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Article

Glyphosate exposure and urinary oxidative stress biomarkers in the Agricultural Health Study

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Abstract

Background: Glyphosate is the most widely applied herbicide worldwide, and its use has been associated with increased risks of certain hematopoietic cancers in epidemiologic studies. Animal and in vitro experiments suggest that glyphosate may induce oxidative stress, a key characteristic of carcinogens; however, evidence in human populations remains scarce. We investigated associations between glyphosate exposure and urinary oxidative stress biomarkers in the Biomarkers of Exposure and Effect in Agriculture study, a molecular epidemiologic subcohort in the Agricultural Health Study.

Methods: This analysis included 268 male farmers selected based on self-reported recent and lifetime occupational glyphosate use and 100 age- and geography-matched male nonfarmers. Concentrations of glyphosate and oxidative stress biomarkers (8-hydroxy-2′-deoxyguanosine [8-OHdG], 8-iso-prostaglandin-F2α, and malondialdehyde [MDA]) were quantified in first-morning-void urine. We performed multivariable linear regression to evaluate associations of urinary glyphosate and self-reported glyphosate use with each oxidative stress biomarker.

Results: Urinary glyphosate concentrations were positively associated with levels of 8-OHdG (highest vs lowest glyphosate quartile; geometric mean ratio = 1.15, 95% confidence interval = 1.03 to 1.28; P_{trend} = .02) and MDA (geometric mean ratio = 1.20, 95% confidence interval = 1.03 to 1.40; P_{trend} = .06) overall. Among farmers reporting recent glyphosate use (last 7 days), use in the previous day was also associated with statistically significantly increased 8-OHdG and MDA levels. Compared with nonfarmers, we observed elevated 8-iso-prostaglandin-F2α levels among farmers with recent, high past 12-month, or high lifetime glyphosate use.

Conclusions: Our findings contribute to the weight of evidence supporting an association between glyphosate exposure and oxidative stress in humans and may inform evaluations of the carcinogenic potential of this herbicide.

Glyphosate is a broad-spectrum herbicide and crop desiccant. Since its commercialization in 1974, glyphosate has become the most widely applied agricultural pesticide in the United States and worldwide (1,2). As of 2012, glyphosate also ranked as the second most commonly used pesticide in US homes and gardens (2). Recently, nationally representative data from the National Health and Nutrition Examination Survey (2013-2014) suggest that approximately 80% of the general US population aged 6 years and older have detectable concentrations of glyphosate in their urine (3,4). Other biomonitoring studies suggest increasing exposure in the general population (5-7) and higher exposure among certain occupations, including farmers (8,9), with dermal contact being the major route of occupational exposure (10).

In 2015, the International Agency for Research on Cancer classified glyphosate as a probable human carcinogen (group 2A), citing limited epidemiologic evidence of an association with non-Hodgkin lymphoma, sufficient evidence of carcinogenicity in experimental animals, and strong mechanistic evidence (mostly in animals and human cells) of genotoxicity and oxidative stress (11,12). However, the relationship between glyphosate exposure and risk of cancer, particularly lymphohematopoietic malignancies, remains inconclusive and controversial (13-15). The Agricultural Health Study (AHS), a prospective cohort of pesticide applicators in Iowa and North Carolina, recently reported a suggestive association between high lifetime use of glyphosate and increased risk of acute myeloid leukemia but not non-Hodgkin lymphoma or other cancers (16). Investigations of intermediate biomarkers of effect can provide timely evidence regarding the carcinogenic potential of this widely used herbicide (17).

Oxidative stress occurs when the production of reactive oxygen species (ROS) and other free radicals exceeds the body's antioxidant defense mechanisms, causing damage to DNA, proteins,

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and lipids (18). Although ROS form as part of normal cellular processes, they may also arise from exposure to exogenous agents, such as pesticides (18). The International Agency for Research on Cancer identified oxidative stress as a key characteristic of carcinogens (19-21), and accumulating evidence supports the role of oxidative stress in the pathogenesis of hematologic cancers (22,23). As such, assessment of glyphosate exposure in relation to markers of oxidative damage may provide insights into potential mechanisms underlying previously observed associations. Glyphosate has been shown to induce oxidative stress in human cells and animal models [reviewed previously (12,24)]; however, research in human populations is scarce. To our knowledge, only 4 studies among agricultural workers have investigated glyphosate exposure in relation to oxidative stress biomarkers (25-28), 2 of which reported positive associations (27,28). Notably, most of these studies had relatively small sample sizes; relied only on self-reported exposures; and lacked details on the timing, frequency, or history of glyphosate use.

In this investigation, we evaluated associations of urinary glyphosate concentrations and self-reported occupational glyphosate use with urinary biomarkers of oxidative DNA damage (8-hydroxy-2′-deoxyguanosine [8-OHdG]) and lipid peroxidation (8-iso-prostaglandin-F2α [8-isoprostane] and malondialdehyde [MDA]) among farmers and nonfarmers in the Biomarkers of Exposure and Effect in Agriculture (BEEA) study.

Methods

Study design and population

The BEEA study is a molecular epidemiologic subcohort nested within the AHS (29,30). Briefly, during 2010-2018, we enrolled 1681 male farmers from the AHS who were aged 50 years and older, resided in Iowa or North Carolina, had never been diagnosed with cancer (except nonmelanoma skin cancer), and completed questionnaires administered at AHS enrollment (1993-1997) and 2 follow-up interviews (1999-2003 and 2005-2010). We additionally enrolled 211 male nonfarmers from Iowa or North Carolina who were aged 50 years and older, had no history of cancer, and had not lived or worked on a farm or held a job that involved handling pesticides within the last 10 years or for more than 12 months since age 18 years. The nonfarmers were identified using voter registration lists and selected to have similar distributions as the BEEA farmers in terms of age, race and ethnicity (Black, White, or other [American Indian or Alaska Native, Asian, and Native Hawaiian or other Pacific Islander]), and state and county of residence (details described in Supplementary Methods, available online). At BEEA enrollment, study staff visited participants' homes to collect first-morning-void urine samples and administer a questionnaire soliciting information on demographics, lifestyle, and medical history, as well as use of specific pesticides (including recency and frequency of use) in the last 12 months. The BEEA protocol was approved by institutional review boards at the National Cancer Institute and other participating institutions. All participants provided written informed consent. The involvement of the Centers for Disease Control and Prevention laboratory did not constitute engagement in human subjects research.

