Resource ID#: 3296

A Case-Control Study of Non-Hodgkin's Lymphoma and the Herbicide 2,4-Dichlorophenoxyacetic Acid (2,4-D) in Eastern Nebraska

Shelia Hoar Zahm,[1] Dennis D. Weisenburger,[2] Paula A. Babbitt,[2] Robert C. Saal,[3] Jimmie B. Vaught,[3] Kenneth P. Cantor,[1] and Aaron Blair[1]

To evaluate the role of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in the development of non-Hodgkin's lymphoma (NHL), we conducted a population-based, case-control study in 66 counties in eastern Nebraska. Telephone interviews were conducted with 201 white men diagnosed with NHL between July 1, 1983, and June 30, 1986, and with 725 controls. There was a 50% excess of NHL among men who mixed or applied 2,4-D (odds ratio [OR] = 1.5; 95% confidence interval = 0.9, 2.5). The risk of NHL increased with the average frequency of use to over threefold for those exposed 20 or more days per year (p for trend = 0.051). Adjusting for use of organophosphate insecticides lowered the risk estimate for frequent users (OR = 1.8), but adjustment for fungicide use increased the risk estimate (OR = 4.5). Simultaneous adjustment for organophosphates and fungicides yielded an OR of 3.1 for farmers who mixed or applied 2,4-D more than 20 days per year. Risk also increased with degree of exposure, as indicated by application method and time spent in contaminated clothing, but not with the number of years of 2,4-D use or failure to use protective equipment. Although other pesticides, especially organophosphate insecticides, may be related to NHL, the risk associated with 2,4-D does not appear to be explained completely by these other exposures. (Epidemiology 1990;1:349–356)

Keywords: agriculture, cancer, 2,4-dichlorophenoxyacetic acid, herbicides, insecticides, non-Hodgkin's lymphoma, occupation, pesticides.

In 1986, a case-control study conducted in Kansas showed an association between the development of non-Hodgkin's lymphoma (NHL) and agricultural use of herbicides (1). Risk for NHL increased with the average number of annual days of exposure to herbicides. Farmers exposed for more than 20 days per year had a sixfold increased risk for NHL. This increased risk seemed to be related specifically to 2,4-dichlorophenoxyacetic acid (2,4-D) use and could not be explained by differential recall, exposure to other pesticides, or other factors. Because of the magnitude of these risks and the widespread potential for exposure to 2,4-D in agriculture, forestry, lawn care, and other uses, we undertook a similar population-based case-control study in Nebraska, another midwestern agricultural state.

Subjects and Methods

Cases of NHL, Hodgkin's disease, multiple myeloma, and chronic lymphocytic leukemia among white men and women, aged 21 years or older, residing in 66 counties in eastern Nebraska, and diagnosed between July 1, 1983, and June 30, 1986, were identified through the Nebraska Lymphoma Study Group and area hospitals. Although not an ongoing population-based cancer registry, special procedures were instituted by the Nebraska Lymphoma Study Group to ascertain all cases in eastern Nebraska. The observed incidence rate for NHL among white males, aged 21 years or older, in eastern Nebraska (18.0/100,000 person-years) was 77% of the rate reported for white men, aged 20 years or older, 1983–1986, by the nearby Iowa component of the National Cancer Institute-sponsored Surveillance, Epidemiology, and End Results program (23.5/100,000 person-years) (L. Ries, personal communication). This report will present data on the white male NHL cases (N = 227).

All cases underwent pathology review and were clas-

[1]Environmental Epidemiology Branch, Division of Cancer Etiology, National Cancer Institute, 6130 Executive Boulevard, Room 418 N, Rockville, MD 20892. [2]Department of Pathology and Microbiology, University of Nebraska Medical Center, 42nd and Dewey Avenue, Omaha, NE 68105. [3]Westat, Inc., 1650 Research Boulevard, Rockville, MD 20850. Address reprint requests to Shelia Hoar Zahm.

This study was supported in part by the National Cancer Institute Contract N01-CP-95618 and State of Nebraska Department of Health Grant LB-506. Preliminary analyses of portions of the data in this report were presented at the annual meeting of the Society for Epidemiologic Research, Vancouver, Canada, June 15–17, 1988.

