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Exposure to Glyphosate-Based Herbicides and Risk for Non-Hodgkin Lymphoma: A Meta-Analysis and Supporting Evidence.

Glyphosate and Non-Hodgkin Lymphoma: An Independent Evaluation

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Abstract

Glyphosate is the most widely used broad-spectrum systemic herbicide in the world. Recent evaluations of glyphosate's carcinogenic potential by various regional, national and international agencies have engendered controversy. We independently investigate

whether there is an association between high cumulative exposures to glyphosate and increased risk of non-Hodgkin lymphoma (NHL) in humans and conduct a new meta-analysis that includes the most recent update of the *Agricultural Health Study* (AHS) cohort in 2018 along with five case-control studies. For comparison, we also perform an additional meta-analysis with the earlier AHS (2005) report and multiple sensitivity tests to assess the validity of our findings. Using the highest exposure groups when available in our meta-analyses, we report the overall meta-relative risk (meta-RR) of NHL in glyphosate-exposed workers is increased by 41% (meta-RR=1.41, 95% CI, confidence interval: 1.13–1.75). Our comparison meta-analysis with the earlier AHS shows an increased meta-RR for NHL of 1.45 (95% CI: 1.11–1.91), which is higher than the meta-RRs previously reported. Sensitivity tests did not reveal meaningful differences from our estimated meta-RR. To contextualize our findings of an increased NHL risk in workers with high glyphosate exposure, we also consider available animal and mechanistic studies. We uncover further support in studies of malignant lymphoma incidence in mice treated with glyphosate, and its potential links to immunosuppression, endocrine disruption, and genetic alterations that are commonly associated with NHL. We recommend that future animal studies investigate the glyphosate-based formulations that most humans are exposed to.

(232/300 words)

Keywords: Glyphosate-based herbicides, pesticide, Roundup, Ranger Pro, carcinogenesis, and meta-analysis.

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; ETC, 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; NHL, non-Hodgkin lymphoma; OR, odds ratio; ppm, parts per million; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analysis; RR, relative risk.

1. Introduction

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 increased dramatically worldwide in recent decades. In the United States alone, usage increased nearly sixteen-fold between 1992 and 2009 [1]. Most of this increase occurred after genetically modified glyphosate-resistant ("Roundup-ready") crops appeared in 1996 [1]. In addition, there have been dramatic 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 with the consequence that crops 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].

1.2 Controversy Surrounding Glyphosate's Carcinogenic Potential

Exposure to glyphosate is reportedly associated with several types of cancer, among which the most-well studied in humans is non-Hodgkin lymphoma (NHL). Some epidemiological studies reported an increased risk of NHL in glyphosate-exposed workers [5-7], while other studies did not confirm this association [8, 9]. Glyphosate has recently undergone a number of regional, national and international carcinogenic evaluations [10-13], which have incited controversy regarding glyphosate's overall carcinogenic potential. Hence, addressing the question of whether or not glyphosate is associated with NHL has

become even more critical. Here, we evaluate the scientific body of research from all published human studies and present the first meta-analysis to include the most recently updated Agricultural Health Study (AHS) cohort [14]. We close with a discussion of the lymphoma-related results reported in experimental animal studies as well as mechanistic considerations in order to integrate our findings with the literature.

1.3 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 may appear to be conflicting, 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 conduct a meta-analysis using published human studies to better understand whether the epidemiological evidence supports an association between glyphosate exposure and increased NHL risk. While three previously published meta-analyses have examined the same association [12, 15, 16], this current meta-analysis differs from earlier ones by focusing on an *a priori* hypothesis targeting exposure magnitude and by including the newly updated AHS study [14].

2. Methods

2.1 A Priori Hypothesis

Our *a priori* hypothesis is that the highest exposure to glyphosate, *i.e*. higher levels or longer durations, 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 [17]. 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 [18] and formaldehyde [19]. The 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 [20]. 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 directly or indirectly exposed to GBHs. Since 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 glyphosate-NHL association exists.

2.2 Agricultural Health Study Update

A recently published update [14] from the large AHS cohort of American pesticide applicators (N>50,000) has been included for the first time in our primary meta-analysis. While the original AHS report [9] was used in previous meta-analyses [12, 15, 16], the 2018 AHS update [14] 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 [9]) and >80% of the total cohort was estimated to be exposed to glyphosate. As the largest and most recently published study, it adds substantial weight to the new meta-analysis [14]. We also performed a comparison meta-analysis using our *a priori* hypothesis with the original AHS report [9] for the purpose of comparing results with our primary meta-analysis (2018 AHS update) and with previous meta-analyses.

2.3 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) [21]. Details of the search terms and strategy are available in Appendix Section A.1; the screening process and results are shown in Figure 1. Briefly, PubMed was searched with various combinations of keywords such as “glyphosate,” “lymphoma” and “farmer.” Overall, 866 studies were initially screened by title and abstract, of which 850 were excluded because they were reports, reviews, irrelevant studies (animal, mechanistic, para-occupational), did not include the exposure or outcome of interest, or were correspondence (Figure 1).

