Letters

RESEARCH LETTER

Excretion of the Herbicide Glyphosate in Older Adults Between 1993 and 2016

The herbicide Roundup is sprayed onto genetically modified crops and applied as a desiccant to most small non-genetically modified grains. Use of this herbicide has increased since 1994 when genetically modified crops were introduced in the United States. Glyphosate, the primary ingredient in the herbicide, is found in these crops at harvest.[1] Environmental exposure through dietary intake of these crops has potential adverse health effects and can be assessed by measuring urinary excretion.[2-4] We measured excretion levels of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) in participants from the Rancho Bernardo Study (RBS) of Healthy Aging.

Methods | The RBS, established in 1972, is a prospective study of 6629 adults older than 50 years residing in Southern California.[5] As of 2016, approximately 1000 participants were active (the primary reason for loss to follow-up was mortality). Of those 1000 participants, 112 had routine morning spot urinary biospecimens obtained at each of 5 clinic visits that took place from 1993 to 1996 and from 2014 to 2016. One hundred of these 112 were randomly selected for this study, which was approved by the University of California, San Diego, institutional review board. All participants gave written informed consent.

Samples were analyzed using high-performance liquid chromatography coupled with mass spectrometry. Limits of detection (LOD) were 0.03 μg/L for glyphosate and 0.04 μg/L for AMPA; assays were linear up to 50 μg/L. Analyses were normalized to each sample's specific gravity, thereby accounting for dilution or concentration effects due to variability in water intake and age-related or other differences in renal function. Changes over time in the proportion of samples above the LOD were assessed using generalized estimating equation models to account for the dependency of observations in repeated measures. A 2-sided significance threshold was set at less than .05. Statistical analyses were performed using R (R Foundation), version 3.3.2.

Results | Among the 100 participants in this study, the mean age in 2014-2016 was 77.7 years (SD, 6.6) and 60% were women. These values were not different from the 112 with urine specimens but were older than the entire group of 1000 active participants in the RBS (mean age, 71.7 years [SD, 12.0]) ($P < .001$).

The mean glyphosate level increased from 0.024 μg/L in 1993-1996 to 0.314 μg/L in 2014-2016, and reached 0.449 μg/L in 2014-2016 for the 70 participants with levels above the LOD (**Table 1**). Mean AMPA levels increased from 0.008 μg/L in 1993-1996 to 0.285 μg/L in 2014-2016, and reached 0.401 μg/L in 2014-2016 for the 71 participants with levels above the LOD.

The prevalence rates of glyphosate samples above the LOD increased significantly over time, from 0.120 (95% CI, 0.064-0.200) in 1993-1996 to 0.700 (95% CI, 0.600-0.788) in 2014-2016 (Wald statistic = 80.5; $P < .001$) (**Table 2**). The prevalence of AMPA samples above the LOD increased significantly from 0.050 (95% CI, 0.016-0.113) in 1993-1996 to 0.710 (95% CI, 0.611-0.796) in 2014-2016 (Wald statistic = 103; $P < .001$).

Discussion | Mean glyphosate and AMPA levels and the proportion of samples with detectable levels increased over time. A 2015 review of nonfarmer US and European adults reported mean urinary glyphosate levels of 1.35 μg/L and 0.215 μg/L, respectively.[6] The values observed in this study fall within this range and were higher than in European adults. Animal and human studies

Table 1. Urinary Excretion Levels of Glyphosate and AMPA Among Rancho Bernardo Study Participants Sampled Between 1993 and 2016

	Glyphosate, μg/L			AMPA, μg/L		
	All Participants[a] (N = 100)	Participants Above LOD[b]		All Participants (N = 100)	Participants Above LOD[b]	
Years	Mean (95% CI), μg/L	No. of Participants	Mean (95% CI), μg/L	Mean (95% CI), μg/L	No. of Participants	Mean (95% CI), μg/L
1993-1996	0.024 (0.010-0.039)	12	0.203 (0.151-0.255)	0.008 (0.001-0.016)	5	0.168 (0.114-0.222)
1999-2000	0.053 (0.033-0.074)	30	0.179 (0.136-0.222)	0.044 (0.020-0.069)	15	0.295 (0.205-0.384)
2001-2002	0.110 (0.075-0.146)	43	0.257 (0.197-0.317)	0.112 (0.071-0.154)	43	0.262 (0.185-0.339)
2004-2005	0.111 (0.070-0.152)	38	0.292 (0.213-0.370)	0.091 (0.057-0.124)	40	0.227 (0.164-0.290)
2014-2016	0.314 (0.235-0.394)	70	0.449 (0.352-0.547)	0.285 (0.217-0.352)	71	0.401 (0.319-0.482)

Abbreviations: AMPA, aminomethylphosphonic acid; LOD, limit of detection.

[a] Participants with levels below the LOD had values set at 0.

[b] The LOD was 0.03 μg/L for glyphosate and 0.04 μg/L for AMPA.

Table 2. Urinary Excretion Prevalence Rates of Glyphosate and AMPA Among Rancho Bernardo Study Participants Sampled Between 1993 and 2016

Years	Prevalence Rate (95% CI)[a] Glyphosate	AMPA
1993-1996	0.120 (0.064-0.200)	0.050 (0.016-0.113)
1999-2000	0.300 (0.212-0.400)	0.150 (0.086-0.235)
2001-2002	0.430 (0.331-0.533)	0.430 (0.331-0.533)
2004-2005	0.390 (0.294-0.493)	0.400 (0.303-0.503)
2014-2016	0.700 (0.600-0.788)	0.710 (0.611-0.796)

Abbreviation: AMPA, aminomethylphosphonic acid.

