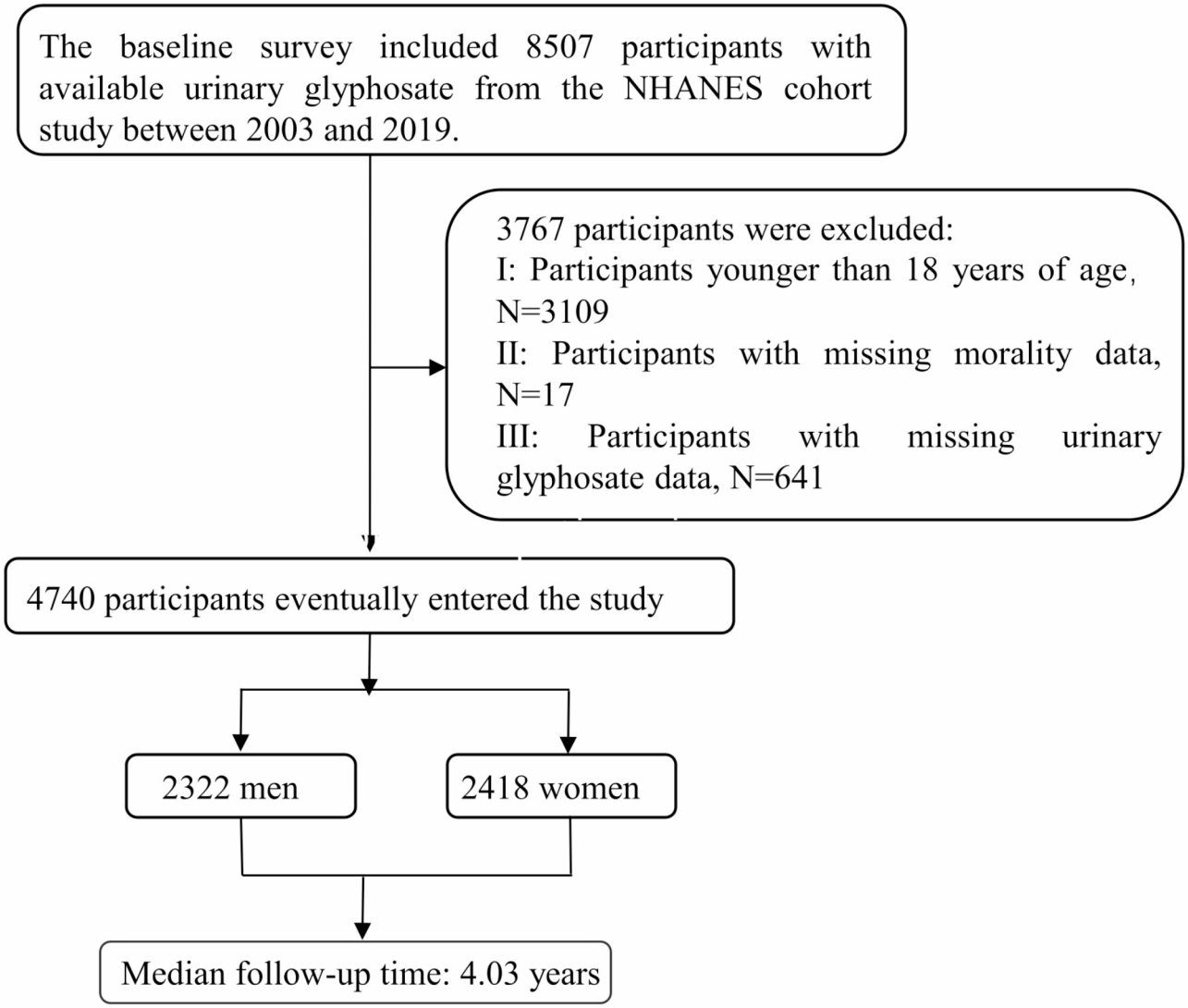

Fig. 1. Flow chart of participants.

Statistical analysis

All statistical analyses in this study were conducted following the CDC guidelines for NHANES data analysis (https://wwwn.cdc.gov/nchs/nhanes/tutorials/default.aspx), with each NHANES participant assigned a sampling weight to ensure the representativeness of the data[42]. To compare baseline characteristics across tertiles of urinary GLY levels, continuous variables were analyzed using weighted linear regression models and presented as means with 95% confidence intervals (CI), while categorical variables were analyzed using weighted chi-squared tests and reported as counts and percentages. The relationship between urinary GLY and mortality (both all-cause and cardiovascular) was evaluated using univariate and multivariate Cox proportional hazards regression models. Three models were constructed: model 1: Unadjusted, model 2: Adjusted for sociodemographic factors, and model 3: Adjusted for all covariates listed in Table 1. To account for potential nonlinear relationships between urinary GLY levels and mortality, cubic spline functions and smooth curve fitting (penalized spline method) were applied in Cox regression models. The Kaplan-Meier method was employed to plot survival curves, comparing mortality rates across different GLY exposure groups. The Log-rank test was used to assess statistical differences between the survival curves. In addition to the main analyses, stratified analyses and interaction tests were conducted to assess the potential modifying effects of various covariates on the relationship between urinary GLY levels and mortality. The following variables were included for stratification: age (< 60 vs. ≥60 years), sex (man vs. woman), race (Mexican American vs. Other Hispanic vs. Non-Hispanic White vs. Non-Hispanic Black vs. other race), educational attainment (< 9th grade vs. 9-11th grade vs. high school vs. college vs. graduate and above), BMI (< 25 vs. ≥25 kg/m2), smoking status (never vs. quit vs. current), alcohol consumption (never vs. 1 ~ 5 drinks/month vs. 5 ~ 10 drinks/month vs. >10 drinks/month vs. unknown), and eGFR (< 60 vs. ≥60 mL/min/1.73 m2), hypertension (yes vs. no), and diabetes (yes vs. no).

