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Review

Exposure to glyphosate-based herbicides and risk for non-Hodgkin lymphoma: A meta-analysis and supporting evidence

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ABSTRACT

Glyphosate is the most widely used broad-spectrum systemic herbicide in the world. Recent evaluations of the carcinogenic potential of glyphosate-based herbicides (GBHs) by various regional, national, and international agencies have engendered controversy. We investigated whether there was an association between high cumulative exposures to GBHs and increased risk of non-Hodgkin lymphoma (NHL) in humans. We conducted a new meta-analysis that includes the most recent update of the *Agricultural Health Study* (AHS) cohort published in 2018 along with five case-control studies. Using the highest exposure groups when available in each study, we report the overall meta-relative risk (meta-RR) of NHL in GBH-exposed individuals was increased by 41% (meta-RR = 1.41, 95% confidence interval, CI: 1.13–1.75). For comparison, we also performed a secondary meta-analysis using high-exposure groups with the earlier AHS (2005), and we calculated a meta-RR for NHL of 1.45 (95% CI: 1.11–1.91), which was higher than the meta-RRs reported previously. Multiple sensitivity tests conducted to assess the validity of our findings did not reveal meaningful differences from our primary estimated meta-RR. To contextualize our findings of an increased NHL risk in individuals with high GBH exposure, we reviewed publicly available animal and mechanistic studies related to lymphoma. We documented further support from studies of malignant lymphoma incidence in mice treated with pure glyphosate, as well as potential links between glyphosate / GBH exposure and immunosuppression, endocrine disruption, and genetic alterations that are commonly associated with NHL or lymphomagenesis. Overall, in accordance with findings from experimental animal and mechanistic studies, our current meta-analysis of human epidemiological studies suggests a compelling link between exposures to GBHs and increased risk for NHL.

1. Background

1.1. Global usage of glyphosate-based herbicides

Glyphosate is a highly effective broad spectrum herbicide that is typically applied in mixtures known as glyphosate-based herbicides (GBHs) and commonly sold under the trade names of *Roundup*® and *Ranger Pro*®. Use of GBHs has dramatically increased worldwide in recent decades (Fig. 1). In the United States alone, usage increased nearly sixteen-fold between 1992 and 2009 [1]. Most of this increase occurred after the introduction of genetically modified glyphosate-resistant "Roundup-ready" crops in 1996 [1]. In addition, there have been significant changes in usage. In particular, the practice of applying GBHs to crops shortly before harvest, so-called "green burndown," began in the early 2000s to speed up their desiccation; as a consequence, crops are likely to have higher GBH residues [2]. By the mid-2000s, green burndown became widespread, and regulatory agencies responded by increasing the permissible residue levels for GBHs [3,4].

Abbreviations: AHS, Agricultural Health Study; c-NHEJ, canonical non-homologous end joining pathway; CI, confidence interval; EDC, endocrine disrupting chemical; EFSA, European Food Safety Authority; EPA, Environmental Protection Agency; ETS, environmental tobacco smoke; GBHs, glyphosate-based herbicides; IARC, International Agency for Research on Cancer; IFN-γ, interferon gamma; IL-2, Interleukin-2; JMPR, Joint Meeting on Pesticide Residues by the Food and Agriculture Organization of the United Nations and World Health Organization; meta-RR, meta-analysis relative risk; mg/kg/day, milligrams per kilogram per day; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; OR, odds ratio; ppm, parts per million; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis; RR, relative risk

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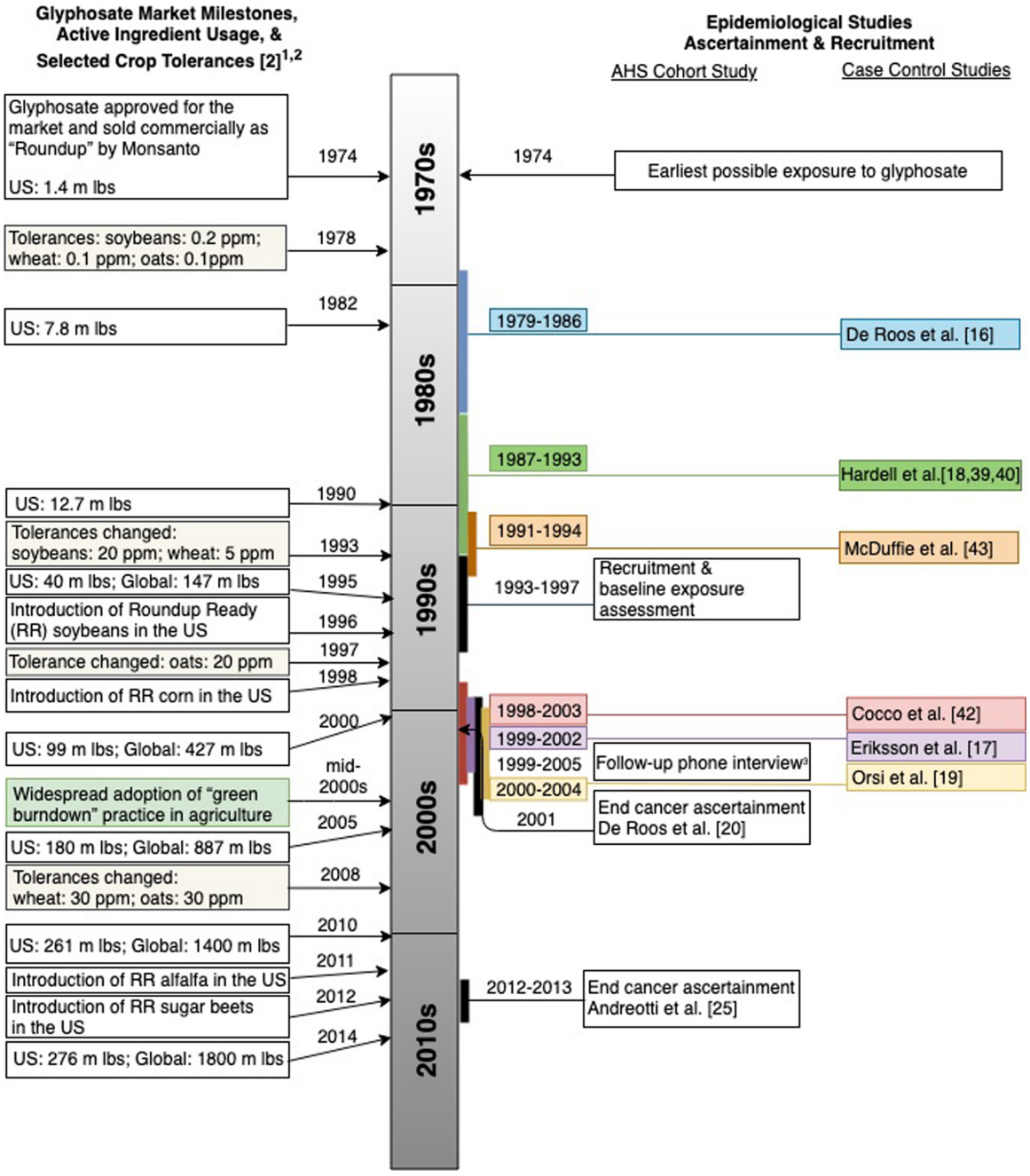

Fig. 1. Timeline of glyphosate use milestones in relation to cohort and case-control study events.
[1]Glyphosate active ingredient usage includes agricultural and non-agricultural applications.
[2]Abbreviations: m = millions; lbs = pounds; ppm = parts per million; RR = Roundup Ready.
[3]Completed by 63% of AHS participants.

1.2. Ubiquitous exposure in humans

Glyphosate and its metabolites persist in food [5–7], water [8], and dust [9], potentially indicating that exposure in the general population is ubiquitous. Non-occupational exposures occur primarily through consumption of contaminated food, but may also occur through contact with contaminated soil [9], dust [9] and by drinking or bathing in contaminated water [8]. In plants, glyphosate may be absorbed and transported to parts used for food; it has been detected in fish [5], berries [6], vegetables, baby formula [7], and grains [10], and its use as a crop desiccant significantly increases residues. GBH residues in food persist long after initial treatment and are not lost during baking [11].

Limited data exist on internal glyphosate levels among GBH-exposed individuals [12]. Average urinary glyphosate levels among occupationally exposed subjects range from 0.26-73.5 μg/L, whereas levels in environmentally exposed subjects have been reported between 0.13–7.6 μg/L [12]. Two studies of secular trends have reported increasing proportions of individuals with glyphosate in their urine over time [13,14]. Given that more than six billion kilograms of GBHs have been applied in the world in the last decade [2], glyphosate may be considered ubiquitous in the environment [15].

1.3. Controversy surrounding the carcinogenic potential of GBHs

Exposure to GBHs is reportedly associated with several types of cancer, among which the most well-studied in humans is non-Hodgkin

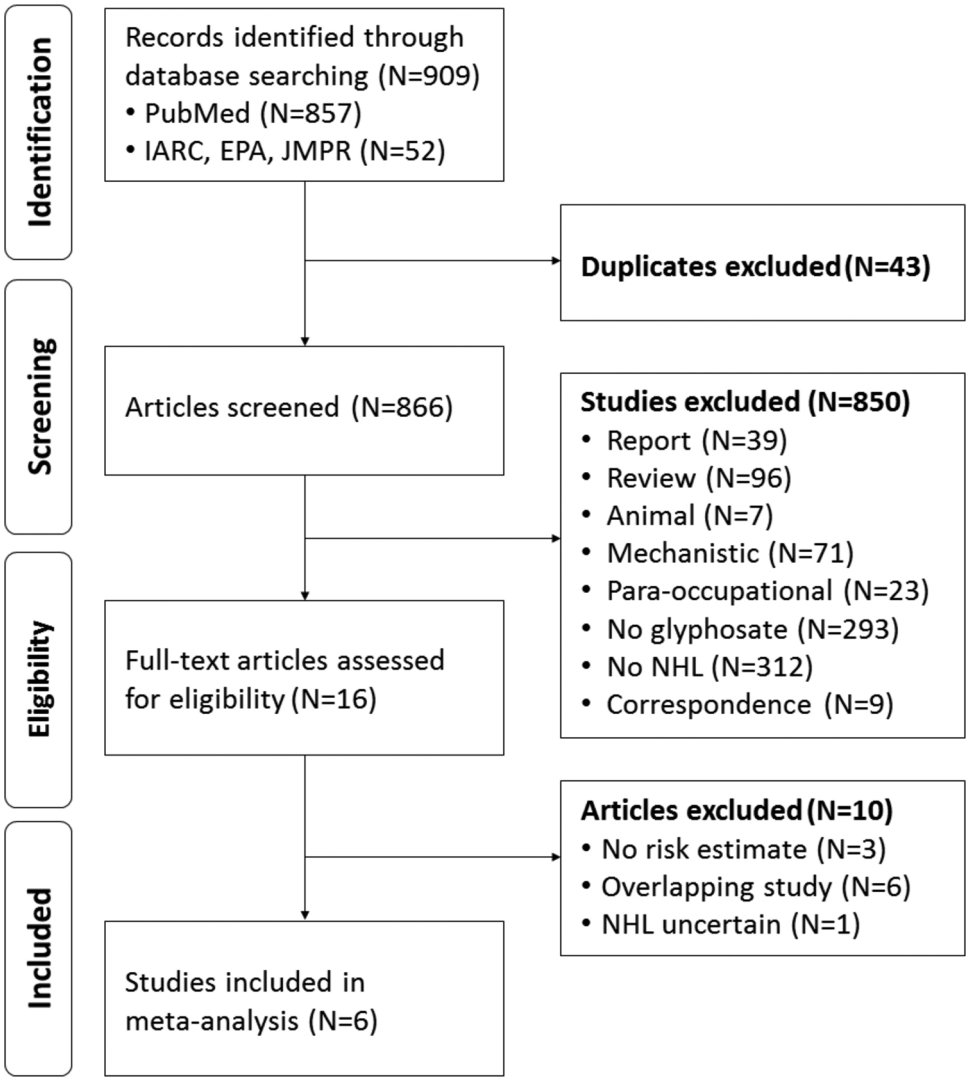

Fig. 2. Study selection process for meta-analysis using PRISMA guidelines.

lymphoma (NHL). Some epidemiological studies have reported an increased risk of NHL in GBH-exposed individuals [16–18]; however, other studies have not confirmed this association [19,20]. GBHs have recently undergone a number of regional, national, and international evaluations for carcinogenicity [21–24], resulting in considerable controversy regarding glyphosate and GBHs' overall carcinogenic potential. Hence, addressing the question of whether or not GBHs are associated with NHL has become even more critical. Here, we evaluated all published human studies on the carcinogenicity of GBHs and present the first meta-analysis to include the most recently updated Agricultural Health Study (AHS) cohort [25]. We also discuss the lymphoma-related results from studies of glyphosate-exposed animals as well as mechanistic considerations to provide supporting evidence for our analysis of the studies of human exposures to GBHs.

