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Association between glyphosate exposure and cognitive function, depression, and neurological diseases in a representative sample of US adults: NHANES 2013–2014 analysis

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ABSTRACT

Glyphosate, the most widely used herbicide globally, has been linked to neurological impairments in some occupational studies. However, the potential neurotoxic effects of glyphosate exposure in the general population are still not fully understood. We conducted analyses on existing data collected from 1532 adults of the 2013–2014 National Health and Nutrition Examination Survey (NHANES) to explore the possible relationship between glyphosate exposure and cognitive function, depressive symptoms, disability, and neurological medical conditions. Our results showed a significant negative association between urinary glyphosate levels and the Consortium to Establish a Registry for Alzheimer's Disease Word List Memory Test (CERAD-WLT) trial 3 recall and delayed recall scores in both models, with ß coefficients of −0.288 (S.E. = 0.111, P = 0.021) and −0.426 (S.E. = 0.148, P = 0.011), respectively. Furthermore, the odds ratio did not show a significant increase with the severity of depressive symptoms with a one-unit increase in ln-glyphosate levels. However, the odds ratio for severe depressive symptoms was significantly higher than for no symptoms (odds ratio = 4.148 (95% CI = 1.009–17.133), P = 0.049). Notably, the odds ratio showed a significant increase for individuals with serious hearing difficulty (odds ratio = 1.354 (95% CI = 1.018–1.800), P = 0.039) with a one-unit increase in ln-glyphosate levels, but not for other neurological medical conditions. In conclusion, our findings provide the first evidence that glyphosate exposure may be associated with neurological health outcomes in the US adult population. Additional investigation is necessary to understand the potential mechanisms and clinical significance of these correlations.

1. Introduction

Glyphosate, the primary active ingredient in the herbicide Roundup, has been in use since 1974. Its effectiveness in controlling weed growth has made it one of the most widely used herbicides in agriculture (Benbrook, 2016). Glyphosate can potentially expose humans through several routes, including skin contact, inhalation, and ingestion of contaminated food and water (Ospina et al., 2022). Available evidence suggests that exposure to glyphosate and glyphosate-based herbicides (GBH) in the United States has shown a temporal trend of increasing frequency and concentration (Mills et al., 2017). Glyphosate and its derivatives have been a topic of concern due to their potential health effects. Studies have linked glyphosate exposure to various health risks, including cancer, birth defects, and reproductive problems (de Araujo et al., 2016). These findings led to the International Agency for Research on Cancer to classify glyphosate as a probable human carcinogen in 2015 (Davoren and Schiestl, 2018).

Because glyphosate has the ability to cross the blood-brain barrier (BBB) (Winstone et al., 2022), research on the potential link between

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Abbreviations

AMPA	Aminomethylphosphonic acid
BBB	Blood-brain barrier
BMI	Body mass index
CERAD-WLT	Consortium to Establish a Registry for Alzheimer's Disease Word List Memory Test
GBH	Glyphosate-based herbicides
DSST	Digit Symbol Substitution test
ETS	Environmental tobacco smoke
LOD	Limit of detection
NHANES	National Health and Nutrition Examination Survey
PHQ-9	Patient Health Questionnaire

glyphosate exposure and the neurological system is ongoing. Glyphosate exposure may affect neuronal growth and development in humans by altering the expression of molecules and genes responsible for these processes, according to in vitro studies (Hao et al., 2019; Martínez et al., 2020). Studies have shown that glyphosate can cause neurotoxic effects in animal models. Exposure during both the early development and adulthood has the potential to cause significant structural and functional changes in the nervous system, as well as alterations in neurotransmission (Ait-Bali et al., 2020; Costas-Ferreira et al., 2022). Glyphosate exposure has also been associated with behavioral changes in rodents, such as decreased locomotion, increased anxiety levels, depression-like behavior, and impaired learning and memory processes (Baier et al., 2017; Luna et al., 2021).

In humans, glyphosate exposure has been associated with adverse effects on the nervous system, such as parkinsonism and encephalopathy from accidental or intentional poisoning (Barbosa et al., 2001; Malhotra et al., 2010). Recent studies suggest that chronic, low-level exposure to glyphosate could pose a significant risk and lead to neurotoxic effects. However, most of the research has focused on occupational exposure. These studies have evaluated the relationship between glyphosate exposure and neurodegenerative (Kamel et al., 2006; Shrestha et al., 2020), neurodevelopmental (Juntarawijit et al., 2020; von Ehrenstein et al., 2019), and neurobehavioral outcomes (Beard et al., 2013, 2014; Fuhrimann et al., 2021), but the results have been inconsistent (Chang et al., 2023). Currently, there is a paucity of epidemiologic studies examining the potential neurologic outcomes of non-occupational residential glyphosate use.

