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Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis

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Background: A recent review by the International Agency for Research on Cancer (IARC) updated the assessments of the > 100 agents classified as Group 1, carcinogenic to humans (IARC Monographs Volume 100, parts A–F). This exercise was complicated by the absence of a broadly accepted, systematic method for evaluating mechanistic data to support conclusions regarding human hazard from exposure to carcinogens.

Objectives and Methods: IARC therefore convened two workshops in which an international Working Group of experts identified 10 key characteristics, one or more of which are commonly exhibited by established human carcinogens.

Discussion: These characteristics provide the basis for an objective approach to identifying and organizing results from pertinent mechanistic studies. The 10 characteristics are the abilities of an agent to *1*) act as an electrophile either directly or after metabolic activation; *2*) be genotoxic; *3*) alter DNA repair or cause genomic instability; *4*) induce epigenetic alterations; *5*) induce oxidative stress; *6*) induce chronic inflammation; *7*) be immunosuppressive; *8*) modulate receptor-mediated effects; *9*) cause immortalization; and *10*) alter cell proliferation, cell death, or nutrient supply.

Conclusion: We describe the use of the 10 key characteristics to conduct a systematic literature search focused on relevant end points and construct a graphical representation of the identified mechanistic information. Next, we use benzene and polychlorinated biphenyls as examples to illustrate how this approach may work in practice. The approach described is similar in many respects to those currently being implemented by the U.S. EPA's Integrated Risk Information System Program and the U.S. National Toxicology Program.

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Introduction

Recently, the International Agency for Research on Cancer (IARC) completed a review of all its Group 1 human carcinogens and updated information on tumor sites and mechanisms of carcinogenesis (IARC Monograph Volume 100A–F) (http://monographs.iarc.fr/ENG/Monographs/PDFs/index.php). About half of the agents classified in Group 1 had been last reviewed > 25 years ago, before mechanistic studies became prominent in evaluations of carcinogenicity. In addition, more recent studies have demonstrated that many cancer hazards reported in earlier studies were later observed to also cause cancer in other organs or through different exposure scenarios (Cogliano et al. 2011).

In compiling and updating the information for Volume 100A–F, two overarching issues became apparent. First, no broadly accepted systematic method for identifying, organizing, and summarizing mechanistic data for the purpose of decision making in cancer hazard identification was readily available. Second, the agents documented and listed as human carcinogens showed a number of characteristics that are shared among many carcinogenic agents. Many human carcinogens act via multiple mechanisms causing various biological changes in the multistage process of carcinogenesis. Indeed, cancer was once described by reference to causative agents, with multistage development of tumors being characterized through the impact of particular chemicals described as initiators and promoters of cancer. Subsequently, multistage development of cancer was identified with morphological change being correlated with genetic alterations. The more recent description by Hanahan and Weinberg of hallmarks of cancer is predicated not on morphology or the impact of carcinogens, but on changes in gene expression and cell signaling (Hanahan and Weinberg 2011). These hallmarks are the properties of cancer cells and neoplasms, and are not characteristic of the agents that cause cancer. Tumors attributable to chemical carcinogens may be distinct by mutational analysis (Westcott et al. 2015), but all neoplasms exhibit the hallmarks. A recent computational toxicology study has shown that chemicals that alter the targets or pathways among the hallmarks of cancer are likely to be carcinogenic (Kleinstreuer et al. 2013). In addition, a series of reviews

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in *Carcinogenesis* by members of the Halifax Project Task Force used the hallmarks framework to identify the carcinogenic potential of low doses and mixtures of chemicals (Harris 2015).

In 2012, participants at two workshops convened by the IARC in Lyon, France, extensively debated the mechanisms by which agents identified as human carcinogens (Group 1) produce cancer. The participants concluded that these carcinogens frequently exhibit ≥ 1 of 10 key characteristics (Table 1). Herein we describe these 10 key characteristics and discuss their importance in carcinogenesis. These characteristics are properties that human carcinogens commonly show and can encompass many different types of mechanistic end points. They are not mechanisms in and of themselves nor are they adverse outcome pathways.

Further, we describe how the 10 key characteristics can provide a basis for systematically identifying, organizing, and summarizing mechanistic information as part of the carcinogen evaluation process. The U.S. Environmental Protection Agency (EPA) and the National Toxicology Program (NTP) in the United States, as well as the IARC internationally, have recognized a need for such an approach (Rooney et al. 2014). The U.S. National Research Council (NRC) emphasized the need for consistent, transparent, systematic approaches for the identification, evaluation, and integration of data in the U.S. EPA's Integrated Risk Information System (IRIS) assessments of carcinogens and elsewhere in human health hazard assessments (NRC 2014).

Progress in the systematic evaluation of published evidence on the adverse health effects of environmental agents has been made through application of methods developed by evidence-based medicine (Koustas et al. 2014). However, mechanistic study databases present a challenge to systematic reviews in that the studies are typically both numerous and diverse, reporting on a multitude of end points and toxicity pathways. One recent example of a systematic approach searched for studies on end points relevant to nine cancer-related mechanistic categories in identifying and presenting mechanistic evidence on di(2-ethylhexyl) phthalate, a chemical with a complex database of > 3,000 research papers (Kushman et al. 2013). In this publication, the categories of mechanistic evidence were identified from a compendium of published reviews. This approach may be difficult to translate to agents with controversial or limited mechanistic evidence. It also would not permit comparisons across agents, including attempts to understand similarities or differences with human carcinogens. Further, it may be biased against the most recent mechanistic and molecular epidemiology studies that have not been the subject of a prior expert review.

To facilitate a systematic and uniform approach to organizing mechanistic data relevant to carcinogens, we propose use of the 10 key characteristics of human carcinogens as a basis for identifying and categorizing scientific findings relevant to cancer mechanisms when assessing whether an agent is a potential human carcinogen. A significant advantage of this approach is that it would encompass a wide range of end points of known relevance to carcinogenesis as identified through examination of the IARC Monographs on Group 1 carcinogens. Mechanistic topics can be included regardless of whether they have been the subject of prior expert reviews of any particular chemical. This should introduce objectivity that could reduce reliance on expert opinion, as well as facilitate comparisons across agents. Moreover, at its essence, the approach may afford a broad consideration of the mechanistic evidence rather than focusing narrowly on independent mechanistic hypotheses or pathways in isolation.

Herein, we demonstrate the applicability of this proposed systematic strategy for searching and organizing the literature using benzene and polychlorinated biphenyls (PCBs) as examples. The mechanistic study database for both of these chemicals is large, comprising > 1,800 studies for benzene and almost 3,900 for PCBs, many with multiple mechanistic end points. We conducted systematic literature searches for end points pertinent to the 10 key characteristics of human carcinogens, using literature trees to indicate the human and experimental animal studies that reported end points relevant to each characteristic. To further indicate their potential contribution to benzene and PCB carcinogenesis, we organized the characteristics into a graphical network representative of an overall mechanistic pathway.

Several recent IARC Monographs (e.g., Guyton et al. 2015; Loomis et al. 2015) have applied the 10 key characteristics described here for a variety of agents and organized the literature search results into flow diagrams. Overall, this categorization facilitated objective consideration of the relevant mechanistic information, thereby advancing analyses of hypothesized mechanisms and toxicity pathways. Because mechanistic data may provide evidence of carcinogenicity, and can play a role in up- or downgrading an evaluation based on cancer findings in animals, we suggest that this systematic approach to organizing the available data will assist future IARC Working Groups and other agencies in evaluating agents as potential human carcinogens, especially in the absence of convincing epidemiological data on cancer in humans.

Description of the Key Characteristics of Carcinogens

The number of ways by which agents contribute to carcinogenesis can be extensive if all biochemical or molecular end points are considered. However, these mechanisms can be grouped into a limited number of categories (e.g., genotoxicity, immunosuppression). Guyton et al. (2009) described 15 types of "key events" associated with human carcinogens that collectively represented many carcinogenic mechanisms. The experts present at the first of the IARC meetings in 2012 originally identified 24 mechanistic end points with several subcategories in each. This number of end points was considered too impractical as a guide for categorizing the literature, and the Working Group merged

Table 1. Key characteristics of carcinogens.

Characteristic	Examples of relevant evidence
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Abbreviations: AhR, aryl hydrocarbon receptor; ER, estrogen receptor; PPAR, peroxisome proliferator–activated receptor. Any of the 10 characteristics in this table could interact with any other (e.g., oxidative stress, DNA damage, and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone.

these categories into 10 at the second meeting in 2012, concluding that human carcinogens commonly show ≥ 1 of the 10 key characteristic properties listed in Table 1. These represent the majority of established properties of human carcinogens as described below.

Characteristic 1: Is Electrophilic or Can Be Metabolically Activated to Electrophiles

Electrophiles are electron-seeking molecules that commonly form addition products, commonly referred to as adducts, with cellular macromolecules including DNA, RNA, lipids, and proteins. Some chemical carcinogens are direct-acting electrophiles, whereas others require chemical conversion within the body (Salnikow and Zhitkovich 2008) or biotransformation by enzymes in a process termed metabolic activation (Miller 1970). Examples of direct-acting electrophilic carcinogens include sulfur mustards and ethylene oxide (Batal et al. 2014; Grosse et al. 2007; IARC 2008; Rusyn et al. 2005). The classic examples of chemical agents that require metabolic activation to become carcinogenic include polycyclic aromatic hydrocarbons, aromatic amines, *N*-nitrosamines, aflatoxins, and benzene, which by themselves are relatively inert (Slaga et al. 1980; Smith 1996). A number of enzymes, including cytochrome P450s, flavin monooxygenase, prostaglandin synthase, and various peroxidases, can biotransform relatively inert chemical compounds to potent toxic and carcinogenic metabolites or reactive intermediates (Hecht 2012; O'Brien 2000). The ability to form adducts on nucleic acids and proteins is a common property of these inherently electrophilic and/or metabolically activated human carcinogens (Ehrenberg 1984).

Characteristic 2: Is Genotoxic

The term "genotoxic" (Ehrenberg et al. 1973) refers to an agent that induces DNA damage, mutation, or both. DNA damage can be spontaneous in origin through errors of nucleic acid metabolism or can be induced by endogenous or exogenous agents. In some cases the exogenous agents may also be generated endogenously, such as formaldehyde and acetaldehyde, producing a background level of DNA damage. Examples of DNA damage include DNA adducts (a molecule bound covalently to DNA), DNA strand breaks (breaks in the phosphodiester bonds), DNA crosslinks, and DNA alkylation. DNA damage by itself is not a mutation and generally does not alter the linear sequence of nucleotides (or bases) in the DNA, whereas a mutation is a change in the DNA sequence and usually arises as the cell attempts to repair the DNA damage (Shaughnessy and DeMarini 2009).

Mutations can be classified into three groups based on their location or involvement in the genome. Gene or point mutations are changes in nucleotide sequence within a gene (e.g., base substitutions, frameshifts, and small deletions/duplications). Chromosomal mutations are changes in nucleotide sequence that extend over multiple genes (e.g., chromosome aberrations, translocations, large deletions, duplications, insertions, inversions, or micronuclei due to chromosome breakage). Genomic mutations involve the duplication or deletion of nucleotide sequences of an entire chromosome, an example of which is aneuploidy or formation of micronuclei that contain a centromere. A large proportion of Group 1 carcinogens are genotoxic, as documented in IARC Monographs Volume 100 A–F.

Characteristic 3: Alters DNA Repair or Causes Genomic Instability

Normal cells avoid deleterious mutations by replicating their genomes with high accuracy. However, the fidelity of DNA replication can vary widely depending on the DNA polymerase involved, introducing the possibility of error. Indeed, most spontaneous mutations are caused by polymerase error (Preston et al. 2010). The nature of the error, the flanking sequence, the presence of DNA damage, and the ability to correct errors all affect the outcome of this process (Arana and Kunkel 2010). As a consequence, defects in processes that determine DNA-replication fidelity can confer strong mutator phenotypes that result in genomic instability. Thus, carcinogens may act not only by producing DNA damage directly, but also by altering the processes that control normal DNA replication or repair of DNA damage. Examples include the inhibition of DNA repair by cadmium (Candéias et al. 2010) and formaldehyde (Luch et al. 2014).

Genomic instability is a well-recognized feature of many cancers (Bielas et al. 2006) and is considered to be one of the enabling characteristics of cancer (Hanahan and Weinberg 2011). Cells exposed to ionizing radiation have genetic instability that is a relatively late-occurring event that appears several cell generations after irradiation and results in a reduced ability to replicate the genotype faithfully (Kadhim et al. 2013). The events indicating genomic instability include chromosome aberrations, gene mutations, microsatellite instability, and apoptosis. These events are observed after exposure to arsenic (Bhattacharjee et al. 2013) and cadmium (Filipic 2012).

Characteristic 4: Induces Epigenetic Alterations

The term "epigenetic" refers to stable changes in gene expression and chromatin organization that are not caused by changes in the DNA sequence itself and can be inherited over cell divisions (Herceg et al. 2013). Epigenetic phenomena, including changes to the DNA methylome and chromatin compaction states, along with histone modification can impact the carcinogenic process by affecting gene expression and DNA repair dynamics (Herceg et al. 2013). A wide range of carcinogens have been shown to deregulate the epigenome, and it has been suggested that their mechanism may involve disruption of epigenetic mechanisms (Pogribny and Rusyn 2013). However, evidence for a causal role of epigenetic changes in cancer caused by Group 1 agents was considered to be limited in Volume 100, and the impact of many agents on the epigenome was considered to be a secondary mechanism of carcinogenesis (Herceg et al. 2013). Herceg et al. (2013) have described a wealth of studies demonstrating the impact of carcinogens on epigenetic mechanisms. Most carcinogens (even those reviewed for Volume 100) were evaluated by IARC Working Groups before new data on their epigenetic effects became available (Chappell et al. 2016). This evolving area will generate new mechanistic data in the years to come.

Characteristic 5: Induces Oxidative Stress

Many carcinogens are capable of influencing redox balance within target cells. If an imbalance occurs, favoring formation of reactive oxygen and/or nitrogen species at the expense of their detoxification, this is referred to as oxidative stress. Reactive oxygen species and other free radicals arising from tissue inflammation, xenobiotic metabolism, interruption of mitochondrial oxidative phosphorylation (Figueira et al. 2013), or reduced turnover of oxidized cellular components may play key roles in many of the processes necessary for the conversion of normal cells to cancer cells. However, oxidative stress is not unique to cancer induction and is associated with a number of chronic diseases and pathological conditions—for example, cardiovascular disease (Kayama et al. 2015), neurodegenerative disease (Chen et al. 2016), and chronic inflammation (Suman et al. 2015). Oxidative stress is also a common occurrence in neoplastic tissue and can be part of the tumor environment (Suman et al. 2015).

Oxidative damage is considered a major factor in the generation of mutations in DNA, and > 100 different types of oxidative DNA damage have been identified (Klaunig et al. 2011). At least 24 base modifications are produced by reactive oxygen species, as well as DNA–protein crosslinks and other lesions (Berquist and Wilson 2012), all potentially leading to genomic instability. Oxidative damage to DNA can lead to point mutations, deletions, insertions, or

chromosomal translocations, which may cause oncogene activation and tumor suppressor gene inactivation, and potentially initiate or promote carcinogenesis (Berquist and Wilson 2012; Klaunig et al. 2011). Thus, the induction of oxygen radical–induced cellular injury is a characteristic of a set of diverse carcinogens, including radiation, asbestos, and carcinogenic infectious agents.

Characteristic 6: Induces Chronic Inflammation

Chronic inflammation from persistent infections, such as that caused by *Helicobacter pylori*, as well as that produced by chemical agents including silica or asbestos fibers, has been associated with several forms of cancer (Grivennikov et al. 2010). Indeed, inflammation has been hypothesized to contribute to multiple aspects of cancer development and progression (Trinchieri 2012) and is an enabling hallmark of cancer (Hanahan and Weinberg 2011). Inflammation acts by both intrinsic and extrinsic pathways. Persistent infection and chronic inflammation disrupt local tissue homeostasis and alter cell signaling, leading to the recruitment and activation of inflammatory cells. These constitute extrinsic pathways linking inflammation to cancer (Multhoff and Radons 2012). On the other hand, intrinsic pathways driven by activation of proto-oncogenes in pre-neoplastic and neoplastic cells recruit host-derived inflammatory cells that accelerate tumor promotion and progression (Grivennikov et al. 2010). Because strong links exist between inflammation and the induction of oxidative stress and genomic instability, it may be difficult to separate out the importance of each of these mechanisms.

Characteristic 7: Is Immunosuppressive

Immunosuppression is a reduction in the capacity of the immune system to respond effectively to foreign antigens, including antigens on tumor cells. Persistent immunosuppression presents a risk of cancer, especially excess risk for lymphoma. For example, immunosuppression poses a significant risk when it is accompanied by continuing exposure to foreign antigens, such as in people with organ transplants, or when it occurs in individuals who are latently infected with a carcinogenic virus (Hartge and Smith 2007; Smith et al. 2004). Immune suppression differs from other mechanisms of carcinogenesis in that agents that cause immunosuppression may not directly transform normal cells into potential tumor cells. Potentially neoplastic cells that arise naturally, or that have been transformed by other carcinogens acting by a mechanism such as genotoxicity or by the various mechanisms of action associated with carcinogenic viruses, escape immune surveillance in immunosuppressed individuals. As a result, survival of these cells and their replication to form tumors is greatly facilitated by immune suppression. Several carcinogens act entirely or largely by immunosuppression, often in concert with other Group 1 agents, especially oncogenic infectious agents. The Group 1 agents that act by immunosuppression include human immunodeficiency virus (HIV-1) and the immunosuppressive drug cyclosporin (Rafferty et al. 2012).

Characteristic 8: Modulates Receptor-Mediated Effects

Numerous carcinogens act as ligands to receptor proteins, including menopausal hormone therapy, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and PCBs (Wallace and Redinbo 2013). Receptor-mediated activation broadly falls into two categories: *a*) intracellular activation, mediated by nuclear receptors that translocate into the nucleus and act on DNA as transcription factors (Aranda and Pascual 2001); and *b*) activation of cell surface receptors that induce signal-transduction pathways resulting in biological responses that involve a variety of protein kinases (Griner and Kazanietz 2007). Most exogenous agents act as agonists by competing for binding with an endogenous ligand; however, there are also receptors for which few or no endogenous ligands have been identified, such as the aryl hydrocarbon (Ah) receptor (Baek and Kim 2014; Ma 2011). Receptor-mediated activation most often results in changes in gene transcription. Molecular pathways that are regulated through ligand-receptor interaction and are most relevant to carcinogenesis include cell proliferation (e.g., stimulation of the normal proliferative pathways, as is the case for estrogen-dependent tissues and hormone therapy), xenobiotic metabolism, apoptosis, as well as modulation of the bioavailability of endogenous ligands by affecting biosynthesis, bioactivation, and degradation (Rushmore and Kong 2002).

Characteristic 9: Causes Immortalization

Several human DNA and RNA viruses, including various human papillomaviruses, Epstein-Barr virus, Kaposi sarcoma–associated herpes virus, hepatitis B virus, hepatitis C virus, HIV, Merkel cell polyomavirus (MCPyV), and human T-lymphotropic virus type 1 (HTLV-1) are carcinogenic to humans (Bouvard et al. 2009). These viruses have evolved multiple molecular mechanisms to disrupt specific cellular pathways to facilitate aberrant replication. Although oncogenic viruses belong to different families, their strategies in human cancer development show many similarities and involve viral-encoded oncoproteins targeting the key cellular proteins that regulate cell growth (Saha et al. 2010). Recent studies show that virus and host interactions also occur at the epigenetic level (Allday 2013). The result of these viral effects is to immortalize the target tissue cells such that they are not subject to the Hayflick limit, the point at which cells can no longer divide due to DNA damage or shortened telomeres (Klingelhutz 1999). For example, the human papilloma virus type 16 (HPV-16) *E6* and *E7* oncogenes are selectively retained and expressed in cervical carcinomas, and expression of *E6* and *E7* is sufficient to immortalize human cervical epithelial cells (Yugawa and Kiyono 2009).

Characteristic 10: Alters Cell Proliferation, Cell Death, or Nutrient Supply

There are at least three scenarios related to carcinogenesis in which alterations in cellular replication and/or cell-cycle control have been described. One invokes the predisposition for unrepaired DNA damage leading to cancer-causing mutations in replicating cells; another has attempted to identify sustained replication as a key mechanistic event; and a third describes the ability of a transformed cell to escape normal cell-cycle control and to continue replication. A component common to all three scenarios is the evasion of apoptosis or other terminal programming, including autophagy, in at least a proportion of the cell population (Ryter et al. 2014).

Necrotic cell death releases pro-inflammatory signals into the surrounding tissue microenvironment, recruiting inflammatory immune cells to the site of trauma, which can enhance cancer-cell proliferation and promote cancer metastasis (Coussens and Pollard 2011; Coussens et al. 2013; Pollard 2008). In contrast, various forms of apoptosis and autophagy (Galluzzi et al. 2015) have the opposite effect by removing potentially cancerous cells from a population before they acquire the changes permitting malignancy. Many agents affect necrosis, apoptosis, and/or autophagy and can have profoundly divergent effects on cancer induction in different tissues.

In addition to cell death caused directly by agent toxicity, cells may die within a tumor as a result of an impaired nutrient supply. Neoplastic cell numbers can increase exponentially, quickly outstripping the supply capabilities of the existing tissue vasculature. Neoangiogenesis, in which new blood vessels grow into a tumor, is key to providing this supply of nutrients. Thus, agents that promote or inhibit angiogenesis will promote or delay tumor growth (Hu et al. 2015).

Cancer cells also usually show quite different cellular energetics, relying on glycolysis for energy even under aerobic conditions (Rajendran et al. 2004). Although a likely

consequence of mutation and altered gene expression rather than a cancer-inducing mechanism, any modification of cellular energetics may reflect an important cancer-relevant switch in the cell's or tissue's metabolic state.

