

# Agricultural risk factors for t(14;18) subtypes of non-Hodgkin's lymphoma

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The t(14;18) translocation is a common somatic mutation in non-Hodgkin's lymphoma (NHL) that is associated with *bcl-2* activation and inhibition of apoptosis. We hypothesized that some risk factors might act specifically along t(14;18)-dependent pathways, leading to stronger associations with t(14;18)-positive than t(14;18)-negative non-Hodgkin's lymphoma. Archival biopsies from 182 non-Hodgkin's lymphoma cases included in a case-control study of men in Iowa and Minnesota (the Factors Affecting Rural Men, or FARM study) were assayed for t(14;18) using polymerase chain reaction amplification; 68 (37%) were t(14;18)-positive. We estimated adjusted odds ratios (OR) and 95% confidence intervals (CI) for various agricultural risk factors and t(14;18)-positive and -negative cases of non-Hodgkin's lymphoma, based on polytomous logistic regression models fit using

the expectation-maximization (EM) algorithm. T(14;18)-positive non-Hodgkin's lymphoma was associated with farming (OR 1.4, 95% CI = 0.9–2.3), dieldrin (OR 3.7, 95% CI = 1.9–7.0), toxaphene (OR 3.0, 95% CI = 1.5–6.1), lindane (OR 2.3, 95% CI = 1.3–3.9), atrazine (OR 1.7, 95% CI = 1.0–2.8), and fungicides (OR 1.8, 95% CI = 0.9–3.6), in marked contrast to null or negative associations for the same self-reported exposures and t(14;18)-negative non-Hodgkin's lymphoma. Causal relations between agricultural exposures and t(14;18)-positive non-Hodgkin's lymphoma are plausible, but associations should be confirmed in a larger study. Results suggest that non-Hodgkin's lymphoma classification based on the t(14;18) translocation is of value in etiologic research. (EPIDEMIOLOGY 2001;12:701–709)

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Many epidemiologic studies have evaluated associations between farming and non-Hodgkin's lymphoma (NHL), but results have been inconsistent.<sup>1</sup> Recent studies have focused on specific agricultural exposures,<sup>2,3</sup> but the re-

lation between farming and NHL is still unclear. While farming obviously encompasses diverse exposures, it is also true that NHL encompasses diverse outcomes,<sup>4–7</sup> and studies have reported stronger associations between risk factors and subtypes of NHL than between the same factors and NHL in the aggregate.<sup>8–14</sup> Unfortunately, subtype definitions do not always coincide, and NHL classification schemes are designed to group cases according to clinical, rather than etiologic, parameters.<sup>15,16</sup> As an alternative, we evaluated associations between exposures and NHL subtypes defined by the t(14;18) chromosomal translocation, a common somatic mutation believed to be an early component step in the pathogenesis of t(14;18)-positive cases.<sup>6,7</sup>

The t(14;18) translocation joins the *bcl-2* gene on chromosome 18 to the immunoglobulin heavy chain gene (*IgH*) on chromosome 14, resulting in increased production of *bcl-2* protein, a potent inhibitor of apoptosis.<sup>17–19</sup> Lymphocytes with t(14;18) as their sole abnormality are not neoplastic,<sup>19–22</sup> but they are effectively immortalized, and t(14;18)-positive cells that develop subsequent oncogenic mutations may not be eliminated through routine cell death mechanisms.<sup>6</sup> The prevalence of t(14;18)-positive lymphocytes varies considerably among individuals of similar age, possibly due to envi-

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ronmental and/or genetic factors.<sup>23-26</sup> If so, such factors would be components in the pathogenesis of t(14;18)-positive NHL.<sup>6,23,24</sup>

Factors that increase the risk of neoplastic transformation among pre-existing t(14;18)-positive lymphocytes may also be associated with t(14;18)-positive NHL; consequently, a t(14;18)-NHL-specific association would not imply a specific mechanism of effect. Nonetheless, because t(14;18)-positive cases share at least one common causal component, they also should share a greater portion of their etiologic basis than NHL cases in the aggregate.<sup>27</sup> Therefore, we reasoned that t(14;18) subgrouping would increase the etiologic specificity of our outcome, and enhance our ability to identify NHL risk factors. To evaluate this approach, we used archival samples and extensive exposure data previously collected from participants in the National Cancer Institute's Factors Affecting Rural Men (FARM) study.<sup>8</sup>

## Subjects and Methods

### CASE SELECTION

The FARM study ascertained 780 cases of newly diagnosed NHL in white men aged 30 or older.<sup>8</sup> Iowa cases diagnosed between March 1981 and October 1983 were identified through the State Health Registry of Iowa. Minnesota cases diagnosed between October 1980 and September 1982 were identified through active surveillance of hospital and pathology laboratory records. Patients residing in major metropolitan areas (Minneapolis, St. Paul, Duluth, Rochester) were excluded to increase the proportion of rural residents. Eighty-nine percent (694) of the 780 ascertained cases were interviewed. A pathology review panel confirmed the NHL diagnosis in 622 cases.

### CONTROL SELECTION

We used data from all 1245 FARM study controls in this analysis. Controls were white males without hemolymphatic cancer, frequency matched to cases on age (within five-year groups), state, and vital status.<sup>8</sup> Potential controls were identified using random digit dialing (controls under age 65, 77% response), Health Care Financing Administration Medicare files (controls 65 and older, 79% response), and state death certificate files (deceased controls, 77% response among next-of-kin). Minnesota residents living in metropolitan areas were ineligible.

