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Article

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Glyphosate Use and Cancer Incidence in the Agricultural Health Study

Gabriella Andreotti, Stella Koutros, Jonathan N. Hofmann, Dale P. Sandler, Jay H. Lubin, Charles F. Lynch, Catherine C. Lerro, Anneclaire J. De Roos, Christine G. Parks, Michael C. Alavanja, Debra T. Silverman, Laura E. Beane Freeman

Affiliations of authors: Occupational and Environmental Epidemiology Branch (GA, SK, JNH, CCL, DTS, LEBF), Biostatistics Branch (JHL), and Formerly of Occupational and Environmental Epidemiology Branch (MCA), Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, MD; Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC (DPS, CGP); Department of Epidemiology, University of Iowa, Iowa City, IA (CFL); State Health Registry of Iowa, Iowa City, IA (CFL); Department of Environmental and Occupational Health, Drexel University Dornsife School of Public Health, Philadelphia, PA (AJDR)

Correspondence to: Laura Beane Freeman, PhD, 9609 Medical Center Drive, Rm 6E136, MSC 9771, Bethesda, MD 20892 (e-mail: freemala@mail.nih.gov).

Abstract

Background: Glyphosate is the most commonly used herbicide worldwide, with both residential and agricultural uses. In 2015, the International Agency for Research on Cancer classified glyphosate as "probably carcinogenic to humans," noting strong mechanistic evidence and positive associations for non-Hodgkin lymphoma (NHL) in some epidemiologic studies. A previous evaluation in the Agricultural Health Study (AHS) with follow-up through 2001 found no statistically significant associations with glyphosate use and cancer at any site.

Methods: The AHS is a prospective cohort of licensed pesticide applicators from North Carolina and Iowa. Here, we updated the previous evaluation of glyphosate with cancer incidence from registry linkages through 2012 (North Carolina)/2013 (Iowa). Lifetime days and intensity-weighted lifetime days of glyphosate use were based on self-reported information from enrollment (1993–1997) and follow-up questionnaires (1999–2005). We estimated incidence rate ratios (RRs) and 95% confidence intervals (CIs) using Poisson regression, controlling for potential confounders, including use of other pesticides. All statistical tests were two-sided.

Results: Among 54 251 applicators, 44 932 (82.8%) used glyphosate, including 5779 incident cancer cases (79.3% of all cases). In unlagged analyses, glyphosate was not statistically significantly associated with cancer at any site. However, among applicators in the highest exposure quartile, there was an increased risk of acute myeloid leukemia (AML) compared with never users (RR = 2.44, 95% CI = 0.94 to 6.32, $P_{trend} = .11$), though this association was not statistically significant. Results for AML were similar with a five-year ($RR_{Quartile\ 4} = 2.32$, 95% CI = 0.98 to 5.51, $P_{trend} = .07$) and 20-year exposure lag ($RR_{Tertile\ 3} = 2.04$, 95% CI = 1.05 to 3.97, $P_{trend} = .04$).

Conclusions: In this large, prospective cohort study, no association was apparent between glyphosate and any solid tumors or lymphoid malignancies overall, including NHL and its subtypes. There was some evidence of increased risk of AML among the highest exposed group that requires confirmation.

Glyphosate was introduced as a broad-spectrum herbicide in 1974, and it quickly became one of the most heavily used herbicides worldwide. With the introduction of genetically engineered glyphosate-tolerant crops, glyphosate use increased dramatically in the late-1990s and 2000s. In addition to agricultural uses, glyphosate is one of the most common residential

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herbicides in the United States. As of 2010, more than 750 products containing glyphosate were on the US market, and it was registered for use in more than 130 countries (2).

Glyphosate is an organophosphorus compound that interferes with the synthesis of aromatic amino acids by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, which is responsible for biosynthesis of the aromatic amino acids phenylalanine, tyrosine, and tryptophan via the shikimate pathway, a mechanism specific to plants. In its 1993 re-registration decision, the US Environmental Protection Agency determined that there were no "unreasonable risks or adverse effects to humans or the environment" and indicated that all uses were eligible for registration (3). However, concerns about glyphosate's possible effects on human health have persisted. In 2015, the International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic to humans." The IARC Working Group cited sufficient evidence for the carcinogenicity of glyphosate in experimental animals, as well as strong evidence that exposure to glyphosate is genotoxic and can induce oxidative stress in experimental animals and in humans in vitro. In addition, they reported limited evidence in humans, noting increased risk of non-Hodgkin lymphoma (NHL) in some epidemiologic studies (4).

In 2005, an evaluation of glyphosate and cancer risk was conducted in the Agricultural Health Study (AHS) (5). This evaluation considered glyphosate use reported at enrollment (1993–1997), and included 2088 cancers diagnosed between enrollment and 2001. No statistically significant associations were found for any cancer sites, including NHL, but there was an increased risk, though not statistically significant, of multiple myeloma in the highest exposure category based on a small number of cases. Here we have updated this early report, extending cancer incidence follow-up through 2012 (North Carolina)/2013 (Iowa) with 7290 incident cancer cases, and included additional exposure information from a follow-up questionnaire.