Using the Key Characteristics to Systematically Identify, Organize, and Summarize Mechanistic Information

Step 1: Identifying the Relevant Information

The starting point for systematic evaluation is to conduct comprehensive searches of the peer-reviewed literature aimed at identifying mechanistic data (Kushman et al. 2013). The searches can be constructed to address a series of study questions in the PECO (population, exposure, comparator, and outcomes) framework (Higgins and Green 2011) wherein end points associated with the key characteristics are identified. Specifically, the question to be answered by the searches is "Does exposure to the agent induce end points associated with one or more specific key characteristic properties of carcinogens?" The population (humans and any relevant experimental systems), exposure (the agent and relevant metabolites), and comparator (the unexposed comparison group or condition) should be sufficiently broad to identify a range of available mechanistic data informative of the overall evaluation of carcinogenic hazard. This approach thus entails comprehensive, targeted literature searches using appropriate medical search heading (MeSH) terms and key words to identify evidence on the 10 key characteristics for the agent(s) or exposure(s) under evaluation.

Additional complementary literature searches may incorporate terms for the agent and its metabolites, alone or in combination with broad terms for carcinogenicity or related effects. For instance, because U.S. EPA IRIS toxicological reviews also encompass a range of non-cancer toxicities, "top-down" broad literature searches aimed at comprehensively identifying studies on all potential toxic effects of an agent are employed (NRC 2014; U.S. EPA 2014). These comprehensive searches of peer-reviewed literature are supplemented by examining past IARC Monographs or other authoritative reviews, databases (e.g., PubChem), and peer-reviewed government reports can also be systematically searched. The search terms used and literature retrieved

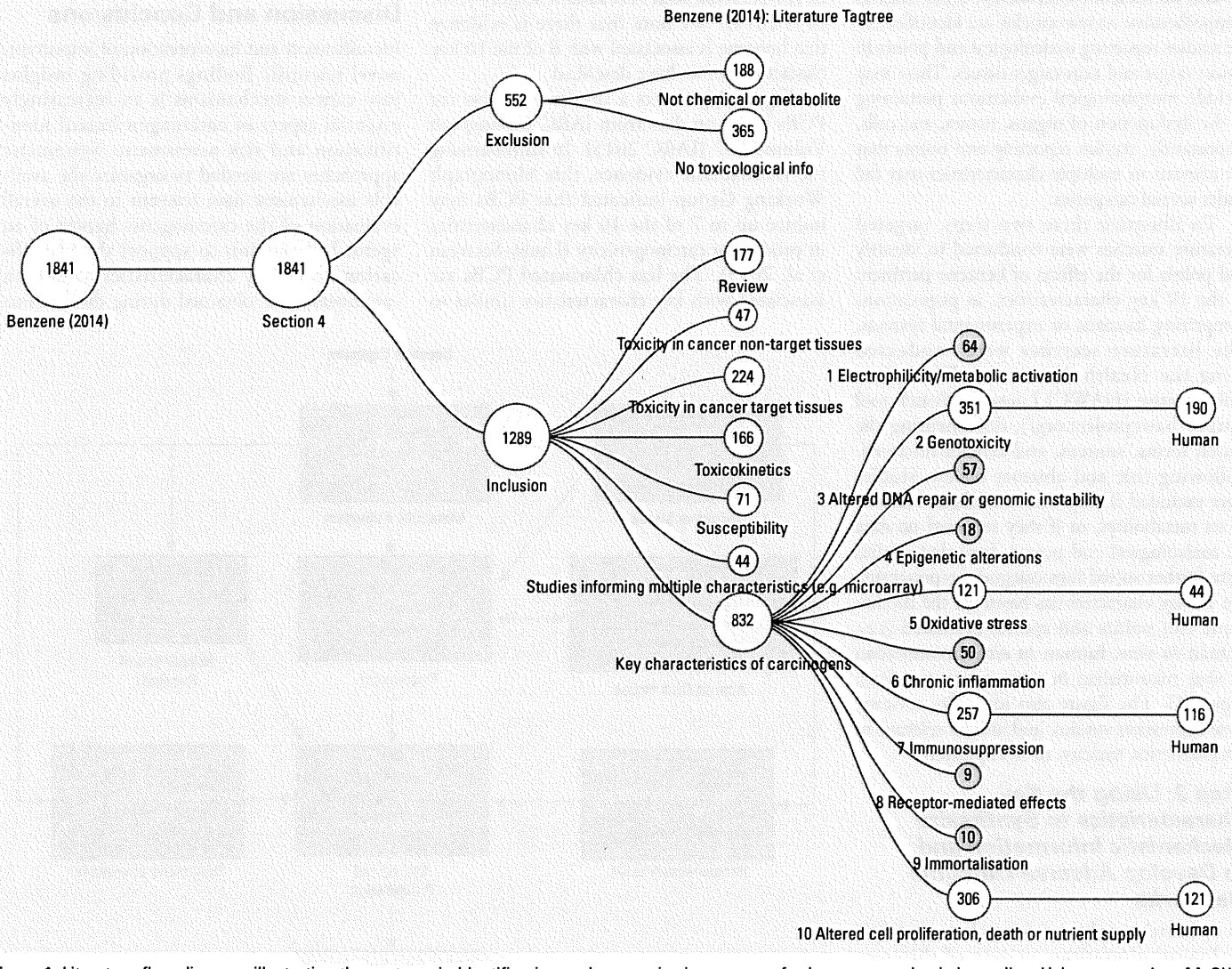

Figure 1. Literature flow diagram, illustrating the systematic identification and categorization process for benzene mechanistic studies. Using appropriate MeSH terms and key words, targeted literature searches were conducted for the 10 key characteristics using online tools available from the HAWC Project (https://hawcproject.org/). Section 4 refers to the location of the discussion of mechanistic data within the IARC Monograph structure (http://monographs.iarc.fr/ENG/Preamble/currentb4studiesother0706.php). All inclusion categories were expanded to document the number of studies attributed to each, down to the individual key characteristic level, which were expanded to illustrate human information when > 100 total studies were identified. Less frequently encountered key characteristic categories (blue-shaded circles) were left unexpanded for clarity. "Human" refers to both humans exposed *in vivo* and human cells exposed *in vitro*.

can be documented (e.g., using MyNCBI, which saves searches of the National Center for Biotechnology database, or https://hawcproject.org/).

Step 2: Screening and Organizing the Results

Based on title and abstract review, studies identified initially are excluded if no data on the chemical or a metabolite are reported, or if no data on toxicological or other cancer-related effects of the chemical are provided. For example, a study on levels of a chemical, but not effects of the chemical, would be excluded. Included studies are then organized by the population (human or experimental systems) and by the end points associated with the 10 key characteristics (Table 1). Studies relevant to toxicokinetics (covering absorption, distribution, metabolism, and excretion) are also identified. Additionally, authoritative, comprehensive review articles are identified, as are studies reporting toxicological end points in cancer target and non-target tissues. These may include morphological evaluations pertaining to the dysfunction of organs, tissues, and cells. Importantly, studies reporting end points that are relevant to multiple characteristics may fall under several categories.

To illustrate these two steps, targeted literature searches were conducted to identify end points for the effects of benzene pertinent to the 10 key characteristics, in populations comprising humans or experimental systems. The literature searches were conducted using the Health Assessment Workplace Collaborative (HAWC) Literature Search tool (https://hawcproject.org/), documenting the search terms, sources, and articles retrieved. Following title and abstract review, studies were excluded if they were not about benzene or its metabolites, or if they reported no data on toxicological end points. Included studies were further sorted into categories representing the 10 key characteristics based on the mechanistic end points and species evaluated (i.e., human *in vivo*, human *in vitro*, mammalian *in vivo*, mammalian *in vitro*, nonmammalian; Figure 1). The figure also identifies reviews, gene expression studies, and articles relevant to toxicokinetics, toxicity, or susceptibility.

Step 3: Using the Key Characteristics to Synthesize Mechanistic Information and to Develop Adverse-Outcome Networks

It is increasingly evident that multiple biological alterations or sets of different perturbations are necessary to convert a normal cell to a transformed cell and ultimately a tumor (Hanahan and Weinberg 2011). Carcinogens appear to affect this complex process in various ways and can act through multiple mechanisms to induce cancer and other adverse health outcomes (Goodson et al. 2015; Guyton et al. 2009). Using the 10 key characteristics as a basis, the collected information can be organized to form hypotheses and evaluate the evidentiary support for mechanistic events as a function of relevant aspects (e.g., dose, species, temporality) (Guyton et al. 2009). The diverse and complex mechanistic end points elicited by benzene can then be organized into an overview inclusive of multiple alterations and any linkages thereof (Figure 2). The resulting overview can provide guidance for further assessments of the literature, including dose relevance, species relevance, and temporality of events. This additional detailed information can then be used to produce proposed mechanisms or adverse outcome pathway networks as described by McHale et al. (2012) and the EPA's NexGen Risk Assessment Report (U.S. EPA 2014). We note that there is evidence that benzene is associated with 8 of the 10 key characteristics we have described.

Figure 3 presents a similar overview for PCBs based on data from IARC Monograph Volume 107 (IARC 2015). In summarizing the mechanistic evidence, this Monograph Working Group indicated that PCBs may induce up to 7 of the 10 key characteristics in producing carcinogenicity (Lauby-Secretan et al. 2013). The less chlorinated PCBs are associated with key characteristics similar to benzene (metabolic activation, DNA damage, cellular proliferation), whereas the dioxin-like PCBs are associated primarily with receptor-mediated activities.

Recently, using this same approach, the Working Groups of IARC Monograph Volume 112 and Volume 113 (in progress) concluded that strong mechanistic evidence exists for five key characteristics being involved in malathion carcinogenicity (i.e., genotoxicity, oxidative stress, inflammation, receptor-mediated effects, and cell proliferation or death), three in DDT carcinogenicity (i.e., immunosuppression, receptor-mediated effects and oxidative stress), and two each for diazinon and glyphosate (i.e., genotoxicity and oxidative stress), providing evidence to support their classification as probable human carcinogens in Group 2A (Guyton et al. 2015; Loomis et al. 2015).

Discussion and Conclusions

Identification and incorporation of important, novel scientific findings providing insights into cancer mechanisms is an increasingly essential aspect of carcinogen hazard identification and risk assessment. Systematic approaches are needed to organize the available mechanistic data relevant to the overall evaluation of the carcinogenic hazard of an agent. Information to support the identification of 10 key characteristics of human carcinogens was obtained during the Volume

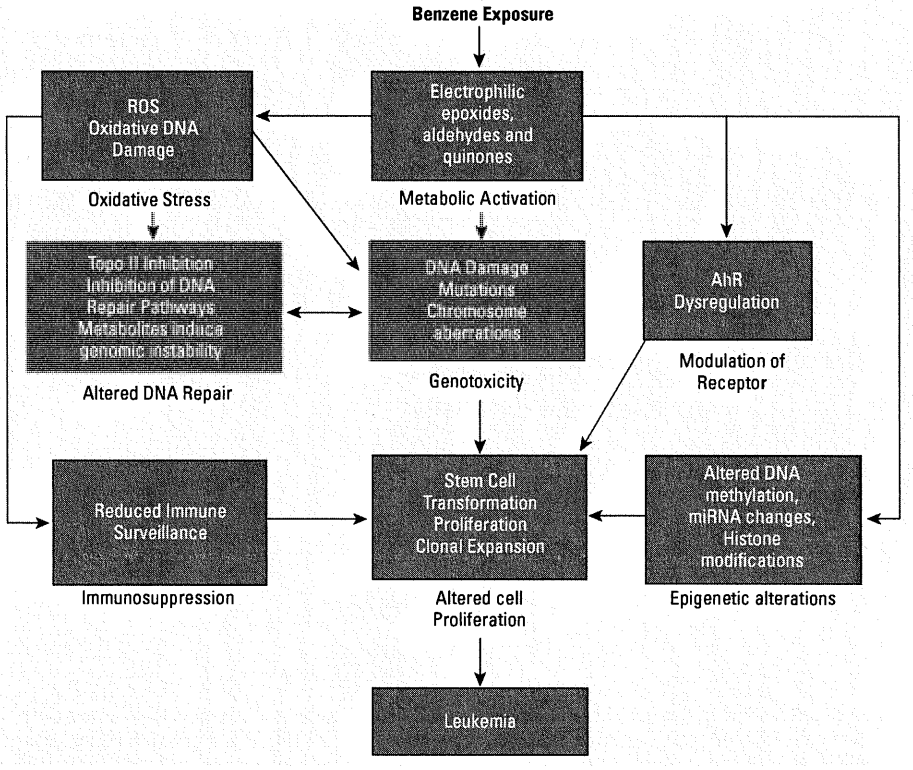

Figure 2. An overview of how benzene induces eight of the key characteristics in a probable mechanism of carcinogenicity. A full review of these mechanistic data is given by McHale et al. (2012), from which this figure was adapted.

100 Monographs and two subsequent expert workshops. These characteristics, although not necessarily representing mechanisms themselves, provide the rationale for an objective approach to identifying and organizing relevant mechanistic data. Using literature collected previously by others as well as by us, we have categorized the literature data according to the 10 characteristics for benzene and PCBs. This approach identified pertinent positive literature for 8 of the 10 key characteristics on benzene and 7 for PCBs, thereby providing a practical, objective method for organizing the large mechanistic literature associated with these chemicals.

This approach also lays the groundwork for a structured evaluation of the strength of the mechanistic evidence base, and therefore its utility in supporting hazard classifications. In the IARC Monographs the strength of the evidence that any carcinogenic effect observed is attributable to a particular mechanism is evaluated using the terms "weak," "moderate," or "strong" (http://monographs.iarc.fr/ENG/Preamble/index.php). In general, the strongest indications that a particular mechanism operates in humans derive from data obtained in exposed humans or in human cells *in vitro*. Data from experimental animals can support a mechanism by findings of consistent results and from studies that challenge the hypothesized mechanism experimentally. Other considerations include whether multiple mechanisms might contribute to tumor development, whether different mechanisms might operate in different dose ranges, whether separate mechanisms might operate in humans and experimental animals, and whether a unique mechanism might operate in a susceptible group. The possible contribution of alternative mechanisms must be considered before concluding that tumors observed in experimental animals are not relevant to humans. An uneven level of experimental support for different mechanisms may reflect that disproportionate resources have been focused on investigating a favored mechanism. All of these factors make assignment of descriptors such as "strong" to the mechanistic evidence challenging; but recent experience with two IARC Monograph meetings suggest that the weighing of the evidence on the basis of the 10 key characteristics focuses the group discussion on the available science and allows rapid consensus to be reached regardless of the strength of the evidence base (Guyton et al. 2015; Loomis et al. 2015).

Because the literature search and categorization approach described herein is comprehensive, it may aid consideration of the overall strength of the mechanistic database according to these principles. In particular, it is inclusive of diverse mechanistic evidence, enabling support for divergent or related mechanisms from human and experimental systems to be identified. Moreover, the literature support for end points relevant to specific mechanisms can be evaluated in an integrated manner when the mechanism is complex. Additionally, comparisons across agents will be facilitated, including evaluation of any similarities or differences in the pattern of key characteristics with agents that are currently classified.

As this approach is carried forward, we hope it will facilitate the objective identification of mechanistic data for consideration in the context of epidemiology, animal bioassay, or other types of evidence (e.g., studies in model organisms or *in vitro* assays) when classifying agents with regard to carcinogenic hazard. Equally important is to consider whether key characteristics of carcinogens are apparent upon exposures that are relevant to human health (Thomas et al. 2013). Overall, these developments will aid advancement of future evaluations of newly introduced agents, including those for which mechanistic data provide the primary evidence of carcinogenicity.

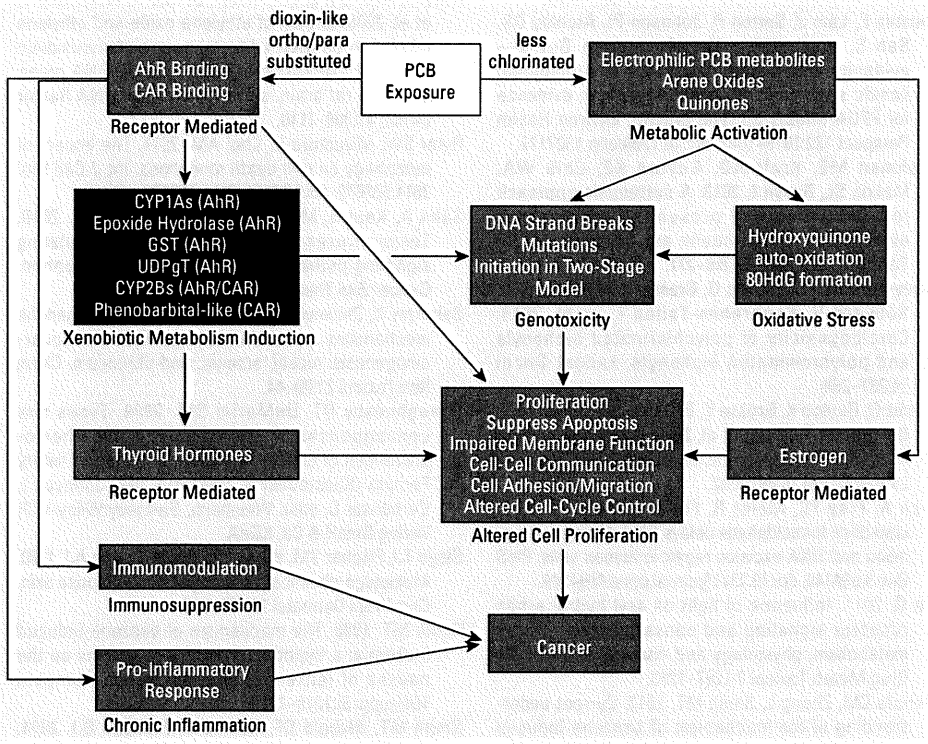

Figure 3. An overview of how polychlorinated biphenyls (PCBs) may induce seven key characteristics in their carcinogenicity (Lauby-Secretan et al. 2013). Highly chlorinated PCBs act as ligands for the aryl hydrocarbon receptor (AhR) and other receptors activating a large number of genes in a tissue- and cell-specific manner that can lead to cell proliferation, apoptosis, and other effects that influence cancer risk. Less chlorinated PCBs can be activated to electrophilic metabolites, such as arene oxides and quinones, which can cause genotoxic effects and induce oxidative stress. Receptor binding to CAR (constitutive androstane receptor) and AhR (a key characteristic) leads to xenobiotic metabolism induction (not a key characteristic; brown box) that in turn leads to genotoxicity and other key characteristics.

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Oxidative stress and oxidative damage in chemical carcinogenesis☆

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ABSTRACT

Reactive oxygen species (ROS) are induced through a variety of endogenous and exogenous sources. Overwhelming of antioxidant and DNA repair mechanisms in the cell by ROS may result in oxidative stress and oxidative damage to the cell. This resulting oxidative stress can damage critical cellular macromolecules and/or modulate gene expression pathways. Cancer induction by chemical and physical agents involves a multi-step process. This process includes multiple molecular and cellular events to transform a normal cell to a malignant neoplastic cell. Oxidative damage resulting from ROS generation can participate in all stages of the cancer process. An association of ROS generation and human cancer induction has been shown. It appears that oxidative stress may both cause as well as modify the cancer process. Recently association between polymorphisms in oxidative DNA repair genes and antioxidant genes (single nucleotide polymorphisms) and human cancer susceptibility has been shown.

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Introduction

Cancer induction by chemicals involves a multi-stage, multi-step process. While this process includes multiple molecular and cellular events to transform a normal cell to a malignant neoplastic cell, evidence in recent years has defined at least three steps in the chemical carcinogenesis process (Klaunig and Kamendulis, 2004). These steps have been identified as initiation, promotion and progression (Fig. 1). Initiation is the step where the normal cell undergoes unrepaired DNA damage and DNA synthesis to produce a mutated, initiated cell. The production of the initiated cell can occur through interaction with physical carcinogens such as UV light and radiation as well as chemical carcinogens that possess DNA damaging or mutagenic properties (genotoxic agents). In addition, recent evidence has shown that during cell proliferation, mutations may be acquired through misrepair of damaged DNA resulting in spontaneous initiated, mutated cells. Following the formation of the initiated cell, chemicals as well as endogenous physiological compounds can cause the selective clonal growth of this initiated cell through the process of tumor promotion. Tumor promotion involves the expansion of the initiated cell to a focal lesion. The tumor promotion process is not a direct DNA reactive or damaging process, but involves modulation of gene expression that results in the increase in cell number through cell division and/or decrease in apoptotic cell death (Klaunig and Kamendulis, 2004). Following continual cell proliferation additional mutations may be acquired in the preneoplastic cells resulting in the production of neoplasms. A third step, progression, involves additional damage to the genome, and unlike the promotion step, is irreversible. This multi-step process has been well defined in rodent systems and evidence has shown that similar processes occur in primates including humans.

The mechanisms by which carcinogens induce their effects have been studied extensively for over a half a century. Using the rodent liver model as an example, the modes of action by which carcinogens induce hepatic cancer can be placed in several categories based upon molecular target and cellular effects (see Table 1). These include genotoxicity and non-genotoxicity, including cytotoxicity, receptor interaction and mitogenic effects.

It is well documented that some agents can induce oxidative stress through either an increase reactive oxidative species generation from endogenous or exogenous sources or a decrease in antioxidant capabilities and oxidative DNA repair (Klaunig and Kamendulis, 2004). In viewing the role that oxidative stress may play in multi-stage process, it is apparent that oxidative DNA damage can have mutagenic effects and result in the formation of the initiated cell during this process. In addition, oxidative stress can modulate the redox potential of the cell and modify gene expression and thus participate at the tumor promotion phase of the cancer process (Benhar et al., 2002). The impact of endogenous as well as exogenous sources of ROS on the cell that if not handled by antioxidants can result in an increase in oxidative stress in the cell. This oxidative stress then, in turn, may damage critical macromolecules resulting in chromosome instability, genetic mutation and/or modulation of cell growth that may result in cancer.

