

Glyphosate use and associations with non-Hodgkin lymphoma major histological sub-types: findings from the North American Pooled Project ¹

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1. *Supplementary files 1 and 2*
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FILE 1: METHODS

Study population

Cases were 19 years of age or older in all studies and controls were selected from the general population in each state/province. Control selection procedures varied by study and included random digit dialing, voters' lists, health insurance records, Medicare listings for those older than 65 years, and state mortality files (deceased cases). Controls were matched to NHL cases in each state/province on the basis of age (± 2 or 5 years). In some states, cases and controls were matched on the additional variables of sex (Nebraska), race (Nebraska), and vital status and year of death for deceased cases (Iowa/Minnesota, Nebraska, Kansas). All studies included men; women were included only in the Nebraska study. Deceased cases and controls were eligible for inclusion in the U.S. studies, but were ineligible for inclusion in the CCSPH. Proxy respondents were used for participants who required assistance due to illness or disability in the Canadian study, and were used for deceased subjects in the U.S. studies. The present analysis used pooled data from both men and women and from alive and deceased NHL cases (N=1,690) and controls (N=5,131).

Data collection

Interviewer-administered questionnaires were conducted by telephone (Kansas and Nebraska) or in person (Iowa/Minnesota) with cases and controls or their proxies (e.g. next of kin) if subjects were deceased or too ill to respond themselves. In Canada, all cases and controls were mailed a questionnaire to complete by themselves (or by their proxies). Participants who indicated that they had used pesticides were subsequently interviewed by telephone for additional details about their pesticide usage. The Canadian postal and telephone questionnaires were modified from the telephone interview questionnaires used in Kansas and Nebraska. The format and content of the questionnaires from all case-control studies were very similar since they shared a common research objective, involved overlapping groups of principal investigators, and were developed during the same time period.

Pesticide exposure classifications were created first using broad categories (e.g. occupations with potential pesticide exposure), then major pesticide classes (e.g. herbicides or insecticides), chemical groups within those classes (e.g. phenoxy herbicides), and finally individual compounds (e.g. glyphosate). For each active ingredient reported, information was collected for agricultural and non-agricultural ever/never use (dichotomous) in all studies. Duration of use (number of years) was collected in studies from Canada, Iowa/Minnesota, and Nebraska. Frequency of personal handling (number of days/year) was collected in studies from Canada and Nebraska. In Kansas, participants were asked an open-ended question to recall the details of their pesticide use, whereas in all other jurisdictions subjects were prompted by a list of chemicals and their trade names. Participants were also asked if they had used any type of personal protective equipment (PPE) in general (Nebraska and Canada) or associated with the use of herbicides (Iowa/Minnesota, and Kansas) or specific individual pesticides (Iowa/Minnesota).

Assessment of glyphosate use

There were 357 participants who had reported that they ever used glyphosate. Seven cases and 21 controls had missing duration information, amounting to approximately 6% (7/109) and 9% (21/237) of cases and controls, respectively, who reportedly ever used glyphosate in states/provinces where duration information was collected. Similarly, 13 cases and 33 controls had missing frequency information, equivalent to nearly 18% (13/74) and 18% (33/182) of cases and controls, respectively, who reportedly ever used glyphosate in locations where frequency data were ascertained. Subjects who reported that they used glyphosate were coded as “ever used” or used/handled for the number of years and days/year that they had reported. In ordinal analyses, increments were selected *a priori* based on the respective distributions of glyphosate duration, frequency, and lifetime-days of use in the NAPP. For comparative purposes, the ordinal duration and frequency of use data were also treated as continuous in additional analyses and associated p-values are reported.

NHL classification

NHL cases in these studies were diagnosed during the 1980s and 1990s. NHL cases were classified in Iowa/Minnesota, and Nebraska according to the Working Formulation (1, 2); in Kansas and Quebec by the International Classification of Diseases for Oncology First Edition (ICD-O-1) (3); and in Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia by ICD-O-2 (4). Multiple myeloma and chronic lymphocytic leukemia were not included in the definition of NHL in these studies, as they are in some current studies. The original histology codes used in each study (led by DDW for the U.S. studies and JJS for the Canadian study) were revisited to classify NHL cases using a single scheme for the NAPP. We used ICD-O-1 to code NHL overall and sub-types in the NAPP since histological sub-types were classified in all jurisdictions according to ICD-O-1. These sub-types were follicular lymphoma (FL), diffuse large B-

cell lymphoma (DLBCL), small lymphocytic lymphoma (SLL), and other sub-types. The “other” category included all cases whose histologies were unknown or could not be classified as one of FL, DLBCL, or SLL. Review by a reference pathologist was conducted on 84% of Canadian cases (5), 87% of Kansas cases (6), and for all interviewed cases in Iowa/Minnesota (7) and Nebraska (8) at the time that the original studies were conducted.