Methods

Study Design

The Agricultural Health Study (AHS) is a prospective cohort of licensed pesticide applicators enrolled in Iowa or North Carolina, which has been described elsewhere (6). Briefly, 57 310 individuals seeking licenses to apply restricted-use pesticides were enrolled between 1993 and 1997. Of the enrolled participants, 63% completed a follow-up phone interview approximately five years after enrollment (1999–2005). The study questionnaires are available on the AHS website (www.aghealth.nih.gov/collaboration/questionnaires.html). Incident cancer diagnoses were ascertained via linkage to cancer registries in Iowa (enrollment through 2013) and North Carolina (enrollment through 2012). Cancer diagnoses were classified according the International Classification of Disease–Oncology, 3rd Revision (7). Subtypes of lymphoid malignancies were defined according to the Surveillance, Epidemiology, and End Results Program Lymphoma Subtype Recodes (http://seer.cancer.gov/lymphomarecode/lymphoma-orig.html). Vital status was ascertained via state mortality registries and the National Death Index, and state of residence was regularly updated. The study has been approved by the Special Studies Institutional Review Board of the National Cancer Institute, and relevant contractors.

Exposure Assessment

Lifetime use of glyphosate and 49 other pesticides was ascertained at enrollment and in the follow-up questionnaire. At enrollment, applicators reported the number of years and days per year each pesticide was used, while at follow-up applicators reported the number of days each pesticide was used in the most recent year farmed. Using this information, three metrics of cumulative lifetime exposure were created for each pesticide: ever/never use, lifetime days of use (days per year $\times$ number of years), and intensity-weighted lifetime days (lifetime days $\times$ intensity score). The intensity score was derived from an algorithm based on literature-based measurements and information provided by the applicator, specifically whether the participant mixed or applied pesticides, repaired pesticide-related equipment, used personal protective equipment, and application method used (8). For participants who did not complete the follow-up questionnaire (37%), a data-driven multiple imputation procedure was used to impute pesticide use since enrollment (9). Factors used to impute pesticide use included demographic data and medical history, as well as factors related to farm characteristics and reported pesticide use at enrollment. Primary results presented here use both self-reported and imputed data to calculate glyphosate exposure metrics.

Statistical Analysis

For this analysis, we excluded individuals who had a history of cancer at enrollment (n = 1096), did not live in North Carolina or Iowa (n = 343), or did not report information regarding glyphosate use at enrollment (n = 1620), resulting in an analytic sample of 54 251 applicators. Individuals accumulated person-time from enrollment until the earliest of the following events: movement out of state, diagnosis of cancer, death, or end of the follow-up period (December 31, 2012 in NC, December 31, 2013 in IA). We used Poisson regression to calculate incidence rate ratios (RRs) and 95% confidence intervals (CIs), and PROC MIANALYZE to obtain the appropriate variance for the imputed data (SAS v.9.3, SAS Institute Inc., Cary, NC). All tests were two-sided and considered to be statistically significant at an α of .05. Risks for total cancer and for cancer sites with at least 20 exposed cases were evaluated. Based on the distribution among all cancer cases, we categorized cumulative lifetime days and intensity-weighted lifetime days of glyphosate exposure into quartiles, tertiles, or the median, such that there were at least five exposed cases in each category. The categories for lifetime days of glyphosate use are as follows: quartiles: 1–13.74, 13.75–38.74, 38.75–108.4, $\geq$108.5; tertiles: 1–19.9, 20.0–61.9, $\geq$62.0; median: 1–38.74, $\geq$38.75. Using the Wald test, linear trend was evaluated using the median of each exposure category as a continuous variable. Risk estimates were adjusted for attained age (continuous), cigarette smoking status (never, former, current), alcohol drinks per month (none, <7 per month, $\geq$7 per month), family history of any cancer (yes, no), state of recruitment (North Carolina, Iowa), and the five pesticides most highly correlated with glyphosate based on lifetime days and intensity-weighted lifetime days ($r > 0.4$: atrazine, alachlor, metolachlor, trifluralin, 2,4-D). We also evaluated lagged exposure. We calculated cumulative exposure for each year of follow-up until cancer diagnosis, death, movement out of state, or end of cohort cancer incidence follow-up; we then subtracted the lag interval of 5, 10, 15, or 20 years. We evaluated other potential confounding factors, including body mass index (BMI; <25, 25–<30, $\geq$30 kg/m^2) and pack-years of cigarettes smoked (tertiles of use among former and current smokers). The number of women and nonwhites was small, precluding adjustment for sex and race for most cancer sites; in sensitivity analyses,

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we assessed the risks in men and whites alone. For lymphohematopoietic cancers, we additionally adjusted for occupational exposure to solvents, gasoline, x-ray radiation, and engine exhaust, and pesticides linked to lymphohematopoietic malignancies in previous AHS analyses (lindane, DDT, diazinon, terbufos, and permethrin) (10,11). We also calculated the risk of NHL excluding multiple myeloma for comparison with previously published studies.

In addition, we conducted sensitivity analyses to evaluate the impact of including additional exposure information. First, we calculated risk estimates including cancer incidence data for the complete follow-up period with only exposure information collected at enrollment. Second, we examined associations excluding imputed exposure data, thereby limiting analyses to participants who completed both the enrollment and follow-up questionnaires. Finally, because the last exposure information was collected between 1999 and 2005, we truncated follow-up at 2005 to coincide with this exposure period.