2. Current meta-analysis of GBHs and NHL

2.1. Meta-analysis objective

Epidemiological studies may vary in several ways, such as by study design, sample size, and exposure assessment methods. Results among individual studies vary and may appear to conflict, which poses challenges in drawing an overall conclusion. Meta-analysis is a quantitative statistical tool that is frequently applied to consolidate the results from similar but separate individual studies so that an overall conclusion about the effects of exposure can be drawn. Here, we conducted a meta-analysis using published human studies to better understand whether the epidemiological evidence supports an association between exposures to GBHs and increased NHL risk. Although three previously published meta-analyses have examined the same association and reported positive meta-risks for GBH-associated NHL [23,26,27], our analysis differs from earlier ones by focusing on an *a priori* hypothesis targeting exposure magnitude and by including the newly updated AHS study [25].

2.2. A priori hypothesis

Our *a priori* hypothesis is that the highest exposure to GBHs, *i.e.*, higher levels, longer durations and/or with sufficient lag and latency, will lead to increased risk of NHL in humans. The hypothesis is based on the understanding that higher and longer cumulative exposures are likely to yield higher risk estimates, given the nature of cancer development [28]. Hence, when cumulative exposure is higher, either due to higher level or longer duration exposures, an elevated association with the cancer of interest is more likely to be revealed if a true association exists. This *a priori* approach has been employed to estimate meta-risks for benzene [29] and formaldehyde [30,31], but not in any of the previous meta-analyses exploring the GBH-NHL association [23,26,27].

Table 1
Epidemiologic studies of GBHs and NHL included in the main meta-analysis.[a]

Author/location	Subject ascertainment	Participation	Exposure assessment	Exposure level	Results for NHL[b]	Weaknesses	Adjustments	Notes
Andreotti *et al.* [25] (Agricultural Health Study) Where: Iowa and North Carolina Design: Prospective cohort Years: 1993-97 to 2012-13 Percent exposed: 82.8%	Who: 54,251 pesticide applicators recruited between 1993-97 Cases: N/A Source of cases: Iowa and North Carolina Cancer Registries, state and national death registries Histologic verification: Not mentioned Controls: N/A Source of controls: N/A Similar demographics (exposed and unexposed): Similar age, sex, race, and smoking. Exposed higher education, alcohol consumption, and family history of cancer Final size: 575 NHL cancers, 53,760 subjects without missing data Follow-up: from enrollment through December 31, 2013 in Iowa and December 31, 2012 in North Carolina (16-20 years)	Exclusions: 3059 excluded (mostly missing data) Percent proxy interviews: None Missing Follow-Up Questionnaire: 37% (Pesticide use imputed)	Collection: Self-administered and take home questionnaire at time of recruitment: 22 specific pesticides application methods, PPE, years of use, and days per use. Review: No Blinded: Prospective design Validation: Similar questions asked 1 year apart in 4088 subjects, agreement on glyphosate ever use = 82%, days per year mixed = 52% [125]	Exposed: Quartiles 1-4 calculated by multiplying lifetime exposure days by an intensity score. Intensity based on (mixing + application method + equipment repair) * PPE in Coble *et al.* 2011 [45] Unexposed: No glyphosate use Lagged: 5, 10, 15, and 20 years	Adjusted Intensity Weighted Cumulative Exposure (Days): RRs for Q1: 1–598.9; Q2: 599–1649.9; Q3: 1650–4339.9; and Q4: ≥4340.0, = 1.0 (Ref), 0.83 (0.59-1.18), 0.83 (0.61-1.12), 0.88 (0.65-1.19), 0.87 (0.64-1.20); p-trend = 0.95; n = 54,251 total, 111 cases in the high exposure group Similar results comparing highest to lowest quartile. Lagged with Adjusted Intensity Weighted Cumulative Exposure: RRs for 20-year lag quartiles: Q1: 1–281.3; Q2: 281.4–895.9; Q3: 896–2609.9; Q4: ≥2610.0 = 1.00 (Ref), 1.22 (0.91-1.64), 1.15 (0.86-1.55), 0.98 (0.71-1.36), 1.12 (0.83-1.51) Lagged with Adjusted Lifetime Cumulative Exposure: RRs for 20-year lag quartiles: Q1: 1-8.74; Q2: 8.75-21.24; Q3: 21.25-59.4; Q4: ≥59.5 = 1.00 (Ref), 1.52 (1.16-2.00), 0.95 (0.68-1.32), 0.97 (0.71-1.33), 1.13 (0.85-1.52) Change with adjustment: No	1. Possible fairly short follow-up for some people 2. Somewhat weak validation 3. Imputed exposure data for participants who did not complete the follow-up questionnaire.	Matched: N/A Adjusted: adjusted for age, state of recruitment, education, cigarette smoking status, alcohol per month, family history of cancer, atrazine, alachlor, metolachlor, trifluralin, 2,4-D. Other: N/A	1. Censored on date the subjects left the state. 2. Increased RR for AML (RR = 2.6, 0.7-9.4); highest exposure group (RR = 2.44, 0.94 - 6.32). 79.3% of all cases ever used glyphosate. 3. Used updated Surveillance Epidemiology End Results (SEER) coding scheme for NHL which includes multiple myeloma [68] 4. Update of De Roos *et al.* [20]
De Roos (2005) [20] (Agricultural Health Study) Where: Iowa and North Carolina Design: Prospective cohort Years: 1993-97 to 2001 Percent exposed: 75.5%	Who: 54,315 pesticide applicators recruited between 1993-97 Cases: N/A Source of cases: Iowa and North Carolina Cancer Registries, state and national death registries Histologic verification: Not mentioned Controls: N/A Source of controls: N/A	Exclusions: 20,802 excluded (mostly missing data) (36.3%) 298 people lost to follow-up Percent proxy interviews: None	Collection: Self-administered and take home questionnaire at time of recruitment: 22 specific pesticides application methods, PPE, years of use, and days per use. Review: No Blinded: Prospective design Validation: Similar questions asked 1 year	Exposed: Ever or upper tertiles Intensity based on (mixing + application method + equipment repair) * PPE in Dosemeci *et al.* 2002 [46] Unexposed: Never or lower tertile Lag: Not mentioned	Ever exposed: Age adjusted: RR = 1.2 (0.7-1.9) Adjusted: RR = 1.1 (0.7-1.9) Intensity-weighted exposure days: For exposures of 0.1-79.5, 79.6-337.1, and 337.2-18241, 1.0 (Ref), 0.6 (0.3-1.1), and 0.8 (0.5-1.4); p-trend = 0.99;	1. Possible fairly short follow-up for some people 2. Large numbers of people excluded from dose-response analysis due to missing data 3. Somewhat weak validation	Matched: N/A Adjusted: Age, education, smoking, alcohol, family cancer history, state, 10 other pesticides most strongly correlated with glyphosate (if RR changes by > 20%) Other: N/A	1. Censored on date the subjects left the state. 2. Increased RR for multiple myeloma (OR = 2.6, 0.7-9.4) but with large change after adjustment (unadjusted OR = 1.1, 0.5-2.4) 3. 75.5% ever used glyphosate

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Table 1 (*continued*)

Author/location	Subject ascertainment	Participation	Exposure assessment	Exposure level	Results for NHL[b]	Weaknesses	Adjustments	Notes
	Similar demographics (exposed and unexposed): Similar age, sex, smoking, alcohol. Exposed higher education and family history of cancer Final size: 92 NHL cancers, 36,509 subjects without missing data Follow-up: from enrollment through Dec. 2001 (5-8 years, median = 6.7 years)		apart in 4088 subjects, agreement on glyphosate ever use = 82%, days per year mixed = 52% [125]		n = 36,823 total, 22 cases in the high exposure group Similar results comparing highest to lowest tertile. Adjusting for other pesticides did not change RR by more than 20% Change with adjustment: No			
De Roos (2003) [16] Where: US Design: Case-control Years: 1979-86 Percent exposed: 5.5%	Who: White men only. Combines data from three NCI case-control studies: Hoar *et al.* [34], Zahm *et al.* [35], and Cantor *et al.* [38]. Cases: Nebraska [35]: White subjects ≥ 21 years, 66 counties in eastern Nebraska, diagnosed 1983-86 Iowa/Minn [38]: White men ≥ 30 years, diagnosed 1980-83. Iowa: entire state. Minnesota: entire state except four large cities Kansas [34]: White men ≥ 21 years diagnosed 1979-81, entire state, 200 of 297 randomly selected Source of cases: Nebraska: Nebraska Lymphoma Study Group and area hospitals, Iowa/Minn: Iowa State Health Registry, surveillance system of Minnesota hospitals and pathology laboratories (not described) Kansas: State wide registry run by the University of Kansas Cancer Data Service,	Exclusions: > 25% (missing data and worked on farm before 18 years old) Interview rates: Kansas Cases: Unclear but likely > 90% Controls: 93% Iowa/Minn Cases: 89% Controls: 76-79% Nebraska Cases: 91% Controls: 85% Percent proxy interviews: 30.9% in cases and 39.7% in controls	Collection: Telephone (Kansas, Nebraska) or in-person (Iowa/Minn) interviews: SES, medical history, smoking, and family history Nebraska: Specific pesticides, number years used, average days used per year, PPE Iowa/Minn: Specific pesticides, first and last year used, method of use, personal application, and PPE Kansas: Open ended question about pesticides used, duration and days per year only for pesticide groups, and PPE Review: No Blinded: Partial (see individual studies) Validation: Kansas: Sought to confirm purchases in 110 subjects, suppliers usually reported fewer purchases, no consistent differences between cases and controls, few details given Iowa/Minn: No Nebraska: No	Exposed: Any reported use, no further details Unexposed: No use of glyphosate Lag: Not mentioned	Unadjusted (calculated): OR = 1.80 (1.18-2.74) Adjusted: OR = 2.1 (1.1-4.0), 36 exposed cases Hierarchical adjustment: OR = 1.6 (0.9-2.8), 36 exposed cases Change with adjustment: Yes Other results: Iowa/Minn [38]: OR = 1.1 (0.7-1.9), 26 exposed cases Kansas: No glyphosate data in Hoar *et al.* [34] Nebraska: No glyphosate data in Zahm *et al.* [35] In non-asthmatics OR = 1.4 (0.98-2.1) in Lee *et al.* [37]	1. Unknown whether there was full case ascertainment in some areas. For example, incidence rates in Nebraska were 77% of those in SEER [35]. Few details provided on Minnesota case surveillance system 2. Only includes White male subjects 3. Large number of proxy interviews 4. No details regarding timing of pesticide use in relation to disease onset	Matched: Race, sex, age, and vital status Adjusted: Age study site, and 47 pesticides. Hierarchical models included pesticide class, and prior knowledge on carcinogenicity from IRIS and IARC Other: Family cancer history, education, and smoking had little impact on results	1. Farmers: 59.9% among controls 2. Exclusions: Subjects who did not work on farms after age 18; subjects with missing data on any of 47 pesticides (about 25% of subjects) 3. In Nebraska, larger percentage of farmers reported no pesticide use 4. Overlapping study with Cantor *et al.* [38][c] and Lee *et al.* [37][d]; both of which were evaluated in the sensitivity analysis.

