Review of:

Hardell L, Eriksson M. A Case-control Study of non-Hodgkin Lymphoma and Exposure to Pesticides. *Cancer* 1999; 85:1353-1360.

By:

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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-methyl phenoxyacetic 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 non-carcinogenic 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, I'll 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 univariate and multivariate 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 1.0-7.0). 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 penetrability[9], 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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