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Pesticides, Chromosomal Aberrations, and Non-Hodgkin's Lymphoma

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Abstract

An excessive incidence of non-Hodgkin's lymphoma (NHL) has been reported among farmers and other occupational groups working with pesticides. Some pesticides exhibit immunotoxic and genotoxic activities. Individuals exposed to pesticides have also been found to have an increased prevalence of chromosomal abnormalities including the t(14;18)(q32;q21), one of the most common chromosomal abnormalities in NHL. Two recent epidemiologic studies reported that the association between pesticide exposures and risk of NHL was largely limited to NHL cases with the chromosomal translocation t(14;18). This review summarizes the findings from these epidemiologic studies, speculates on implications, and suggests the research needed to clarify the role of pesticides in NHL.

Keywords

Chromosomal abnormalities; epidemiology; lymphoma; pesticides; t(14; 18)

INTRODUCTION

Non-Hodgkin's lymphoma (NHL) is a cancer of the immune system. It includes many different subtypes with a variety of different molecular characteristics. The incidence of NHL in the United States increased more than 3.5% a year between 1973 and 1990. Although the overall NHL incidence rates began to stabilize in the late 1990s, it has been one of the most rapidly increasing malignancies over the past four decades in the United States and other developed countries.¹ Although some of the steep rise in NHL incidence is due to the human immunodeficiency virus (HIV) infection, HIV infection cannot account for the earlier increases or all of the more recent increases.¹ It has been suggested that the initial large increase of NHL incidence and later stabilized incidence might be related to certain environmental factors such as pesticides, solvents, and other chemicals.² This paper summarizes the findings from

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epidemiologic studies on pesticide use and risk of NHL, discusses possible role for the chromosomal translocation t(14;18)(q32;q21), and suggests the research needed to clarify the role of pesticides in NHL.

PESTICIDES AND NHL

Pesticides encompass a heterogeneous group of chemicals developed to control a variety of pests. Pesticides are sometimes classified according to the type of pests such as insecticides, herbicides, or fungicides. Each is a broad category with a wide diversity of chemicals having different properties and different effects. Carcinogenic pesticides exist among herbicides, insecticides, and fungicides as well as in several chemical classes (e.g., triazines, organophosphates, organochlorines).³

Several lines of evidence suggest that pesticides may have contributed to some of the increase of NHL in the United States. First, the United States cancer mortality patterns for NHL for 1950–1994 show a significant increase in the central part of the United States, a predominantly agricultural area, which suggests a possible role for agricultural exposures. Second, the increasing or decreasing exposures to pesticides in the United States and many developed countries mimic the changing trend of the incidence of NHL (i.e., a substantial increase from the 1960s to early 1990s followed by a leveling off afterward). Pesticides such as organochlorines (e.g., DDT, chlordane, lindane, etc.) were introduced to the United States in the 1940s. The highest exposure to persistent organic pollutants such as dioxins, chlorophenols, and polychlorinated biphenyls (PCBs) among the general population occurred during the 1970s. Since then, chlorinated compounds in the environment have been tightly regulated during the 1970s and 1980s. As a result, serum samples from the U.S. general population in 2003 were found to have substantially lower levels of certain organic pollutants (e.g., PCBs, dioxins, dibenzofurans, etc.) than those obtained in 1973.⁴ Although it is not clear whether or not the exposure patterns for these chemicals are linked to the risk of NHL, they do indicate a need for further evaluation. Thirdly, several epidemiologic studies have associated pesticides with a higher risk of NHL and results have been summarized in recent reviews.^{3,5} Pesticides associated with NHL include phenoxyacetic acids (particularly 2,4-dichlorophenoxyacetic acid [2,4-D]), organochlorines (particularly chlordane, DDT, lindane, and toxaphene), organophosphates such as diazinon, dichlorvos, and malathion. Of note, however, is that risk estimates vary widely among studies and in some studies the risk was not increased at all.^{1,3,5} Possible explanations for the inconsistencies have been discussed.³

PESTICIDES AND CHROMOSOMAL ABNORMALITIES

Pesticides may exert their carcinogenic effects through a variety of mechanisms, including genotoxicity, tumor promotion, hormonal action, and immunotoxicity.³ For example, several compounds, such as lindane, mevinphos, and fosetyl-aluminium, were found to have genotoxic effect on the rat or human hematopoietic system.⁶ In addition, 2,4-D, a pesticide that has been associated with the risk of NHL, is associated with lymphocyte replication and chromosomal aberrations in cytogenetic studies.⁷ In a study of farmers, 2,4-D was associated with reductions in lymphocyte subsets, including circulating helper and suppressor T cells, cytotoxic T lymphocytes, natural killer cells, and lymphocyte mitogenic responses.⁸ Furthermore, organophosphates inhibit serine esterases, which are critical components in the cytolytic activities of T lymphocytes and natural killer cells.⁹