When the final 16 qualified epidemiological studies of glyphosate and NHL were identified, 10 studies were further excluded because: (1) they did not report relative risk (RR) estimates, odds ratios (OR), or the data needed to calculate them [22-24]; (2) the

cohort overlapped with another study [9, 25-29]; or (3) they did not specify whether the lymphomas were specifically NHL [30]. For studies including overlapping cohorts, we used results from the most complete and updated analysis with the greatest number of participants. Additionally, though overlapping, we kept the earlier AHS 2005 cohort study for comparison with updated AHS 2018 and with previous meta-analyses. Sensitivity analyses were performed to evaluate the impact of these exclusions on the results.

Table A.1 summarizes the results and characteristics of all the studies evaluated in this meta-analysis, including both versions of the AHS report (n = 6+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 and category, results, and statistical adjustments. We evaluated the strengths and weaknesses of the individual studies used in the meta-analysis (Table A.1), and conducted a quality assessment of the cohort and case-control studies detailed in Appendix Section A.2 (Tables A.2 and A.3).

2.4 Selection of the Most Highly Exposed Category

Based on our *a priori* hypothesis, when multiple RRs or ORs were given in original studies, we selected the result for the highest exposure category 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. In both AHS reports [9, 14], cumulative exposure was calculated as an

intensity-weighted exposure (lifetime exposure days multiplied by an intensity score) [31, 32]. RR estimates that adjusted for other pesticide use were selected over their unadjusted counterparts to mitigate potentially substantial confounding by other pesticide use.

We prioritized highest cumulative exposure based on evidence of glyphosate's persistence in the environment [33-35] and because chronic disease, including cancer, is usually the result of cumulative exposures [36]. We selected longest lag or latency, as decades may be needed for the health effects of many environmental toxicants to manifest as detectable cancers. If no high exposure data were available, we used ever-exposure. Given the relatively few human studies published to date on the topic, we did not want to exclude potentially relevant human data even with the risk of underestimating any association, if it exists, through possible inclusion of minimally-exposed individuals. 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.* [9] was used; the ever-RR estimate from Andreotti *et al.* [14] was not available). We summarize the risk estimate data selected from each original study and the study weights used in the meta-analyses in Table 1.

In total, we included one cohort study [14] and five case-control control studies [5-8, 37] in our primary 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 A.1).

2.5 Statistical Analysis

We calculated the meta-analyzed summary relative risk (meta-RR) and confidence intervals using both the fixed effects inverse variance method [20] and the random effects method [38]. 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. We report only the fixed effects model estimates unless heterogeneity was present. We evaluated heterogeneity, defined as the X^2-test statistic for heterogeneity being greater than its degrees of freedom, using the summary variance method [39]. If heterogeneity was present [39], then we report both the results for the fixed effects model and the random effects model (see Table 2).

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 [39]. However, a problem with 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 [39]. For these reasons, our results focus on the fixed effects model.

Publication bias was evaluated through funnel plots, Egger's test, and Begg's test [40, 41]. All statistical analyses were conducted with Stata IC 15.1 [42] and Microsoft Excel 2013 [43].

3. Results

3.1 Meta-Analysis Findings

Table 2 includes the results from our two meta-analyses, which include the primary analysis using the most recently updated AHS cohort [14], and the comparison analysis using the original report [9]. Using the updated AHS results [14], we observe 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 glyphosate exposure. With the original AHS 2005 cohort results, we observe 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 (Figure 2A-B) and Funnel plots (Figure 2C-D) from these two major meta-analyses are reported in Figure 2.

We observe little evidence of publication bias in the Funnel plots (Figure 2C-D), Eggers (p=0.185), and Beggs tests (p=0.851). Overall, however, we cannot exclude the potential of publication bias, given the limited number of studies.

We also assessed the effect of *a priori* selection of the longest exposure duration to compare with 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 2).

3.2 Sensitivity Analyses

We conducted several sensitivity analyses to evaluate the impact of excluding or including different studies (Tables 2 and 3). When we excluded the only cohort study and

limited our analysis to the case-control study design (Table 2), there was little inter-study heterogeneity and we estimated a doubling of the NHL risk (meta-RR=**1.84**, 95% CI: 1.33-2.55) from 41% to 84%. While the RR of the only cohort study was not statistically significant (RR = 1.12, 95% CI: 0.83-1.51), the upper 95% CI overlapped with the CI of the case-control meta-RR estimate. Although our primary meta-analysis included six studies, there was a possibility to include a seventh study [30]. We excluded this study from the primary analysis because it included all B-cell lymphomas, which account for approximately 85% of all NHL [44], however, not all four cases were confirmed to be NHL. When we added Cocco *et al.* [30] to the meta-analysis (n=7, Table 2), the resulting RR remained fairly similar at **1.43** (95% CI: 1.15-1.78).