Results

Among 54 251 participants, 44 932 (82.8%) reported ever using glyphosate at enrollment or follow-up. Among the participants who used glyphosate, the median lifetime days of use was 48 (interquartile range [IQR] = 20–166 days), and the median lifetime years of use was 8.5 (IQR = 5–14 years). A total of 7290 incident cancers were diagnosed during the follow-up period. Among the participants who used glyphosate and were diagnosed with cancer during follow-up (n = 5779), the median lifetime days of use was 38.75 (IQR = 13.75–108.5 days), and the median lifetime years of use was 8.0 (IQR = 3.5–13.0). Selected characteristics of the study participants by glyphosate use are presented in Table 1. Those with the median or greater lifetime days of glyphosate use were younger and more likely to be male and NC residents than those with less than the median lifetime days of use and never users of glyphosate. Also, those with higher use were more likely to have a higher education level, drink alcohol more frequently, and have a family history of cancer.

Risk ratios for unlagged intensity-weighted lifetime days of glyphosate use and cancer risk are shown in Table 2. Glyphosate use was not associated with total cancer ($RR_{Quartile\ 4}$ = 0.99, 95% CI = 0.91 to 1.08, P_{trend} = .91) or with lymphohematopoietic malignancies (n = 543 exposed cases; $RR_{Quartile\ 4}$ = 1.00, 95% CI = 0.74 to 1.34, P_{trend} = .43). There also was no evidence for associations with NHL or any NHL subtypes. The rate ratio in the top exposure quartile was 0.87 for NHL (n = 440 exposed cases; 95% CI = 0.64 to 1.20, P_{trend} = .95) and 0.87 for multiple myeloma (n = 88 exposed cases; 95% CI = 0.45 to 1.69, P_{trend} = .84). The association for NHL was not meaningfully changed when multiple myeloma was excluded (RR = 0.85, 95% CI = 0.62 to 1.18, P_{trend} = .94; data not shown). Although not statistically significant, we observed an increased risk of acute myeloid leukemia (AML; n = 57 exposed cases) among applicators in the highest quartile of intensity-weighted glyphosate use compared with never users (RR = 2.44, 95% CI = 0.94 to 6.32, P_{trend} = .11). These findings were unchanged in sensitivity analyses, including further adjustment for additional potential confounders, or by exclusion of women and nonwhites. Results based on lifetime days of glyphosate use and cancer risk were similar to the results for intensity-weighted lifetime days (Supplementary Table 1, available online).

We evaluated the impact of lagging exposure on risk estimates for lymphohematopoietic cancers. Results for five- and 20-year lags are presented in Table 3. Overall, the patterns of risk for lagged exposures were similar to those for unlagged exposures. For all lymphohematopoietic cancers, the rate ratio in the highest quartile of intensity-weighted lifetime days of

Table 1. Selected characteristics of the Agricultural Health Study population by glyphosate use

Characteristics*	Never-used glyphosate No. (%)	Lifetime days of glyphosate use†	
		< Median No. (%)	≥ Median No. (%)
Total	9319 (100.0)	19 714 (100.0)	24 727 (100.0)
Age at enrollment, y			
<30	814 (8.7)	1726 (8.8)	2372 (9.6)
30–39	1730 (18.6)	4293 (21.8)	6612 (26.7)
40–49	2217 (23.8)	5304 (26.9)	7437 (30.1)
50–59	2051 (22.0)	4261 (21.6)	4759 (19.2)
60–69	1797 (19.3)	3043 (15.4)	2738 (11.1)
70+	710 (7.6)	1087 (5.5)	809 (3.3)
Sex			
Male	8887 (95.4)	19 220 (97.5)	24 203 (97.9)
Female	432 (4.6)	494 (2.5)	524 (2.1)
Race			
White	8838 (94.8)	19 128 (97.0)	24 267 (98.1)
Black and other	441 (4.7)	538 (2.7)	404 (1.6)
Missing	40 (0.4)	48 (0.2)	56 (0.2)
State of recruitment			
Iowa	6692 (71.8)	12 668 (64.3)	15 756 (63.7)
North Carolina	2627 (28.2)	7046 (35.7)	8971 (36.3)
Applicator type			
Private (farmer)	8476 (91.0)	18 717 (94.9)	21 932 (88.7)
Commercial	843 (9.0)	997 (5.1)	2795 (11.3)
Highest level of education			
High school or less	6528 (70.1)	11 409 (57.9)	12 005 (48.6)
Beyond high school	2569 (27.6)	7884 (40.0)	12 213 (49.2)
Missing	222 (2.4)	421 (2.1)	509 (2.1)
Body mass index, kg/m^2			
<25	1656 (17.8)	3779 (19.2)	4168 (16.9)
25–<30	3044 (32.7)	7123 (36.1)	8492 (34.3)
30+	1435 (15.4)	3175 (16.1)	3985 (16.1)
Missing	3184 (34.2)	5637 (28.6)	8082 (32.7)
Cigarette smoking status			
Never	4987 (53.5)	10 371 (52.6)	12 876 (52.1)
Former	2621 (28.1)	6004 (30.5)	7295 (29.5)
Current	1526 (16.4)	3147 (16.0)	4355 (17.6)
Missing	185 (2.0)	192 (1.0)	201 (0.8)
Cigarette smoking pack-years			
Never	4987 (53.5)	10 371 (52.6)	12 876 (52.1)
Former, tertile 1	896 (9.6)	2004 (10.2)	2471 (10.0)
Former, tertile 2	791 (8.5)	1865 (9.5)	2198 (8.9)
Former, tertile 3	741 (8.0)	1748 (8.9)	2109 (8.5)
Current, tertile 1	548 (5.9)	1037 (5.3)	1513 (6.1)
Current, tertile 2	453 (4.9)	975 (4.9)	1399 (5.7)
Current, tertile 3	461 (4.9)	1076 (5.5)	1376 (5.6)
Missing	442 (4.7)	638 (3.2)	785 (3.2)
Usual number of alcohol drinks per month in year prior enrollment			
Never	3150 (33.8)	6406 (32.5)	6946 (28.1)
≤6/mo	3036 (32.6)	6646 (33.7)	8240 (33.3)
≥7/mo	2492 (26.7)	5631 (28.6)	8646 (35.0)
Missing	641 (6.9)	1030 (5.2)	895 (3.6)