Pesticides may also contribute to the etiology of NHL through chromosomal aberrations (a hallmark of NHL). NHL can arise when reciprocal rearrangements of B-cell immunoglobulin or T-cell receptor genes occur with oncogenes within immature lymphoid cells in the bone marrow or more mature cells in the peripheral lymphoid organs. These chromosomal translocations often result in the overexpression of oncogenes and cause the cells to proliferate

in an uncontrolled manner. One of the most common chromosomal abnormalities in NHL is the t(14;18)(q32;q21), which occurs in 70% to 90% of cases of follicular lymphoma, 20% to 30% of diffuse large B-cell lymphoma, and 5% to 10% of other less common subtypes.¹⁰ The t(14;18) translocation joins the *BCL2* gene on chromosome 18 to the immunoglobulin heavy chain gene on chromosome 14, leading to an inhibition of programmed cell death through *BCL2* overexpression and, consequently, prolonged survival of the affected B cells.¹⁰ Other chromosomal abnormalities commonly found in NHL include the t(2;18)(p11;q21) and t(18;22)(q21;q11) involving the *BCL2* proto-oncogene in follicular lymphoma and diffuse large B-cell lymphoma; t(3;14)(q27;q32) and other translocations of 3q27 involving the *BCL6* proto-oncogene in follicular lymphoma and diffuse large B-cell lymphoma; t(8;14)(q24;q32), t(2;8)(p11;q24) and t(8;22)(q24;q11) involving the *C-MYC* proto-oncogene in Burkitt lymphoma and diffuse large B-cell lymphoma; and the t(11;14)(q13;q32) involving the *BCL1* proto-oncogene in mantle cell lymphoma.

Several lines of evidence suggest that pesticides may be causally related to chromosomal abnormalities or genetic mutations in NHL. Farmers who are exposed to pesticides have an increased prevalence of the t(14;18) translocation during the high pesticide use periods. Several studies reported that the use of pesticides (e.g., 2,4-D) is more common among t(14;18)-positive healthy individuals.^{11,12} In one study of fumigant applicators,¹¹ breakpoint analysis showed that four chromosome bands (i.e., 1p13, 2p23, 14q32, and 21q12) had a significant excess of breaks in the exposed group, but no breaks in the control groups. Chromosome 14q32 is also involved in rearrangements of the *BCL6* gene and other oncogenes. Finally, in a study of 12 herbicide applicators spraying 2,4-D,¹² the urinary concentration of 2,4-D was associated with increased peripheral blood lymphocyte replicative index scores. Findings from these studies suggest that cytogenetic and molecular studies of individuals exposed to a wide range of pesticides seem promising in terms of revealing the role of pesticide exposures in the induction of chromosomal rearrangements, particularly the t(14;18) translocation.

POSSIBLE LINK BETWEEN PESTICIDES, CHROMOSOMAL ABERRATIONS, AND NHL

Two recent epidemiologic studies (one in Nebraska by Chiu et al.¹³ and the other in Iowa/Minnesota by Schroeder et al.¹⁴) evaluated risk of NHL from pesticide exposures and other factors among cases with and without chromosomal aberrations (i.e., the t(14;18) translocation). Results from these two studies have been summarized in a recent review.¹⁵ Briefly, the study conducted by Schroeder and colleagues¹⁴ used pesticide data derived from a population-based, case-control study conducted in Iowa and Minnesota between 1981 and 1983. The parent study included 622 cases and 1245 controls and was limited to men. Tumor blocks were retrieved for 248 of the 622 cases (40%) in the parent case-control study and the presence of the t(14;18) translocation in tumor tissue was determined by polymerase chain reaction. One hundred eighty-two of the 248 blocks (73%) were successfully assayed and 37% (68) of these cases were t(14;18)-positive, whereas 63% (114) were t(14;18)-negative. Schroeder and colleagues¹⁴ found that the t(14;18)-positive NHL cases tended to have larger relative risks from agricultural exposures than t(14;18)-negative cases. Specifically, t(14;18)-positive NHL was associated with farming (odds ratio [OR] = 1.4; 95% confidence interval [CI] = 0.9–2.3) and exposures to fungicides (OR = 1.8; 95% CI = 0.9–3.6) as well as a few specific pesticides, including dieldrin (OR = 3.7; 95% CI = 1.9–7.0), lindane (OR = 2.3; 95% CI = 1.3–3.9), toxaphene (OR = 3.0; 95% CI = 1.5–6.1), and atrazine (OR = 1.7, 95% CI = 1.0–2.8).