To maximize statistical power and reduce potential bias from excluding observations with missing covariate data, we employed a robust approach to handle missing values. Multiple imputation was used for continuous

Characteristics	*N*[b]	Missing [c]	Glyphosate (ng/mL, Urine)		*P*-value
			Low group (< 0.33)	High group (0.33–8.21)	
N [a]			2368	2372	
Sex	723,907,169	0			< 0.001
Male			45.39 (43.13 ,47.68)	51.62 (48.28 ,54.95)	
Female			54.61 (52.32 ,56.87)	48.38 (45.05 ,51.72)	
Age, years	723,907,169	0	45.88 (44.86 ,46.90)	48.05 (47.11 ,48.98)	0.001
Race, %	723,907,169	0			< 0.001
Mexican American			9.83 (7.60 ,12.62)	8.02 (6.07 ,10.53)	
Other Hispanic			6.90 (5.40 ,8.79)	6.22 (4.90 ,7.87)	
Non-Hispanic White			62.40 (58.26 ,66.36)	65.01 (60.44 ,69.32)	
Non-Hispanic Black			10.07 (8.07 ,12.50)	12.79 (10.21 ,15.92)	
Other race			10.81 (8.92 ,13.03)	7.96 (6.85 ,9.23)	
Education level, %	723,907,169	0			0.467
< 9th grade			4.22 (3.47 ,5.13)	4.53 (3.72 ,5.51)	
9–11th grade			7.99 (6.65 ,9.57)	9.23 (7.44 ,11.40)	
High school			22.90 (20.29 ,25.75)	22.76 (20.53 ,25.16)	
College			31.96 (28.89 ,35.20)	33.21 (30.37 ,36.17)	
Graduate or above			32.92 (28.47 ,37.70)	30.27 (27.25 ,33.46)	
PIR	669,181,295	54,725,874	3.11 (2.99 ,3.23)	2.94 (2.80 ,3.08)	0.031
Physical Activity [d]	723,907,169	0			0.597
Inactive			44.38 (41.65 ,47.15)	46.46 (42.59 ,50.37)	
Moderately active			32.52 (29.48 ,35.72)	31.40 (28.37 ,34.60)	
Highly active			23.10 (20.81 ,25.55)	22.14 (19.69 ,24.80)	
Smoking Status, %	723,907,169	0			0.644
Never Smoking			58.52 (55.46 ,61.52)	57.65 (54.84 ,60.42)	
Quit Smoking			19.99 (17.74 ,22.43)	20.85 (18.81 ,23.04)	
Current Smoking			21.49 (19.18 ,24.00)	21.44 (19.25 ,23.81)	
Unknow			0.00 (0.00 ,0.00)	0.06 (0.01 ,0.28)	
Drinking status, %	723,907,169	0			< 0.001
Never Drinking			13.41 (10.41 ,17.12)	17.44 (15.35 ,19.74)	
1–5 drinks/month			27.22 (24.22 ,30.43)	34.36 (31.76 ,37.07)	
5–10 drinks/month			4.76 (3.90 ,5.79)	6.16 (5.04 ,7.51)	
10 + drinks/month			9.95 (7.38 ,13.27)	11.31 (9.50 ,13.41)	
Unknown			44.67 (40.12 ,49.31)	30.73 (27.47 ,34.20)	
BMI [e], kg/m^2	720,018,844	3,888,325	28.96 (28.53 ,29.39)	29.67 (29.21 ,30.14)	0.009
Hypertension [f], %	723,907,169	0			0.033
No			64.92 (61.71 ,67.99)	60.92 (58.30 ,63.47)	
Yes			35.08 (32.01 ,38.29)	39.08 (36.53 ,41.70)	
Diabetes [g], %	723,907,169	0			< 0.001
No			90.44 (89.19 ,91.56)	85.13 (83.13 ,86.93)	
Yes			9.56 (8.44 ,10.81)	14.87 (13.07 ,16.87)	
ALT, U/L	699,645,877	24,261,292	23.81 (23.06 ,24.56)	25.08 (23.97 ,26.19)	0.053
SCR, umol/	699,593,948	24,313,221	75.66 (74.51 ,76.82)	79.61 (78.31 ,80.91)	< 0.001
BUN, mg/dL	699,304,649	24,602,520	4.90 (4.80 ,5.01)	5.29 (5.17 ,5.42)	< 0.001
eGFR [h]	699,593,948	24,313,221	96.65 (95.23 ,98.07)	93.38 (92.17 ,94.59)	< 0.001
UA, umol/L	699,134,538	24,772,632	320.28 (315.50 ,325.05)	321.58 (316.76 ,326.40)	0.684
Continued					

Characteristics	N[b]	Missing [c]	Glyphosate (ng/mL, Urine)		P-value
			Low group (< 0.33)	High group (0.33–8.21)	
TC, mmol/L	699,423,837	24,483,333	5.06 (4.98 ,5.13)	4.89 (4.83 ,4.95)	< 0.001
Triglyceride, mmol/L	700,624,321	23,282,848	1.68 (1.57 ,1.80)	1.71 (1.64 ,1.77)	0.681
HDL-C, mmol/L	702,434,526	21,472,643	1.43 (1.40 ,1.47)	1.36 (1.34 ,1.39)	< 0.001
FBG, mmol/L	699,356,578	24,550,591	5.39 (5.32 ,5.45)	5.74 (5.64 ,5.84)	< 0.001
HbA1c, %	707,770,890	16,136,279	5.56 (5.52 ,5.59)	5.73 (5.68 ,5.77)	< 0.001

Table 1. Characteristics of study population. Continuous variables were described by means (95%CI) and *P*-values were calculated by weighted linear regression model. Categorical variables were described by percentages (95%CI) and *P*-values was calculated by weighted Chi-square test. *PIR* poverty income ratio, *BMI* body mass index, *ALT* alanine aminotransferase, *SCR* serum creatinine, *BUN* blood urea nitrogen, *eGFR* estimated glomerular filtration rate, *UA* uric acid, *TC* total cholesterol, *HDL-C* high density lipoprotein cholesterol, *FBG* fasting blood glucose, *HbA1c* hemoglobin A1c. [a] Unweighted number of observations in dataset, [b] Weighted number, [c] Weighted number of missing data. [d] Inactive is defined as engaging in no moderate- or vigorous-intensity physical activity; Moderately active refers to engaging in moderate- or vigorous-intensity physical activity for more than 10 min on 1 to 3 days per week; Highly active is defined as engaging in moderate- or vigorous-intensity physical activity for more than 10 min on 4 to 7 days per week. [e] BMI was calculated as the body weight in kilograms divided by the square of the height in meters. [f] Hypertension was defined by ≥ 1 of the following criteria: systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 or self-reported physician diagnosis of hypertension. [g]. Diabetes was defined as self-reported physician diagnosis of diabetes or a fasting glucose concentration > 126 mg/dL. [h] Estimated using the newly developed Chronic Kidney Disease Epidemiology Collaboration equation: $eGFR = 141 \times \min(SCR/\kappa, 1)^{\alpha} \times \max(SCR/\kappa, 1)^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] × 1.159 [if black], where Scr is serum creatinine, κ is 0.7 for females and 0.9 for males, α is -0.329 for females and − 0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

variables, ensuring that the missing data were replaced with plausible values based on other available information. For categorical variables, dummy variables were introduced to account for missing data. All statistical analyses were conducted using R software (version 4.2.2) and EmpowerStats (http://www.empowerstats.com, X&Y Solutions, Inc., Boston, MA). A two-sided P-value < 0.05 was considered statistically significant.

