3. Literature Review of Carcinogenicity Publications

Over the 40 year product history of glyphosate based herbicides, regulatory expert and other authoritative review panels have evaluated multiple data sets to evaluated glyphosate safety, including potential for carcinogenicity. These multiple reviews over the decades have consistently drawn the same conclusion; glyphosate is not carcinogenic. These conclusions include those of the U.S. Environmental Protection Agency in 1993 and 1997 (Category E, evidence of non-carcinogenicity for humans -- based on the lack of convincing evidence of carcinogenicity in adequate studies); the European Commission's Health and Consumer Protection Directorate-General in 2002 (no evidence of carcinogenicity); the U.S. Forest Service (based on standard animal bioassays for carcinogenic activity *in vivo*, there is no basis for asserting that glyphosate is likely to pose a substantial risk); Canadian regulators (no evidence that glyphosate causes cancer); the World Health Organization and Food and Agriculture Organization of the United Nations in 2004 (long-term studies of toxicity and carcinogenicity were conducted in mice and rats. In the study of carcinogenicity in mice, no toxic effects were observed at up to the highest dose tested (1000 mg/kg bw per day), and there was no evidence of carcinogenicity).

A number of epidemiology studies over the last decade have focused on pesticide exposure and associated health outcomes. Publications vary in the specificity of their conclusions regarding pesticides in general, classes of pesticides and in some cases individual insecticides, herbicides or fungicides. While some of these publications specifically mention glyphosate, few draw tenable associations with any specific cancer outcome. Publications suggesting glyphosate is associated with any cancer outcome are discussed below.

One publication (George et al., 2009) utilized a 2-stage cancer model in mice to evaluate a glyphosate formulation for tumor promotion. A known tumor promoter, 12-o-tetradecanoyl-phorbol-13-acetate (TPA) was used for a positive control/comparator after exposure to a tumor initiator, 7, 12-dimethylbenz[a]anthracene. Proteomics were later applied to extrapolate a basis for glyphosate formulation tumor promotion. This study is discussed in more detail below.

An essential consideration in both, risk assessment and interpreting the relevance of toxicology data is exposure assessment. An inherent low level of confidence exists for epidemiological studies where tenuous links to exposure exist. Suggested associations between health outcomes and any possible causative agent are merely speculation if exposures are not identifiable. Pivotal to the understanding of glyphosate exposure are data published by Acquavella et al. (2004; 2005), which quantified human systemic glyphosate exposure levels in farmer applicators and their families. The geometric mean systemic dose for farmers applying glyphosate, some of whom applied glyphosate to areas up to 400 acres, was 0.0001 mg/kg/day, approximately 0.03% of the current EU glyphosate acceptable operator exosure Level (AOEL). The highest systemic dose, skewed well above the geometric mean, was 0.004 mg/kg/day, which is 1.95% of current EU glyphosate AOEL and 1.3% of the current EU glyphosate attapcable daily intake (ADI). Not surprisingly, even lower systemic doses were determined for spouses and children, 0.00004 mg/kg and 0.0008 mg/kg, respectively. Interestingly, the current European ADI is based on the NOAEL (highest dose tested) in an old 2-year rat carcinogenicity study; multiple carcinogenicity studies have since been conducted by numerous glyphosate registrants demonstrating NOAELs of at least ten-fold higher than the highest dose tested in the study driving the current EU ADI calculation.

The largest epidemiological study of pesticide exposure and health outcomes in the United States is the Agricultural Health Study (AHS), which included glyphosate. Dozens of publications have resulted from data generated in this study of approximately 57,000 enrolled farmer applicators. Blair et al. (2009) provided an overview of cancer endpoints associated with different agricultural chemicals reported in earlier AHS publications. Glyphosate was not reported to be associated with leukemia, melanoma, or cancers of the prostate, lung, breast, colon or rectum. De Roos et al. (2005) reported AHS data evaluating glyphosate use and multiple cancer endpoints; no association was noted for glyphosate with all cancers, including cancer of the lung, oral cavity, colon, rectum, pancreas, kidney, bladder, prostate, melanoma, all lymphohematopoietic cancers, non-Hodgkin's lymphoma (NHL) and leukemia. In an earlier publication based on another data set, however, De Roos et al., (2003) reported an association between NHL and glyphosate use. McDuffie et al. (2001) reported a non-significant positive association between self-

reported glyphosate exposure and NHL in a Canadian study. Blair et al. (2009) did not report an association between glyphosate use and NHL in the AHS data, but a "possible association" between glyphosate use and multiple myeloma was mentioned. The AHS publication reporting this refers to a "suggested association" between glyphosate use and multiple myeloma (De Roos et al., 2005), yet it did not demonstrate significant increase in relative risk for multiple myeloma. Both De Roos papers will be discussed in more detail below. Interestingly, a subsequent AHS review paper for the President's Cancer Panel (Freeman, 2009) specifically references De Roos (2005) as providing no observed incidents of cancers of any type being associated with glyphosate.

Lee et al. (2005) reported a glyphosate association with gliomas, with the odds ratio differing between self-respondents (OR = 0.4) and proxy respondents (OR = 3.1). The authors expressed concern that higher positive associations observed for proxy respondents with glyphosate and several other pesticides, and suggested perhaps more accurate reporting of proxies for cases, and underreporting by proxies for controls; proxy respondents were spouses in 62% of cases versus 45% of controls, lending to lower reported incidents in the control group.

The follow epidemiology publications report a lack of association between glyphosate and specific cancer types.

- Alavanja et al. (2003) reported on prostate cancer associations with specific pesticide exposures in the AHS; glyphosate did not demonstrate a significant exposure-response association with prostate cancer.
- Multigener et al, (2008) also reported a lack of association between glyphosate use and prostate cancer. This data appears to have also been reported by Ndong et al. (2009).
- The lack of association between glyphosate use and prostate cancer was also supported recently in an epidemiology study of Farmers in British Columbia, Canada by Band et al. (2011).
- Lee et al. (2004) reported a lack of association between glyphosate use and stomach and esophageal adenocarcinomas.
- Carreon et al. (2005) reported epidemiological data on gliomas and farm pesticide exposure in women; glyphosate had no association with gliomas.
- Engel et al. (2005) reported AHS data on breast cancer incidence among farmers' wives, with no association between breast cancer and glyphosate.
- Flower et al (2004) reported AHS data on parental use of specific pesticides*a* and subsequent childhood cancer risk among 17,280 children, with no association between childhood cancer and glyphosate.
- Andreotti et al. (2009) reported AHS data where glyphosate was not associated with pancreatic cancer.
- Landgren et al. (2009) reported AHS data on monoclonal gammopathy of undetermined significance (MGUS), showing no association with glyphosate use.
- Karunanayake et al. (2011) reported a lack of association between glyphosate and Hodgkin's lymphoma.
- Pahwa et al. (2012) reported a lack of association between glyphosate and multiple myeloma.

In summarizing AHS publications, Weichenthal et al. (2010) noted that increased rates in the following cancers were not associated with glyphosate use; overall cancer incidence, lung cancer, pancreatic cancer, colon or rectal cancer, lymphohematopoietic cancers, leukemia, NHL, multiple myeloma, bladder cancer, prostate cancer, melanoma, kidney cancer, childhood cancer, oral cavity cancers, stomach cancer, esophagus cancer and thyroid cancer.

Monge et al (2007) investigated associations between parental pesticide exposures and childhood Leukaemia in Costa Rica. Results are not interpretable for glyphosate as exposure was estimated with "other pesticides", including paraquat, chlorothalanil and "others". No association was noted for paternal exposures, but elevated leukaemias were associated with maternal exposures to "other pesticides" during

pregnancy. Similarly, glyphosate is captured under "other pesticides" being associated with NHL by Fritschi et al. (2005) and therefore should not be interpreted as an association with glyphosate.

Non-Hodgkin's Lymphoma (NHL)

Non-Hodgkin's lymphoma is not a specific disease, but rather a grouping of all lymphoma types, other than Hodgkin's lymphoma. This is a large group of different cancers of the immune system including Burkitt lymphoma, diffuse large B-cell lymphoma (NLPHL), follicular lymphoma, immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, mantle cell lymphoma, mycosis fungoides, anaplastic large cell lymphoma, and precursor T-lymphoblastic lymphoma (National Cancer Institute, http://cancer.gov/cancertopics/wyntk/non-hodgkin-lymphoma.pdf). Risk factors associated with NHL include weakened immune system (such as from an inherited condition or certain drugs used after an organ transplant), infections (Epstein-Barr virus, EBV; Human immunodeficiency virus, HIV; *Helicobacter pylori* bacteria; Human T-cell leukemia/lymphoma virus, HTLV-1; Hepatitis C virus; age). There are many different types of Non-Hodgkin's lymphomas, which are different lymphomas arising from different pathogeneses, and as such, should not be clustered together as a single disease with a common etiology for epidemiological investigation. When clustered together in epidemiological studies, further investigation to identify both the specific type of lymphoma and any underlying risk factors associated with individual reports of HNL is necessary.

Author(s)	Year	Study title
Hardell, L. Eriksson, M.	1999	A Case-Control Study of Non-Hodgkin Lymphoma and Exposure to Pesticides. Cancer Volume: 85 Number: 6 Pages: 1353-1360

Abstract*

BACKGROUND. The incidence of non-Hodgkin lymphoma (NHL) has increased in most Western countries during the last few decades. Immunodefective conditions are established risk factors. In 1981, the authors reported an increased risk for NHL following exposure to certain pesticides. The current study was designed to further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL.
METHODS. A population-based case–control study in northern and middle Sweden encompassing 442 cases and twice as many controls was performed. Exposure data were ascertained by comprehensive questionnaires, and the questionnaires were supplemented by telephone interviews. In total, 404 cases and 741 controls answered the questionnaire. Uni-variate and multi-variate analyses were performed with the SAS statistical data program.
RESULTS. Increased risk for NHL was found for subjects exposed to herbicides (odds ratio [OR], 1.6; 95% confidence interval [CI], 1.0 –2.5) and fungicides (OR, 3.7; 95% CI, 1.1–13.0). Among herbicides, the phenoxyacetic acids dominated (OR, 1.5; 95% CI, 0.9 –2.4); and, when subclassified, one of these, 4-chloro-2-methyl phenoxyacetic acid (MCPA), turned out to be significantly associated with NHL (OR, 2.7; 95% CI, 1.0–6.9). For several categories of herbicides, it was noted that only exposure during the most recent decades before diagnosis of NHL was associated with an increased risk of NHL. Exposure to impregnating agents and insecticides was, at most, only weakly related to NHL.
CONCLUSIONS. Exposure to herbicides in total, including phenoxyacetic acids, during the decades before NHL diagnosis resulted in increased risk for NHL. Thus, the risk following exposure was related to the latency period. Fungicides also increased the risk for NHL when combined, but this group consisted of several different agents, and few subjects were exposed to each type of fungicide.
* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Various herbicides, insecticides, fungicides, impregnating agents, organic solvents
Active substance(s):	Glyphosate, phenoxyacetic acid, MCPA, 2,4-D, 2,4,5-T, DDT, Pyrethrins, mercurial seed dressing, chlorophenols, pentachlorophenol, arsenic, creosote
Description:	Not reported
Source of test medium:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

Species:	Human

Age of test persons:	≥25
Sex:	Males

4. Test system:

Study type:	A Case-Control Study of Non-Hodgkin Lymphoma and Exposure to Pesticides
Guideline:	None
GLP:	No
Guideline deviations:	Not applicable
Collection of data:	Questionnaire
Total No. of cases analysed:	442
Total No. of controls:	741
No. of exposed cases to glyphosate:	4
No. of controls for glyphosate	3

5. Observations/analyses:

Working history:	All subjects
Additional information:	Smoking habits, previous diseases, and certain food habits were assessed.
Detailed assessment of exposure:	Years and total number of days for exposure to various pesticides were assessed for all subjects.
Parameters determined:	Tumour induction period (time from first exposure to diagnosis), time span (time from last exposure to diagnosis). NHLs with different pathogeneses were not distinguished.
Statistics:	Conditional logistic regression analysis for matched studies was performed with the SAS statistical program. Thereby, odds ratios (OR) and 95% confidence intervals (95% CI) were obtained. All 95% CIs were rounded outward, e.g., a 95% CI of 1.07– 4.52 is written 1.0 – 4.6. Both uni-variate and multi-variate analyses were performed. When exposure to different pesticides was analyzed, subjects with no pesticide exposure were taken as unexposed.