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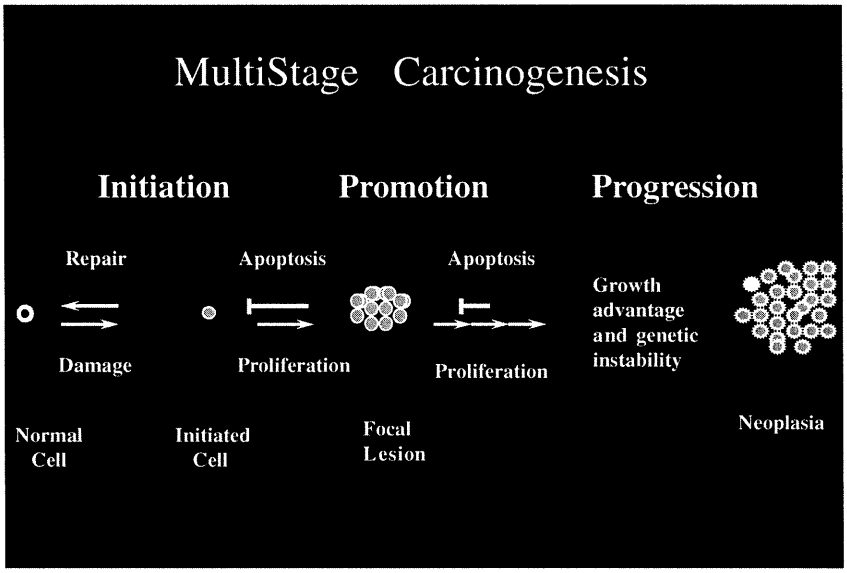

Fig. 1. Multistage carcinogenesis.

Sources of ROS

Experimental evidence indicates critical roles of ROS in tumor development (Guyton and Kensler, 1993; Petros et al., 2005; Ishikawa et al., 2008; Kumar et al., 2008). While the exact mechanisms of ROS production and human cancer development have not been fully defined, it is known that ROS can be produced from both endogenous and exogenous sources. Endogenous sources include mitochondria, peroxisomes, and inflammatory cell activation (Klaunig and Kamendulis, 2004). A wide array of exogenous sources is also documented in the literature, including environmental agents, radiation, therapeutic agents, and tobacco smoke (Table 2).

Exogenous generation of ROS

ROS may arise from several external sources including ionizing radiation and xenobiotics. Ionizing radiation can cause damage to living cells including DNA damage and gene mutation, cell death, and cancer (Riley, 1994). Most of the toxic effects of ionizing radiation are mediated by ROS (Tulard et al., 2003). ROS are generated rapidly through radiolysis of water molecules, as well as from secondary reactions leading to increased levels of ROS, which can persist and diffuse within the cell resulting in delayed toxic effects (Riley, 1994; Leach et al., 2001). Ionizing radiation is a proven carcinogen in humans and has been shown to induce cancer in multiple target organs.

Table 1
Modes of action (MOA) of rodent hepatic carcinogens.

Genotoxicty
Cytotoxicity
Receptor mediated
PPAR alpha (peroxisome proliferator)
CAR
Estrogen
Ah
Mitogenic
Oxidative stress
Porphyria
Metal overload (Cu Fe)
Increase in target cell number
(increased cell proliferation/decreased apoptosis)
P450 induction

Xenobiotics including environmental agents also have been shown to generate ROS in cells either metabolizing directly to primary radical intermediates or by activating endogenous sources of ROS (Rice-Evans and Burdon, 1993; Klaunig et al., 1997). The induction of oxidative stress and damage has been observed following exposure to xenobiotics of varied chemical structures and modes of action (Table 2). Chlorinated compounds, radiation, metal ions, barbiturates, phorbol esters, and some peroxisome proliferating compounds are among the classes of compounds that have been shown to induce oxidative stress and oxidative damage in vitro and in vivo (Klaunig et al., 1997).

Endogenous cellular generation of ROS

The well established endogenous sources of ROS include mitochondria, peroxisomes, and inflammatory cell activion (Klaunig and Kamendulis, 2004). The mitochondria are the major source of ROS in the cell. Since it was first reported by Loschen et al. (1971) that mitochondria generate superoxide radicals, extensive studies have been conducted to elucidate the mechanism of mitochondrial ROS generation and the physiological and toxicological significance of the mitochondrial ROS. Interest in mitochondrial bioenergetics and biogenesis and mitochondrial ROS has been renewed in recent years, linking mitochondrial ROS to tumor development (Gottlieb and Tomlinson, 2005; Guzy et al., 2008; Ishikawa et al., 2008). However, the role of ROS in tumor development and progression is still controversial, largely due to the fact that the exact mechanisms of mitochondrial ROS generation are not fully defined. It has been known that ROS production in mitochondria is species and tissue and cell cycle specific (Ku et al., 1993; Sohal et al., 1995). In addition, more ROS is produced in mitochondria from aging cells compared to younger counterparts (Ku et al., 1993), and in general higher in cancer cells than normal cells (Trachootham et al., 2006). The biological significance of these differences of mitochondrial ROS generation remains defined.

The ROS produced are by-products of mitochondrial oxidative phosphorylation. It is estimated that during mitochondrial respiration, 1–2% of electrons released from electron transfer chain (ETC) to form superoxide, although this value is controversial (St-Pierre et al., 2002). The major sites are defined to be mitochondrial complex I, NADH–ubiquinone oxidoreductase, and complex III, the ubiquinol–cytochrome c oxidoreductase, both responsible for much of superoxide production in mitochondria.

Table 2
Environmental and pharmaceutical carcinogens that can induce oxidative stress and damage.

Chemicals	Experimental models	ROS or effects	Reference
Genotoxic			
N-nitroso compounds	Murine	MDA, 8OHdG	(Bartsch et al., 1989; Srinivasan and Glauert, 1990; Chung and Xu, 1992)
Bisphenol A	Rats	8OHdG	(Cho et al., 2009)
BaP	Mice	8OHdG	(Mauthe et al., 1995)
AFB-1	Rats	8OHdG	(Shen et al., 1995)
Heterocyclic amines	In vitro	·OH	(Sato et al., 1992)
MMC and 2-acetylaminofluoene	In vitro	·OH	(Komiyama et al., 1982; Srinivasan and Glauert, 1990)
KBrO3	Rats	8OHdG	(Umemura et al., 1995)
Nongenotoxic			
2-Butoxyethanol	Mice		(Siesky et al., 2002)
Acrylonitrile	Rats; in vitro	MDA, 8OHdG	(Whysner et al., 1998; Kamendulis et al., 1999; Pu et al., 2009)
Chlorinated compounds (TCDD, dieldrin, DTT, lindane)	Murine; in vitro	lipid peroxidation, $O2^{\cdot -}$, etc.	(Videla et al., 1990; Junqueira et al., 1991; Alsharif et al., 1994)
Phenobarbital	Murine	·OH, 8OHdG, lipid peroxidation	(Junqueira et al., 1991)
Metal (nickel, BrCl, chromium, Fe-NTA iodobenzene)	Murine	·OH, 8OHdG, MDA, NO	(Klein et al., 1991; Sai et al., 1992; Bagchi and Stohs, 1993; Iqbal et al., 1995)
Peroxisome proliferator (DEHP, WY-14643, clofibrate, ciprofibrate, PFDA)	Murine	·OH, 8OHdG, etc.	(Srinivasan and Glauert, 1990; Tamura et al., 1990; Wada et al., 1992; Cattley and Glover, 1993; Huang et al., 1994)
CCl4		Trichloromethyl peroxyl radical	(Brattin et al., 1985)
Phorbol ester (TPA, PMA)	Murine, in vitro	·OH, 8OHdG	(Witz, 1991)
Quinones	V79 cells	8OHdG	(Dahlhaus et al., 1995)

The mechanism of ROS generation at mitochondrial complex III has been well characterized, which involves in the ubiquinone cycle of complex III (Betteridge, 2000). The mitochondrial complex III-dependent ROS generation has been implicated in cancer development and progression in recent studies. For example, hypoxia plays a causal role in pathological progression of cancer. It has been suggested that ROS generated at the ubiquinone cycle of complex III regulates hypoxic activation of hypoxia-inducible factors (HIFs), a family of transcription factors, including a broad range of cellular functions including cell proliferation and angiogenesis which is implicated in tumor development an progression (Bell et al., 2007). Mitochondrial complex I is the other site for ROS production. ROS generation at mitochondrial complex I has also been implicated into the mechanism of cancer progression (Ishikawa et al., 2008; Koshikawa et al., 2009; Sun et al., 2009). In addition, mitochondrial complex II, succinate: ubiquinone oxidoreductase, has been demonstrated to be another source of ROS production in mitochondria, which receives increasing attention in relation to tumorigenesis (Yankovskaya et al., 2003; Gottlieb and Tomlinson, 2005; Guzy et al., 2008).

Peroxisomes are another important cellular source of ROS generation. These cellular organelles consume oxygen to generate hydrogen peroxide and superoxide. The production of ROS involves a battery of peroxisomal oxidases including acyl-CoA oxidase and xanthine oxidase (see review; Schrader and Fahimi, 2006), which generate hydrogen peroxide and superoxide. The amount of oxidases and H_2O_2 produced varies among cells and tissues. In rat liver, peroxisomes produce about 35% of all H_2O_2 which accounts for about 20% of total oxygen consumption (Schrader and Fahimi, 2006). Although antioxidant enzymes such as catalase, glutathione peroxidase (Asayama et al., 1994), copper zinc superoxide dismutase (Dhaunsi et al., 1992), epoxide hydrolase, and peroxiredoxin I (Immenschuh et al., 2003) are present in peroxisomes, peroxisomes still contribute to a net-production of cellular ROS. Induction of peroxisomal ROS has been suggested to be implicated in chemical induced carcinogenesis. Peroxisome proliferators including hypolipidemic drugs, phthalate esters and halogenated solvents all lead to tumor development (Reddy et al., 1980; Reddy et al., 1983; Moody et al., 1991). Although a causal link has not been established between peroxisome proliferator-induced ROS and tumorigenesis (Rose et al., 1999), ROS has been associated with liver tumor induction (Klaunig and Kamendulis, 2004).

Inflammatory cells including neutrophils, eosinophils, and macrophages are an additional endogenous source of ROS and contribute significantly to the cellular ROS load. These phagocytes produce ROS using NADPH oxidase, a complex composed of two membrane bound subunits gp91phox and p22phox, and three regulatory cytosolic components $p47^{pho}x$, $p67^{phox}$, and Rac (Babior, 1999). Upon activation by a variety of endogenous and exogenous stimuli, phagocytes undergo a respiratory burst leading to transient increase in oxygen uptake resulting in generation of ROS through NADPH oxidase that catalyzes the one electron reduction of oxygen, using NADPH as the electron donor (Griendling et al., 2000). The O_2^- generated in this reaction can be further dismutated by superoxide dismutase to hydrogen peroxide. These reactive oxidative species play an important role in killing bacteria. Besides acting as cellular defense mechanism, recent studies suggest that these phagocyte-dependent ROS may also be involved in the development of a variety of cancers. However, it should be noted here, NADPH oxidase is not unique to inflammatory cells, it also presents in other non-phagocytes particularly in vascular cells. The importance of NADPH oxidase is increasingly being recognized in cancer cells as well. In a study on regulation of angiogenesis Xia et al. (2007) found that knockout of NADPH oxidase subunit $p47^{phox}$ diminishes ROS generation leading to decreased expression of VEGF and HIF-1α and tumor angiogenesis, indicative of critical role of endogenous ROS produced by NADPH oxidase in tumorigenesis.

Kupffer cells, the resident macrophages of the liver, have been increasingly recognized in the role of hepatocarcinogenesis. It has been well documented that the Kupffer cell oxidant production is critically involved in peroxisome proliferator-induced neoplasia (Rose et al., 1999). Mechanistic studies suggest that the Kupffer cell may be involved in the promotion stage of carcinogenesis since activation of Kupffer cells with LPS resulted in an increase in focal volume and DNA synthesis within diethylnitrosamine-induced hepatic foci, whereas inactivation of Kupffer cells using dietary glycine ablated the LPS-induced effects on liver cell growth (Klaunig and Kamendulis, 2004).

Interaction of ROS and biological macromolecules

Lipid peroxidation

Polyunsaturated fatty acids (PUFA), containing two or more double bonds, are readily oxidized by ROS to produce lipid peroxyl radicals and lipid hydroperoxides, a process called lipid peroxidation (Rice-Evans and Burdon, 1993). Once the process of lipid peroxidation is initiated, it proceeds as a free radical-mediated chain reaction involving initiation, propagation, and termination (Gago-Dominguez et al., 2005). Initiation of lipid peroxidation is started by the abstraction of hydrogen atom from polyunsaturated fatty acid moiety of membrane phospholipids by the attack of reactive species (Gago-Dominguez et al., 2005). The fatty acid radicals formed in the initiation step will react with the neighboring lipid molecules and generate new free radicals. The propagation phase can repeat many times until it is stopped by chain breaking antioxidants (Rice-Evans and Burdon, 1993; Foy, 1999; Niki et al., 2005).

The breakdown of lipid peroxidation products results in the formation of many reactive aldehydes, such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE) (Tuma, 2002). These aldehydes have shown reactivity with protein and DNA and form adducts with these macromolecules (Kikugawa et al., 1987; Nicholls et al., 1992; Uchida and Stadtman, 1993; Klaunig et al., 1998). MDA and MDA-MDA dimmers are mutagenic (Spalding, 1988; Klaunig and Kamendulis, 2004).

Isoprostanes, prostaglandin like compounds, are generated from the free radical-initiated peroxidation of arachidonic acid (Morrow et al., 1990; Liu et al., 1999; Morrow and Roberts, 2002). They are formed in vivo and can be detected in plasma, tissue and urine, and are elevated by oxidative stress inducers such as chloroform, carbon tetrachloride, and cigarette smoking (Morrow et al., 1995; Kadiiska et al., 2005a; Kadiiska et al., 2005b; Delannoy et al., 2009).

Oxidative DNA damage

Oxidative DNA damage is a major source of the mutation load in living organisms (Lu et al., 2001). Over 100 oxidative DNA adducts have been identified (von Sonntag, 1987; Dizdaroglu, 1992; Demple and Harrison, 1994; Brown et al., 2009). The estimated frequency of oxidative DNA damage is at 10^4 lesions/cell/day in humans (Fraga et al., 1990; Lu et al., 2001). Free radicals can attack both purine and pyrimidine bases, as well as the deoxyribose backbone. ROS-induced DNA damage includes single- or double-strand breakage, base modifications, deoxyribose modification, and DNA cross-link. If DNA damage is not properly repaired prior to or during replication, it may result in cell death, mutation, or induction of transcription, induction of signaling pathways, replication errors, and genomic instability, all of which have been associated with the carcinogenesis process (Marnett, 2000; Cooke, 2003; Klaunig and Kamendulis, 2004; Valko et al., 2006).

Several ROS are capable of producing oxidative DNA damage. Intermediates from the lipid and protein oxidation process may react with DNA and form DNA adducts (Kikugawa et al., 1987; Nicholls et al., 1992; Uchida and Stadtman, 1993; Klaunig et al., 1998; Lu et al., 2001; Tuma, 2002). However, the hydroxyl free radical is considered the major ROS that interacts with DNA bases, deoxyribose and free nucleotides (Lu et al., 2001). The hydroxyl free radical is highly reactive and has short half-life. Thus its migration in the cell is limited and reacts with cellular components quickly in close vicinity (Klaunig and Kamendulis, 2004). Hydrogen peroxide, a precursor to hydroxyl radical, is less reactive and more readily diffusible and thus more likely to be involved in the formation of oxidized bases (Guyton and Kensler, 1993; Barber and Harris, 1994). Peroxynitrite, formed from the reaction of nitric oxide and superoxide, is also diffusible between cells. During inflammation, activated macrophages produce nitric oxide and superoxide, which in turn may give rise to peroxynite and modify DNA bases. This may explain the reported association between inflammation and mutation (Marnett, 2000; Klaunig and Kamendulis, 2004).

There have been at least 24 bases modifications related to ROS attack of DNA that have been identified to date (Wilson et al., 2003). Among these modified bases, 8-hydroxy-2′-deoxyguanosine (8OHdG) is the predominant adduct. 8OHdG is formed through the oxidation of guanine at the C8 position in guanine base (Kasai et al., 1984; Dizdaroglu, 1985). This oxidative DNA adduct has been detected in different tissues and urine and it is the most commonly used biomarker for oxidative DNA damage as well oxidative stress both in vitro and in vivo. A variety of environmental agents have been reported to induced elevated levels of 8OHdG, including ionizing radiation; cigarette smoking; metals such as arsenic, iron, and cadmium; and organic chemicals such as carbon tetrachloride and chloroform (Kasprzak, 2002; Kadiiska et al., 2005a,b). Elevated levels of 8OHdG have also been detected in some disease conditions, including diabetes, Parkinson's disease, Alzheimer's disease, and chronic hepatitis C infection (Owen et al., 1996; Kato et al., 2001; Kakimoto et al., 2002; Moreira et al., 2005).

Oxidative damage to RNA

Compare to oxidative modifications of DNA by ROS, the extent and distribution of oxidative damage to RNA is not as well understood. There is evidence that shows that purified RNA possesses greater oxidative stability than DNA (Thorp, 2000). Parallel experiments on chemical cleavage of DNA and RNA by enediynes and rhodium (III) photoxidants revealed that C-H bond cleavage might be more difficult in RNA than in DNA (Chow et al., 1992; Kappen and Goldberg, 1995; Thorp, 2000). 2-Nitropropane treatments resulted in 3.6-fold increase in 8OHdG in DNA versus 11-fold increase on 8-hydroxyguanosine in RNA in rat liver (Fiala et al., 1989). Administration of oxidant doxorubicin to Fisher-344 rats resulted in a significant increase in liver RNA oxidation, but no significantly increased DNA oxidation (Hofer et al., 2006). Oxidative RNA damage include modifications of bases and ribose, base excision, and strand break (Li et al., 2006). Several oxidative RNA adducts including 8-oxoguanosine, 8-hydroxyadenine, and 5-hydroxycitosine have been reported (Yanagawa et al., 1992; Schneider et al., 1993). Oxidative damage to protein-coding RNA or non-coding RNA may potentially cause errors in protein synthesis or dysregulation of gene expression, and such non-acutely lethal insults to cells might be associated with underlying mechanisms of several human diseases. Oxidative RNA damage has been described in several neurodegenerative diseases (Li et al., 2006; Nunomura et al., 2007).

Oxidative damage to protein

Reactive species can react directly with protein or they can react with sugars and lipids, generating products that in turn react with the protein (Klaunig et al., 1997; Freeman et al., 2009). Within the protein, either the peptide bond or the sidechain may be targeted. Basic mechanisms involved in the oxidation of proteins by ROS were elucidated by studies in which amino acids, peptides, and proteins were exposed to ionizing radiations under conditions where •OH or a mixture of •OH and $O^{2-\bullet}$ are formed (Stadtman, 2004). It has been demonstrated that the attack by hydroxyl free radical leads to an abstraction of a hydrogen atom from the protein polypeptide backbone and form a carbon-centered radical (Stadtman, 2004).

Oxidative damage to proteins ROS may have significant biological consequences. It can result in modification of enzyme activity (stimulation or inhibition) (White et al., 1976; Bellomo et al., 1983). Damage to the membrane transport proteins may produce cellular ionic homeostasis and lead to alterations in intercellular calcium and

potassium that will trigger a series of changes in cells (Kerr et al., 1992; Klaunig et al., 1998). Changes to receptor proteins and gap junction proteins may also modify signal transfer in cells. In selective cases alteration of protein structure may allow the target protein to be further attacked by proteinases (Klaunig et al., 1998).

Effects of ROS on DNA mutation

Epidemiological studies indicated that chronic oxidative stresses are strongly associated with carcinogenesis (Hwang and Bowen, 2007). For example, ulcerative colitis has long been linked with high incidence of colorectal cancer, and chronic gastritis, such as HP infection, has been associated with a high incidence of gastric cancer (Seril et al., 2003; Konturek et al., 2006). Oxidative damage and modifications to DNA bases lead to changes in the genomic information. This damage may include point mutations, deletions, insertions, or chromosomal translocations which may cause oncogene activation and tumor suppressor gene inactivation, and potentially lead to initiation of carcinogenesis (Toyokuni, 2006). $P15^{INK4B}$ and $p16^{INK4A}$ tumor suppressor genes have been reported to be the major target gene of ROS-induced renal cell carcinoma in rats (Tanaka et al., 1999). Reports have shown that ROS and oxidative DNA damage may be involved in AFB1-induced *p53* and *ras* gene mutations in hepatocarcinogenesis (Shen and Ong, 1996), and in human skin cancers in sun exposed areas and in UV-induced mouse skin cancers (Nishigori et al., 2004).

In vitro systems have been used to study the mutagenic effects of DNA damage induced by ROS including H_2O_2, $^1O_2$, $O_2^{-\bullet}$, HOC1, and $HO^{\bullet}$. Mutants can be identified phenotypically, and DNA isolated from phage-exhibiting mutant phenotypes is then sequenced, which allow the determination of the frequency and types of mutations within the target gene (McBride et al., 1991). The most frequent mutations that result from ROS-induced damage to DNA in bacteria are C to T transitions (Feig et al., 1994). A tandem CC to TT double substitution has been shown to be induced by ROS generated by a variety of systems.

The most extensively studied, and also the most abundant oxidative DNA lesion produced is 8OHdG, which is highly mutagenic due to mispairing with adenine during DNA replication (Cheng et al., 1992). Numerous studies have demonstrated that the 8OHdG level is elevated in various human cancers (Tanaka et al., 2008; Valavanidis et al., 2009), and in animal models of tumors (Muguruma et al., 2007; Harvilchuck et al., 2009; Pu et al., 2009). These studies strongly supported that oxidative DNA damage is involved in the etiology of cancer. Based on this evidence, 8OHdG has been widely used as a biomarker of oxidative DNA damage, and measurement of 8OHdG level is applied to evaluate the load of oxidative stress (Hwang and Bowen, 2007; Valavanidis et al., 2009). The assessment of oxidative DNA damage products in various biological matrices, such as serum and/or urinary 8OHdG, could be important to understanding the role of oxidative stress and subsequently devising proper intervention strategies. In addition, RNS, produced during the process of chronic inflammation, can cause nitrative DNA damage to form 8-nitroguanine. The formation of 8-nitroguanine has been observed in various human samples, and experimental evidence has suggested that 8-nitroguanine is a mutagenic DNA lesion, which preferentially leads to G→T transversions (reviewed by Kawanishi and Hiraku (2006)). Therefore, 8-nitroguanine could also be used as a potential biomarker to evaluate the risk of inflammation, during which high levels of ROS are usually produced, related carcinogenesis.