To further expand our understanding of the relationship between glyphosate levels and neurological health, we analyzed data from the 2013–2014 National Health and Nutrition Examination Survey (NHANES), which includes comprehensive data on urinary glyphosate levels and neurological health through multiple questionnaires. The primary objective of this study was to assess the potential correlation between glyphosate exposure and cognitive function, depressive symptoms, disability, and neurological disease in a representative sample of the adult population in the United States.

2. Materials and methods

2.1. Study population

The NHANES is a biennial nationwide survey that recruits a representative sample of the population. Comprehensive information about the survey methodology and participant eligibility documents can be found on the official NHANES website (CDC, 2016c). Because many of the key neurological system questionnaires used to assess neurological function in NHANES are only available to adults, we restricted our study population to those 18 years of age or older. In NHANES 2013–2014, we included individuals 18 years of age or older who had measures of glyphosate exposure, available demographic data, and had information on cognitive function tests or questionnaires related to depression, disability, or medical conditions in our study. A total of 1523 subjects aged 18–80 years were included in this study. A flowchart of the algorithm is shown in Fig. 1.

2.2. Measurement of urinary glyphosate levels

In the NHANES 2013–2014 study, a subset of individuals aged 6 years and older had their urinary glyphosate levels analyzed, representing for one-third of the total participants. For our analysis, we obtained data from individuals aged 18 years and older. The glyphosate measurements and imputation were performed as part of the NHANES 2013–2014 study, which has been previously published (Schütze et al., 2021). For levels below the limit of detection (LOD), NHANES provided an imputed value (LOD divided by the square root of 2). The analytical method is available on the NHANES website (CDC, 2022).

2.3. Cognitive function test

The NHANES 2013–2014 included a cognitive assessment for participants aged 60 years and older that included the Consortium to Establish a Registry for Alzheimer's Disease Word List Memory Test (CERAD-WLT), the Animal Fluency Test, and the Digit Symbol Substitution Test (DSST). The CERAD-WLT assesses new verbal learning ability using three consecutive learning trials and a delayed recall, with a maximum score of 10 per trial. This test has been used in numerous epidemiological studies in diverse populations (Fillenbaum et al., 2008). The Animal Fluency test assesses categorical verbal fluency and has been used in large-scale screening and epidemiological research. Participants are asked to name as many animals as possible in 1 min, with a score of one point is assigned for each animal correctly identified (Clark et al.,

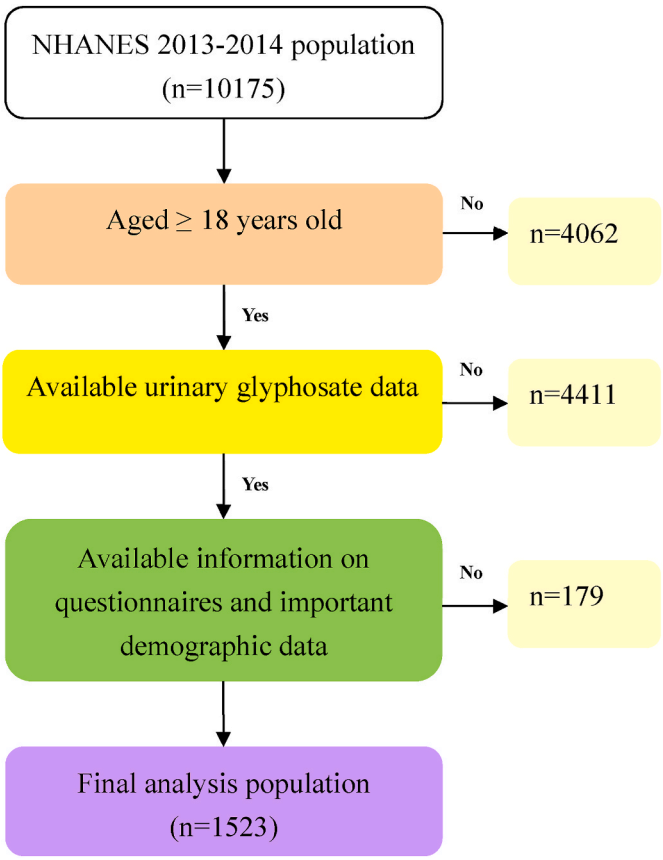

Fig. 1. Flow chart algorithm.

2009). The DSST test measures processing speed, sustained attention, and working memory by requiring participants to match corresponding symbols in 133 boxes within 2 min, with scores based on correct matches (Proust-Lima et al., 2007). A detailed methodology is available on the NHANES website (CDC, 2017).