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comment: Study prone to selection and recall bias. No evidence of relevant glyphosate exposures. Medical history was assessed, but not reported.

2. Relevance of study: **Not relevant** (Exposure to multiple chemicals and though glyphosate exposure data were convincing (7/1145 subjects) and statistically non-significant positive associations reported.

3. Klimisch code: **3**

Response 1 – Review by Mark R. Cullen, MD, Professor of Medicine and Epidemiology, Yale University School of Medicine, June 21 1999

This study is part of an ongoing effort of the investigators and their team to unravel the cause(s) of NHL, which has been increasing in incidence in Sweden and most developed countries for at least 2 decades. The premise, that the increase suggests an environmental cause or causes, is certainly correct.

The basic approach, the case control study using the superb existing tumor and population registries of Sweden, is appropriate to this challenge, and the investigators seem to have a clear grasp of the basic approach to such studies. Inclusion criteria for cases appear well considered, and the ability to recruit almost all is a strong plus for the study. The criteria for including controls, including the matching on vital status for comparability of information regarding past exposures is laudable, though, as discussed below, possibly unsuccessful despite careful consideration. The response of the subjects is encouragingly high.

Unfortunately the approach to exposure assessment for agricultural chemicals is very problematic. First, as I believe the data themselves ultimately demonstrate, it is not at all clear that even living subjects, let alone relatives of dead ones, can meaningfully assess or quantify exposure to herbicides and pesticides. It appears from the small number of phone interviews conducted (itself a problem, see below) that almost every subject provides different information or expanded information when directly contacted by phone. It is not at all obvious that the respondents can easily evaluate their exposures, which in many cases amount to an occasional use of a product many years before the survey, nor is it obvious that the surrogate measure of dose, i.e., days of use, is meaningful, especially given the remarkable difference which exists in actual biological exposure depending on how the products are used, information which was not even attempted here. In other words, the first problem is the degree to which this study classifies subjects in any biologically relevant way, or validly.

As if this were not problematic enough, there is evidence within the study results to suggest significant information or recall bias. When they were contacted because of ambiguous or missing information, a high proportion, possibly all subjects reported a positive history of exposure -- it is unclear from the report just how many such were contacted overall, but it appears that most were contacted to confirm positive histories, despite the evidence that the negative histories were more likely unreliable. I would worry greatly that cases, clearly aware of their disease status even if not the underlying hypothesis here, might be more thorough in their recollection of these distant events, whose recall is likely more subtle than recall of major industrial chemicals which likely would have involved (unforgettable) daily work exposures, unlike the chemical use with doses averaging about a month! The authors would have done well to interview everybody given this spareness, and the ubiquity of recall bias in such studies.

The third problem with the exposure assessment relates to co-linearity. For obvious reasons people exposed to one agricultural chemical have a non-independent (true) chance of exposure to another, and that recollection of one is likely to interact with recollection of others. The data presented are consistent with this, though the actual degree of overlapping exposures in the data are not fully disclosed. In any event, the effort to tease them apart using multi-variate regression unlikely gets at the fundamental issue, which is that information is hopelessly confounded. Even if one were not concerned about the other issues vitiating the exposure assessment, the attempt to distinguish one exposure from another within the herbicide category is, in my view, fatuous, though the investigators have drawn some rather sweeping inferences from it, and from the latency analysis which I believe suffers from the same recall issues.

One final comment, which I fear may betray a range of the authors preconceived ideas, is the inclusion of glyphosate in the uni-variate and multi-variate analyses, despite the fact that only 7 of 1145 subjects in the study gave exposure histories to this agent, and for a mean duration of what appears to be a few days! Since there is zero possibility that exposure to glyphosate could explain the Swedish excess of NHL which is the premise of the study, and since it is biologically absurd to imagine a few days exposure to virtually any short lived compound, let alone one with so little oncogenic potential based on its toxicologic profile, the inclusion of these data and the highlighting of them in the discussion - with a very biased review of the tox literature-- undermines even further the report.

In the end I think this study adds little to our overall knowledge of the cause(s) of NHL, though it continues to appear that farmers have increased risk, certainly an important clue for follow-up. However, it is unlikely that the roles of infection, other biological factors, UV light, diet and lifestyle issues or agricultural chemicals will be successfully unraveled by studies of this design. In particular, the evidence

regarding glyphosate in relation to NHL is meaningless, and it would be highly inappropriate to construe this as a positive study in that regard.

Response 2 – Review by Hans-Olav Adami, Professor of Epidemiology, Harvard School of Public Health and Dimitrios Trichopoulos, Vincent L. Gregory Professor of Cancer Prevention, Department of Epidemiology, Harvard School of Public Health.

We have classified our comments into those concerning study design and those concerning data analysis and interpretation, and we have concluded our evaluation with a short commentary and overall assessment.

Study design

The study base comprises men 25 years of age or older and living in any of seven Swedish counties from January 1, 1987 to December 31, 1990. The cases were divided according to their vital status at a time when the actual data collection took place. Of the 442 cases, 192 were deceased. The date of vital status ascertainment is not clearly indicated, as it should have been. Since, however, data were collected from 1993 to 1995, we assume that vital status was determined in 1993 or earlier.

The authors state that they have conducted a population-based study, but they have chosen their controls in a way that violates the defining characteristics of these studies. Sampling from the population register took place sometime after 1990, so that people who had migrated out of the area after the diagnosis of the corresponding case would have been incorrectly ineligible, whereas those who had migrated into the area after the diagnosis of the corresponding case would have been incorrectly eligible. Migration is generally related to socio-economic status, which is a plausible predictor of exposure to pesticides. Thus, important bias may have been introduced.

There are other issues that should have been addressed in the study design. Is it really possible to blind interviewers as to the case or control status of the interviewed person, so as to minimize interviewer-related information bias? And, what assurance is there that the substantial difference in response proportion between cases and controls did not introduce interviewee-related selection bias? It is certainly disturbing that all 17 reported odds ratios (Table 1 of the authors) were higher than the null value of 1, even though only marginally significant results were reported. It is also astonishing that there is no category of missing or unknown in any of the tables, even though about half of the exposure information was provided by proxy responders and this information was concerning compounds as complicated as 2,4-D/2,4,5-trichlorophenoxyacetic acid.

Analysis

The analysis is in many ways superficial and shows a surprising disregard to confounding. The authors appear so eager to report significant results, that when multi-variate analysis, *that is the proper analysis*, reduces all reported odds ratios to essentially non-significant values (table 7), they make the amazing statement that "regarding lymphomagenesis, the uni-variate analysis may be more informative than the multi-variate analysis". Moreover, they pay little attention to the multiplicity of comparisons and they attempt causal inferences with unacceptable disregard of the statistical limitations of their study. For example, for glyphosate, the p value is no less than 0.35 and for phenoxyacetic acids the multi-variate odds ratio has a p value of 0.25.

There are several other issues in the analysis. Although most of them are trivial, one deserves more attention. Non-Hodgkin lymphoma has been reported to be more common in some rural occupations. Exposure to pesticides is a possible explanation, but there are other plausible explanations, including exposure to infectious agents of animal origin and delayed establishment of herd immunity with concomitant increase in the average age at exposure to possible critical agents (the classical paradigm of paralytic polio has been invoked by several investigators in the study of the etiology of multiple sclerosis,

leukemias and lymphomas). In the latter two instances, occupation should be adjusted for in the analysis, in order to control for confounding.

Conclusion

This is a study that has limited power, was inadequately designed, poorly analysed and confusingly reported. Every epidemiological investigation should meet basic standards concerning selection bias, information bias, confounding and power. The investigation by Hardell and Eriksson does not provide reasonable confidence that it is free of information and selection bias, shows clear signs of uncontrolled confounding and lacks the power necessary to document agent-specific effects when several agents are inter-correlated, as they are in this situation. There is also evidence that the results were selectively interpreted by the investigators. For these reasons, the study cannot provide reliable information concerning possible associations between exposures to pesticides and risk for non-Hodgkin lymphoma

Response 3 – Monsanto Review by John Acquavella, PhD and Donna Farmer, PhD

Executive Summary

Hardell and Erikkson conducted a case control study to look for associations between reported pesticide use and non-Hodgkin's lymphoma (NHL). The study included 404 NHL cases and 741 controls. The measure of association in this study was the odds ratio (OR), a statistic that estimates of the ratio of disease rates (in this case NHL rates) for exposed and unexposed populations.

The authors reported statistically significant associations for NHL with: reported use of any herbicide (OR = 1.6), reported use of any fungicide (OR = 3.7), and reported use of 4-chloro-2-methylphenoxyacetic acid (OR = 2.7). The major limitations of this study were: the reliance on reported pesticide use (not documented exposure) information, the small number of subjects who reported use of specific pesticides, the possibility of recall bias, the reliance on secondary sources (next-of-kin interviews) for approximately 43% of the pesticide use information, and the difficulty in controlling for potential confounding factors, given the small number of exposed subjects.

The authors also reported a moderately elevated OR of 2.3 for glyphosate. This OR was not statistically significant and was based on only four "exposed" cases and three "exposed" controls. This finding needs to be evaluated in light of the limitations of the study, mentioned above, and the wealth of toxicologic information that has resulted in glyphosate being judged to be non-mutagenic and noncarcinogenic by the U.S. Environmental Protection Agency and the World Health Organization. Systematic error or chance seem the most likely explanations for the findings reported for glyphosate in this study.