### CASE-SUBTYPE ASCERTAINMENT

We determined the translocation status of FARM study cases using DNA extracted from archival paraffin-embedded tumor blocks. The State Health Registry of Iowa and the Environmental and Occupational Health Division of the University of Minnesota School of Public Health requested blocks beginning in 1997. A histopathologist (GD) reviewed newly-cut sections and classified them according to an approximation of the Revised European-American Lymphoma (REAL) system.<sup>15</sup>

To identify t(14;18)-positive cases, we used a polymerase chain reaction (PCR) assay designed to amplify DNA spanning the most common region of t(14;18) chromosome fusion.<sup>28</sup> All laboratory work was conducted at the University of North Carolina (RB), without knowledge of sample histologic subtype or exposure status. DNA was extracted from deparaffinized sections, using a phenol:chloroform extraction procedure. All reactions included the J<sub>H</sub> consensus primer (5'-ACCT-GAGGAGACGGTGAGC-3') for IgH<sup>28</sup>, and two primers corresponding to *bcl-2* segments near the major breakpoint region, MBR1: 5'-GAG AGTTGCTT-TACGTGGCCT G-3';<sup>28</sup> and MBR2: 5'-CGCTT-GACTCCTTTACGTGCTG-3'.<sup>17</sup> A segment of the  $\beta$ -globin gene was amplified to confirm that samples could produce a 175-base pair control product.<sup>29</sup> DNA degradation may have been sufficient to prevent  $\beta$ -globin- but not t(14;18)-amplification in some cases, since many t(14;18) products were shorter than the  $\beta$ -globin control. Consequently, we classified ten t(14;18)-positive/ $\beta$ -globin-negative cases as t(14;18)-positive, while 66 cases that failed to amplify either t(14;18) or  $\beta$ -globin (27% of those assayed) were unclassified.

PCR products were isolated using gel electrophoresis, denatured, and transferred to a nylon membrane using Southern blotting. Amplification was confirmed using radio-labeled probes for *bcl-2* segments adjacent to each *bcl-2* primer (MBR1 probe: 5'-CAACACAGAC-CCACCCAGAGC-3'; and MBR2 probe: 5'-GATG-GCTTTGCTGAGAGGTTT-3'). A subset of cases was probed for IgH (5'-GGGTBCCWTGGCCCCAG-3', B = GCT, W = AT). Fifty-seven t(14;18)-positive cases were identified in the first round of PCR. Samples that were t(14;18)-negative but  $\beta$ -globin-positive (N = 125) were subjected to a nested PCR assay, using interior primers corresponding to the probes above. Eleven additional t(14;18)-positive cases were identified using the nested assay.

Precautions were taken during all procedures to prevent sample contamination. *Bcl-2* and *IgH* breakpoints vary, and random nucleotides are added when the breakpoints are fused;<sup>30</sup> consequently, amplification products should vary. We monitored amplification products for size variation, and cloned and sequenced 20 t(14;18)-positive samples to confirm that products were unique.

### EXPOSURE ASSESSMENT

In-person structured interviews were administered between August 1981 and May 1984. One-third (193 cases, 423 controls) were conducted with next-of-kin proxies. Information was collected about sociodemographic characteristics, tobacco and alcohol use, hobbies, pets, medical history, occupational history (including specific exposures at jobs held at least one year), and non-occupational exposures of *a priori* interest that occurred at least once a month for 1 year.<sup>8,31</sup>

Participants who worked on farms for at least 6 months after age 18 were considered farmers. Farmers were asked for detailed information regarding agricul-

tural exposures, including specific pesticides (23 livestock insecticides, 34 crop insecticides, 38 herbicides, and 16 fungicides used in the region during the time of interest), specific crops and average acres planted, specific livestock and average herd sizes, and the timing and cumulative duration of employment on each farm.<sup>8</sup> We categorized pesticide exposure based on any reported use where participants worked, on personal handling of products, and according to use of protective equipment. Pesticides were grouped by chemical characteristics or evaluated individually. We also assessed exposure to fumigants used to preserve grain (carbon tetrachloride, carbon disulfide, methyl bromide, ethylene dibromide, phosphine),<sup>32</sup> consumption of unpasteurized dairy products, and exposure to livestock diagnosed with brucellosis or leukemia. Unexposed (referent) categories included non-farmers and unexposed farmers.

#### DATA ANALYSIS

Many archival samples were not available, and over 70% of the FARM study cases could not be classified as t(14;18)-positive or -negative. Missing case-subtype information was associated with state of origin and related exposures, and was obviously associated with being a case. Consequently, ignoring unclassified cases may have biased estimates for case-subtypes compared with controls. To address this problem, we used the Expectation-Maximization (EM) algorithm, a statistical missing-data technique that maximizes the likelihood function using all available data, including exposure data from unclassified cases.<sup>33</sup> In brief, we estimated the probability of each case-subtype conditional on being a case, based on a polytomous logistic regression model of the observed data. Next, we constructed pseudo-data with observations missing case-subtype data apportioned to case-subtypes according to the probabilities for their covariate stratum. Then we modeled these data to determine new maximum likelihood probabilities, which were used to assign new pseudo-data. These steps were repeated until models converged. Variances were calculated based on the observed-data likelihood. Application of the EM method in this context has been described in detail elsewhere, and simulation studies demonstrated that this technique will prevent bias and improve precision relative to results obtained when unclassified cases are ignored, as long as absence is unrelated to the true case-subtype within covariate strata.<sup>33a</sup> Case-only unconditional logistic regression models were used to compare associations between case-subtypes. Analyses were performed using Stata, Release 6.0 (Strata Corporation, College Park, TX).<sup>34</sup>

We ignored exposures with fewer than 10 exposed cases, and report subtype-specific estimates only when there were at least four exposed cases in the subtype. All models included the frequency matching factors age (upper and lower tail-restricted quadratic splines<sup>35</sup>) and state, but not vital status, which had little impact on effect estimates. Livestock, crop, and pesticide exposures were highly correlated, so they were modeled separately.

**TABLE 1. Number of t(14;18)-Positive and -Negative Non-Hodgkin's Lymphoma Cases, and Proportion of t(14;18)-Positive Cases in Revised European American Lymphoma (REAL) Histologic Subtypes\***

REAL subtype	t(14;18)- Negative	t(14;18)- Positive	% t(14;18)- Positive
Chronic Lymphocytic†	20	3	13.0
Follicular	30	24	46.3
Diffuse Large (B) Cell‡	39	25	39.0
Burkitt's/Burkitt-like	10	4	28.6
Other	4	2	66.7
Unclassified	11	10	52.4
Total	114	68	37.4

\* REAL subtypes are an approximation based on morphology of newly cut sections and information contained in pathology reports, without knowledge of translocation status.

† Restricted to cases of chronic lymphocytic lymphoma. REAL subtype also includes chronic lymphocytic leukemia.

‡ B-cell status assumed based on morphology, but not confirmed.