TABLE 1. Distribution of Non-Hodgkin's Lymphomas by Histologic and Immunologic Type in Interviewed White Men

Histology	Number	Percent
Low grade		
A. Small lymphocytic	14	(7)
B. Follicular, predominantly small cleaved cell	20	(10)
C. Follicular, mixed small cleaved and large cell	22	(11)
Intermediate grade		
D. Follicular, predominantly large cell	15	(8)
E. Diffuse, small cleaved cell	23	(11)
F. Diffuse, mixed small and large cell	16	(8)
G. Diffuse, large cell	51	(25)
High grade		
H. Large cell, immunoblastic	30	(15)
I. Lymphoblastic	1	(<1)
J. Small noncleaved cell	4	(2)
Miscellaneous*	5	(3)
	201	
Immunologic type		
T	20	(10)
B	160	(80)
Indeterminant	11	(5)
Not available	10	(5)
	201	

* Composite lymphomas were assigned to the follicular component if the follicular and diffuse components had the same cell type and to the most indolent cell type if the follicular and diffuse components differed.

sified according to the Working Formulation (2) (Table 1). Only histologically confirmed cases (N = 220) were included. The review also included immunologic phenotyping of the NHL. All follicular lymphomas were considered to be B-cell lymphomas. The diffuse lymphomas were phenotyped using the monoclonal antibodies L26 and UCHL1 (DAKO Corporation, Santa Barbara, CA) that mark B cells and T cells, respectively, in paraffin-embedded tissues (3,4).

Control subjects were selected from residents of the same 66-county area by 3:1 frequency matching by race, sex, vital status, and age (±2 years) to the combined age distribution of the four cancer case series (NHL, Hodgkin's disease, multiple myeloma, and chronic lymphocytic leukemia). For living cases under age 65 (N = 73), controls were selected by two-stage random digit dialing (5). For living cases aged 65 or older (N = 67), controls were selected from the Health Care Financing Administration (Medicare) records. For deceased cases (N = 80), controls were selected from the Nebraska state mortality files using the additional matching factor of year of death. Persons with an underlying cause of death of NHL, Hodgkin's disease, multiple myeloma, leukemia, malignancy of unknown site, aplastic anemia, suicide, homicide, or legal intervention were excluded as controls. A total of 831 white male controls were selected.

Telephone interviews were conducted with 201 NHL cases and 725 controls, or with their next-of-kin, between May, 1986, and October, 1987. The interviewers were not aware of the subjects' case-control status. The response rates for the cases and controls were 91% (living: 93%; deceased: 89%) and 87% (living: 89%; deceased: 85%), respectively. The overall control response rate was 85% and consisted of a weighted average accounting for the refusals in the household census phase of the random digit dialing procedure and the refusals of the randomly selected eligible controls to provide interviews.

This investigation covers the findings related to the association between NHL and agricultural exposure to 2,4-D. The interview questions on agricultural practices included those regarding the herbicides and insecticides used, the application method used most often, use of protective equipment, duration of time wearing work clothes after handling pesticides, cattle raising, and use of fungicides, rodenticides, fumigants, wood preservatives, and fertilizers. For each herbicide and insecticide, the years of use, the average annual number of days of use on the farm, and the average annual number of days the pesticides were personally handled were obtained. The interviewer noted whether the response about each pesticide was volunteered in answer to an open-ended question or reported only after a probe naming the specific pesticide.

All odds ratio (OR) estimates were adjusted for age by stratification (21–59, 60–69, 70–79, and greater than 80 years). Maximum likelihood estimates of a uniform odds ratio and 95% confidence intervals (CI) were computed by Gart's method (6). We assessed duration- and dose-response relationships by means of Mantel's one-tailed linear trend test (7). Logistic regression was also used for the data from farmers to evaluate the effects of several pesticide factors simultaneously (8).

Results

There was no overall excess of NHL among persons who had ever lived or worked on a farm; however, a 50% excess risk of NHL was found among men who mixed or applied 2,4-D (Table 2). Men who lived or worked on farms where 2,4-D was used, but who did not personally handle 2,4-D, had an OR of 1.2 (CI = 0.3, 4.2).

Among men who personally handled 2,4-D, risk in-

TABLE 2. Number of White Men with Non-Hodgkin's Lymphoma, Number of Controls, and Odds Ratios by Farming History

Farming History	Cases	Controls*	OR (95% CI)†
Never lived or worked on farm	54	184	1.0
Ever lived or worked on farm	147	539	0.9 (0.6,1.4)
Insecticides used on farm	104	321	1.1 (0.7,1.6)
Herbicides used on farm	75	203	1.3 (0.8,2.0)
Mixed or applied 2,4-D	43	98	1.5 (0.9,2.5)

* Two controls had unknown values for ever having lived or worked on a farm.
† OR (95% CI) = Age-adjusted odds ratio (95% confidence interval).