The second study was conducted by Chiu and colleagues¹³ using data from a population-based, case-control study conducted in Nebraska between 1983 and 1986. The parent case-control study included 385 cases and 1432 controls and included both men and women. The

researchers¹³ obtained 175 tumor blocks for the 385 cases (45.5%) that participated in the parent case-control study. They used fluorescence in situ hybridization (FISH) analysis to determine the t(14;18) translocation. FISH analysis was successfully conducted on 172 of the 175 cases (98.3%) and 37% (64) of the cases were t(14;18)-positive, while 62% (108) were t(14;18)-negative. Chiu and colleagues¹³ also found that agricultural exposures were associated with a greater risk of NHL among the t(14;18)-positive cases than the t(14;18)-negative cases. The risk of t(14;18)-positive NHL was significantly elevated among farmers who used animal insecticides (OR = 2.6; 95% CI = 1.0–6.9), crop insecticides (OR = 3.0; 95% CI = 1.1–8.2), herbicides (OR = 2.9; 95% CI = 1.1–7.9), and fumigants (OR = 5.0; 95% CI = 1.7–14.5), compared with farmers who never used pesticides. None of these categories of pesticides were associated with t(14;18)-negative NHL. The risk of t(14;18)-positive NHL associated with insecticides and herbicides increased with longer duration of use. In this Nebraska study, the risk of t(14;18)-positive NHL was also elevated among farmers using dieldrin (OR = 2.4; 95% CI = 0.8–7.9), toxaphene (OR = 3.2; 95% CI = 0.8–12.5), and lindane (OR = 3.5; 95% CI = 1.4–8.4) compared with nonfarmers.¹³

Of note is that the Iowa/Minnesota study¹⁴ used nonfarmers and farmers without exposures as the referent group, whereas the Nebraska study¹³ used farmers who never used pesticides as the referent group. When the same definition for the referent group was applied to the Nebraska data, the researchers found that the ORs for t(14;18)-positive NHL became smaller but remained statistically significant, whereas the ORs for t(14;18)-negative NHL were essentially unchanged. When analysis was conducted in the Nebraska study according to major histologic subtypes that commonly exhibit the t(14;18) translocation (i.e., follicular lymphoma and diffuse large B-cell lymphoma), the researchers¹⁵ found that agricultural pesticide use was not significantly associated with either follicular NHL or diffuse large B-cell NHL.

RESEARCH NEEDS

The consistent findings for NHL, pesticides, and the t(14;18) translocation from these two epidemiologic studies^{13,14} suggest that pesticides might contribute to the development of NHL through pathways involving the t(14;18) translocation. However, due to the relatively small size and natural limitation of the study design (i.e., case-control study), it remains unclear whether pesticides caused the t(14;18) translocation or provide a second or later hit in lymphoid cells with the t(14;18) translocation that ultimately leads to the occurrence of NHL. The presence of the t(14;18) translocation is not sufficient for the development of NHL because it can also be detected at a low level in peripheral blood and lymphoid tissues of a high proportion of healthy individuals.^{10,16} The causes of the t(14;18) translocation and the relationship between t(14;18) translocation and progression to NHL remain largely unknown,¹⁶ although associations with environmental genotoxins such as chemicals or ionizing radiation have been suggested. It has been proposed that some t(14;18)-bearing cells are “long-lived” memory cells that proliferate in response to antigens.¹⁶ Observations from these two epidemiologic studies^{13,14} provide clues for additional research regarding potential exposures that may be important in the long-term evolution of t(14;18)-positive clones.

Farmers might be exposed to several pesticides during a lifetime, and they might apply many pesticides together during the growing season. This poses a challenge for identifying the effect of a specific pesticide. In addition, as previously discussed, pesticides have diverse chemical and different biological modes of action. It is well recognized that chemical characteristics cannot be used to identify or accurately predict which pesticides might be carcinogenic.³ Unfortunately, the small sample size in these two NHL case-control studies of t(14;18) translocation and agricultural exposure does not allow the evaluation of multiple pesticides or specific pesticides. Future epidemiologic studies need to be larger to investigate the relationship of individual pesticides or specific combinations of pesticides and risk of NHL.