Similar to our inclusion of the Cocco *et al.* [30] study which evaluated all B-cell lymphomas (Table 2), another cell-type specific study evaluated all cases of hairy cell leukemia (HCL), a subtype of NHL [28]. It is one of two studies [27, 28] included in the Hardell *et al.* [7] analysis, with the other study examining NHL only [27]. Excluding HCL cases had no effect on the meta-RR **1.41** (95 % CI: 1.13-1.77, Table 3). Similarly, using only hairy-cell leukemia cases from Hardell *et al.* [7] (reported in Nordstrom *et al.* [28] did not impact the meta-RR (1.43, 95% CI: 1.14-1.78).

Additional sensitivity analyses are described in Table 3. When evaluating studies with only the highest levels of exposure [6, 14, 37], the meta-analysis relative risk (meta-RR) was **1.36** (95% CI: 1.06-1.75, Table 3). In studies that combined all exposures as ever exposed [5-9, 37], the meta-RR was **1.30** (95% CI: 1.03-2.64). While the higher exposure group was used in the main analysis, Eriksson *et al.* [6] also provided results for greater than 10 years latency, which contributed to a meta-RR of **1.40** (95% CI: 1.13-

1.75). Studies in North America [5, 14, 37] had a meta-RR of **1.38** (95% CI: 1.08-1.76), while European studies [6-8] had a meta-RR of **1.53** (95% CI: 0.93-2.52). On average, when studies adjusted for other pesticide use [5-7, 9] 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). Note: AHS 2018 did not provide ever-exposure, so AHS 2005 was used to calculate this statistic and *ever* exposure above.

Consistent with the two previous meta-analyses by IARC [12] and Schinasi and Leon [15] discussed in Section 4.1 below, we selected the RR estimated using the more traditional logistic regression over the hierarchical regression estimate in the case-control study by De Roos *et al.* [5], and found there was little impact of this selection (meta-RR=**1.36**, 95% CI: 1.09-1.70). When Cantor *et al.* [26] or Lee *et al.* [25] were used instead of De Roos *et al.* [5], 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.* [29] instead of McDuffie *et al.* [37] caused the meta-RR to decrease to **1.23** (95% CI: 0.99-1.53). Excluding each of the case-control studies slightly lowered the risk estimate, except for Orsi *et al.* [8], where the meta-RR increased to **1.46** (95% CI: 1.16-1.83).

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 they all nominally lowered the meta-RR (Table 3), except for the exclusion of Orsi *et al.* [8], where the meta-RR increased to **1.46** (1.16-1.83).

4. Discussion

Our primary meta-analysis including the new AHS 2018 study and our *a priori* hypothesis suggest that there is an increased risk of NHL in individuals highly exposed to glyphosate/GBHs (meta-RR = 1.41, 95% CI: 1.13-1.74). The corresponding estimate, using the original AHS 2005 study is 1.45 (95% CI: 1.11-1.91; Table 2 and Figure 2B). In this section, we compare our findings to previous meta-analyses exploring the same association. Further, we evaluate the strengths and limitations of our meta-analyses, as well as of the cohort study and the case-control studies utilized. Lastly, we integrate our findings from the human studies with relevant animal and mechanistic data.

4.1 Comparison with Previous Meta-Analyses

Previous meta-analyses of NHL in relation to glyphosate exposure reported lower, albeit positive, risk estimates. In contrast to our work, these analyses did not focus on the highest exposed groups. The major results from the three published meta-analyses [12, 15, 16] are summarized in Table 4.

Schinasi and Leon [15] first reported a meta-RR of **1.45** (95% CI: 1.08-1.95). While their selection criteria stated 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 [6, 7]. The IARC Working Group subsequently corrected this discrepancy in an otherwise identical meta-analysis [12] resulting in a meta-RR of **1.30** (95% CI: 1.03 -1.65). Most recently, Chang and Delzell [16] 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.

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 available in only three studies [6, 9, 37]. In our comparison meta-analysis with the same six studies (including AHS 2005), we found an additional 0.15-0.18 higher NHL relative risk than previous meta-RRs [12, 16] (not including Schinasi and Leon, since 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 increase in NHL relative risk to the previous meta-RRs [12, 16]. Overall, the meta-RR obtained using our *a priori* hypothesis suggests increased risk of NHL in workers highly exposed to glyphosate.

4.2 Strengths and Limitations of Our Meta-Analysis

The strengths of these meta-analyses 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 response effect. The current meta-analysis is also the first study to include the newly updated AHS.

Weaknesses of the analysis include the availability of limited data for inclusion, given the relatively few published studies to date, and the imbalance in study design; among only six included studies, five were case-control and one was a cohort. Findings in the cohort study suggested no excess risk [14], in contrast to the evidence suggested by most of the case-control studies [5-7, 37].

4.3 Strengths and Limitations of the AHS Cohort Study

Our new meta-analysis is the first to include the AHS 2018 update, the largest, newest, and most heavily weighted study (>50%, Table 1). Given the magnitude of the study and the recency of its findings, several aspects of the AHS study are discussed below.