(continued)

Table 1. (continued)

Characteristics*	Never-used glyphosate No. (%)	Lifetime days of glyphosate use† < Median No. (%)	Lifetime days of glyphosate use† ≥ Median No. (%)
Family history of cancer			
No	5452 (58.5)	10 846 (55.0)	13 866 (56.1)
Yes	3226 (34.6)	7700 (39.1)	9876 (39.9)
Missing	641 (6.9)	1168 (5.9)	985 (4.0)

*Data from the enrollment questionnaire.
†Based on median cumulative lifetime days of glyphosate use among all cancer cases (38.75 days)

glyphosate was 1.00 (95% CI = 0.77 to 1.31, P_{trend} = .43) for the five-year lagged exposure (n = 524 exposed cases) and 1.14 (95% CI = 0.87 to 1.50, P_{trend} = .37) for the 20-year lagged exposure (n = 270 exposed cases). For total NHL, the rate ratio in the highest quartile was 0.87 for the five-year lagged exposure (95% CI = 0.64 to 1.17, P_{trend} = .76) with 425 exposed cases and 1.12 for the 20-year lagged exposure (95% CI = 0.83 to 1.51, P_{trend} = .62) with 221 exposed cases. For AML, the rate ratio in the highest quartile of exposure was 2.32 (95% CI = 0.98 to 5.51, P_{trend} = .07) with a five-year lag (n = 56 exposed cases). The rate ratio was elevated and the trend statistically significant with a 20-year lag and tertiles of exposure (to satisfy our reporting criteria due to a smaller number of exposed cases; n = 32 exposed cases; RR = 2.04, 95% CI = 1.05 to 3.97, P_{trend} = .04). The risk estimates for lymphohematopoietic cancers and intensity-weighted lifetime days of glyphosate lagged by 10 and 15 years were similar to the other lagged results (Supplementary Table 2, available online). Also, the risk estimates for lymphohematopoietic cancers with lagged lifetime days of glyphosate use were similar to the unlagged results (Supplementary Table 3, available online).

In primary analyses, we included exposure information reported at two time points, at enrollment and at a follow-up interview five years later. Among the 54 251 applicators in this analysis, 44 932 (82.8%) reported ever using glyphosate, with 40 987 (75.6%) reporting use prior to enrollment. We conducted several sensitivity analyses evaluating the impact of including exposure data obtained at the two time points. When restricted to exposure reported at enrollment, the patterns of risk were the same as analyses that considered glyphosate use reported at enrollment and follow-up. For example, when using only exposure information reported at enrollment, the rate ratio in the highest exposure quartile was 0.82 (95% CI = 0.62 to 1.80, P_{trend} = .82) for NHL (n = 428 exposed cases) and 2.62 (95% CI = 1.14 to 6.07, P_{trend} = .03) for AML (n = 61 exposed cases; data not shown). To evaluate the impact of using imputed exposure data for participants who did not complete the follow-up questionnaire, we limited the analysis to 34 698 participants who completed both questionnaires, reducing the total number of cancer cases to 4699. Glyphosate use was not associated with NHL (n = 306 total cases; $RR_{Quartile\ 4}$ = 0.90, 95% CI = 0.63 to 1.27, P_{trend} = .54), and there was a non–statistically significantly elevated risk for AML (n = 35 exposed cases; $RR_{Tertile3}$ = 2.64, 95% CI = 0.78 to 6.86, P_{trend} = .18; data not shown). Finally, when we truncated the follow-up period to 2005 to be concurrent with the latest exposure information, we had even fewer total cancer cases (n = 2588 exposed cases). For NHL (n = 193 exposed cases), the $RR_{Quartile4}$ was 1.04 (95% CI = 0.70 to 1.57, P_{trend} = .83); for AML (n = 26 exposed cases), the $RR_{Tertile3}$ was 1.56 (95% CI = 0.44 to 5.57, P_{trend} = .49; data not shown).

