Pesticides and non-Hodgkin Lymphoma: An Overview for the Clinician

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Non Hodgkin Lymphoma (NHL) represents a heterogeneous group of malignant diseases arising from the lymphatic tissues. Grossly, those disease entities can be subdivided into T-and B-cell lymphomas. Several systems have been developed over the last decades in order to further subclassify NHL. The **World Health Organization** (**WHO**) recognizes greater than 40 pathological subtypes of NHL. As molecular techniques evolve, the heterogeneity of this group of diseases is expanding further.

There were an estimated 56,390 new cases and 19,200 deaths from NHL in 2005. It was the 6th most common cancer among men and the 5th most common cancer among women.[1] The incidence of NHL increased at a rate of 3-4% per year during the 1970s and 1980s and stabilized in the 1990s.[2] Initially, better diagnostic techniques, changes in the classification patterns of NHL and the AIDS epidemic were potential explanations for this rise. However, more recent data revealed that the changes in NHL incidence are not fully explained by those phenomena and suggest that increasing exposures to not fully defined environmental factors, like diet or occupational exposures seem to be of importance.

The incidence of NHL is not homogenous throughout the **United States** (US). NHL-related mortality is higher in the central, more agricultural areas of the US. Hence, agricultural occupational exposures to pesticides have been investigated as causes for the development of NHL in numerous laboratory and epidemiological studies. Because of the large variety of potentially toxic substances, the corresponding wide spectrum of chemical structures and the sophisticated statistical techniques employed in the epidemiological investigations, the literature on the subject of the association of the development of NHL and use of pesticides is not straightforward.

What is a Pesticide?

The **United States Environmental Protection Agency** (**US EPA**) defines pesticides as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating insects, rodents or other animals, unwanted plants or weeds, fungi or microorganisms like bacteria or viruses. Approximately 890 pesticide compounds are marketed in the US in the form of more than 20,000 different products.

Methodological Difficulties with the Available Studies

Many studies tried to link farm life with the increased incidence of NHL. This does not consider whether the individual diagnosed with NHL participated in farming or was exposed to any of the toxic substances in question. Other investigations focused on the association of pesticide use and the development of NHL. Those studies often did not sufficiently address the question of the intensity of the exposure, its tenure, which pesticide was used and which type of lymphomas were observed.

Along with those more principal problems, most of those studies were retrospective cohorts or had a cross-sectional case-control design, which are generally associated with various types of biases, including coverage, sampling, selection, and recall bias.

Pesticides Frequently Used in the USA

Phenoxyacetic acid herbicides PAH

This group of pesticide compounds encompasses 2,4,5-T, which was found to contain a direct dioxin derivative that was banned in most European countries and the US in 1977, and 2,4-D, which is one of the most frequently used broadleaf pesticides in the US.

Early case control studies suggested an association between a professional PAH exposure and an increased incidence of NHL. Although most follow-up studies revealed a similar association, the findings have not always been statistically significant. An early interview-based Swedish case-control study from 1981 reported a 6-fold increase of NHL in individuals using PAH.[3] Subsequent studies from Kansas, Nebraska and New Zealand demonstrated an elevated incidence among PAH-using farmers.[4,5] Those studies also suggested that increased, unprotected and prolonged use of those pesticides seemed to increase the risk for NHL development. More recent studies showed a trend towards increased NHL incidence in the PAH using farming population without reaching statistical significance.[6] A large-scale international retrospective cohort study (SMR = 1.39, 95% CI 0.89-2.06) and a large retrospective effort from the Netherlands (RR=1.7, 95% CI 0.2-16.5) on the increase of NHL risk in occupational PAH exposure both showed trends towards an increase in NHL incidence in those populations.[7,8]

In summary, the methodological difficulties of those large scale studies notwithstanding, PAH appears to increase the development of NHL and prolonged and more intense use seems to strengthen this association.

Agent Orange

Agent Orange, used mainly for warfare, is a dioxin-contaminated mixture of PAH. It has been extensively investigated as a cause of NHL.[9,10] Although uncontrolled studies revealed an increase in NHL incidence in Vietnam veterans, ongoing large-scale Vietnam based studies are still examining the association of increased NHL incidence and exposure to Agent Orange.

Thus far, there is insufficient evidence to conclude whether there is an increased incidence of NHL in Vietnam veterans who were exposed to Agent Orange.

Chlorophenol herbicides (CPH)

The CPH are a group of nineteen different compounds which are chemically related to the PAH and which have the potential for dioxin contamination. They are mainly used in wood preservation and have been banned in many European countries since the 1970s but large amounts may still exist in soil and sedi-

ments even after the discontinuation of their use. Chlorophenols are still in use in several industries in the US. Most studies did identify a trend for increased NHL incidence in CPH exposed individuals without statistical significance and often large confidence intervals for the proposed association odds ratio.[7,11] Another international project identified a not statistically significant trend for the development of NHL with prolonged CPH exposure.[8] In addition, a recently published German study suggested an increased incidence of CPH and indolent as well as aggressive lymphoma subtypes.[12]

In conclusion, CPHs are a less well studied group of pesticides. However, their structural similarity to PAH and the possibility for contamination with carcinogenic dioxins, makes them, in the light of the evidence, potential lymphomagenic substances.