4.3.1 Exposure Quantification

The risk estimates generated from the follow-up AHS 2018 report depended on "multiple imputation" to generate glyphosate exposure information for the 37% of participants who did not complete the follow-up questionnaire [14]. Their imputation method relied exclusively on the reported pesticide use and other data, including demographics, medical history at baseline, and farming characteristics at enrollment [45]. The imputations did not use the NHL or any other cancer outcome information reported by Andreotti *et al.* [14]. This approach is problematic because it is known that multiple imputation of a covariate (*e.g*. the exposure variable) in a model that omits the outcome variable to be used in the inference leads to attenuation of the effect estimate for that covariate [46]. Since 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.* [47]. 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 "imputed" (i.e. simulated) in a model that does not take the NHL outcome into account, the uncertainty in the "imputed" exposure behaves like classical measurement error and thus will bias the effect estimate towards the null.

4.3.2 Exposure Misclassification

Non-differential misclassification occurs when exposure status is equally misclassified among exposed cases and unexposed controls. Differential misclassification is unlikely in a cohort study since exposure is assessed prior to the disease occurrence. Non-differential misclassification may occur in the context of a ubiquitous exposure, as it is hard for participants to know to what extent or how long they have been exposed [48]. Glyphosate's ubiquity in the environment leads to profound concerns that even "unexposed" individuals in the cohort are likely to have been exposed to glyphosate; consequently, the magnitude of any potential association 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 carry some cancer risk and are not the ideal true reference group in studies of smoking and tobacco related cancers [49]. These instances of non-differential misclassification are likely to attenuate measures of association, and bias the RR toward the null of 1.0 [50]. Although it is difficult to ascertain exactly, the extent of non-differential misclassification in cohort studies can be estimated through smaller-scale validation studies [50].

4.3.3 Latency

A median latency range of 15-20 years for chronic, low level exposures is expected for NHL [51]. The follow-up period (median=6.7 years) in the 2005 AHS study [9] may have been too short for a sufficient number of exposure-related cancer events to manifest. Given that participants had been exposed to glyphosate 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, while 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 report added an additional 11-12 years of follow-up for all study participants and an additional 483 cases of NHL, increasing the RR for those most highly exposed from **0.8** (95% CI: 0.5-1.4) [9] to **1.12** (95% CI: 0.83-1.51) [14] listed in Table 1. Epidemiologic studies often lag exposures to account for disease latency under the assumption that recent exposure has very little impact on disease development. Theoretically, even longer exposure durations and/or lags would present a higher NHL risk in the most highly exposed groups, as is indicated in the study-specific estimates from the case-control studies. However, given the challenge in interpreting the new AHS study results (37% of participants did not respond to the follow-up questionnaire and thus had their follow-up simulated), it is difficult to discern how much additional information was added by the longer follow-up duration in this study.

Overall, both the impact of exposure simulation and the high probability of non-differential misclassification may have played a role in the weaker trend of the large highly-weighted AHS study, which in turn diluted the meta-RR of the primary meta-analysis.

4.4 Strengths and Limitations of Case-Control Studies

Five of the six studies included in this meta-analysis are case-control designs. 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 better by cases and underreported by controls. Cases may have been more motivated to recall glyphosate exposure, and the exposures may be more vivid or meaningful due to awareness of the risk factors for their disease. With differential misclassification, the OR can be artificially inflated (if cases are more likely to report exposure) or deflated (if cases are less likely to report exposure).

Despite these limitations, case-control studies are the optimal choice when studying a rare disease, such as NHL. For example, the only cohort study of glyphosate 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, while the 5 case-control studies assembled 2,836 NHL cases among all participants (N=8,868) in a much shorter period of time (Tables 1 and A.1). 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 2) and reported similar findings pointing away from null.

4.5 Summary of the Glyphosate and NHL Association

Overall, the results from our new meta-analysis employing the *a priori* hypothesis and including the updated AHS 2018 study: 1) demonstrate a significantly increased NHL risk in highly glyphosate-exposed individuals (meta-RR=**1.41**, 95% CI: 1.13-1.75; Table 2 and Figure 2A); 2) confirm similar findings (Table 4) from previous meta-analyses [12, 16]; 3) reveal an additional 11-14% and 15-18% increase in NHL relative risk due to high

levels of glyphosate exposure (Table 4) 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 glyphosate, more precisely to GBHs, is associated with a statistically significant increased risk of NHL.

As 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 NHL manifested as a result of exposure to the mixture or a different ingredient in the formulation. To investigate causal inference regarding the association between glyphosate exposure and NHL, we briefly discuss whether or not the association identified from epidemiological studies could be further supported by experimental animal and mechanistic studies.