2.4. Patient Health Questionnaire (PHQ = 9)

The 2013–2014 NHANES collected information on depression using the PHQ-9, a nine-item screening tool. The questionnaire was administered to individuals aged 18 years and older to assess the frequency of depressive symptoms experienced during the previous two weeks. Response options for the questionnaire ranged from "not at all" to "nearly every day," and each response was assigned a score from 0 to 3. The PHQ-9 incorporates diagnostic criteria for depression as outlined in the Diagnostic and Statistical Manual of Mental Disorders-IV (Spitzer et al., 1999). To determine the severity of depressive symptoms, the total scores obtained were divided into five categories. A score of 0–4 indicates no depressive symptoms, while 5–9 indicates mild depressive symptoms, 10–14 indicates moderate depressive symptoms, 15–19 indicates moderately severe depressive symptoms, and 20–27 indicates severe depressive symptoms (Kroenke et al., 2001). The NHANES website provides a detailed description of the methodology used for the PHQ-9 assessment (CDC, 2016a).

2.5. Hearing, seeing, and concentrating disability

The 2013–2014 NHANES survey included a disability questionnaire that assessed serious hearing difficulty, serious seeing difficulty, and serious difficulty with concentration. Participants aged 18 years or older were included in our analysis. More details about the disability questionnaire are available on the NHANES website (CDC, 2015).

2.6. Medical condition in neurological system

NHANES 2013–2014 collected information from participants aged 20 years and older about whether they had ever been diagnosed with a stroke by a medical professional. Moreover, individuals aged 60 years and older were assessed for cognitive function, specifically asking about any difficulties with thinking or memory in the past year, as well as any recent difficulties with memory retention. Those who answered "never" were considered to have no difficulty in remembering, while those who answered otherwise were classified as experiencing difficulty in remembering. The sleep disorder questionnaire was available to participants 16 years of age and older, and we obtained data from individuals 18 years of age and older. Those who answered in the affirmative were classified as having a sleep disorder. The full methodology is available on the official NHANES website (CDC, 2015a).

2.7. Covariates

The NHANES website states that data collection was conducted at all study sites by trained personnel using standardized procedures. During the household interview, sociodemographic information such as age, sex, and race/ethnicity was collected. Based on the results of a smoking questionnaire, participants were classified as active smokers, environmental tobacco smoke (ETS) exposed, or non-smokers (CDC, 2016b). Alcohol consumption was assessed using a questionnaire that asked whether the participant had consumed at least 12 alcoholic drinks in the previous year, and responses were dichotomized. The definitions of body mass index (BMI), hypertension, diabetes mellitus, and hyperlipidemia used in this study were based on previous scientific research (Lin et al., 2022).

2.8. Statistics

In this study, CDC glyphosate measurements in NHANES 2013–2014 were expressed as ng/mL or μg/g creatinine units. The natural logarithm of glyphosate was calculated to determine the exponential mean and SD for various subgroups. Statistical analyses were performed using two-tailed Student's t-test and one-way analysis of variance. A complex general linear model was used to evaluate the relationship between cognitive test scores (dependent variable) and ln-glyphosate (independent variable). The selection of covariates was guided by previous research and clinical knowledge of risk factors for neurological disorders, including hypertension, diabetes mellitus, and hyperlipidemia (Kim et al., 2019). The model was adjusted for age, sex, ethnicity, BMI, smoking, alcohol consumption, household income, urinary creatinine, hypertension, diabetes mellitus, and hyperlipidemia. Urinary creatinine was considered as a separate independent variable based on previous literature, rather than as an adjustment for hydration (Barr et al., 2005; O'Brien et al., 2016). For logistic regression analyses examining the potential association between PHQ-9, neurological disease, and glyphosate, the model was used for covariate adjustment in the complex sample. Sample weights were applied according to the recommended methodology on the NHANES website (CDC, 2005). The natural logarithm of glyphosate and urinary creatinine were used in the analysis because these variables were not normally distributed. Statistical analyses were performed using SPSS version 20 (SPSS Inc., Chicago, Illinois, USA). The threshold for statistical significance was set at $P < 0.05$.