For this investigation, we selected 4 subgroups of BEEA participants (N = 368) based on their reported glyphosate use: 1) recently exposed farmers with occupational glyphosate use during the 7 days prior to urine collection, regardless of lifetime use (n = 98); 2) high lifetime-exposed farmers who were in the top 80th percentile of cumulative lifetime occupational glyphosate use but reported no use in the last 7 days (n = 70); 3) farming controls with minimal lifetime occupational glyphosate use (never used or have not used since after the 1999-2003 interview and in the lowest tertile of cumulative lifetime use) (n = 100); and 4) nonfarming controls with no home or garden use of glyphosate in the last 7 days (n = 100). The farming and nonfarming control groups were frequency matched to the glyphosate-exposed farmers (recently and high lifetime-exposed combined) by age (50-60, 61-70, older than 70 years), state (Iowa, North Carolina), and season of enrollment (April-September, October-March [off-season]). Details of the questionnaire-based glyphosate exposure assessment and study group definitions are described in Supplementary Methods (available online).

Laboratory measurements

Urinary glyphosate concentrations were quantified at the Centers for Disease Control and Prevention (Atlanta, GA, USA) using ion chromatography isotope-dilution tandem mass spectrometry as described previously (31) and in Supplementary Methods (available online). The limit of detection (LOD) was 0.2 μg/L; concentrations below the LOD (n = 45; 12.2%) were assigned a value of LOD/$\sqrt{2}$ (32). Oxidative stress biomarkers were quantified in urine using enzyme-linked immunosorbent (for 8-OHdG and 8-isoprostane) and thiobarbituric acid reactive substances (for MDA) assays at Cayman Chemical (Ann Arbor, MI, USA), as detailed in Supplementary Methods (available online). To account for urinary dilution, creatinine was quantified using an enzymatic method at the University of Minnesota Advanced Research and Diagnostic Laboratory (Minneapolis, MN, USA).

For each of these analyses, samples from participants in each of the 4 study groups were distributed evenly across all batches. To assess reproducibility of measurements, we included blinded quality control duplicate samples interspersed within and across batches. For glyphosate, 8-OHdG, 8-isoprostane, and MDA, respectively, the within-batch coefficients of variation were 2.9%, 16.8%, 15.8%, and 11.0%, and intraclass correlation coefficients were 0.997, 0.76, 0.80, and 0.95.

Statistical analysis

For our main analysis, we performed multivariable linear regression to evaluate associations between urinary glyphosate concentrations (quartiles) and natural log-transformed concentrations of each oxidative stress biomarker, overall and within each study group. Basic models adjusted for age (continuous) and urinary creatinine concentration (continuous; natural log transformed). Fully adjusted models additionally included study design–related variables (state, season, and time of urine collection), lifestyle and medical factors suggested to influence oxidative stress (body mass index, smoking status, alcohol consumption, recent nonsteroidal anti-inflammatory drug use, recent infection, history of diabetes, and history of hypertension and/or heart disease) (33-36), as well as occupational use of 2,4-dichlorophenoxyacetic acid (2,4-D), a commonly applied herbicide for which there is some prior evidence of associations with oxidative stress biomarkers (37) and the only pesticide used recently by more than 10% of farmers in this investigation. We reported associations as geometric mean ratios (GMRs) with 95% confidence intervals (CIs). GMRs were calculated by exponentiating the parameter estimates from linear regression models and interpreted as the ratio of geometric mean oxidative stress biomarker concentration for each glyphosate quartile relative to the lowest quartile. Tests for linear trend across quartiles were conducted by modeling within-quartile median values of glyphosate concentration as a

continuous variable. Additionally, we evaluated associations between continuous glyphosate concentration (log_2 transformed) and oxidative stress biomarkers.

We also evaluated associations between recent (last 7 days) occupational glyphosate use and oxidative stress biomarker concentrations compared with either farming or nonfarming controls as the referent category. Farmers with recent use were further classified by number of days since last use (≤1, 2-4, 5-7 days). To evaluate potential effects of longer-term or chronic exposure, we estimated associations of past 12-month and lifetime occupational glyphosate use (tertiles of intensity-weighted days of use; described in Supplementary Methods, available online) with oxidative stress biomarkers among glyphosate-exposed farmers and compared with each of the 2 control groups.

We performed several sensitivity analyses to further assess potential confounding: restricting to White participants, Iowa residents, participants enrolled during farming season (April-September), or farmers without recent occupational 2,4-D use. To assess the impact of outliers and highly concentrated or diluted urine, we ran models excluding participants whose urinary oxidative stress biomarker concentrations were more than 3 standard deviations above the mean or those with creatinine concentrations outside the World Health Organization's reference range (30-300 mg/dL) (38). To assess the influence of nonoccupational exposure on findings, we also conducted analyses excluding participants with home and/or garden glyphosate use.

Statistical analyses were performed using SAS, v9.4 (Cary, NC, USA). All tests were 2-sided, with statistical significance evaluated at a P value less than .05.