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Table 1 (*continued*)

Author/location	Subject ascertainment	Participation	Exposure assessment	Exposure level	Results for NHL[b]	Weaknesses	Adjustments	Notes
	mandatory cancer reporting in the state Histologic verification: Yes, in all three studies (Kansas 90%) Controls: Randomly selected from the same areas Source of controls: Random digit dialing, Medicare, and state mortality records for deceased cases Similar demographics: Similar education; family history higher in cases; few other variables described Final size: 650 cases and 1933 controls							
Eriksson *et al.* [17] Where: Sweden Design: Case-control Years: 1999-2002 Percent exposed: 1.8%	Who: Population based, men and women Cases: Age 18-74 diagnosed 1999-2002 Source of cases: 4 of 7 health service regions in Sweden associated with four University Hospitals, from physicians and pathologists Histologic verification: Yes Controls: Randomly selected from the same health regions, matched on age and sex Source of controls: Sweden population registry for the same health service regions Similar demographics: Data not provided Final size: 910 cases and 1016 controls	Exclusions: 134 of 1163 (11.5%) with medical conditions or deceased Interview rates: Cases: 91% Controls: 92% Percent proxy interviews: Deceased cases were not included (n = 88)	Collection: Mailed questionnaire on work history, specific pesticides, number of years, days per year, hours per day, with follow-up telephone interviews as needed Review: No Blinded: Partial, interviewers blinded to case-control status Validation: No	Exposed: One full day, or median number of days exposed in the controls. Unexposed: Unexposed to any included pesticide Lag: Exposures in the year of and the year before diagnosis disregarded	Unadjusted: Not provided Adjusted: ≥1 day (univariate): OR = 2.02 (1.10-3.71) ≤10 days: OR = 1.69 (0.70-4.07) > 10 days: OR = 2.36 (1.04-5.37, n = 17 exposed cases) ≥1 day adjusted for other pesticides (multivariate): OR = 1.51 (0.77-2.94) Lag (≥1 day) 1-10 years: OR = 1.11 (0.24-5.08) > 10 years: OR = 2.26 (1.16-4.40) Change with adjustment: Yes	1. Deceased cases not included 2. True participation rates may be lower 3. Use of PPE not assessed 4. Population based: not a high exposure group 5. Adjustments for other pesticides not completely clear	Matched: Age and sex Adjusted: Age, sex, and year of diagnosis. Adjusted for other pesticides in some analyses (MCPA, mercurial seed dressing…) Other: N/A	1. Authors state that all lymphoma treating clinics and all lymphoma pathologists in the study regions were covered by the study. 2. Also gives RR by subtype 3. Percent farmers unknown, but only 51 controls (5.0%) used herbicides
Hardell *et al.* [18] Where: Sweden Design: Case-control Years: 1987-90 Percent exposed: 0.7%	Combines two published studies, one of NHL [39] and one of hairy cell leukemia (HCL) [40] Who: Population based, males ≥25 years old	Exclusions: Deceased HCL cases excluded, numbers unknown. No other obvious major exclusions Interview rates NHL Cases: 91%	Collection: Mailed questionnaire: complete working history, exposure to specific chemicals (years and total number of days). Supplemented with phone interviews as	Exposed: Minimum exposure of 1 day (8 hours) Unexposed: No reported pesticide exposure Lag: At least one year	NHL and HCL combined: Unadjusted: OR = 3.04 (1.08-8.52) Adjusted: OR = 1.85 (0.55-6.20), 8 exposed cases NHL only: [39] Unadjusted OR = 2.3	1. Large change in ORs with adjustments, and changes were in opposite directions for NHL only vs. NHL and HCL combined 2. Adjustment factors not listed in some analyses	Matched: Age, county, and year of death (in deceased) Adjusted: Study (NHL vs. HCL), study area, vital status, (unclear, but it seems likely the multivariate analysis adjusted for 2-methyl-4-chlorophenoxyacetic acid, 2,4-	1. Percent farmers unknown. 184/1141 controls (16.1%) used insecticides so probably low

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Table 1 (*continued*)

Author/location	Subject ascertainment	Participation	Exposure assessment	Exposure level	Results for NHL[b]	Weaknesses	Adjustments	Notes
	NHL cases (n = 404): All male cases, living or deceased, diagnosed 1987-1990 from 7 Swedish counties HCL cases (n = 121): All living male cases in whole country 1987-92 Source of cases: Regional cancer registries (compulsory reporting) Histologic verification: Yes Controls: 2-4 per case matched on age, county, and year of death (if deceased). Those closest in age of birth to case were selected Source of controls: National Population Registry and National Registry for Causes of Death Similar demographics: Data not provided Final size: 515 cases and 1141 controls	Controls: 84% HCL Cases: 91% Controls: 83% Percent proxy interviews: Approximately 43.4% of NHL cases and controls. HCL study only living subjects used.	needed. Review: No Blinded: Subjects blinded to hypothesis, interviewers blinded to case status Validation: None		(0.4-1.3), 4 exposed cases Adjusted OR = 5.8 (0.6-54). Adjustment factors and sample sizes not given HCL only: [40] Unadjusted OR = 3.1 (0.8-12), 4 exposed cases Adjusted results not given. Age adjustment decreases OR for "herbicides" (2.9 to 1.8) Change with adjustment: Yes	3. Cut-off for defining exposure is very low 4. Population based: not a high exposure group 5. Small numbers of exposed cases 6. Demographic data not provided 7. Large number of proxy interviews	dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid, and other herbicides)	
McDuffie *et al.* [43] Where: Canada Design: Case-control Years: 1991-94 Percent exposed: 8.8%	Who: Population based, males 19 years and older Cases: ICD9 200, 202 newly diagnosed 1991-94 Source of cases: Provincial cancer registries, except Quebec (hospitals) Histologic verification: Partial (84%) Controls: Men age 19 or older randomly selected matched on 2-year age groups Source of controls: Randomly selected from provincial health insurance records, telephone listings, or voter lists Similar demographics: Yes for age, farm, smoking, and missing	Exclusions: 68 cases in pilot study, all deceased cases (% unknown) Interview rates: Cases: 67.1% Controls: 48.0% Percent proxy interviews: Deceased cases excluded	Collection: Mailed questionnaires: demographics, medical history, family cancer history, lifetime job history, exposure to specific substances, accidental spills, and protective equipment. Phone interviews in those with ≥10 hours/year of cumulative exposure to all pesticides combined. Asked about exposure to pesticides and number of days per year Review: No Blinded: Not mentioned Validation: In a pilot sample of 27 farmers, compared questionnaire data to pesticide purchases. "Excellent concordance" but no actual numbers given	Exposed: Not provided Unexposed: No reported exposure to glyphosate Lagged: Not mentioned	Any exposure Unadjusted: OR = 1.26 (0.87-1.80), n = 51 exposed cases (age and province adjusted) Adjusted: OR = 1.20 (0.83-1.74) Dose-response: ORs for 0, > 0-≤2, and > 2 days per year = 1.00 (Ref), 1.00 (0.63-1.57), and 2.12 (1.20-3.73; n = 23 exposed cases) (adjusted only for age and province) Unadjusted in high exposure group (calculated) OR = 1.88 (1.01-3.21) Change with adjustment: No Other: Glyphosate only: OR = 0.92 (0.54-1.55) Glyphosate and malathion: OR = 2.10	1. Unclear if full case ascertainment in Quebec 2. Is registry compulsory 3. Fairly low participation rates, and difference seen between cases and controls 4. Average exposure in the highest group not given. Duration of exposure not given 5. Deceased cases excluded	Matched: Age and province Adjusted: Age, province, measles, mumps, cancer allergy shots, and family cancer. Factors with p ≤ 0.05 retained in the models. Unclear what factors included in the high exposure analysis	1. Farmers: 44.7% in controls had residence on farm 2. Similar rural/urban make-up between respondents and non-respondents but other differences not assessed. 3. See Kachuri *et al.* [36] for multiple myeloma data 4. Overlapping study with Hohenadel *et al.* [41][e] which was excluded from the main meta analysis.

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Table 1 (*continued*)

Author/location	Subject ascertainment	Participation	Exposure assessment	Exposure level	Results for NHL[b]	Weaknesses	Adjustments	Notes
	data. Cases less likely to have mumps, measles, and allergy shots/tests; more likely to have previous cancer Final size: 517 cases and 1506 controls				(1.31-3.37) Hohenadel *et al.* [41]			
Orsi *et al.* [19] Where: France (6 cities) Design: Case-control Years: 2000-04 Percent exposed: 5.5%	Who: Population-based, males age 20-75 years old Cases: Diagnosed in one of the main hospitals in the 6 cities, ICD-0-3 codes (listed in their Table 1) Source of cases: Hospitals Histologic verification: Yes Controls: Hospital controls Source of controls: Men from the same hospitals, mostly orthopedic and rheumatology. Unclear if randomly selected. Similar demographics: Similar for SES, education, rural vs. urban Final size: 244 cases and 436 controls	Exclusions: History of immuno-suppression (% unknown) Interview rates: Cases: 95.7% Controls: 91.2% Percent proxy interviews: Not mentioned	Collection: Self-administered questionnaire: all jobs, years, tasks and products handled (open-ended); followed by structured personal interview including non-occupational and occupational use (in farmers) of pesticides, mixing or spraying, number and duration of applications Review: Questionnaires reviewed by occupational hygienist and agronomist Blinded: Interviewer and subject blind to hypothesis, and reviewer blind to case status. Unclear if interviewer was blinded Validation: Partial (see "Review"), pesticides compared to annual directories that list recommended pesticides by crop and pest	Exposed: Any, possible or definite; duration greater than the median in the exposed Unexposed: Never exposed to glyphosate, similar results with "never used any pesticide" Lag: Not mentioned or assessed for glyphosate	Unadjusted: OR = 0.89 (0.44-1.81) Adjusted: OR = 1.0 (0.5-2.2), 12 exposed cases for any exposure Change with adjustment: No	1. Deceased cases probably not included 2. Private clinics not included 3. Unknown if control selection is population based 4. Population based: not a high exposure group or high risk group	Matched: Center and age Adjusted: Age and center Other: Rural vs. urban, type of housing, education, infection, family history, skin characteristics, smoking, and alcohol had little impact on results	1. Also has multiple myeloma results (OR = 2.4; 0.8-7.3) 2. Results for a few subtypes also given but with small numbers 3. Farm, agriculture, or forestry work in 92 of 426 controls (21.1%)

Abbreviations: HCL, Hairy Cell Leukemia; IARC, International Agency for Research on Cancer; ICD, International Classification of Disease; IRIS, US Environmental Protection Agency Integrated Risk Information System; Minn, Minnesota; N/A, not applicable; NCI, National Cancer Institute; OR, odds ratio; PPE, personal protective equipment; Ref, reference; RR, relative risk; SEER, Surveillance Epidemiology End Results; SES, socio-economic status.

[a] Although there is no overlapping study used in the main analysis, Cocco *et al.* [42] was excluded because only results for all B-cell lymphomas combined were reported (two cases of NHL, one case of multiple myeloma, and one unspecified B-cell lymphoma; n = 4). It is evaluated in the sensitivity analysis.

[b] 95% confidence intervals in parentheses.

[c] Cantor *et al.* [38] was excluded because it was combined with two other U.S. case-control studies in De Roos *et al.* [16].

[d] Lee *et al.* [37] was excluded because it presents results comparing asthmatics to non-asthmatics and results are not adjusted for other pesticide use. It is evaluated in the sensitivity analysis.

[e] Hohenadel *et al.* [41] was excluded because it presents results in subjects exposed and unexposed to malathion, which has not been consistently linked to NHL; the OR for glyphosate only was used in the sensitivity analysis.