Results

Baseline characteristics of the study population

Overall, this study included 4740 individuals (median follow-up time: 4.03 years) (Fig. 1). The social demographic characteristics and other covariates of the selected participants are shown in Table 1. The ranges of urinary GLY content for the two groups are < 0.33 and 0.33-8.21ng/mL, respectively. Except for education level, physical activity, smoking status, ALT, UA and Triglyceride, there were significant statistical differences between both groups for all included characteristics. Compared to the low group, the high group participants are more likely to be male, older, non-Hispanic white and black and have a history of hypertension and diabetes, have lower levels of PIR, eGFR, TC and HDL-C; and have a higher level of more than 10 drinks per month, BMI, SCR, BUN, FBG and HbA1c.

Association of urinary GLY with all-cause, cardiovascular mortality and cancer mortality

As indicated by Table 2 and Table S1, there were 238 deaths from all causes, 75 deaths from cardiovascular disease and 52 deaths from cancer during the follow-up period of 19,092.33 person-years. We constructed three models to analyze the independent role of urinary GLY in all-cause, cardiovascular mortality and cancer mortality. Overall, regardless of adjusting for confounding factors, the urinary GLY of all participants was significantly positively correlated with all-cause mortality. In the unadjusted model, the all-cause mortality increased by 49% with each 1 ng/mL increase in the urinary GLY (HR 1.49, 95% CI 1.24–1.79). This positive relationship in Model 2 (HR 1.31, 95% CI 0.99–1.71) and Model 3 (HR 1.40, 95% CI 1.09–1.80) remained robust after adjusting for the confounding factors. All-cause mortality was significantly higher in the High group as compared to the Low group. In Model 3, the corresponding HRs for all-cause mortality was 1.50 (95% CI, 1.05–2.14). Moreover, in Model 1, mortality from cardiovascular disease was positively correlated with urinary GLY, with corresponding mortality HRs of 1.44 (95% CI, 1.21–1.71). While the effect values in Models 2 and 3 were still more prominent and the association's direction remained constant across all models, the relationships in those models did not attain statistical significance. Adjusting for additional factors may have diminished statistical significance, but generally the association between cardiovascular mortality and urinary GLY appears to be robust. Cancer mortality and urine GLY had a neutral correlation in Model 1, with corresponding mortality HRs of 1.07 (95% CI, 0.67–1.72) 0.775. Although not statistically significant, there was a negative connection between cancer mortality and urine GLY in Models 2 and 3, with corresponding mortality HRs of 0.66 (95% CI, 0.37–1.18) and 0.77 (95% CI, 0.42–1.44). We reanalyzed the relationship between urinary GLY and mortality using post-interpolation data, and the results were not qualitatively different (Table 3 and Table S2). The fully adjusted smooth curve fitting results also support the prior findings (Fig. 2 and Figure S1). Additionally, we

Glyphosate (ng/mL, urine)	Person-y	No. of events	Mortality rate (per 1000 person-y)	Adjusted HR (95% CI) [a], *P* Value		
				Model 1	Model 2	Model 3
All-cause mortality						
Continuous	19,092.33	238	12.47	1.49 (1.24–1.79) < 0.001	1.31 (0.99–1.71) 0.055	1.40 (1.09–1.80) 0.008
Low group	8,761.60	86	9.82	reference	reference	reference
High group	9,972.28	152	15.24	1.77 (1.28-2,44) < 0.001	1.38 (0.98–1.95) 0.066	1.50 (1.05–2.14) 0.026
Cardiovascular mortality						
Continuous	19,092.33	75	3.93	1.44 (1.21–1.71) < 0.001	1.11 (0.83–1.48) 0.491	1.16 (0.89–1.52) 0.278
Low group	8,761.60	20	2.28	reference	reference	reference
High group	9,972.28	55	5.52	3.06 (1.38–6.78) 0.006	2.11 (0.84–5.31) 0.112	2.00 (0.72–5.53) 0.181

Table 2. Association of urine glyphosate with All-cause and cardiovascular mortality. Model 1: adjusted for none. Model 2: adjusted for sex, age, race, education status, and PIR. Model 3: adjusted for sex, age, race, education status, PIR, smoking and drinking status, Physical Activity, hypertension and diabetes, BMI, ALT, SCR, eGFR, UA, TC, Triglyceride, FBG, and HbA1c. *HR* hazard ratio, *CI* confidence interval, *PIR* poverty income ratio, *BMI* body mass index, *ALT* alanine aminotransferase, *SCR* Serum creatinine, *BUN* blood urea nitrogen, *eGFR* estimated glomerular filtration rate, *UA* uric acid, *TC* total cholesterol, *FBG* fasting blood glucose, *HbA1c* hemoglobin A1c. [a]Weighted Cox proportional hazards models were used to estimate HRs and 95% CIs.

Glyphosate (ng/mL, urine)	HR (95% CI) [a]									
	MI.1		MI.2		MI.3		MI.4		MI.5	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
All-cause mortality										
Continuous	1.49 (1.24–1.79)	1.31 (1.02–1.69)	1.49 (1.24–1.79)	1.33 (1.03–1.72)	1.49 (1.24–1.79)	1.35 (1.05–1.74)	1.49 (1.24–1.79)	1.33 (1.03–1.72)	1.49 (1.24–1.79)	1.34 (1.05–1.72)
Low group	reference	reference	reference	reference	reference	reference	reference	reference	reference	reference
High group	1.77 (1.28–2.44)	1.39 (1.01–1.93)	1.77 (1.28–2.44)	1.39 (1.01–1.91)	1.77 (1.28–2.44)	1.41 (1.04–1.92)	1.77 (1.28–2.44)	1.39 (1.01–1.91)	1.77 (1.28–2.44)	1.37 (1.00-1.90)
Cardiovascular mortality										
Continuous	1.44 (1.21–1.71)	1.10 (0.84–1.45)	1.44 (1.21–1.71)	1.16 (0.90–1.50)	1.44 (1.21–1.71)	1.15 (0.89–1.47)	1.44 (1.21–1.71)	1.14 (0.87–1.48)	1.44 (1.21–1.71)	1.16 (0.90–1.50)
Low group	reference	reference	reference	reference	reference	reference	reference	reference	reference	reference
High group	3.06 (1.38–6.78)	1.99 (0.81–4.89)	3.06 (1.38–6.78)	2.03 (0.86–4.81)	3.06 (1.38–6.78)	2.05 (0.86–4.86)	3.06 (1.38–6.78)	2.00 (0.85–4.74)	3.06 (1.38–6.78)	2.04 (0.85–4.86)