Results

Distributions of participant characteristics were generally similar across the 4 study groups, except for lower prevalence of diabetes and more common occupational 2,4-D use among recently and high lifetime glyphosate-exposed farmers (Table 1). Additionally, as expected, recently exposed farmers were more likely to be enrolled during farming season than other groups. We also noted lower prevalence of hypertension and/or heart disease and more common 2,4-D use among participants in higher urinary glyphosate quartiles (Supplementary Table 1, available online).

Urinary glyphosate concentrations were statistically significantly elevated among recently exposed farmers (geometric mean = 0.89 μg/L) compared with high lifetime-exposed farmers (0.59 μg/L) and farming (0.46 μg/L) and nonfarming (0.39 μg/L) controls (all $P < .01$), whereas no statistically significant differences in 8-OHdG, 8-isoprostane, or MDA concentrations were observed across groups (Supplementary Table 2, available online). The 3 oxidative stress biomarkers were moderately correlated with one another (Spearman correlation coefficients = ∼0.6-0.7), although correlations were attenuated for creatinine-corrected concentrations (Supplementary Table 3, available online).

Urinary concentrations of each oxidative stress biomarker increased with increasing quartiles of urinary glyphosate among all participants (Figure 1). In fully adjusted models, we observed statistically significant positive associations between urinary glyphosate and 8-OHdG (highest vs lowest quartile: GMR = 1.15 [95% CI = 1.03 to 1.28], $P_{trend} = .02$) and MDA (GMR = 1.20 [95% CI = 1.03 to 1.40], $P_{trend} = .06$) but not 8-isoprostane (Table 2). Modest positive associations with 8-OHdG and MDA were also observed when urinary glyphosate was modeled as a continuous log_2-transformed variable. Patterns of associations were generally similar when stratified by study group, particularly among recently exposed farmers (8-OHdG: GMR = 1.23 [95% CI = 0.97 to 1.57], $P_{trend} = .03$; MDA: GMR = 1.19 [95% CI = 0.85 to 1.66], $P_{trend} = .43$) and nonfarming controls (8-OHdG: GMR = 1.29 [95% CI = 1.00 to 1.67], $P_{trend} = .06$; MDA: GMR = 1.17 [95% CI = 0.81 to 1.68], $P_{trend} = .39$) (Supplementary Table 4, available online).

Table 3 presents fully adjusted associations between recent occupational glyphosate use (last 7 days) and urinary oxidative stress biomarkers (models only adjusted for age and creatinine shown in Supplementary Table 5, available online). Among recently exposed farmers, glyphosate use within 1 day (vs 5-7 days) of urine collection was associated with elevated concentrations of 8-OHdG (GMR = 1.20 [95% CI = 1.01 to 1.42]) and MDA (GMR = 1.28 [95% CI = 1.02 to 1.60]); comparisons with farming or nonfarming controls showed similar patterns but were not statistically significant. Furthermore, compared with nonfarmers, recent glyphosate use (regardless of further classification by days since last use) was associated with increased 8-isoprostane levels (GMR = 1.23 [95% CI = 1.03 to 1.47]).

In analyses examining longer-term occupational glyphosate use (Table 4 and Supplementary Table 6, available online), we found an association between high use in the last 12 months and elevated urinary 8-isoprostane levels in the fully adjusted model (tertile 3 of intensity-weighted days vs nonfarming controls: GMR = 1.21 [95% CI = 1.02 to 1.44]); a similar association was observed for high intensity-weighted lifetime days of use. We found no associations between these metrics and 8-OHdG or MDA. Stratified analyses showed similar results for recently exposed and high lifetime-exposed (but not recently) farmers (Supplementary Table 7, available online).

Each sensitivity analysis (described in "Methods") yielded similar results as our main analysis. Notably, urinary glyphosate remained positively associated with 8-OHdG and MDA across all analyses, and associations with MDA became slightly stronger after excluding participants with extreme MDA or creatinine values or restricting to those enrolled during farming season or those with no home or garden glyphosate use (Supplementary Table 8, available online).

Discussion

In this investigation among male farmers and demographically similar nonfarmers in Iowa and North Carolina, we observed associations between exposure to glyphosate and certain biomarkers of oxidative stress. Specifically, urinary glyphosate concentrations, as well as occupational glyphosate use in the previous day, were positively associated with urinary 8-OHdG and MDA. Compared with nonfarmers, we also observed elevated 8-isoprostane levels among farmers with occupational glyphosate use in the last 7 days and those with high past 12-month or lifetime use.

To our knowledge, only one previous study has evaluated occupational glyphosate exposure in relation to 8-OHdG, a promutagenic DNA lesion formed in response to ROS (25). Among 80 pesticide sprayers of an agricultural community in Greece, those who applied glyphosate at least once in the last spraying season were 1.5 times as likely to have high 8-OHdG levels (>75th percentile) in whole blood as those who did not; however, the association was based on univariate analysis and not statistically significant (25). Given that 8-OHdG reflects oxidative stress–induced DNA damage, our findings for 8-OHdG also support the genotoxic potential of glyphosate in humans (12) and strengthen existing evidence from studies that have reported associations

Table 1. Selected characteristics of glyphosate-exposed farmers and farming and nonfarming controls in the BEEA study