Risk estimates, including relative risks (RRs) and odd ratios (ORs), in high exposure groups are less likely to be dominated by confounding or other biases compared to RRs or ORs from groups experiencing average or low exposure [32]. Furthermore, including people with very low exposure in the exposed group can dilute risk estimates. Studying the most highly exposed group is also useful to ensure an adequate exposure contrast, given the potential that most people have been exposed either directly or indirectly to GBHs. Because our main goal is to determine whether there is an exposure effect and not to conduct a precise dose-response assessment or to evaluate risks in people with low exposures, we assert that this *a priori* hypothesis is appropriate for testing whether or not a GBH-NHL association exists.

2.3. Agricultural Health Study (AHS) update

A recently published update [25] from the large AHS cohort of pesticide applicators (N > 50,000) has been included for the first time in our primary meta-analysis. Although the original AHS report [20] was used in previous meta-analyses [23,26,27], the 2018 AHS update [25] contributes 11–12 additional years of follow-up with over five times as many NHL cases (N = 575 compared to N = 92 in the original study [20]), and > 80% of the total cohort was estimated to be exposed to GBHs. As the largest and most recently published study, it adds substantial weight to the new meta-analysis [25]. We also performed a secondary comparison analysis using our *a priori* hypothesis with the original AHS report [20] for the purpose of comparing results with: 1) our primary analysis (using AHS 2018); and, 2) other meta-analyses published previously.

2.4. Identifying relevant human studies

The literature search was conducted according to the guidelines of the *Preferred Reporting Items for Systematic Reviews and Meta-Analysis* (PRISMA) [33]. The screening process and results are shown in Fig. 2. We conducted a systematic electronic literature review using PubMed in November 2017, and we updated it in March 2018 and again in August 2018. We used the following keywords: (glyphosat* OR pesticide [MeSH] or herbicides [MeSH]) AND (lymphoma, non-Hodgkin [MeSH] OR lymphoma [tiab] OR non–Hodgkin [tiab] OR non–hodgkins [tiab] OR lymphoma[tiab] OR lymphomas[tiab] OR NHL OR cancer OR cancers) AND ("occupational exposure"[MeSH] OR occupational exposure[tiab] OR occupational exposures[tiab] OR farmers [MeSH] OR farmer OR applicators OR applicator OR agricultural workers OR agricultural worker or workers or worker).

Searches included all cohort, case-control, and cross-sectional studies. No language restrictions were applied, although non-English language articles needed to be obtained in full and translated completely in order to be eligible for inclusion. From the PubMed search, we identified 857 studies. Additionally, we identified 52 studies from the IARC [23] evaluation of the carcinogenicity of glyphosate, the U.S. EPA [21] review of glyphosate, and the WHO JMPR [22] report on glyphosate, for a total of 909 studies.

After 43 duplicates were excluded, 866 studies were initially screened by title and abstract, of which 850 were excluded because they were reports, correspondence, reviews, irrelevant studies (animal, mechanistic, para-occupational), or did not include the exposure or outcome of interest (Fig. 2). When the final 16 qualified epidemiological studies of GBHs and NHL were identified, 10 studies were further excluded because (1) they did not report RRs, ORs, or the data needed to calculate either [34–36], (2) the cohort overlapped with another study [20,37–41], or (3) they did not specify whether the lymphomas were specifically NHL [42]. For studies including overlapping cohorts, we used results from the most complete and updated analysis with the greatest number of participants. Although overlapping, we kept the earlier AHS (2005) [20] for comparison with our primary meta-analysis (using the updated AHS 2018 publication) and with previous meta-analyses. The impact of selecting these studies was evaluated in sensitivity analyses (Section 3.2).

2.5. Review and assessment of selected human studies

2.5.1. Data collection and extraction

In total, six studies (one cohort [25] and five case-control control studies [16–19,43]) with nearly 65,000 participants were eligible for inclusion in the meta-analysis. Two studies were conducted in the United States, one study was from Canada, two studies were from Sweden, and one study was from France. All six studies reported NHL risks (RRs or ORs) above or close to 1.0, three of which were statistically significant in the original analyses (Table 1). From each study, we abstracted information on study design, location, dates, sample size, participation rates, age, sex, case/control source, diagnosis, histologic verification, exposure assessment, results, and statistical adjustments. Table 1 summarizes key aspects of the design and exposure assessment, the results, strengths, and weaknesses of all the studies evaluated in this meta-analysis, including both versions of the AHS report ($n = 6 + 1$). As described above, the early AHS data [20] were also evaluated in Table 1 and in the secondary comparison meta-analysis described later.

2.5.2. Study quality evaluation

The methodological quality of the cohort (Table 2) and case-control studies (Table 3) included in the meta-analyses was assessed independently by two co-authors using the Newcastle Ottawa Scale (NOS) [44]. Studies were evaluated based on selection, comparability, and outcome or exposure (in nine categories).

Cohort studies were evaluated based on (1) representativeness of the cohort, (2) selection of non-exposed, (3) ascertainment of exposure, (4) demonstration that the outcome of interest was not present at the start of study, (5) comparability of cohort on the basis of controlling for other pesticide use and (6) age, (7) assessment of NHL outcome, (8) sufficiency of follow-up length, and (9) response rate.

Case-control studies were evaluated on (1) the validation of cases, (2) representativeness of cases, (3) selection of controls, (4) absence of disease in the controls, (5) whether the study controlled for other pesticide use and (6) age, (7) exposure assessment, (8) concordance of method among cases and controls, and (9) similarity of response rate among both groups. Each study was awarded a maximum of one point for every item that was satisfied, with a total of 9 available points.

According to our quality assessment (Tables 2 and 3), the highest quality study in either design category was the AHS 2018 cohort [25]. The highest quality case-control study was Eriksson *et al.* [17], while the lowest quality studies were McDuffie *et al.* [43] and Orsi *et al.* [19].

2.6. Selection of the most highly exposed category

Based on our *a priori* hypothesis, when multiple RRs or ORs were given in the original studies, we selected estimates in the following order: (1) highest cumulative exposure and longest lag (the time period preceding NHL onset, which is excluded from the exposure estimate) or latency (time between first lifetime exposure and NHL diagnosis); (2) highest cumulative exposure; (3) longest exposure duration and longest lag or latency; (4) longest exposure duration; (5) longest lag or latency; and (6) ever-exposure. The definition of cumulative exposure includes duration and intensity. As we discuss in more detail later in Section 5.2, cumulative exposure in both AHS reports [20,25] was calculated as an intensity-weighted exposure (lifetime exposure days multiplied by an intensity score) [45,46].

We prioritized highest cumulative exposure based on evidence of glyphosate's persistence in the environment [47–49] and because chronic disease, including cancer, is usually the result of cumulative exposures [50]. We selected the longest lag or latency because decades may be needed for the health effects of many environmental toxicants to manifest as detectable cancers. If no high exposure data were

Table 2
Quality assessment of the cohort studies in meta-analysis.[a]

Study	Selection				Comparability		Outcome			Overall Quality Scores
	Representativeness of Exposed	Selection of Non-Exposed	Exposure Assessment	NHL Absent at Start	Controls for Other Pesticides	Controls for Age	Assessment of Outcome	Follow-up Length	Adequacy of Follow-up	
Andreotti *et al.* [25]	1	1	1	1	1	1	1	1	0	8
De Roos (2005) [20]	1	1	1	1	1	1	1	0	0	7

[a] The study quality was assessed according to the Newcastle Ottawa Quality assessment scale for cohort studies [43]. One point was awarded for yes, and zero points were awarded for no, unable to determine, or inadequate.

Table 3
Quality assessment of the case-control studies in meta-analysis.[a]

Study	Selection				Comparability		Exposure			Overall Quality Scores
	Adequate Case Definition	Representativeness of Cases	Control Selection	Definition of Controls	Controls for Other Pesticides	Controls for Age	Exposure Assessment	Method Consistency	Non-response Rate	
De Roos (2003) [16]	1	1	1	0	1	1	0	1	0	6
Eriksson *et al.* [17]	1	1	1	0	1	1	0	1	1	7
Hardell *et al.* [18]	1	1	1	0	1	1	0	1	0	6
McDuffie *et al.* [43]	1	1	1	1	0	1	0	0	0	6
Orsi *et al.* [19]	1	1	0	1	0	1	0	0	1	2

[a] The study quality was assessed according to the Newcastle Ottawa Quality assessment scale for case-control studies [44]. One point was awarded for yes, and zero points were awarded for no, unable to determine, or inadequate.

Table 4
Description and weight of studies selected for the current meta-analyses.

Study (Author, Year)	Case Number (Exposed/Total)	Exposure Category	Risk Estimate [a] (95% CI)	Weight (%) [b]	
				AHS 2018	AHS 2005
AHS Cohort					
Andreotti *et al.*, 2018 [25]	55/575	≥2610 d/l [c,d]	1.12 (0.83, 1.51)[e]	54.04	–
De Roos *et al.*, 2005 [20]	22/92	≥337.2 d/l [c]	0.8 (0.5, 1.4)[f]	–	28.43
Case-Control					
De Roos *et al.*, 2003 [16]	36/650	Ever, log	2.10 (1.10, 4.00)	11.61	18.08
Eriksson *et al.*, 2008 [17]	17/910	> 10 d/y	2.36 (1.04, 5.37)	7.18	11.18
Hardell *et al.*, 2008 [18]	8/515	Ever	1.85 (0.55, 6.20)	3.30	5.14
McDuffie *et al.*, 2001 [43]	23/517	> 2 d/y	2.12 (1.20, 3.73)	15.05	23.43
Orsi *et al.*, 2009 [19]	12/244	Ever	1.0 (0.5, 2.2)	8.82	13.73

Abbreviations: AHS, Agricultural Health Study; CI, confidence interval; d, days; l, lifetime; log, logistic regression; y, year.
[a] Relative risk (RR) reported in both AHS analyses and odds ratio (OR) reported in all case-control studies.
[b] Weight calculated for each study using the fixed-effects model.
[c] Intensity-weighted lifetime exposure days (cumulative exposure days multiplied by intensity score).
[d] 20 years or more lag (time between study recruitment and NHL onset).
[e] Reference group is unexposed.
[f] Reference group is lowest exposed.

available, we used the ever-exposure estimate. Given the relatively few human epidemiological studies published to date on the topic, we made this decision because we did not want to exclude any potentially relevant data, even though the inclusion of minimally exposed individuals in the "exposed" category could attenuate any potential association of interest.

Although there are different perspectives on the best way to account for other pesticide exposures, we selected RR estimates that adjusted for other pesticide use over their unadjusted counterparts to mitigate potentially substantial confounding. Five of the seven studies adjusted for a combination of different pesticides [16–18,20,25]. However, if these multiple pesticides acted synergistically or on different points along a pathway, this approach to adjustment may no longer be the appropriate, and alternatives such as interaction analysis should be considered. Reanalysis of the raw data, which is beyond the scope of this paper, would be helpful to address this possibility.

We evaluated the impact of our *a priori* exposure selection criteria in sensitivity analyses. We also conducted a separate meta-analysis of all ever-exposed individuals to assess the magnitude of potential bias caused by adding subjects with low exposures (ever-RR from De Roos *et al.* [20] was used; the ever-RR estimate from Andreotti *et al.* [25] was not available). In Table 4 we summarize the risk estimates selected from each original study and the study weights used in the meta-analyses.

2.7. Statistical methods

We calculated the meta-analysis summary relative risk (meta-RR) and confidence intervals (CI) using both the fixed-effects inverse-variance method [32] and the random-effects method [51]. In the fixed-effects model, the weights assigned to each study are directly proportional to study precision, whereas in the random-effects model, weights are based on a complex mix of study precision, relative risk (RR), and meta-analysis size. One benefit of the random-effects model is the ability to incorporate between-study variance into the summary-variance estimate and confidence intervals, which may help prevent artificially narrow confidence intervals resulting from use of the fixed-effects model in the presence of between-study heterogeneity [52]. However, a feature of the random-effects model is that study weighting is not directly proportional to study precision, and greater relative weight is given to smaller studies, which may result in summary estimates that are less conservative than the fixed-effects model [53]. For these reasons, our primary results focus on the fixed-effects model, although the random-effects model estimates are also reported. We further estimated between-study heterogeneity, defined as the X^2-test statistic for heterogeneity being greater than its degrees of freedom (number of studies minus one), using the summary-variance method [52].