4.6 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 [52] 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 [11]. EFSA [52] reported results from five unpublished studies: four in CD-1 [53-56] and one in Swiss albino mice [57], while JMPR [11] also reported data from a study in female CD-1 mice [58] (see Appendix Section A.3). Each study reported four glyphosate doses, and

corresponding lymphoma incidence in males and females, except for Takahashi [58], where the only data available in the public domain was for female mice [11].

Table A.4 documents these data in detail and also includes glyphosate exposure as ppm (parts per million) and a dose (mg/kg/day), as reported by these agencies. In summarizing these studies, EFSA [52] noted that Sugimoto [54] and Wood *et al.* [55] showed statistically significant dose-response in males according to the Cochran-Armitage test for linear trend, while Kumar [57] showed statistically significant Z-test for both males and females. In agreement, JMPR [11] noted that Sugimoto [54] and Wood *et al.* [55] showed a statistically significant trend in males, and that Kumar [57] reported statistically significant increases in malignant lymphoma in high dose groups of both males and females. JMPR [11] further reported Takahashi [58] 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.

One challenge with these studies is that at face value they appear to be inconsistent because some show statistically significant findings while others do not. However, consistent with the framework provided by EPA in its Cancer Guidelines, evidence of increased lymphoma incidence should not be discounted based on non-standard considerations for hazard assessments of long-term carcinogenicity studies (i.e. the high doses were too high, there was lack of statistical significance in trend and/or pairwise comparison tests, and the incidence was consistent with levels seen in historical controls) [59]. 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 species.

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 [60-62]. GBH mixtures, which contain a number of adjuvants, have been reported to exert synergistic toxic effects in mechanistic studies (Section 4.7). Therefore, we recommend chronic carcinogenicity studies of animals exposed to GBHs should be conducted to better capture representative exposure of humans.

4.7 Potential Mechanistic Context

There are several possible mechanistic explanations for the increased NHL risk in humans and lymphomas in animals. While the etiology of NHL remains largely unknown, potential risk factors include autoimmune diseases, infection with viruses and/or bacteria, immunosuppressant medications, and exposures to some pesticides [63, 64]. Although not a formally recognized risk factor for NHL, endocrine disruptors have recently been associated with risk of B-cell neoplasms [65] most of which are NHL [44]. 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 [66] and in pesticide-exposed farmers [67, 68]. Hence, immunosuppression, viral/bacterial infections, endocrine disruption, and genetic alterations have been suspected as key underlying mechanisms in the development of lymphoma (lymphomagenesis).

4.7.1 Immunosuppression/Inflammation

The strongest factors known to increase NHL risk are congenital and acquired states of immunosuppression [69]. Several studies suggest that glyphosate alters the gut microbiome [60, 70] and cytokine, IFN-γ and IL-2 production [71]. These changes could impact the immune system, associate with chronic inflammation [72], and contribute to susceptibility of invading pathogens, such as *H. pylori* [73].

4.7.2 Endocrine Disruption

Disruption of sex hormones may contribute to lymphomagenesis or NHL [74]. Glyphosate may act as an endocrine disrupting chemical (EDC), as it has been found to alter sex hormone production [75-77], increase mammary gland development and estrogen receptor (ESR1) in males [78], and decrease estradiol production in ovarian granulosa cells [79].

4.7.3 Genetic Alterations

Several studies report that glyphosate can induce single and double strand DNA breaks [80-83], purine and pyrimidine oxidation [81], increased comet tail moment [84], and activation of the canonical non-homologous end joining pathway (c-NHEJ) [82] that stimulates DNA repair. Glyphosate was also reported to induce micronuclei [85-91], sister chromatid exchanges [90], and chromosomal aberrations [92], but other studies found no change in these parameters [93-97].

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 pathways.

5. Conclusion

The rise of glyphosate as the most widely used herbicide raises serious health concerns, given potential links with NHL. Using our high-exposure *a priori* hypothesis and including the recently updated AHS cohort in a meta-analysis for the first time, we report that glyphosate exposure is associated with increased risk of NHL in humans. Our findings are consistent with results reported from prior meta-analyses but show higher NHL risk, likely due to our focus on the highest exposure groups. Malignant lymphoma incidence in a few studies of glyphosate-exposed mice support this association in humans and, although the underlying mechanisms remain unknown, biological studies of glyphosate-induced immunosuppression/inflammation, endocrine disruption, and genetic alterations suggest plausible links between glyphosate exposure and NHL development. The overall evidence from human, animal, and mechanistic studies presented here supports glyphosate's carcinogenic potential in mediating NHL. Given that humans are exposed to adjuvant-containing mixtures known to provoke synergistic toxic effects *in vivo* and *in vitro*, future studies of GBHs in experimental animals should be conducted.

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.

Conflict 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

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Figure Captions

Figure 1. Study Selection Process for Meta-Analysis using PRISMA Guidelines.