Characteristics[a]	Recently exposed (n = 98)	High lifetime-exposed (n = 70)	Farming controls (n = 100)	Nonfarming controls (n = 100)
Age, mean (SD), y	63.2 (8.3)	62.6 (9.4)	64.4 (10.1)	63.5 (8.7)
BMI, mean (SD), kg/m^2	29.2 (5.3)	28.6 (4.4)	30.1 (5.9)	30.0 (6.1)
State				
Iowa	77 (78.6)	49 (70.0)	76 (76.0)	76 (76.0)
North Carolina	21 (21.4)	21 (30.0)	24 (24.0)	24 (24.0)
Race				
Black	0 (0.0)	0 (0.0)	2 (2.0)	1 (1.0)
White	97 (99.0)	70 (100.0)	96 (96.0)	98 (98.0)
Other[b]	1 (1.0)	0 (0.0)	2 (2.0)	1 (1.0)
Season of urine collection				
April-September	92 (93.9)	29 (41.4)	71 (71.0)	63 (63.0)
October-March (off-season)	6 (6.1)	41 (58.6)	29 (29.0)	37 (37.0)
Time of urine collection				
Before 4:00 AM	22 (22.4)	8 (11.4)	14 (14.0)	16 (16.0)
4:00-5:59 AM	33 (33.7)	29 (41.4)	40 (40.0)	38 (38.0)
6:00 AM or later	43 (43.9)	33 (47.1)	46 (46.0)	46 (46.0)
Smoking status				
Never	65 (66.3)	37 (52.9)	65 (65.0)	48 (48.0)
Former	28 (28.6)	31 (44.3)	32 (32.0)	45 (45.0)
Current	5 (5.1)	2 (2.9)	3 (3.0)	7 (7.0)
Alcohol consumption (last 7 d)[c]				
None	42 (42.9)	36 (51.4)	54 (54.0)	41 (41.0)
1-6 servings	41 (41.8)	20 (28.6)	27 (27.0)	31 (31.0)
≥7 servings	15 (15.3)	14 (20.0)	19 (19.0)	28 (28.0)
Recent NSAID use (last 7 d)[d]				
No	31 (31.6)	28 (40.0)	41 (41.0)	33 (33.0)
Yes	67 (68.4)	42 (60.0)	59 (59.0)	67 (67.0)
Recent infection (last 7 d)[e]				
No	90 (91.8)	60 (85.7)	88 (88.0)	88 (88.0)
Yes	8 (8.2)	10 (14.3)	12 (12.0)	12 (12.0)
History of diabetes				
No	90 (91.8)	66 (94.3)	84 (84.0)	77 (77.0)
Yes	8 (8.2)	4 (5.7)	16 (16.0)	23 (23.0)
History of hypertension and/or heart disease				
No	48 (49.0)	38 (54.3)	44 (44.0)	45 (45.0)
Yes	50 (51.0)	32 (45.7)	56 (56.0)	55 (55.0)
Home and/or garden glyphosate use				
Did not use in the last 12 mo	61 (62.2)	42 (60.0)	58 (58.0)	56 (56.0)
Used in the last 12 mo	37 (37.8)	28 (40.0)	42 (42.0)	44 (44.0)
Occupational 2,4-D use				
Did not use in the last 12 mo	22 (22.4)	22 (31.4)	87 (87.0)	–[f]
8-365 days ago	55 (56.1)	39 (55.7)	13 (13.0)	–[f]
≤7 days ago	21 (21.4)	9 (12.9)	0 (0.0)	–[f]

[a] Presented as frequencies and percentages (%) unless otherwise specified. 2,4-D = 2,4-dichlorophenoxyacetic acid; BEEA = Biomarkers of Exposure and Effect in Agriculture; BMI = body mass index; NSAID = nonsteroidal anti-inflammatory drug.
[b] American Indian or Alaska Native (n = 1) or not reported (n = 3).
[c] Number of servings of alcoholic beverages in the last 7 days. One serving of an alcoholic beverage was defined as 12 fluid ounces of beer, 5 fluid ounces of wine, or 1.5 fluid ounces of hard liquor.
[d] Use of any aspirin- or ibuprofen-containing products in the last 7 days.
[e] Having a cold, flu, or other infection during the last 7 days.
[f] Not applicable.

between glyphosate exposure and increased DNA damage, assessed as DNA strand breaks (39) or micronucleus formation (40). The associations we observed with MDA, an end product of ROS reaction with polyunsaturated fatty acids, further suggest that glyphosate may induce oxidative injury to cell membrane lipids (41) and are consistent with a recent study among 180 maize farmers in Thailand which reported a positive association between urinary glyphosate and serum MDA levels following glyphosate application (28). Conversely, another study of 93 Thai farmers who used glyphosate found no difference between pre- and postwork urinary MDA levels (26). Compared with most previous studies relying on self-reported use or geographic proximity to spraying, our analyses based on urinary glyphosate measurements may be more relevant to the effects of the internal glyphosate dose. Furthermore, although our study focused on occupational exposure in farmers, we also observed associations between urinary glyphosate and oxidative stress biomarkers, particularly 8-OHdG, among nonfarmers, suggesting these effects may apply more broadly to the general population who are primarily exposed through ingestion of contaminated food and water or residential applications (8). Two prior general population studies, one conducted among pregnant women (42) and the other among children (43), have examined glyphosate and oxidative stress biomarkers, both reporting positive associations for urinary glyphosate or its main metabolite, aminomethylphosphonic acid (AMPA).

Contrary to our results for 8-OHdG and MDA, we observed no associations between urinary glyphosate and 8-isoprostane,