Table 3. Association of urine glyphosate with All-cause and cardiovascular mortality based on multiple imputed data. [a] Weighted Cox proportional hazards models were used to estimate HRs and 95% CIs. Model 1: adjusted for none. Model 2 adjusted for sex, age, race, education status, PIR, smoking and drinking status, Physical Activity, hypertension and diabetes, BMI, ALT, SCR, eGFR, UA, TC, Triglyceride, FBG, and HbA1c. *HR* hazard ratio, *CI* confidence interval, *PIR* poverty income ratio, *BMI* body mass index, *ALT* alanine aminotransferase, *SCR* Serum creatinine, *BUN* blood urea nitrogen, *eGFR* estimated glomerular filtration rate, *UA* uric acid, *TC* total cholesterol, *FBG* fasting blood glucose, *HbA1c* hemoglobin A1c.

investigated urinary GLY's threshold effect analysis on all-cause mortality, cardiovascular mortality and cancer mortality (Table 4 and Table S3). To fit the relationship between urinary GLY and mortality, we employed the Cox proportional hazards model and the 2-segment Cox proportional hazards model, respectively. Table 4 and Table S3 demonstrates a linear correlation between urinary GLY and all-cause mortality, but not with urinary GLY and cardiovascular mortality and cancer mortality ($P < 0.05$ for the log likelihood ratio test). We discovered that urinary GLY has an inflection point of 0.38 ng/mL. When urinary GLY < 0.38 ng/mL, an increase in urinary GLY is substantially associated with an increased risk of cardiovascular mortality (HR = 73.08; 95% CI, 2.12-2523.35; $P = 0.018$). Conversely, when urinary GLY > 0.38 ng/mL, there is no statistically significant association between increased urinary GLY and cardiovascular mortality. Furthermore, the results of the unadjusted Kaplan Meier curve indicated that the High group had a higher risk of all-cause, cardiovascular mortality and cancer mortality as compared to the Low group (Fig. 3 and Figure S2).

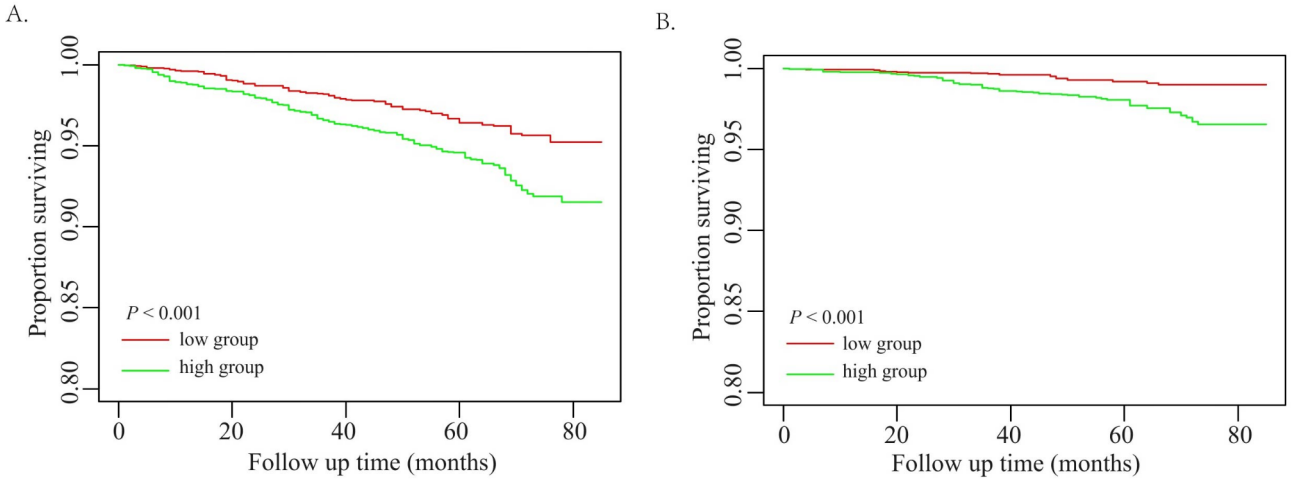

Fig. 2. Kaplan–Meier survival curve for mortality by glyphosate (weighted and unadjusted). (**A**) All-cause mortality, (**B**) Cardiovascular mortality.

	Adjusted HR (95% CI) [a], *P* Value	
	All-cause mortality	**Cardiovascular mortality**
Fitting by standard Cox proportional hazards model	1.47 (1.08, 2.00) 0.014	1.41 (0.86, 2.30) 0.176
Fitting by 2-piecewise Cox proportional hazards model		
Inflection point	0.57	0.38
< Inflection point	2.47 (0.81, 7.56) 0.113	73.08 (2.12, 2523.35) 0.018
> Inflection point	1.26 (0.80, 1.98) 0.319	0.95 (0.51, 1.78) 0.874
Log likelihood ratio	0.344	0.019

Table 4. Threshold effect analysis of urine glyphosate on All-cause and cardiovascular mortality in US adults. Adjust for adjusted for sex, age, race, education status, PIR, smoking and drinking status, physical activity, hypertension and diabetes, BMI, ALT, SCR, eGFR, UA, TC, Triglyceride, FBG, and HbA1c. *HR* hazard ratio, *CI* confidence interval, *PIR* poverty income ratio, *BMI* body mass index, *ALT* alanine aminotransferase, *SCR* Serum creatinine, *BUN* blood urea nitrogen, *eGFR* estimated glomerular filtration rate, *UA* uric acid, *TC* total cholesterol, *FBG* fasting blood glucose, *HbA1c* hemoglobin A1c. [a]Cox proportional hazards models were used to estimate HRs and 95% CIs.

Subgroup analyses

We stratified the primary covariates and performed subgroup analysis to further confirm the results' reliability in the presence of confounding variables and to determine whether there are any factors that could change the connection between urinary GLY and mortality. Other than gender and hypertension, no other covariates—such as age, PIR, diabetes, BMI, and eGFR—had a statistically significant effect on the association between GLY and mortality (all p interactions > 0.05) (Fig. 4 and Figure S3). The significant interaction (p-value of 0.03 for the interaction) was found between all-cause mortality and sex, suggesting that sex may be an important factor influencing the effects of urinary GLY, with female detriment the most (HR 1.59, 95% CI 1.20–2.10). Cancer mortality and hypertension have a significant interaction (p-value of 0.022 for the interaction), indicating that urine GLY may have a greater impact on cancer mortality in persons without hypertension (HR 2.31, 95% CI 0.87–6.13). The urinary GLY effects, nevertheless, were highly consistent for other important factors, such as age, PIR, diabetes, BMI, and eGFR subgroups; none of the interaction effects, however, reached statistical significance (P for all interactions > 0.05).