Non-agricultural exposures were evaluated as confounders if age- and state-adjusted case-subtype:control odds ratios were above 1.5 or below 0.65. These included a history of hemolymphatic cancer in a parent, sibling, or child; any tobacco use; pet cats; marital status; hair dye use; and composite variables representing occupational factors associated with each case subtype. We dropped these covariates from final models, since they had little influence on the estimates of interest.

Statistical interactions were modeled using indicator variables for independent or joint exposure, and a common referent group with neither exposure. Joint odds ratios were compared with those predicted for additive or multiplicative relations, and interaction contrast ratios (ICRs) were derived to quantify departures from the additive null.<sup>36,37</sup> Joint exposures were evaluated among major pesticide classes; between major pesticide classes and livestock or crops; for agricultural exposures and use of tobacco or cigarettes; and for agricultural exposures and a family history of hemolymphatic cancer.

#### Results

Tumor blocks were retrieved for 40% (248) of the 622 FARM study cases, and 29% (182) were successfully assayed. State of origin was an influential determinant of retrieval success; 58% of assayed cases were from Iowa, compared with 47% of the total cases. Assayed cases also were more likely to have represented themselves at interview (76% vs. 67%). The distribution of assayed cases among NHL histologic subtypes was similar to that of all FARM cases. Sixty-eight (37%) assayed cases were t(14;18)-positive and 114 were t(14;18)-negative. Proportions of t(14;18) cases overall and within Revised European American Lymphoma subtypes (Table 1) were comparable with previous reports.<sup>15,38</sup> Participants in different state, age, and vital status categories were proportionately distributed between t(14;18)-positive and -negative cases.

Use of hair dye and a positive family history of hemolymphatic cancer were both associated with NHL (Table 2), as previously reported.<sup>12,39</sup> Having been di-

**TABLE 2. Frequency of Selected Characteristics Among Controls, Factors Affecting Rural Men Study Non-Hodgkin's Lymphoma Cases, and t(14;18)-Positive and -Negative Cases; and Adjusted Case:Control and Case:Case Odds Ratios\***

	FARM study cases vs. controls				t(14;18)-positive vs. t(14;18)-negative			
	Control % N = 1245	Case % N = 622	OR	95% CI	Negative % N = 114	Positive % N = 68	OR	95% CI
Education								
More than 12 years	29	30	1.0		28	22	1.0	
12 years or less	71	70	1.1	0.9–1.3	72	78	1.4	0.7–2.9
Used tobacco daily	77	81	1.3	1.0–1.7	79	85	1.5	0.7–3.5
Marital status								
Married/cohabiting	79	82	1.0		81	79	1.0	
Widowed	4	8	0.8	0.5–1.1	4	9	0.1	0.0–1.1
Divorced/separated	11	4	1.1	0.7–1.8	11	2	2.4	0.6–8.8
Never married	6	5	0.9	0.6–1.4	4	10	2.8	0.8–10.2
Kept cats	44	50	1.2	1.0–1.5	54	63	1.4	0.7–2.6
Any hair dye use†	5	9	2.0	1.3–2.9	15	13	0.9	0.4–2.1
Family history‡	4	8	2.0	1.3–2.9	10	6	0.5	0.1–1.5

\* Adjusted for frequency matching factors (age (restricted quadratic splines), state, and vital status).

† Based on personal or occupational use of hair dyes or tints.

‡ History of hemolymphatic cancer in a parent, sibling, or child.

voiced, separated, or never married was more strongly related to translocation-positive cases. Having had a family history of hemolymphatic cancer and having been a widower were more strongly related to translocation-negative cases.

Farming was related more strongly to translocation-positive NHL [odds ratio (OR) 1.4, 95% confidence interval (CI) = 0.9–2.3] (Table 3) than t(14;18)-negative NHL (OR 1.0, 95% CI = 0.8–1.4). Farm operators were at increased risk for translocation positive NHL (OR 1.7, 95% CI = 1.1–2.9), but not translocation negative NHL (OR 0.9, 95% CI = 0.6–1.3). Estimates for t(14;18)-positive NHL were similar for different time periods and categories of farming duration.

Neither NHL subgroup was associated with specific crops, with the possible exception of soybean cultivation and t(14;18)-positive NHL (OR 1.4, 95% CI = 0.9–2.1). Associations of similar magnitude (ORs 1.3–1.4) were found for t(14;18)-positive NHL and work on farms with dairy cattle, pigs, sheep, or horses, mules, or donkeys (Table 4). Meaningful dose-response trends were not evident when crop and livestock exposures were categorized by amount or duration. Livestock and crops were not associated with t(14;18)-negative NHL in general, but exposure to pigs diagnosed with brucellosis was associated with t(14;18)-negative NHL (OR 5.2, 95% CI = 1.4–19), based on a small number of cases (Table 4). Consumption of unpasteurized dairy products or ex-

**TABLE 3. Adjusted Odds Ratios for Non-Hodgkin's Lymphoma Case Subtype:Control and Case:Case Comparisons by Farming and Years of Employment as a Farmer, Relative to Non-Farmers\***

Exposure	Controls	t(14;18)-Positive NHL vs. Controls			t(14;18)-Negative NHL vs. Controls			t(14;18)-Positive vs. -Negative NHL	
	N	N	OR	95% CI	N	OR	95% CI	OR	95% CI
Nonfarmer	547	24	1.0		49	1.0			
Farmer	698	44	1.4	0.9–2.3	65	1.0	0.8–1.4	1.4	0.7–2.8
Type of work									
Farm operator	523	37	1.7	1.1–2.9	43	0.9	0.6–1.3	1.9	1.0–4.0
Ever farm hand	175	7	0.9	0.4–2.0	22	1.3	0.9–2.0	0.7	0.2–1.9
First year									
Before 1930	326	18	1.2	0.6–2.4	39	1.3	0.8–2.1	1.0	0.4–2.8
1930 to 1940	171	12	1.3	0.7–2.5	16	0.8	0.5–1.3	1.8	0.7–4.8
1941 or later	200	14	1.6	0.8–3.0	19	1.0	0.6–1.6	1.5	0.6–3.7
Last year									
Before 1950	276	13	1.3	0.7–2.4	20	0.9	0.6–1.4	1.4	0.6–3.5
1950 to 1969	205	16	1.6	0.8–2.9	21	1.1	0.7–1.7	1.5	0.7–3.6
1970 or later	212	15	1.4	0.8–2.5	23	1.1	0.7–1.6	1.4	0.6–3.3
Total years									
0.5 to 9	229	14	1.6	0.9–2.8	15	0.8	0.5–1.4	1.9	0.8–4.6
10 to 39	292	19	1.4	0.8–2.5	31	1.1	0.8–1.6	1.3	0.6–2.9
40 or more	172	11	1.2	0.6–2.6	18	1.1	0.6–1.7	1.2	0.4–3.4
Years prior†									
2 to 20	279	18	0.8	0.4–1.6	33	1.2	0.8–2.0	0.9	0.4–2.2
21 to 30	340	25	1.5	0.7–3.1	41	1.2	0.7–2.1	1.1	0.4–2.8
31 or more	612	34	0.9	0.9–1.6	58	0.9	0.6–1.3	1.0	0.4–2.2