Triazine pesticides

Atrazine, cyanazine and metribuzin are members of the triazine pesticide group. These chemicals were introduced in the 1950s and are used to control germinating weeds. Atrazine is one of the most heavily used herbicides in the US, used extensively in corn, citrus, nut, sugarcane, sorghum, and cotton cultivation. Its use was restricted in the 1990s but despite the restrictions it is still significantly utilized. A Kansas case-control study demonstrated a statistically significant association of agricultural atrazine use and increased NHL incidence (OR=2.7, 95% CI 1.2-5.9). Several subsequent studies demonstrated equivocal or contradictory results.[13] A recent evaluation from the agricultural health study cohort, including 53,943 participants, could not establish an association of increased NHL incidence and agricultural atrazine use.[14]

In summary, atrazine has not been consistently associated with increased NHL incidence, whilst a synergistic effect with other pesticides, appears possible.

Glyphosate

Glyphosate (and its trade product Roundup®) is one of the most frequently used herbicides in the US. Although preclinical studies suggested an impact on the liver metabolism of prenatal rodents, a recent literature review concluded that glyphosate is not toxic to humans. Subsequent reports raised the concern that other compounds within the traded herbicide are more toxic than glyphosate itself.

One study from the Netherlands suggested an association of glyphosate exposure and hairy cell leukemia.[7] Other studies could not establish such a relationship.[11] Further, the large scale prospective agricultural health study was not able to detect an association between NHL incidence and glyphosate exposure.[15]

In summary, at this time we feel that there is no sufficient evidence that glyphosate induces NHL in farmers.

Organochlorine Insecticides (OCI)

The OCIs are a heterogenous group of insecticides that encompass DDT, DDD among others. The OCIs exert their toxic effect by virtue of opening sodium channels and hence generating steady action potentials. Due to their long half-lives the OCIs have a strong tendency for bioaccumulation in adipose tissues. Most of the OCI compounds are banned in the US because laboratory studies on rodents revealed carcinogenicity.

OCIs have been investigated for their potential to increase NHL incidence, with lindane and DDT being the most widely studied compounds. Although an early study from Kansas with few lindane exposed cases showed an increase of NHL with lindane use in the agricultural setting (OR=6.1, 95% CI 1.3-29),[8] most subsequent studies demonstrated only non-statistically significant trends towards an increase in incidence of NHL with exposure to OCI.[6,13] In conclusion, there is insufficient evidence at this time to determine if OCIs are associated with NHL.

Organophosphate Insecticides (OPI)

OPIs are the most broadly used insecticides in the US. They were originally developed as byproducts of nerve-gas production in Nazi Germany. They function as neurotoxins by virtue of irreversibly inhibiting the enzyme acetylcholinesterase. The OPIs easily degrade with sunlight, prohibiting significant bioaccumulation. However, small amounts can be detected in food and drinking water.

Several studies have investigated the impact of OPIs on the development of NHL. The Nebraska study[16] and a Canadian study[6] established a statistically significant increase in NHL incidence and the use of OPIs. Most other investigations established a similar trend, although the findings were not statistically significant.[4,13]

Overall, the OPI compounds can be regarded as potential culprits for lymphomagenesis.

Carbamates

The carbamates represent a heterogenous group of compounds that are used as herbicides and insecticides. They belong to the same class as the clinically used compounds neostigmine and rivastigmine, whose chemical structure is based on the natural alkaloid physostigmine.

A study from Nebraska described a statistically significant association of carbamate exposure and the development of NHL.[16] Several later North American studies showed a positive association, which held statistical significance for only certain subclasses of carbamate insecticides and herbicides,[16,17] which were not statistically significantly associated with the development of NHL in other studies.

Fungicides

Fungicides make up less than 10% of the pesticides used in the US. Association of fungicide use with the incidence of NHL in users has been studied over more than two decades. Although in a Canadian study[6] sulfur compound fungicides have been statistically significantly linked to an increased incidence of NHL, most other studies that studied sulfur compound and other fungicide use demonstrated either protective effects or lost statistical significance of an association when corrected for other pesticide use.[11,17]

Lymphoma subtypes

The inability of several of the above outlined studies to detect a statistically significant association between pesticide exposure and NHL development may partially be related to the fact that the incidence of NHL in general was examined as the outcome variable. As discussed previously, NHLs are a highly heterogeneous group of diseases. Even within the same subtype of NHL, the genetic profiles observed often vary widely. This prompts the question if lymphomas, harboring specific genetic abnormalities, are more likely to be associated with pesticide exposures than others.