Discussion

In this large prospective analysis, we discovered a strong positive connection between urinary GLY and all-cause mortality, which persisted even after controlling for confounding variables. Furthermore, we discovered that the relationship between urinary GLY and all-cause mortality may vary depending on a person's sex in the stratification and interaction study. The findings demonstrated a substantial positive correlation between the urinary GLY by female individuals and all-cause mortality, but not a significant correlation between the urinary GLY by male individuals and all-cause mortality.

GLY is an efficient and broad-spectrum herbicide[29]. It works well in fields with a variety of crops, including cotton, corn, soybeans, etc[5,43,44]. Broad-leaved weeds like amaranth, quinoa, and purslane, as well as annual and perennial weeds like barnyard grass, sagebrush, cowweed, and horseweed, can all be successfully prevented

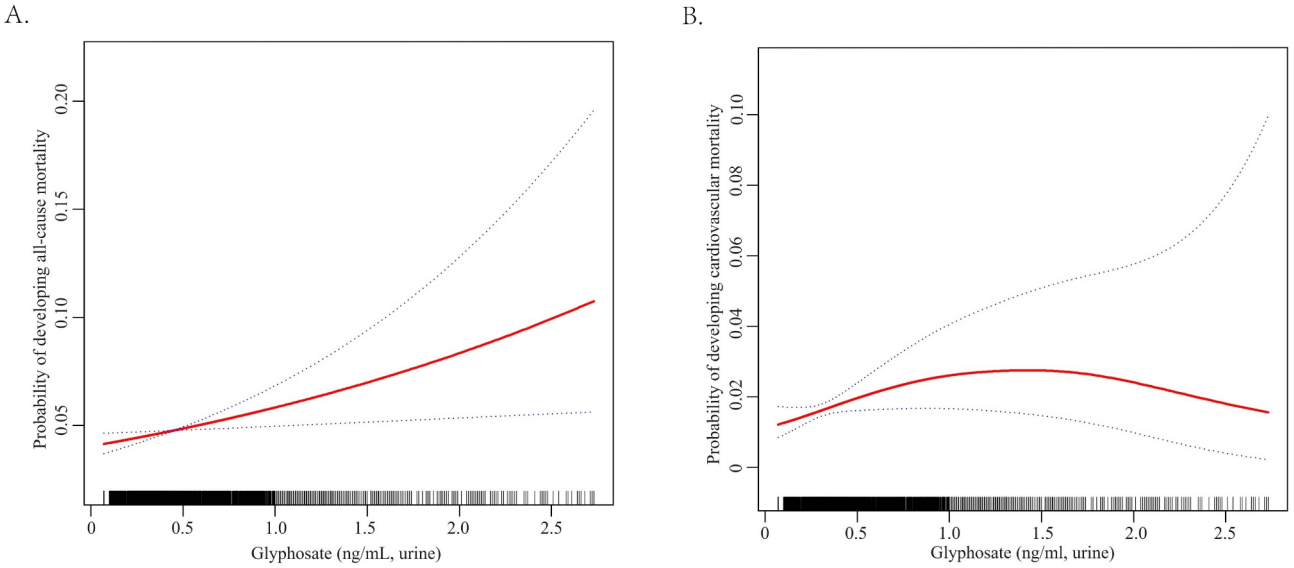

Fig. 3. Association between glyphosate and all-cause and cardiovascular mortality. (**A**) All-cause mortality, (**B**) Cardiovascular mortality. The solid and dotted lines represent the estimated values and their corresponding 95% confidence intervals, respectively. Adjustment factors included sex, age, race, education status, PIR, physical activity, smoking and drinking status, BMI, hypertension and diabetes, ALT, SCR, BUN, eGFR, UA, TC, TG, HDL-C, FBG, and HbA1c. *PIR* poverty income ratio, *BMI* body mass index, *ALT* alanine aminotransferase, *SCR* Serum creatinine, *BUN* blood urea nitrogen, *eGFR* estimated glomerular filtration rate, *UA* uric acid, *TC* total cholesterol, *TG* triglyceride*HDL-C* high density lipoprotein cholesterol, *FBG* fasting blood glucose, *HbA1c* hemoglobin A1c.

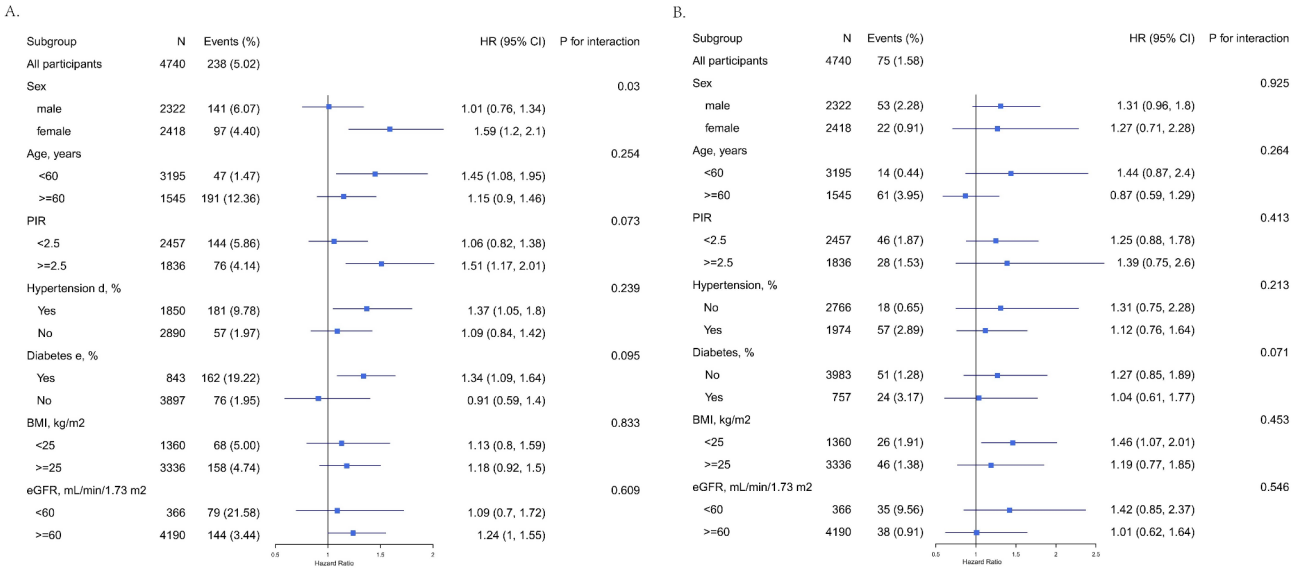

Fig. 4. Stratifying analyses by potential modifiers of the association between glyphosate and all-cause mortality and cardiovascular mortality. Each subgroup analysis adjusted for sex, age, PIR, BMI, hypertension and diabetes and eGFR except for the stratifying variable.