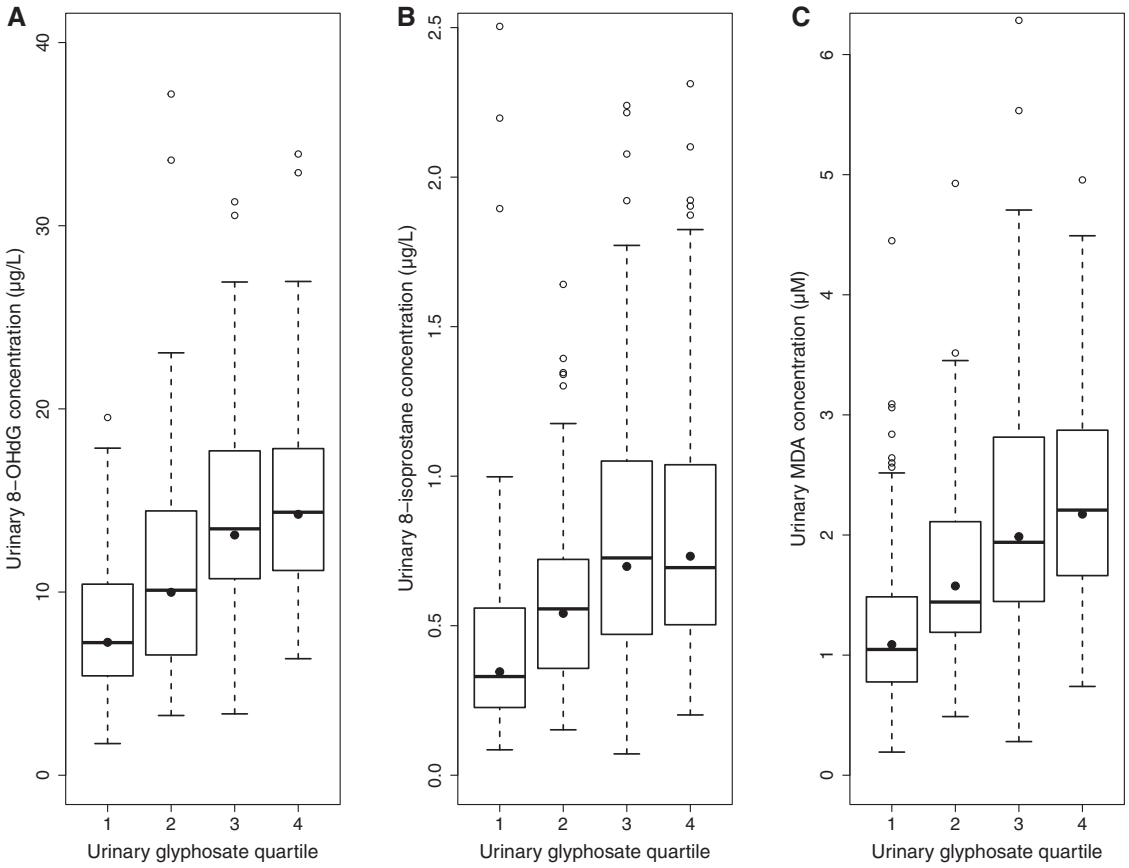

Figure 1. Box and whisker plots of urinary **(A)** 8-OHdG, **(B)** 8-isoprostane, and **(C)** MDA concentrations across quartiles of urinary glyphosate concentration among all 368 participants (quartile 1 = <LOD-0.289 µg/L; quartile 2 = 0.290-0.506 µg/L; quartile 3 = 0.507-0.933 µg/L; quartile 4 = 0.934-35.2 µg/L). The **top and bottom edges** of the boxes represent the upper (75th percentile) and lower (25th percentile) quartiles of the oxidative stress biomarker, respectively, and the **whiskers** above and below the boxes indicate the range of the data that lie within 1.5 times the IQR (ie, 1.5 IQR above the 75th percentile for the upper whisker and the minimum observed value for the lower whisker). The **thick horizontal lines** represent the median, and the **solid circles** represent the geometric mean concentration of the oxidative stress biomarker. For ease of visualization, the y-axis was truncated at a value of 3 IQRs above the 75th percentile for each oxidative stress biomarker. 8-isoprostane = 8-iso-prostaglandin-F2α; 8-OHdG = 8-hydroxy-2′-deoxyguanosine; IQR = interquartile range; LOD = limit of detection; MDA = malondialdehyde.

although levels of this marker were elevated among farmers with recent or high longer-term glyphosate use compared with non-farmers. Like MDA, 8-isoprostane is a widely assessed biomarker of lipid peroxidation and has been suggested to be more stable within individuals over time than other oxidative stress markers (44,45). It is possible that the associations with 8-isoprostane reflect chronic effects of long-term glyphosate exposure, which may not be detected by markers reflecting immediate or short-term responses to environmental stressors (46). This may also explain the associations we observed between urinary glyphosate, a marker of recent exposure given its short half-life in humans (approximately 5-10 hours) (47,48), as well as reported glyphosate use in the previous day, and 8-OHdG and MDA but not 8-isoprostane. Our findings are somewhat consistent with those from a study of 120 Brazilian agricultural and nonagricultural workers, where plasma 8-isoprostane levels were elevated among farmers reporting regular glyphosate use, although the specific time window of exposure was unclear (27). A study among 227 pregnant women in Puerto Rico reported suggestive positive associations between urinary glyphosate and 8-isoprostane (42); however, potential differences in glyphosate toxicokinetics may complicate comparisons across such different populations (49).

Findings from our study and several other human population studies to date (27,28,42,43) agree with in vitro and animal studies that have together provided strong evidence for the potential of glyphosate to induce oxidative stress (12,24). In particular, rodent studies have shown increased lipid peroxidation upon glyphosate exposure, as indicated by elevated MDA levels in blood or tissues of glyphosate-treated animals relative to untreated animals (12,24). In vitro and animal experiments evaluating glyphosate genotoxicity also suggest that glyphosate may contribute to formation of oxidative DNA adducts, including 8-OHdG (50). In addition, glyphosate has been shown to induce oxidative stress by altering antioxidant enzyme activity and/or levels of glutathione or other endogenous antioxidants in rodents (12). Using untargeted metabolomics profiling, a recent study in China identified statistically significant alterations in pathways related to glutathione metabolism among workers from glyphosate manufacturing facilities compared with participants from the general population, further suggesting that glyphosate exposure may disrupt the oxidant-antioxidant balance in humans (51).