Mitochondrial DNA (mtDNA) is more susceptible to oxidation than nuclear DNA (Inoue et al., 2003; Brandon et al., 2006). Evidence exists that oxidative mtDNA damage is involved in the development of many human cancers including colon (Polyak et al., 1998), liver (Nishikawa et al., 2001), breast (Tan et al., 2002), lung (Suzuki et al., 2003), bladder (Chen et al., 2004), prostate (Petros et al., 2005), esophageal cancer (Tan et al., 2006), ovarian (Van Trappen et al., 2007), head and neck (Zhou et al., 2007), and nasopharyngeal (Pang et al., 2008). Mutant mtDNA has been reported to be 220 times more abundant than a mutated nuclear DNA marker of cancer cells (Czarnecka et al., 2006). The mtDNA mutations in cancer could either arise in female germ line (oncogenic germline mutations) and predispose to cancer or arise in the mtDNA of the tissue (tumor-specific mutations) and participate in the tumor progression process (Brandon et al., 2006). mtDNA mutations in tumors generally fall into two main classes: tumorigenic and adaptive. Tumorigenic mtDNA mutations are mutations that inhibit oxidative phosphorylation (OXPHOS) and impede electron flow down the mitochondrial electron transfer chain, resulting in increased ROS production and contribute to cancer promotion and progression. Adaptive mtDNA mutations are milder mutations that facilitate tumor survival under adverse environments (Wallace, 2005; Brandon et al., 2006). It is, therefore, plausible to hypothesize that a positive feedback loop may exist between ROS, mtDNA mutation and tumor development.

ROS effects on gene expression

Most of the effort on examining the effects of ROS has been focused on oxidative DNA damage and mutation; however, the presence of epigenetic effects of ROS has also been examined (Evans et al., 2004). It is well established that upon exposure to oxidants (or oxidative stress-inducing agents), mammalian cells express stress-induced genes, which encode antioxidant defense. Although increases in ROS production may lead to the induction of apoptosis or necrosis, low levels of oxidants, through interaction and modification of genome DNA, may alter gene expression, particularly growth factors and proto-oncogenes (Frenkel, 1992). Researchers investigating the effects of ROS on cell proliferation demonstrated that the induction of cell proliferation occurred only at exposure to low concentrations or transient exposure to ROS (Fiorani et al., 1995). The effect of ROS on cell growth also depends on the cell type; it may promote normal cell proliferation but kill tumor cells (Laurent et al., 2005).

As a signaling messenger, ROS is able to activate critical target molecules such as PKC, which is relevant to tumor progression (Wu, 2006). The effects of cellular oxidants have also been related to activation of downstream transcription factors. The most significant effects of oxidants on signaling pathways have been observed in the nuclear factor erythroid 2-related factor 2 (NF-E2/rf2 or Nrf2) (Kensler et al., 2007), mitogen-activated protein (MAP) kinase/AP-1 (Benhar et al., 2002), and NF-κB pathways (Pantano et al., 2006); hypoxia-inducible transcription factor 1α (HIF-1α) is also activated (Rankin and Giaccia, 2008). The activation of these transcription factors is involved in both cell survival and apoptosis. The cellular concentration of ROS appears to influence the selective activation of these transcription factors and therefore may help explain the observation that either cell death or cell proliferation may result from exposure to oxidative stress.

PKC

PKC (protein kinase C) is a family of serine/threonine kinases that are involved in controlling the function of other proteins through the phosphorylation of hydroxyl groups of serine and threonine amino acid residues on these proteins. Studies have shown that ROS induces the release of calcium from intracellular stores, resulting in the activation of PKC, that regulates a variety of cell functions including proliferation, cell cycle, differentiation, cytoskeletal organization, cell migration, and apoptosis (Wu, 2006). While PKC can also be activated by ROS (Frank and Eguchi, 2003), the activation of PKC is required for generating ROS in several systems (Lin and Takemoto, 2005). A recent report demonstrated that ROS-mediated sustained activation of PKC signaling pathways plays a critical role in migration of human HepG2

cells (Wu et al., 2006). PKC signaling pathway was also reported to be involved in mitochondrial ROS mediated *HIF-1α* gene activation in lung carcinoma cell line (Koshikawa et al., 2009).

Interestingly, the activation of PKC seems to be differentially regulated by cellular oxidants: oxidation at the NH2-terminal regulatory domain activates PKC, whereas oxidation at the COOH terminal inactivates PKC (Gopalakrishna and Anderson, 1989). Because these pathways regulate cellular mitogenesis, migration, proliferation, survival, and death responses, their aberrant activation has been suggested to be a potential mechanism of ROS-induced carcinogenesis.

Nrf2

Nrf2 is a basic region-leucine zipper (bZip)-type transcription factor, which belongs to cap "n" collar family (Moi et al., 1994) and is located in the chromosome 17q21.3 (Chan et al., 1995). Nrf2 heterodimerizes with members of small Maf family of transcription factors, and then binds to ARE (antioxidant response element), leading to the transcriptional expression of ARE-regulated genes (Itoh et al., 1997). Under basal unstressed conditions, Keap1 (Kelch ECH associating protein 1), a cytosolic repressor protein, binds to Nrf2 and promotes its proteasomal degradation through Cullin 3 (Cul-3)-based E3 ligase (Cullinan et al., 2004). Upon exposure to environmental stressors such as ROS or electrophiles, keap1 undergoes conformational changes, liberating Nrf2 from keap1, Nrf2 then translocates into nucleus to bind to ARE and activate gene transcription (Itoh et al., 1999). The Nrf2-keap1 system has been observed in virtually all vertebrates, including humans, mouse, rats, chicken, and fish, suggesting that Nrf2 is a highly conservative cellular defense mechanism (Kobayashi and Yamamoto, 2006).

The mechanisms for activating of Nrf2 have been intensively investigated since its isolation in 1994 (Moi et al., 1994). A number of stressors including both endogenous and exogenous agents have been reported to activate Nrf2; these stressors included ROS, RNS, lipid aldehydes, 15-dexoy-D12,14-prostaglandin J2, electrophilic xenobiotics and their metabolites (Dinkova-Kostova et al., 2005; Osburn and Kensler, 2007). Recently, Nrf2 was reported to be activated in defending metals, such as chromium (Cr) (VI) (He et al., 2007), and cadmium (Ali et al., 2008; Liu et al., 2009), induced reactive stress by transactivating ARE-driven genes, and reduction of ROS production. Activation of Nrf2 by Cr (VI) was accompanied by the nuclear translocation and deubiquitination of Keap1 indicating the recycling of Keap1 in Nrf2 signaling (He et al., 2007). Two independent mechanisms have been previously demonstrated (reviewed by Osburn and Kensler (2007)) to be responsible for the dissociation of Nrf2 from Keap1: 1) Keap1 contains reactive cysteines (C273 and C288) that form protein–protein crosslinks through intermolecular disulfide bonds upon exposure to electrophiles or oxidant stress; the resulting conformation change leads to the disruption of Keap1–Nrf2 interaction and liberation of Nrf2 (Wakabayashi et al., 2004). 2) Secondary sensor proteins and activation of protein kinases (such as PKC) signaling pathway are involved, resulting in phosphorylation of Nrf2, which enhanced the stability and/or release of Nrf2 from Keap1 (Huang et al., 2002). Most recently, a hypothetic model of Nrf2-mediated redox signaling was brought up (Li and Kong, 2008). In this model, two pools of Nrf2 proteins exist: free floating Nrf2 (fNrf2) and Keap1-binding Nrf2 (kNrf2). Under homeostatic conditions, kNrf2 binds to a Keap1 which destines the Nrf2 proteins to proteasomal degradation, and there is only a small pool of fNrf2, contributing to basal activation. Upon oxidative stress, the conformation change of the Keap1 leads to the inhibition of proteasomal degradation, and Keap1 is saturated by undegraded kNrf2. At the same time, the pool of fNrf2 expands. fNrf2 can sense the change of redox milieu and transmit redox signals to cell nucleus via gradient nuclear translocation. In this model, Keap1 plays a gatekeeper role and dictates the pool size of fNrf2, thus regulating the overall redox sensitivity (Li and Kong, 2008).

The activation of Nrf2 results in transcriptional expression of a broad spectrum of protective enzymes including xenobiotic detoxification, antioxidative response, and proteome maintenance, all favoring cell survival (Kensler et al., 2007). The major antioxidant enzymes have been identified to be including glutathione reductase, peroxiredoxin, thioredoxin and thioredoxin reductase, catalase, copper/zinc superoxide dismutase and glutathione peroxidase (Osburn and Kensler, 2007). Low levels of Nrf2 or loss of Nrf2 activity appear to increase ROS production and DNA damage, and predispose cells to tumorigenesis. For example, disruption of Nrf2 has been shown to increase ROS generation and DNA damage which promote prostate tumorigenesis (Frohlich et al., 2008). Interestingly, emerging data provided evidence that elevated activity may also play a role in the evolution of cancer. Nrf2 and its downstream genes are overexpressed in many cancer cell lines and human cancer tissues, which render cancer cells an advantage for survival and growth (Hayes and McMahon, 2006; Lau et al., 2008). In two separate studies, mutations in Keap1 gene were found to provide a growth advantage for lung cancer cells (Ohta et al., 2008) and breast cancer cells (Nioi and Nguyen, 2007). Biallelic inactivation of Keap1 gene was reported to be a frequent genetic event in non-small cell lung cancer. Loss of function of Keap1 led to constitutive activation of Nrf2-mediated gene expression, favoring the cancer cell survival against chemotherapeutic agents (Singh et al., 2006), suggesting a potential mechanism for chemoresistance in certain cancers.

AP-1

AP-1 protein was first identified as a transcription factor that contributes both to basal gene expression (Lee et al., 1987), as well as phorbol ester (TPA)-inducible gene expression (Angel et al., 1987). Ever since, the gene has been intensively studied. AP-1 is a collection of dimeric bZip proteins that belong to the Jun (c-Jun, JunB, JunD), Fos (FosB, Fra-1, Fra-2), Maf (musculoaponeurotic fibrosarcoma), and ATF (activating transcription factor) subfamilies, all of which can bind TPA or cAMP response elements (Chinenov and Kerppola, 2001). c-Jun, a potent transcriptional regulator, often forms stable heterodimers with Jun proteins, which aid the binding of Jun to DNA (Kouzarides and Ziff, 1988), and is positively autoregulated by its product, Jun/AP-1 (Angel et al., 1988). AP-1 activity is induced in response to H_2O_2 as well as several cytokines and other physical and chemical stresses. In addition, in vitro transcriptional activity of AP-1 is regulated by the redox state of a specific cysteine 64 located at the interface between the two c-Jun subunits, highlighting the importance of redox status on gene transcription (Klatt et al., 1999). The induction of AP-1 by ROS, cytokines, and other stressors is mediated mainly by JNK and p38 MAP kinase cascades (Chang and Karin, 2001). Once activated, JNK proteins translocate to the nucleus and phosphorylate c-Jun and ATF2, enhancing transcriptional activities (Gupta et al., 1995; Karin, 1995). ROS such as H_2O_2 can activate MAP kinases and thereby AP-1 in several manners. One involves the apoptosis signal regulating kinase (ASK1) (Tobiume et al., 2001). Oxidation of thioredoxin, which is an endogenous inhibitor of ASK1, by H_2O_2, resulted in ASK1 activation (Liu et al., 2000; Tobiume et al., 2001). The second mechanism involves oxidant-mediated inhibition of MAP kinase phosphatases, which leads to increased MAP kinase activation. In addition, ROS may activate MAP kinase via PKC pathway (Wu, 2006). Whichever mechanism dominates, activation of MAP kinases directly leads to increased AP-1 activity.

One common effect of AP-1 activation is an increased cell proliferation. In particular, it has been demonstrated that c-fos and c-jun are positive regulators of cell proliferation (Shaulian and Karin, 2001). One of the genes regulated by AP-1 is cyclin D1. AP-1 binding sites have been identified in the cyclin D1 promoter and AP-1

activates this promoter, resulting in activation of cyclin-dependent kinase, which promotes entry into the cell division cycle (Brown et al., 1998). c-Jun also stimulates the progression into the cell cycle both by induction of cyclin D1 and suppression of p21waf, a protein that inhibits cell cycle progression (Bakiri et al., 2000). JunB, considered a negative regulator of c-jun-induced cell proliferation, represses c-jun-induced cyclin D1 activation by the transcription of p16INK4a (Passegue and Wagner, 2000). Although JunD exhibits high sequence homology to c-Jun, its biological consequences of expression and activity are distinct from that of c-Jun (Castellazzi et al., 1991). While most functions of JunD reported so far are related to decrease in cellular oxidative stress. It is recently reported that JunD inhibits intestinal epithelial cell proliferation through the activation of p21 promoter (Li et al., 2002), and reduces tumor angiogenesis by protecting cells from oxidative stress (Gerald et al., 2004). Therefore, the effect of AP-1 activation is dependent on the relative abundance of AP-1 subunits, the composition of AP-1 dimers, cell types, stimuli, as well as cellular environment (Hess et al., 2004).

NF-κB

NF-κB is a nuclear transcription factor that was first identified by Sen and Baltimore (1986). It is ubiquitously expressed and participates in a wide range of biological processes involved in cell survival, differentiation, inflammation, and growth (Sethi et al., 2008). This dimeric transcription factor is composed of different members of the Rel family, consisting of p50 (NF-kB1), p52 (NF-kB2), c-Rel, v-Rel, Rel A (p65), and Rel B (Baeuerle and Baltimore, 1996). Normally, NF-κB dimmers are sequestered in the cytoplasm in an inactive state through binding to inhibitory IkB proteins (IkBa, IkBb and IkBe). Activation of NF-κB occurs in response to a wide spectrum of extracellular stimuli, including cytokines, oxidative stress, oncogenes, and DNA damage, which promote the dissociation of IkBs by sequential phosphorylation and proteolytic degradation, a process that depends on the IkB kinase (IKK) complex, of these inhibitors, thereby allowing the entry of NF-κB into nucleus and binds κB-regulatory elements (Hacker and Karin, 2006; Wu and Miyamoto, 2007). NF-κB has been known to be redox regulated and is a direct target for oxidation that can affect its ability to bind to DNA (Pantano et al., 2006). NF-κB activation has been linked to the carcinogenesis process because of its critical roles in inflammation, differentiation and cell growth (Okamoto et al., 2007). Experimental evidence has demonstrated that NF-κB activation 1) is required for growth factor mediated cell proliferation, 2) promotes tumor cell survival, 3) mediates tumor cell invasion, 4) is needed for angiogenesis, and 5) is involved in tumor cell metastasis (Sethi et al., 2008). It is therefore reasonable that NF-κB serves as a potential molecular target for chemoprevention and therapy (Sarkar and Li, 2008; Shen and Tergaonkar, 2009).

While it is widely accepted that NF-κB is a tumor promoting transcription factor, recent emerging data have suggested an tumor suppressor like effect of NF-κB in carcinogenesis (Chen and Castranova, 2007). As a tumor suppressor, NF-κB functions in DNA repair to preserve genome integrity and senescent state in mouse and human fibroblast senescence models (Wang et al., 2009). Further investigations using different cellular and animal models and human tumor tissues as well are needed to establish the tumor suppressor effect of NF-κB. And caution should also be taken in regard with blocking NF-κB pathway in treating cancers.

HIF-1

HIF-1 is a heterodimeric transcription factor that plays an important role in signaling the cellular oxygen levels. HIF-1 consists of two subunits, HIF-1α (120 kDa) and HIF-1α (91–94 kDa), which belong to the basic-helic-loop-helix (bHLH) proteins of the PAS family. HIF-1α (also known as ARNT) is expressed constitutively in all cells and does not respond to changes in oxygen tension, is essential for hypoxia-induced transcriptional changes mediated by the HIF-1 heterodimer (Wang et al., 1995). The level of HIF-1α is tightly control by the cellular oxygen level. HIF-1α is made continuously and accumulates in hypoxic cells, but is rapidly degraded and is almost absent in normoxic cells. The oxygen-dependent degradation of HIF-1α is sensed by prolyl hydroxylases (PHDs). Following hydroxylation, HIF-1α is then recognized by the von Hippel–Lindau (pVHL, the E3 ubiquitin protein ligase) and subjected to proteasomal degradation (Ivan et al., 2001). Recently, HIF-1α has also been shown to be up-regulated under normal oxygen conditions in response to in response to growth factor (Richard et al., 2000).

HIF-1 has been implicated in the in ROS-induced carcinogenesis in a variety of human tumors, including bladder, breast, colon, glial, hepatocellular, ovarian, pancreatic, prostate, and renal tumors (Talks et al., 2000; Galanis et al., 2008). Elevated HIF-1 expression has been shown to be correlated with poor outcome in patient with head and neck cancer, nasopharyngeal carcinoma, colorectal, pancreatic, breast, cervical, osteosarcoma, endometrial, ovarian, bladder, glioblastoma, and gastric carcinomas (for review, see Rankin and Giaccia, 2008). Taken together, these findings highlight that HIF1 activation is a common event in cancer and suggest that HIF-1 may play a role in tumorigenesis. Emerging evidence indicates that ROS generated by mitochondria are required for stabilization and hypoxic activation of HIF-1α (Simon, 2006; Klimova and Chandel, 2008). Thus, ROS is considered the direct activator of HIF-1 in hypoxic tumors.

Activation of transcription factors is clearly stimulated by signal transduction pathways that are activated by ROS, such as H_2O_2, and other cellular oxidants. Through the ability to stimulate cell proliferation and either positive or negative regulation of apoptosis, transcription factors can mediate many of the documented effects of both physiological and pathological exposure to H_2O_2, or chemicals that induce ROS and/or other conditions that favor increased cellular oxidants. Through regulation of gene transcription factors, and disruption of signal transduction pathways, ROS are intimately involved in the maintenance of concerted networks of gene expression that may interrelate with neoplastic development.

Polymorphisms in oxidative stress related genes

Human genetic variation is very common and single nucleotide polymorphisms (SNPs), which are defined as a variation in a single nucleotide pair which occurs at a population frequency of at least 1%, contribute to the majority of the variants. It is estimated that there are approximately 10 million SNPs in humans (Kruglyak and Nickerson, 2001). While many of these variants are silent (or "neutral") and without functional consequences on gene expression and protein function (Fay et al., 2002), a small portion of these variants are in coding and regulatory region of genes, contribute to the phenotypic change, and are functionally important (Brookes, 1999). The relationship between genetic susceptibility and human cancers has been intensively studied during the last 2 decades, especially after the completion of human genome sequence (Dong et al., 2008). Recent advance in genotyping technologies, for example, the genome wide association studies (GWAS), has led to a rapid increase in available data on common genetic variants and phenotypes and numerous discoveries of new loci associated with risks of human cancers as well as other complex human diseases (Lin et al., 2006; Khoury et al., 2009).

Cancer is a complex disease attributed to the integrated outcome of carcinogen activation or detoxification, DNA repair capacity, and other known or unknown factors. Individual responses to a chemical carcinogenic agent depend on polymorphisms of enzymes responsible for metabolic activation/detoxification of the carcinogen, DNA repair, and apoptosis, as well as promotion and progression in malignantly transformed cells (Belitsky and Yakubovskaya, 2008). In

this review, we focus on a panel of oxidative stress related genes that control the levels of cellular ROS and oxidative DNA damage, including genes involved in carcinogen metabolism, antioxidants, and DNA repair pathways. Polymorphisms in these genes may alter the production of ROS and therefore modified risk of cancer.

Polymorphisms in carcinogen metabolizing genes

As has been discussed in the previous section, xenobiotics including various chemical carcinogens can generate ROS either directly through metabolism to primary radical intermediates or indirectly by activating endogenous sources of ROS (Rice-Evans and Burdon, 1993; Klaunig et al., 1997). For example, ethanol is mainly metabolized by CYP2E1 and is known to enhance the activity of this enzyme, leading to a burst of ROS production that damage with consequent toxicity and carcinogenicity in small rodents (Parke, 1994). Aflatoxin B1 (AFB1), a known liver carcinogen, induces ROS production accompanied by its activation via CYP3A4 and /or detoxification via GSTs and EPHX (Shen et al., 1996; Alpsoy et al., 2009). And it has been demonstrated that genetic polymorphisms in these enzymes have been associated with modified liver cancer risk because of AFB1 exposure (McGlynn et al., 2003).

The metabolism of carcinogens has been traditionally categorized into two major phases. Following exposure to a carcinogen, the dominating reactions are mediated by microsomal oxidases encoded by cytochrome P450 (*CYP*) gene superfamily, but other enzymes are included too (Belitsky and Yakubovskaya, 2008). The other enzymes such as epoxide hydrolase 1 (*EPHX1*) use a different chemistry than cytochrome P450. They all use oxygen in some form, mostly from water or molecular oxygen, and generate free chemical groups which can be detoxified through conjugation with phase II enzymes, such as glutathione S-transferase (GST) and N-acetyltransferase-2 (NAT2), into water-soluble chemical groups such as a sugar, amino acid or sulfate molecule. Most of the carcinogen metabolizing genes have been shown to be polymorphic which may alter the activity of an enzyme, and thus, modify individual cancer risk (Hayes et al., 2005; McIlwain et al., 2006; Agundez, 2008; Belitsky and Yakubovskaya, 2008).