according to chromosomal aberrations. The small size in these two case-control studies on NHL and t(14;18) translocation also limit the opportunity to evaluate pesticide-NHL associations by chromosomal aberrations and histologic subtypes simultaneously, which might provide information on whether or not defining NHL subgroups by t(14;18) status is more specific than histologic subtypes for etiologic research. In the Nebraska study,¹⁵ the researchers also classified the 175 cases from whom tumor blocks were available according to the new World Health Organization classification. They found that agricultural pesticide use was not significantly associated with follicular NHL or diffuse large B-cell NHL, two histologic types that commonly exhibit t(14;18) translocation. However, when subgroups of NHL were grouped by t(14;18) status, the same study reported that pesticide use was associated with t(14;18)-positive NHL but not t(14;18)-negative NHL. Unfortunately, the sample size is not large enough to compare ORs between t(14;18)-defined subgroups within follicular lymphoma as well as ORs between t(14;18)-defined subgroups within diffuse large B-cell lymphoma. Consequently, although data from these two case-control studies of t(14;18)-defined subgroups of NHL and pesticide exposure provide additional information on the pathogenesis of NHL, the etiologic significance of grouping NHL according to t(14;18) status remains to be determined.

In addition to pesticides, farmers might also be exposed to animals, infectious agents, dusts, radiation, or other chemicals. In the Nebraska study,¹³ insecticides, herbicides, and fumigants were all associated with the risk of t(14;18)-positive NHL. These findings (i.e., lack of variability among pesticide classes) suggest that some chemicals within these pesticide classes or unknown factors related to pesticide use might be important. For example, many pesticides contain benzene and other organic solvents. Benzene has been shown to reduce both the number and function of B and T lymphocytes. Exposure to solvents and benzene has also been associated with risk of NHL.^{3,5} There is some evidence suggesting that benzene and organic solvents may be specifically associated with histologic subtypes of NHL that commonly exhibit the t(14;18) translocation. For example, Blair and colleagues¹⁷ found that high levels of exposure to benzene are associated with a higher risk of follicular lymphoma and diffuse lymphoma. Another study¹⁸ reported a positive association between aromatic and chlorinated hydrocarbons and risk of diffuse large cell and small lymphocytic lymphoma. Interestingly, the prevalence of the t(14;18) translocation has also been associated with benzene,¹⁹ even though another study found no association.²⁰ Taken together, these findings suggest that benzene and solvents may be related to the development of NHL through pathways involving the t(14;18) translocation. Future analytic epidemiologic studies evaluating the association of benzene, solvents, and farming activities with NHL risk might benefit from defining subgroups of NHL according to t(14;18) status.

The two studies reviewed in this paper represent the first epidemiologic studies that focused on a specific pathway to evaluate the association of pesticides and NHL. Despite a small sample size that limited analytic details, the findings from these studies are intriguing and suggest that pesticides might involve the development of NHL through a t(14;18) pathway. Future epidemiologic studies with larger sample sizes are warranted to evaluate the effect of individual pesticides, specific sets of chemicals, or other agricultural exposures on risk of NHL according to t(14;18) status. Further research investigating other chromosomal aberrations, in addition to the t(14;18) translocation, could be informative because t(14;18)-negative NHL remains a molecularly heterogeneous group. Furthermore, epidemiologic studies with repeated measures of pesticide exposures, biomarkers of pesticide exposures, and chromosomal abnormalities are needed to delineate the effects of pesticides in molecularly defined subgroups of NHL, and consequently, improving our understanding on the pathogenesis of NHL.