Table 2. Cancer incidence in relation to intensity-weighted lifetime days of glyphosate use in the Agricultural Health Study

Cancer site*	Glyphosate use†	No.	RR (95% CI)‡	P_{trend}‡
All cancers				
	None	1511	1.00 (reference)	
	Q1	1445	0.99 (0.91 to 1.07)	
	Q2	1443	0.99 (0.91 to 1.07)	
	Q3	1440	1.04 (0.96 to 1.13)	
	Q4	1451	0.99 (0.91 to 1.08)	.91
Oral cavity				
	None	33	1.00 (reference)	
	Q1	36	0.95 (0.56 to 1.60)	
	Q2	35	0.92 (0.54 to 1.57)	
	Q3	35	0.96 (0.56 to 1.65)	
	Q4	35	0.84 (0.48 to 1.46)	.54
Colon				
	None	116	1.00 (reference)	
	Q1	104	1.00 (0.74 to 1.35)	
	Q2	102	1.03 (0.76 to 1.39)	
	Q3	102	1.06 (0.78 to 1.44)	
	Q4	96	1.01 (0.74 to 1.38)	1.00
Rectum				
	None	50	1.00 (reference)	
	Q1	43	0.81 (0.51 to 1.28)	
	Q2	55	1.16 (0.76 to 1.76)	
	Q3	39	0.80 (0.50 to 1.29)	
	Q4	46	0.84 (0.52 to 1.34)	.43
Pancreas				
	None	25	1.00 (reference)	
	Q1	42	1.80 (1.05 to 3.08)	
	Q2	42	1.69 (0.98 to 2.94)	
	Q3	24	1.09 (0.59 to 2.02)	
	Q4	23	1.06 (0.57 to 1.97)	.14
Lung				
	None	144	1.00 (reference)	
	Q1	117	0.92 (0.70 to 1.22)	
	Q2	138	1.19 (0.91 to 1.56)	
	Q3	159	1.39 (1.07 to 1.82)	
	Q4	131	1.00 (0.76 to 1.33)	.78
Melanoma				
	None	56	1.00 (reference)	
	Q1	59	1.00 (0.67 to 1.50)	
	Q2	67	1.18 (0.80 to 1.74)	
	Q3	69	1.12 (0.75 to 1.67)	
	Q4	78	1.17 (0.78 to 1.74)	.53
Prostate				
	None	579	1.00 (reference)	
	Q1	571	0.99 (0.87 to 1.12)	
	Q2	564	0.95 (0.83 to 1.08)	
	Q3	559	1.03 (0.91 to 1.18)	
	Q4	571	0.99 (0.86 to 1.13)	.89
Testicular				
	None	7	1.00 (reference)	
	T1	17	1.28 (0.49 to 3.34)	
	T2	12	0.74 (0.26 to 2.09)	
	T3	11	0.57 (0.20 to 1.67)	.07
Bladder				
	None	66	1.00 (reference)	
	Q1	86	1.29 (0.91 to 1.82)	
	Q2	68	1.04 (0.72 to 1.51)	
	Q3	66	1.09 (0.75 to 1.59)	
	Q4	79	1.26 (0.87 to 1.82)	.42

(continued)

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Table 2. (continued)

Cancer site*	Glyphosate use†	No.	RR (95% CI)‡	P_{trend}‡
Kidney				
	None	54	1.00 (reference)	
	Q1	54	1.13 (0.74 to 1.71)	
	Q2	50	0.91 (0.59 to 1.41)	
	Q3	45	0.87 (0.55 to 1.38)	
	Q4	53	1.03 (0.66 to 1.61)	.95
Lymphohematopoietic				
	None	161	1.00 (reference)	
	Q1	136	0.87 (0.64 to 1.19)	
	Q2	126	0.88 (0.66 to 1.17)	
	Q3	137	0.93 (0.71 to 1.23)	
	Q4	144	1.00 (0.74 to 1.34)	.43
Hodgkin lymphoma				
	None	7	1.00 (reference)	
	M1	7	0.59 (0.17 to 2.11)	
	M2	11	0.90 (0.25 to 3.24)	.94
Non-Hodgkin lymphoma				
	None	135	1.00 (reference)	
	Q1	113	0.83 (0.59 to 1.18)	
	Q2	104	0.83 (0.61 to 1.12)	
	Q3	112	0.88 (0.65 to 1.19)	
	Q4	111	0.87 (0.64 to 1.20)	.95
Non-Hodgkin lymphoma B cell				
	None	128	1.00 (reference)	
	Q1	102	0.79 (0.55 to 1.13)	
	Q2	93	0.76 (0.56 to 1.05)	
	Q3	106	0.88 (0.64 to 1.21)	
	Q4	103	0.86 (0.62 to 1.19)	.86
Chronic lymphocytic lymphoma, small lymphocytic leukemia				
	None	36	1.00 (reference)	
	Q1	28	0.75 (0.40 to 1.41)	
	Q2	26	0.76 (0.41 to 1.41)	
	Q3	26	0.90 (0.50 to 1.62)	
	Q4	27	0.87 (0.48 to 1.58)	.71
Diffuse large B cell lymphoma				
	None	27	1.00 (reference)	
	Q1	28	1.11 (0.60 to 2.07)	
	Q2	23	0.94 (0.49 to 1.80)	
	Q3	30	1.13 (0.59 to 2.17)	
	Q4	22	0.97 (0.51 to 1.85)	.83
Marginal-zone lymphoma				
	None	4	1.00 (reference)	
	M1	6	0.39 (0.06 to 2.45)	
	M2	5	0.44 (0.09 to 2.17)	.67
Follicular lymphoma				
	None	16	1.00 (reference)	
	T1	21	0.89 (0.37 to 2.15)	
	T2	11	0.61 (0.23 to 1.60)	
	T3	20	0.85 (0.36 to 2.03)	.95
Multiple myeloma				
	None	30	1.00 (reference)	
	Q1	19	0.70 (0.36 to 1.36)	
	Q2	26	0.94 (0.50 to 1.76)	
	Q3	19	0.78 (0.39 to 1.56)	
	Q4	24	0.87 (0.45 to 1.69)	.84
Non-Hodgkin lymphoma T cell				
	None	2	1.00 (reference)	
	M1	14	4.25 (0.73 to 24.64)	
	M2	6	1.53 (0.23 to 10.38)	.31
Acute myeloid leukemia				
	None	9	1.00 (reference)	
	Q1	13	1.62 (0.60 to 4.38)	
	Q2	14	1.70 (0.61 to 4.73)	
	Q3	12	1.46 (0.49 to 4.37)	
	Q4	18	2.44 (0.94 to 6.32)	.11
Chronic myeloid leukemia				
	None	7	1.00 (reference)	
	M1	5	0.36 (0.09 to 1.43)	
	M2	11	0.82 (0.23 to 2.98)	.36