CYP constitutes a superfamily of monooxygenases which are responsible for the phase I metabolism of many endogenous as well as exogenous compounds such as drugs and xenobiotic compounds (Lewis et al., 2004). The main CYPs in humans that metabolize carcinogens are CYP1A1, CYP2A6, CYP3A4, CYP1B1, and CYP2E1 (Belitsky and Yakubovskaya, 2008). These enzymes have specificities for various classes of carcinogens and genetic polymorphism has been identified for most of them (Guengerich et al., 1991; Guengerich, 1994; Ingelman-Sundberg, 2004). The individual differences in expression may be due to the genetic polymorphisms or the extent of their induction. Numerous studies have investigated the associations of CYP polymorphisms and many human cancers (Agundez, 2004; Dong et al., 2008).

Glutathione *S*-transferases (GSTs), a major superfamily of dimeric phase II metabolic enzymes, metabolize a variety of environmental carcinogens with a large overlap in substrate specificity. GST enzymes catalyze the conjugation of toxic and carcinogenic electrophilic molecules with glutathione and thereby protect cellular macromolecules against toxic foreign chemicals and oxidative stress (Hayes and Strange, 2000). Human GSTs are divided into three major families, the cytosolic, mitochondrial, and microsomal (now referred to as membrane-associated proteins in eicosanoid and glutathione, MAPEG) (Hayes et al., 2005). Cytosolic GSTs represent the largest family of such transferases and are further divided into eight subclasses: Alpha, Pi, Mu, Omega, Sigma, Theta, Zeta and Kappa, they are all dimeric with subunits of 199–244 amino acids in length (Mannervik et al., 1992; Strange et al., 2001). The chromosomal localization of these genes is reviewed elsewhere (McIlwain et al., 2006). Most of the cytosolic GSTs have been reported to be polymorphic which may contribute to the interindividual difference in response to xenobiotics, and hence distinct cancer risk (Hayes et al., 2005).

Polymorphisms in antioxidant genes

Antioxidant enzymes consist one of the major cellular protective mechanisms against oxidative stress in human body. Malignant transformation may be accompanied by either reduced antioxidant activity or increased levels of ROS (Oberley and Oberley, 1988). Many of the antioxidant genes are known to be polymorphic which lead to altered enzyme activity and regulatory efficiency on ROS level, and finally modify the risk of ROS-induced carcinogenesis. Copper-zinc superoxide dismutase 1 (*CuZnSOD* or *SOD1*) occurs as a dimer of identical 16 KDa subunits. Mutations in *SOD1* have been known to cause 5% of all amyotrophic lateral sclerosis cases (Rosen, 1993). More than 100 mutations have been identified and arise in all five exons of *SOD1* (Andersen et al., 2003). A recent study reported that SNPs in *SOD1* were associated with adult glioma risk (Rajaraman et al., 2009). Several other reports investigated the relationship between common polymorphisms of *SOD1* and risk of breast and prostate cancer, but no significant association was found (Cebrian et al., 2006; Udler et al., 2007).

Manganese superoxide dismutase, MnSOD or SOD2, is a mitochondrial enzyme that catalyzes the formation of H_2O_2 from superoxide radicals generated in human body. The variant allele of *MnSOD* has been associated with elevated risk of breast (Bewick et al., 2008), brain (Rajaraman et al., 2008), prostate (Mikhak et al., 2008), lung (Liu et al., 2004), ovarian (Olson et al., 2004) cancers, and non-Hodgkin lymphoma (Wang et al., 2006).

Superoxide dismutase 3 (SOD3) is a major extracellular antioxidant enzyme expressed in the extracellular matrix of many tissues and especially blood vessels (Marklund, 1984). *SOD3* gene contains three exons with coding region in exon 3. A common genetic variant *SOD(R213G)* with a substitution in the heparin-binding domain was recently reported to be associated with brain tumor (Rajaraman et al., 2008) but not prostate cancer risk (Kang et al., 2007).

Glutathione peroxidase (GPX) is a family of selenium-dependent enzyme with at least four isoenzymes identified so far. GPX is encoded by different genes in various cellular locations. *GPX1*, located on chromosome 3p21.3, is the first identified and the most abundant selenoprotein in mammals (Kiss et al., 1997), and is ubiquitously expressed in humans, protecting cells against oxidative damage by reducing hydrogen peroxide and a wide range of organic peroxides (Arthur, 2000). A SNP with proline–leucine at codon 198 of human *GPX*1 has been identified and associated with many human cancer risks, such as breast (Ravn-Haren et al., 2006), prostate (Arsova-Sarafinovska et al., 2008), lung (Raaschou-Nielsen et al., 2007), and bladder cancer (Ichimura et al., 2004), but are not consistent in all populations (Ahn et al., 2005; Cebrian et al., 2006; Udler et al., 2007).

Glutathione synthase (GS), glutamyl-cysteinyl synthase (GCS) and glutathione reductase (GR) are important enzymes involved in the production and recycling of glutathione; genetic variations in these genes may affect the glutathione levels in human body and thus contribute to oxidative stress (Forsberg et al., 2001a), so it is plausible to hypothesize that changes in these genes may influence cancer risk.

Catalase (CAT) is an endogenous antioxidant enzyme that neutralizes ROS by converting H_2O_2 into H_2O and O_2, and can be up-regulated by oxidative stress (Hunt et al., 1998). A common catalase-262C/T polymorphism has been identified in the promoter region of the human *CAT*, and the variant of this gene affects transcriptional activity and catalase levels in red blood cells (Forsberg et al., 2001b). Because of the importance of this enzyme in regulating ROS levels in human body and the clear role of ROS in tumorigenesis, genetic polymorphisms of this gene are believed to play a role in ROS-induced

carcinogenesis. Several epidemiologic studies have investigated the relationship between SNPs of this gene and human cancer risks, however, results remain inconclusive. Polymorphisms of *CAT* was not associated with lung cancer risk in a Chinese population (Ho et al., 2006), non-Hodgkin's lymphoma in the UK (Lightfoot et al., 2006), and prostate cancer in the US (Choi et al., 2007). A recent report suggested that a *CAT* variant allele is associated with a decreased risk of acoustic neuroma (Rajaraman et al., 2008), while this result needs to be confirmed by further investigations.

Polymorphisms in DNA repair genes

As discussed in the previous section, 8OHdG is the most abundant and by far the most intensively studied lesion caused by oxidative stress (Cooke et al., 2003). Several pathways are involved in the removal, or repair, of this lesion from damaged DNA. It is preferentially repaired by base excision repair (BER) enzymes, including 8-oxoguanine DNA glycosylase (OGG1), human endonuclease nei-like glycosylase 1 (NEIL1), and MutY homologue (MUTYH) (Evans et al., 2004). In addition, nucleotide excision repair (NER) may also participate in the process of removing the 8OHdG lesion (Patel et al., 2007). Recently, the human apurinic/apyrimidinic endonuclease (APE1) and xeroderma pigmentosum complementation group C (XPC), a NER pathway enzyme, and NEIL1 proteins have been shown to enhance the activity of OGG1 (Mokkapati et al., 2004; D'Errico et al., 2006; Sidorenko et al., 2007).

hOGG1 (*human 8-oxoguanine DNA N-glycosylase 1 gene*), located at 3p26.2 of the human chromosome, encodes OGG1. Several SNPs within *hOGG1* have been reported (Kohno et al., 1998). Thus, polymorphisms in this gene that alter glycosylase function and an individual's ability to repair oxidatively damaged DNA, possibly resulting in genetic instability that may contribute to carcinogenesis (Boiteux and Radicella, 2000; Ide and Kotera, 2004; Shao et al., 2006). A most frequently found polymorphism is a serine (Ser) to cysteine (Cys) substitution at position 326 of the OGG1 protein. Functional study of this polymorphic enzyme using human cell extracts revealed that cells homozygous for the Cys variant have an almost 2-folder lower 8OHdG DNA glycosylase activity compared with cells with Ser variant (Bravard et al., 2009). Consistent with the enzyme activity, the Cys/Cys cells displayed an increased genetic instability and reduced in vivo 8OHdG repair rates (Bravard et al., 2009). While epidemiologic studies investigating the associations between the SNPs of *OGG1* have led to conflicting results. The variant allele of this *OGG1* was shown to be associated with significantly increased risk a number of human cancers, including lung (Hung et al., 2005; Li et al., 2008), esophageal (Xing et al., 2001), prostate (Xu et al., 2002), and gastric (Farinati et al., 2008) cancer. However, no association was found for polymorphisms of this gene and risk of squamous cell carcinoma of the head and neck (SCCHN) (Zhang et al., 2004), squamous oral carcinomas (Gorgens et al., 2007), and pancreatic cancer (McWilliams et al., 2008). The difference in cancer risks may depend on the exposure of diverse environmental factors (Weiss et al., 2005).

A total of 18 polymorphisms in *APE1* have been reported, among which, *Gln51His* and *Asp148Glu* are the two most common SNPs. Associations between polymorphisms in *APE1* and increased risk of lung, colon, breast, SCCHN, prostate, pancreatic and colorectal cancer have been reported, but with mixed results (Goode et al., 2002; Zhang et al., 2004; Hung et al., 2005; Jiao et al., 2006; Kasahara et al., 2008). SNPs of *MUTYH* gene were also reported and have been associated with risks of lung, colorectum, and head and neck cancer in different populations (Ali et al., 2008; Kasahara et al., 2008; Tao et al., 2008; Miyaishi et al., 2009; Sliwinski et al., 2009). In addition, at least two polymorphic sites for *NEIL1* gene were identified , which may be involved in the pathogenesis of gastric cancer (Shinmura et al., 2004).

Concluding remarks

ROS has been well recognized for playing a dual role as both beneficial and deleterious species (Valko et al., 2007). As discussed in this review, overproduction of ROS via various sources can cause damage to both nuclear and mitochondrial DNA, which have been associated with a number of human cancers. ROS act as secondary messengers in multiple intracellular pathways that confer carcinogenic effects, while ROS can also induce apoptosis and promote cellular senescence, therefore functioning as anticarcinogenic species (Mates et al., 2008). Low levels of ROS involve in cellular defense against infectious agents and ROS-mediated activation of Nrf2 transcriptional expression of antioxidant enzymes protect cells against ROS-induced oxidative stress, a mechanism to re-establish cellular redox homeostasis. Furthermore, individual responses to chemical carcinogens also depend on polymorphisms of enzymes responsible for metabolic activation/detoxification of the carcinogen, producing/reducing ROS, and DNA repair. Future studies should address functional changes of these polymorphic genes and how they are related to cancer risk. Individualized prevention/therapeutic strategy of a cancer should also be developed considering not the specific exposure but also the polymorphism profile of the patient.

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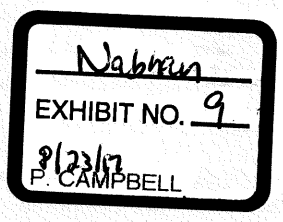
Baseline determination in social, health, and genetic areas in communities affected by glyphosate aerial spraying on the northeastern Ecuadorian border



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Abstract

The northeastern Ecuadorian border has undergone aerial spraying with an herbicide mix that contains surfactants and adjuvants, executed by the Colombian Government. The purpose of this study was to diagnose social, health, and genetic aspects of the people affected by glyphosate. For this objective to be achieved, 144 people were interviewed, and 521 medical diagnoses and 182 peripheral blood samples were obtained. Genotyping of GSTP1 Ile105Val, GPX-1 Pro198Leu, and XRCC1 Arg399Gln polymorphisms were analyzed, using PCR-RFLP technique. The assessment of chromosomal aberrations was performed, obtaining 182 karyotypes. Malnutrition in children was 3%. Of the total population, 7.7% had children with malformations, and the percentage of abortions was 12.7%. Concerning genotyping, individuals with GSTP1 Val/Val obtained an odds ratio of 4.88 ($p<0.001$), and Ile/Val individuals, together with Val/Val individuals, had an odds ratio of 2.6 ($p<0.05$). In addition, GPX-1 Leu/Leu individuals presented an odds ratio (OR) of 8.5 ($p<0.05$). Regarding karyotyping, the 182 individuals had normal karyotypes. In conclusion, the study population did not present significant chromosomal and DNA alterations. The most important social impact was fear. We recommend future prospective studies to assess the communities.

Keywords: Arg399Gln; GPX-1; GSTP1; Ile105Val; Pro198Leu; XRCC1.

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Introduction

Glyphosate (N-phosphonometyl glycine) is a nonselective, broad spectrum, postmergence organophosphorus herbicide effective in controlling annual, biennial, and perennial herb species, pastures, and broadleaf weeds (1). Glyphosate is one of the world's most widely used herbicides with 20,000 tons year used in Europe and 51,000 tons year in the USA (2, 3). The glyphosate activity is primarily due to the inhibition of 5-enolpyruvylshikimate-3-phosphate synthase, resulting in a retardation of the shikimate pathway that is involved in the synthesis of aromatic amino acids in plants and microorganisms (4, 5). The herbicide is commonly formulated with surfactants that decrease the surface tension of the solution and increase penetration into the tissues (6). Roundup® (Monsanto, St. Louis, MO, USA) is an aqueous solution of the isopropylamine salt of glyphosate with a polyethoxylated tallowamine surfactant (POEA) and the adjuvant Cosmoflux 411F (Monsanto, St. Louis, MO, USA) (7, 8).

Several research studies worldwide demonstrated that the use of glyphosate formulations develops high and low levels of toxicity in different organisms. Glyphosate can interfere with certain enzymatic functions in animals, but the symptoms of poisoning depend on the dose and exposure time. In humans, Roundup® is toxic in placental and embryonic cells and sexual steroid biosynthesis (9). This pesticide mixed with adjuvants was cytotoxic through alteration of succinate dehydrogenase and was toxic to human peripheral blood mononuclear cells (10). The results of four case-control studies suggested an association between glyphosate and the risk of non-Hodgkin's lymphoma (11–14). In amphibians, *Rana pipiens* Schreber tadpoles showed decreased snout-vent length at metamorphosis and increased time for metamorphosis to occur, tail damage, and gonadal abnormalities. Pesticide toxicity is often proposed as a contributing factor to the worldwide decline of amphibian populations (15, 16). In sea urchin eggs development, glyphosate prevents the hatching enzyme transcription synergistically and activates the DNA damage checkpoint CDK1/cycline B of the first cell cycle of development for commitment to cell death by apoptosis (9, 17, 18). In rabbits, glyphosate treatment resulted in a decline in body weight, sperm concentration, and semen osmolality (19). In isolated rat liver mitochondria, Roundup® depresses the mitochondrial complexes II, III and is able to induce a dose-dependent formation of DNA adducts in the kidney and the liver (20).

Among the research studies showing a low toxicity of glyphosate, an outstanding study conducted by Bolognesi

et al. (8) executed a cytogenetic analysis of agricultural workers from five Colombian regions; a study conducted by Sanin et al. (21) proved a non-association between glyphosate and the prolongation of pregnancy in women; and another study proved the genotoxicity of glyphosate at a low-risk level in the environment, compared with the harmful products used during cocaine production in Colombia (22).

During the period 2000–2007, the Ecuadorian northern border suffered from repeated aerial spraying with an herbicide mix composed of high doses of glyphosate, the surfactant polyethoxylated tallowamine (POEA), and the adjuvant Cosmoflux 411F. After analyses were conducted in 2004 and 2006, in which an increase in DNA damage and genetic risk was detected, biomonitoring established a baseline for social, health, genetic, and environmental areas in the Ecuadorian communities bordering Colombia, to determine what occurred at the biological level once aerial spraying with a broad spectrum herbicide was suspended two years after the last aerial spraying with a herbicide mix with glyphosate.

Experimental

Area of study

This research was carried out in the province of Sucumbios located in the Ecuadorian Amazon basin bordering Colombia. Baseline determination in social, health, and genetic areas was performed in the following communities: Chone-2, Yanamarum, Playera Oriental, Fuerzas Unidas, Puerto Escondido, Corazon Orense, Santa Marianita, San Francisco, and Las Salinas 5 de Agosto in the province of Sucumbíos (Figure 1).

Biological samples and field data collection

Subjects (n=144) were interviewed, and 521 medical diagnoses of men (47.8%) and women (52.2%) were obtained. The origin of the population from the study area corresponds to 53.4% of those born in the Amazonian region, 46.6% come from other Ecuadorian regions, and 16.1% are Colombian immigrants; the presence of immigrants from said country has increased over the last 10 years, when aerial spraying of illegal crops in Colombia started. Psychological assessment in children from different schools belonging to the study communities consisted of

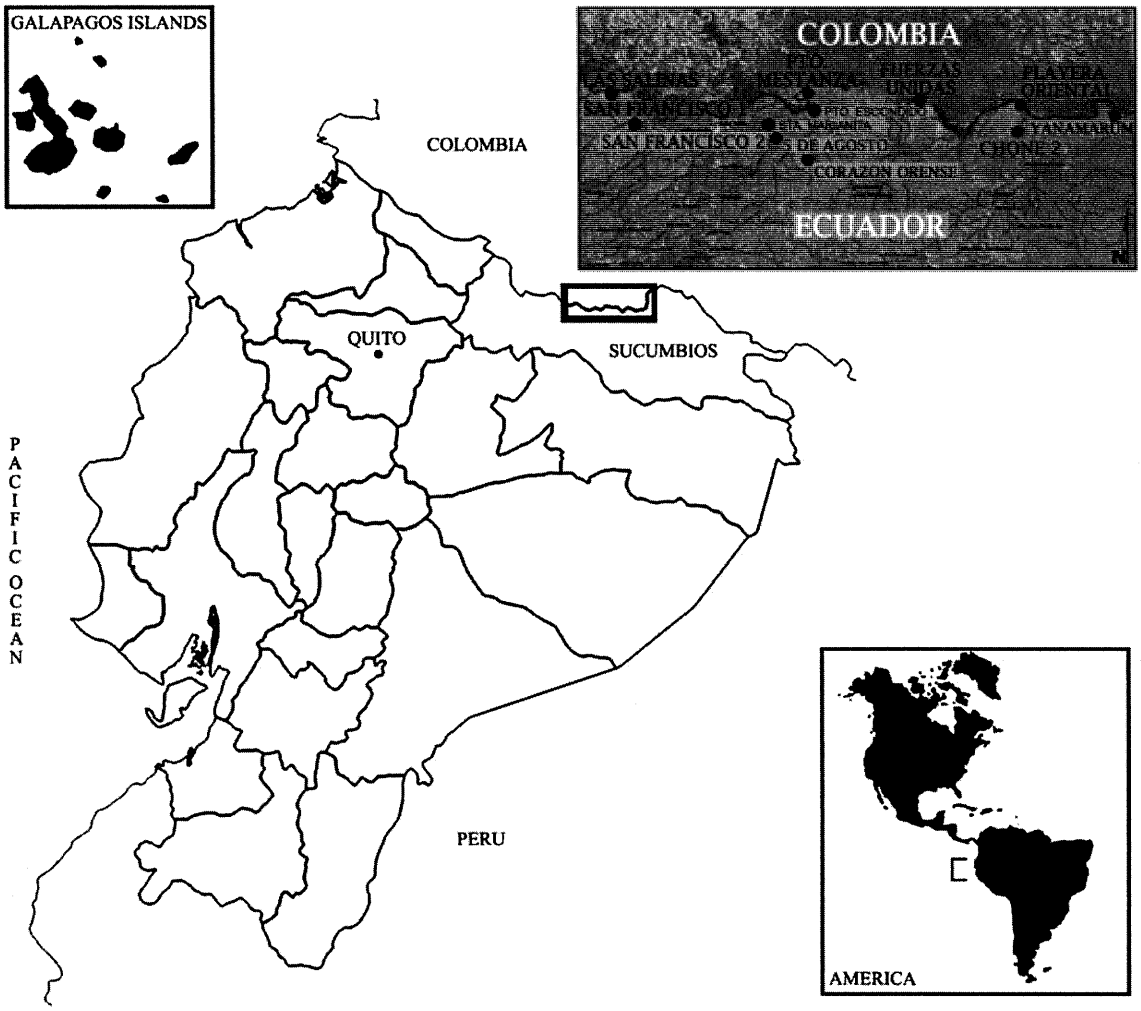

Figure 1 Studied communities in Ecuador.

the analysis of drawings made by the children, for which the following formal features were considered – transparency, contrast, proportionality, symmetry, support base, concealment, confusion, motion, rigidity, lines, presentation, chromatic expression, outline, and texture.

For the analysis of chromosomal aberrations and the study of GSTP1 (glutathione S-transferase pi 1), GPX-1 (glutathione peroxidase 1), and XRCC1 (X-ray repair cross-complementing group 1) genes, 92 peripheral blood samples in vacutainer tubes with heparin and EDTA were obtained from individuals exposed to the aerial spraying of an herbicide mix with glyphosate. The genetic study also required the analysis of 90 DNA samples from healthy individuals who belonged to several provinces of the country who did not have a background of smoking or exposure to genotoxic substances, such as hydrocarbons, X-rays, or pesticides. Each one of these study individuals signed their corresponding informed consent.