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References

1. Chiu BC, Weisenburger DD. An update of the epidemiology of non-Hodgkin's lymphoma. *Clin Lymphoma* 2003;4:161–168. [PubMed: 14715098]
2. Hardell L, Eriksson M. Is the decline of the increasing incidence of non-Hodgkin lymphoma in Sweden and other countries a result of cancer preventive measures? *Environ Health Perspect* 2003;111:1704–1706. [PubMed: 14594618]
3. Zahm S.; Ward, MH.; Blair, A. Pesticides and cancer. In: Keifer, MC., editor. *Human Health Effects of Pesticides*. Philadelphia, PA: Hanley & Belfus; 1997. p. 269-289.
4. Schecter A, Päpke O, Tung KC, Joseph J, Harris TR, Dahlgren J. Polybrominated diphenyl ether flame retardants in the U.S. population: current levels, temporal trends, and comparison with dioxins, dibenzofurans, and polychlorinated biphenyls. *J Occup Environ Med* 2005;47:199–211. [PubMed: 15761315]
5. Alexander DD, Mink PJ, Adami HO, Chang ET, Cole P, Mandel JS, Trichopoulos D. The non-Hodgkin lymphomas: a review of the epidemiologic literature. *Int J Cancer* 2007;120 (Suppl 12):1–39. [PubMed: 17405121]
6. Parent-Massin D, Thouvenot D. In vitro study of pesticide hematotoxicity in human and rat progenitors. *J Pharmacol Toxicol Methods* 1993;30:203–207. [PubMed: 8123901]
7. Soloneski S, González NV, Reigosa MA, Larramendy ML. Herbicide 2,4-dichlorophenoxyacetic acid (2,4-D)-induced cytogenetic damage in human lymphocytes in vitro in presence of erythrocytes. *Cell Biol Int* 2007;31:1316–1322. [PubMed: 17606385]
8. Faustini A, Settini L, Pacifici R, Fano V, Zuccaro P, Forastiere F. Immunological changes among farmers exposed to phenoxy herbicides: preliminary observations. *Occup Environ Med* 1996;53:583–585. [PubMed: 8882113]
9. Newcombe DS. Immune surveillance, organophosphorus exposure, and lymphomagenesis. *Lancet* 1992;339:539–541. [PubMed: 1346889]
10. Janz S, Potter M, Rabkin CS. Lymphoma- and leukemia-associated chromosomal translocations in healthy individuals. *Genes Chromosomes Cancer* 2003;36:211–223. [PubMed: 12557221]
11. Garry VF, Danzl TJ, Tarone R, Griffith J, Cervenka J, Krueger L, Whorton EB Jr, Nelson RL. Chromosome rearrangements in fumigant applicators: possible relationship to non-Hodgkin's lymphoma risk. *Cancer Epidemiol Biomarkers Prev* 1992;1:287–291. [PubMed: 1303128]
12. Figs LW, Holland NT, Rothman N, Zahm SH, Tarone RE, Hill R, Vogt RF, Smith MT, Boysen CD, Holmes FF, VanDyck K, Blair A. Increased lymphocyte replicative index following 2,4-dichlorophenoxyacetic acid herbicide exposure. *Cancer Causes Control* 2000;11:373–380. [PubMed: 10843448]
13. Chiu BC, Dave BJ, Blair A, Gapstur SM, Zahm SH, Weisenburger DD. Agricultural pesticide use and risk of t(14;18)-defined subtypes of non-Hodgkin lymphoma. *Blood* 2006;108:1363–1369. [PubMed: 16621961]
14. Schroeder JC, Olshan AF, Baric R, Dent GA, Weinberg CR, Yount B, Cerhan JR, Lynch CF, Schuman LM, Tolbert PE, Rothman N, Cantor KP, Blair A. Agricultural risk factors for t(14;18) subtypes of non-Hodgkin's lymphoma. *Epidemiology* 2001;12:701–709. [PubMed: 11679800]
15. Chiu BC, Lan Q, Dave BJ, Blair A, Zahm SH, Weisenburger DD. The utility of t(14;18) in understanding risk factors for non-Hodgkin lymphoma. *J Natl Cancer Inst Monogr* 2008;39:69–73. [PubMed: 18648007]
16. Roulland S, Lebailly P, Lecluse Y, Heutte N, Nadel B, Gauduchon P. Long-term clonal persistence and evolution of t(14;18)-bearing B cells in healthy individuals. *Leukemia* 2006;20:158–162. [PubMed: 16307019]

17. Blair A, Linos A, Stewart PA, Burmeister LF, Gibson R, Everett G, Schuman L, Cantor KP. 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. [PubMed: 8427258]
18. Miligi L, Costantini AS, Benvenuti A, Kriebel D, Bolejack V, Tumino R, Ramazzotti V, Rodella S, Stagnaro E, Crosignani P, Amadori D, Mirabelli D, Sommani L, Belletti I, Troschel L, Romeo L, Miceli G, Tozzi GA, Mendico I, Vineis P. Occupational exposure to solvents and the risk of lymphomas. *Epidemiology* 2006;17:552–561. [PubMed: 16878041]
19. Zhang L, Rothman N, Li G, Guo W, Yang W, Hubbard AE, Hayes RB, Yin S, Lu W, Smith MT. Aberrations in chromosomes associated with lymphoma and therapy-related leukemia in benzene-exposed workers. *Environ Mol Mutagen* 2007;48:467–474. [PubMed: 17584886]
20. McHale CM, Lan Q, Corso C, Li G, Zhang L, Vermeulen R, Curry JD, Shen M, Turakulov R, Higuchi R, Germer S, Yin S, Rothman N, Smith MT. Chromosome translocations in workers exposed to benzene. *J Natl Cancer Inst Monogr* 2008;39:74–77. [PubMed: 18648008]