*Cancer sites are based and presented in order of Surveillance, Epidemiology, and End Results Site Recode ICD-O-3. CI = confidence interval; RR = rate ratio.
†Quartiles: Q1: 1–598.9; Q2: 599–1649.9; Q3: 1650–4339.9; Q4: ≥4340.0. Tertiles: T1: 1–866.24; T2: 866.25–2963.9; T3: ≥2964.0. Median: M1: 1–1649.9; M2: ≥1650.0.
‡Poisson regression was used to model rate ratios and confidence intervals, and P values were calculated using a two-sided Wald test. All models adjusted for age, state of recruitment, education, cigarette smoking status, alcohol per month, family history of cancer, atrazine, alachlor, metolachlor, trifluralin, 2,4-D.

Discussion

In this updated evaluation of glyphosate use and cancer risk in a large prospective study of pesticide applicators, we observed no associations between glyphosate use and overall cancer risk or with total lymphohematopoietic cancers, including NHL and multiple myeloma. However, there was some evidence of an increased risk of AML for applicators, particularly in the highest category of glyphosate exposure compared with never users of glyphosate.

Like other hematological malignancies, AML is thought to result from multiple genetic and environmental factors (12). Occupational farming and general pesticide exposure have long been linked to leukemia (13). In 2007, a meta-analysis of occupational pesticide exposure found a statistically significant risk of AML when restricting to cohort studies (meta RR = 1.55, 95% CI = 1.02 to 2.34) (14), although specific chemicals were not evaluated. One case–control study that evaluated glyphosate use found no evidence of an association with leukemia overall based on 15 exposed cases and did not report results for AML (15). Similarly, in the previous AHS analysis, there was no association with leukemia overall based on 32 exposed cases, and AML was not evaluated (5). To our knowledge, our study is the first to report a possible association between glyphosate use and AML.

Risk estimates were similar in magnitude between the unlagged and lagged exposure analyses for all sites evaluated. For AML, there were elevated risks in the highest exposure categories, and statistically significant or borderline significant tests of trend for unlagged and lagged analyses. The latent period between relevant exposure and AML diagnosis is unknown, and it may vary by type of exposure and population characteristics (12). Most studies of established AML risk factors, such as benzene, suggest a relatively short latency period (less than five years) (16), as do studies of therapy-induced AML (five to seven years) (17). Long-term studies of radiation-exposed populations have reported elevated risks of AML up to 55 years after exposure (18).

The IARC Working Group noted strong evidence of genotoxicity and oxidative stress effects from glyphosate exposure (4). In particular, they highlighted two studies in communities exposed to glyphosate through aerial spraying that showed

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Table 3. Cancer incidence in relation to lagged intensity weighted lifetime days of glyphosate use in the Agricultural Health Study