TABLE 3. Number of White Men with Non-Hodgkin's Lymphoma, Number of Controls, and Odds Ratios by Characteristics of Exposure to 2,4-Dichlorophenoxyacetic Acid (2,4-D)

Use of 2,4-D	Cases	Controls	OR (95% CI)*
Never lived or worked on farm	54	184	1.0
Days/year mixing or applying 2,4-D:			
1–5	16	44	1.2 (0.6,2.4)
6–20	12	25	1.6 (0.7,3.6)
21+	3	4	3.3 (0.5,22.1)
Unknown days/year	12	25	—
Chi for trend = 1.639, p = 0.051			
Years 2,4-D used on farm:			
1–5	3	12	0.9 (0.2,3.6)
6–15	11	15	2.8 (1.1,7.1)
16–20	3	18	0.6 (0.1,2.1)
21+	13	33	1.3 (0.6,2.7)
Unknown years	15	29	—
Chi for trend = 0.601, p = 0.274			
First year of 2,4-D use:			
Prior to 1945	8	21	1.4 (0.5,3.5)
1946–1955	13	39	1.1 (0.5,2.3)
1956–1965	5	8	2.1 (0.6,7.7)
1965–1986	4	12	1.3 (0.3,4.9)
Unknown year	13	18	—
Chi for trend = 0.955, p = 0.170			

* OR (95% CI) = age-adjusted odds ratio (95% confidence interval).

creased according to the average annual number of days spent mixing or applying 2,4-D in comparison with men who never lived or worked on a farm (Table 3). Risk increased to more than threefold for those with 21 or more days of exposure per year (p = 0.051). There was no consistent increase in risk with the number of years of 2,4-D use while the subjects lived or worked on a farm or with the first year of 2,4-D use.

Several characteristics of pesticide use that indicate potential for exposure were evaluated. Among men who personally handled 2,4-D, risk varied by the method used most often to apply herbicides. Tractor-mounted spraying was associated with an OR of 1.4 (CI = 0.8, 2.6; 27 cases, 62 controls) and handheld spraying with an OR of 1.7 (CI = 0.4, 6.7; 4 cases, 9 controls). Risk increased substantially the longer farmers usually waited to change into clean work clothes after handling pesticides (Table 4). Farmers who changed immediately, at the end of the work day, or the following day or later (presumably, these farmers wore the clothes for more than one work day but did not sleep in them) had ORs of 1.1, 1.5, and 4.7, respectively (p for trend = 0.015). Risk did not increase if the farmers reported that they usually failed to use any protective equipment (eg, rubber gloves, rubber boots, mask, spray suit) when handling pesticides. Among farmers who mixed or applied 2,4-D, those who typically used protective equipment while handling any pesticide had an OR of 1.7 (CI = 0.9, 3.1; 25 cases, 48 controls), whereas farmers who did not had an OR of 1.2 (CI = 0.6, 2.4; 16 cases, 49 controls).

Possible confounding of the results for 2,4-D by use of other pesticides was evaluated. The risks associated with use of any phenoxyacetic acid herbicide (ever and average annual number of days) were identical to the risks for 2,4-D alone. All 13 cases and 27 controls who handled 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (ever handled 2,4,5-T: OR = 1.6, CI = 0.7, 3.6; average days per year of exposure 1–5: OR = 1.1; 6–20: OR = 6.4, 4 cases, 2 controls) were also 2,4-D users. None of the subjects who handled 2,4-D more than 20 days per year was a 2,4,5-T user. Excluding the 2,4,5-T users did not change the risks for handling 2,4-D (ever handled 2,4-D: OR = 1.5, CI = 0.8, 2.6; days per year 1–5: OR = 1.1; 6–20: OR = 1.3; 21+: OR = 3.3). Restricting the analysis to farmers and adjusting for the use of other herbicides by class (triazines, amides, benzoics, carbamates, trifluralins, and other) resulted in no meaningful changes in the ORs for those who ever handled 2,4-D or in the positive trend associated with average annual days of exposure to 2,4-D. Adjustments for the use of insecticides by class (chlorinated hydrocarbons, carbamates, organophosphates, metals, and other) also resulted in no meaningful changes in the risk estimates for 2,4-D, except for the use of organophosphates. Adjusting for or-

TABLE 4. Number of White Men with Non-Hodgkin's Lymphoma and Controls Who Mixed and Applied 2,4-Dichlorophenoxyacetic Acid (2,4-D) by Timing of Change to Clean Work Clothes after Handling Pesticides

When Subject Usually Changed to Clean Work Clothes	Cases	Controls*	OR (95% CI)†
Never lived or worked on farm	54	184	1.0
Immediately after handling pesticides	6	19	1.1 (0.4,3.1)
At end of work day	31	73	1.5 (0.8,2.6)
Following day or later	6	4	4.7 (1.1,21.5)
Chi for trend = 2.166, p = 0.015			

* Two controls who personally handled 2,4-D had unknown values.
† OR (95% CI) = age-adjusted odds ratio (95% confidence interval).