3. Results

The study population had a mean age (SD) of 48.15 (18.32) years and a mean BMI (SD) of 29.15 (7.25) kg/m^2. The majority of participants were women (51.5%), while the most common ethnicity was non-Hispanic white (47.1%). Regarding socioeconomic status, 53.7% of participants reported a household income of $\geq$ $4500 per year. Additionally, 37.2% of participants had a body mass index of $\geq$30 kg/m^2. The proportion of individuals with detectable levels of glyphosate was 80.4%. The mean levels (SD) of glyphosate were 0.55 (0.55) μg/L. Table 1 shows the geometric mean of glyphosate in various subgroups and indicates that urinary glyphosate levels were higher in men, older individuals, non-Hispanic blacks, higher BMI, hypertension, and diabetes mellitus. Additionally, creatinine-corrected glyphosate levels were higher in women, older individuals, non-Hispanic whites, non-smokers, hypertension, diabetes mellitus, and hyperlipidemia. Table 2 shows the geometric means of glyphosate in various neurological conditions. The results show that individuals with severe hearing and visual impairment had higher mean glyphosate concentrations, whereas individuals with severe hearing impairment had higher creatinine-corrected glyphosate levels. Furthermore, individuals with sleep disorders had elevated mean glyphosate and creatinine-corrected glyphosate levels. The regression analyses in Table 3 indicate that a one-unit increase in ln-glyphosate levels was negatively correlated with CERAD-WLT trial 3 recall and delayed recall, with ß coefficients of −0.288 (S.E. = 0.111, $P = 0.021$) and −0.426 (S.E. = 0.148, $P = 0.011$), respectively. Fig. 2 provides an overview of CERAD-WLT scores across quartiles of urinary glyphosate in multiple linear regression models. The results of the study indicate that neither trial 3 recall nor delayed recall showed a statistically significant increase with increasing glyphosate quartiles. Nevertheless, both CERAD-WLT scores showed a significant reduction in the highest quartile compared to the lowest quartile ($P = 0.015$ for trial 3 recall and $P = 0.047$ for delayed recall, respectively).

Table 4 illustrates the associations between PHQ-9 categories and a unit increase in natural log-transformed urinary glyphosate in logistic regression models. Our results indicate that for a one-unit increase in ln-glyphosate levels, the odds ratio does not show a significant increase with the severity of depressive symptoms. However, the odds ratio for severe depressive symptoms is significantly higher than for no symptoms

Table 1
The geometric means (S.E.) of urinary glyphosate levels in different demographic subgroups.

	No. (%)	Glyphosate (μg/L)	*P* value	Glyphosate (μg/g creatinine)	*P* value
Total	1523 (100.0)	0.41 (1.02)		0.43 (1.02)	
Sex			0.002		<0.001
Men	739 (48.5)	0.43 (1.03)		0.37 (1.03)	
Women	784 (51.5)	0.38 (1.03)		0.49 (1.03)	
Age (years)			<0.001		<0.001
18-40	542 (35.6)	0.38 (1.03)		0.35 (1.03)	
40-59	505 (33.2)	0.38 (1.03)		0.43 (1.03)	
≥60	476 (31.2)	0.47 (1.04)		0.55 (1.04)	
Ethnicity			<0.001		<0.001
Mexican-American	198 (13.0)	0.38 (1.05)		0.39 (1.06)	
Other Hispanic	132 (8.7)	0.38 (1.07)		0.43 (1.06)	
Non-Hispanic white	717 (47.1)	0.41 (1.03)		0.47 (1.03)	
Non-Hispanic black	276 (18.1)	0.47 (1.04)		0.37 (1.05)	
Non-Hispanic Asian	152 (10.0)	0.34 (1.07)		0.43 (1.07)	
Other ethnicity	48 (3.1)	0.38 (1.11)		0.34 (1.12)	
Household income (USD per year)			0.566		0.821
<4500	705 (46.3)	0.41 (1.03)		0.43 (1.03)	
≥4500	818 (53.7)	0.40 (1.03)		0.43 (1.03)	
Body mass index (kg/m^2)			0.013		0.379
<25	473 (31.1)	0.38 (1.03)		0.45 (1.04)	
25-30	483 (31.7)	0.41 (1.04)		0.43 (1.04)	
≥30	567 (37.2)	0.43 (1.03)		0.42 (1.03)	
Smoking status			0.115		0.004
Non-smoker	926 (60.8)	0.41 (1.03)		0.45 (1.03)	
ETS	222 (14.6)	0.37 (1.05)		0.39 (1.05)	
Current smoker	375 (24.6)	0.42 (1.04)		0.40 (1.04)	
Alcohol consumption (drink/year)			0.836		0.221
<12	456 (29.9)	0.40 (1.04)		0.45 (1.04)	
≥12	1067 (70.1)	0.41 (1.02)		0.42 (1.02)	
Hypertension			0.001		<0.001
Yes	573 (37.6)	0.44 (1.03)		0.48 (1.03)	
No	950 (62.4)	0.39 (1.02)		0.40 (1.03)	
Diabetes mellitus			<0.001		<0.001
Yes	258 (16.9)	0.49 (1.05)		0.52 (1.05)	
No	1265 (83.1)	0.39 (1.02)		0.41 (1.02)	
Hyperlipidemia			0.539		0.001
Yes	792 (52.0)	0.40 (1.03)		0.46 (1.03)	
No	731 (48.0)	0.41 (1.03)		0.40 (1.03)	

Student's 2-tailed *t*-test is used to compare means between two groups. One-way analysis of variance is used to compare means between more than two groups. ETS - environmental tobacco smoke.