\* Case-subtype:control estimates were from polytomous logistic regression models fit using the EM algorithm. Case:case estimates were from binary logistic regression models restricted to assayed cases. All estimates are adjusted for state and age (restricted quadratic splines), unless otherwise indicated.

† Time period of employment as a farmer relative to date of diagnosis or interview. Estimates are adjusted for farming during other time periods.

**TABLE 4. Adjusted Odds Ratios for t(14;18) Non-Hodgkin's Lymphoma Case Subtype:Control or Case:Case Comparisons and Livestock Production or Brucellosis in Livestock\***

Exposure	Controls		t(14;18)-Positive NHL vs. Controls		t(14;18)-Negative NHL vs. Controls			t(14;18)-Positive vs. -Negative NHL	
	N	N	OR	95% CI	N	OR	95% CI	OR	95% CI
Chickens	634	38	1.2	0.8-1.9	59	1.0	0.8-1.4	1.1	0.6-2.2
Beef cattle	463	27	0.9	0.6-1.4	52	1.2	0.9-1.6	0.7	0.4-1.4
Dairy cattle	618	38	1.4	0.9-2.2	57	1.0	0.7-1.4	1.2	0.6-2.4
Pigs	638	41	1.4	0.9-2.2	60	1.0	0.8-1.4	1.4	0.7-2.7
Sheep	208	15	1.3	0.8-2.2	15	0.7	0.4-1.1	1.7	0.8-3.9
Horses, mules, or donkeys	488	28	1.4	0.8-2.2	33	0.7	0.5-0.9	1.9	1.0-3.9
Brucellosis†									
In cattle	107	4	1.0	0.4-2.3	8	0.9	0.5-1.6	1.0	0.3-3.7
In pigs	4	1	nc		5	5.2	1.4-19.4	nc	

\* Case-subtype:control estimates were from polytomous logistic regression models fit using the EM algorithm. Case:case estimates were from binary logistic regression models restricted to assayed cases. All estimates are adjusted for state and age (restricted quadratic splines), unless otherwise indicated. The referent category included all participants (farmers and non-farmers) without exposure.

† Exposure to livestock diagnosed with Brucellosis by a veterinarian.

nc = not calculated because of insufficient data.

posure to cattle or poultry diagnosed with leukemia viruses was not materially associated with either NHL subtype.

Aggregate exposure to insecticides was not clearly associated with either case-subtype (Table 5), but cyclo-diene chlorinated hydrocarbons were associated with t(14;18)-positive NHL, particularly dieldrin (OR 3.7, 95% CI = 1.9-7.0) and toxaphene (OR 3.0, 95% CI = 1.5-6.1). Only one t(14;18)-negative case reported exposure to these products. Chlordane was weakly associ-

ated with both NHL subtypes. Lindane, a non-cyclo-diene chlorinated hydrocarbon, was associated only with t(14;18)-positive NHL (OR 2.3, 95% CI = 1.3-3.9). Nicotine livestock insecticide was associated with t(14;18)-negative NHL (OR 2.0, 95% CI = 1.2-3.4).

Neither NHL subtype was associated with herbicides overall, but t(14;18)-positive NHL was associated with atrazine (a triazine herbicide, OR 1.7, 95% CI = 1.0-2.8) (Table 5). Fungicides were associated with t(14;18)-positive NHL (OR 1.8, 95% CI = 0.9-3.6), particularly

**TABLE 5. Adjusted Odds Ratios for t(14;18) Non-Hodgkin's Lymphoma Case Subtype:Control and Case:Case Comparisons by Use of Agricultural Pesticides on Farms Where Participants Worked\***

Exposure	Controls		t(14;18)-Positive NHL vs. Controls		t(14;18)-Negative NHL vs. Controls			t(14;18)-Positive vs. -Negative NHL	
	N	N	OR	95% CI	N	OR	95% CI	OR	95% CI
Insecticides	588	37	1.3	0.8-2.0	56	1.0	0.7-1.3	1.4	0.7-2.6
Chlorinated hydrocarbons	284	20	1.2	0.8-2.0	27	1.1	0.7-1.5	1.2	0.6-2.4
DDT	216	13	1.1	0.6-1.9	22	1.2	0.8-1.7	0.9	0.4-1.9
Lindane	104	14	2.3	1.3-3.9	12	1.0	0.5-1.7	2.1	0.9-5.1
Cyclodienes	146	15	1.6	1.0-2.8	15	0.9	0.5-1.5	1.9	0.8-4.3
Aldrin	109	11	1.5	0.8-2.7	10	0.7	0.4-1.4	1.9	0.7-4.9
Chlordane	63	8	1.4	0.7-2.9	13	1.5	0.9-2.6	1.0	0.4-2.5
Dieldrin	33	7	3.7	1.9-7.0	1	nc		nc	
Toxaphene	30	5	3.0	1.5-6.1	1	nc		nc	
Organophosphates	161	15	1.4	0.8-2.5	20	1.2	0.8-1.8	1.4	0.6-3.0
Malathion	91	9	1.4	0.7-2.7	13	1.1	0.7-1.9	1.3	0.5-3.2
Carbamates	92	10	1.3	0.7-2.5	12	0.9	0.5-1.5	1.5	0.6-3.8
Arsenicals	80	6	1.1	0.5-2.5	11	1.5	0.9-2.5	0.9	0.3-2.6
Nicotine	52	1			12	2.0	1.2-3.4		
Fly spray	414	25	1.0	0.6-1.7	39	0.9	0.7-1.3	1.0	0.5-2.0
Herbicides	344	22	1.0	0.7-1.7	38	1.1	0.8-1.5	1.0	0.5-1.2
Phenoxy acids	266	17	0.9	0.5-1.5	30	1.1	0.7-1.5	0.9	0.4-1.8
Triazines	172	15	1.5	0.9-2.5	17	0.9	0.6-1.4	1.6	0.7-3.5
Atrazine	143	15	1.7	1.0-2.8	16	1.0	0.6-1.5	1.7	0.8-3.8
Amides	148	11	1.2	0.7-2.1	14	0.9	0.6-1.4	1.3	0.6-3.3
Benzoic Acids	122	12	1.4	0.8-2.5	12	0.8	0.4-1.3	2.1	0.8-5.3
Dinitroanilines	122	11	1.4	0.8-2.5	11	0.8	0.5-1.4	1.9	0.7-4.9
Fungicides	62	7	1.8	0.9-3.6	6	0.9	0.4-1.8	2.1	0.7-7.0
Phthalimides	18	4	2.9	1.1-7.5	1	nc		nc	
Fumigants	198	14	1.2	0.7-2.0	20	1.0	0.7-1.5	1.2	0.5-2.5