ganophosphate use on the farm yielded an OR of 1.1 for men who ever handled 2,4-D and ORs of 0.9, 1.3, and 1.8 for men exposed to 2,4-D for 1–5, 6–20, and more than 20 days per year (p for trend = 0.246) relative to farmers with no 2,4-D exposure. Adjustments using more detailed measures of organophosphate exposure (eg, duration and average annual days spent mixing or applying) also resulted in approximately twofold increased risks of NHL among the most frequent handlers of 2,4-D. Analysis of organophosphate use, adjusted for use of 2,4-D, showed an independent association with NHL (ever: OR = 2.4; days per year 1–5: OR = 1.7; 6–20: OR = 1.8; 21+: OR = 3.1) and will be described more thoroughly in a future report. The risk among 2,4-D users compared with nonusers, excluding all organophosphate users, was similar to the adjusted 2,4-D risk for ever use (OR = 1.1) and for the two lower use categories (days per year 1–5: OR = 0.7; 6–20: OR = 1.5). There were no cases exposed to 2,4-D for 21 or more days who were unexposed to organophosphates. Adjustments for the use of fungicides led to increases in the risk estimates associated with 2,4-D exposure (OR = 1.8, CI = 1.1, 3.0) and with average annual days of exposure to 2,4-D (1–5 days: OR = 1.6; 6–20 days: OR = 2.2; 21+ days: OR = 4.5; p for trend = 0.003). Simultaneous adjustment for use of organophosphates, fungicides, and age resulted in ORs of 0.8, 1.3, and 3.1 for farmers who mixed or applied 2,4-D 1–5, 6–20, and more than 20 days per year, respectively. The results of logistic regression analyses, restricted to farmers and including the variables age and use of 2,4-D, organophosphates, and fungicides, were consistent with the stratified analyses. Use of organophosphate insecticides (ever used on farm: OR = 2.4) and 2,4-D (handled 21+ days per year: OR = 2.1) were independent risk factors for NHL.

Approximately two-thirds of both the exposed cases (63%) and controls (64%) volunteered the history of 2,4-D use on the farms where they lived or worked, whereas about one-third of the exposed cases (37%) and controls (36%) reported 2,4-D use only after a specific probe. Risk estimates were similar among the two groups for the use of 2,4-D on the farm (volunteers: OR = 1.5; probes: OR = 1.5), personal handling of 2,4-D (volunteers: OR = 1.5; probes: OR = 1.5), and more than 20 days per year exposure to 2,4-D (volunteers: OR = 2.5, 1 case, 2 controls; probes: OR = 3.8, two cases, 2 controls).

The risk of NHL associated with personal handling of 2,4-D was higher among persons with proxy interviews (1–5 days per year: OR = 2.2; 6–20 days: OR = 2.2; 21+ days: OR = 2.4) than among self-respondents (1–5 days per year: OR = 1.0; 6–20 days: OR = 1.6; 21+ days: OR = 1.4).

Histology, tumor grade, degree of maturation, and immunologic type of the NHLs were evaluated. The association with 2,4-D did not appear to be specific to any subgroup of NHL, although small numbers limited the reliability of the risk estimates. There was a slight suggestion that risk may be higher in intermediate grade NHL (Working Formulation groups D–G, Table 1) (ever: OR = 1.7; 21+ days per year: OR = 5.0, 2 cases, 4 controls), follicular center cell NHL (Working Formulation groups B–D, F–G, Table 1) (ever: OR = 1.7; 21+ days per year: OR = 6.4, 2 cases, 4 controls), large cell NHL (Working Formulation groups G–H) (ever: OR = 1.5; 21+ days per year: OR = 6.2, 1 case, 4 controls), and blastic NHL (Working Formulation groups D, G, and J) (ever: OR = 2.3; 21+ days per year: OR = 9.3, 1 case, 4 controls). Personally handling 2,4-D was associated with both T-cell (OR = 2.0; CI = 0.5, 7.3) and B-cell (OR = 1.5; CI = 0.9, 2.6) lymphomas; however, the trend with days per year was significant (p = 0.045) for B-cell lymphomas only. The ORs for B-cell lymphomas were 1.1, 1.6, and 4.3 for persons exposed to 2,4-D for 1–5, 6–20, and 21 or more days per year, respectively. There were no T-cell lymphoma cases who were exposed to 2,4-D more than 20 days per year.

None of the other factors covered in the interviews, including family history of cancer, prior radiation treatment, other aspects of the medical history, tobacco consumption, or use of hair coloring products, was responsible for the observed 2,4-D associations.