		5-y lag			20-y lag		
Cancer sites*	Glyphosate use†	No. of cases	RR (95% CI)‡	P_{trend}‡	No. of cases	RR (95% CI)‡	P_{trend}‡
Lymphohematopoietic							
	None	180	1.00 (reference)		434	1.00 (reference)	
	Q1	133	0.92 (0.69 to 1.24)		73	1.19 (0.90 to 1.55)	
	Q2	114	0.85 (0.65 to 1.12)		61	1.07 (0.81 to 1.41)	
	Q3	142	1.05 (0.79 to 1.39)		66	1.14 (0.87 to 1.51)	
	Q4	135	1.00 (0.77 to 1.31)	.43	70	1.14 (0.87 to 1.50)	.37
Hodgkin lymphoma							
	None	9	1.00 (reference)		17	1.00 (reference)	
	M1	7	0.48 (0.17 to 1.33)		4	1.51 (0.48 to 4.70)	
	M2	9	0.60 (0.21 to 1.69)	.73	4	1.27 (0.35 to 4.62)	.82
Non-Hodgkin lymphoma							
	None	150	1.00 (reference)		354	1.00 (reference)	
	Q1	113	0.92 (0.66 to 1.28)		63	1.22 (0.91 to 1.64)	
	Q2	92	0.79 (0.59 to 1.06)		55	1.15 (0.86 to 1.55)	
	Q3	119	1.03 (0.75 to 1.41)		48	0.98 (0.71 to 1.36)	
	Q4	101	0.87 (0.64 to 1.17)	.76	55	1.12 (0.83 to 1.51)	.62
Non-Hodgkin lymphoma B cell							
	None	141	1.00 (reference)		326	1.00 (reference)	
	Q1	100	0.87 (0.61 to 1.22)		57	1.17 (0.86 to 1.60)	
	Q2	85	0.76 (0.56 to 1.03)		49	1.15 (0.85 to 1.57)	
	Q3	112	1.05 (0.75 to 1.45)		48	1.06 (0.76 to 1.47)	
	Q4	94	0.86 (0.63 to 1.18)	.93	52	1.14 (0.84 to 1.55)	.49
Chronic lymphocytic leukemia, small lymphocytic lymphoma							
	None	42	1.00 (reference)		88	1.00 (reference)	
	Q1	26	0.85 (0.47 to 1.56)		15	1.18 (0.65 to 2.15)	
	Q2	22	0.72 (0.40 to 1.32)		13	1.16 (0.63 to 2.11)	
	Q3	32	1.13 (0.64 to 2.00)		14	1.25 (0.68 to 2.29)	
	Q4	21	0.74 (0.40 to 1.35)	.61	13	1.19 (0.65 to 2.18)	.60
Diffuse large B cell lymphoma							
	None	31	1.00 (reference)		80	1.00 (reference)	
	Q1	26	1.09 (0.61 to 1.94)		11	0.89 (0.44 to 1.80)	
	Q2	25	0.99 (0.53 to 1.84)		13	1.24 (0.68 to 2.26)	
	Q3	25	1.03 (0.55 to 1.92)		11	0.90 (0.44 to 1.81)	
	Q4	23	1.02 (0.55 to 1.89)	.90	15	1.35 (0.76 to 2.41)	.31
Marginal-zone lymphoma							
	None	4	1.00 (reference)		10	1.00 (reference)	
	M1	6	0.48 (0.11 to 2.01)		2	0.77 (0.16 to 3.73)	
	M2	5	0.57 (0.13 to 2.40)	.80	3	1.16 (0.29 to 4.65)	.78
Follicular lymphoma							
	None	16	1.00 (reference)		41	1.00 (reference)	
	T1	22	1.19 (0.58 to 2.45)		10	1.11 (0.49 to 2.51)	
	T2	11	0.62 (0.26 to 1.47)		9	1.35 (0.64 to 2.86)	
	T3	19	1.03 (0.47 to 2.25)	.96	8	0.90 (0.37 to 2.19)	.82
Multiple myeloma							
	None	33	1.00 (reference)		72	1.00 (reference)	
	Q1	19	0.70 (0.36 to 1.33)		13	1.36 (0.74 to 2.53)	
	Q2	21	0.80 (0.43 to 1.48)		14	1.51 (0.84 to 2.69)	
	Q3	25	1.06 (0.57 to 1.95)		9	0.89 (0.43 to 1.87)	
	Q4	20	0.74 (0.38 to 1.44)	.82	10	0.96 (0.48 to 1.91)	.69
Non-Hodgkin lymphoma T cell							
	None	4	1.00 (reference)		12	1.00 (reference)	
	M1	12	1.86 (0.57 to 6.03)		9	2.97 (1.20 to 7.31)	–
	M2	6	0.96 (0.25 to 3.72)	.36	1	–	
Acute myeloid leukemia							
	None	10	1.00 (reference)		34	1.00 (reference)	
	Q1/T1	12	1.35 (0.55 to 3.31)		8	1.26 (0.57 to 2.76)	
	Q2/T2	13	1.59 (0.63 to 4.01)		9	1.33 (0.62 to 2.84)	
	Q3/T3	13	1.47 (0.54 to 3.96)		15	2.04 (1.05 to 3.97)	.04
	Q4	18	2.32 (0.98 to 5.51)	.07	–	–	

(continued)

Table 3. (continued)

Cancer sites*	Glyphosate use†	5-y lag			20-y lag		
		No. of cases	RR (95% CI)‡	P_{trend}‡	No. of cases	RR (95% CI)‡	P_{trend}‡
Chronic myeloid leukemia							
	None	8	1.00 (reference)		16	1.00 (reference)	
	M1	4	0.31 (0.07 to 1.29)		3	0.58 (0.13 to 2.63)	
	M2	11	1.00 (0.32 to 3.18)	.29	4	0.87 (0.24 to 3.23)	.91