Genotyping

DNA from individuals exposed to an herbicide mix with glyphosate and that of healthy individuals, stored in the nucleic acid data bank of the Biomedical Research Institute at the Universidad de las Américas, was extracted from peripheral blood samples using PureLink™ Genomic DNA Kit (Invitrogen). The mean concentration of the DNA samples was 100 ng mL^{-1} measured in a Qubit® Fluorometer (Invitrogen). Because the affected communities had a background involving spraying with an herbicide mix with glyphosate, we proceeded to study single nucleotide polymorphisms (SNPs) in the GSTP1 (Ile105Val), GPX-1 (Pro198Leu), and XRCC1 (Arg399Gln) genes. Genotyping was performed through the polymerase chain reaction–restriction fragment length polymorphism technique (PCR-RFLP). For GSTP1, GPX-1, and XRCC1 genes amplification, a PCR final volume of 50 µL was prepared, containing 4 µL of DNA template, 34 µL H_2O Milli-Q, 0.4 µM of forward and reverse primers, 1.5 mM $MgCl_2$, 5 µL 10× buffer (200 mM Tris-HCl pH 8.4, 500 mM KCl), 0.2 µM each deoxynucleotide triphosphate (dNTPs), and 2.5 U Taq DNA polymerase (Invitrogen). For the 177 bp fragment amplification and the analysis of the Ile105Val polymorphism found in chromosome 11, codon 105, exon 5, we used the following primers: FW 5′-ACCCCAGGGCTCTATGGGAA-3′ and RV 5′-TGAGGGCACAAGAAGCCCCT-3′. Once the PCR reaction was obtained, the samples were placed in the MultiGene Thermal Cycler TC9600-G for amplification (Labnet, Edison, NJ, USA). The initial denaturation lasted 5 min at 95°C, followed by 35 cycles of 45 s at 94°C, 30 s at 62°C, 30 s at 72°C, and 1 min at 72°C. Digestion of the amplified fragment was performed during 2 h at 37°C with 5 U of the Alw26I (Promega, Madison, WI, USA) restriction enzyme. Electrophoresis analysis revealed homozygous individuals (Ile/Ile), (Val/Val) or heterozygous (Ile/Val) (23). For a 191 bp fragment amplification and the analysis of the Pro198Leu polymorphism found in chromosome 3, we used FW 5′-AAGGTGTTCCTCCCTCGTAGGT-3′ and RV 5′-CTACGCAGGTACAGCCGCCGCT-3′ primers (24, 25). In the thermal cycler, the initial denaturation step lasted 10 min at 95°C, then 35 cycles of 30 s at 56°C, 30 s at 56°C, 45 s at 72°C and 3 min at 72°C were needed. Digestion of PCR product was carried out during 2 h at 37°C with the ApaI (Promega) restriction enzyme. The PCR-RFLP test revealed homozygous individuals (Pro/Pro), (Leu/Leu) or heterozygous (Pro/Leu) (25, 26), whereas for a 242 bp fragment amplification and the analysis of the Arg399Gln polymorphism found in chromosome 19, codon 399, exon 10, the following primers FW 5′-CCCCAAGTACAGCCAGGTC-3′ and RV 5′-TGCCCCGCTCCTCTCAGTAG-3′ were used (27). The initial denaturation step lasted 5 min at 95°C, then 35 cycles of 45 s at 94°C, 1 min at 59°C, 30 s at 72°C and 3 min at 72°C. Digestion of amplicon was performed during 2 h at 37°C with the MspI (Promega) restriction enzyme. The analysis revealed homozygote individuals (Arg/Arg), (Gln/Gln), or heterozygote individuals (Arg/Gln) (27).

Karyotyping

For the cytogenetic analysis, we used techniques that we modified in our laboratory from previously standardized protocols (28, 29). The 92 individuals belonging to the 10 communities in the study area in Ecuador's northern border area were karyotyped to assess the existence of chromosomal alterations, according to the 'An International System for Human Cytogenetic Nomenclature' (30). Peripheral blood (5 mL) in vacutainer tubes with heparin was extracted, the samples were cultured at 37°C using RPMI 1640 medium (Gibco Laboratories, Grand Island, NY, USA), complemented with 10% phytohemagglutinin, 15% fetal bovine serum, 0.5 mL L-glutamine, 1.5 mL penicillin-streptomycin, and 1.5 mL HEPES buffer for the stimulation of cell division. After 48 h, 200 µL of colcemid was placed in the culture medium to collect metaphase cells. For harvesting the cells: we used hypotonic solutions (KCl) to increase cell volume, which spreads apart the chromosomes, and methanol-acetic acid to fix them for study. The fixed cells were dropped onto slides, stained with Giemsa 8% diluted with buffer solution (KH_2PO_4 0.025 M, pH 6.8) and ready to be observed with an Olympus BX51 microscope at 100×. The CytoVision® System (Applied Imaging, Santa Clara, CA, USA) allowed us to order the chromosomes in homologous pairs to obtain the karyotyping of the individuals.

Statistical analysis

The allelic and genotypic frequencies of each single nucleotide polymorphism were calculated from the information provided by the genotypes, and the Hardy-Weinberg equilibrium was determined by using software available on the Internet (http://www.genes.org.uk/software/hardy-weinberg.shtml). All the information obtained from the individuals studied was compiled in a database, and the statistical analysis was carried out using PASW Statistical 17 for Windows (SPSS, Chicago, IL, USA). The allelic and genotypic frequencies of the GSTP1, GPX-1, and XRCC1 genes were calculated. The chi-square (χ^2) analysis was performed to determine significant differences between the presence of Ile105Val, Pro198Leu, and Arg399Gln polymorphisms and the studied population. The relative risk of dysfunction in the DNA detoxification or repair process, in the presence of the polymorphisms in individuals exposed and non-exposed to the aerial spraying with glyphosate, was determined using the odds ratio test (OR). The data were analyzed using a 2×2 contingency table.

Results

Social and health analysis

A descriptive study was conducted to determine the population baseline in the social and health areas. The health and housing general conditions in the communities studied here are not very appropriate for the environment found in the Amazon Basin. Houses are built with zinc roofs, 73.9% of the houses are barely open to the air, and 43.3% have no awning that protects them from vectors. The population consumes water that comes mainly from such natural sources as rivers, marshes, or springs (38.8%), whereas 25.2% of water comes

from rain, 21.58% from open wells, and drinking water consumption represents only 14.42%. Of the families, 38.4% have facilities for the elimination of feces, whereas 61.4% eliminate feces in the open land.

As for the global nutritional status (weight-for-age), 2 years after the last aerial spraying with pesticide (2007), we observed that the global malnutrition status of children aged between 6 and 17 years old decreased from 10.3% to 3%, and the risk of slight malnutrition diminished from 36.3% to 23.2%. The group of children under 6 years old had the largest percentage of malnutrition (14.9% in boys and 13.6% in girls); the percentage of malnutrition decreased markedly in the group of children between 6 and 11 years old (2% boys and 1.8% girls); whereas the percentage of malnutrition in the group between 12 and 17 years old increased to 3.8% in boys and 6.7% in girls. Concerning chronic malnutrition (height-for-age), we found numbers very similar to those obtained in young people between the ages of 6 and 17 years old in 2006, when the percentage of chronic malnutrition decreased from 29% to 28%. The most malnourished group is the one between the ages of 12 and 17 years old (41%), in comparison with the group of children under 6 years old (30%), and the group of children between 6 and 11 years old (22%). Regarding acute malnutrition (weight-for-height), a slight change is seen now if we compare the data obtained in 2006, in which the percentage of acute malnutrition decreased from 1.87% to 1% and the risk went down from 7.17% to 5.8%. The body mass index (BMI) in adults demonstrates that, after 2 years without aerial spraying, no malnutrition occurred in adults over 18 years old, but rather a surge in the tendency to obesity in women (29.7%) and in men (7.8%). As for family health, we observed that during the aerial spraying the percentage of abortions rose from 8.4% to 12.7%, whereas in the same period the percentage of child mortality decreased from 12% to 9.1%. The main causes of child mortality were diseases (40%), unknown reasons (17%), labor (13%), violence (9%), malaria (6%), aerial spraying with glyphosate (5%), cancer (4%), traffic accidents and congenital malformations (2%), and finally, pesticides and snakebites (1%).

Concerning the health conditions caused by aerial spraying with glyphosate, we found that in 84.7% of families, an individual fell ill during the spraying, and the symptoms were respiratory, digestive, and ophthalmological problems, cephalea, and skin conditions, whereas a little after the spraying, the latter became the most important problem. Psychological tests determined that 84.86% of the population had psychological manifestations, with fear being the most frequent reaction (51.3%). After the spraying, fear diminished and concern about the future of the crops rose (18.6%), as well as depression (16.7%).

Genotyping

Table 1 shows the Hardy-Weinberg equilibrium and the genotypic and allelic frequency of the studied polymorphisms. Table 2 shows the statistical analysis through χ^2 and OR tests. The study population was found in Hardy-Weinberg equilibrium. Regarding the GSTP1 Ile105Val polymorphism, we observed that the frequency of the Val allele was higher in exposed individuals (0.48) than in control individuals (0.28) (Table 1). The presence of the Val/Val variant was associated with a 4.88-fold risk of acquiring detoxification problems (OR=4.88, 95% CI, 2.0–11.8, $p<0.001$), whereas the combination of the Ile/Val and Val/Val alleles was associated with a 2.6-fold risk of presenting a GSTP1 gene dysfunction (OR=2.6, 95% CI, 1.4–4.8, $p<0.05$) (Table 2). As for the GPX-1 Pro198Leu polymorphism, we observed that the Leu allele had a higher frequency in exposed individuals (0.41), unlike control individuals (0.32) (Table 1). The presence of the Leu/Leu variant was associated with an 8.5-fold risk of having problems in the function of the GPX-1 gene (OR=8.5, 95% CI, 1.8–39.9, $p<0.05$) (Table 2). Concerning the XRCC1 Arg399Gln polymorphism, we observed that the frequency of the Gln allele was higher in control individuals (0.98), unlike the population exposed to glyphosate (0.54) (Table 1). None of the variables of the Arg399Gln polymorphism presented a significant OR (Table 2).

Chromosomal analysis

After analyzing the metaphases and karyotyping the 92 individuals who belonged to the different communities of the province of Sucumbios located in Ecuador's northeastern border, we observed that all the analyzed women obtained a normal karyotype (46, XX). We also observed that 33% of the 92 individuals with normal karyotype had a low percentage of chromosomal fragility (<5%), whereas 67% of the individuals did not present this feature. All the studied population came within the normal parameters considered for studies of chromosomal fragility (30) (Table 3).

Discussion

During the years 2000–2007, the communities located in the Ecuadorian northern area bordering Colombia suffered from involuntary exposure to aerial spraying with a broad spectrum herbicide mix containing high doses of glyphosate (main herbicide), as well as surfactants and adjuvants to strengthen its power. The aerial spraying with this herbicide mix is part of a program provided by the Colombian National Police (DIRAN-CNP) to eliminate cocaine production (*Erythroxyum coca*) in Colombia. Involuntary exposure to this herbicide mix with high doses of glyphosate has triggered a political, social, and economic conflict between the two countries. Therefore, the Instituto de Investigaciones Biomédicas at the Universidad de las Américas has conducted a descriptive study to determine the baseline on the aerial spraying system and its impact on the social, health, genetic, and environmental areas in the communities located along Ecuador's northeastern border, affected by the aerial spraying with an herbicide mix containing high doses of glyphosate.

The communities studied here do not have health and housing general conditions appropriate for the environment found in the Amazon basin because many lack ventilation systems, as well as protection systems against vectors. The

Table 1 Genotype distribution and allelic frequency of GSTP1 Ile105Val, GPX-1 Pro198Leu and XRCC1 Arg399Gln polymorphism.

Genes	Genotype	Genotypic frequency			Allelic frequency			HWE (χ^2)
		Case	Control	All	Case	Control	All	
GSTP1 Ile105Val	Ile/Ile	0.32	0.54	0.43	0.52	0.72	0.62	0.04^{NS}
	Ile/Val	0.40	0.36	0.38				
	Val/Val	0.28	0.10	0.19	0.48	0.28	0.38	
GPX-1 Pro198Leu	Pro/Pro	0.35	0.38	0.36	0.59	0.68	0.63	0.03^{NS}
	Pro/Leu	0.48	0.6	0.54				
	Leu/Leu	0.17	0.02	0.1	0.41	0.32	0.37	
XRCC1 Arg399Gln	Arg/Arg	0.07	0.01	0.04	0.46	0.02	0.25	0.01^{NS}
	Arg/Gln	0.79	0.01	0.41				
	Gln/Gln	0.14	0.98	0.55	0.54	0.98	0.75	

HWE, Hardy-Weinberg equilibrium of all study population; NS, no significant difference.

Table 2 Statistical analysis of case and control individuals.

Genes	Genotype	Cases (n=92) No. (%)	No. (%) of control (n=90)	OR	95% CI	p-Value
GSTP1 Ile105Val	Ile/Ile	29 (32)	49 (54)	1.0 (reference)		
	Ile/Val	37 (40)	32 (36)	1.95	1.0–3.8	0.07^{NS}
	Val/Val	26 (28)	9 (10)	4.88	2.0–11.8	<0.001[a]
	Ile/Val+Val/Val	63 (58)	41 (37)	2.6	1.4–4.8	<0.05[a]
GPX-1 Pro198Leu	Pro/Pro	32 (35)	34 (38)	1.0 (reference)		
	Pro/Leu	44 (48)	54 (60)	0.87	0.5–1.6	0.77^{NS}
	Leu/Leu	16 (17)	2 (2)	8.5	1.8–39.9	<0.05[a]
	Pro/Leu+Leu/Leu	60 (55)	56 (50)	1.14	0.6–2.1	0.79^{NS}
XRCC1 Arg399Gln	Arg/Arg	6 (7)	1 (1)	1.0 (reference)		
	Arg/Gln	73 (79)	1 (1)	12.2	0.7–219.8	0.4^{NS}
	Gln/Gln	13 (14)	88 (98)	0.03	0.003–0.2	<0.001[a]
	Arg/Gln+Gln/Gln	86 (79)	89 (80)	0.2	0.02–1.4	0.1^{NS}

[a]Significant difference. NS, no significant difference.

water consumed by the population comes mainly from natural sources, such as rivers, marshes or springs that are highly prone to be polluted by chemical substances.

Concerning nutritional status, 2 years after the last aerial spraying with an herbicide mix, we observed that the global malnutrition status of children aged between 6 and 17 years decreased from 10.3% to 3%, whereas the risk of slight malnutrition diminished from 36.3% to 23.2%. As for chronic malnutrition, we observed that this percentage decreased from 29% to 28%, and acute malnutrition diminished from 1.87% to 1%, in comparison with the studies carried out by Acción Ecológica in 2006 (31). Likewise, the body mass index in adults demonstrated no malnutrition in adults over 18 years old; yet, with a tendency to obesity in women (29.7%) and in men (7.8%). This information clearly indicates that during the aerial spraying, the population had nutritional problems due to the broad spectrum herbicides that caused harm in the agricultural products essential for the population feeding, whereas the analyses obtained 2 years after the last aerial spraying confirmed improvement in the general nutritional status of the population.

Regarding family health, we noticed that the percentage of abortions rose during the aerial spraying with an herbicide mix with glyphosate, whereas child mortality decreased. According to the data compiled in the communities bordering Colombia, 5% of child mortality was caused by health complications due to exposure to the aerial spraying with an herbicide mix. Of the interviewed families, 84.7% had an ill relative during the spraying who presented the following symptoms: respiratory, digestive, ophthalmological problems, cephalea, or skin conditions. Regarding the psychological study, one of the most important impacts developed by the aerial spraying was fear. Fear is a feeling that has lasted until now, and 7.7% of the interviewed subjects manifested their fear as nightmares, abnormal behavior, developmental disorders, and stuttering. In the psychological study consisting of drawings made by the children, the pictures reflected sensitivity, creativity, expression capability, adaptation to environmental demands, and in turn, anguish, caution, and paranoid tendencies, where the need for protection and safety was evident.

Genetic assessment consisted of the analysis of DNA damage through the presence of chromosomal aberrations or

Table 3 Chromosomal fragmentation and karyotypes.

Individuals (n=92)	62	2	14	1	2	1	7	1	1	1
Percentage	0	1	1.2	1.4	1.5	1.9	2	2.4	2.5	2.8
Karyotype	46, XX			n=92				100%		

DNA variation through the presence of polymorphisms in the GSTP1, XRCC1, and GPX-1 genes in women of different ages who present a major susceptibility to hepatic toxins due to the variety of physiological processes. In 2006, DNA damage in 24 Ecuadorian individuals exposed to the aerial spraying with an herbicide mix with glyphosate was assessed by means of the comet assay technique, which has a high use in studies with genotoxic substances, such as hydrocarbons, X-rays, and pesticides (32–34). The results showed that DNA in the exposed individuals was highly damaged (comet length=35.5 μm), in comparison with the control group (comet length=25.94 μm). Thus, the results suggest that the individuals exposed to the broadspectrum herbicide suffered a genotoxic effect (35). Two years after the last aerial spraying, none of the studied population had any type of chromosomal alteration, being their normal karyotype (46, XX), and the percentage of chromosomal fragility was within normal parameters. Regarding genetics, the GSTP1 gene encodes proteins that are believed to function in xenobiotic metabolism and play the role as regulator of apoptosis (36–38). We observed a higher frequency of the valine allele in exposed individuals (0.48) than in healthy ones (0.28). Glutathione peroxidase (GPX-1), one of the most important antioxidant enzymes in humans, is responsible for the detoxification of hydrogen peroxide and is part of the enzymatic antioxidant defense system preventing oxidative DNA damage (38). A Pro198Leu polymorphism has been associated with the risk of developing lung, breast, and bladder cancer (23, 25, 39, 40). We observed a higher frequency of the leucine allele in exposed individuals (0.41) than in healthy ones (0.32). Those individuals presenting the GSTP1 Val/Val and GPX-1 Leu/Leu variables may have a higher risk of acquiring problems in the detoxification functions as in the case of the Ecuadorian population with bladder cancer (25). The protein encoded by the XRCC1 gene is involved in the maintenance of the structural integrity of DNA in the face of damage arising from environmental abuse, as well as from normal metabolic processes (41). The Arg allele was found mainly in the population exposed to the glyphosate. The OR test determined no significant risk in the population bearing the Arg399Gln polymorphism. The genetic analyses, carried out during the aerial spraying with an herbicide mix containing glyphosate, showed that the population had suffered DNA fragmentation (35), whereas the cytogenetic assessment executed 2 years after the last aerial spraying with the same herbicide proved that the studied population had no chromosomal alterations.

Several research studies related to glyphosate exposure have been conducted in Colombia by Bolognesi et al. (8), Sanin et al. (21), and Solomon et al. (22), which state that the studied populations have low genotoxic risk associated with glyphosate. Regarding our study, we obtained results showing no chromosomal alterations in the analyzed individuals. Nevertheless, the aerial spraying had a socially and psychologically negative impact on the Ecuadorian communities. Carrying out studies in the short and long term is very important for taking control of population health and for monitoring possible disease development in the coming future.

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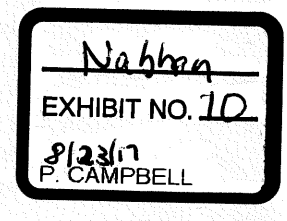
Biomonitoring of Genotoxic Risk in Agricultural Workers from Five Colombian Regions: Association to Occupational Exposure to Glyphosate



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In order to assess possible human effects associated with glyphosate formulations used in the Colombian aerial spray program for control of illicit crops, a cytogenetic biomonitoring study was carried out in subjects from five Colombian regions, characterized by different exposure to glyphosate and other pesticides. Women of reproductive age (137 persons 15–49 yr old) and their spouses (137 persons) were interviewed to obtain data on current health status, history, lifestyle, including past and current occupational exposure to pesticides, and factors including those known to be associated with increased frequency of micronuclei (MN). In regions where glyphosate was being sprayed, blood samples were taken prior to spraying (indicative of baseline exposure), 5 d after spraying, and 4 mo after spraying. Lymphocytes were cultured and a cytokinesis-block micronucleus cytome assay was applied to evaluate chromosomal damage and cytotoxicity. Compared with Santa Marta, where organic coffee is grown without pesticides, the baseline frequency of binucleated cells with micronuclei (BNMN) was significantly greater in subjects from the other four regions. The highest frequency of BNMN was in Boyacá, where no aerial eradication spraying of glyphosate was conducted, and in Valle del Cauca, where glyphosate was used for maturation of sugar cane. Region, gender, and older age (≥35 yr) were the only variables associated with the frequency of BNMN measured before spraying. A significant increase in frequency of BNMN between first and second sampling was observed in Nariño, Putumayo, and Valle immediately (<5 d) after spraying. In the post-spray sample, those who reported direct contact with the eradication spray showed a higher quantitative frequency of BNMN compared to those without glyphosate exposure. The increase in frequency of BNMN observed immediately after the glyphosate spraying was not consistent with the rates of application used in the regions and there was no association between self-reported direct contact with eradication sprays and frequency of BNMN. Four months after spraying, a statistically significant decrease in the mean frequency of BNMN compared with the second sampling was observed in Nariño, but not in Putumayo and Valle del Cauca. Overall, data suggest that genotoxic damage associated with glyphosate spraying for control of illicit crops as evidenced by MN test is small and appears to be transient. Evidence indicates that the genotoxic risk potentially associated with exposure to glyphosate in the areas where the herbicide is applied for coca and poppy eradication is low.

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Glyphosate (*N*-phosphonomethyl glycine), a nonselective herbicide, is the active ingredient of a number of herbicide formulations and one of the most widely used pesticides on a global basis (Baylis, 2000; Woodburn, 2000; Duke & Powles, 2008). It is a postemergence herbicide, effective for the control of annual, biennial, and perennial species of grasses, sedges, and broadleaf weeds. The relatively high water solubility and the ionic nature of glyphosate retard penetration through plant hydrophobic cuticular waxes. For this reason, glyphosate is commonly formulated with surfactants that decrease the surface tension of the solution and increase penetration into the tissues of plants (World Health Organization International Program on Chemical Safety, 1994; Giesy et al., 2000).

A large number of glyphosate-based formulations are registered in more than 100 countries and are available under different brand names. One of the most commonly applied glyphosate-based products is Roundup, containing glyphosate as the active ingredient (AI) and polyethoxylated tallowamine

(POEA) as a surfactant. Glyphosate and its formulations have been extensively investigated for potential adverse effects in humans (Williams et al., 2000). This pesticide was reported to exert a low acute toxicity to different animal species. Experimental evidence showed that glyphosate did not bioaccumulate in any animal tissues (Williams et al., 2000). Chronic feeding studies in rodents did not find evidence of carcinogenic activity or any other relevant chronic effects (U.S. EPA, 1993; World Health Organization International Program on Chemical Safety, 1994).