Oxidative stress has been implicated in lymphomagenesis and leukemogenesis (22,23), with in vivo evidence of oxidative stress–induced bone marrow injury upon exposure to known

Table 2. Associations between urinary glyphosate and oxidative stress biomarker concentrations in the BEEA study (n = 368)

		8-OHdG		8-isoprostane		MDA	
Urinary glyphosate concentration (μg/L)	No.	Age and creatinine adjusted[a] GMR[c] (95% CI)	Fully adjusted[b] GMR[c] (95% CI)	Age and creatinine adjusted[a] GMR[c] (95% CI)	Fully adjusted[b] GMR[c] (95% CI)	Age and creatinine adjusted[a] GMR[c] (95% CI)	Fully adjusted[b] GMR[c] (95% CI)
Quartile 1 (<LOD-0.289)	93	1 (Referent)	1 (Referent)	1 (Referent)	1 (Referent)	1 (Referent)	1 (Referent)
Quartile 2 (0.290-0.506)	91	1.02 (0.93 to 1.12)	1.04 (0.95 to 1.14)	1.03 (0.90 to 1.18)	1.01 (0.89 to 1.16)	1.08 (0.95 to 1.23)	1.11 (0.97 to 1.26)
Quartile 3 (0.507-0.933)	92	1.13 (1.02 to 1.24)	1.14 (1.03 to 1.26)	1.04 (0.90 to 1.19)	1.08 (0.94 to 1.25)	1.16 (1.01 to 1.33)	1.19 (1.03 to 1.37)
Quartile 4 (0.934-35.2)	92	1.12 (1.01 to 1.24)	1.15 (1.03 to 1.28)	0.96 (0.83 to 1.11)	1.02 (0.88 to 1.19)	1.16 (1.01 to 1.34)	1.20 (1.03 to 1.40)
P_{trend}[d]		.03	.02	.33	.89	.10	.06
Continuous[e]	368	1.02 (0.99 to 1.05)	1.03 (1.00 to 1.06)	0.98 (0.94 to 1.02)	1.00 (0.96 to 1.04)	1.02 (0.98 to 1.06)	1.03 (0.99 to 1.07)

[a] Adjusted for age (continuous; years) and natural log-transformed urinary creatinine concentration (continuous; mg/dL). 8-isoprostane = 8-iso-prostaglandin-F2α; 8-OHdG = 8-hydroxy-2′-deoxyguanosine; BEEA = Biomarkers of Exposure and Effect in Agriculture; CI = confidence interval; GMR = geometric mean ratio; LOD = limit of detection; MDA = malondialdehyde.

[b] Adjusted for age (continuous; years), natural log-transformed urinary creatinine concentration (continuous; mg/dL), state (Iowa, North Carolina), season of urine collection (April-September, October-March), time of urine collection (before 4:00 AM, 4:00-5:59 AM, 6:00 AM or later), body mass index (continuous; kg/m^2), smoking status (never, former, current), alcohol consumption (0, 1-6, ≥7 servings in the last 7 days), nonsteroidal anti-inflammatory drug use in the last 7 days (no, yes), infection in the last 7 days (no, yes), history of diabetes (no, yes), history of hypertension and/or heart disease (no, yes), and occupational 2,4-dichlorophenoxyacetic acid use (did not use in the last 12 months, 8-365 days ago, ≤7 days ago).

[c] GMR represents the ratio of geometric mean urinary oxidative stress marker concentration compared with the reference group and was calculated by exponentiating the parameter estimate (e^{β}) from linear regression model with natural log-transformed urinary oxidative stress marker concentration as the dependent variable.

[d] Calculated by modeling within-quartile median values as a continuous variable (0.204, 0.385, 0.677, and 1.435 μg/L for quartiles 1, 2, 3, and 4, respectively).

[e] Per 1-unit increase in $\log_2$-transformed urinary glyphosate concentration, corresponding to a doubling in urinary glyphosate concentration.

Table 3. Associations between recent occupational glyphosate use (last 7 days) and urinary oxidative stress biomarker concentrations in the BEEA study

			Fully adjusted GMR (95% CI)[a]		
Glyphosate use	No.	Geometric mean concentration (95% CI)	Compared with nonfarming controls	Compared with farming controls	Among recently exposed only
8-OHdG (μg/L)					
Nonfarming controls	100	10.2 (9.2 to 11.3)	1 (Referent)	–	–
Farming controls	100	10.9 (9.8 to 12.2)	–	1 (Referent)	–
Recently exposed	98	10.7 (9.7 to 11.8)	0.98 (0.84 to 1.14)	0.97 (0.85 to 1.10)	–
Days since last use					
5-7	42	9.4 (7.8 to 11.2)	0.92 (0.79 to 1.09)	0.92 (0.80 to 1.06)	1 (Referent)
2-4	35	11.0 (9.5 to 12.7)	0.98 (0.82 to 1.18)	0.96 (0.83 to 1.13)	1.04 (0.90 to 1.20)
≤1	21	13.5 (11.8 to 15.4)	1.11 (0.92 to 1.35)	1.08 (0.91 to 1.29)	1.20 (1.01 to 1.42)
8-isoprostane (μg/L)					
Nonfarming controls	100	0.53 (0.46 to 0.62)	1 (Referent)	–	–
Farming controls	100	0.55 (0.47 to 0.64)	–	1 (Referent)	–
Recently exposed	98	0.57 (0.50 to 0.64)	1.23 (1.03 to 1.47)	1.15 (0.96 to 1.38)	–
Days since last use					
5-7	42	0.53 (0.43 to 0.64)	1.25 (1.03 to 1.51)	1.17 (0.95 to 1.44)	1 (Referent)
2-4	35	0.57 (0.46 to 0.70)	1.21 (0.98 to 1.49)	1.14 (0.91 to 1.43)	0.95 (0.80 to 1.12)
≤1	21	0.65 (0.50 to 0.85)	1.22 (0.96 to 1.53)	1.11 (0.86 to 1.43)	0.98 (0.81 to 1.19)
MDA (μM)					
Nonfarming controls	100	1.57 (1.38 to 1.78)	1 (Referent)	–	–
Farming controls	100	1.66 (1.47 to 1.88)	–	1 (Referent)	–
Recently exposed	98	1.69 (1.52 to 1.88)	0.98 (0.80 to 1.21)	1.03 (0.86 to 1.22)	–
Days since last use					
5-7	42	1.51 (1.26 to 1.80)	0.95 (0.76 to 1.18)	0.98 (0.80 to 1.20)	1 (Referent)
2-4	35	1.61 (1.37 to 1.90)	0.92 (0.72 to 1.17)	0.95 (0.77 to 1.19)	0.96 (0.79 to 1.17)
≤1	21	2.29 (1.83 to 2.88)	1.18 (0.91 to 1.55)	1.25 (0.98 to 1.60)	1.28 (1.02 to 1.60)