Hardell and Eriksson[1] conducted an epidemiologic study to look for associations between self-reported pesticide use and non-Hodgkin's lymphoma (hereafter NHL). The rationale for conducting this research was previous studies by the first author[2,3] and by investigators at the U.S. National Cancer Institute[4,5], which found associations between reported use of phenoxyacetic acids (primarily 2,4-D) and NHL. The results of these studies were determined to be inconclusive by a special Science Advisory Panel convened in the early 1990s by the U.S. Environmental Protection Agency (EPA).[6]

The present study presents new data about phenoxyacetic acids and other commonly used pesticides. Herein, we review the methods and results of this recent study.

Study design

Hardell and Eriksson employed a case control design for their research. In case control studies, subjects are selected on the basis of their disease status. Those with the disease of interest (in this case those with NHL) are the cases; disease free study participants are the controls. Information about presumptive etiologic factors are collected from cases and controls using similar methodology.

The controls in a case control study provide an estimate of the exposure prevalence (in this case the prevalence of self-reported pesticide use) in the base population that gave rise to the cases and controls[7]. The exposure odds for the cases is then compared to the exposure odds for the controls. The resulting ratio of exposure odds - called the odds ratio (OR) - estimates the ratio of disease rates for exposed versus unexposed subjects[8]. The ratio of disease rates is the fundamental measure of association in epidemiologic studies.

The interpretation of the OR is straightforward. An OR of 1.0 implies that the disease rate (in this case the rate of NHL) is the same for exposed members of the base population and for unexposed members and indicates no association between exposure and disease. An OR greater than 1.0 or less than 1.0 implies that the disease rate is different for the exposed population than for the unexposed population and, if valid, may indicate an exposure disease relationship. Exposure disease relationships can be "positive" (viz. the OR is greater than 1.0) - where exposure is associated with increased rates of disease - or inverse (viz. the OR is less than 1.0) - where exposure is associated with decreased rates of disease (viz. exposure prevents disease). For example, an OR of 2.0 is consistent with a disease rate among exposed persons that is twice the disease rate for unexposed persons; likewise, an OR of 0.5 is consistent with a disease rate for exposed persons that is half the disease rate for unexposed persons.

Interpreting ORs at face value requires the assumption that there is no confounding or other bias in a study. Much of the evaluation of epidemiologic studies hinges on whether there are discernible sources of bias or potential for bias, which, if present, compromise the validity of findings. Often it is not possible to pinpoint specific sources of bias, but methodologic limitations can usually be identified and the results interpreted accordingly.

A major validity concern in case control studies is recall bias: that is when cases or their next-of-kin are more likely to recall (real or imagined) specific exposures than are controls. This can result in differential exposure misclassification whereby cases are more likely to be classified as exposed than are controls, despite no real difference in exposure prevalence. Recall bias is particularly an issue in cancer studies; cancer being a disease that stimulates introspection about presumptive causes. Other important validity concerns are selection bias (cases or controls as selected are unrepresentative) or uncontrolled confounding factors. Proper reporting of an epidemiologic study requires consideration of potential biases and their likely impact on study results.

Finally, findings are also evaluated according to how likely they are to have occurred by chance alone if there is not, in fact, a true relationship between exposure and disease. This is evaluated by calculating a probability (called a p-value) for seeing results at least as extreme as those observed if the null hypothesis of no true effect is true. By convention, only findings where the p value is less than 0.05 are considered "statistically significant." Hardell and Erikkson did not actually calculate p values in their study. Instead, they calculated 95% confidence intervals for the OR. The 95% CI is defined as the range of values that are consistent with the data observed in a study with 95% confidence. For example, a CI of 0.4 to 13.0 means the data are consistent with an OR as low as 0.4 (implying a 60% reduced rate with exposure) or as high as 13.0 (implying a 13-fold elevated rate with exposure). A finding is statistically significant when the OR of 1.0 is not included in the 95% CI.

Study subjects

The study included 404 NHL cases, diagnosed during the period 1987-1990, from the four most northern counties of Sweden. These cases (or their next-of-kin when cases were deceased) and 741 controls (or their next-of-kin when controls were deceased) were sent a mailed 18 page questionnaire that addressed a variety of (self-reported, viz. undocumented) factors including pesticide use, work history and chemical exposures, smoking habits, previous diseases, and certain dietary habits.

Controls were selected to be similar to cases in terms of age and vital status (i.e. living cases were matched to living controls and deceased cases were matched to deceased controls). Matching subjects on vital status was intended to minimize recall bias to the extent that the fact of death, but not death from a

specific cause, might affect recollections of pesticide use. Approximately 43% of cases were deceased, hence next-of-kin information a significant component of this study.

Exposure Assessment

There was no exposure assessment, per se, in this study. Exposure was presumed based on reported use of specific pesticides. This can be an inaccurate indicator of exposure for two reasons: 1) inaccurate recall or 2) negligible exposure from use. An example of the latter would be glyphosate which has very low skin penetrability[9], so reported use is not equivalent to (meaningful) exposure. A recent study of forestry sprayers by Lavy et al. found indications of significant dermal exposure, but no indication, based on biomonitoring, of an absorbed dose of glyphosate.[10]

Statistical analysis

The data analysis involved standard techniques to estimate the OR and control, in a very limited sense, for coincident pesticide exposures as potential confounding factors. These statistical techniques included uni-variate and multi-variate logistic regression analysis. The analysis was primarily restricted to a crude dichotomous classification of reported pesticide use (ever use versus never use). There were too few "exposed" subjects to conduct dose response analyses for most specific chemicals. The authors also estimated 95% CIs as a measure of the statistical variability of the ORs.

Results

The authors found modest, though statistically significant, associations between NHL and reported use of any herbicide (OR = 1.6, 95% CI 1.0-2.5) reported use of any fungicide (OR = 3.7, 95%
CI 1.1-13.0) and reported use of 4-chloro-2-methyl phenoxyacetic acid (MCPA) (OR = 2.7, 95% CI 1.07.0).
Through various analyses, the authors concluded that only exposure in the two decades preceding diagnosis was associated with increased risk.

The authors also reported findings for glyphosate, none of which were statistically significant. The overall OR for glyphosate was 2.3 (95% CI 0.4-13.0) based on 4 cases (1% of cases) and 3 controls (0.4% of controls) reporting glyphosate use. The authors also mentioned an additional analysis where glyphosate and phenoxyacetic acids were considered jointly in attempt to control for confounding from phenoxyacetic acids on the glyphosate/NHL association. In this instance, the OR for glyphosate was 5.8 (95% CI 0.6-54.0) and the OR for phenoxyacetic acids was 1.4 (95% CI 0.8-2.2). The description of this analysis was insufficient to know what the authors actually did or even to know the number of cases who reported using glyphosate. But it was clear that there was no systematic attempt to assess the association between glyphosate and NHL while controlling for exposures other than phenoxyacetic acids.

Authors' conclusions

The authors interpreted their results as supportive of a role for chemical pesticides in the etiology of NHL. They speculated, since NHL is known to be related to immunosuppression from studies of transplant patients[11], that phenoxyacetic acids might produce NHL by an immunosuppressive mechanism. In fact, they interpreted selected papers from the literature as supportive of an immunotoxic effect for phenoxyacetic acids and chlorophenols.[12,13,14]

The authors reached less definite conclusions about other pesticides and specifically about glyphosate. They noted the elevated OR for glyphosate, an elevated OR for glyphosate from another study of theirs[15] concerning hairy cell leukemia (OR = 3.1, 95% CI 0.8-12.0, based on 4 cases who reported use of glyphosate), and selected toxicologic data[16-21] as indicative that glyphosate is, at least, deserving of further epidemiologic study.

The authors considered several potential biases in interpreting their results. They ruled out selection bias by arguing that they had good response rates from cases and controls and included most cases who were diagnosed during the study period. They felt they minimized recall bias by matching cases and controls on vital status and collecting information from all study subjects using similar (blinded) methodology.

Critique

This study has several important limitations: no exposure assessment, dependence on next-of-kin's recollections of study subjects' pesticide use for approximately 43% of study subjects, potential recall bias, and the very small number of subjects who reported using specific herbicides. The latter leads to findings that are statistically imprecise. Due to the potential for bias and the statistical imprecision, the results of this study are not convincing.

In epidemiologic studies results can be:

- real (viz. disease is due to exposure)
- biased (viz. the results are invalid)
- due to chance (viz. the association is unbiased, but non causal).

It is by exclusion of the latter two possibilities and application of generally accepted criteria for causality[22] that scientists come to believe that an exposure disease association is causal. The most important causal criteria are strength of association (judged by the size of the OR), dose response (judged by whether the OR increases or decreases with increasing exposure), temporality (exposure should precede the onset of disease by an appropriate induction/latent period), consistency of findings across studies, and biological plausibility. I'll return to each of these criteria subsequently.

The major potential sources of bias in this study are recall bias, confounding bias, and selection bias. Recall bias is a major concern in cancer case control studies because cancer cases, and especially their next-of-kin, tend to scrutinize their lives hoping to understand the cause(s) of their disease. Hardell and Eriksson's matching of study subjects on vital status does not address the specific recall bias issue for cancers. Other investigators have found elevated ORs for the popular herbicide 2,4-D based on next-of-kin responses, but not based on responses of direct informants.23 Results based on a substantial number of next-of-kin respondents are usually considered less persuasive than data from actual study subjects. It would have been informative had Hardell and Erikkson analyzed their data separately for next-of-kin respondents to see whether the elevated ORs were determined primarily by next-of-kin responses. That would be difficult in the present study due to the limited number of cases who reported using most specific pesticides.

A second important limitation of the study was the inability to control for potential confounding factors. Confounding refers to finding spurious exposure-disease associations resulting from other correlated factors. The confounding factor must also be a risk factor for the disease in question. Relatively little is known about the etiology of NHL, other than there seems to be a relationship with immunosuppression.[24] It is difficult to control for confounding factors when little is known about etiologic factors. In addition, in light of the high correlation between reported use of various pesticides, it is difficult in such a study, given the small number of exposed subjects, to separate the putative effects of one pesticide from another. Therefore, associations reported for any specific pesticide might be due to effects from other pesticides.

The final source of bias to be considered is selection bias. There is no way to know whether the cases or controls who participated in the study were a biased sample, but the relatively high participation rates for cases and controls would make selection bias a less likely explanation for the findings in this study.

Specific results in an epidemiology study can be due to chance, especially when many statistical associations have been evaluated. The convention is that a p value of 0.05 or less is considered unlikely to have occurred by chance and is therefore "statistically significant." The p values for the glyphosate findings are well in excess of 0.05, approximately 0.30 or greater by my estimation, so neither of the

elevated ORs for glyphosate are close to the conventional criterion for statistical significance. They could easily be chance findings. It is noteworthy that if even one exposed case was misclassified, the OR would be approximately 1.8 (95% CI 0.6-9.9, p value 0.43); two misclassified exposed cases would give an OR of 1.2 (95% CI 0-6.2, p value 0.99). Hence, the elevated OR for glyphosate hinges on the classification of a single case or two and an exposure assessment methodology of questionable accuracy.