We evaluated publication bias through funnel plots, Egger's test, and Begg's test [53,54]. All statistical analyses were conducted with Stata IC 15.1 [55] and Microsoft Excel 2013 [56].

3. Meta-analysis findings

3.1. Increased meta-relative risk of NHL

Table 5 reports the results from our two meta-analyses, which included the primary analysis using the most recently updated AHS cohort [25] and the secondary comparison analysis using the original study [20]. With the AHS results [25], we observed a meta-RR of 1.41 (95% CI: 1.13–1.75), which indicates a statistically significant increased risk (41%) of NHL following high cumulative GBH exposure. Although our results focus on the fixed-effects model, using the random-effects model resulted in a meta-RR of 1.56 (95% CI: 1.12–2.16) shown in Table 5. With the original AHS 2005 cohort results, we observed a meta-RR of 1.45 (95% CI: 1.11–1.91) for NHL. The results did not change appreciably when comparing the fixed-effects model to the random-effects model.

Forest plots (Fig. 3A,B) and Funnel plots (Fig. 3C,D) from these two major meta-analyses are reported in Fig. 3. We observed little evidence of publication bias in the Funnel plots (Fig. 3C,D), Egger's ($p = 0.185$), and Begg's tests ($p = 0.851$).

3.2. Sensitivity analyses

We conducted several sensitivity analyses to evaluate the impact of excluding or including different studies as well as using different RRs/ORs from original studies (Tables 5 and 6). In general, results were similar across our sensitivity analyses, demonstrating the robustness of our findings.

3.2.1. Alternative exposure criteria

As a sensitivity analysis, we also conducted a meta-analysis using the longest exposure duration results to compare with our primary analysis using the highest cumulative exposure results. When RRs corresponding to exposures with the longest duration were selected from the AHS 2018, the meta-RR remained the same at 1.41 (95% CI: 1.13–1.74). When the AHS 2005 report was included, the meta-RRs increased to 1.56 (95% CI: 1.17–2.06) (Table 5).

Table 5
Major findings from current meta-analyses.

Analysis	N	Fixed-Effects meta-RR (95% CI)	Random-Effects meta-RR (95% CI)	Heterogeneity[a] X^2	p
Highest cumulative exposure					
AHS (2018) [25]	6	1.41 (1.13, 1.75)	1.56 (1.12, 2.16)	8.26	0.14
AHS (2005) [20][b]	6	1.45 (1.11, 1.91)	1.52 (1.00, 2.31)	10.59	0.06
Longest exposure duration					
AHS (2018) [25]	6	1.41 (1.13, 1.74)	1.56 (1.12, 2.16)	8.21	0.15
AHS (2005) [20][b]	6	1.56 (1.17, 2.06)	1.57 (1.06, 2.26)	7.81	0.17
Study design					
Case-control [16,17,18,19,43]	5	1.84 (1.33, 2.55)	1.86 (1.39, 2.48)	3.36	0.50
Cohort (AHS 2018) [25]	1	1.12[c] (0.83, 1.51)	–	–	–

Abbreviations: AHS, Agricultural Health Study; meta-RR, meta-relative risk; N, number of studies.
[a] Heterogeneity is present when X^2 heterogeneity statistic is greater than degrees of freedom (number of studies minus 1).
[b] De Roos *et al.* [20] used instead of Andreotti *et al.* [25] for comparison. See Table 4 for clarifications about the risk estimates used.
[c] Since there was only one cohort study, the RR is presented instead of a meta-RR.

When evaluating studies with only the highest levels of exposure [17,25,43], the meta-RR was 1.36 (95% CI: 1.06–1.75, Table 6). In studies that combined all exposures as ever exposed [16–20,43], the meta-RR was 1.30 (95% CI: 1.03–2.64). Although the higher exposure group was used in the main analysis, Eriksson *et al.* [17] also provided results for greater than 10 years latency, which contributed to a meta-RR of 1.40 (95% CI: 1.13–1.75). [Note: AHS 2018 did not provide ever-exposure, so AHS 2005 was used to calculate this statistic and ever exposure above].

3.2.2. Study inclusion

When we limited our analysis to case-control studies (Table 5), there was little inter-study heterogeneity. We estimated a doubling of the NHL risk (meta-RR = 1.84, 95% CI: 1.33–2.55) from 41% to 84% compared to the estimate that included the cohort study.

To ensure that one individual study was not artificially inflating the meta-risk estimate, we excluded the case-control studies one at a time and found that they all nominally lowered the meta-RR, except for the exclusion of Orsi *et al.* [19], where the meta-RR increased to 1.46 (1.16–1.83) (Table 6).

3.2.3. NHL vs. cell-type specific lymphomas

Although our primary meta-analysis included six studies, there was a possibility to include a seventh study [42]. We excluded this study from the primary analysis because it included all B-cell lymphomas (4 cases), which account for approximately 85% of all NHL [57]; however, not all four cases were confirmed to be NHL. When we added Cocco *et al.* [42] to the meta-analysis (n = 7, Table 6), the resulting RR remained fairly similar at 1.43 (95% CI: 1.15–1.78).

Similar to our inclusion of the Cocco *et al.* [42] study, another cell-type specific study evaluated all cases of hairy cell leukemia (HCL), a subtype of NHL [40]. It was one of two studies [39,40] included in the

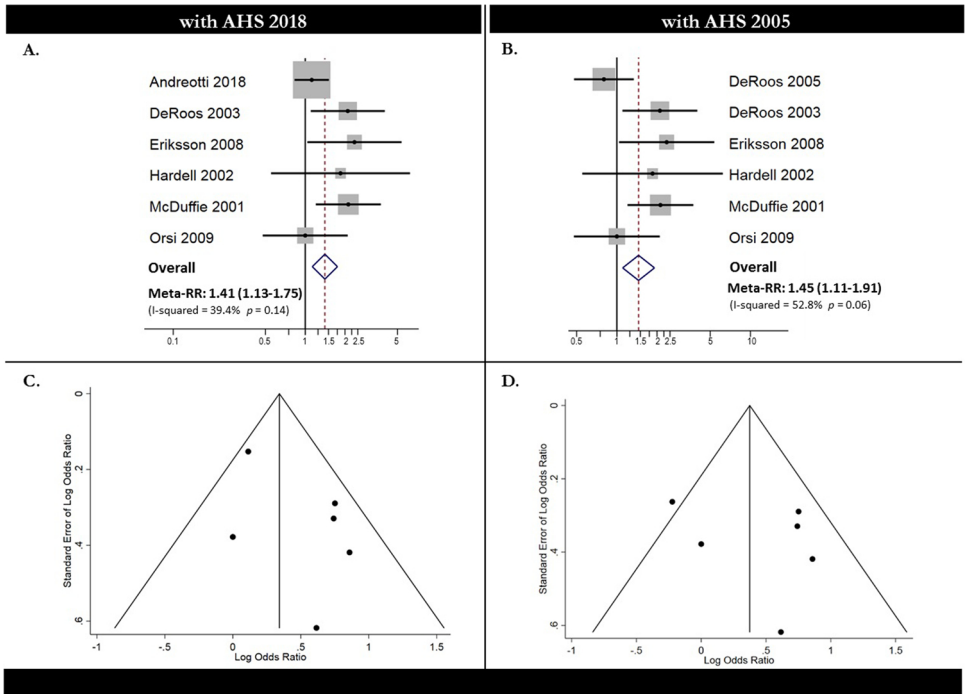

Fig. 3. Findings from major meta-analyses. 3A-B: Forest plots for meta-analyses using AHS 2018 (3A) and AHS 2005 (3B); 3C-D: Funnel plots for meta-analyses using AHS 2018 (3C) and AHS 2005 (3D).

Table 6
Results from sensitivity analyses.

Analysis	N	Fixed-Effects	Random-Effects	Heterogeneity[1]	
		meta-RR (95% CI)	meta-RR (95% CI)	X^2	*p*
Alternate exposure categories					
High level[2]	3	1.36 (1.06, 1.75)	1.63 (0.97, 2.76)	5.70	0.06
Ever (AHS 2005)	6	1.30 (1.03, 1.64)	1.26 (1.07, 1.48)	3.73	0.59
Latency[3]	6	1.40 (1.13, 1.75)	1.54 (1.12, 2.13)	8.01	0.16
Cell type specific					
Add Cocco *et al.* [42][4]	7	1.43 (1.15, 1.78)	1.59 (1.16, 2.18)	9.10	0.17
Exclude HCL [18][5]	6	1.41 (1.13, 1.77)	1.61 (1.11, 2.34)	9.58	0.09
Only use HCL [18][6]	6	1.43 (1.14, 1.78)	1.62 (1.14, 2.31)	9.36	0.10
Study location					
North America	3	1.38 (1.08, 1.76)	1.61 (0.99, 2.60)	5.70	0.06
Europe	3	1.53 (0.93, 2.52)	1.55 (0.88, 2.71)	2.43	0.30
Other pesticides[7]					
Adjusted (AHS 2005)	4	1.46 (1.05, 2.02)	1.43 (1.06, 1.92	2.61	0.46
Unadjusted (AHS 2005)	4	1.69 (1.29, 2.23)	1.70 (1.26, 2.30)	3.47	0.33
De Roos *et al.* [16]					
Hierarchical OR[8]	6	1.36 (1.09, 1.70)	1.46 (1.08, 1.96)	6.80	0.24
Cantor *et al.* [38][9]	6	1.29 (1.04, 1.59)	1.36 (1.02, 1.80)	7.07	0.22
Lee *et al.* [37][10]	6	1.35 (1.11, 1.65)	1.41 (1.09, 1.82)	6.63	0.25
Other					
Hohenadel [41] vs. McDuffie [43][11]	6	1.23 (0.99, 1.53)	1.30 (0.96, 1.76)	7.34	0.20
Exclude one study[12]					
Andreotti *et al.* [25]	5	1.84 (1.33, 2.55)	1.86 (1.39, 2.48)	3.36	0.50
De Roos *et al.* [16]	5	1.34 (1.06, 1.69)	1.47 (1.02, 2.11)	6.59	0.16
Eriksson *et al.* [17]	5	1.35 (1.08, 1.70)	1.47 (1.04, 2.07)	6.62	0.16
Hardell *et al.* [18]	5	1.40 (1.12, 1.75)	1.56 (1.08, 2.24)	8.06	0.09
McDuffie *et al.* [43]	5	1.31 (1.03, 1.66)	1.43 (1.01, 2.03)	5.90	0.21
Orsi *et al.* [19]	5	1.46 (1.16, 1.83)	1.69 (1.16, 2.45)	7.36	0.12

Abbreviations: CI, confidence interval; HCL, hairy cell leukemia; meta-RR, meta-relative risk.
[1] Heterogeneity is present when X^2 heterogeneity statistic is greater than degrees of freedom (number of studies minus 1).
[2] Risk estimates for the most highly exposed group available in the three studies that stratify by exposure level.
[3] Eriksson *et al.* [17] results for any glyphosate exposure > 10 years latency was used instead of the higher exposure group used in the main analysis.
[4] The study combined all B-cell lymphomas and is added to the analysis on highest cumulative exposure (AHS 2018).
[5] Hairy cell leukemia cases excluded—results presented in Hardell and Eriksson [39].
[6] NHL cases excluded; only HCL results used—results presented in Nordstrom *et al.* [40].
[7] Studies that provided RRs that are both adjusted and not adjusted for other pesticide use for ever exposure, or reported that adjusting for pesticide use had little impact on the RR estimate. AHS (2018) did not report ever exposure, so AHS (2005) was used instead.
[8] Hierarchical model RR used instead of the standard logistic regression model RR.
[9] Cantor *et al.* [38] used instead of De Roos *et al.* [16]. Cantor *et al.* [38] was the only of the three studies combined by De Roos *et al.* [16] that presented data for glyphosate.
[10] Lee *et al.* [37] used instead of De Roos *et al.* [16]. Lee *et al.* [37] used same subjects as De Roos *et al.* [16] but did not adjust for other pesticide exposure, did not exclude those with missing data on other pesticide use, and used only non-asthmatics.
[11] Hohenadel *et al.* [41] used same subjects as McDuffie *et al.* [43] but presented results in subjects exposed to glyphosate but not malathion (OR = 0.92; 95% CI: 0.54–1.55).
[12] One study excluded at a time to evaluate the impact of each individual study on the overall meta-RR.