Statistical analyses

History of living or working on a farm or ranch, and diagnosis with select medical conditions related to immune suppression (any allergies, food allergies, drug allergies, asthma, hay fever, mononucleosis, arthritis, or tuberculosis; ever received chemotherapy or radiation) (9-11), were selected *a priori* but were not found to be important confounders in this analysis because these variables did not alter OR^a by more than 10% and they were not significantly associated with NHL in the NAPP as independent variables or collectively as the variable “any medical condition”.

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FILE 2: RESULTS

Sensitivity analyses excluding proxy respondents

Proxy respondents were used for deceased cases and controls (U.S studies only), and for living cases who were too ill to respond themselves (U.S. and Canadian studies). A sensitivity analysis excluding cases and controls with data provided by proxy respondents was performed (Table A1) for comparison with the main analysis presented in Tables 2-5. Similar to results reported for the full dataset, OR estimates tended to be larger from the frequency and lifetime-days exposure metrics as compared to duration of exposure. Adjustment for use of 2,4-D, dicamba and malathion tended to reduce ORs and weaken trends for all metrics of glyphosate use, as with the full dataset.

With the exclusion of proxy respondents, the trends for associations with NHL overall and DLBCL were statistically significant with increased days/year of glyphosate usage (p-trends for $OR^a=0.006$ and 0.02 , respectively), but became statistically non-significant upon adjustment for other pesticides (p-trends for $OR^b=0.3$ and 0.2 , respectively). For SLL, the trend was statistically significant for longer duration of glyphosate use (p-trend for $OR^a=0.05$); this trend diminished upon adjustment for other pesticides (p-trend for $OR^b=0.2$). For ordinal lifetime-days, even after adjustment for use of 2,4-D, dicamba and malathion, the OR and trend tests for SLL were statistically significant ($OR^b=1.08$, 95% CI: 1.01-1.16, p-value=0.03). Compared to the main analysis, trends of increasing odds ratios for SLL in association with longer duration, greater frequency and lifetime-days of categorical glyphosate use were marginally stronger when data from proxy respondents were excluded. This pattern was observed for results both adjusted and unadjusted for other pesticides.

Between-study heterogeneity and differences in odds ratios between NHL major histological sub-types

There was no apparent heterogeneity between the case-control studies comprising the NAPP based on the analyses of ever/never glyphosate use and ORs for NHL overall (OR^a: I²=9%, 95% CI: 0-86%, p=0.35; OR^b: I²=0%, 95% CI: 0-85%, p=0.60).

There were no statistically significant differences between ORs for each NHL sub-type (FL, DLBCL, SLL, and other) in association ever/never glyphosate use when ORs were not adjusted for other pesticide uses (i.e. OR^a).

Table S1: Ever/never, duration, frequency, and lifetime-days of glyphosate use and associations with NHL overall and by major histological sub-types among men and women in the NAPP (proxy respondents excluded)

	Controls (N=3372)	Overall (N=1140)			FL (N=398)			DLBCL (N=384)			SLL (N=131)			Other (N=227)		
		Cases	OR ^a (95% CI)	OR ^b (95% CI)	Cases	OR ^a (95% CI)	OR ^b (95% CI)	Cases	OR ^a (95% CI)	OR ^b (95% CI)	Cases	OR ^a (95% CI)	OR ^b (95% CI)	Cases	OR ^a (95% CI)	OR ^b (95% CI)
Glyphosate used																
Never used	3154	1051	1	1	373	1	1	350	1	1	116	1	1	212	1	1
Ever used	218	89	1.22 (0.92, 1.61)	0.95 (0.69, 1.32)	25	0.93 (0.59, 1.46)	0.66 (0.38, 1.13)	34	1.40 (0.93, 2.11)	1.09 (0.68, 1.76)	15	1.89 (1.03, 3.49)	1.80 (0.86, 3.78)	15	1.13 (0.64, 2.03)	0.96 (0.49, 1.89)
Duration (years used)																
0	2723	977	1	1	348	1	1	319	1	1	110	1	1	200	1	1
>0 and ≤3.5	101	51	1.46 (1.02, 2.10)	1.17 (0.79, 1.74)	13	0.97 (0.53, 1.80)	0.71 (0.36, 1.39)	21	1.83 (1.10, 3.03)	1.50 (0.85, 2.62)	6	1.60 (0.66, 3.87)	1.44 (0.54, 3.81)	11	1.89 (0.96, 3.71)	1.56 (0.74, 3.28)
>3.5	96	33	0.99 (0.65, 1.50)	0.78 (0.49, 1.24)	10	0.85 (0.42, 1.69)	0.61 (0.29, 1.31)	11	0.97 (0.50, 1.88)	0.79 (0.39, 1.62)	8	2.08 (0.92, 4.69)	0.91 (0.76, 4.80)	4	0.65 (0.23, 1.82)	0.54 (0.18, 1.62)
p-trend ^c			0.4	0.5		0.7	0.2		0.4	0.9		0.05	0.2		0.97	0.6
Ordinal I (5 years)	2920	1061	1.13 (0.93, 1.36)	1.03 (0.84, 1.26)	371	0.97 (0.68, 1.38)	0.86 (0.58, 1.29)	351	1.18 (0.91, 1.52)	1.08 (0.82, 1.43)	124	1.38 (0.98, 1.96)	1.31 (0.90, 1.90)	215	1.07 (0.71, 1.60)	0.97 (0.62, 1.52)