Discussion

This population-based case-control study conducted in eastern Nebraska found a 50% excess of NHL associated with mixing or applying 2,4-D. The risk for NHL increased with the average frequency of use to more than threefold among those exposed more than 20 days per year. These findings are consistent with those of a previous case-control study conducted in Kansas (1), although the risk estimates are lower in the present study. The difference in risks in the two states may be explained by statistical variation, since the confidence intervals for risk estimates obtained in Nebraska (CI = 0.5, 22.1) and Kansas (CI = 1.8, 32.3) show considerable overlap.

Some, but not all, variables that indicated the degree of exposure to 2,4-D were related to an increased risk of NHL. In addition to the average annual number of days mixing or applying 2,4-D, the potential for dermal exposure of the usual method of herbicide application (9,10) and the time of change to clean work clothes after handling pesticides were both related to increased risk. However, the number of years of 2,4-D use while the subject lived or worked on the farm was not consistently related to an increased risk for NHL. Interestingly, a similar lack of association with years of use was observed in the Kansas study (1). Computing years of use as a measure of exposure assumes that the level of exposure is similar throughout the year and from year to year. Pesticide use, however, is sporadic, not continuous, throughout the work year, and the amount used may vary considerably from year to year depending on the need and on the use of other farm workers to mix and apply the pesticides. Annual frequency of exposure is more strongly correlated with risk than years of use and may be a better surrogate for delivered dose.

In contrast to the findings of the Kansas study (1), failure to use protective equipment regularly was inversely associated with an increased risk of NHL among 2,4-D users. The elevated risks for users and nonusers of protective equipment were not substantially different from one another. Certainly, one should not discourage the use of protective equipment based on the present study's results.

Exposure to other pesticides affected risk estimates from exposure to 2,4-D. Adjustment for the use of organophosphate insecticides reduced the observed risk associated with 2,4-D exposure, while adjustment for fungicide use increased the risk. Simultaneous adjustment for both resulted in risk estimates for average annual days of exposure similar to the values adjusted for age alone. Logistic regression analyses also indicated independent effects of 2,4-D and organophosphates. Because of the small number of subjects and the high proportion of subjects with multiple exposures, it is not possible in this study to entirely disentangle these relationships. There may be some residual confounding. Case-control studies of larger populations with detailed data on more variable patterns of exposures are needed.

This study relied upon study subjects or their next-of-kin to recall complicated lifetime exposure histories. While there is a great need to improve methods for estimating exposure to pesticides in epidemiologic studies (11), exposure misclassification is not likely to have created spurious risks in this study or in the Kansas study (12). The similarity of the proportions of cases and controls who volunteered histories of 2,4-D use in response to an open-ended question as compared with those who responded to a specific probe for 2,4-D use and the increased risks among frequent users in both the subjects and proxy respondents suggest that recall bias did not occur in this study. Corroboration of a sample of the exposure histories in the Kansas study (1) and methodologic studies of industrial workers (13) observed little difference in accuracy of reports from cases and controls and suggest that the exposure misclassification in this study is likely to be independent of case-control status. Such misclassification tends to decrease risk estimates and reduce exposure-response gradients (14). Thus, misclassification in the Nebraska study is likely to result in an underestimate of the true risk associated with 2,4-D exposure. In addition, increasingly detailed measures of exposure to organophosphates did not further reduce the adjusted OR for 2,4-D exposure, suggesting that misclassification of organophosphate exposure did not lead to an artificial inflation of the risk estimate for 2,4-D.

The large proportion of farmers with no known history of pesticide use in this study (37% of the controls) suggests inaccurate recall by the study subjects. The study definition of farmers, however, included anyone who had ever lived or worked on a farm. This definition includes dependents of farmers and persons who farmed for only brief periods of time. Their opportunity to use pesticides would have been considerably less than for career farmers. Also, some of the older study subjects farmed several decades ago when pesticide use was much less common than in recent years. In addition, some subjects who reported no use of pesticides probably used them. Such misclassification would result in some exposed farmers being classified as nonexposed. In the presence of a positive association, these improperly classified "nonexposed" farmers would reduce the true risk estimates for farmers as a group and lower risk estimates for frequent users of 2,4-D. In fact, the farmers who reported no exposure to 2,4-D had an odds ratio of 0.8

(CI = 0.5, 1.2). This deviation from 1.0 could result from random variation or uncontrolled negative confounding. If confounding were the explanation, the odds ratios reported for the exposed farmers are likely to be underestimates of the true risks.