\* Case-subtype:control estimates were from polytomous logistic regression models fit using the EM algorithm. Case:case estimates were from binary logistic regression models restricted to assayed cases. All estimates are adjusted for state and age (restricted quadratic splines), unless otherwise indicated. The referent category included all participants (farmers and non-farmers) who did not report use of product on farms where they were employed.

nc = not calculated because of insufficient data.

**TABLE 6.** Adjusted\* Joint and Separate Odds Ratios and Interaction Contrast Ratios (95% Confidence Intervals) for t(14;18)-positive non-Hodgkin's Lymphoma in Association with Fumigants and Other Agricultural Exposures

	Separate Exposure†		Joint Exposure‡				
	N	OR	N	OR	95% CI	ICR§	95% CI
Fumigants and Farming	4	0.7	10	2.1	1.0–4.0	1.3	–0.1, 2.6
Fumigants and Dairy Cattle	6	0.8	8	2.3	1.1–4.7	1.4	–0.1, 3.0
Fumigants and Chickens	30	1.1	7	2.3	1.0–4.4	1.3	–0.2, 2.8
Fumigants and Pigs	7	0.8	8	2.1	1.0–4.2	1.0	–0.5, 2.4
Fumigants and Soybeans	31	1.1	7	2.4	1.1–5.1	1.4	–0.4, 3.2
Fumigants and Corn	6	0.9	8	1.8	0.9–3.6	0.8	–0.5, 2.2
Fumigants and Fungicides	8	0.8	4	6.9	2.3–21	6.1	–1.5, 14
Fumigants and Organophosphates	4	1.0	5	3.1	1.2–7.6	2.0	–0.7, 4.8
Fumigants and Chlorinated HC	10	0.9	6	1.8	0.8–4.1	0.8	–0.8, 2.4
	9	1.3					
	8	1.0					
	14	1.2					

\* Results from separate EM polytomous regression models including controls and both case subtypes, with exposures categorized according to separate or joint exposure using dummy variables, adjusted for state and age (restricted quadratic splines).

† OR compares t(14;18)-positive NHL among those exposed to one of the covariates in each exposure pair, with those without either exposure.

‡ OR compares t(14;18)-positive NHL among those exposed to both covariates in each exposure pair, with those without either exposure.

§ Interaction Contrast Ratio (ICR). ICR = 0 suggests that the relative risk associated with joint exposure is compatible with the additive null when averaged over the population.

phthalimides (Captan, Captifol) (OR 2.9, 95% CI = 1.1–7.5, 4 exposed cases). Odds ratios were comparable with those shown when we restricted pesticide-exposed categories to include only farmers who personally handled products or farmers who did not use personal protective equipment.

Additive and multiplicative expectations were similar for the joint exposures evaluated, and all joint estimates for t(14;18)-negative NHL were compatible with these expectations (data not shown). Most joint estimates for t(14;18)-positive NHL were also close to expected values. Joint exposure to farming and fumigants, however, was associated with a greater than additive increase in t(14;18)-positive NHL (joint OR 2.1, 95% CI = 1.0–4.0; ICR 1.3, 95% CI = –0.1, 2.6), and similar joint estimates were noted for fumigants combined with soybean, chicken, dairy, or hog production (Table 6). Larger but less precise departures from additivity were found for fumigants combined with fungicides or organophosphate insecticides.

## Discussion

We found a consistent pattern of greater relative strength for t(14;18)-positive compared with t(14;18)-negative associations for many agricultural exposures. Weak associations may have resulted from confounding, since we were unable to model individual agricultural exposures simultaneously. Nevertheless, we noted relatively strong associations between t(14;18)-positive NHL and chlorinated hydrocarbon insecticides, particularly cyclodienes and lindane. Cyclodienes were weakly associated with all NHL in a previous analysis of FARM data (OR 1.3), while the odds ratio for lindane used on

crops was 2.0.<sup>8</sup> We noted a moderate association between atrazine herbicide and t(14;18)-positive NHL, which contrasted with previous negative findings for all NHL.<sup>8,40</sup> There was a small relative increase in t(14;18)-positive NHL associated with livestock production, which was ubiquitous among farmers in this population. Previous reports have suggested that livestock producers, abattoir workers, and veterinarians may be at increased risk of NHL,<sup>9,10,41–45</sup> and work in meat packing has been specifically associated with follicular lymphoma (OR 1.6, 95% CI: 1.0–2.6), a predominantly t(14;18)-positive histologic subtype.<sup>10</sup>

In theory, causal associations with t(14;18)-positive NHL could result from exposures that affect the incidence of t(14;18),<sup>23,24</sup> if other cofactors required to complete pathogenesis are present.<sup>46</sup> Studies of pesticide applicators suggest that pesticides might increase the risk of t(14;18) translocations. The prevalence of chromosomal gaps and breaks among peripheral blood lymphocytes was increased during the peak spraying season,<sup>47</sup> and peripheral lymphocytes from pesticide applicators had increased double-strand DNA breaks at 18q21 and 14q32, the chromosomal regions involved in t(14;18).<sup>48</sup> Published studies have not, to our knowledge, specifically evaluated relations between pesticides and t(14;18)-positive lymphocytes.