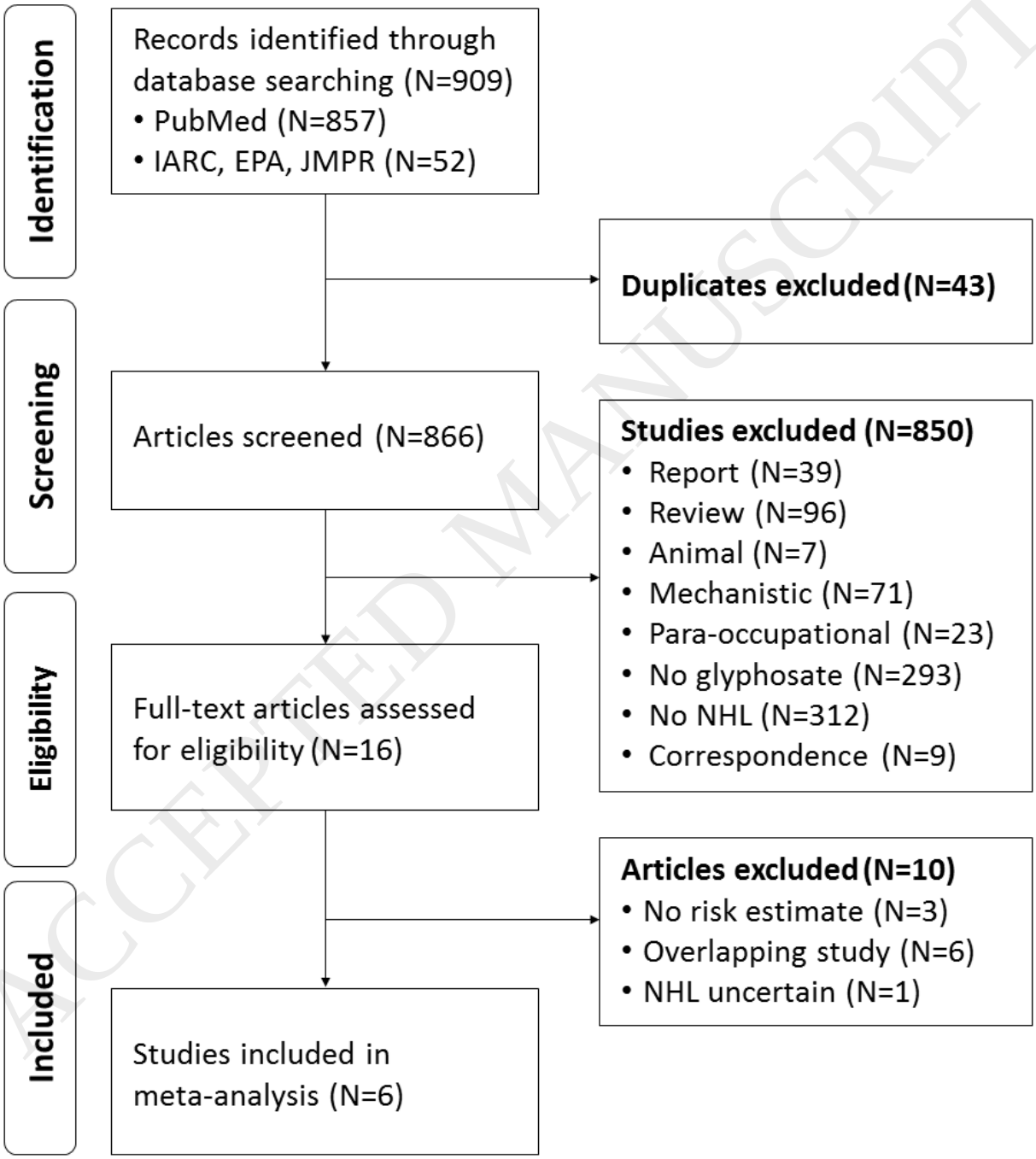
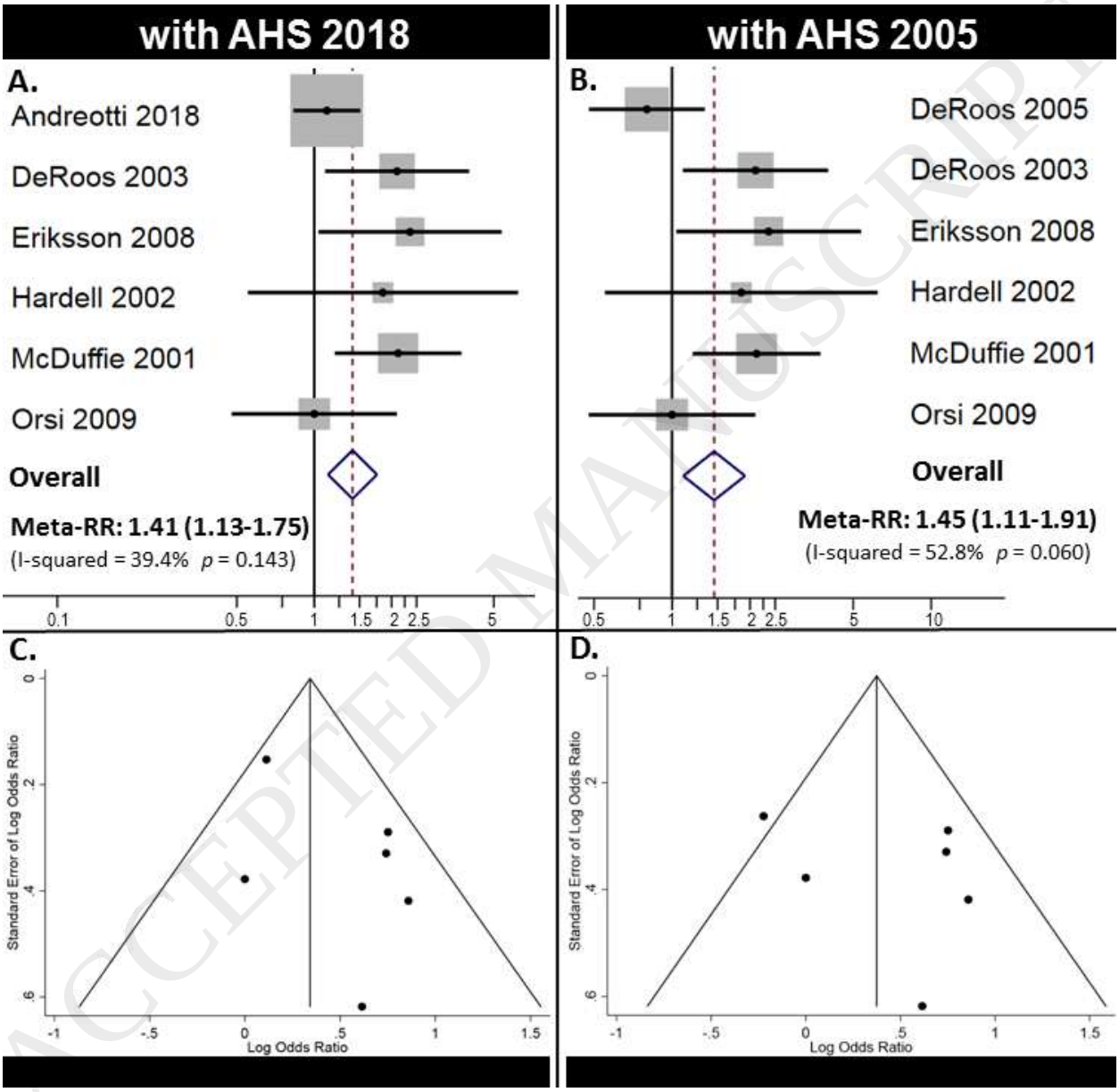