(odds ratio = 4.148 (95% CI = 1.009–17.133), $P = 0.049$). Table 5 shows the associations between neurological conditions and a unit increase in natural log-transformed urinary glyphosate in logistic regression models. Our results indicate that for a one-unit increase in ln-glyphosate levels, the odds ratio shows a significant increase in individuals with serious hearing difficulty (odds ratio = 1.354 (95% CI = 1.018–1.800), $P = 0.039$).

4. Discussion

Our study used a cohort representative of the U.S. adult general population and found a significant negative correlation between urinary glyphosate levels and cognitive function test scores. Additionally, our findings suggest that the odds of having severe depressive symptoms were significantly higher than having no symptoms in individuals with higher glyphosate levels, as measured by the PHQ-9. We also observed an increased odds ratio for individuals reporting serious hearing difficulty with increasing urinary glyphosate levels.

The current study examined 1523 individuals aged 18–80 years and found detectable levels of glyphosate in 80.4% of participants. Given that the elimination half-life of glyphosate has been previously reported to be between 5.5 and 10 h, the substantial detection rate of glyphosate in the U.S. population raises concerns about potential sources of exposure that are currently unaccounted for (Zoller et al., 2020). This finding suggests that there may be unrecognized and potentially frequent instances of high glyphosate exposure during routine daily activities. According to a NHANES study, glyphosate levels may differ depending on whether an individual has fasted or not, and a separate study found that consumption of whole-grain bread may be associated with higher levels of glyphosate in urine samples. These findings suggest that dietary intake may be a significant contributor to our exposure to glyphosate (Ashley-Martin et al., 2023; Ospina et al., 2022). Individuals with elevated glyphosate levels may have been exposed to a diet that is frequently exposed to glyphosate as a result of herbicide application.

Multiple in vitro studies have shown that glyphosate exposure can cross the BBB and appears to effect the maturation and progression of neurons in the human central nervous system by altering the expression of molecules associated with these processes (Hao et al., 2019; Martinez and Al-Ahmad, 2019). Additionally, glyphosate exposure and its metabolites have been shown to decrease the expression of genes responsible for the synthesis of proteins that make up the neuronal cytoskeleton and axonal growth cones (Martínez et al., 2020). Several research studies have examined the effects of glyphosate or GBH in animal models and comprehensively documented their effects on the nervous system. These effects include alterations in developmental and neurotransmission processes, oxidative stress, neuroinflammation, and neuronal death (Ait Bali et al., 2017; Bali et al., 2019b). Most of these studies indicate that exposure to glyphosate during early development results in significant neurotoxic effects (Ait-Bali et al., 2020). However, exposure during adulthood can also cause significant changes in the structural and functional characteristics of the nervous system (Baier et al., 2017; Costas-Ferreira et al., 2022). Additionally, glyphosate exposure can cause behavioral changes in rodents, such as decreased locomotion, increased anxiety, and depression-like behavior, which may be related to changes in neurotransmission caused by the substance (Baier et al., 2017; Hernández-Plata et al., 2015). One animal study showed that exposure to GBH can lead to elevated levels of extracellular glutamate, which may result in neurotoxicity in the hippocampus of immature rats (Cattani et al., 2014). Other studies have also shown that glyphosate exposure can negatively affect cognitive function, possibly related to changes in the functioning of the cholinergic system. This could manifest as difficulties with memory and learning (Ait-Bali et al.,

Table 2
The geometric means (S.E.) of urinary glyphosate levels in different neurological conditions.