Causal associations with t(14;18)-positive NHL also could result if exposures affect the prevalence, rather than the incidence, of t(14;18).<sup>6,23,24</sup> Livestock producers are exposed to dusts that may contain non-specific B-lymphocyte mitogens.<sup>49,50</sup> In theory, such exposures could multiply risks by stimulating the clonal expansion of t(14;18)-positive lymphocytes, thereby increasing the

number of cells susceptible to t(14;18)-positive NHL.<sup>6,23</sup> In addition, immunologic activation triggers a somatic hypermutation process that could theoretically increase the risk of oncogenic mutations in immortalized t(14;18)-positive cells.<sup>51</sup> In this scenario, t(14;18) would be a cause of increased susceptibility to an exposure, rather than a direct effect of exposure.

Pesticide-induced T-lymphocyte suppression and secondary viral infection has been proposed as a cause of NHL,<sup>52</sup> but NHL risk factors that cause immune dysfunction (HIV infection, therapeutic or hereditary immune suppression) tend to be associated with histologic subtypes that are predominantly t(14;18)-negative.<sup>53,54</sup> Agricultural factors associated with t(14;18)-negative NHL in this analysis included brucellosis in swine (probably *Brucella suis*) and nicotine livestock insecticide. Brucellosis is a reportable zoonotic disease associated with livestock production, abattoir and veterinary work, and consumption of unpasteurized dairy products. Over 90% of human cases of brucellosis in the United States may be undiagnosed.<sup>55,56</sup> If swine-derived human infection is a relevant factor, then the true risk of t(14;18)-negative NHL could be much higher than suggested by the odds ratio of 5.2 for exposure to pigs diagnosed with brucellosis.

We noted greater than additive joint odds ratios for t(14;18)-positive NHL and fumigant exposure combined with agricultural exposures. This finding might indicate biologic interactions, or could be related to a particular mode of fumigant use, for example, in enclosed feed storage facilities. Fumigant exposures may be associated with NHL among workers in the flour industry.<sup>32</sup>

Some concerns have been raised about the ability of farmers to recall accurately their use of specific pesticides. This problem may have led to exposure misclassification in our study, but would have been more likely to mask associations than create spurious ones.<sup>57,58</sup> It is unlikely that exposure misclassification, recall bias, or interviewer bias would have been differentially associated with case-subtypes.

One-third of study participants were represented by next-of-kin respondents, and these were more likely to be classified as unexposed to pesticides, or to be missing data on specific agricultural exposures.<sup>58</sup> Effect estimates for t(14;18)-positive NHL tended to increase slightly when proxy data were excluded, but this change may have been due to combined effects of improved exposure classification and the exclusion of cases with more rapidly fatal disease. The use of deceased controls for deceased cases is also a concern, since exposures associated with early mortality (e.g., cigarette smoking) may be over-represented in such controls.<sup>59,60</sup> We do not know the extent to which this would have affected risk estimates for agricultural exposures; however, adjusting for vital status had little impact on model results.

Some false-negative outcome classification was likely, due to t(14;18) breakpoints outside the range of PCR primers. The MBR1:<sub>H</sub> consensus primer set has been reported to miss approximately 20% of t(14;18) breakpoints.<sup>29</sup> The sensitivity of our assay may have been

improved by the addition of the MBR2 primer, which increased the range of *bcl-2* breakpoints we could detect; nine of our t(14;18)-positive cases had breakpoints outside the range of MBR1. A sensitivity analysis demonstrated that false-negative classification of t(14;18)-positive cases would have biased estimates for t(14;18)-negative NHL toward those for -positive NHL, while estimates for t(14;18)-positive NHL would have been unaffected, as long as misclassification was independent of exposure. We were careful to avoid PCR contamination, a possible cause of false-positive t(14;18) amplification. Analyses that excluded cases most likely to have been contaminated ( $\beta$ -globin negative cases and cases identified via nested PCR) were comparable with results for all assayed cases.

We were unable to classify over two-thirds of the FARM study NHL cases, and the uneven distribution of these cases with regard to state of origin and related characteristics could have led to biased estimates if unclassified cases were ignored in our analysis. Instead, we used the EM method of model-fitting,<sup>33</sup> which we have previously shown will reduce bias related to missing data, as long as unclassified cases are not related to case-subtypes within covariate strata.<sup>33a</sup> Translocation status would not affect the likelihood of diagnostic biopsy for NHL, and neither block availability nor sample adequacy was associated with tumor histology, grade, or anatomic site. Deceased cases were more likely to be unclassified, but vital status was not associated with translocation status among assayed cases. Therefore, we believe that EM method assumptions were met.

Our use of exposure data and archival samples from a previous study was efficient, but estimates were unstable because of small numbers in case-subtype groups, and our ability to use detailed exposure data was limited. Although confounding by unmeasured factors cannot be ruled out, established risk factors (HIV<sup>61</sup> or HTLV infection,<sup>62</sup> immune suppression) are unlikely to have been associated with NHL in the FARM study population. We could not adjust estimates for shared agricultural exposures, however. Our control group undoubtedly included participants with increased t(14;18)-positive lymphocytes, and the magnitude of relative effect estimates for exposures acting at this intermediate step would have been reduced in proportion to the number of such controls. Although this misclassification is an obvious limitation in our study, it is one that is probably common to all studies of multi-factorial diseases.

In conclusion, we found weak to relatively strong associations between many agricultural exposures and t(14;18)-positive, but not t(14;18)-negative NHL. Our findings support the hypothesis that outcome subtypes defined by t(14;18) have greater etiologic specificity than NHL in the aggregate. This outcome classification was based on a single pathogenic component that may act in multiple complex causal pathways. More substantial increases in specificity might be achieved in a larger study with case subgroups defined by multiple markers of underlying pathogenic mechanisms.<sup>27</sup> In addition, a longitudinal study of t(14;18)-positive NHL risk factors,

t(14;18)-positive lymphocytes, and t(14;18)-positive NHL might help clarify the pathogenic process,<sup>24</sup> and identify steps in NHL pathogenesis that are susceptible to intervention.