It is helpful at this point to assess how the findings in the present study for glyphosate (and for most of the other herbicides) match up with the causal criteria generally accepted by epidemiologists.
Specifically:

- strength of association - the findings of the present study show a weak to moderate non significant association between glyphosate use and NHL. The association is statistically imprecise and, even assuming an absence of bias, is not convincing.
- temporality - in this study, the presumed exposures would precede disease onset satisfying, in general, the temporality criterion. However, the authors did not have enough exposed subjects to consider issues of disease induction/latency as they tried to do for the phenoxyacetic acids.
- dose response - there was insufficient data in this study to consider dose response. Also, in light of glyphosate's very low skin penetrability9, one can question whether any meaningful range of
- exposure occurred among study subjects.
- consistency - there are no other studies that have reported an association between glyphosate and NHL. Hence the consistency criterion cannot be met.
- biological plausibility - Hardell and Erikkson characterized the available glyphosate toxicologic data as showing: excess mutations and chromosome aberrations in studies with mouse lymphoma cells[16-19], excess sister chromatid exchanges (SCEs) in cultures of human lymphocytes[20], and a somewhat increased incidence of various cancers in one carcinogenicity study of mice.21 However, five of the six references cited did not use glyphosate as the test material.[16-19,21] In these studies the test material was sulfosate - the trimesium salt of glyphosate. Sulfosate has a somewhat different toxicology profile than glyphosate. Nonetheless, it is worth pointing out that Hardell and Erikkson's assessment of these studies is not shared by regulatory agencies. For example, the U.S. Environmental Protection Agency (EPA) considered the mouse lymphoma findings[16-19] to be false positives due to sulfosate's acidity; sulfosate was not mutagenic in this assay when the pH was adjusted to a physiological level.[25] Also, EPA characterized the sulfosate mouse carcinogenicity study[21] as showing "… no evidence of carcinogenicity … at the doses tested" and classified sulfosate as category E - no evidence for carcinogenicity in humans.[25]

The one glyphosate toxicology study cited[20] showed weak positive findings for sister chromatid exchange in human lymphocytes in vitro. This study had many limitations and numerous, more specific, mutagenicity assays have not shown positive results for glyphosate.[26] Extensive reviews of the available toxicologic data have been completed recently by the U.S. Environmental Protection Agency[27,28] (EPA) and the World Health Organization.[29] These agencies concluded that glyphosate is not mutagenic or carcinogenic. EPA classified glyphosate as category E.[27,28] This would argue against the biological plausibility of the findings reported by Hardell and Erikkson.

In conclusion, the study by Hardell and Eriksson found a modest association between NHL and several chemical pesticides - most notably for MCPA and the collective group of fungicides. The reported weak to moderate associations for glyphosate are not statistically significant and could be due to chance or to recall or confounding bias. It is clear, however, that the widespread use of glyphosate and concerns about pesticide related health effects for farmers and their families will raise the "index of concern" for glyphosate in future agricultural epidemiologic studies.

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Author(s)	Year	Study title
Hardell, L. Eriksson, M. Nordstrom, M.	2002	Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: Pooled analysis of two Swedish case-control studies. Leukemia & Lymphoma Volume: 43 Number: 5 Pages: 1043-1049

Abstract*

Increased risk for non-Hodgkin's lymphoma (NHL) following exposure to certain pesticides has previously been reported. To further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL a pooled analysis was performed on two case-control studies, one on NHL and another on hairy cell leukemia (HCL), a rare subtype of NHL. The studies were population based with cases identified from cancer registry and controls from population registry. Data assessment was ascertained by questionnaires supplemented over the telephone by specially trained interviewers. The pooled analysis of NHL and HCL was based on 515 cases and 1141 controls. Increased risks in uni-variate analysis were found for subjects exposed to herbicides (OR 1.75, CI 95% 1.26-2.42), insecticides (OR 1.43, CI 95% 1.08-1.87), fungicides (OR 3.11, CI 95% 1.56-6.27) and impregnating agents (OR 1.48, CI 95% 1.11-1.96). Among herbicides, significant associations were found for glyphosate (OR 3.04, CI 95%

1.08-8.52) and 4-chloro-2-methyl phenoxyacetic acid (MCPA) (OR 2.62, CI 95% 1.40-4.88). For several categories of pesticides the highest risk was found for exposure during the latest decades before diagnosis. However, in multi-variate analyses the only significantly increased risk was for a heterogeneous category of other herbicides than above.

* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Various herbicides, insecticides, fungicides, impregnating agents, organic solvents
Active substance(s):	Glyphosate, phenoxyacetic acid, MCPA, 2,4-D, 2,4,5-T, DDT, Pyrethrins, mercurial seed dressing, chlorophenols, pentachlorophenol, arsenic, creosote
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

(in the following data only presented for exposures to glyphosate and total number of subjects)

Species:	Human
Age of test persons:	≥25
Sex:	Males

4. Test system:

Study type:	Epidemilogical study for Non-Hodgkin´s Lymphoma (NHL) and Hairy cell Leukemia (HCL)
Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Collection of data:	Questionnaire & telephone interviews
No. of exposed persons with NHL or HCL:	NHL study: 404 HCL study:111 Total: 515
No. of control persons:	NHL study: 404 HCL study:111 Total: 515
No. of persons with NHL or HCL exposed to glyphosate:	8
No. of persons in control group:	8

5. Observations/analyses:

Working history:	All subjects
Detailed assessment of exposure:	Years and total number of days for exposure to various pesticides were assessed for all subjects. For analysis only subjects with a minimum exposure of 1 working day (8h) and a

tumour induction period of at least one year were included.

Parameters determined: Tumour induction period (time from first exposure to diagnosis), time span (time from last exposure to diagnosis). NHLs with different pathogeneses were not distinguished.

Statistics. Conditional logistic regression analysis for matched studies was performed with SAS statistical program. Odds ratios and 95% confidence intervals were obtained. Both uni-variate and multi-variate analyses were done. In the pooled analysis an adjustment was made for study, study area and vital status. When risk estimates for different pesticide exposures were analysed only subjects with no pesticide exposure were taken as unexposed, whereas subjects exposed to other pesticides were disregarded.

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comment: This publication combines the results of two previous studies by the authors on HNL (Hardell and Eriksson, 1999) and HCL (Nordstrom,et al., 1998). No information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc). Study documentation is insufficient for assessment.

2. Relevance of study: **Not relevant** (Due to reliability of data set drawn from Hardell and Eriksson, 1999)

3. Klimisch code: **3**

Response – GTF

- This study pools NHL data from the previously reviewed publication by Hardell and Eriksson (1999) with HCL data from Nordstrom et al. (1998). Therefore the responses to Hardell and Eriksson (1999), the methodology and data issues, also apply to the NHL data set used in Hardell et al. (2002). It is of interest to note that Hardell was also a coauthor of Nordstrom et al. (1998).
- Each individual study reported non-statistically significant associations between glyphosate and NHL or HCL.
- Each study was based on few exposed cases, 4 each. The pooled analysis combined these cases.
- The uni-variate odds ratio was similar to those in the two individual studies (OR = 3.04; 95% CI: 1.08–8.52), the multi-variate adjusted odds ratio was attenuated (OR = 1.85; 95% CI: 0.55–6.20)
- These data fail to demonstrate convincing evidence for an association between glyphosate and NHL or HCL.

Author(s)	Year	Study title
Fritschi, L. Benke, G. Hughes, A. M. Kricker, A. Turner, J. Vajdic, C. M. Grulich, A. Milliken, S. Kaldor, J. Armstrong, B. K.	2005	Occupational exposure to pesticides and risk of non-Hodgkin's lymphoma American Journal of Epidemiology Volume: 162 Pages: 849-857

Abstract*

Pesticide exposure may be a risk factor for non-Hodgkin's lymphoma, but it is not certain which types of pesticides are involved. A population-based case-control study was undertaken in 2000-2001 using detailed methods of assessing occupational pesticide exposure. Cases with incident non-Hodgkin's lymphoma in two Australian states (n = 694) and controls (n = 694) were chosen from Australian electoral rolls. Logistic regression was used to estimate the risks of non-Hodgkin's lymphoma associated with exposure to subgroups of pesticides after adjustment for age, sex, ethnic origin, and residence. Approximately 10% of cases and controls had incurred pesticide exposure. Substantial exposure to any pesticide was associated with a trebling of the risk of non-Hodgkin's lymphoma (odds ratio = 3.09, 95% confidence interval: 1.42, 6.70). Subjects with substantial exposure to organochlorines, organophosphates, and "other pesticides" (all other pesticides excluding herbicides) and herbicides other than phenoxy herbicides had similarly increased risks, although the increase was statistically significant only for "other pesticides." None of the exposure metrics (probability, level, frequency, duration, or years of exposure) were associated with non-Hodgkin's lymphoma. Analyses of the major World Health Organization subtypes of non-Hodgkin's lymphoma suggested a stronger effect for follicular lymphoma. These increases in risk of non-Hodgkin's lymphoma with substantial occupational pesticide exposure are consistent with previous work.

* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Organophosphates, organochlorines, phenoxy herbicides, other herbicides, and other pesticides
Active substance(s):	Glyphosate and others
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

Species:	Human
Age of test persons:	20-74
Sex:	Males and females

4. Test system:

Study type:	Occupational exposure study to assess exposure to pesticides and risk of non-Hodgkin’s lymphoma

Guideline:	Non
GLP:	No
Guideline deviations:	Not applicable
Collection of data:	Questionnaire Histopathological confirmation of NHL was done by an experienced pathologist.
No. of exposed persons with NHL:	694
No. of control persons:	694
Pesticide use frequency:	Not reported

5. Observations/analyses:

Working history:	All subjects
Detailed assessment of exposure:	The questionnaire included a diary with a detailed lifetime history of each job the subject had held for 1 year or more. Information obtained on each job included job title, employer, industry, start and finish years, number of hours worked per day, and number of days worked per week.
Parameters determined:	A pesticide-crop matrix was developed for assistance with exposure assessment. Levels of exposure were considered according to time-weighted average threshold limit values. Frequency of exposure was allocated as number of 8-hour days per year and was calculated using responses to the task questions. If no data on frequency of exposure were available (n=4), subjects were assumed to have been exposed for 2 days per year.
Statistics:	Logistic regression was used to calculate odds ratios (as estimates of relative risk) for non-Hodgkin's lymphoma associated with exposure to any pesticide and exposure to each pesticide subtype in each amount category (substantial or nonsubstantial), with adjustment for age, sex, ethnic origin, and state of residence. In addition, logistic regression analyses were carried out for exposure to any pesticide after restricting the sample to males only and after excluding cases that were not on the electoral roll. We also examined the odds of non-Hodgkin's lymphoma using the following metrics of exposure to any pesticide: maximum exposure level (low, medium, high); ever being exposed before 1985 (yes, no); maximum frequency of exposure (0, ≤4, or >4 days/year); and total number of years exposed (0, ≤5, or >5 years). For the latter two metrics, 4 days per year and 5 years were the median frequency and duration, respectively, in control subjects. All *p* values were two-sided.