[a] Adjusted for age (continuous; years), natural log-transformed urinary creatinine concentration (continuous; mg/dL), state (Iowa, North Carolina), season of urine collection (April-September, October-March), time of urine collection (before 4:00 AM, 4:00-5:59 AM, 6:00 AM or later), body mass index (continuous; kg/m^2), smoking status (never, former, current), alcohol consumption (0, 1-6, ≥7 servings in the last 7 days), nonsteroidal anti-inflammatory drug use in the last 7 days (no, yes), infection in the last 7 days (no, yes), history of diabetes (no, yes), history of hypertension and/or heart disease (no, yes), and occupational 2,4-dichlorophenoxyacetic acid use (did not use in the last 12 months, 8-365 days ago, ≤7 days ago). 8-isoprostane = 8-iso-prostaglandin-F2α; 8-OHdG = 8-hydroxy-2′-deoxyguanosine; BEEA = Biomarkers of Exposure and Effect in Agriculture; CI = confidence interval; GMR = geometric mean ratio; MDA = malondialdehyde.

carcinogens (52-54). Additionally, several case-control studies have reported higher urinary or blood levels of oxidative stress biomarkers, including 8-OHdG (55,56), MDA (57-59), and 8-isoprostane (60), among newly diagnosed hematologic cancer patients compared with healthy controls. As such, our findings provide mechanistic insights and biological plausibility for the potential role of glyphosate in the development of certain hematologic malignancies (12,16,61,62).

A distinctive feature of our study was the comprehensive exposure assessment, including urinary glyphosate measurements and well-characterized recent and lifetime occupational pesticide exposure histories, as well as the inclusion of farming and nonfarming controls. Other strengths included the larger sample size compared with other human studies, availability of information on a range of potential confounders, and detailed sensitivity analyses that confirmed the robustness of our findings. Our study also had several limitations. Given the cross-sectional study design, biomarker measurements were from a single time point, which precluded the assessment of longitudinal associations, although we were able to explore potential temporal relationships based on timing and recency of glyphosate use. Although self-reported pesticide use may be subject to misclassification of exposure, reported use among AHS participants has been shown to be reliable (63,64), and questionnaire-assessed exposures and intensity metrics have been correlated with pesticide biomarkers in the AHS (65). Furthermore, we did not measure urinary concentrations of AMPA as an additional marker of exposure. However, AMPA is generally detected less frequently and at similar or lower concentrations than glyphosate because of limited glyphosate metabolism in humans (8,49,66), and AMPA may form as a breakdown product of other phosphonate-containing compounds (eg, detergents) besides glyphosate (67). Recent research also suggests that people may be primarily exposed to AMPA through food and water and to a lesser extent from metabolism of glyphosate (49). Nevertheless, future studies may consider assessing AMPA given experimental evidence of its potential role in oxidative stress (12) and associations observed with oxidative stress biomarkers in 2 recent general population studies (42,43). Lastly, although we measured 3 established and representative biomarkers of oxidative DNA damage or lipid peroxidation (21), they may not reflect the full extent of oxidative stress responses; future work using untargeted approaches may uncover additional oxidative stress–related metabolites or pathways associated with glyphosate exposure (51,68).

In conclusion, our findings suggest that glyphosate exposure may be positively associated with certain urinary biomarkers of oxidative stress. Although the observed associations mainly appear to reflect effects of recent occupational exposure, there was also some evidence of associations with longer-term exposure. Our study contributes to accumulating evidence supporting the role of glyphosate in oxidative stress among humans and provides insights into potential mechanisms underlying previously observed associations with some hematopoietic cancers. Future investigations including additional biomarkers of oxidative stress or other intermediate endpoints related to cancer development (eg, genotoxicity, epigenetic alterations) may further inform the evaluation of the carcinogenic potential of this herbicide.

Table 4. Associations of occupational glyphosate use in the last 12 months and cumulative lifetime occupational glyphosate use with urinary oxidative stress biomarker concentrations in the BEEA study