Other Epidemiologic Studies

There have been many epidemiologic studies evaluating the relation of pesticides to cancer which, at first glance, appear to report inconsistent results. The studies generally have not evaluated the same chemicals with the same measures of exposure, however. Only the Kansas study (1) appears comparable with the Nebraska study, ie, based on days per year of agricultural exposure to 2,4-D. Other case-control studies of NHL and herbicides have either treated the phenoxyacetic acid herbicides as a group, with no specific information on 2,4-D and/or lacked information on the number of days per year of exposure (15–21). Case-control studies in Sweden, however, have also noted excess risks for NHL among persons having contact with phenoxyacetic acid herbicides (15,16,21), with an indication in one study (15) that excess risks were present among persons exposed to 2,4,5-T and those exposed only to phenoxys considered unlikely to be contaminated by polychlorinated dibenzodioxins and dibenzofurans, such as 2,4-D and 4-chloro-2-methyl phenoxyacetic acid (MCPA). A study in western Washington state (22) observed a small, but significant, excess risk of NHL among farmers, but the risk did not increase with duration in farming occupations nor with estimated level of exposure in other occupations to 2,4-D; however, no data on the annual number of days of exposure were available. Pearce (19), who found no association between duration or frequency of herbicide use and lymphoma among New Zealand applicators, was studying workers exposed almost entirely to 2,4,5-T. Exposure to 2,4,5-T was not associated with an elevated risk of NHL in the Kansas study, but was associated with a nonsignificant increased risk in the Nebraska study. The results of the Kansas and the Nebraska studies indicate that evaluating risk by job title or duration of exposure only may be inadequate, missing important information. It is apparent that considerable variation of exposure occurs among farmers and that personal exposure histories must be obtained in such studies.

Cohort studies of manufacturers and applicators have also been subject to the problems of mixed exposures. Most of the cohorts exposed to 2,4-D have also been exposed to either 2,4,5-T (23–25) or MCPA (26–29). These investigations have generally not observed excesses of NHL, but the small number of subjects in these studies has limited their usefulness in examining NHL, a rare cause of death (23,24). A recent cohort study of farmers in Canada reported that the risk of NHL increased with the number of acres sprayed with herbicides, particularly in smaller farming operations of less than 1,000 acres (30). Bond et al (31) studied a group of 878 chemical workers who were potentially exposed to several agricultural chemicals, including 2,4-D, and observed a nonsignificant excess of lymphatic and hematopoietic cancers. This excess occurred exclusively among workers who were employed in the 2,4-D plant (5 deaths observed, relative risk = 3.1, $p \leq 0.05$). Two of the five lymphatic and hematopoietic cancers were non-Hodgkin's lymphomas.

Experimental Studies

There is little evidence that 2,4-D is mutagenic or genotoxic (32,33). A 2-year animal feeding study of 2,4-D resulted in a statistically significant excess of astrocytomas in male rats at the highest dose level (Industry Task Force on 2,4-D research data, as cited in Bond et al [31]). The International Agency for Research on Cancer (34) recently concluded that there is inadequate evidence of animal carcinogenicity for 2,4-D. 2,4-D has been associated with increased rates of sister chromatid exchanges and other chromosomal aberrations in vitro (35–37) and in vivo (37,38). The possibility that 2,4-D may be carcinogenic, not by mutagenic activity, but by excessive production of hydrogen peroxide and the proliferation of peroxisomes has been suggested (39).

Immunosuppression, a well-established strong risk factor for NHL (40), could be a possible mechanism by which 2,4-D might increase the risk of NHL. Acute exposure of female mice to high levels of 2,4-D resulted in suppression of antibody production against sheep red blood cells; however, subacute exposure, more comparable with human occupational exposures, did not affect antibody production but, rather, enhanced B- and T-lymphocyte proliferative responses (41). 2,4-D has rarely been reported to be contaminated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (42), the dioxin congener that is a frequent contaminant of some other phenoxy herbicides and that has been reported to be both immunosuppressive and carcinogenic (43–49).

The fact that the mechanism for 2,4-D's putative action is unknown should not detract from the strength and consistency of the results in Kansas and Nebraska concerning risk by days per year of herbicide use. Based on the positive results in these two studies and the likelihood that any exposure misclassification has probably decreased the risk estimates and diluted exposure-response gradients, we believe that the weight of evi-

dence indicates that the use of 2,4-D in an agricultural setting increases the risk of NHL among persons handling the chemical frequently.

Acknowledgments

The authors acknowledge the cooperation of the Nebraska Lymphoma Study Group, and Robert Hoover and Joseph F. Fraumeni, Jr for their helpful reviews of the manuscript.