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## References

- Scherr P, Mueller N. Non-Hodgkin's lymphomas. In: Schottenfeld D, Fraumeni J, eds. *Cancer Epidemiology and Prevention*. Second ed. New York: Oxford University Press, 1996:920-945.
- Pearce N, Reif J. Epidemiologic studies of cancer in agricultural workers. *Am J Ind Med* 1990;18:133-148.
- Zahm S, Blair A. Pesticides and non-Hodgkin's lymphoma. *Cancer Res* 1992;52(suppl):5485s-5488s.
- Jaffe ES, Raffeld M, Medeiros L, Stetler-Stevenson M. An overview of the classification of non-Hodgkin's lymphomas: an integration of morphological and phenotypical concepts. *Cancer Res* 1992;52(suppl):5447s-5452s.
- Weisenburger D. Pathological classification of non-Hodgkin's lymphoma for epidemiological studies. *Cancer Res* 1992;52(suppl):5456s-5462s.
- Magrath I. Molecular basis of lymphomagenesis. *Cancer Res* 1992;52(suppl):5529s-5540s.
- Potter M. Pathogenetic mechanisms in B-cell non-Hodgkin's lymphoma in humans. *Cancer Res* 1992;52(suppl):5522s-5528s.
- Cantor K, Blair A, Everett G, Gibson R, Burmeister L, Brown L, Schuman L, Dick F. Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. *Cancer Res* 1992;52:2447-2455.
- Amadori D, Nanni O, Falcini F, Saragoni A, Tison V, Callea A, Scarpi E, Ricci M, Riva N, Buiatti E. Chronic lymphocytic leukemias and non-Hodgkin's lymphomas by histologic type in farming-animal breeding workers: a population-based case-control study based on job titles. *Occup Environ Med* 1995;52:374-379.
- Tatham L, Tolbert P, Kjeldberg C. Occupational risk factors for subgroups of non-Hodgkin's lymphoma. *Epidemiology* 1997;8:551-558.
- Scherr PA, Hutchinson G, Neiman R. Non-Hodgkin's lymphoma and occupational exposure. *Cancer Res* 1992;52(suppl):5503s-5509s.
- Pottern LM, Linet M, Blair A, Dick F, Burmeister L, Gibson R, Schuman L, Fraumeni J. Familial cancers associated with subtypes of leukemia and non-Hodgkin's lymphoma. *Leukemia Res* 1991;15:305-314.
- Schroeder J, Savitz D. Lymphoma and multiple myeloma mortality in relation to magnetic field exposure among electric utility workers. *Am J Ind Med* 1997;32:392-402.
- Herrinton L. Epidemiology of the Revised European-American Lymphoma classification subtypes. *Epidemiol Rev* 1998;20:187-203.
- Harris N, Jaffe E, Stein H, Banks P, Chan J, Cleary M, Delsol G, De Wolf-Peters C, Falini B, Gatter K, Grogan T, Isaacson P, Knowles D, Mason D, Muller-Hermelink H, Pileri S, Piris M, Ralfkiaer E, Warnke R. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994;84:1361-1392.
- The Non-Hodgkin's Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphoma. Summary and description of a Working Formulation for clinical usage. *Cancer* 1982;49:2112-2135.
- Cleary M, Smith S, Sklar J. Cloning and structural analysis of cDNAs for bcl-2 and a hybrid bcl-2/immunoglobulin transcript resulting from the t(14,18) translocation. *Cell* 1986;47:19-28.
- Graninger WB, Seto M, Boutain B, Goldman P, Korsmeyer SJ. Expression of bcl-2 and bcl-2 fusion transcripts in normal and neoplastic cells. *J Clin Invest* 1987;80:1512-1515.
- Reed J, Cuddy M, Sliabak T, Croce C, Nowell P. Oncogenic potential of bcl-2 demonstrated by gene transfer. *Nature* 1988;336:259-261.
- Hockenbery D, Nunez G, Millman C, Schreiber R, Korsmeyer S. Bcl-2 in an inner mitochondrial membrane protein that blocks programmed cell death. *Nature* 1990;348:334-336.
- Vaux D, Cory S, Adams J. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature* 1988;335:440-442.
- McDonnell T, Deane N, Platt F, Nuñez G, Jaeger U, McKearn J, Korsmeyer S. Bcl-2-immunoglobulin transgenic mice demonstrate extended B-cell survival and follicular lymphoproliferation. *Cell* 1989;57:79-88.
- Liu Y, Hernandez A, Shibata D, Cortopassi G. BCL2 translocation frequency rises with age in humans. *Proc Natl Acad Sci USA* 1994;91:8910-8914.
- Fuscoe J, Setzer R, Collard D, Moore M. Quantification of t(14,18) in the lymphocytes of healthy adult humans as a possible biomarker for environmental exposures to carcinogens. *Carcinogenesis* 1996;17:1013-1020.
- Ji W, Qu G, Ye P, Zhang X-Y, Halabi S, Erlich M. Frequent detection of bcl-2/JH translocations in human blood and organ samples by a quantitative polymerase chain reaction assay. *Cancer Res* 1995;55:2876-2882.
- Bell D, Liu Y, Cortopassi G. Occurrence of bcl-2 oncogene translocation with increased frequency in the peripheral blood of heavy smokers. *J Natl Cancer Inst* 1995;87:223-224.
- Weiss N, Liff J. Accounting for the multicausal nature of disease in the design and analysis of epidemiologic studies. *Am J Epidemiol* 1983;117:14-18.
- Crescenzi M, Seto M, Herzig G, Weiss P, Griffith R, Korsmeyer S. Thermostable DNA polymerase chain amplification of t(14,18) chromosome breakpoints and detection of minimal residual disease. *Proc Natl Acad Sci USA* 1988;85:4869-4873.
- Liu J, Johnson R, Traweck S. Rearrangement of the BCL-2 gene in follicular lymphoma. Detection by PCR in both fresh and fixed tissue samples. *Diag Molec Pathol* 1993;2:241-247.
- Bakshi A, Wright J, Graininger W, Seto M, Owens J, Cossman J, Jensen J, Goldman P, Korsmeyer S. Mechanism of the t(14,18) chromosomal translocation: Structural analysis of both derivative 14 and 18 reciprocal partners. *Proc Natl Acad Sci USA* 1987;84:2396-2400.
- Blair A, Linos A, Stewart P, Burmeister L, Gibson R, Everett G, Schuman L, Cantor K. Evaluation of risks for non-Hodgkin's lymphoma by occupation and industry exposures from a case-control study. *Am J Ind Med* 1993;23:301-312.
- Alavanja M, Blair A, Masters M. Cancer mortality in the US flour industry. *J Natl Cancer Inst* 1990;82:840-848.
- Dempster A, Laird N, Rubin D. Maximum likelihood from incomplete data via the EM algorithm. *J R Statist Soc B* 1977;39:1-38.
- Schroeder JC, Weinberg CR. Use of missing data methods to correct bias and improve precision in case-control studies where cases are subtyped but subtype information is incomplete. *Am J Epidemiol* (in press).
- StataCorp. *Stata Statistical Software*. 6.0 ed. College Station, TX: Stata Corporation, 1999.
- Greenland S. Dose-response and trend analysis in epidemiology: alternatives to categorical analysis. *Epidemiology* 1995;6:356-365.
- Greenland S, Rothman K. *Concepts of Interaction*. In: Rothman K, Greenland S, eds. *Modern Epidemiology*. Second ed. Philadelphia: Lippincott-Raven, 1998:329-342.
- Hosmer D, Lemeshow S. Confidence interval estimation of interaction. *Epidemiology* 1992;3:452-456.
- Fifth International Workshop on Chromosomes in Leukemia-Lymphoma. Correlation of chromosome abnormalities with histologic and immunologic characteristics in non-Hodgkin's lymphoma and adult T cell leukemia-lymphoma. *Blood* 1987;70:1554-1564.
- Cantor K, Blair A, Everett G, VanLier S, Burmeister L, Dick F, Gibson R, Schuman L. Hair dye use and risk of leukemia and lymphoma. *Am J Public Health* 1988;78:570-571.
- Zahm SH, Weisenburger D, Cantor K, Holmes F, Blair A. Role of the herbicide atrazine in the development of non-Hodgkin's lymphoma. *Scand J Work Environ Health* 1993;19:108-114.
- Persson B, Fredriksson M, Olsen K, Boeryd B, Axelson O. Some occupational exposures as risk factors for malignant lymphomas. *Cancer* 1993;72:1773-1778.
- Johnson E, Fischman HR, Matanowski GM, Diamond E. Cancer mortality among white males in the meat industry. *J Occup Med* 1986;28:23-32.
- Burmeister L, Everett G, van Lier S, Isaacson P. Selected cancer mortality and farm practices in Iowa. *Am J Epidemiol* 1983;118:72-77.
- Pearce NE, Smith AH, Reif JS. Increased risk of soft tissue sarcoma, malignant lymphoma and acute myeloid leukemia in abattoir workers. *Am J Ind Med* 1988;14:63-72.
- Blair A, Hayes H. Mortality patterns among US veterinarians, 1947-1977; an expanded study. *Int J Epidemiol* 1982;11:391-397.
- Limpens J, deJong D, vanKrieken J, Price C, Young B, vanOmmen G, Kluin P. Bcl-2/JH rearrangements in benign lymphoid tissues with follicular hyperplasia. *Oncogene* 1991;6:2271-1532.
- Yoder J, Watson M, Benson W. Lymphocyte chromosome analysis of agricultural workers during extensive exposure to pesticides. *Mutat Res* 1973;21:335-340.
- Garry VF, Tarone RE, Long L, Griffith J, Kelly JT, Burroughs B. Pesticide applicators with mixed pesticide exposure: G-banded analysis and possible relationship to non-Hodgkin's lymphoma. *Cancer Epidemiol Biomarkers* 1996;5:11-16.
- Simpson JC, Niven RM, Pickering CA, Oldham LA, Fletcher AM, Francis HC. Comparative personal exposures to organic dusts and endotoxin. *Ann Occup Hyg* 1999;43:107-115.