Hardell *et al.* [18] analysis, with the other study examining NHL only [39]. Excluding HCL cases had no effect on the meta-RR (1.41, 95% CI: 1.13–1.77, Table 6). Similarly, using only hairy cell leukemia cases from Hardell *et al.* [18] (reported in Nordstrom *et al.* [40]) did not impact the meta-RR (1.43, 95% CI: 1.14–1.78).

3.2.4. Study location and covariate adjustment

Studies in North America [16,25,43] had a meta-RR of 1.38 (95% CI: 1.08–1.76), whereas European studies [17–19] had a meta-RR of 1.53 (95% CI: 0.93–2.52). On average, when studies were adjusted for other pesticide use [16–18,20], the meta-RR for ever-exposure was lower than unadjusted risk estimates from the same studies (meta-$RR_{adjusted}$ = 1.46, 95% CI: 1.05–2.02; meta-$RR_{unadjusted}$ = 1.69, 95% CI: 1.29–2.23).

3.2.5. Logistic vs. hierarchical regression

Consistent with the two previous meta-analyses by IARC [23] and Schinasi and Leon [26] discussed in Section 4 below, we selected the RR estimated using the more traditional logistic regression over hierarchical regression in the case-control study by De Roos *et al.* [16] and found that there was little impact of this selection (meta-RR = 1.36, 95% CI: 1.09–1.70). The De Roos (2003) [16] study included pooled data from two separate studies [37,38]. When Cantor *et al.* [38] or Lee *et al.* [37] was used instead of De Roos *et al.* [16], the meta-RR decreased to 1.29 (95% CI: 1.04–1.59) and 1.35 (95% CI: 1.11–1.65), respectively. Similarly, using Hohenadel *et al.* [41] instead of McDuffie *et al.* [43] caused the meta-RR to decrease to 1.23 (95% CI: 0.99–1.53).

4. Comparison with previous meta-analyses

Three meta-analyses of NHL in relation to GBH exposure have been published [23,26,27], all of which report lower, albeit also positive, risk estimates. In contrast to our work, these analyses did not focus on the highest exposed groups. Table 7 summarizes the major results from all GBH-NHL meta-analyses conducted to date, including the current one.

Schinasi and Leon [26] first reported a meta-RR of 1.45 (95% CI: 1.08–1.95). Although their selection criteria stated that they used the most adjusted effect estimate for the dichotomously defined exposure with the greatest number of exposed cases, they did not use adjusted effect estimates in the two Swedish studies [17,18]. The IARC Working

Table 7
Comparison of current meta-analysis to other published meta-analyses.

Studies	Schinasi and Leon [26][a]	IARC [23]	Chang and Delzell [27][a,b]	Current Meta-Analysis [RR (95% CI)]	
(Author, Year)	RR (95% CI)	RR (95% CI)	RR (95% CI)	with AHS 2005 [20]	with AHS 2018 [25]
Andreotti *et al.*, 2018 [25]	N/A	N/A	N/A	N/A	1.12 (0.83-1.51)
De Roos *et al.*, 2005 [20]	1.1 (0.7, 1.9)	1.1 (0.7, 1.9)	1.1 (0.7, 1.9)	0.8 (0.5, 1.4)	N/A
De Roos *et al.*, 2003 [16]	2.1 (1.1, 4.0)	2.1 (1.1, 4.0)	1.6 (0.9, 2.8)	2.1 (1.1, 4.0)	2.1 (1.1, 4.0)
Eriksson *et al.*, 2008 [17]	2.0 (1.1, 3.7)	1.51 (0.77, 2.94)	1.51 (0.77, 2.94)	2.36 (1.04, 5.37)	2.36 (1.04, 5.37)
Hardell *et al.*, 2002 [18]	3.0 (1.1, 8.5)	1.85 (0.55, 6.20)	1.85 (0.55, 6.20)	1.85 (0.55, 6.20)	1.85 (0.55, 6.20)
McDuffie *et al.*, 2001 [43]	1.2 (0.8, 1.7)	1.20 (0.83, 1.74)	1.20 (0.83, 1.74)	2.12 (1.20, 3.73)	2.12 (1.20, 3.73)
Orsi *et al.*, 2009 [19]	1.0 (0.5, 2.2)	1.0 (0.5, 2.2)	1.0 (0.5, 2.2)	1.0 (0.5, 2.2)	1.0 (0.5, 2.2)
meta-RR (95% CI)	**1.45 (1.08, 1.95)**[c]	**1.30 (1.03, 1.64)**	**1.27 (1.01, 1.59)**	**1.45 (1.11, 1.91)**	**1.41 (1.13, 1.75)**

Abbreviations: AHS, Agriculture Health Study; CI, Confidence Interval; IARC, International Agency for Research on Cancer; N/A, not available; meta-RR, meta-relative risk; RR, relative risk.

[a] In their published reports, meta-RRs and their 95% confidence intervals were rounded to one digit right of the decimal point.

[b] Findings from Model 1, the primary analysis, are reported here..

[c] Random-effects model.

Group subsequently corrected this discrepancy in an otherwise identical meta-analysis [23], resulting in a meta-RR of 1.30 (95% CI: 1.03–1.65). Although both studies are listed in Table 7 for completeness, we consider IARC 2015 to be the most accurate and updated version of this meta-analysis.

Most recently, Chang and Delzell [27] reported a meta-RR of 1.27 (95% CI: 1.01–1.59) in their primary analysis (model one). For each included study, the authors selected the most fully adjusted RR from the publication with the most recent and complete study population with the largest number of exposed cases. (In their publication, the meta-RR was rounded to one digit to the right of the decimal point.)

Whereas the three previous meta-analyses focused on general exposure (ever versus never), our new meta-analysis differs primarily because of our *a priori* selection of risk estimates from the most highly exposed groups when available (from three studies [17,20,43]). In our secondary comparison meta-analysis with the same six studies (including AHS 2005), we document an additional 0.15-0.18 (or 15–18%) higher NHL RR than previous meta-RRs [23,27] (not including Schinasi and Leon, because it was corrected in IARC 2015). Similarly, in our primary analysis with AHS 2018, our meta-RR estimate adds an additional 0.11-0.14 (11–14%) increase in NHL relative risk to the previous meta-RRs [23,27]. Overall, the meta-RR obtained using our *a priori* hypothesis, while generally consistent with previous analyses, gave somewhat higher estimates and suggested increased risk of NHL in individuals highly exposed to GBHs.

5. Strengths and limitations

In this section, we evaluate the strengths and limitations of our meta-analysis, as well as of the cohort and the case-control studies utilized.

5.1. Current meta-analyses

The strengths of this meta-analysis are the inclusion of the updated AHS 2018 study and our novel *a priori* hypothesis. By using the highest exposure group in each study when it was reported, we maximized the ability to detect the presence of an exposure-disease association. The current meta-analysis is also the first study to include the newly updated AHS.

There are several weaknesses of our analysis that should be noted, however. First, there were only limited published data available for inclusion. Although meta-analysis prevents overemphasis on any single study [58], we cannot exclude the potential for publication bias, given the relatively few published studies to date. Second, there was imbalance in study design: among the six included studies, five were case-control and one was a cohort. The collection of NHL findings from the cohort study was consistent with a wide range of risks [25], while, by contrast, most of the case-control studies did suggest an increased risk [16–18,43]. There were also important differences in the comparison group utilized in the studies; some used the lowest exposure group as the reference, while others used the unexposed group. Because of this heterogeneity, and because no statistical tests can confirm elimination of publication bias or heterogeneity in a meta-analysis [59], our results should be interpreted with caution. Finally, as depicted in Fig. 1 illustrating key milestones related to glyphosate use in society and in epidemiological studies, none of the available studies capture the effects of the significant increased usage of glyphosate that began with the introduction of “green burndown” in the mid-2000s.

5.2. AHS cohort study

In general, cohort studies are considered the gold standard among observational studies because of their ability to estimate exposure before disease occurrence (which allows for clarity of temporality and can minimize recall bias), to estimate incidence, to examine multiple outcomes, and for some target populations, to study a large number of exposed subjects. Our current meta-analysis is the first to include the AHS 2018 update, which is the largest, newest, and most heavily weighted study (> 50%, Table 4). Given its importance and because it was the only cohort study in our analyses, we discuss below several aspects of the AHS 2018 [25] and compare it with the results reported in AHS 2005 [20]. Key differences between the AHS 2018 and AHS 2005 are summarized in Table 8.

5.2.1. Exposure assessment and quantification

Exposures were self-reported using questionnaires. AHS 2005 used the exposures reported at baseline only, whereas AHS 2018 supplemented this information with responses to a follow-up questionnaire returned by 63% of AHS participants.

The risk estimates generated from the follow-up AHS 2018 report depended on a “multiple imputation” approach with multiple steps to generate GBH exposure information for the 37% of participants who did not complete the follow-up questionnaire [25]. A standard imputation model captures the full distribution of the exposure by relying on two parts of a model: the regression or predictable part and the residual error part. The validity of the imputed exposures and the resulting risk estimates relies on the validity of both parts of the imputation model. The AHS imputation method for ever/never pesticide use conditioned on the reported pesticide use and other data, including demographics, medical history at baseline, and farming characteristics at enrollment, with some covariates chosen by stepwise regression (see Table 2 in Heltshe *et al.* [60]). Based on their analysis of a 20% holdout dataset, the prevalence of glyphosate use was underreported by 7.31%,

Table 8
Key differences between AHS 2005 and AHS 2018, with an emphasis on exposure quantification.

Exposure assessment	AHS 2005 [20] Self-report at baseline			AHS 2018 [25] Self-report at baseline & follow-up questionnaire with exposure simulation[1]				
Exposure quantification	Ever/never	Cumulative exposure days	Intensity-weighted exposure days[2]	Ever/never[3]	Cumulative exposure days[3]	Intensity-weighted exposure days[2]		
Lag period	Unlagged	Unlagged	Unlagged	Unlagged	Unlagged	Unlagged	5-year lag	20-year lag[4]
Reference group	Unexposed	Lowest exp. (T1)	Lowest exp. (T1)	Unexposed	Unexposed	Unexposed	Unexposed	Unexposed
Exposed groups[5] (day)	Ever exposed	T1: 1–20; T2: 21–56; T3: 57–2678	T1: 0.1–79.5; T2: 79.6–337.1; T3: 337.2–18,241	Ever exposed	T1: 1–19.9; T2: 20.0–61.9; T3: > 62.0	Q1: 1–598.9; Q2: 599–1649.9; Q3: 1650–4339.9; Q4: ≥4340.0	Q1: 1–530.9; Q2: 531.0–1511.9; Q3: 1512.0–4063.4; Q4: ≥4063.5	Q1: 1–281.3; Q2: 281.4–895.9; Q3: 896–2609.9; Q4: ≥2610.0
Exposure duration[6] (year)	Max range: 20–24;[7] Actual max: 7.3[8]; Median: N/A; IQR: N/A			Max range: 26-32;[9] Actual maximum: N/A; Median: 8.5; IQR: 5–14			Max range: 21–27;[9] Actual max: N/A; Median: 4.1;[10] IQR: N/A	Max range: 6–12;[9] Actual max: N/A Median: 2.5;[10] IQR: N/A
Potential misclassification	Differential misclassification unlikely; Non-differential misclassification likely			Differential misclassification possible; Non-differential misclassification likely				
Follow-up (years)	Median: 6.7; Maximum possible: 9[11]			Median: N/A; Maximum possible: 20[11]				
Outcome inclusion	Multiple myeloma not included in NHL cases			Multiple myeloma included in NHL cases				

Abbreviations: AHS, Agricultural Health Study; IQR, interquartile range; max, maximum; N/A, not applicable; NHL, non-Hodgkin lymphoma; T1, tertile one.