and controlled by it[45–48]. GLY can be used before to planting or during crop growth to enhance crop quality and production by establishing a conducive growing environment[49]. Additionally, GLY works well against some harmful plants that are hard to eradicate, such reeds, tiny flying awns, etc[50,51]. Furthermore, GLY finds extensive use in horticulture, forestry, and other domains[52,53]. However, GLY has been abused precisely because of its potent weed control action. Extensive and prolonged use of GLY may alter the diversity and activity of soil microbes, diminish soil fertility, and harm soil structure[54–56]. GLY may be dangerous to aquatic life if it gets into a body of water[57,58]. Elevated levels of GLY have the potential to impede the development of aquatic

vegetation and impact the equilibrium of aquatic environments[59,60]. Fish and shellfish are examples of aquatic species that may be toxically affected by GLY[61–63]. Furthermore, while gathering nectar and pollen, insects like bees and butterflies may come into contact with GLY and become poisoned as a result[64–67]. In northwestern Germany, Liebing et al. discovered that two-thirds of the examined samples for Phasianus colchicus hens that were free-ranged showed positive results for GLY[68]. A retrospective analysis of all suspected cases of livestock poisoning revealed that GLY was the cause in cases involving dogs, cats, horses, goats, and sheep[69]. Moreover, it is important to consider how GLY affects aquatic organisms, as we have previously mentioned. Agbohesi et al. discovered that GLY damages the liver of Clarias gariepinus, an African catfish[70]. According to Ames et al., GLY can harm zebrafish embryonic larvae, resulting in teratogenicity, heart defects, and even death[71]. When adult zebrafish exposed to GLY, Sulukan et al. observed that the progeny had decreased blood flow and heart rate, delayed hatching, increased physical malformations, and a worse survival rate[72,73]. GLY was discovered by Lu et al. to have an impact on zebrafish body length shortening, improper hatching, and embryo death[74]. Zebrafish embryos exposed to GLY, on the other hand, showed cardiac anomalies such as ventricular dilatation, ventricular wall thinning, and irregular rhythms[74]. A study by Pompermaier et al. indicated that exposure to GLY lowers the survival rates of zebrafish, causes hyperactivity and anti-anxiety behavior, affects larval anti-predation behavior negatively, and raises acetylcholinesterase activity[75]. In zebrafish larvae, Lanzarin et al. observed that GLY triggers oxidative stress, inflammation, and cell death[76]. Moreover, embryos exposed to high concentrations of GLY showed lower heart rate and hatching rate, as well as increased mortality and number of abnormalities[76]. On the other hand, exposure to amounts that do not result in teratogenic effects caused a dose-dependent drop in heart rate without significantly altering development[77]. Nonetheless, the larvae exposed to these quantities did not exhibit any alterations in histology[77]. According to DíazMartín et al., zebrafish embryos exposed to GLY for a brief period of time may develop skeletal, craniofacial, and motor disorders[78]. Bridi et al. showed that GLY shortens the zebrafish larvae's eye distance and decreases the adult zebrafish's walking distance, average speed, and crossing line[79]. It was discovered by Roy et al. that GLY causes neurotoxicity in zebrafish[80]. Research by Flach et al. showed that GLY has an impact on the Xenopus laevis embryos' development of the heart and nervous system[81]. Bullfrogs (Lithobates catesbeiana) tadpoles' metabolic processes and behavioral performance are affected by GLY exposure, according to research done by Costa et al.[82]. Furthermore, research has shown that GLY's lethal and sublethal effects on non-target plants may contribute to the decline of biodiversity in natural forest remnants submerged in agricultural settings[83]. All of these instances suggest that GLY has infiltrated the ecosystem and might stay there, potentially endangering the ecosystem's stability and equilibrium.

In this work, we first examined the association between urine GLY and mortality using representative large sample prospective data. We have made some significant new discoveries. First off, there is a strong link between the urine GLY and all-cause mortality according to our research. Additionally, the Kaplan Meier curve supports our findings. People with high urine GLY have comparatively greater rates of all-cause and cardiovascular mortality when compared to those with low urinary GLY. GLY has been shown to be harmful to the heart in numerous investigations. According to a retrospective study, patients who are exposed to GLY poisoning may have certain arrhythmia, such as I degree atrioventricular block, intraventricular conduction delay, and prolonging of the QTc interval[84]. Case reports from recent years have shown demonstrated that ingesting GLY can harm the cardiovascular system to varied degrees[85–89]. The majority of patients received gastric lavage, norepinephrine and vasopressin infusion, intubation and mechanical breathing, and substantial intravenous fluid replacement, despite the fact that the quantity of GLY ingested varied. Per Calderon et al., prolonged exposure to the GLY environment may have an impact on women's development of cardiovascular disease[90]. On a cardiac organoid model produced from human pluripotent stem cells, Sun et al. discovered that GLY had developmental toxicity[91]. In the study by Maia et al., GLY can lead to atherosclerosis regardless of exposure route and concentration[92]. However, Printemps et al. contend that Roundup ® A herbicide formulated with GLY and adjuvants can induce severe cardiac toxicity by blocking the CaV1.2 channel, leading to worsening cardiac contractility and arrhythmia, which cannot be attributed to GLY[93]. Lee et al. discovered that infusion of isopropylamine (IPA) salt (IPAG) containing GLY can alter the hemodynamics of piglets and cause piglet death, while GLY has no such impact[94]. Maybe as a result of the active ingredient GLY's interaction with other ingredients in herbicide formulations, or maybe because of their increased cytotoxic activity. In order to determine the true health concerns associated with occupational and environmental exposure, more formulation research is necessary when creating herbicides based on GLY. Notably, this research shows for the first time the unique biological significance of urinary GLY concentration in the low threshold range, meaning that a small increase in urinary GLY concentration is significantly positively correlated with the risk of cardiovascular mortality when the concentration is below 0.38 ng/mL. This finding challenges the conventional toxicology's linear "dose-response" cognitive framework and raises the prospect of non-monotonic dose-response relationships or critical threshold effects at very low exposure levels. Based on our research data and analysis process, urinary GLY concentration is very likely to become a potential biomarker for cardiovascular mortality risk which may be more significant in the low concentration range, even though there are currently no epidemiological studies reporting similar phenomena. The finding offers fresh concepts and avenues for investigation in the area of risk assessment for cardiovascular disease. Further investigation into the inherent connection between variations in low-level urine GLY concentration and the risk of cardiovascular mortality may be feasible in the future, thereby providing more valuable information for the prevention and management of cardiovascular disease.