50. Hammarstrom L, Bird AG, Smith CI. Mitogenic activation of human lymphocytes: a protein A plaque assay evaluation of polyclonal B-cell activators. *Scand J Immunol* 1980;11:1-13.
51. Kelsoe G. V(D)J hypermutation and receptor revision: coloring outside the lines. *Curr Opin Immunol* 1999;11:70-75.
52. Newcombe D. Immune surveillance, organophosphorus exposure, and lymphomagenesis. *Lancet* 1992;339:539-541.
53. Gaidano G, Carbone A, Dalla-Favera R. Pathogenesis of AIDS-related lymphomas. Molecular and histogenetic heterogeneity. *Am J Pathol* 1998;152:623-630.
54. Biemer. Malignant lymphomas associated with immunodeficiency states. *Ann Clin Lab Sci* 1990;20:175.
55. Corbel M. Brucellosis: an Overview. *Emerg Infect Dis* 1997;3:213-221.
56. Centers for Disease Control. Brucellosis outbreak at a pork processing plant—North Carolina, MMWR 1992. 1994;43:113-116.
57. Blair A, Zahm SH. Methodologic issues in exposure assessment for case-control studies of cancer and herbicides. *Am J Ind Med* 1990;18:285-293.
58. Blair A, Zahm S. Patterns of pesticide use among farmers: implications for epidemiologic research. *Epidemiology* 1993;4:55-62.
59. Gordis L. Should dead cases be matched to dead controls? *Am J Epidemiol* 1982;115:1-5.
60. McLaughlin J, Blot W, Mehl E, Mandel J. Problems in the use of dead controls in case-control studies. I. General results. *Am J Epidemiol* 1985;121:131-139.
61. Gail M, Pluda J, Rabkin C, Biggar R, Goedert J, Horn J, Sondik E, Yarchoan R, Broder S. Projections of the incidence of non-Hodgkin's lymphoma related to Acquired Immunodeficiency Syndrome. *J Natl Cancer Inst* 1991;83:695-701.
62. Mueller N. The epidemiology of HTLV-I infection. *Cancer Causes Control* 1991;2:37-52.