References

1. Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R, Fraumeni JF Jr. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. JAMA 1986;256:1141–7.
2. The Non-Hodgkin's Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: Summary and description of a Working Formulation for Clinical Usage. Cancer 1982;49:2112–35.
3. Linder J, Ye Y, Harrington DS, Armitage JO, Weisenburger DD. Monoclonal antibodies marking T lymphocytes in paraffin-embedded tissue. Am J Pathol 1987;127:1–8.
4. Linder J, Ye Y, Armitage JO, Weisenburger DD. Monoclonal antibodies marking B-cell non-Hodgkin's lymphoma in paraffin-embedded tissue. Modern Pathol 1988;1:29–34.
5. Waksberg J. Sampling methods for random digit dialing. J Am Stat Assoc 1978;73:40–6.
6. Gart JJ. Point and interval estimation of the common odds ratio in the combination of 2 × 2 tables with fixed marginals. Biometrika 1970;57:471–5.
7. Mantel N. Chi square test with 1 degree of freedom, extension of the Mantel-Haenszel procedure. Am Stat Assoc J 1963;58:690–700.
8. Engelman L. Stepwise logistic regression. In: Dixon WJ, ed. BMDP statistical software. Berkeley, CA: University of California Press, 1983:330–44.
9. Lavy TL, Shepard JS, Mattice JD. Exposure measurements of applicators spraying (2,4,5-trichlorophenoxy) acetic acid in the forest. J Agric Food Chem 1980;28:626–30.
10. Lavy TL, Norris LA, Mattice JD, Marx DB. Exposure of forestry ground workers to 2,4-D, picloram, and dichlorprop. Environ Toxicol Chem 1987;6:209–24.
11. Blair A, Zahm SH, Cantor KP, Stewart PA. Estimating exposure to pesticides in epidemiologic studies of cancer. In: Wang RGM, Franklin CA, Honeycutt RC, et al, eds. Biological monitoring for pesticide exposure—measurement, estimation, and risk reduction. Washington, DC: American Chemical Society, ACS Symposium Series 382, 1989:38–46.
12. Blair A, Zahm SH. Herbicides and cancer: A review and discussion of methodologic issues. In: Band P, ed. Recent results in cancer research: Occupational cancer epidemiology. Vancouver, Canada: 1989;120:132–45.
13. Bond GG, Bodner KM, Sobel W, Shellenberger RJ, Flores GH. Validation of work histories obtained from interviews. Am J Epidemiol 1988;128:343–51.
14. Marshall JR, Priore R, Graham S, Brasure J. On the distortion of risk estimates in multiple exposure level case-control studies. Am J Epidemiol 1981;113:464–80.
15. Hardell L, Eriksson M, Lenner P, Lundgren E. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: A case-control study. Br J Cancer 1981;43:169–76.
16. Hardell L. Relation of soft-tissue sarcoma, malignant lymphoma and colon cancer to phenoxy acids, chlorophenols and other agents. Scand J Work Environ Health 1981;7:119–30.
17. Pearce NE, Smith AH, Howard JF, Sheppard RA, Giles HJ, Teague CA. Non-Hodgkin's lymphoma and exposure to phenoxyherbicides, chlorophenols, fencing work, and meat works employment: A case-control study. Br J Ind Med 1986;43:75–83.
18. Pearce NE, Sheppard RA, Smith AH, Teague CA. Non-Hodgkin's lymphoma and farming: An expanded case-control study. Int J Cancer 1987;39:155–61.
19. Pearce N. Phenoxy herbicides and non-Hodgkin's lymphoma in New Zealand: Frequency and duration of herbicide use. Br J Ind Med 1989;46:143–44.
20. Woods JS, Polissar L, Severson RK, Heuser LS, Kulander BG. Soft tissue sarcoma and non-Hodgkin's lymphoma in relation to phenyoxyherbicide and chlorinated phenol exposure in Western Washington State. J Natl Cancer Inst 1987;78:899–910.
21. Persson B, Dahlander A-M, Fredriksson M, Brage HN, Ohlson CG, Axelson O. Malignant lymphoma and occupational exposures. Br J Ind Med 1989;46:516–20.
22. Woods JS, Polissar L. Non-Hodgkin's lymphoma among phenoxy herbicide-exposed farm workers in Western Washington State. Chemosphere 1989;18:401–6.
23. Riihimaki V, Asp S, Hernberg S. Mortality of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid herbicide applicators in Finland: First report of an ongoing prospective cohort study. Scand J Work Environ Health 1982;8:37–42.
24. Riihimaki V, Asp S, Pukkala E, Hernberg S. Mortality and cancer morbidity among chlorinated phenoxyacid applicators in Finland. Chemosphere 1983;12:779–84.
25. Bond AG, Bodner KM, Cook RR. Phenoxy herbicides and cancer: Insufficient epidemiologic evidence for a causal relationship. Fund Appl Toxicol 1989;12:172–88.
26. Lynge E. Background and design of a Danish cohort study of workers in phenoxy herbicide manufacture. Am J Indus Med 1987;11:427–37.
27. Lynge E. A follow-up study of cancer incidence among workers in manufacture of phenoxy herbicides in Denmark. Br J Cancer 1985;52:259–70.
28. Wiklund K, Dich J, Holm L-E. Risk of malignant lymphoma in Swedish pesticide appliers. Br J Cancer 1987;56:505–8.
29. Wiklund K, Lindefors B-M, Holm L-E. Risk of malignant lymphoma in Swedish agricultural and forestry workers. Br J Ind Med 1988;45:19–24.
30. Wigle DT, Semenciw RM, Wilkins K, et al. Mortality study of Canadian male farm operators: Non-Hodgkin's lymphoma mortality and agricultural practices in Saskatchewan. J Natl Cancer Inst 1990;82:575–82.
31. Bond GG, Wetterstroem NH, Rousch GJ, McLaren EA, Lipps TE, Cook RR. Cause-specific mortality among employees engaged in the manufacture, formulation, or packaging of 2,4-dichlorophenoxyacetic acid and related salts. Br J Ind Med 1988;45:98–105.
32. Canadian Centre for Toxicology. Expert panel report on carcinogenicity of 2,4-D. Guelph, Ontario: 1987.
33. Seiler JP. The genetic toxicology of phenoxy acids other than 2,4,5-T. Mutat Res 1978;55:197–226.
34. International Agency for Research on Cancer. IARC monographs on the evaluation of carcinogenic risks to humans. Overall evaluation of carcinogenicity: An update of IARC monographs volumes 1 to 42. Lyon, France: IARC, 1987:156–60.
35. Korte C, Jalal SM. 2,4-D induced clastogenicity and elevated rates of sister chromatid exchanges in cultured human lymphocytes. J Heredity 1982;73:224–6.
36. Turkula TE, Jalal SM. Increased rates of sister chromatid exchanges induced by the herbicide 2,4-D. J Heredity 1985;76:213–4.
37. Mustonen R, Kangas J, Vuojolahti P, Linnainmaa K. Effects of phenoxyacetic acids on the induction of chromosome aberrations in vitro and in vivo. Mutagenesis 1986;1:241–5.
38. Yoder J, Watson M, Benson WW. Lymphocyte chromosome analysis of agricultural workers during extensive occupational exposure to pesticides. Mutation Res 1973;21:335–40.