Several investigations have indicated that GLY may have carcinogenic properties. Dal'Bó et al. discovered that while GLY can have a major cytotoxic effect on cells, it can also have a major proliferative effect, particularly on papillary thyroid cancer cells[95]. An increased risk of breast cancer may be linked to exposure to aminomethyl phosphonic acid (AMPA), the primary metabolite of GLY, according to a prospective study by Franke et al.[96]. Herbicides based on GLY have been shown by Silva et al. to decrease autophagy and enhance energy metabolism in C6 glioma cell lines[97]. Martínez et al. established that exposure to GLY in the environment is problematic by

demonstrating how GLY and AMPA cause oxidative stress, development, and cell death in human neuroblastoma cell line SH-SY5Y neurons via the pathways of necrosis, autophagy, and apoptosis[98]. Malatesta et al. showed that hepatic cancer tissue culture (HTC) cells' metabolic pathways can be disrupted by low doses of GLY[99]. Conversely, Parajuli and colleagues showed that the combination of methoxyacetic acid (MAA) and AMPA can induce apoptosis in prostate cancer cells, indicating its potential as a prostate cancer treatment medication[100]. The main source of the debate on GLY's carcinogenicity is an IARC assessment. GLY was categorized as a "possible human carcinogen" (Class 2 A) by the IARC in 2015. This classification is based on findings from in vitro research, animal trials, and epidemiological studies, some of which have linked GLY to non-Hodgkin's lymphoma (NHL). But it's important to keep in mind that the IARC's designation of GLY as "possibly carcinogenic" does not imply that it causes cancer; rather, it suggests that there is enough evidence to support the possibility that it may cause cancer, but the findings are conflicting or the evidence is insufficient. The degree and mode of exposure must also be taken into account when assessing GLY's carcinogenic consequences. The amounts that people who are directly exposed to GLY, such farmers, gardeners, and nearby residents, receive from GLY exposure or spraying are significantly higher than those that they consume through food. These populations may therefore be at greater risk for health problems. However, there isn't any solid proof that GLY causes cancer directly, even in these populations. It's also important to note that GLY may be present in trace amounts in plants themselves. This does not imply that all plants are GLY-contaminated; rather, it indicates that because GLY is so pervasive in the environment, plants may absorb traces of the chemical while they are growing. Whether these small levels of GLY are harmful to human health is still up for debate, though. The results of our research indicated that, even after controlling for confounding variables, there was a negative correlation between urine GLY content and cancer mortality; however, this relationship was not statistically significant. Furthermore, the protective effect of GLY against cancer is not yet supported by any trustworthy scientific data. We consider that the incredibly complicated influencing factors of cancer mortality may be the cause of this outcome. Despite our best efforts to account for known confounding variables, the results may still be imprecise due to unmeasured or insufficiently controlled confounding variables. To further confirm whether GLY has carcinogenic effects or its possible health impacts, more carefully planned and sizable sample investigations are therefore still required in the future, particularly long-term tracking and large-scale population research.

There is mounting evidence that GLY may also harm different organs to differing degrees. According to a recently released study, there is a positive linear link between non-alcoholic fatty liver disease (NAFLD) and GLY exposure[101]. Another prospective study discovered that early exposure to GLY and AMPA during childhood may raise the risk of metabolic diseases related to the liver and heart in early adulthood[102]. Tang et al. showed that GLY significantly harmed rats' livers and resulted in an imbalance in the concentration of different mineral elements in the rats' various organs[103]. Liu et al. demonstrated that GLY can worsen liver toxicity by blocking the Nrf2/GSH/GPX4 axis, which causes iron death in the liver cells[104]. The work of Gasnier et al. discovered that GLY can harm liver cell lines intracellularly at various levels; however, human cell lines can be somewhat shielded from this pollution by a combination of Dig1 medicinal plant extract[105]. Urine KIM-1 is the most effective early biomarker for kidney injury, according to research by Wunnapuk et al. who also showed that GLY-induced nephrotoxicity can occur[106]. Furthermore, GLY appears to have some effect on the neurological system. Oliveira et al. reported that GLY has an age- and tissue-specific impact on the hypothalamus pituitary thyroid axis[107]. Adewale et al. observed that in the brains of Wistar rats, GLY can trigger markers of oxidative stress, inflammation, and cell death[108]. Winstone et al. showed that GLY penetrates the brain, causes a dose-dependent disruption of the transcriptome, and raises the expression of TNF α and soluble A β[109]. Cattani noticed that GLY may cause extracellular glutamate levels to become too high, which would then cause oxidative stress and glutamate excitotoxicity in the rat hippocampal tissues[110]. According to Gui et al., Parkinson's disease may be linked to exposure to commonly used GLY[111]. Numerous studies have offered sufficient data to demonstrate the possible risk of urinary GLY, despite the fact that there is still considerable debate and doubt about the connection between this chemical and damage to human organs. Thus, given the possible health hazards associated with GLY, we need to take proactive preventive actions, such as tightening laws governing the use of pesticides, increasing public awareness of safety issues, lowering exposure outside of the workplace, and promoting the adoption of greener substitutes. In order to lessen or even reverse the harm that GLY does to organs, people that are already impacted by it must receive prompt medical monitoring and intervention. In summary, the application of GLY needs to be prudent and careful in order to protect human health and safety while preserving agricultural output efficiency. To strengthen the scientific foundation for protecting human health, additional comprehensive and long-term studies are required in the future to elucidate the precise mechanism and extent of urinary GLY's impact on organ damage.