With in vitro studies with tissue cultures or aquatic organisms, several of the formulated products are more toxic than glyphosate AI (Giesy et al., 2000; Williams et al., 2000). Differences in the response of test organisms to the AI and the commercial formulation, e.g., Roundup, are likely due to the toxicity of different formulants and surfactants contained in commercial products. There is a general agreement that adjuvants may be more toxic for animals than glyphosate itself (Giesy et al., 2000; Williams et al., 2000; Richard et al., 2005). Cytotoxicity of the commercial formulation Roundup to human peripheral mononuclear cells was 30-fold higher (LC_{50} = 56 mg/L) than for the AI (LC_{50} = 1640 mg/L) (Martinez et al., 2007). Several in vitro and in vivo studies with parallel testing of glyphosate AI and Roundup showed that only the commercial formulation was genotoxic (Rank et al., 1993; Bolognesi et al., 1997b; Gebel et al., 1997; Grisolia 2002). Cytotoxic and genotoxic effects were observed with Roundup and other formulations of glyphosate, but not with glyphosate AI alone in comparative studies involving different experimental systems (Peluso et al., 1998; Richard et al., 2005; Dimitrov et al., 2006). The observed differences were attributed to some ingredients of Roundup, mainly surfactants, and/or to a synergic effect of glyphosate and components of the formulation (Sirisattha et al., 2004; Peixoto 2005).

Epidemiological studies generally showed no consistent or strong relationships between human exposure to glyphosate or glyphosate-containing products and health outcomes in human populations. No statistically significant association in humans was found with spontaneous abortion, fetal deaths, preterm birth, neural tube defects (Rull et al., 2006), and cancer incidence overall, although a suggested association between cumulative exposure to glyphosate and the risk of multiple myeloma was reported (De Roos et al., 2005).The epidemiologic evidence is insufficient to verify a cause–effect relationship for childhood cancer (Wigle et al., 2008). Four case-control studies suggested an association between reported glyphosate use and the risk of non-Hodgkin's lymphoma (NHL) in age groups from 20 to 70 yr (Hardell & Eriksson, 1999; McDuffie et al., 2001; Hardell et al., 2002; De Roos et al., 2003; Eriksson et al., 2008).

Glyphosate AI and Roundup were extensively tested for genotoxicity in a wide range of in vitro and in vivo systems evaluating different genetic endpoints (gene mutation, chromosome mutation, DNA damage and repair) using bacteria and mammalian somatic cells (Williams et al., 2000). The active ingredient did not induce any relevant genotoxic effects such as gene mutations in a variety of in vitro bacterial assays including the *Salmonella typhimurium* reversion assay, with and without metabolic activation (Wildeman & Nazar 1982; Moriya et al., 1983; Li & Long, 1988) and *Escherichia coli* WP-2 (Moriya et al., 1983; Li & Long, 1988). The active ingredient was also negative in the Chinese hamster ovary cell HGPRT gene mutation assay and in primary hepatocyte DNA repair assay (Li & Long, 1988). The genotoxic potential of the formulation Roundup was investigated in a number of studies evaluating various genetic endpoints in different biological systems and was (1) negative in the *S. typhimurium* reversion assay (Kier et al., 1997), (2) negative in the sex-linked recessive lethal assay with *Drosophila melanogaster* (Gopalan & Njagi, 1981), and (3) negative for in vivo micronucleus (MN) induction in mouse bone marrow (Rank et al., 1993; Kier et al., 1997; Dimitrov et al., 2006). The Roundup formulation was reported in a number of studies to exert weak genotoxic effects in short-term assays.

Differences in the response of test organisms to the active ingredient glyphosate and the commercial formulation Roundup might be due to the toxicity of different co-formulants and surfactants contained in commercial products. Several studies with parallel testing of glyphosate and Roundup showed that only the commercial formulation was genotoxic (Rank et al., 1993; Bolognesi et al., 1997b; Gebel et al., 1997; Grisolia 2002). A recent study on the genotoxic potential of glyphosate formulations found that in some cases the genotoxic effects were obtained under exposure conditions that are not relevant for humans (Heydens et al., 2008).

An in vitro study described a concentration-dependent increase of DNA single-strand breaks (SSB), evaluated by comet assay, in two different human cell lines treated with glyphosate at sublethal concentrations (Monroy et al., 2005). Roundup formulations were shown to affect the cell cycle by inhibiting the G2/M transition and DNA synthesis leading to a genomic instability (Marc et al., 2004a, 2004b). Evidence of DNA damage in peripheral lymphocytes from a small group of subjects potentially exposed to glyphosate was reported in a recent paper (Paz-y-Miño et al., 2007). The number of subjects (21 control and 24 exposed) was small and there were 23 females and only 1 male in the exposed group, making interpretation of the results difficult.

Frequency of MN in human lymphocytes has been widely used for biomonitoring exposure to pesticides (Bolognesi, 2003; Costa et al., 2006; Montero et al., 2006). The MN test, an index of chromosomal damage, is one of the most appropriate biomarkers for monitoring a cumulative exposure to genotoxic agents. Chromosomal damage, as a result of inefficient or incorrect DNA repair, is expressed during the cell

division and represents an index of accumulated genotoxic effects. The cytokinesis-block micronucleus (CBMN) methodology (Fenech & Morley, 1985) allows a distinction to be made between a mononucleated cell that did not divide and a binucleated cell that has divided once, expressing any genomic damage associated to recent exposure. The test in its comprehensive application, as was proposed by Fenech (2007) including a set of markers of gene amplification, cellular necrosis, and apoptosis, allows evaluation of genotoxic and cytotoxic effects induced by exposure to a genotoxic agent.

Colombia's anti-drugs strategy includes a number of measures ranging from aerial spraying of a mixture of a commercial formulation of glyphosate (Glyphos) and an adjuvant, Cosmo-Flux (Solomon et al., 2007b), to manual eradication, including alternative development and crop substitution programs (UNODC, 2007). In order to assess the potential genotoxic risk associated with the aerial spraying program with the glyphosate mixture, a cytogenetic biomonitoring study was carried out in subjects from five Colombian regions, characterized by different exposure to glyphosate formulations and other pesticides.

MATERIALS AND METHODS

The study was carried out in five regions of Colombia, with different potential exposure to glyphosate as reported by Sanin et al. (2009). Briefly, the characteristics of the study areas are described here:

Sierra Nevada de Santa Marta—where organic coffee is grown without use of pesticides.

Boyacá—an area of illicit crops, where manual eradication is performed and the use of pesticides and other chemical agents is common.

Putumayo and Nariño—where aerial spraying of glyphosate is performed for coca and poppy eradication. The aerial application rate for eradication of coca is 3.69 kg glyphosate a.e. (acid equivalents)/ha (Solomon et al., 2007b). In order to maximize penetration and effectiveness of the spray formulation, Glyphos is tank-mixed with an adjuvant (Cosmo-Flux® 411F; Cosmoagro, Bogotá).

Valle del Cauca—where glyphosate is applied through aerial spraying for sugar cane maturation. Roundup 747 is the most commonly used product and is applied at a rate of 1 kg a.e./ha, and has no additional adjuvant (personal communication, ASOCAÑA, the Colombian Association for Sugar Growers, December 2008).

Study Population

Two hundred and seventy-four individuals were included in the study. The objective was to sample 30 couples of reproductive age in each area and, where possible, the same couples in the study conducted by Sanin et al. (2009) were sampled. In Putumayo, Nariño, and Valle del Cauca, the population was selected based on the scheduled aerial spraying of glyphosate. This schedule was confidential and provided exclusively for the purpose of the study by the Antinarcotics Police (Putumayo and Nariño) or ASOCAÑA (Valle del Cauca). In Valle del Cauca, a sample size of 30 couples could not be achieved because spraying was not carried out in populated areas of the study region. Most spraying during the study period was carried out on sugar cane crops where no inhabitants were found. All reported areas to be sprayed in Valle del Cauca were visited to search for couples; however, only 14 could be included.

In Sierra Nevada de Santa Marta and Boyacá, the same areas investigated in a previous study (Sanin et al., 2009) were identified, although, due to the instability of the population and high migration, most couples from the previous study were not located. In all regions, the same strategy as described before (Sanin et al., 2009) was followed, visiting household by household until completing 30 couples who fulfilled the inclusion criteria, women of reproductive age (15–49 yr of age) and their spouses, who voluntarily accepted to participate in the study.

Field Data Collection

Field data collection was carried out between October 2006 and December 2007. Epidemiologists and interviewers in the five regions who participated in the Sanin et al. (2009) study were informed about the objectives of the study and trained for data collection. The Ethical Committee of Fundacion Santa Fe de Bogotá approved the study protocol and the informed consent forms used for the study. All the subjects were informed about the aims of the study. All of them gave their informed consent and volunteered to donate blood for sampling. They did not self-report illness at the time of blood sampling and interviews. Every volunteer was interviewed with a standardized questionnaire, designed to obtain relevant details about the current health status, history, and lifestyle. This included information about possible confounding factors for chromosomal damage: smoking, use of medicinal products, severe infections or viral diseases during the last 6 mo, recent vaccinations, presence of known indoor/outdoor pollutants, exposure to diagnostic x-rays, and previous radio- or chemotherapy. A simplified food frequency questionnaire that had already been used in other regions of Colombia was also applied, in order to evaluate dietary folic acid intake. Folic acid intake was characterized because of the role of folic acid deficiency in baseline genetic damage in human lymphocytes (Fenech & Rinaldi, 1994). Specific information about exposure at the time of aerial spraying in Putumayo, Nariño, and Valle del Cauca was addressed in the questionnaire.

Blood Sampling and Cell Culture

Blood samples were collected twice in Boyacá, at the beginning of the study and 1 mo after the first survey, and at 3 different times in Nariño, Putumayo, and Valle del Cauca: immediately before spraying, within 5 d after spraying, and 4 mo later. A sample of 10 ml whole blood was collected from each subject, by venipuncture, using heparinized Vacutainer tubes kept at room temperature and sent within 24 h for the establishment of the lymphocyte cultures. The samples were coded before culturing. The modified cytokinesis-blocked method of Fenech and Morley (1985) was used to determine frequency of MN in lymphocytes. Whole blood cultures were set up for cytogenetic analysis in Bogotá (Colombia) by personnel specifically trained by cytogeneticists from Environmental Carcinogenesis Unit of the National Cancer Research Institute (Genoa, Italy).

Three sterile cultures of lymphocytes were prepared. A 0.4-ml aliquot of whole blood was incubated at 37°C in duplicate in 4.6 ml RPMI 1640 (Life Technologies, Milano, Italy) supplemented with 10% fetal bovine serum (Gibco BRL, Life Technologies SrL, Milano, Italy), 1.5% phytohemoagglutinin (Murex Biotech, Dartford, UK), 100 units/ml penicillin, and 100 µg/ml streptomycin. After 44 h, cytochalasin B (Sigma, Milano, Italy) was added at a concentration of 6 µg/ml. At the end of incubation at 37°C for 72 h, cells were centrifuged (800 × g, 10 min), then treated with 5 ml of 0.075 m*M* KCl for 3 min at room temperature to lyse erythrocytes. The samples were then treated with pre-fixative (methanol:acetic acid 3:1) and centrifuged . The cellular pellets were resuspended in 1 ml methanol. At this step the samples were sent to the Environmental Carcinogenesis Unit (National Cancer Research Institute, Genoa, Italy). All the samples were centrifuged in methanol. Treatment with fixative (methanol:acetic acid, 5:1) followed by centrifugation was repeated twice for 20 min. Lymphocytes in fresh fixative were dropped onto clean iced slides, air-dried, and stained in 2% Giemsa (Sigma, Milano, Italy). MN analysis was performed blind only on lymphocytes with preserved cytoplasm. On average, 2000 cells were analyzed for each subject. Cells were scored cytologically using the cytome approach to evaluate viability status (necrosis, apoptosis), mitotic status (mononucleated, binucleated, multinucleated) and chromosomal damage or instability status (presence of micronuclei, nucleoplasmic bridges, nucleoplasmic buds) (Fenech 2007). The proliferation index (PI) was calculated as follows:

$$PI = (\text{number of mononucleated cells} + 2 \times \text{number of binucleated cells} + 3 \times \text{number of polynucleated cells}) / \text{total number of cells}.$$

Statistical Analysis

Continuous variables were characterized using mean and standard deviation, while categorical variables were expressed as proportions. Dependent variables, micronuclei per binucleated cell (BNMN), and differences in MN between sampling were square-root transformed where required to comply with the required assumptions of normal distribution and equal variances. Comparison of MN between areas was made by one-way analysis of variance (ANOVA). A significance level at 5% was used to assess differences among areas. For multiple comparisons, the Bonferroni test was applied ($\alpha = .05$). Significance of differences in frequency of BNMN between first and second, and second and third sampling were tested by the unpaired *t*-test with equal variances. Difference and 95% confidence interval were used to compare between samplings.

Bivariate analysis between dependent variables and putative risk factors was performed by one-way ANOVA, comparing exposed and nonexposed subjects. In cases where risk factor was continuous, such as age, folic acid intake, alcohol consumption, and coffee consumption, the correlation coefficient was used.

A multiple linear regression was conducted to assess association with BNMN at the first sampling with different variables: region, age (as continuous variable as well as categorical age), ethnicity as a dichotomous variable, exposure to genotoxic products as defined earlier, gender (female vs. male), and intake of folic acid (categorized in quartiles). Regression analysis was conducted with transformed variables, with square root transformation of BNMN and natural logarithm of age, to obtain a normal distribution.

RESULTS

Demographic characteristics and habits of the study groups are described in Table 1. The study population comprised 274 subjects (137 female and 137 male; average age 30.4 ± 7.8 yr). The mean age of the subjects was similar in the different regions. A large part of the studied population was mestizo, with the exception of the Nariño area consisting of individuals of African origin. In the total population, 38% of interviewees had not completed primary education. Putumayo had the largest proportion with education and Valle del Cauca the lowest as shown in Table 1. Only 10% of all subjects were smokers, (20% in Putumayo); a large majority of subjects were drinkers of beer or liquor with a consistent consumption of guarapo (traditional alcoholic beverage prepared by fermentation of maize) in Santa Marta and Boyacá. No statistically significant differences of folic acid intake were observed between different regions (the mean values ranged from 750 and 1189 µg/wk).

One hundred and nine (39.8%) of 274 participants reported current use of pesticides in their occupation or other activities. Nariño (76.6%) and Putumayo (61.7%) were the two regions where prevalence of use of genotoxic pesticides was higher; Boyacá (24.2%) and Valle del Cauca (28.6%) reported lower use. None of the study subjects in Santa Marta reported use of pesticides. No data regarding quantity of pesticide used were available. Fifty (18.3%) out of 273 who gave information

TABLE 1
Demographic Characteristics and Possible Confounding Exposures in the Study Populations

Area	Santa Marta	Boyacá	Putumayo	Nariño	Valle del Cauca
Number of subjects	60	62	60	64	28
Age (mean (SD))	27.0 (5.6)	29.1 (8.8)	31.4 (7.2)	32.5 (7.4)	33.4 (8.7)
Ethnicity (%)					
Mestizo	100	100	88.3	3.1	60.7
African			6.7	96.9	39.3
Indian			5.0		
Education (%)					
None		4.8	1.7		
Primary incomplete	26.7	38.7	53.3	42.2	21.4
Primary complete	21.7	29.0	20.0	23.4	32.1
High school incomplete	25.0	8.1	20.0	25.0	28.6
High school complete	26.7	19.4	3.3	9.4	17.9
Technical			1.7		
Occupation (%)					
Agriculture	10.0	41.9	60.0	62.5	7.1
Housewife	40.0	50.0	38.3	34.4	50.0
Other	50.0	8.1	1.7	3.1	42.9
Health insurance (%)					
Uninsured	50.0	9.7	36.7	71.9	7.1
Subsidized	38.3	83.9	60.0	18.7	50.0
Insured	11.7	6.4	3.3	9.4	42.9
Coffee consumption (cups/day)					
Mean (SD)	1.8 (2.3)	1.7 (0.8)	2.3 (4.1)	1.3 (0.4)	1.7 (1.2)
Percent of population	80.0	67.7	88.3	76.6	82.1
Smoking (%)					
Nonsmokers	91.7	95.2	80.0	87.5	92.9
Alcohol (%)					
Liquor	28.3	25.8	53.3	78.1	78.6
Beer	51.6	67.7	63.1	82.8	64.3
Guarapo	6.7	59.7	1.7	3.2	10.7
Users of illicit drugs (%)	6.7	0	5.0	7.8	0
Diet					
Folic acid intake (µg/wk)	1189	873	750	1160	812

about x-ray examination reported to having been exposed at some time; however, only 21 out of 46 who gave information on dates of x-ray reported exposure in the last 6 mo before the interview and first blood sample. Sixty-one percent of population reported viral infections, the highest prevalence in Nariño (89.5%) and the lowest in Putumayo (49.2%). However, 89.3% of viral infections were the common cold and 6.1% dengue fever. Hepatitis was reported by six interviewees without any specification of the type of the infection.

The means and standard deviations of frequency of MN and related parameters according to regions are shown in Table 2 and presented graphically in Figure 1. Compared with Santa Marta, where people grow organic coffee without the use of pesticides and which is considered as a reference area, the baseline frequency of BNMN was significantly greater in subjects from the other four regions. The highest frequency of BNMN was in Boyacá, where no aerial eradication spraying of glyphosate was carried out, and Valle del Cauca, where aerial spraying was for maturation of sugar cane. There was no significant difference between mean frequency of BNMN in Boyacá and Valle del Cauca. There was no significant difference in frequency of BNMN between Putumayo and Nariño,

TABLE 2

Mean (SD) Frequency of Binucleated Cells with Micronuclei (BNMN), Total Micronuclei (MNL) per 1000 Binucleated Peripheral Lymphocytes, Frequency of Mononucleated Cells per 1000 Lymphocytes (MNMO), and Proliferation Index (PI) by Region before the Exposure (Phase 1), 5 d after Spraying (Phase 2) and 4 mo Later (Phase 3)

Region	Santa Marta	Boyacá	Putumayo	Nariño	Valle del Cauca
Phase 1					
Number of subjects	60	62	58	63	28
BNMN	1.83 (0.97)	5.64 (1.72)	3.61 (1.51)	4.12 (1.65)	5.75 (2.48)
MNL	1.97 (1.05)	6.16 (1.91)	3.90 (1.66)	4.36 (1.85)	6.02 (2.50)
MNMO	0.41 (0.44)	0.99 (0.64)	0.47 (0.51)	0.51 (0.39)	1.12 (0.88)
PI	1.54 (0.14)	1.45 (0.14)	1.68 (0.15)	1.47 (0.12)	1.51 (0.15)
Phase 2					
Number of subjects	ND	55	53	55	27
BNMN		4.96 (2.00)	4.64 (2.45)	5.98 (2.03)	8.64 (2.81)
MNL		5.41 (2.25)	5.02 (2.95)	6.35 (2.18)	8.98 (2.93)
MNMO		0.87 (0.65)	0.44 (0.46)	0.70 (0.45)	1.65 (0.62)
PI		1.72 (0.14)	1.66 (0.20)	1.40 (0.18)	1.51 (0.14)
Phase 3					
Number of subjects	ND	ND	50	56	26
BNMN			5.61(3.08)	3.91 (1.99)	7.38 (2.41)
MNL			5.96 (3.23)	4.13 (2.20)	8.17 (2.72)
MNMO			0.82 (0.54)	0.55 (0.42)	0.98 (0.60)
PI			1.43 (0.17)	1.41 (0.14)	1.45 (0.20)

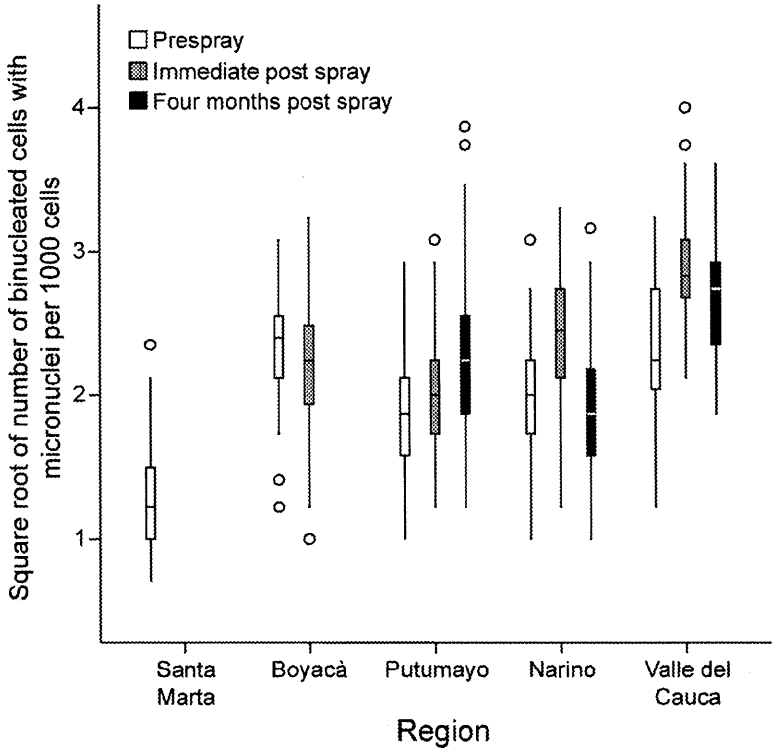

FIG. 1. Box plot of frequency of BNMN in the five study regions with samples taken prespray, 4–5 d post-spray, and 4 mo post-spray. Box plots: The center horizontal line marks the median of the sample. The length of each box shows the range within which the central 50% of the values fall, with the top and bottom of the box at the first and third quartiles. The vertical T-lines represent intervals in which 90% of the values fall. The O symbols show outliers. See text for description of statistically significant differences.

although Boyacá and Valle del Cauca showed a significantly higher frequency than Nariño and Putumayo. A higher frequency of BNMN in Boyacá was also observed in a second sampling 1 mo later.

There were differences in frequency of BNMN between sampling periods. A statistically significant difference in frequency of BNMN between first and second sampling was observed in Valle, Putumayo, and Nariño immediately (<5 d) after spraying. Four months after spraying in Nariño, there was a statistically significant decrease in the mean frequency of BNMN compared with the second sampling, but in Valle del Cauca the decrease was not significant nor was the increase observed in Putumayo significant (Figure 1 and Table 2).