39. Vainio H, Nickels J, Linnainmaa K. Phenoxy acid herbicides cause peroxisome proliferation in Chinese hamsters. Scand J Work Environ Health 1982;8:70–73.
40. Greene MH. Non-Hodgkin's lymphoma and mycosis fungoides. In: Schottenfeld D, Fraumeni JF, Jr., eds. Cancer epidemiology and prevention. Philadelphia: WB Saunders Company, 1982:754–78.
41. Blakely BR, Schiefer BH. The effect of topically applied n-butylester of 2,4-dichlorophenoxyacetic acid on the immune response in mice. J Appl Toxicol 1986;6:291–5.
42. Hagenmaier H. Determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin in commercial chlorophenols and related products. Fresenius Z Anal Chem 1986;325:603–6.
43. Vos JG, Moore JA, Zinkl JG. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the immune system of laboratory animals. Environ Health Perspect 1973;5:149–62.
44. Harris MW, Moore JA, Vos JG, Gupta BN. General biological effects of TCDD in laboratory animals. Environ Health Perspect 1973;5:101–9.
45. Kociba RJ, Keyes DG, Beyer JE, Carreon RM, Wade CE, Dittenber DA, Kalnins RP, Frauson LE, Park CN, Barnard SD, Hummel RA, Humiston CG. Results of a two-year chronic toxicity and oncogenicity study of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rats. Toxicol Appl Pharmacol 1978;46:279–303.
46. Kociba RJ, Keyes DG, Beyer JE, Carreon RM, Gehring PJ. Long-term toxicologic studies of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in laboratory animals. Ann NY Acad Sci 1979;320:397–404.
47. Van Miller JP, Lalich JJ, Allen JR. Increased incidence of neoplasms in rats exposed to low levels of 2,3,7,8-tetrachlorodibenzo-p-dioxin. Chemosphere 1977;10:625–32.
48. National Toxicology Program. (Report 80-31) Carcinogenesis bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin (CAS N. 1746-01-6) in Osborne-Mendel rats and B6C3F1 mice (Gavage study), technical report series 209, National Institutes of Health publication 82-1765. Research Triangle Park, NC, Dept of Health and Human Services, 1982.
49. National Toxicology Program. (Report 80-32) Carcinogenesis bioassay of 2,3,7,8-tetrachlorodibenzo-p-dioxin (CAS No. 1746-01-6) in Swiss-Webster mice (Dermal study), technical report series 209, National Institutes of Health publication 82-1757. Research Triangle Park, NC, Dept of Health and Human Services, 1982.