It's crucial to keep in mind that GLY has also been linked to some degree of reproductive system impairment. According to Chianese et al., GLY can activate estrogen receptors and cause cell death in prostate cells. It also functions as a heteroestrogen. Hormonal changes that follow could reduce fertility[112]. Lu et al. discovered that GLY, which may be harmful to reproduction, stimulates iron death and suppresses testosterone synthesis via ferritin autophagy mediated by NCOA4[113]. Long-term dietary exposure to GLY in chickens has been demonstrated by Estienne et al. to cause the accumulation of GLY in egg yolks, which causes severe early embryo mortality and delayed embryo development in survivors[114]. These negative effects go away after two weeks of GLY exposure[114]. Ganesan et al. revealed that ovarian mitochondria and oxidative stress proteins are changed in female C57BL6 mice exposed to GLY[115,116]. Cai et al. determined that even very low concentrations (0.9 ppm) of GLY are detrimental to pre-implantation development in cattle [117]. They also reported that exposure to agricultural recommended dosages of GLY can result in stunted in vitro growth and fast loss of bovine embryos[117]. Additionally, Cavalli et colleagues demonstrated that GLY may affect male fertility[118]. GLY has been shown by Razi et al. to impact spermatogenesis, motility of sperm, and anomalies in rat testicular tissue, all of which may result in infertility[119]. Benachour et al. reported that in human umbilical cord, embryonic,

and placental cells, GLY causes necrosis and apoptosis[120]. Dallegrave and colleagues found that GLY causes embryonic bone development to be delayed and is hazardous to the mother of Wistar rats[121]. In conclusion, it is impossible to overlook the possible harm that GLY could cause to the developing reproductive system. Numerous studies have alerted us to the danger, even though there is still some debate on its precise impact. It is additionally essential to keep in consideration that the majority of GLY research is based on animal studies, and that the dosage of GLY in animal studies may differ significantly from the amounts of exposure in humans. This necessitates analyzing research findings with caution and a scientific mindset. To investigate the possible toxicity of GLY under high exposure, animal tests are usually carried out in harsh environments. The results of animal trials cannot be directly applicable to humans because these concentrations are frequently far greater than the amounts that people may encounter in their daily lives. Additionally, GLY exposure in humans typically occurs through a variety of pathways, including the food chain, air, and water, whereas in animals, trials are typically carried out in highly controlled situations and the sources of GLY that animals come into contact with are single and obvious. It is challenging to determine the precise amount of GLY that humans are exposed to due to the intricacy of these exposure pathways. Therefore, in order to have a more thorough picture of the actual state of human exposure to GLY, we must rely on epidemiological studies and environmental monitoring data. Moreover, species differences may also play a role. Different animal species may react, tolerate, and metabolize GLY quite differently than humans do. Certain animals may have a high resistance to GLY and will not react similarly to people, even at high doses, while other animals may be more sensitive to the chemical and need higher doses to have effects comparable to those seen in humans. Therefore, the amount and concentration of GLY in animal studies cannot be directly compared to human exposure. Thus, we should completely take into account variables like exposure pathways, dosages, length, and individual characteristics when describing the distinctions between exposure in humans and animal study. In order to more precisely evaluate the possible effects of GLY on human health, we need also keep an eye out for fresh research findings. In conclusion, even though the dosages used in animal tests and human exposure differ, these studies nevertheless give us important insights into how to use GLY more safely and shield people from any potential risks.

Secondly, we note that sex and hypertension may affect the relationship between urinary GLY and mortality. In subgroup analysis of sex, we observed that female individuals had lower rates of all-cause mortality. It is true that there is a correlation between gender and mortality, but this correlation is highly complex and influenced by a variety of factors, such as work, environment, behavior and lifestyle, and heredity. Furthermore, we observed that the fraction of low concentration urine GLY in females is larger than in males, which could be one of the causes of the lower all-cause death rate in females (Table 1). Additionally, GLY may have a higher effect on cancer mortality in patients without hypertension, according to our subgroup study. We speculate that it might be because the toxic effects of GLY are more likely to appear in non-hypertensive patients with relatively simple health status, whereas the numerous illness loads and medication interference of hypertensive patients may obscure the impact of GLY. It is important to remember that these conclusions are merely theories, and additional clinical data will be needed to validate our research findings. As of yet, there is no known cure for GLY poisoning, and the only available treatment is prompt systemic support. More research could lead to the development of GLY inhibitors that are more potent in the future. However, the primary way to solve the issue is to stop the misuse of herbicides like GLY. Therefore, we should strengthen policy supervision and regulatory enforcement, strictly formulate and implement usage norms, enhance agricultural technology guidance and training, and raise public environmental awareness, in order to bring the minimum negative impact while maximizing the benefits of GLY.

We must recognize that this study has significant drawbacks even though it is a huge sample size and thoroughly illustrates the intricate stratified sampling methodology of NHANES. First off, despite our best efforts to account for confounding variables, lifestyle, medication, occupational characteristics, and other factors that may be known or unmeasured confounding factors cannot be completely ruled out as potential sources of bias in the research findings. In addition, there may be complex interactions between confounding variables and between confounding variables and research factors, which can make the impact of confounding factors more complex. There may be a synergistic effect between GLY exposure and lifestyle factors such as smoking and alcohol consumption, which collectively affect human health. However, it is difficult to accurately separate and effectively control the effects of this interaction separately in statistical analysis. Moreover, in studying the effects of long-term GLY exposure on human health, confounding variables such as dietary habits and living environment of research subjects may change during the study period. For instance, an individual's water intake can have a big impact on how diluted their urine is, which can change the target substance's detection concentration. The accuracy of detection results may be hampered by diets that contain compounds that physically resemble urine GLY or that alter the body's metabolism of GLY. Furthermore, statistical analysis can often only be controlled based on data from a specific time point or limited time period, making it difficult to consider these dynamic changes in a real-time and comprehensive manner. Second, the majority of our findings are derived from questionnaire surveys, and self-reporting by participants could contain bias. It is noteworthy, however, that questionnaire surveys constitute a significant part of the National Health and Nutrition Survey, and that a great deal of research has been done using the data from these surveys. Thirdly, results should not be extended to other nations or age groups because the population we covered consisted of adult Americans who are 18 years of age or older. In conclusion, before the aforementioned results are applied in clinical settings, more clinical research is required to confirm these results.

Conclusion

Overall, this study investigated the relationship between urinary GLY and mortality, and discovered that there might be an association between urinary GLY and all-cause mortality, which we observed that this correlation is

more pronounced in female populations. Furthermore, urine GLY may have a higher effect on cancer mortality in people without hypertension.

Data availability

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

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Author contributions
YC and ZW conceived and designed the study; YC drafted the manuscript and participated in the literature search, data analysis, and interpretation. ZW collected the data and contributed to the statistical analysis. All authors contributed to the review/editing of key intellectual content of the manuscript. ML and YW provided key revisions. All authors contributed to the article and approved the submitted version.

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Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
The studies involving human participants were reviewed and approved by Ethics Review Committee of the National Center for Health Statistics. The patients/participants provided their written informed consent to participate in this study.

Additional information
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