			Fully adjusted GMR (95% CI)[a]		
Glyphosate use	No.	Geometric mean concentration (95% CI)	Compared with nonfarming controls	Compared with farming controls	Glyphosate-exposed farmers only
8-OHdG (μg/L)					
Nonfarming controls	100	10.2 (9.2 to 11.3)	1 (Referent)	–	–
Farming controls	100	10.9 (9.8 to 12.2)	–	1 (Referent)	–
Last 12-month use[b]					
Tertile 1	56	11.0 (9.5 to 12.6)	1.04 (0.92 to 1.18)	1.02 (0.91 to 1.15)	1 (Referent)
Tertile 2	56	11.0 (9.7 to 12.5)	0.97 (0.85 to 1.11)	0.96 (0.85 to 1.08)	0.92 (0.82 to 1.04)
Tertile 3	56	11.2 (9.9 to 12.7)	1.06 (0.93 to 1.21)	1.05 (0.93 to 1.18)	1.00 (0.89 to 1.13)
P_{trend}[c]			.36	.40	.56
Lifetime use[d]					
Tertile 1	56	10.2 (8.8 to 11.9)	1.01 (0.89 to 1.16)	0.99 (0.88 to 1.12)	1 (Referent)
Tertile 2	56	12.3 (11.1 to 13.7)	1.06 (0.93 to 1.20)	1.04 (0.93 to 1.17)	1.05 (0.93 to 1.18)
Tertile 3	55	10.6 (9.3 to 12.1)	0.99 (0.86 to 1.13)	0.98 (0.87 to 1.12)	0.97 (0.86 to 1.10)
P_{trend}[c]			.75	.85	.44
8-isoprostane (μg/L)					
Nonfarming controls	100	0.53 (0.46 to 0.62)	1 (Referent)	–	–
Farming controls	100	0.55 (0.47 to 0.64)	–	1 (Referent)	–
Last 12-month use[b]					
Tertile 1	56	0.54 (0.44 to 0.65)	1.13 (0.97 to 1.32)	1.10 (0.93 to 1.29)	1 (Referent)
Tertile 2	56	0.60 (0.51 to 0.70)	1.14 (0.96 to 1.34)	1.11 (0.93 to 1.33)	1.00 (0.87 to 1.15)
Tertile 3	56	0.59 (0.49 to 0.70)	1.21 (1.02 to 1.44)	1.17 (0.98 to 1.40)	1.03 (0.90 to 1.19)
P_{trend}[c]			.08	.13	.63
Lifetime use[d]					
Tertile 1	56	0.53 (0.45 to 0.64)	1.19 (1.01 to 1.41)	1.14 (0.95 to 1.35)	1 (Referent)
Tertile 2	56	0.61 (0.52 to 0.72)	1.10 (0.94 to 1.29)	1.08 (0.91 to 1.28)	0.94 (0.82 to 1.10)
Tertile 3	55	0.57 (0.48 to 0.69)	1.20 (1.00 to 1.42)	1.17 (0.97 to 1.40)	1.03 (0.88 to 1.20)
P_{trend}[c]			.19	.20	.51
MDA (μM)					
Nonfarming controls	100	1.57 (1.38 to 1.78)	1 (Referent)	–	–
Farming controls	100	1.66 (1.47 to 1.88)	–	1 (Referent)	–
Last 12-month use[b]					
Tertile 1	56	1.65 (1.44 to 1.88)	1.01 (0.85 to 1.20)	1.00 (0.85 to 1.18)	1 (Referent)
Tertile 2	56	1.67 (1.44 to 1.95)	0.94 (0.78 to 1.13)	0.95 (0.80 to 1.13)	0.92 (0.78 to 1.07)
Tertile 3	56	1.75 (1.52 to 2.01)	1.07 (0.89 to 1.29)	1.06 (0.90 to 1.26)	1.03 (0.88 to 1.20)
P_{trend}[c]			.26	.34	.40
Lifetime use[d]					
Tertile 1	56	1.62 (1.38 to 1.91)	1.01 (0.84 to 1.22)	1.02 (0.86 to 1.20)	1 (Referent)
Tertile 2	56	1.72 (1.51 to 1.96)	0.98 (0.83 to 1.17)	0.98 (0.83 to 1.15)	0.95 (0.81 to 1.12)
Tertile 3	55	1.74 (1.52 to 1.98)	1.05 (0.87 to 1.28)	1.04 (0.87 to 1.25)	1.04 (0.88 to 1.24)
P_{trend}[c]			.59	.67	.48

[a] Adjusted for age (continuous; years), natural log-transformed urinary creatinine concentration (continuous; mg/dL), state (Iowa, North Carolina), season of urine collection (April-September, October-March), time of urine collection (before 4:00 AM, 4:00-5:59 AM, 6:00 AM or later), body mass index (continuous; kg/m^2), smoking status (never, former, current), alcohol consumption (0, 1-6, ≥7 servings in the last 7 days), nonsteroidal anti-inflammatory drug use in the last 7 days (no, yes), infection in the last 7 days (no, yes), history of diabetes (no, yes), history of hypertension and/or heart disease (no, yes), and occupational 2,4-dichlorophenoxyacetic acid use (did not use in the last 12 months, 8-365 days ago, ≤7 days ago). 8-isoprostane = 8-iso-prostaglandin-F2α; 8-OHdG = 8-hydroxy-2′-deoxyguanosine; BEEA = Biomarkers of Exposure and Effect in Agriculture; CI = confidence interval; GMR = geometric mean ratio; MDA = malondialdehyde.

[b] Intensity-weighted days of occupational glyphosate use in the last 12 months among glyphosate-exposed farmers (tertile 1 = 0-512; tertile 2 = >512-1320; tertile 3 = >1320-11 375), calculated by multiplying the number of days of use in the last 12 months by an exposure intensity score that accounts for factors known to influence pesticide exposure.

[c] Calculated by modeling within-tertile median values as continuous variables.

[d] Intensity-weighted lifetime days of occupational glyphosate use among glyphosate-exposed farmers (tertile 1 = 1321-11 440; tertile 2 = >11 440-23 071; tertile 3 = >23 071-244 237), calculated by multiplying the total number of lifetime days of use by an exposure intensity score that accounts for factors known to influence pesticide exposure. One participant had missing data on lifetime glyphosate use and was excluded from the analysis.

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Data availability

The data underlying this investigation will be provided upon request as described for the BEEA subcohort on the AHS website: https://aghealth.nih.gov/collaboration/studies.html. For more information on the process of requesting access to these data, including any administrative and human subjects requirements, please contact the Principal Investigator of the BEEA study (Dr Jonathan Hofmann) at hofmannjn@mail.nih.gov.

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