Figure 2. Major meta-analysis results. A) Forest plot for meta-analysis using AHS 2018 and B) using AHS 2005. C) Funnel plot for meta-analysis using AHS 2018 and D) using AHS 2005.



Tables

Table 1: Description and weight of studies selected for the current meta-analyses.

Study (Author, Year)	Case No. (Exp/Tot)	Exposure Category	Risk Estimate[a] (95% CI)	Weight[b]	
				AHS 2018	AHS 2005
AHS Cohort					
Andreotti *et al.* [14]	55/575	≥2610 d/l [c,d]	1.12 (0.83, 1.51)	54.04	--
De Roos (2005) [9]	22/92	≥337.2 d/l [c]	0.8 (0.5, 1.4)	--	28.43
Case-Control					
De Roos (2003) [5]	36/650	Ever, log	2.10 (1.10, 4.00)	11.61	18.08
Eriksson *et al.* [6]	17/910	>10 d/y	2.36 (1.04, 5.37)	7.18	11.18
Hardell *et al.* [7]	8/515	Ever	1.85 (0.55, 6.20)	3.30	5.14
McDuffie *et al.* [37]	23/517	>2 d/y	2.12 (1.2, 3.73)	15.05	23.43
Orsi *et al.* [8]	12/244	Ever	1.0 (0.5, 2.2)	8.82	13.73

Abbreviations: AHS, Agricultural Health Study; d, days; exp, exposed; l, lifetime; log, logistic regression; tot, total; y, year.

[a] Relative risk (RR) reported in both AHS analyses and odds ratio (OR) reported in all case-control studies.

[b] Weight given to each study in 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).

Table 2. Major Findings from Current Meta-Analyses

		Fixed Effects		Random Effects[a]		Heterogeneity	
Analysis	**N**	**meta-RR (95% CI)**		**meta-RR (95% CI)**		X^2	*p*
Highest cumulative exposure							
AHS (2018) [14]	6	1.41 (1.13, 1.75)		1.56 (1.12, 2.16)		8.26	0.14
AHS (2005) [9][b]	6	1.45 (1.11, 1.91)		1.52 (1.00, 2.31)		10.59	0.06
Longest exposure duration							
AHS (2018) [14]	6	1.41 (1.13, 1.74)		1.56 (1.12, 2.16)		8.21	0.15
AHS (2005) [9][b]	6	1.56 (1.17, 2.06)		1.57 (1.06, 2.26)		7.81	0.17
Study design							
Case-control [5-8, 37]	5	1.84 (1.33, 2.55)				3.36	0.50
Cohort (AHS 2018) [14]	1	1.12[c] (0.83, 1.51)					
Other							
Add Cocco *et al.* [30][d]	7	1.43 (1.15, 1.78)		1.59 (1.16, 2.18)		9.10	0.17

Abbreviations: AHS, Agricultural Health Study; meta-RR, meta-relative risk; N, number of studies.