	No. (%)	Glyphosate (μg/L)	*P* value	Glyphosate (μg/g creatinine)	*P* value
PHQ-9			0.635		0.373
Total	1515 (100.0)	0.41 (1.02)		0.43 (1.02)	
No (0–4)	1137 (75.0)	0.40 (1.02)		0.42 (1.02)	
Mild (5–9)	227 (15.0)	0.43 (1.05)		0.43 (1.05)	
Moderate (10–14)	105 (6.9)	0.40 (1.07)		0.48 (1.07)	
Moderately-severe (15–19)	35 (2.3)	0.40 (1.15)		0.39 (1.15)	
Severe (20–27)	11 (0.8)	0.52 (1.37)		0.55 (1.33)	
Serious hearing difficulty			<0.001		<0.001
Total	1522 (100.0)	0.41 (1.02)		0.43 (1.02)	
Yes	128 (8.4)	0.53 (1.08)		0.55 (1.07)	
No	1394 (91.6)	0.40 (1.02)		0.42 (1.02)	
Serious seeing difficulty			0.004		0.435
Total	1523 (100.0)	0.41 (1.02)		0.43 (1.02)	
Yes	95 (6.2)	0.50 (1.08)		0.46 (1.09)	
No	1428 (93.8)	0.40 (1.02)		0.43 (1.02)	
Serious concentrating difficulty			0.064		0.388
Total	1523 (100.0)	0.41 (1.02)		0.43 (1.02)	
Yes	146 (9.6)	0.45 (1.07)		0.45 (1.08)	
No	1377 (90.4)	0.40 (1.02)		0.43 (1.02)	
Stroke			0.133		0.060
Total	1449 (100.0)	0.41 (1.02)		0.43 (1.02)	
Yes	46 (3.2)	0.48 (1.12)		0.54 (1.12)	
No	1403 (96.8)	0.40 (1.02)		0.43 (1.02)	
Difficulties in thinking or remembering in past 1 years			0.605		0.721
Total	475 (100.0)	0.47 (1.04)		0.55 (1.04)	
Yes	72 (15.2)	0.49 (1.11)		0.56 (1.10)	
No	403 (84.8)	0.46 (1.04)		0.54 (1.04)	
Trouble in remembering in past 7 days			0.086		0.062
Total	476 (100.0)	0.47 (1.04)		0.55 (1.04)	
Yes	215 (45.2)	0.44 (1.06)		0.51 (1.06)	
No	261 (54.8)	0.49 (1.05)		0.58 (1.05)	
Sleep disorder			0.040		<0.001
Total	1523 (100.0)	0.41 (1.02)		0.43 (1.02)	
Yes	447 (29.3)	0.43 (1.04)		0.48 (1.04)	
No	1076 (70.7)	0.40 (1.02)		0.41 (1.02)	

Student's 2-tailed *t*-test is used to compare means between two groups. One-way analysis of variance is used to compare means between more than two groups. PHQ-9 - Patient Health Questionnaire.

Table 3
Linear regression coefficients (standard error) of CERAD-WLT scores with a unit increase in ln-urinary glyphosate in multiple linear regression models, with results weighted for sampling strategy.

	Unweighted no./Population size	Glyphosate (ng/mL)	
		Adjusted β (SE)	*P* value
CERAD-WLT			
Score trial 1 recall	461/57434081	−0.144 (0.132)	0.292
Score trial 2 recall	460/57370125	−0.180 (0.155)	0.266
Score trial 3 recall	460/57370125	−0.288 (0.111)	0.021
Score delayed recall	460/57370125	−0.426 (0.148)	0.011
Animal fluency score	458/57403207	0.587 (0.345)	0.109
DSST score	441/55880173	−0.032 (1.009)	0.975

Model adjusted for age, gender, ethnicity, BMI, smoking status, drinking status, household income, urinary creatinine, hypertension, diabetes mellitus, and hyperlipidemia. CERAD-WLT - Consortium to Establish a Registry for Alzheimer's Disease Word List Memory Test. DSST - Digit Symbol Substitution Test.

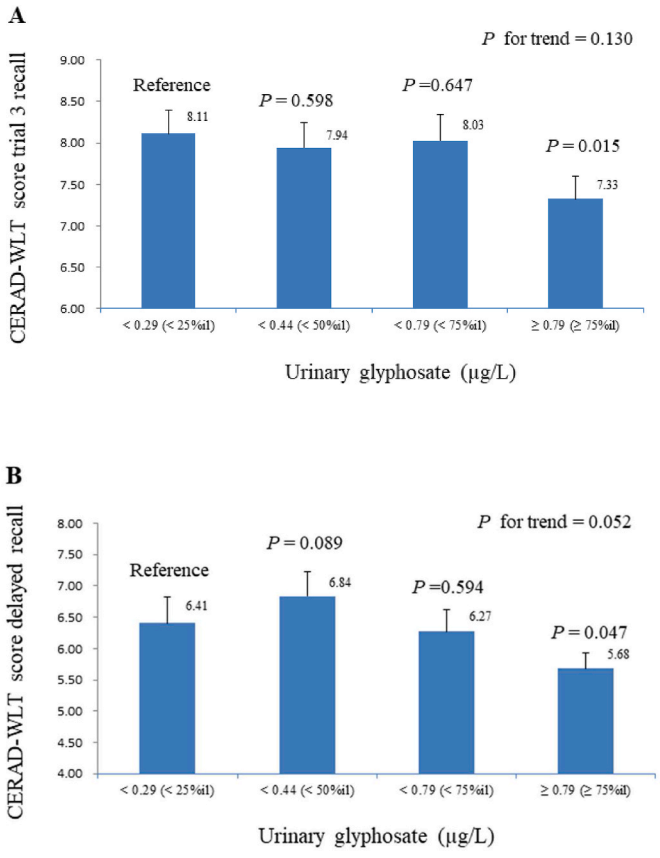

Fig. 2. CERAD-WLT scores across quartiles of urine glyphosate in multiple linear regression models, with results weighted for sampling strategy. A: Score trial 3 recall. B: Score delayed recall.