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comment: No information about exposure duration, used glyphosate products, exposure duration and application rates. Documentation is insufficient for assessment.

2. Relevance of study: **Not relevant** (Multiple pesticide exposures. No definitive association between NHL and glyphosate can be made.)

3. Klimisch code: **3**

Author(s)	Year	Study title
De Roos, A. J. Zahm, S. H. Cantor, K. P. Weisenburger, D. D. Holmes, F. F. Burmeister, L. F. Blair, A.	2003	Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. Occupational and Environmental Medicine Volume: 60 Number: 9 Pages: -E11

Abstract*

Background: An increased rate of non-Hodgkin's lymphoma (NHL) has been repeatedly observed among farmers, but identification of specific exposures that explain this observation has proven difficult.

Methods: During the 1980s, the National Cancer Institute conducted three case-control studies of NHL in the midwestern United States. These pooled data were used to examine pesticide exposures in farming as risk factors for NHL in men. The large sample size (n = 3417) allowed analysis of 47 pesticides simultaneously, controlling for potential confounding by other pesticides in the model, and adjusting the estimates based on a prespecified variance to make them more stable.

Results: Reported use of several individual pesticides was associated with increased NHL incidence, including organophosphate insecticides coumaphos, diazinon, and fonofos, insecticides chlordane, dieldrin, and copper acetoarsenite, and herbicides atrazine, glyphosate, and sodium chlorate. A subanalysis of these "potentially carcinogenic" pesticides suggested a positive trend of risk with exposure to increasing numbers.

Conclusion: Consideration of multiple exposures is important in accurately estimating specific effects and in evaluating realistic exposure scenarios.

* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Various herbicides, insecticides (in total 47)
Active substance(s):	Glyphosate and 46 others
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

Species:	Human
Age of test persons:	≥ 21
Sex:	Males

4. Test system:

Study type:	Epidemiological studies for Non-Hodgkin´s Lymphoma (NHL) in male farm workers exposed to pesticides

	Pooled data from three population based cased control studies conducted in Nebraska, Iowa and Minesota and Kansas.
Guideline:	None
GLP / GCP:	No
Guideline deviations:	Not applicable
Selection of test persons:	Nebraska: Persons identified by Nebraska Lymphoma Study Group and area hospitals (Time of diagnosis: July 1983 – June 1986). Iowa and Minesota: Ascertained from records of the Iowa State Health Registry; Surveillance system of Minnesota hospitals and pathology laboratories (Time of diagnosis: 1980 - 1983) Kansas: A random sample of cases from the statewide cancer registry run by the University of Kansas Cancer Data Service (Time of diagnosis: 1979 – 1981)
Selection of control persons:	Randomly; Same geographical areas as the cases; Frequency matched to cases by race, sex, age, and vital status at the time of interview
Collection of data:	Questionnaire / Interview

(in the following data only presented for exposures to glyphosate and total number of subjects)

No. of exposed persons with NHL:	870
No. of control persons:	2569
No. of persons with NHL or HCL exposed to glyphosate:	36
No. of persons in control group:	61
Pesticide use frequency:	≥ 20 per person

5. Observations/analyses:

Working history:	All subjects
Detailed assessment of exposure:	Years and total number of days for exposure to various pesticides were assessed for all subjects. For analysis only subjects with a minimum exposure of 1 working day (8h) and a tumour induction period of at least one year were included. No analysis of actual exposure duration or frequency was included.
Parameters determined:	Tumour induction period (time from first exposure to diagnosis), time span (time from last exposure to diagnosis)
Analyses and statistics:	Standard logistic regression (maximum likelihood estimation); Hierarchical regression, calculating odds ratios to estimate the relative risk associated with each pesticide Models included variables for age (coded as a quadratic spline variable with one knot at 50 years) and indicator variables for study site Other factors known or suspected to be associated with NHL, including first degree relative with haematopoietic cancer, education, and smoking, were evaluated and found not to be important confounders of the associations between NHL and pesticides

Conditional logistic regression analysis for matched studies was performed with SAS statistical program. Odds ratios and 95% confidence intervals were obtained. Both uni-variate and multi-variate analyses were done. In the pooled analysis an adjustment was made for study, study area and vital status. When risk estimates for different pesticide exposures were analysed only subjects with no pesticide exposure were taken as unexposed, whereas subjects exposed to other pesticides were disregarded.

The standard logistic regression models did not assume any prior distribution of pesticide effects, in contrast to the hierarchical regression modelling

KLIMISCH EVALUATION

1. Reliability of study: **Not reliable**

Comment: No useful information about exposure duration, exposure concentration, as well as medical history, lifestyle factors (e.g. smoker, use of prescribed drugs etc were reported. Specific lymphomas are not identified (NHL captures all types of lymphoma other than Hodgkin's lymphoma). Documentation is insufficient to associate exposures with specific NHL diseases.

2. Relevance of study: **Not relevant** (No report of identifying various types of lymphoma under the NHL umbrella; no definite association between specific NHL diseases and glyphosate can be made)

3. Klimisch code: **3**

Response – GTF

- The authors pooled data from three case-control studies conducted in Iowa and Minnesota, Nebraska, and Kansas
- The data available in this study did not permit analyses of duration or frequency of use.
- No consideration of types of NHL of varying pathogeneses was presented.
- The reported logistic regression analysis noted, a statistically significant odds ratio for ever use of glyphosate and NHL (OR = 2.1; 95% CI: 1.1–4.0).
- The reported hierarchical regression did not find a statistically significant odds ratio for ever use of glyphosate and NHL (OR = 2.1; 95% CI: 1.1–4.0) (OR = 1.6; 95% CI: 0.9–2.8).
- Authors introduce the phraseology "a possible increase" in NHL incidence establishing their criteria for this category as OR >1.3 and lower confidence limit >0.8.

Author(s)	Year	Study title
De Roos, A.J. Blair, A. Rusiecki, J.A. Hoppin, J.A. Svec, M. Dosemeci, M. Sandler, D.P. Alavanja, M.C.	2005	Cancer Incidence among Glyphosate-Exposed Pesticide Applicators in the Agricultural Health Study Environmental Health Perspectives Volume: 113 Number: 1 Pages: 49-54

Abstract*

Glyphosate is a broad-spectrum herbicide that is one of the most frequently applied pesticides in the world. Although there has been little consistent evidence of genotoxicity or carcinogenicity from *in vitro* and animal studies, a few epidemiologic reports have indicated potential health effects of glyphosate. We evaluated associations between glyphosate exposure and cancer incidence in the Agricultural Health Study (AHS), a prospective cohort study of 57,311 licensed pesticide applicators in Iowa and North Carolina. Detailed information on pesticide use and other factors was obtained from a self-administered questionnaire completed at time of enrolment (1993–1997). Among private and commercial applicators, 75.5% reported having ever used glyphosate, of which > 97% were men. In this analysis, glyphosate exposure was defined as *a*) ever personally mixed or applied products containing glyphosate; *b*) cumulative lifetime days of use, or "cumulative exposure days" (years of use × days/year); and *c*) intensity-weighted cumulative exposure days (years of use × days/year × estimated intensity level). Poisson regression was used to estimate exposure–response relations between glyphosate and incidence of all cancers combined and 12 relatively common cancer subtypes. Glyphosate exposure was not associated with cancer incidence overall or with most of the cancer subtypes we studied. There was a suggested association with multiple myeloma incidence that should be followed up as more cases occur in the AHS. Given the widespread use of glyphosate, future analyses of the AHS will allow further examination of long-term health effects, including less common cancers.

* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Various pesticides
Active substance(s):	Glyphosate and 50 others
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

Species:	Human
Age of test persons:	Up to 70 years
Sex:	Males and females

4. Test system:

Study type:	Prospective cohort study
Data collection:	Self-administered enrolment questionnaire
Guideline:	None
GLP:	No
Guideline deviations:	Not applicable
No. of persons analyzed:	54315

5. Observations/analyses:

Working history:	All subjects
Detailed assessment of exposure:	Collected comprehensive-use data on 22 pesticides, ever/never use information for 28 additional pesticides, and general information on pesticide application methods, personal protective equipment, pesticide mixing, and equipment repair. Data were also collected on basic demographic and lifestyle factors. Glyphosate exposure metrics for this analysis: *a*) ever personally mixed or applied products containing glyphosate (ever/never); *b*) cumulative lifetime days of use, or "cumulative exposure days" (years of use × days per year, categorized in tertiles among users: 1–20, 21–56, 57–2,678); and *c*) intensity-weighted cumulative exposure days (years of use × days per year × intensity level, categorized in tertiles: 0.1–79.5, 79.6–337.1, 337.2–18,241).
Parameters determined:	The median time of follow-up for occurring cancers was 6.7 years.
Statistics:	Differences between the exposure groups were tested using the chi-square statistics and associated *p*-values. Poisson regression analyses were carried out for all cancers combined and specific cancer sites to estimate rate ratios (RRs) and 95% confidence intervals (CIs) associated with glyphosate exposure metrics; the effect of each metric was evaluated in a separate model for each cancer. Tertile exposure variables were analyzed in separate models using either the lowest tertile–exposed or never-exposed subjects as the reference category. For each exposure metric, RRs were adjusted for emographic and lifestyle factors, including age at enrolment (continuous), education (dichotomous: ≤ high school graduate or GED/education beyond high school), pack-years of cigarette smoking [indicator variables: never, pack-years at or below the median (12 pack years), pack-years above the median], alcohol consumption in the past year [indicator variables: none, frequency at or below the median (72 drinks), frequency above

the median], family history of cancer in first-degree relatives (dichotomous: yes/no), and state of residence (dichotomous: Iowa/North Carolina).

Potential confounding from exposure to other pesticides was explored by adjusting for the five pesticides for which cumulative exposure-day variables were most highly associated with glyphosate cumulative exposure days [(2,4-dichlorophenoxy)acetic acid (2,4-D), alachlor, atrazine, metolachlor, trifluralin].

Tests for trend across tertiles were conducted by creating a continuous variable with assigned values equal to the median value of cumulative exposure days (or intensity weighted exposure days) within each tertile; the *p*-value for the trend test was that from the Poisson model coefficient for this continuous variable. **P**-values < 0.10 were considered as indicative of a trend.

Additional analyses were conducted for cancers for which we observed elevated RRs, and for NHL (non-Hodgkin lymphoma) because of its association with glyphosate in previous studies. These included analyses stratified by state and analyses across quartiles and quintiles (where numbers allowed) of exposure day's metrics.