[a] Random effects model was only presented when X^2 heterogeneity statistic was greater than degrees of freedom (number of studies minus 1)

[b] De Roos *et al.* [9] used instead of Andreotti *et al.* [14] for comparison.

[c] Since there was only one cohort study, the RR is presented instead of a meta-RR.

[d] The study combined all B-cell lymphomas and is added to the analysis on cumulative exposure duration (AHS 2018).

Table 3. Sensitivity tests for meta-analysis

		Fixed Effects		Random Effects[1]		Heterogeneity	
Analysis	**N**	**meta-RR (95% CI)**		**meta-RR (95% CI)**		**X^2**	***p***
Exposure							
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)				3.73	0.59
Latency[3]	6	1.40 (1.13, 1.75)		1.54 (1.12, 2.13)		8.01	0.16
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[4]							
Adjusted (AHS 2005)	4	1.46 (1.05, 2.02)				2.61	0.46
Unadjusted (AHS 2005)	4	1.69 (1.29, 2.23)		1.70 (1.26, 2.30)		3.47	0.33
Hardell *et al.* [7]							
Exclude HCL[5]	6	1.41 (1.13, 1.77)		1.61 (1.11, 2.34)		9.58	0.09
Only use HCL[6]	6	1.43 (1.14, 1.78)		1.62 (1.14, 2.31)		9.36	0.10
De Roos *et al.* [5]							
Hierarchal OR[7]	6	1.36 (1.09, 1.70)		1.46 (1.08, 1.96)		6.80	0.24
Cantor *et al.* [26][8]	6	1.29 (1.04, 1.59)		1.36 (1.02, 1.80)		7.07	0.22
Lee *et al.* [25][9]	6	1.35 (1.11, 1.65)		1.41 (1.09, 1.82)		6.63	0.25
Other							
Hohenadel vs. McDuffie[10]	6	1.23 (0.99, 1.53)		1.30 (0.96, 1.76)		7.34	0.20
Exclude one study[11]							
Andreotti *et al.* [14]	5	1.84 (1.33, 2.55)					
De Roos *et al.* [5]	5	1.34 (1.06, 1.69)		1.47 (1.02, 2.11)		6.59	0.16
Eriksson *et al.* [6]	5	1.35 (1.08, 1.70)		1.47 (1.04, 2.07)		6.62	0.16
Hardell *et al.* [7]	5	1.40 (1.12, 1.75)		1.56 (1.08, 2.24)		8.06	0.09
McDuffie *et al.* [37]	5	1.31 (1.03, 1.66)		1.43 (1.01, 2.03)		5.90	0.21
Orsi *et al.* [8]	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. Random effects model is only provided if heterogeneity is present, defined as X^2 heterogeneity statistic > 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.* [6] results for any glyphosate exposure >10 years latency was used instead of the higher exposure group used in the main analysis.
4. 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.
5. Hairy cell leukemia cases excluded—results presented in Hardell and Eriksson [27].
6. NHL cases excluded; only HCL results used—results presented in Nordstrom *et al.* [28].
7. Hierarchical model RR used instead of the standard logistic regression model RR.
8. Cantor *et al.* [26] used instead of De Roos *et al.* [5]. Cantor *et al.* [26] was the only of the three studies combined by De Roos *et al.* [5] that presented data for glyphosate.
9. Lee *et al.* [25] used instead of De Roos *et al.* [5]. Lee *et al.* [25] used same subjects as De Roos *et al.* [5] but did not adjust for other pesticide exposure, did not exclude those with missing data on other pesticide use, and used only non-asthmatics.
10. Hohenadel *et al.* [29] used same subjects as McDuffie *et al.* [37] but presented results in subjects exposed to glyphosate but not malathion (OR=0.92; 95% CI: 0.54-1.55).
11. One study excluded at a time to evaluate the impact of each individual study on the overall meta-RR.

Table 4. Comparison of current meta-analysis to other published meta-analyses

Studies	Schinasi and Leon [15][a]	IARC [12]	Chang and Delzell [16][a]	Current Meta-Analysis	
				with AHS 2005 [9]	with AHS 2018 [14]
	RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)
Andreotti *et al.* [14]	N/A	N/A	N/A	N/A	1.12 (0.83-1.51)
De Roos (2005) [9]	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 (2003) [5]	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.* [6]	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.* [7]	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.* [37]	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.* [8]	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: CI, confidence interval; 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.