p-value ^d			0.2	0.8		0.9	0.5		0.2	0.6		0.07	0.2		0.8	0.9
Frequency (days per year handled)																
0	1961	581	1	1	189	1	1	218	1	1	50	1	1	124	1	1
>0 and ≤2	97	25	0.96 (0.59, 1.55)	0.66 (0.39, 1.12)	7	0.88 (0.38, 2.03)	0.52 (0.21, 1.30)	9	0.87 (0.41, 1.81)	0.61 (0.27, 1.37)	4	1.53 (0.49, 4.82)	1.48 (0.42, 5.20)	5	1.13 (0.42, 3.04)	0.81 (0.28, 2.33)
>2	36	26	2.49 (1.46, 4.25)	1.77 (0.99, 3.17)	7	2.15 (0.91, 5.09)	1.31 (0.50, 3.40)	12	2.92 (1.44, 5.90)	2.22 (1.04, 4.75)	3	2.66 (0.75, 9.46)	2.58 (0.66, 10.10)	4	2.23 (0.74, 6.70)	1.57 (0.49, 5.05)
p-trend ^c			0.006	0.3		0.2	0.9		0.02	0.2		0.1	0.2		0.2	0.6
Ordinal (5 days/year)	2094	632	1.18 (1.00, 1.39)	1.09 (0.91, 1.30)	203	1.21 (0.98, 1.48)	1.10 (0.88, 1.38)	239	1.15 (0.92, 1.43)	1.07 (0.85, 1.36)	57	1.22 (0.92, 1.61)	1.18 (0.88, 1.59)	133	1.02 (0.64, 1.62)	0.91 (0.52, 1.61)
p-value ^d			0.06	0.4		0.08	0.4		0.2	0.6		0.2	0.3		0.9	0.8
Lifetime days (years used*days per year handled)																
0	1964	584	1	1	190	1	1	220	1	1	50	1	1	124	1	1
>0 and ≤7	68	20	1.15 (0.68, 1.95)	0.82 (0.46, 1.44)	6	1.12 (0.46, 2.73)	0.69 (0.27, 1.81)	8	1.15 (0.53, 2.50)	0.81 (0.35, 1.88)	2	1.35 (0.30, 6.06)	1.29 (0.27, 6.27)	4	1.51 (0.51, 4.48)	1.09 (0.35, 3.41)
>7	62	28	1.53 (0.94, 2.49)	1.06 (0.62, 1.81)	7	1.27 (0.55, 2.96)	0.74 (0.29, 1.88)	11	1.48 (0.73, 2.99)	1.08 (0.50, 2.32)	5	2.38 (0.83, 6.83)	2.35 (0.72, 7.63)	5	1.56 (0.57, 4.26)	1.11 (0.37, 3.26)
p-trend ^c			0.09	0.99		0.6	0.4		0.3	0.96		0.1	0.2		0.3	0.8
Ordinal (10 years*days/year)	2094	632	1.06 (1.00, 1.11)	1.04 (0.99, 1.09)	203	1.06 (1.01, 1.12)	1.05 (1.00, 1.11)	239	1.01 (0.93, 1.11)	0.99 (0.90, 1.10)	57	1.09 (1.02, 1.16)	1.08 (1.01, 1.16)	133	1.03 (0.94, 1.13)	1.01 (0.91, 1.12)
p-value ^d			0.04	0.1		0.03	0.08		0.8	0.9		0.01	0.03		0.5	0.9

FL: follicular lymphoma; DLBCL: diffuse large B-cell lymphoma; SLL: small lymphocytic lymphoma

^aORs adjusted for age, sex, state/province, lymphatic or hematopoietic cancer in a first-degree relative, use of any personal protective equipment

^bORs adjusted for age, sex, state/province, lymphatic or hematopoietic cancer in a first-degree relative, use of any personal protective equipment, use of 2,4-D, use of dicamba, use of malathion

^cp-trend values derived from treating duration, frequency, and lifetime days variables as continuous in statistical analyses

^dp-value derived from treating duration, frequency, and lifetime days ordinal variables as continuous in statistical analyses

Note: Data for duration (years) of glyphosate use were not collected in Kansas. Data for frequency (days/year) of glyphosate handling were not collected in Kansas, Iowa, and Minnesota.