[1] This was referred to as "multiple imputation" by study authors; see manuscript text for further details.

[2] The algorithm for calculating "intensity-weighted exposure days" was updated between 2005 and 2018. Key differences include rescaling of scores by a factor of 10 and altering the weights for mixing, certain pesticide application techniques, and the use of chemically resistant gloves [45]. Therefore, these metrics cannot be directly compared.

[3] Ever/never and cumulative exposure days were only presented in the AHS 2018 supplement but are presented here to facilitate comparisons with AHS 2005.

[4] Results and quartiles for 10- and 15-year lags are presented in the AHS 2018 supplement.

[5] Exposure group abbreviations are as follows: Tertiles = "T"; Quartiles = "Q".

[6] The values provided in this row are based on the subset of individuals who reported using glyphosate.

[7] This theoretical maximum duration value was calculated based on the year that glyphosate entered the market (1974) and the end of AHS enrollment (1993–1997), since AHS 2005 used only baseline exposure information.

[8] This value was calculated based on the upper bound of the cumulative exposure days tertiles.

[9] These theoretical maximum duration values were calculated based on the year that glyphosate entered the market (1974) and the end of AHS follow-up exposure questionnaire (1999–2005), with the appropriate adjustments for the lag times as indicated.

[10] These medians were calculated using the information provided in the footnote in Table 3 of the AHS 2018 publication.

[11] These follow-up times were calculated based on timing of study enrollment and follow-up.

suggesting some lack of validity in the predictable part of the imputation model that may in turn affect the NHL risk estimates. The imputations of days of use per year and most recent year of farming activity relied upon a stratified sampling with replacement approach, with values sampled from Phase 2 respondents based on strata defined using Phase 1 information.

The imputations did not use the NHL or any other cancer outcome information reported by Andreotti *et al.* [25]. This approach is problematic because of how the residual error part of the imputation model is handled. It is known that multiple imputation of a covariate (*i.e.,* glyphosate exposure) in a model that omits the outcome variable to be used in the inference leads to attenuation of the effect estimate for that covariate due to lack of correlation with the outcome in the residual error part of the imputed exposures [61]. As we discuss further in the next paragraph, this approach effectively "bakes into the results" the null hypothesis of no increased risk of NHL exposure due to glyphosate risk.

Because the NHL outcome information was not used in the imputation procedure, the exposure "imputation" method used in the AHS 2018 report can be better named "exposure simulation" as described by Gryparis *et al.* [62]. This term gives a much more accurate understanding of the impact of the imputation of the data on the risk estimates because when exposure is simulated in a model that does not take the outcome into account, the uncertainty in the "imputed" exposure behaves like classical measurement error and, thus, will bias the effect estimate towards the null [63].

AHS 2018 authors argue that their imputation approach "likely did not materially impact risk estimates" [64]. However, their argument has to do with the impact on the average change in the number of predicted events in an outcome-augmented imputation model and not the role of classical measurement error in the imputed exposure estimates.

There was also a subtle yet important difference in the categorization and quantification of exposure data between AHS 2005 and 2018. As depicted in Table 8, both studies classified exposure based on (1) ever/never, (2) cumulative exposure days, and (3) intensity-weighted exposure days. However, the algorithm utilized to calculate intensity-weighted exposure days was updated between 2005 and 2018. Key differences include rescaling of scores by a factor of 10 and altering the weights for mixing, certain pesticide application techniques, and the use of chemically resistant gloves [45]. Therefore, these metrics cannot be directly compared.

Additionally, it is crucial to highlight the difference in reference groups between these two studies, which further limits the comparability of their estimates. AHS 2005 utilized the lowest exposed tertile as the comparison group for risk estimation. They justified this decision as an attempt to control residual confounding, because of the presence of significant differences in key characteristics between the never-exposed and lowest-exposed groups. By contrast, AHS 2018 utilized the unexposed group as the reference group even though our comparison of the demographics reported in each paper does not suggest there is substantially better comparability between groups in AHS 2018. Furthermore, because the exposure information by which these groups were classified was based on their imputation procedure, the limitations of which are highlighted above, the actual comparability between groups may differ from the values reported. Not only would it be helpful to be able to compare directly the risk estimates across the two papers, it would also be useful to investigate whether there was residual confounding introduced into the AHS 2018 analysis by the use of the "unexposed" group as the reference.

5.2.2. Exposure misclassification

Differential misclassification is unlikely in a cohort study when exposure is assessed prior to the disease occurrence. In AHS 2018, however, we suspect there is some potential for differential misclassification. Sixty-three percent of the original cohort provided updated exposure information by questionnaire one time between the years of 1999 and 2005. Although details are not provided, it is likely that some of the cases reported their exposure after disease occurrence, allowing for potential differential misclassification in the self-reported exposures in this cohort similar to general concerns with case-control studies. Furthermore, noting large societal trends in GBH exposure between initial exposure ascertainment and the follow-up questionnaire, and the 7.3% under-prediction of glyphosate exposures in the holdout dataset [60], the prediction part of the imputation modeling may be differentially under-predicting exposures.

Non-differential misclassification occurs when exposure status is equally misclassified among exposed cases and unexposed controls [65]. The approach in AHS 2018 to exposure imputation is one theoretically well-understood source of non-differential misclassification. In addition, it may be more problematic in the context of a ubiquitous exposure because it is hard for participants to know to what extent or how long they have been exposed. Glyphosate's ubiquity in the environment leads to profound concerns that even "unexposed" individuals in the cohort are likely to have been exposed to GBHs; consequently, the magnitude of any potential association relative to the unexposed group may be attenuated due to this misclassification. This problem is encountered with other environmental exposures such as environmental tobacco smoke (ETS): never smokers with ETS exposure carry some cancer risk and are not the ideal true reference group in studies of smoking and tobacco-related cancers [66]. As we noted above, non-differential misclassification is likely to attenuate measures of association, biasing the RR towards the null of 1.0 [67]. Although it is difficult to ascertain exactly, the extent of this source of non-differential misclassification can be estimated through smaller-scale validation studies [67].

5.2.3. Disease classification and latency

The updated AHS 2018 included multiple myeloma (MM) in their NHL cases, but the previous AHS 2005 did not. Although MM traditionally did not belong to NHL, WHO recently revised the classification of lymphoid neoplasms and suggested some types of MM (e.g., IgM mutation-related MM) are related more closely to lymphomas, including NHL, than to myelomas [68].

There is much uncertainty surrounding the latency period for NHL. The latency period for short-term high-dose exposures to carcinogens may be as short as two years, but it may also be as long as 15 years or more. Low-dose long-term exposures are expected to have longer median latencies between 15 to 20 years for NHL [69,70]. It is possible that different NHL subtypes may also have different latencies. Given the uncertainty surrounding NHL latency, it is possible that the follow-up period (median = 6.7 years) in the 2005 AHS study [20], which was unlagged, may have been too short for a sufficient number of exposure-related cancer events to manifest. Given that participants had been exposed to GBHs prior to enrolling in the study (median = 8 years; mean = 7.5 years; SD = 5.3 years), participants could have had an exposure duration ranging from as low as 0 years to as high as 18 years at the time of enrollment, assuming a normal distribution. Hence, although some AHS members may have had sufficient exposure durations to develop NHL, many fell short of the median 15–20 years of expected NHL latency.

The 2018 AHS publication added 11–12 further years of follow-up for all study participants, an additional 483 cases of NHL, and considered five, ten, fifteen, and twenty year exposure lags, which was not possible in AHS 2005 due to its short follow-up duration. Epidemiologic studies often lag exposures to account for disease latency under the assumption that recent exposures have little impact on disease development. Theoretically, longer exposure durations and/or lags would present more biologically plausible associations with NHL. For AHS 2018 specifically, not only are the risk estimates associated with longer lag times more plausible than unlagged risk estimates in AHS 2005 and 2018, but the twenty-year exposure lag, specifically, may also be free of the bias caused by exposure imputation described above, given that at this lag exposure information may have been derived exclusively from the baseline questionnaire.

5.2.4. Summary

Overall, the cohort study features highlighted above related to exposure assessment and quantification, misclassification, and latency

and lag suggest caution in direct comparisons between AHS 2005 and 2018. Additionally, the limitations with AHS 2018 with regard to exposure simulation, potential residual confounding, and misclassification may have accounted for the weaker meta-RR estimate that we obtained when incorporating this study into the meta-analysis.

5.3. Case-control studies

Although cohort studies are the gold standard in observational epidemiology, they are often challenging to conduct due to the small number of incident cases for rare diseases such as NHL. Case-control studies can be more efficient for evaluation of rare diseases. For example, the AHS had to recruit tens of thousands of participants (N = 53,760) and follow them for more than a decade in order to gather 575 new cases of NHL, whereas the 5 case-control studies assembled 2836 NHL cases among all participants (N = 8868) in a much shorter period of time (Tables 1 and 4). Though the case-control studies are smaller and carry less weight than the large cohort study, it is worth noting that results from multiple case-control studies displayed little heterogeneity (Table 5) and reported similar findings pointing away from null (Table 4).

However, there are other challenges and concerns relevant to the case-control studies utilized in our meta-analysis, which we briefly discuss below.

5.3.1. Control selection and exposure quantification

Four of the five case-control studies utilized here are population-based, while one is hospital-based [19]. There may be important differences between hospital-based controls and population-based controls that could impact the interpretability and comparability of the resulting risk estimates. Of relevance to this concern is that, as noted above in our sensitivity analyses, exclusion of Orsi *et al.* [19] (the hospital-based case-control study) resulted in an increased meta-RR of 1.46 (95% CI: 1.16–1.83) shown in Table 6, while sequential exclusion of each of the population-based case control studies produced decreased meta-RRs.

Exposure was also quantified differently between the selected case-control studies, further impacting their comparability. While all the studies considered in our meta-analysis conducted exposure assessment based on self-reported questionnaire data, some studies considered ever/never exposure, while others evaluated exposure based on number of days per year (see Tables 1 and 4). Some studies also relied on proxy respondents such as next of kin.

5.3.2. Exposure misclassification

It is always possible for the internal validity of case-control studies to be threatened by recall bias, a form of differential exposure misclassification that occurs when exposures are remembered differently by cases (or their proxies) and controls. Cases may have been more motivated to recall GBH exposure, and the exposures may be more vivid or meaningful due to awareness of the risk factors for their disease. While differential misclassification can bias the OR in either direction, differential misclassification due to cases being more likely to report exposure tends to artificially inflate the OR.

5.3.3. Latency and lag

As discussed in Section 5.2.3, the latency for NHL is uncertain and could be anywhere from 2 years to greater than 15 years. There were differences in how the case-control studies considered and incorporated latency and lag into their analyses. For example, De Roos *et al.* [16] and McDuffie *et al.* [43] do not mention these considerations; by contrast, Hardell *et al.* [18], Orsi *et al.* [19], and Eriksson *et al.* [17] each incorporate latency and lag, albeit differently. These differences suggest caution in the integration of these results.

6. Summary of the GBH and NHL association in humans

Overall, the results from our new meta-analysis employing the *a priori* hypothesis and including the updated AHS 2018 study (1) demonstrate a statistically significant increased NHL risk in highly GBH-exposed individuals (meta-RR = **1.41**, 95% CI: 1.13–1.75; Table 5 and Fig. 3A), (2) are aligned with findings from previous meta-analyses [23,26,27] (Table 7), and (3) reveal an additional 11–14% and 15–18% increase in NHL relative risk due to high levels of GBH exposure (Table 7) when using the AHS 2018 and the AHS 2005 cohort, respectively.

Together, all of the meta-analyses conducted to date, including our own, consistently report the same key finding: exposure to GBHs are associated with an increased risk of NHL.