*Cancer sites are based and presented in order of Surveillance, Epidemiology, and End Results Site Recode ICD-O-3. CI = confidence interval; RR = rate ratio.
†Five-year lag quartiles: Q1: 1–530.9; Q2: 531.0–1511.9; Q3: 1512.0–4063.4; Q4: ≥4063.5. Five-year lag tertiles: T1: 1–787.4; T2: 787.5–2795.9; T3: ≥2796.0. Five-year lag median: M1: 1–1511.9; M2: ≥1512.0. Twenty-year lag quartiles: Q1: 1–281.3; Q2: 281.4–895.9; Q3: 896–2609.9; Q4: ≥2610.0. Twenty-year lag tertiles: T1: 1–409.4; T2: 409.5–1819.9; T3: ≥1820.0. Twenty-year lag median: M1: 1–895.9; M2: ≥896.0.
‡Poisson regression was used to model rate ratios and confidence intervals, and *P* values were calculated using a two-sided Wald test. All models were adjusted for age, state of recruitment, education, cigarette smoking status, alcohol per month, family history of cancer, atrazine, alachlor, metolachlor, trifluralin, 2,4-D.

evidence of DNA damage, including strand breaks (19) and micronuclei (20). A third study, where blood was collected up to two years after putative glyphosate exposure, showed no effects (21). Several in vitro studies also reported genotoxic effects (4). There were no human studies evaluating oxidative stress, but multiple in vitro studies have reported an increase in these markers in a number of different cell types (4).

In our study, we observed no associations between glyphosate use and NHL overall or any of its subtypes. This lack of association was consistent for both exposure metrics, unlagged and lagged analyses, after further adjustment for pesticides linked to NHL in previous AHS analyses, and when we excluded multiple myeloma from the NHL grouping. The lack of association between glyphosate and NHL is also consistent with the previous AHS analysis (5). However, three case–control studies reported increased risks of NHL with glyphosate exposure (22–24). Another study reported a statistically significant association between glyphosate and NHL, but the association was attenuated when controlling for other pesticides (25). Two other case–control studies evaluated glyphosate and NHL risk, but had limited power (n = 12 and n = 4 exposed cases) (26,27). All of these studies, including the AHS, relied on self-reported pesticide use for exposure assessment, and registry- or hospital-based cancer diagnoses. However, some study design differences are important to note. First, the AHS is a prospective cohort study, while the others are case–control studies. While exposure misclassification is possible in all studies, recall bias should not occur in the AHS because pesticide use was ascertained prior to cancer diagnosis. Second, this AHS analysis includes only licensed pesticide applicators who have been shown to reliably report their pesticide use (28,29). In this analysis, we controlled for the use of correlated pesticides, which was not possible in all previous studies. At least one study showed evidence of confounding by the use of other pesticides (25). In our study, controlling for other pesticides did not change the risk estimates. Finally, it is important to note that these studies have been conducted in different time periods; changing agricultural practices, such as pesticide application methods and use of personal protective equipment, may impact actual exposure levels. In addition, if changing product formulations or amounts used are associated with risk, this may also impact results.

The non–statistically significant increase in multiple myeloma noted in the previous AHS analysis (RR = 2.1, 95% CI = 0.6 to 7.0 for the highest intensity-weighted exposure category) was based on 19 exposed cases with a median follow-up of 6.7 years (5). This association was not evident in this update, with an extended follow-up of 17.5 years and 88 exposed cases. Three case–control studies, one in Iowa, (30), one in France (26), and one in Canada (31), also suggested possible positive associations between glyphosate and multiple myeloma. Subsequently, a pooled analysis of the Iowa and Canadian studies reported a null association with multiple myeloma based on 32 exposed cases (odds ratio = 0.94, 95% CI = 0.44 to 1.99 for the highest exposure) (32).

In this prospective cohort study, we expanded a previous analysis of glyphosate use and cancer risk with more than 11 years of additional follow-up and more than four times the number of glyphosate-exposed cancer cases (n = 5779 compared with n = 1324). We also included additional information on pesticide use from a follow-up questionnaire that was administered five years after enrollment and completed by 63% of the participants. We imputed glyphosate exposure information for participants who did not complete the follow-up questionnaire to evaluate cancer risk in the full cohort. Results for the full cohort (including imputed data) were similar to those that did not include imputed data, but only included people who completed the follow-up questionnaire. Finally, we truncated cancer incidence follow-up in 2005 to be concurrent with the last exposure information. Based on 26 exposed cases, the association with AML was attenuated, but still elevated.

This evaluation has some limitations that should be acknowledged. First, despite the specific information provided by the applicators about use of glyphosate, some misclassification of exposure undoubtedly occurred. Given the prospective design, however, any misclassification should be nondifferential and lead to attenuated risk estimates. Second, because we evaluated many cancer sites for potential associations with glyphosate use, we cannot dismiss the possibility that these results were observed by chance, and thus should be interpreted with caution. The fact that no other studies have reported an association between glyphosate and AML risk also calls for cautious interpretation. However, the observed consistent pattern of increasing risk with increasing exposure and the statistically significant trend with lagged exposure of 10 or more years is concerning.

In conclusion, we found no evidence of an association between glyphosate use and risk of any solid tumors or lymphoid malignancies, including NHL and its subtypes. However, we found some evidence of a possible association between glyphosate use and AML. This association was consistent across different exposure metrics and for unlagged and lagged exposure. Given the prevalence of use of this herbicide worldwide, expeditious efforts to replicate these findings are warranted.

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