[a] *P* value was less than .001.

suggest that chronic exposure to glyphosate-based herbicides can induce adverse health outcomes.[3] Animals consistently fed an ultra-low dosage of the herbicide with a 50-ng/L glyphosate concentration show hepatotoxicity consistent with nonalcoholic fatty liver disease and its progression to steatohepatosis.[4] In July 2017, in accordance with the Safe Drinking Water and Toxic Enforcement Act of 1986, the state of California listed glyphosate as a probable carcinogen.

Limitations of this study include that the cohort lived in Southern California, which might have different exposures than other states, only a subset of RBS participants were studied, urinary levels represent recent exposure, urinary-specific gravity is reduced with age, and data on clinical outcomes were not evaluated. Future studies of the relationships between chronic glyphosate exposure and human health are needed.

Paul J. Mills, PhD
Izabela Kania-Korwel, PhD
John Fagan, PhD
Linda K. McEvoy, PhD
Gail A. Laughlin, PhD
Elizabeth Barrett-Connor, MD

Author Affiliations: Family Medicine and Public Health, University of California, San Diego, La Jolla (Mills, Laughlin); Health Research Institute Laboratories, Fairfield, Iowa (Kania-Korwel, Fagan); Radiology, University of California, San Diego, La Jolla (McEvoy); Family Medicine, Internal Medicine, and Public Health, University of California, San Diego, La Jolla (Barrett-Connor).

Correction: This article was corrected for an omission in the Conflicts of Interest section on April 3, 2018.

Accepted for Publication: July 31, 2017.

Corresponding Author: Paul J. Mills, PhD, University of California, San Diego, 9500 Gilman Dr, La Jolla, CA 92093-0725 (pmills@ucsd.edu).

Author Contributions: Dr Mills had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Concept and design: Mills, Laughlin.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Mills, Fagan.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Mills, Fagan.
Obtained funding: Mills, McEvoy, Barrett-Connor.
Administrative, technical, or material support: Korwel, Fagan, Laughlin.
Supervision: Mills, Fagan, Barrett-Connor.

Conflict of Interest Disclosures: The authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Mills reported launching a citizen science crowdfunding site to raise funds for additional research that offers free glyphosate testing with a donation of $130, with tests being run by Health Research Institute (HRI) Laboratories, which processed the samples for the study, and proceeds going to the University of California, San Diego. Dr Fagan reported being founder, chairman of the board, and senior scientist at HRI Laboratories, a 501(c)(3) nonprofit research organization, which is conducting a citizen science research program in which individuals complete a lifestyle and diet survey, provide a urine sample, and partially cover the cost of testing the urine sample for glyphosate ($99/sample). Dr McEvoy reported receiving a grant from the National Institutes of Health. No other disclosures were reported.

Funding/Support: The Rancho Bernardo Study was funded by grant AA021187 from the National Institute on Alcohol Abuse and Alcoholism, grants AG028507 and AG007181 from the National Institute on Aging, and grant DK31801 from the National Institute of Diabetes and Digestive and Kidney Diseases.
Additional support for this study was provided by the Solomon Dutka Fund in the New York Community Trust, the Caroline Young Foundation, and the Westreich Foundation.

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

1. Bøhn T, Cuhra M, Traavik T, Sanden M, Fagan J, Primicerio R. Compositional differences in soybeans on the market. *Food Chem*. 2014;153:207-215.

2. von Soosten D, Meyer U, Hüther L, et al. Excretion pathways and ruminal disappearance of glyphosate and its degradation product aminomethylphosphonic acid in dairy cows. *J Dairy Sci*. 2016;99(7):5318-5324.

3. Myers JP, Antoniou MN, Blumberg B, et al. Concerns over use of glyphosate-based herbicides and risks associated with exposures. *Environ Health*. 2016;15:19.

4. Mesnage R, Renney G, Séralini GE, Ward M, Antoniou MN. Multiomics reveal nonalcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide. *Sci Rep*. 2017;7:39328.

5. Barrett-Connor E. The Rancho Bernardo Study. *Glob Heart*. 2013;8(2).

6. Niemann L, Sieke C, Pfeil R, Solecki R. A critical review of glyphosate findings in human urine samples and comparison with the exposure of operators and consumers. *J Fur Verbraucherschutz Leb.* 2015;10:3-10. https://link.springer.com/article/10.1007/s00003-014-0927-3. Accessed September 13, 2017.

COMMENT & RESPONSE

Prediabetes Prevalence in China

To the Editor Mr Wang and colleagues[1] estimated that the overall prevalence of prediabetes was 35.7% among Chinese adults in 2013 and concluded that the difference from previous estimates may be due to an alternate method of measuring hemoglobin A_{1c} (HbA_{1c}). The previous estimate by Xu et al[2] in 2010 was 50.1% (52.1% in men and 48.1% in women).

However, the study by Wang and colleagues might still have overestimated the prevalence of prediabetes in China. In a nationally representative sample of 46 239 adults in 2008, the prevalence of prediabetes was 15.5% (16.1% among men and 14.9% among women).[3] In contrast, the prevalence of diabetes in the 2008 study was 9.8%, which is close to the 10.2% in the current study. Wang and colleagues used HbA_{1c} levels as the criterion for prediabetes. Although the increased prediabetes prevalence could be due to improved sensitivity of screening with HbA_{1c} measurement, the increase from 15.5% in 2008 to 35.7% in 2013 is suspect.

Wang and colleagues used HbA_{1c} levels of 5.7% to 6.4% to diagnose prediabetes, as recommended in the United States. However, this criterion, developed in the US population, has not been validated in a Chinese population. In addition, a study on the relationship between HbA_{1c} level and the oral glucose tolerance test among Chinese adults found that the accuracy of HbA_{1c} measurement for detecting diabetes defined with an