Because most people in these epidemiological studies were not exposed to pure glyphosate, but rather glyphosate-based formulations (e.g. Roundup® or Ranger Pro®) with a number of adjuvants, it could be argued that the NHL manifested as a result of exposure to the mixture or an ingredient other than glyphosate in the formulation. To investigate causal inference regarding the association between glyphosate exposure and NHL, we discuss briefly whether or not the association identified from epidemiological studies could be supported further by experimental animal and mechanistic studies related to lymphoma.

7. Animal data: lymphoma prevalence in glyphosate-exposed mice

The animal study outcome most closely linked to human NHL is malignant lymphoma. We identified six unpublished glyphosate and lymphoma studies in mice that are in the public domain from two sources: a presentation by the European Food Safety Authority (EFSA) [71] at the EPA FIFRA Scientific Advisory Panel on Carcinogenic Potential of Glyphosate and a report by The Food and Agriculture Organization of the United Nations and World Health Organization Joint Meeting on Pesticide Residues (JMPR) [22]. EFSA [71] reported results from five unpublished studies: four in CD-1 [72–75] and one in Swiss albino mice [76], whereas JMPR [22] also reported data from an additional study in female CD-1 mice [77]. Each study reported four glyphosate doses and corresponding lymphoma incidence in males and females except for Takahashi [77], where the only data available in the public domain was for female mice [22].

7.1. Results of mouse lymphoma studies

Results from all studies (n = 6) of malignant lymphomas in mice available in the public domain are presented in Table 9. Study durations ranged from 1.5 to 2 years. All studies administered glyphosate through the diet [72–77], and the concentrations tested ranged from 100 ppm to 50,000 ppm [22]. EFSA [71] and JMPR [22] reported slightly different doses, with JMPR [22] further stratifying by sex. Lymphoma incidence was abstracted from EFSA [71], with slightly different numbers for one study [72]. Table 9 provides the dietary concentration of glyphosate (reported in ppm), the doses (reported in mg/kg/day) provided by EFSA [71] and JMPR [22], and lymphoma incidence in males and females. One study [74] reported food consumption, which was recorded for each treatment group, and weekly mean achieved-dose levels were averaged to calculate actual doses for males and females. Information on how doses were calculated for the other studies [72,73,75,73–77] was not available.

In summarizing these studies, EFSA [71] noted that Sugimoto [73] and Wood *et al.* [74] showed statistically significant dose-response in males according to the Cochran-Armitage test for linear trend, whereas Kumar [76] showed a statistically significant Z-test for both males and females. In agreement, JMPR [22] noted that Sugimoto [73] and Wood *et al.* [74] showed a statistically significant trend in males and that Kumar [76] reported statistically significant increases in malignant lymphoma in high-dose groups of both males and females. JMPR [22] further reported Takahashi [77] had a statistically significant increased incidence in lymphoma among females by their trend test. The remaining two studies did not report evidence of a statistically significant dose-response effect.

Table 9
Data from publicly available studies of malignant lymphomas in mice exposed to glyphosate.[a]

Study	Strains	Study Duration	Concentration in Diet (ppm)	Dose (mg/kg/day)		Incidence[b] (%)	
				EFSA [71]	JMPR [22][c]	Male	Female
Wood *et al.* [74]	CD-1	1.52 years (79 weeks)	0	0	0, 0	0/51 (0)	11/51 (22)
			500	71	71.4, 97.9	1/51 (2)	8/51 (16)
			1500	234	234.2, 299.5	2/51 (4)	10/51 (20)
			5000	810	810, 1081.2	5/51(10)*	11/51 (22)
Kumar [76]	Swiss Albino	1.5 years	0	0	0, 0	10/50 (20)	18/50 (36)
			100	15	14.5, 15.0	15/50 (30)	20/50 (40)
			1000	151	149.7, 151.2	16/50 (32)	19/50 (38)
			10000	1460	1453, 1466.8	19/50 (38)*	25/50 (50)*
Sugimoto [73]	CD-1	1.5 years	0	0	0, 0	2/50 (4)	6/50 (12)
			1600	153	165, 153.2	2/50 (4)	4/50 (8)
			8000	787	838.1, 786.8	0/50 (0)	8/50 (16)
			40000	4116	4348, 4116	6/50 (12)*	7/50 (14)
Atkinson *et al.* [75]	CD-1	2 years	N/A	0	0	4/50 (8)	14/50 (28)
			N/A	100	100	2/50 (4)	12/50 (24)
			N/A	300	300	1/50 (2)	9/50 (18)
			N/A	1000	1000	6/50 (12)	13/50 (26)
Knezevich and Hogan [72]	CD-1	2 years	0	0	0, 0	2/48 (4)	6[d]/50 (12)
			1000	157	157, 190	5[d]/49 (10)	6/48 (13)
			5000	814	814, 955	4/50 (8)	7[d]/49 (14)
			30000	4841	4841, 5874	2/49 (4)	11[d]/49 (22)
Takahashi [77]	CD-1	1.5 years	0	N/A	0, 0	N/A	3/50 (6)
			500		67.6, 93.2		1/50 (2)
			5000		685, 909		4/50 (8)
			50000		7470, 8690		6/50 (12)*

Abbreviations: EFSA, European Food Safety Authority; JMPR, Joint Meeting on Pesticide Residues; N/A, not available.
[a] Data sources: EFSA [71] and JMPR [22] for both males and females.
[b] Number of lymphomas / total mice in group.
[c] Data for male, female mice.
[d] Reported slightly differently in JMPR [22] ($N \pm 1$).
* $p_{trend} < 0.05$ reported by at least one test for trend in EFSA [71] or JMPR [22].

7.2. Additional considerations and recommendations

One challenge with these studies is that at face value they appear to be inconsistent because some show statistically significant findings whereas others do not. However, based on EPA's Cancer Guidelines, evidence of increased lymphoma incidence should not be discounted due to lack of statistical significance in trend and/or pairwise comparison tests. Additional factors that should not be used to exclude study findings are the use of high doses and/or incidence rates that are consistent with levels seen in historical controls [78].

Another consideration is that the study lengths in these animal experiments may have been insufficient for development of lymphoma. There are proposals that the standard timeframe of two years for a cancer bioassay to approximate long-term cancer incidence in humans should be extended to account for potentially longer latencies. Eighty percent of all human cancers occur after the age of sixty. A two-year-old rat approximates a human of 60–65 years, indicating a traditional two-year bioassay may not be sufficient for late-developing tumors [79].

Future work should combine the results from these six studies into an overall pooled analysis to give a more robust assessment of the evidence. A pooled analysis would take into account the varying study durations (of 18 or 24 months) as well as other between-study differences in dose regimens and mouse strains.

These studies, in which mice were exposed to only glyphosate, may have underreported incidence of malignant lymphoma given evidence of increased toxicity of GBHs compared to glyphosate alone [80–82]. GBH mixtures, which contain a number of adjuvants, have been reported to exert synergistic toxic effects in mechanistic studies (discussed below). Therefore, we also recommend the evaluation of GBHs in chronic animal carcinogenicity studies to better capture representative exposure of humans.

8. Potential mechanistic context

There are several possible mechanistic explanations for the increased NHL risk in humans and lymphomas in animals. The etiology of NHL remains largely unknown; however, potential risk factors include autoimmune diseases, infection with viruses and/or bacteria, immunosuppressant medications, and exposures to some pesticides [83,84]. Although not a formally recognized risk factor for NHL, endocrine disruptors have been associated recently with risk of B-cell neoplasms [85], most of which are NHL [57]. Furthermore, a genetic hallmark of NHL is the recurrence of chromosomal translocations, such as t(14;18), involving the immunoglobulin heavy chain gene fusion (*BCL2-IGH*), which are frequently detected in subgroups of NHL patients [86] and in pesticide-exposed farmers [87,88]. Hence, we discuss immunosuppression/inflammation, endocrine disruption, genetic alterations, and oxidative stress as potential underlying mechanisms for the development of lymphoma. Genetic alterations (genotoxicity) and oxidative stress were previously identified as two glyphosate-related key characteristics of carcinogens [126]. Although not specifically linked to NHL, oxidative stress is a general mechanism of carcinogenesis that could contribute to lymphomagenesis.

8.1. Immunosuppression/inflammation

The strongest factors known to increase NHL risk are congenital and acquired states of immunosuppression [89]. Several studies suggest that glyphosate alters the gut microbiome [80,90] and cytokine IFN-γ and IL-2 production [91]. These changes could impact the immune system,

promote chronic inflammation [92], and contribute to susceptibility of invading pathogens, such as *H. pylori* [93].

8.2. Endocrine disruption

Disruption of sex hormones may contribute to lymphomagenesis/NHL [94]. Glyphosate may act as an endocrine disrupting chemical (EDC) because it has been found recently to alter sex hormone production. Several *in vivo* studies of male rats exposed to glyphosate have reported significantly lower testosterone levels [95–97], spermatid numbers [95], altered sperm and testicular morphology [95,96], greater development of the mammary gland [98], and a surge in mast cell infiltration and proliferation accompanied by increased estrogen receptor (ESR1) [98]. In ovarian granulosa cells, glyphosate exposure resulted in decreased cell proliferation and estradiol production [99], which may contribute to lymphomagenesis [94].

8.3. Genetic alterations

Several studies report that glyphosate can induce single- and double-strand DNA breaks [100–103], purine and pyrimidine oxidation [101], increased comet tail moment [104], and activation of the canonical non-homologous end-joining pathway (c-NHEJ) [102] that stimulates DNA repair. Glyphosate was also reported to induce micronuclei [105–111], sister chromatid exchanges [110], and chromosomal aberrations [112], but other studies found no change in these parameters [113–117]. Conclusions on the genotoxicity of glyphosate remain controversial in the debate on its carcinogenic potential [118]. A recent review reported that this discrepancy could be attributed to differences in the literature analyzed (published versus unpublished), exposure type (glyphosate versus GBHs), and exposure magnitude (low everyday exposures versus higher exposure groups) [119].

8.4. Oxidative stress

Numerous studies indicate glyphosate causes oxidative stress [120–123]. Biomarkers of oxidative stress have been reported in a number of tissues in rats and mice, including liver, skin, kidney, brain, and plasma. In a study of *albino male rats*, levels of hepatic reduced glutathione were significantly decreased in GBH-exposed animals (1.64 mmol/g) compared to controls (2.64 mmol/g) [81]. A different study in glyphosate-exposed *Wistar rats* reported increased lipid peroxidation across all tissues studied and reactive nitrogen species in the brain and plasma [120]. A proteomic analysis of *Swiss albino mice* reported overexpression of carbonic anhydrase 3, a cytoplasmic protein that plays a role in cellular response to oxidative stress [124].

Generally speaking, these mechanisms, among others, provide evidence of biological plausibility for the observed link between glyphosate exposure and human NHL, though further work is needed to better understand these factors.

9. Conclusions and future directions

The rise of GBHs as the most widely used herbicide raises serious health concerns, given its potential links with NHL. Using our *a priori* hypothesis and including the recently updated AHS cohort in a meta-analysis for the first time, we report that GBH exposure is associated with increased risk of NHL in humans. Our findings are consistent with results reported from prior meta-analyses but show higher risk for NHL because of our focus on the highest exposure groups. However, given the heterogeneity between the studies included, the numerical risk estimates should be interpreted with caution. Additionally, as noted above and depicted in Fig. 1, the available studies do not capture the possible effects of increased population exposures due to secular increases in use. For example, “green burndown” practices that became widespread in the mid-2000s may be a particularly important source of population exposures. The totality of the evidence from six studies of glyphosate-exposed mice support this association in humans. Although the underlying mechanisms remain unknown, mechanistic studies of glyphosate-induced immunosuppression/inflammation, endocrine disruption, genetic alterations, and oxidative stress suggest plausible links between GBH exposure and NHL development. The overall evidence from human, animal, and mechanistic studies presented here supports a compelling link between exposures to GBHs and increased risk for NHL.

Declaration of interest

All authors have no financial conflicts of interest to declare. We disclose Drs. Zhang, Taioli, and Sheppard served as Science Review Board Members of the US EPA FIFRA Scientific Advisory Panel (SAP) Meeting that evaluated glyphosate in December 2016.

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