KLIMISCH EVALUATION

1. Reliability of study: **Reliable without restrictions**

Comment: Well documented publication. Study included glyphosate exposure, as well as demographic and lifestyle factors. However, adjusted relative risk calculations eliminated a significant proportion of the data set without justification.

2. Relevance of study: **Relevant** (Evaluation focussed on glyphosate, although other pesticides were also considered in the data evaluation)

3. Klimisch code: **2**

Response 1 – summary from Letter to the Editor by Donna Farmer, PhD (Monsanto), Timothy Lash, PhD (Boston University) and John Acquavella PhD (Monsanto)

- Authors provided an incomplete genotoxicity review which was inconsistent with opinions of regulatory agencies and experts around the world, that glyphosate is not genotoxic. An extensive toxicology review of glyphosate was cited by the authors, mentioning a lack of carcinogenicity with glyphosate exposures, yet neglected to cite the extensive genotoxicity review in the same publication by Williams et al. (2000)
- Biological plausibility of a cancer effect should be considered in the light of exposure. Acquavella et al (2004) reported the maximum systemic dose to resulting from application of glyphosate to areas as large as 400 acres was 0.004 mg/kg, and the geometric mean systemic dose was 0.0001 mg/kg in farmers. If these glyphosate applications and exposures continued daily over the course of a lifetime, the systemic dose would be at least 250,000-fold lower than the cancer no-effect level in rodents.

- The authors were requested to further evaluate their models for confounding and selection bias in the multiple myeloma analysis.

  Note: Farmer et al. (2005) is referenced in Doc L Table 3 and included in Doc K.

Response 2 – summary from Lash (2007)

- Table 2 of De Roos et al. (2005) noted 32 cases of multiple myeloma associated with "ever-use" of glyphosate and when compared with "never-use" (adjusted for age only) yielded a rate ratio of 1.1 (95% CI 0.5-2.4). However, when the data set was adjusted for age, demographic and lifestyle factors and other pesticide use, the rate ratio increased to 2.6 (95% CI 0.7-9.4).
- The adjusted estimate merits careful inspection and can only be undertaken with access to the primary data, not made available by the authors.
- Bias analysis was conducted, accounting for confounding and exposure misclassification.
- Adjustment for confounders in De Roos et al. (2005), which resulted in limiting the data set by 25% because of missing data on the adjustment variables, likely introduced selection bias and produced the a rate ratio of 2.6 that was substantially biased.

  Note: Lash (2007) was captured in the literature search, is referenced in Doc L Table 2 and included in Doc K.

Author(s)	Year	Study title
Eriksson, M. Hardell, L. Carlberg, M. Akerman, M.	2008	Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis International Journal of Cancer Volume: 123 Pages: 1657-1663

Abstract*

We report a population based case-control study of exposure to pesticides as risk factor for non-Hodgkin lymphoma (NHL). Male and female subjects aged 18-74 years living in Sweden were included during December 1, 1999, to April 30, 2002. Controls were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total 910 (91 %) cases and 1016 (92%) controls participated. Exposure to herbicides gave odds ratio (OR) 1.72, 95% confidence interval (CI) 1.18-2.51. Regarding phenoxyacetic acids highest risk was calculated for MCPA; OR 2.81, 95% CI 1.27-6.22, all these cases had a latency period >10 years. Exposure to glyphosate gave OR 2.02, 95% CI 1.10-3.71 and with >10 years latency period OR 2.26, 95% CI 1.16-4.40. Insecticides overall gave OR 1.28, 95% CI 0.96-1.72 and impregnating agents OR 1.57, 95% CI 1.07-2.30. Results are also presented for different entities of NHL. In conclusion our study confirmed an association between exposure to phenoxyacetic acids and NHL and the association with glyphosate was considerably strengthened.

* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Various herbicides, insecticides, fungicides, rodenticides, and impregnating agents
Active substance(s):	Glyphosate and others
Description:	Not reported
Source of test item:	Not reported
Lot/Batch #:	Not reported
Purity:	Not reported

2. Vehicle and/or positive control: Not applicable

3. Test group:

(in the following data only presented for exposures to glyphosate and total number of subjects)

Species:	Human
Age of test persons:	18-74
Sex:	Males and females

4. Test system:

Study type:	Epidemological study for pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis
Guideline:	None
GLP / GCP:	No
Guideline deviations:	Not applicable
Collection of data:	Questionnaire

No. of exposed persons with NHL:	910
No. of control persons:	1016
No. of persons with Non-Hodgkin lymphoma (NHL) exposed to glyphosate:	29
No. of persons in control group:	18
Pesticide use frequency:	Glyphosate exposed / control group ≤ 10 days: 1/9 persons ≥ 10 days: 17/9 persons
Application rates:	Not reported

5. Observations/analyses:

Working history:	All subjects
Other:	Smoking habits, medications, leisure time activities, proximity from home to certain industrial installations (these factors were not reported)
Detailed assessment of exposure:	Questionnaire included a total work history with in depth questions regarding exposure to pesticides, organic solvents and several other chemicals. For all pesticides not only numbers of years and numbers of days per year, but also approximate length of exposure per day were questioned. Since most work with pesticides was performed in an individualized manner, no job-exposure matrix was judged to be applicable.
Parameters determined:	Regarding phenoxy herbicides and glyphosate an analysis was made taken the latency period for exposure into account
Statistics:	Unconditional logistic regression analysis (Stata/SE 8.2 for Windows) was used to calculate odds ratios (OR) and 95% confidence intervals (CI). Adjustment was made for age, sex and year of diagnosis (cases) or enrolment (controls). In the uni-variate analysis, different pesticides were analyzed separately and the unexposed category consisted of subjects that were unexposed to all included pesticides. When analyzing subgroups of NHL all controls were used in the separate analyses. In the dose-response calculations made for agents with at least 20 exposed subjects, median number of days of exposure among controls was used as cut-off. Latency period calculations and multi-variate analyses included agents with statistically significant increased OR, or with an OR > 1.50 and at least 10 exposed subjects

KLIMISCH EVALUATION

1. Reliability of study:	**Not reliable**
Comment:	Multiple avenues for bias were introduced in study design, execution and data processing. No information about exposure duration, used glyphosate products and application rates. Other factors (i.e. smoking habits, medication etc.) were assessed but not included in the evaluation.
2. Relevance of study:	**Relevant** with reservation

3. Klimisch code: 3

Response –Review by Professor Pamela Mink, PhD, Rollins School of Public Health, Emory University, Atlanta Georgia, USA

Study Overview and Main Findings

The authors (Eriksson et al. 2008) conducted a population-based case-control study of exposure to a variety of pesticides and non-Hodgkin lymphoma (NHL), including separate analyses of histopathological categories of NHL. Study subjects were males and females, ages 18-74, living in Sweden between December 1, 1999 and April 30, 2002. The final study group included 910 cases and 1016 controls. Exposure, ascertained via an interviewer-administered questionnaire, focused on pesticide and other chemical agents, and included a total work history (although a job-exposure matrix was not used). For pesticide exposure, information on number of years, number of days per year, and approximate length of exposure per day was also obtained. A minimum of one full day of exposure was required for categorization as "exposed."

The authors reported a statistically significant positive association between "herbicide exposure" and NHL (OR = 1.72; 95% CI: 1.18-2.51). Glyphosate exposure was reported by 29 cases and 18 controls, and the corresponding odds ratio (OR) was 2.02 (95% CI:
1.10-3.71). The ORs for glyphosate exposure of <10 days and >10 days were 1.69 (95% CI: 0.70-4.07) and 2.36 (1.04-5.37), respectively. The ORs for glyphosate were 1.11 (95% CI: 0.24-5.08) and 2.26 (95% CI: 1.16-4.40) for "latency" periods of 1-10 years and >10 years, respectively. In analyses of glyphosate and type of NHL, statistically significant positive associations were observed for small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) (OR = 3.35; 95% CI: 1.42-7.89) and for "unspecified NHL" (OR = 5.63; 95% CI: 1.44-22.0). Odds ratios for the other types (total B-cell lymphomas, grade I-III follicular lymphoma, diffuse large B-cell lymphoma, other specified B-cell lymphoma, unspecified B-cell lymphoma, and T-cell lymphomas) were above 1.0, but were not statistically significant (i.e., the 95% confidence intervals were relatively wide and included the null value of 1.0).

The authors concluded, "Glyphosate was associated with a statistically significant increased OR for lymphoma in our study, and the result was strengthened by a tendency to dose-response effect…" (p. 1662). The authors suggested that their findings are consistent with results of a previous case-control study (Hardell and Eriksson 1999) and pooled analysis (Hardell et al. 2002) that they conducted. In the case-control study, an OR of 2.3 (95% CI: 0.4-13.0), based on 4 exposed cases and 3 exposed controls, was reported for glyphosate and NHL. In the pooled analysis of two case-control studies, which included data from Hardell and Eriksson (1999), an OR of 3.04 (95% CI: 1.08- 8.52) was reported, based on 8 exposed cases and 8 exposed controls. The authors also cited three studies (De Roos et al. 2003; McDuffie et al. 2001; De Roos et al. 2005) by other groups as being consistent with their results in that they "also associate glyphosate with different B-cell malignancies such as lymphomas and myelomas." It should be noted, however, that the relative risk (RR) reported by De Roos et al. (2005) for the highest versus lowest category of cumulative exposure days of glyphosate and NHL in the prospective Agricultural Health Study was 0.9.

Interpretation Issues

Identification of Cases and Potential Referral Bias. It is noteworthy that the cases in the current analysis were identified from some of the same hospitals as the authors' prior publication; thus, referral bias may have been an issue. In particular, the researchers approached the patients after diagnosis if the physicians deemed it appropriate. Therefore, if the physicians were concerned that their patient's NHL was associated with agricultural exposures, they may have suggested participation in the study.

Participation Rates and Potential Selection Bias. The authors report a participation rate of 91% and 92% for cases and controls, respectively; however, these figures are based on completed questionnaires out of those who had previously said they would participate in the study. The number of eligible patients (i.e., prior to physician approval to "approach") was not reported, so the computation of an exact participation

rate is difficult. Based on information provided in the paper, participation among cases is estimated to be about 80%. Nonparticipation is a concern for several reasons. First, in a case-control study, an odds ratio will be an accurate representation of the exposure-disease association when the cases are representative of all cases and the controls are representative of the exposure experience of the population that gave rise to the cases. If the final study sample is not representative of this "target population" then measures of effect (e.g., the odds ratio) may not be valid. In addition, one must be concerned about selection bias. Selection bias occurs in a case-control study when the exposure distribution for cases and controls differ for those who participate in the study compared to those who are eligible but do not participate in the study. It is not possible to determine whether there is selection bias without information about nonparticipants.

