Review of:

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

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

In a recent study, Hardell and Erikkson[1] found a non-significant association between their study subjects' reported use of glyphosate and non-Hodgkin's lymphoma. The authors interpreted this result conservatively due to the low prevalence of reported glyphosate use among study subjects (4 cases and 3 controls) and other methodologic limitations of their study. However, they considered the association to be worthy of concern citing toxicologic findings for glyphosate of: excess mutations and chromosome aberrations in studies with mouse lymphoma cells[2-5], excess sister chromatid exchanges (SCEs) in cultures of human lymphocytes[6], and a somewhat increased incidence of various cancers in one carcinogenicity study of mice.[7]

Hardell and Erikkson's summary of the relevant toxicology data included six studies, five of which did not use glyphosate as the test material.[2-5,7] 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 the U.S. Environmental Protection Agency (EPA) considered the mouse lymphoma findings[2-5] 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.[8] Also, EPA characterized the sulfosate mouse carcinogenicity study[7] as showing "… no evidence of carcinogenicity … at the doses tested" and classified sulfosate as category E - no evidence for carcinogenicity in humans.[8]

Hardell and Erikkson also did not address the weight of evidence for glyphosate that is contrary to their view. The one glyphosate toxicology study cited[6] showed a weak positive SCE finding in human lymphocytes in vitro. This study had many limitations and numerous, more specific, mutagenicity assays have not shown positive results for glyphosate.[9] Extensive reviews of the available toxicologic data have been completed recently by the EPA[10,11] and the World Health Organization.[12] These agencies concluded that glyphosate is not mutagenic or carcinogenic. EPA classified glyphosate as category E.[10,11]

Finally, we note that the exposure classification methodology used by Hardell and Erikkson, based on study subjects' reported glyphosate use, is not likely to be meaningful. Agricultural or residential uses do not result in appreciable inhalation exposure due to glyphosate's extremely low vapor pressure. Exposure opportunity is almost exclusively through dermal contact. Glyphosate, however, has been shown to have very low skin penetrability in experimental studies.[13] A 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.[14] This raises the question of whether reports of glyphosate use, even if accurate, equate to any meaningful exposure.

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Review of Hardell and Eriksson, A case control study of non-Hodgkin's lymphoma and exposure to pesticides, Cancer 1999; 85: 1353-60.

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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 problem 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 collinearity. 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 multivariate 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 univariate and multivariate 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.

Review of Nordstrom et al. Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukemia evaluated in a case control study. British Journal of Cancer 1998; 77: 2048-52.

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This study comes from the same research group as the above reference NHL study, and focuses on one variant malignancy, hairy cell leukemia (HCL). This disorder is a lymphoproliferative disease which has been classified by some as a variant NHL, though others, probably rightly treat it s a related disorder of B-cells with likely different pathogenesis and etiology. Living cases were ascertained, along with living controls, using the tumor and population registries which have been the foundation for the work of this Swedish group. As with the NHL study above, the selection and recruitment of cases and controls is exemplary, and this may be one of the largest available studies of this relatively uncommon disease.

As with the previous study, the major concern is with exposure assessment, done to questionnaires and, when necessary (not well specified) follow-up interviews by phone. A wide range of factors, including agricultural products, industrial materials, UV light and smoking were simultaneously under investigation.

The systematic excesses found for virtually every occupational and environmental factor other than smoking makes the likelihood of uncontrollable recall bias the most likely overall interpretation of the findings. There also appears to be a serious problem of collinearity among agricultural exposures, with comparable odds ratios for a widely divergent range of exposures, and suggestion that exposure to one group of hazards was strongly associated with the others. This renders any interpretation of data within the agricultural category highly suspect, so that the effects of animal exposure cannot readily be disentangled from the effects of chemicals used. The absence of meaningful dose- response relationships further undermines any confidence in the importance of these observations.

As with the Hardell study, above, the authors have chosen to comment on the role of glyphosate, despite the reported use of this product by only 9 of 605 subjects. It is not at all

clear what the purpose of this presentation is, as well as that of several of the industrial chemicals, which were similarly restricted to a handful of cases and controls. It is grossly inappropriate to consider the study of any value in determining the etiologic role of glyphosate or any of these other rare exposures. As noted, the fact that virtually every tested factor proved positive -- inconceivable biologically -- speaks to simpler interpretation, namely differential reporting by cases and controls. The authors are to be congratulated for mentioning this, as well as the possible roles of collinearity and multiple comparisons, but I believe they have underestimated the fatal effects these biases have introduced.

Review of the study by Hardell and Erikson on

Non-Hodgkin Lymphoma and Exposure to Pesticides

Cancer 1999;85:1353-60

by Hans-Olav Adami and Dimitrios Trichopoulos

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 socioeconomic 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 multivariate 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 univariate analysis may be more informative than the multivariate 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 multivariate 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 intercorrelated, 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.