Table 4
Associations between categories of Patient Health Questionnaire and a unit increase in natural log-transformed urinary glyphosate in logistic regression models, with results weighted for sampling strategy (n = 1507).

PHQ-9 Depressive symptoms	Population size (%)	Ln-glyphosate (ng/mL)			
		OR	95% confidence interval	*P* value	*P* for trend
No (0–4)	165296204 (76.9)	Reference			0.274
Mild (5–9)	29300004 (13.6)	0.983	0.603–1.604	0.942	
Moderate (10–14)	15314369 (7.1)	0.965	0.764–1.217	0.745	
Moderately-severe (15–19)	3631494 (1.7)	1.379	0.713–2.669	0.315	
Severe (20–27)	1352087 (0.6)	4.158	1.009–17.133	0.049	

Model adjusted for age, gender, ethnicity, BMI, smoking status, drinking status, household income, urinary creatinine, hypertension, diabetes mellitus, and hyperlipidemia. PHQ-9 - Patient Health Questionnaire.

Table 5
Associations between neurological condition and a unit increase in natural log-transformed urinary glyphosate in logistic regression models, with results weighted for sampling strategy.

Medical condition	Unweighted no./ Population size	Glyphosate (ng/mL)		
		OR	95% confidence interval	*P* value
Serious hearing difficulty	1513/ 215272381	1.354	1.018–1.800	0.039
Serious seeing difficulty	1514/ 215327722	1.407	0.873–2.268	0.148
Serious concentrating difficulty	1514/ 215327722	1.134	0.742–1.734	0.537
Stroke	1440/ 207890584	0.769	0.407–1.453	0.769
Difficulties in thinking or remembering in past 1 years	469/57806397	1.375	0.735–2.572	0.295
Trouble in remembering in past 7 days	470/57906844	0.840	0.522–1.351	0.446
Sleep disorder	1514/ 215327722	1.085	0.863–1.366	0.459

Model adjusted for age, gender, ethnicity, BMI, smoking status, drinking status, household income, urinary creatinine, hypertension, diabetes mellitus, and hyperlipidemia.

2020; Bali et al., 2019a; Luna et al., 2021).

Studies of the effects of glyphosate exposure in humans have focus primarily on the effects of intentional or accidental poisoning. Intoxication has been associated with adverse effects on the neurological system, particularly parkinsonism and encephalopathy (Barbosa et al., 2001; Madani and Carpenter, 2022; Malhotra et al., 2010). Moreover, current research on the presence of glyphosate in the ecosystem suggests that chronic, low-level exposure could pose a significant risk and lead to neurotoxic effects. However, most of the existing research has focused on occupational exposure and has produced conflicting results. Several occupational studies have examined the association between glyphosate exposure and neurodegenerative outcomes. Results from the Agricultural Health Study cohort do not support a statistically significant association between glyphosate use and either Parkinson's disease or mortality from amyotrophic lateral sclerosis (Kamel et al., 2006, 2012; Shrestha et al., 2020). Nevertheless, glyphosate exposure was found to be positively associated with macular degeneration in the highest exposure category (Montgomery et al., 2017). Several epidemiologic studies have also examined the potential association between glyphosate exposure and neurodevelopmental outcomes. Research conducted in Thailand found no significant correlation between glyphosate use and developmental delay in children (Juntarawijit et al., 2020). In contrast, a case-control study conducted in an agricultural area in the U.S. found a positive correlation between prenatal glyphosate exposure and a higher likelihood of developing autism, particularly in cases of infant exposure with concurrent intellectual disability (von Ehrenstein et al., 2019).

4.1. Cognitive function

Regarding the association between glyphosate use and neurobehavioral outcomes, such as depression and cognitive function, inconsistent results have been reported in various occupational studies. Two cohort studies from the Agricultural Health Study, one of men and one of women, found no association between glyphosate use and risk of depression (Beard et al., 2013, 2014). However, a study of Ugandan farmers reported an association between glyphosate use and poorer visual memory, although no significant association with other neurobehavioral outcomes was observed (Fuhrimann et al., 2021). The results of our study indicate a statistically significant inverse association between urinary glyphosate levels and cognitive function test parameters, specifically Trial 3 Recall and Delayed Recall. The highest quartile scores showed a significant reduction compared to the lowest quartile. This observation is consistent with previous animal research suggesting that glyphosate exposure may impair memory and learning ability, possibly as a result of changes to the cholinergic system (Ait-Bali et al., 2020; Bali et al., 2019a; Luna et al., 2021). The fact that only two parameters of the CERAD-WLT are negatively correlated with glyphosate levels, it suggests that glyphosate exposure may specifically affect certain aspects of memory and learning related to the retrieval of previously learned information.