Strengths and Limitations of Using Living Cases Only versus All Cases (Living + Dead).
The authors noted that 88 potential cases died before they could be interviewed and were therefore excluded from the study. It is also stated in the Discussion that restricting the study to living cases and controls was an "advantage" of the study, as interviewing cases and controls directly compared to interviewing next-of-kin was preferable. While it is generally true that this would be an advantage, the following statement by the authors, therefore, is not accurate, "The study covered all new cases of NHL during a specified time" (p. 1660). The study did not include all new cases; it included only those cases who survived until the time of the interview. Thus, while there may have been an advantage to restricting the study to living cases, there was a trade-off in that the study population did not represent all cases, specifically those cases with more aggressive disease. This disadvantage was not discussed by the authors, nor was the potential bias that could have resulted from excluding many eligible cases.

Exposure Measurement and Information Bias. Exposure was ascertained via a questionnaire oriented towards pesticide and other chemical agents. In addition, interviewers collected information by telephone if "important" data were lacking, incomplete, or unclear. It is unknown what is meant by "important," and the proportion of cases and controls who received phone calls was not reported. Thus, information bias may be a concern. Even though interviewers were blinded to case and/or control status, they may have been able to determine this information during the course of the interview. Furthermore, recall bias may be an issue because exposure information was based on participant response and cases and controls may recall and/or report past pesticide exposures differently. No exposure validation techniques were implemented, nor did an industrial hygienist (or any other type of personnel trained in assessing occupational exposures) independently validate/estimate the frequency and/or intensity of exposure. The authors assumed that "some misclassification regarding quantity of exposure has probably occurred, but such misclassification would most probably be nondependent of case/control status, and therefore only weaken any true risks" (p. 1660). They do not provide any explanation as to why they believe that exposure misclassification would be "most probably" nondifferential. If NHL cases believe that pesticides may be related to their disease, then it is certainly possible that they may recall and/or report pesticide exposure differently than NHL-free controls, which could result in odds ratios that are inflated as a result of bias.

Interpretation of "dose-response" analyses. The referent group in the statistical analyses consisted of participants who were unexposed to all pesticides. The dose-response analyses were based on a dichotomy of the median number of days exposed to a particular agent. It is difficult to analyze "dose-response" when only two exposure categories are considered. Furthermore, the dose-response analyses were based on median values of exposure but heterogeneity of cut-points is evident across agents. For example, glyphosate was analyzed as < 10 days and > 10 days, whereas, "other" herbicides were analyzed as < 32 days and > 32 days. Although analytical cut-points were data driven, interpretation across the wide variety of exposures is complicated by the variability in exposure cut-points. In addition, even though the OR for the higher category of exposure days was greater than the OR for the lower category, the two 95% confidence intervals were wide and overlapped considerably (0.70-4.07 and 1.04-5.37).
Thus, it is not clear whether the two point estimates reported (1.69 and 2.36) are significantly different from each other. Finally, this result cited in the "dose-response" analyses may have been confounded by exposure to other herbicides. In Table II (Eriksson et al. 2008), the authors observed elevated associations for other herbicides, including MCPA, 2,4,5-T and/or 2,4-D. The correlation between exposure to glyphosate and other herbicides was not provided nor were analyses of glyphosate-exposed individuals

after accounting for the collinear relation between this agent and other agents. The odds ratio for "ever" exposure to glyphosate was attenuated after additional adjustment for other pesticides (Table VII, Eriksson et al. 2008), but multi-variate -adjusted estimates for the "dose-response" odds ratios were not reported.

Unusual Pattern of Positive Associations. The authors conducted multiple comparisons, and one would expect a certain proportion of their findings to be statistically significant (whether in the positive or inverse direction) simply as a result of chance. It is somewhat surprising, therefore, that the vast majority of the ORs presented in this manuscript are greater than 1.0, regardless of the statistical significance. The authors do note that for some of the analyses (e.g., latency), only chemicals for which ORs were greater than 1.5 and for which there were at least 10 exposed cases, or for which there was a statistically significant OR were evaluated. On the other hand, dose-response was evaluated based on the number of exposed subjects and not on the strength or significance of the findings. The authors do not address this directly, but do state in their Discussion, "…several pesticides are chemically related and may exert their effects on humans through a similar mechanism of action, which may explain the wide range of pesticides that have been related to NHL over time in different countries and with different exposure conditions" (p. 1661). On the other hand, this pattern of positive findings could be a result of bias, including recall bias (or other information bias), selection bias, uncontrolled confounding, or a combination of these and other factors.

Interpretation of Eriksson et al. (2008) in Context of Other Studies. Despite the statement by the authors that, "Recent findings from other groups also associate glyphosate with different B-cell malignancies such as lymphomas and myeloma" (p. 1662), most multi-variate analyses of glyphosate and NHL do not report statistically significant associations (De Roos et al. 2005; Cantor et al. 1992; De Roos et al. 2003; Hardell and Eriksson 1999; Hardell et al. 2002; Lee et al. 2004; McDuffie et al. 2001; Nordstrom et al. 1998) (Tables A and B). It is notable that Hardell et al. (2002) reported a significant positive association between glyphosate association and NHL, but the multi-variate -adjusted odds ratio was attenuated and not statistically significant. Similar findings were reported by Eriksson et al. (2008). Specifically, the association reported by the authors in the abstract (OR = 2.02; 95% CI: 1.10-3.71) was adjusted for age, sex and year of diagnosis or enrollment. When other pesticides were added to that model (i.e., agents with statistically significant increased odds ratios, or with an odds ratio greater than 1.5 and with at least 10 exposed subjects), the adjusted odds ratio was 1.51 (95% CI: 0.77-2.94). Thus, the authors' final statement, "Furthermore, our earlier indication of an association between glyphosate and NHL has been considerably strengthened" is questionable. Their previous findings showed a non-significant association after multi-variate adjustment (OR = 1.85; 95% CI: 0.55-6.20). The 2008 study similarly reported a statistically non-significant association between glyphosate and NHL after multi-variate adjustment (OR = 1.51; 95% CI: 0.77-2.94). The results reported for analyses of duration of exposure and latency of exposure did not adjust for other pesticides, and one would expect that those ORs would also be attenuated.

Summary of Findings: Cohort and Case-Control Studies of Exposure to Glyphosate and Non-Hodgkin Lymphoma

Table A. Cohort Studies

Author Year	Description	No. of Exposed Cases	Type of Relative Risk Estimate	Relative Risk Estimate	95% Confidence Limits	Variables Included in Statistical Model
De Roos et al. 2005	57-2,678 vs. 1-20 Cumulative Exposure Days[a]	17	RR	0.9	0.5-1.6	Age at enrollment, education, pack-years of cigarette smoking, alcohol consumption in the past year, family history of cancer in first-degree relatives, and state of residence Also adjusted for other pesticides
	337.2-18,241 vs. 0.1-79.5 Intensity-Weighted Exposure Days[b]	22	RR	0.8	0.5-1.4	

[a] Years of use x days per year; categorized by tertiles
[b] Years of use x days/year x estimated intensity level; categorized by tertiles

Table B. Case Control Studies

Author Year	Exposure Evaluated	Subgroup Description	No. of Exposed Cases	No. of Exposed Controls	OR	95% CI	Variables Included in Statistical Model
Cantor et al. 1992	Agricultural exposure based on ever living or working on a farm	Nonfarmer	266	547	1.0	Referent	Vital status, state, age, smoking, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures
		Farmer	356	698	1.2	1.0-1.5	
	Farmers with specific pesticide exposures (ever mixing, handling, or applying) compared to nonfarmers	Glyphosate	26	49	1.1	0.7-1.9	
De Roos et al. 2003	Ever exposure to specific pesticide; men only (all 47 pesticides were regressed simultaneously)	Glyphosate (Logistic Regression)	36	61	2.1	1.1-4.0	Age, study site and other pesticides
		Glyphosate (Hierarchical Regression)	36	61	1.6	0.9-2.8	Second-level model incorporated what was known about each true effect parameter prior to seeing the study data
Hardell and Eriksson 1999	Exposure to specific pesticides (ever/never exposed to the specific pesticide vs. no exposure to any pesticide)	Glyphosate (conditional logistic regression; uni-variate analysis)	4	3	2.3	0.4-13	Age and country (matching factors)
		Glyphosate (conditional logistic regression; multi-variate analysis)	4	3	5.8	0.6-54	Multi-variate variables not listed by authors
Hardell et al. 2002	Exposure to specific pesticides (ever/never exposed to the specific pesticide vs. no exposure to any pesticide)	Glyphosate (conditional logistic regression; uni-variate analysis)	8	8	3.04	1.08-8.52	Age and county (matching factors); study, study area (county), and vital status
		Glyphosate (conditional logistic regression; multi-variate analysis)	8	8	1.85	0.55-6.20	Multi-variate variables not listed by authors
Lee et al. 2004a	Exposure to individual pesticides	Glyphosate use, Non-asthmatics	53	91	1.4	0.98-2.1	Age, state, vital status
		Glyphosate use, Asthmatics	6	12	1.2	0.4-3.3	

McDuffie et al. 2001	Exposure to individual active chemicals	Glyphosate (Round-Up)	51	133	1.26	0.87-1.80	Strata for age and province of residence
		Glyphosate (Round-Up)	NR	NR	1.20	0.83-1.74	Plus statistically significant medical variables
Nordstrom et al. 1998	Exposure to specific herbicides, insecticides, and fungicides	Glyphosate	4	5	3.1	0.8-12	Age and country (matching factors)
Eriksson et al. 2008	Exposure to specific herbicides regardless if they also had been exposed to phenoxyacetic acids or not	Glyphosate	29	18	2.02	1.10-3.71	Age, sex, and year of diagnosis or enrollment
			29	18	1.51	0.77-2.94	Age, sex, and year of diagnosis or enrollment and pesticides with statistically significant increased odds ratios, or with an odds ratio greater than 1.5 and with at least 10 exposed subject
	Exposure to herbicide stratified by median number of days among exposed controls	Glyphosate ≤ 10 days	12	9	1.69	0.70-4.07	Age, sex, and year of diagnosis or enrollment
		Glyphosate >10 days	19	9	2.36	1.04-5.37	
	Exposure to specific herbicides according to different lymphoma entities	Glyphosate: B-Cell lymphomas	NR	NR	1.87	0.998-3.51	Age, sex, and year of diagnosis or enrollment
		Lymphocytic lymphoma/B-CLL	NR	NR	3.35	1.42-7.89	
		Follicular grade I-III	NR	NR	1.89	0.62-5.79	
		Diffuse large B-cell Lymphoma	NR	NR	1.22	0.44-3.35	
		Other specified B-cell lymphoma	NR	NR	1.63	0.53-4.96	
		Unspecified B-cell Lymphoma	NR	NR	1.47	0.33-6.61	
		T-cell lymphomas	NR	NR	2.29	0.51-10.4	
		Unspecified NHL	NR	NR	5.63	1.44-22.0	

Author(s)	Year	Study title
George, J. Prasad, S. Mahmood, Z. Shukla, Y.	2010	Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach Journal of Proteomics Volume: 73 Pages: 951-964

Abstract*

Glyphosate is a widely used broad spectrum herbicide, reported to induce various toxic effects in non-target species, but its carcinogenic potential is still unknown. Here we showed the carcinogenic effects of glyphosate using 2-stage mouse skin carcinogenesis model and proteomic analysis. Carcinogenicity study revealed that glyphosate has tumor promoting activity. Proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry showed that 22 spots were differentially expressed (>2 fold) on glyphosate, 7, 12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoyl-phorbol-13-acetate (TPA) application over untreated control. Among them, 9 proteins (translation elongation factor eEF-1 alpha chain, carbonic anhydrase III, annexin II, calcyclin, fab fragment anti-VEGF antibody, peroxiredoxin-2, superoxide dismutase [Cu–Zn], stefin A3, and calgranulin-B) were common and showed similar expression pattern in glyphosate and TPA-treated mouse skin. These proteins are known to be involved in several key processes like apoptosis and growth-inhibition, anti-oxidant responses, etc. The up-regulation of calcyclin, calgranulin-B and down-regulation of superoxide dismutase [Cu–Zn] was further confirmed by immunoblotting, indicating that these proteins can be good candidate biomarkers for skin carcinogenesis induced by glyphosate. Altogether, these results suggested that glyphosate has tumor promoting potential in skin carcinogenesis and its mechanism seems to be similar to TPA.