One commonly detected genetic abnormality in NHL is a the translocation 14;18 or t(14;18). This genetic event corresponds with the activation of Bcl-2, an anti-apoptotic protein. Cells with this mutation can be immortalized by virtue of the activity of this cell cycle relevant protein and this event has been directly linked with lymphomagenesis, mainly follicular lymphoma. Several preliminary retrospective studies identified a possible link between lymphomas containing t(14;18) and pesticide exposure.[18]

Two large population-based studies have specifically evaluated the association of t(14;18)-positive lymphomas in occupational pesticide exposure. In the first study by Schroeder and colleagues,[18] the aggregate exposure OR for insecticides in t(14;18) showed a weakly positive trend without reaching statistical significance (OR 1.3, 95% CI 0.8-2.0). However, when examining specific OCI exposures, the associations observed were much stronger and reached statistical significance. A second population-based study by Chiu and colleagues found an association between pesticide exposure and t(14;18)-positive NHL.[19] The association retained statistical significance for 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). No association between pesticide exposure and t (14;18)-negative NHL was found.

Conclusions

Several key points can be highlighted based on our review. First, the literature on the subject is complicated by the fact that several pesticides are often used simultaneously. This might obscure pathogenic relationships between certain pesticides and NHL development. Second, most of the epidemiological studies are biased secondary to retrospective cohort and case-control designs. For better study of the exposure disease relationship further prospective studies are required. Third, in spite of several methodological shortcomings, the literature suggests an association between the exposure to several pesticides and the development of NHL. Fourth, several other compounds such as carbamates, fungicides and glyphosate have no sufficient evidence on such a relationship. Lastly, the evolving knowledge about the molecular diversity and pathogenesis of lymphomas might allow for a more specific study of associations between pesticide exposures and certain NHL subtypes.

References

1. American Cancer Society. Cancer Facts and Figures 2005. Atlanta, 2005.
2. Clarke CA, Glaser SL. Changing incidence of non-Hodgkin lymphomas in the United States. *Cancer* 2002; 94: 2015-23.
3. Hardell L, Eriksson M, et al. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids. *Br J Cancer* 1981; 43: 169-76.
4. Hoar SK, Blair A, et al. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. *JAMA* 1986; 256: 1141-7.
5. Pearce NE, Sheppard RA, et al. Non-Hodgkin's lymphoma and farming. *Int J Cancer* 1987; 39: 155-61.
6. McDuffie HH, Pahwa P, et al. Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. *Cancer Epidemiol Biomarkers Prev* 2001; 10: 1155-63.
7. Hooiveld M, Heederik DJ, et al. Second follow-up of a Dutch cohort occupationally exposed to phenoxy herbicides, chlorophenols, and contaminants. *Am J Epidemiol* 1998; 147: 891-901.
8. Kogevinas M, Becher H, et al. Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. *Am J Epidemiol* 1997; 145: 1061-75.
9. Frumkin H. Agent Orange and cancer. *CA Cancer J Clin* 2003; 53: 245-55.
10. Michalek JE, Wolfe WH, Miner JC. Health status of Air Force veterans occupationally exposed to herbicides in Vietnam. II. Mortality. *JAMA* 1990; 264: 1832-6.
11. Hardell L, Eriksson M. A case-control study of non-Hodgkin lymphoma and exposure to pesticides. *Cancer* 1999; 85: 1353-60.
12. Richardson DB, Terschuren C, Hoffmann W. Occupational risk factors for non-Hodgkin's lymphoma. *Am J Ind Med* 2008; 51: 258-68.
13. Miligi L, Costantini AS, et al. Non-Hodgkin's lymphoma, leukemia, and exposures in agriculture. *Am J Ind Med* 2003; 44: 627-36.
14. Rusiecki JA, De Roos A, et al. Cancer incidence among pesticide applicators exposed to atrazine in the Agricultural Health Study. *J Natl Cancer Inst* 2004; 96: 1375-82.
15. De Roos AJ, Blair A, et al. Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. *Environ Health Perspect* 2005; 113: 49-54.
16. Zahm SH, Weisenburger DD, et al. A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. *Epidemiol* 1990; 1: 349-56.
17. Zheng T, Zahm SH, et al. Agricultural exposure to carbamate pesticides and risk of non-Hodgkin lymphoma. J Occup *Environ Med* 2001; 43: 641-9.
18. Schroeder JC, Olshan AF, et al. Agricultural risk factors for t(14;18) subtypes of non-Hodgkin's lymphoma. *Epidemiol* 2001; 12: 701-9.
19. Chiu BC, Dave BJ, et al. Agricultural pesticide use and risk of t(14;18)-defined subtypes of non-Hodgkin lymphoma. *Blood* 2006; 108: 1363-9.

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