4.2. Depression

We also observed that the odds of severe depressive symptoms were significantly higher than no symptoms in individuals with higher glyphosate levels. This finding suggests that the effect of glyphosate on depression may be dose-dependent, with higher levels of exposure leading to more severe symptoms of depression. Numerous animal studies have provided evidence that glyphosate exposure may disrupt the homeostasis of neurotransmitters in the brain, particularly levels of serotonin and dopamine, which are important for regulating mood and emotion (Baier et al., 2017; Hernández-Plata et al., 2015). Glyphosate exposure may contribute to the development of depression by activating of oxidative stress and inflammation, processes that have been implicated in the pathogenesis of this psychiatric disorder (Ait Bali et al., 2017; Bali et al., 2019b). In addition, glyphosate exposure may disrupt the gut microbiome, which may play a role in regulating mood and mental health (Capuco et al., 2020; Madani and Carpenter, 2022; Puigbò et al., 2022). It is important to note that our study shows an association, not necessarily a causal relationship, between glyphosate exposure and cognitive function and depression. Further research is needed to establish a definitive causal relationship. Nevertheless, our research provides preliminary evidence of an association between glyphosate exposure and neurobehavioral outcomes in the U.S. adult population.

4.3. Hearing impairment

In addition to the aforementioned neurological sequelae, a prospective cohort study of Chinese farmers found no significant correlation between glyphosate exposure and increased susceptibility to health complications, including nerve conduction abnormalities (Zhang et al., 2016, 2018). Nevertheless, a separate investigation reported a positive association between glyphosate use and olfactory dysfunction (Shrestha et al., 2021). In the present study, we observed an increased likelihood

of experiencing severe hearing impairment associated with higher levels of glyphosate exposure. It is noteworthy that our research was conducted using a sample of adult participants in the United States, and thus the differences in findings between our study and the Chinese farmer cohort study could be attributed to various factors, including differences in exposure levels, genetic background, lifestyle, and environmental factors. Additionally, the positive association between glyphosate use and olfactory dysfunction in the study by Shrestha et al. suggests that glyphosate may have different effects on different neurological systems (Shrestha et al., 2021). If the association is casual, the underlying mechanisms behind the association between glyphosate exposure and serious hearing difficulty observed in our study remain unclear and warrant further investigation.

The article has several strengths, including providing the first preliminary evidence linking glyphosate exposure to neurological diseases in the U.S. adult population. If a causal relationship is established, it raises significant concerns about the potential impact of glyphosate exposure on the neurological health of American adults. The importance of the study lies in its use of reliable and comprehensive data from the NHANES database and its inclusion of a representative sample of American adults aged 18 years and older. However, it is important to recognize the weaknesses and limitations of this study when interpreting the results. First, the sample size was limited to glyphosate and a few questionnaires from NHANES 2013–2014, which may have hindered the ability to conduct a comprehensive analysis and may have compromised the reliability of the results. For example, while our sample size was 1523, the primary finding on cognition was based on approximately 460 subjects aged 60 years and older, which may have reduced the power of our analysis. In addition, the cross-sectional design of this investigation makes it unsuitable for making causal inferences. Third, we did not consider other pollutants that may have been co-exposed with glyphosate or that may have confounded the results. For example, aminomethylphosphonic acid (AMPA) is a major metabolite of glyphosate and is formed by microbial degradation in soil (Soukup et al., 2020). AMPA has comparable toxicity to glyphosate and is therefore considered to be of similar toxicological concern (Tresnakova et al., 2021). However, it is important to note that the NHANES survey does not include measurements of AMPA. Therefore, we were unable to assess the association between AMPA levels and neurological disorders in our study. Fourth, although seasonality has been reported to affect neurological function (Zhang and Volkow, 2023), the NHANES data collection does not provide information on the season of the participant's interview. Future studies should consider including information on seasonality to better control for this potential confounding factor. Finally, the study participants were all adults from the United States, which limits the generalizability of the conclusions to other age groups and geographic regions.

5. Conclusions

In conclusion, our study provides important evidence of an association between urinary glyphosate levels and adverse neurological outcomes in a representative cohort of U.S. adult population. Specifically, we observed lower cognitive function scores, greater odds of severe depressive symptoms, and increased risk of serious hearing difficulty in individuals with higher glyphosate exposure. While the directionality and clinical significance of these associations require further investigation, our findings underscore the need for continued research on the potential neurological effects of glyphosate exposure in adults. Such research can inform public health policy and regulatory decisions regarding glyphosate use and ultimately contribute to the protection of human health.

Author's contributions

Ching Chung Hsiao and An-Ming Yang formulated the theoretical framework and prepared the initial draft of the manuscript. Chi-Kang Wang assisted in conducting the analytical calculations. Chien-Yu Lin contributed to the development of the theoretical framework, recorded and analyzed covariates, engaged in critical discussions, and contributed to the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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