* Quoted from article

MATERIALS AND METHODS

1. Test material:

Test item:	Roundup Original ®
Active substance(s):	Glyphosate
Source:	Monsanto Company St. Louis, USA (obtained from a local market)
Lot/Batch #:	Not reported
Purity:	360 g/L glyphosate salt equivalent as the isopropylamine salt
Co-formulants:	The formulation contained 15% POEA (polyethoxylated tallow amine)

2. Vehicle and positive controls:

50% ethanol
12-o-tetradecanoylphorbol-13-acetate (TPA);
7, 12-dimethylbenz[a]anthracene (DMBA).

3. Test animals:

Species:	Mice
Strain:	Swiss albino
Source:	Indian Institute of Toxicology Research (IITR)
Age of test animals at study initiation:	Not reported
Sex:	Male
Body weight:	12-15 g
Acclimation period:	1 week

Diet/Food:	Synthetic pellet basal diet (Ashirwad, Chandigarh, India), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Not reported
Environmental conditions:	Temperature: 23 ± 2°C
	Humidity: 55 ± 5%
	Air changes: Not reported
	Light/dark cycle Not reported

4. Test system:

Study type:	**Proteomic study in mouse skin**
Guideline:	No
GLP:	No
Guideline deviations:	Not applicable
Duration of study:	32 weeks
Dose groups:	**Group I** – Untreated control (No treatment). **Group II** – Glyphosate alone (25 mg/kg bw, topically 3 times per week). **Group III** – DMBA+TPA (Single topical application of DMBA, 52 µg/mouse followed 1 week later by thrice a week application of TPA, 5 µg/mouse). **Group IV** – Glyphosate (s)+TPA (Single topical application of glyphosate, 25 mg/kg bw followed 1 week later by TPA application as in group III). **Group V** – Glyphosate (m)+TPA (Thrice a week topical application of glyphosate, 25 mg/kg bw for 3 weeks [total of 9 applications], followed1 weeklater byTPA application as in group III). **Group VI** – DMBA (Single topical application of DMBA, 52 µg/ mouse). **Group VII** – TPA (Thrice a week topical application of TPA, 5 µg/mouse). **Group VIII** – DMBA+glyphosate (Single topical application of DMBA [as in group III], followed 1 week later by topical treatment of glyphosate, 25mg/kg bw thrice per week).
Animals per dose group:	8 groups of 20 animals each

Study type:	**Proteomic study**
Guideline:	No
GLP:	No
Guideline deviations:	Not applicable
Dose groups:	**Group I** – Untreated controls (No treatment). **Group II** – Glyphosate (Single topical application, 50 mg/kg bw/mouse). **Group III** – DMBA (Single topical application of DMBA, 104 µg/mouse). **Group IV** – TPA (Single topical application of TPA, 10 µg/mouse).
Animals per dose group:	4 groups of 4 animals each

Sampling and sample preparation:	24 h after application animals were sacrificed and skin tissues from the treatment site were excised. Hair and subcutaneous fat was removed, and small pieces of cleaned skin tissues of each mouse from all the groups were then homogenised (10 % w/v) individually, in 2-DE lysis buffer. The lysed samples were soniocated, centrifuged and pooled for the respective group. After quantification of proteins by Lowry´s method, the supernants were stored at -80°C until use fro electrophoresis.

5. Observations/analyses:

Carcinogenicity study in mouse skin

Body weight:	Measured weekly
Development:	Examined weekly
Gross morphological changes:	Volume of squamous cell papillomas (tumors) locally on the skin was examined during the entire study period. Tumors larger than 1 mm diameter were included in the total number of tumors.
Mortality:	Not reported
Clinical signs:	Not reported
Food- and water consumptions:	Not reported
Test substance intake:	Not reported
Haematology:	Not reported
Clinical chemistry:	Not reported
Urine analysis:	Not reported
Sacrifice/pathology:	Not reported
Organ weights:	Not reported
Histopathology:	Not reported

Proteomic study

Protein quantification:	Quantification of proteins in the supernants prepared for 2-DE by Lowry's method.
Protein expression profile:	2-D electrophoresis (2-DE) IEF was carried out using commercially dedicated equipment, Protean IEF. IEF was performed for each individual sample to a total of 45.5 kVh. All IEF steps were carried out at 20 °C. After the first-dimensional IEF, focused IPG strips were placed in an equilibration solution. Separation in the second dimension was carried out using Protean II xi electrophoresis equipment. Each experiment was performed in triplicate to obtain the reproducible results. After completion of the second-dimension electrophoresis, the gels were fixed and stained by using a fast silver staining protocol with neutral silver nitrate. Analysis of the 2D-gels including background subtraction, spot detection, volume normalization and differences in protein

	expression levels among samples were analyzed by using PDQuest software Ver. 7.4.0. To determine the variation, 3 gels were prepared for each sample. The protein spots that varied >2 fold change and were specific for the test groups and the control group were manually labeled and considered for MS analysis.
Protein identification	Matrix-assisted laser desorption/ ionization time-of-flight (MALDI-TOF/TOF) and liquid chromatography mass spectrometry (LC-MS) Differential protein spots of interest were excised manually and washed with deionised water. After in-gel digestion, trypsinised samples were dissolved and mixed with matrix (α-cyano-4-hydroxy cinnamic acid). Following drying the peptides were spotted on ground steel plate and subjected to Bruker Ultraflex MALDI-TOF/TOF and 2D Nano LC-ESI-Trap (Agilent) for mass spectrometric identification. Data acquisition and analysis was performed using flex control and flex analysis/biotools version 2.2 software, respectively. Data was acquired in reflectron positive mode using 15–18% laser power. Mass tolerance and monoisotopic values (50 ppm/100 ppm for peptide mass fingerprint and peptide mass tolerance of 2 Da for MS/MS spectra) were used for searching. The datasets of the MS spectra, including peptide sequence information, were searched against the SWISS-PROT and NCBInr database using Mascot Daemon as a client attached to the Mascot search protocol.
Verification of calcyclin, calgranulin-B and SOD 1:	The differential proteins screened with 2-DE were confirmed by Western blot analysis. Skin tissue samples were lysed in lysis buffer, resolved on a 12-15% polyacrylamid gel, and electro-transferred onto polyvinylidene fluoride membranes. After blocking, the membranes were immunoblotted with antibodies of calcyclin, calgranulin-B and superoxide dismutase (SOD 1) and beta-actin at dilutions recommended by the suppliers. Horse radish conjugated secondary antibodies and chemiluminescence kit, were used for detection. Protein expression was visualized by Versa Doc Imaging System. The intensity was given in terms of relative pixel density for each band normalized to band of beta-actin. The intensity of the bands was measured using software UNSCAN-IT automated digital system version.
Statistics:	The skin tumour incidence was analyzed by one-way analysis of variance (ANOVA) test in untreated control and treated groups, $p<0.05$ value was considered as significant. Protein expression data for untreated control and treated groups are expressed as the mean±SD of 3 replicate gels for fold changes of normalized spot volumes. For the statistical analysis of data, Student-t-test was used and $p<0.05$ was considered as significant. Hierarchical clustering analysis using Ward's minimum variance was performed by NCSS software.

KLIMISCH EVALUATION

1. Reliability of study: **Reliable with restrictions**

Comment: Non-guideline mechanistic study. Scientifically acceptable study with deficiencies (controls with glyphosate alone, and co-formulants were not included)

2. Relevance of study: **Relevant with restrictions** (Glyphosate formulation not glyphosate alone was tested.)

3. Klimisch code: **2**

Response – GTF

It is important to note that the authors use glyphosate as a synonym for what is really a glyphosate based formulated product. Doses in this study are not representative of human exposures to glyphosate or glyphosate based formulations. Mice in the tumor promoting group VIII received topical applications of concentrated glyphosate formulated product three times per week for over thirty weeks without washing after an initial treatment with the potent tumor initiator DMBA. Glyphosate had been shown to have very low dermal absorption, even in formulated products, and since is non-volatile, would likely accumulate on mouse skin. Surfactants are typically irritating and non-volatile. Given the irritation potential of the unwashed exposed mouse skin over the course of thirty or more weeks, tumor promotion may be a physical response to substantial localized dermal irritation. Epidemiological studies reported above note no association with glyphosate and either skin or lip cancers.

Label directions outline appropriate personal protective equipment such as gloves and long sleeves. Furthermore, any dermal exposure of concentrated product to human skin would prove irritating and prompt handlers to wash off soon after dermal exposure.

Human *in vitro* dermal absorption studies reported in Section IIA 5.9.9 for a range of glyphosate based formulations containing different surfactant systems all demonstrate extremely low dermal absorption of glyphosate active ingredient for concentrated products, of less than 0.2%. Test material recovery in each of the four reported dermal absorption studies was very good, close to 100%. Most of the glyphosate was removed during skin surface washing at either eight or twenty four hours of in vitro human skin exposure. This also suggests significant potential for accumulation of glyphosate on the surface of the mice skin in George et al. (2010).

Proteomics is an emerging science, not yet yielding validated test methods for establishing human health endpoints. The up-regulation / down-regulation of protein expression reported after a single dermal dose of a glyphosate formulated product (proteomics experiment, group II), while interesting, does not demonstrate any toxicological endpoint. Rather, perturbations may well represent normal homeostatic fluctuations and be a natural response to insult. Further research and validation in this field will be necessary before such studies may prove useful in human health risk assessment.