The frequency of mononucleated cells with micronuclei (MOMN) was used as an index of background level of chromosomal damage accumulated in vivo (Table 2). The lowest frequency of MOMN for the first sampling was observed in Santa Marta; however, there was no marked difference in frequency of MOMN in Santa Marta, Putumayo, and Nariño and no statistically significant difference between Valle and Boyacá. However, Valle and Boyacá had a significantly higher frequency of MOMN than Putumayo, Nariño, and Santa Marta at first sampling. Immediately after spraying, Valle showed a significantly higher frequency of MOMN compared to Putumayo and Nariño, and Nariño was also higher than Putumayo. Between first and second sampling, the increase in frequency of MOMN in Nariño and Valle was statistically significant, but there was no difference in Putumayo nor in Boyacá 4 mo after the first sampling. Data suggest greater exposure to genotoxic agents in these populations is independent of the exposure to glyphosate products.

The proliferation index (PI) in all the studied groups was in the range of normal values described in the literature. No significant reduction of PI was observed in association with environmental exposures in groups of subjects from the different regions. A statistically significant correlation coefficient (0.288) between PI values from the first and the second samplings was observed, confirming the association with individual characteristics and not with any toxicity related to the exposure or to the culture techniques. Due to the low frequency observed, data with respect to other nuclear alterations, including in cytome analysis (Fenech, 2007), are not described in Table 2: the mean frequency of nucleoplasmic bridges (NPB) for all subjects was 0.010 per 1000 cells, that of nuclear buds was 0.022 per 1000 cells, and only rare necrotic and apoptotic cells were found in some samples.

Gender was the most important demographic variable affecting the BNMN index. Frequencies of BNMN in females were greater than those in males (mean 4.43 ± 2.36 vs. 3.61 ± 1.82, respectively, in total population) (Table 3). The groups of subjects were evenly matched for gender by including only couples in the study. No association was found between frequency of MN and age as a categorical variable, nor was there an association with smoking, but prevalence of smoking was low (~10% in the total population). A higher baseline frequency of MN was observed in subjects of African origin, suggesting greater susceptibility. Other lifestyle factors such as alcohol, coffee consumption, or illicit drug intake were not associated with initial measures of BNMN and MOMN.

One hundred and thirty-four of the 152 subjects in Nariño, Putumayo, and Valle reported information on contact with Glyphos and Cosmo-Flux after eradication spraying. The other 18 did not provide information in the second survey or blood samples were inadequate for testing micronuclei. Sixty-six (49.2.0%) reported no contact with the spray and 68 (50.8%) reported coming into contact with the spray because they entered sprayed fields or reported contact with the spray droplets. The mean BNMN in Nariño and Putumayo was greater in respondents who self-reported exposure, but differences were not statistically significant (Table 4). In Valle, only one respondent reported contact with glyphosate.

Region, gender, and older age (≥35 yr) were the only variables associated with the frequency of BNMN before spraying (Table 5). In fact, using Santa Martha, where no use of pesticides was reported, as reference, Boyacá, Valle del Cauca, Putumayo, and Nariño showed a statistically significant higher mean frequency of BNMN. There were also significant differences between Boyacá and Valle and Putumayo and Nariño. Females had a statistically higher mean frequency of BNMN than males after adjusting for all other variables. Greater age was also associated with greater frequency of BNMN. Neither exposure to genotoxic products, nor ethnicity, nor intake of folic acid was associated with frequency of BMMN at the first sampling. The multiple linear regression analysis of difference between second and first sampling only demonstrated statistically significant association with region after adjusting for all other variables, indicating that Putumayo, Nariño, and Valle had significantly greater differences between second and first sampling than Boyacá.

DISCUSSION

The main objective of this study was to test whether there was an association between aerial spraying of glyphosate and cytogenetic alterations, evaluated as frequency of MN in peripheral leukocytes. Biomonitoring was carried out in three regions of Colombia in populations exposed to aerial spraying of glyphosate: Putumayo and Nariño, where the application was performed for eradication of coca and poppy, and Valle del Cauca where the herbicide was used for maturation of sugar cane. Two control populations not exposed to aerial spraying of glyphosate were also selected: the first one from Sierra Nevada de Santa Marta, where organic coffee is grown without the use of any pesticides, and the other from Boyacá, with a region of illicit crops, where manual eradication is performed and subjects were potentially exposed to several pesticides but not glyphosate for aerial eradication. The ex vivo analysis of leukocytes in the presence of cytochalasin B, added 44 h after the

TABLE 3
Association of Mean (SD) Frequency of Binucleated Cells (First Sampling) with Micronuclei (BNMN/1000 Binucleated Lymphocytes) and Demographic Variables

Variable	Santa Marta	Boyacá	Putumayo	Nariño	Valle del Cauca	Total
Sex						
Females	1.98 (1.03)	6.22 (1.79)	3.91 (1.71)	4.57(1.77)	6.45 (2.82)	4.43 (2.36)
Males	1.68 (0.90)	5.06 (1.46)	3.31 (1.25)	3.66 (1.39)	5.05 (1.94)	3.61 (1.82)
p	.236	.007	.131	.028	.138	.002
Age						
18–24 yr	2.00 (1.14)	5.50 (1.96)	3.32 (1.25)	3.64 (1.72)	6.19 (2.15)	3.67 (2.16)
25–34 yr	1.66 (0.87)	5.70 (1.66)	3.53 (1.17)	4.20 (1.77)	4.20 (0.76)	3.97 (2.08)
35 yr and older	1.93 (0.67)	5.62 (1.73)	3.84 (1.86)	4.25 (1.52)	6.04 (2.84)	4.41 (2.19)
p	.438	.929	.574	.564	.313	.093
Ethnicity						
Mestizo	1.83 (0.97)	5.64 (1.72)	3.72 (1.52)	4.75 (1.06)	5.82 (2.44)	3.94(2.24)
Africa and Indian	0	0	2.86 (1.31)	4.10 (1.66)	5.64 (2.65)	4.20(1.90)
p			.162	.588	.850	.368
Smoking						
Yes	2.00 (1.06)	5.33 (0.76)	3.31 (1.00)	4.77 (1.51)	4.50 (1.41)	3.83 (1.60)
No	1.82 (0.97)	5.65 (1.76)	3.80 (1.56)	4.03 (1.66)	5.90 (2.57)	4.07 (2.20)
p	.693	.756	.395	.233	.459	.592
Folic acid intake (quartiles)						
1	1.92 (0.99)	6.11 (1.95)	3.23 (1.12)	4.50 (1.75)	5.86 (2.34)	3.89 (2.23)
2	1.64 (0.66)	5.70 (1.75)	3.47 (1.49)	3.80 (1.47)	5.86 (2.74)	3.97 (2.21)
3	1.69 (0.92)	5.69 (1.82)	4.00 (1.37)	3.85 (2.04)	6.58 (2.84)	4.47 (2.22)
4	1.94 (1.20)	4.94 (1.13)	3.69 (2.429)	4.28 (1.51)	4.63 (2.05)	3.75 (1.89)
p	.779	.399	.515	.645	.612	.220

TABLE 4
Mean Frequency of Binucleated Cells with Micronuclei (BNMN) at the Second Sampling per 1000 Binucleated Lymphocytes and Self-Reported Exposures to the Glyphosate Spray in Three Areas Where Aerial Application Had Occurred

	Nariño (*n* = 55)		Putumayo (*n* = 53)		Valle del Cauca (*n* = 26)	
Route of exposure	*n*	Mean BNMN (SD)	*n*	Mean BNMN (SD)	*n*	Mean BNMN (SD)
No exposure	28	5.81 (1.85)	13	3.84 (1.30)	25	8.56 (2.90)
Spray in air	5	7.30 (0.57)	1	5.50 (0)		
Spray on skin	8	5.62 (1.60)	15	4.90 (1.87)	1	9.50 (0)
Entered sprayed field	14	6.06 (2.77)	24	4.87 (3.18)		
p Value (ANOVA)		0.472		0.612		0.760
Any exposure	27	6.16 (2.22)	40	4.90 (2.69)	1	9.50 (0)
p Value (no exposure vs. any exposure)		0.525		0.181		0.760

Note. The data comprise respondents in the second survey from which blood samples were obtained.

TABLE 5
Multiple Linear Regression Analysis Adjusted for Region, Age, Gender, Ethnicity, and Folic Acid Intake

Variable	Coefficient	*p*	95% CI
Region			
Boyacá	3.75	≤.0001	3.19, 4.31
Putumayo	1.58	≤.0001	1.00, 2.16
Nariño	2.06	≤.0001	1.49, 2.64
Valle del Cauca	3.65	≤.0001	2.92, 4.39
Age (yr)			
25–34	0.28	.250	–0.20, 0.76
35 and older	0.75	.008	0.20, 1.31
Gender			
Females	1.00	≤.0001	0.60, 1.40

start of cultivation, made it possible to distinguish between non-dividing mononucleated cells—as an index of accumulated chromosomal damage—and binucleated cells, which had completed one nuclear division during in vitro culture and expressed MN associated with recent exposure to genotoxic agents.

The baseline level of chromosomal damage, evaluated as frequency of BNMN, was associated with the different regions considered in our study. The frequency of BNMN before spraying was also associated with region, gender, and age. Gender difference in the background incidence of MN in peripheral leukocytes, with the frequency being consistently higher in females, and a strong correlation between MN frequency and increasing age are well documented (Bonassi et al., 1995, 2001; Bolognesi et al., 1997a).

Data demonstrated no significant effect of smoking, confirming findings from the literature (Bonassi et al., 2003) although prevalence of smoking in our study population was small (7–20%, Table 1). No association with alcohol consumption was observed. A higher susceptibility of people of African origin compared to the mestizo group was suggested by a greater baseline frequency of BNMN and increased frequency at the second sampling period.

There was some indication of an association between BNMN and exposure to pesticides in general. The lowest frequency of BNMN was observed in Sierra Nevada de Santa Marta, where people self-reported that they did not use pesticides. The mean frequency of BNMN in this group of subjects (1.83 ± 0.97) was similar to that observed in healthy unexposed subjects for the same range of age (Bolognesi et al., personal communication). The higher mean frequency of BNMN observed in Boyacá and Valle del Cauca (5.64 ± 1.72 and 5.75 ± 2.48, respectively) and that in Nariño and Putumayo (4.12 ± 1.65 and 3.65 ± 1.51, respectively), compared to Santa Marta, are in agreement with similar biomonitoring studies carried out in subjects exposed to pesticides using the MN test or other genetic endpoints (Bolognesi, 2003; Bull et al., 2006).

There was no clear relationship between BNMN and the reported use of pesticides classified as genotoxic. Participants in Boyacá and Valle del Cauca showed higher frequency of BNMN than those in Putumayo and Nariño. However, a greater proportion of participants in the latter regions self-reported the use genotoxic pesticides (76.6% in Nariño and 61.7% in Putumayo). There is no information available on other relevant factors such as frequency of use, rate applied, time of exposure, and protective measures used, and we could therefore not characterize exposures to explain the differences. There were further inconsistencies; for example, in Boyacá, where more frequent use of pesticides was expected, only 24.2% of participants self-reported use, compared with the greater values in Nariño and Putumayo. However, it is possible that in areas such as Boyacá, individuals might be potentially exposed to persistent pesticides applied in the past and still present in the environment.

There was no evidence of an association between BNMN and folic acid deficiency. An assessment of folic acid intake from the semiquantitative food frequency questionnaire showed that, according to accepted recommendations (Herbert, 1987), the diet of the study populations was not deficient in folic acid and there were only small differences between regions. Consistent with these data, no association was found between MN and folic acid intake, either as a continuous variable or by quartiles.

The frequency of BNMN increased after spraying with glyphosate but not consistently. The results obtained with a second sampling, carried out immediately after the glyphosate spraying, showed a statistically significant increase in frequency of BNMN in the three regions where glyphosate was sprayed. However, this was not consistent with the rates of application use in the regions. The increase in frequency of BNMN in Valle (application rate = 1 kg a.e. glyphosate/ha) was greater than that in Nariño and Putumayo (3.69 kg a.e. glyphosate/ha).

There was no significant association between self-reported direct contact with eradication sprays and frequency of BNMN. The frequency of BNMN in participants who self-reported that they were exposed to glyphosate because they entered the field immediately after spraying (to pick the coca leaves), felt spray drops in their skin, or they thought they were exposed because they had contact with the chemical in the air, was not significantly greater than in subjects living in the same areas but who were not present during spraying. Decreases in frequency of BNMN in the recovery period after glyphosate spraying were not consistent. The third sampling, 4 mo after spraying, demonstrated a statistically significant decrease in frequency of BNMN only in Nariño.

Overall, these results suggest that genotoxic damage associated with glyphosate spraying, as evidenced by the MN test, is small and appears to be transient. The frequencies of BNMN in Nariño and Putumayo during the second and the third sampling fell within the range of values observed in Boyacá, an area

where people were exposed to a complex mixture of different pesticides (including glyphosate). A greater increase in frequency of BNMN was observed in Valle del Cauca, but it cannot be attributed only to the glyphosate exposure, because the application rate of the herbicide in this area was one-third compared with that in Nariño and Putumayo. This conclusion is further supported by the frequency of MN in mononucleated cells (MOMN), which provides an indication of the background level of chromosome/genome mutations accumulated in vivo (Manteuca et al., 2006). A statistically significant increase of MOMN was observed in Boyacá and Valle del Cauca before and after the aerial spraying, suggesting exposure to other genotoxic compounds in these populations was independent of the exposure to glyphosate. Evidence indicates that the genotoxic risk potentially associated with exposure to glyphosate in the areas where the herbicide is applied for eradication of coca and poppy is of low biological relevance. One of the strengths of our study was the detection of a transient chromosomal damage, evaluated as MN frequency in peripheral blood of the exposed subjects, since it was possible to compare the baseline before spraying with the effects detected immediately after spraying. Glyphosate persists in the environment for only a short time (half-life for biological availability in soil and sediments is hours, and 1-3 d in water; Giesy et al., 2000), is rapidly excreted by mammals and other vertebrates (Williams et al., 2000; Acquavella et al., 2004) and chronic effects, if any, would not be expected.

One of the major drawbacks of environmental epidemiology studies is the characterization of exposures to the agents being investigated. In this study two approaches were used to characterize exposures to glyphosate: ecological and self-reported. In the ecological study design, frequency of BNMN in participants was compared from regions with different patterns of pesticide use. As previously discussed (Sanin et al., 2009), this ecological design may result in misclassification of exposures (Arbuckle et al., 2004), but as an exploratory assessment of exposure it is useful (Ritter et al., 2006).

Others have attempted to improve assessment of exposure to pesticides in epidemiological studies. One study used a self-administered questionnaire for the assessment of exposure to glyphosate, which was defined as (a) ever personally mixed or applied products containing glyphosate; (b) cumulative lifetime days of use, or "cumulative exposure days" (years of use times days/year); and (c) intensity-weighted cumulative exposure days (years of use times days/year times estimated intensity level) (De Roos et al., 2005). A pesticide exposure score based on self-reported work practices was recently developed to estimate annual exposure level (Firth et al., 2007). Based on an algorithm to estimate lifetime exposure to glyphosate from questionnaire information, a moderate correlation was found with concentrations of glyphosate in urine and no significant correlation with self-reported exposure (Acquavella et al., 2004).

In our study, questions related to whether there was direct contact with the spray were used but this did not consider area of skin exposed, region of skin exposed, differences in rates of penetration, or personal hygiene.

Given the situation, the best approach possible, a prospective cohort, was used but the need to use better procedures to estimate the exposure is acknowledged. Based on the applicable Bradford–Hill guidelines (Hill, 1965), it is not possible to assign causality to the increases in frequency of BNMN observed in our study. There was a smaller frequency of BNMN and MOMN in the region of no pesticide use compared with the regions where pesticides (including glyphosate) were used, which is consistent with other reports in the literature. Although temporality was satisfied in the increase in frequency of BNMN after spraying, this response did not show strength as it was not consistently correlated with the rate of application. Recovery was also inconsistent with decreases in frequency of BNMN in the areas of eradication spraying but not in the area where lower rates were applied on sugar cane.

Further studies are needed to better characterize the potential genotoxic risk associated with the application of glyphosate for sugar cane maturation. The smaller number of subjects recruited in this study and small amount of information about the exposure precluded any conclusions. Many pesticides are used in conventional agriculture in Colombia and many pesticides are used in the production of coca (Solomon et al., 2007a, 2007b); however, there is not sufficient information to correlate the frequency of MN to the pesticide exposure.

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Non-Hodgkin's Lymphoma and Specific Pesticide Exposures in Men: Cross-Canada Study of Pesticides and Health[1]

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Abstract

Our objective in the study was to investigate the putative associations of specific pesticides with non-Hodgkin's Lymphoma [NHL; International Classification of Diseases, version 9 (ICD-9) 200, 202]. We conducted a Canadian multicenter population-based incident, case (n = 517)-control (n = 1506) study among men in a diversity of occupations using an initial postal questionnaire followed by a telephone interview for those reporting pesticide exposure of 10 h/year or more, and a 15% random sample of the remainder. Adjusted odds ratios (ORs) were computed using conditional logistic regression stratified by the matching variables of age and province of residence, and subsequently adjusted for statistically significant medical variables (history of measles, mumps, cancer, allergy desensitization treatment, and a positive history of cancer in first-degree relatives). We found that among major chemical classes of herbicides, the risk of NHL was statistically significantly increased by exposure to phenoxyherbicides [OR, 1.38; 95% confidence interval (CI), 1.06–1.81] and to dicamba (OR, 1.88; 95% CI, 1.32–2.68). Exposure to carbamate (OR, 1.92; 95% CI, 1.22–3.04) and to organophosphorus insecticides (OR, 1.73; 95% CI, 1.27–2.36), amide fungicides, and the fumigant carbon tetrachloride (OR, 2.42; 95% CI, 1.19–5.14) statistically significantly increased risk. Among individual compounds, in multivariate analyses, the risk of NHL was statistically significantly increased by exposure to the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D; OR, 1.32; 95% CI, 1.01–1.73), mecoprop (OR, 2.33; 95% CI, 1.58–3.44), and dicamba (OR, 1.68; 95% CI, 1.00–2.81); to the insecticides malathion (OR, 1.83; 95% CI, 1.31–2.55), 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT), carbaryl (OR, 2.11; 95% CI, 1.21–3.69), aldrin, and lindane; and to the fungicides captan and sulfur compounds. In additional multivariate models, which included exposure to other major chemical classes or individual pesticides, personal antecedent cancer, a history of cancer among first-degree relatives, and exposure to mixtures containing dicamba (OR, 1.96; 95% CI, 1.40–2.75) or to mecoprop (OR, 2.22; 95% CI, 1.49–3.29) and to aldrin (OR, 3.42; 95% CI, 1.18–9.95) were significant independent predictors of an increased risk for NHL, whereas a personal history of measles and of allergy desensitization treatments lowered the risk. We concluded that NHL was associated with specific pesticides after adjustment for other independent predictors.

Introduction

NHL[4] has been epidemiologically associated with farming (1–8), with certain farm practices (9), with pesticide exposure (10–13), and with certain other occupations (14–17). The term pesticide is used to denote a wide variety of chemicals used to destroy weeds (herbicides), insects (insecticides), and mold (fungicides). Such chemicals are widely used in agriculture, horticulture, and forestry, and in the secondary processing of the products of these primary industries. Many of the NHL and pesticide case-control or cohort studies focused either on a small geographical area (1, 2, 4) or on one occupational group (2, 4, 5, 9). Our study encompassed six provinces of Canada with diverse agricultural practices and a number of different types of occupational and nonoccupational exposures to pesticides. Non-Hodgkin's lymphoma incidence rates have been increasing in Canada for the last 25 years reflecting a worldwide trend (18) that has not been explained by improved diagnostic (19) methods or record-keeping (20).

Materials and Methods

Study Population. We conducted a population-based case-control study among men resident in six Canadian provinces to

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[3] Dr. Choi was a collaborator who is now deceased.

[4] The abbreviations used are: NHL, non-Hodgkin's lymphoma; DDT, 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane; STS, soft tissue sarcoma; HD, Hodgkin's disease; MM, multiple myeloma; 2,4-D, 2,4-dichlorophenoxyacetic acid; MCPA, 4-chloro-2-methylphenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; OR, odds ratio; OR_{adj}, adjusted OR; 95% CI, 95% confidence interval.

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test the pesticide-exposure hypothesis related to four rare tumors. Incident cases among men, ages 19 years or over, with a first diagnosis of STS, HD, NHL [International Classification of Diseases, version 9 (ICD-9), code 200 or 202], or MM diagnosed between September 1, 1991, and December 31, 1994, were eligible. To balance the number of cases by geographical regions, each province was assigned a target number of cases in each tumor category. Each province ceased to ascertain cases when their preassigned target was reached. This report is based solely on cases diagnosed with NHL. Cases were ascertained from provincial Cancer Registries except in Quebec, for which hospital ascertainment was used. The Cancer Registries and hospitals provided information, including pathology reports, to confirm the diagnosis. Pathological material was reviewed and classified according to the working formulation by the reference pathologist. Misclassified and ineligible (*e.g.*, Kaposi's sarcoma, known HIV-positive) cases were excluded. Subjects for whom pathological material was unavailable remained in the study. After physician consent was received, postal questionnaires and informed consent forms were mailed to potential cases. Surrogates for deceased cases were not contacted.

Men, ages 19 years and older, selected at random within age constraints from the provincial Health Insurance records (Alberta, Saskatchewan, Manitoba, Quebec), computerized telephone listings (Ontario), or voters' lists (British Columbia) were potential controls. The random control subject selection was stratified by age ± 2 years to be comparable with the age distribution of the entire case group (STS, HD, NHL, and MM) within each province. Postal questionnaires and informed consent forms were mailed to potential controls. Surrogates for deceased persons were ineligible as controls. All of the participating control subjects were used in the statistical analyses of each cancer site.

Pilot Study. We conducted a pilot study (21) in each provincial region to test study procedures and to determine an operational definition of pesticide exposure to distinguish between environmental (which includes bystander and incidental) and more intensive exposure. Nonoccupational use of pesticides (home, garden, hobby) was included. There were few individuals who were completely free of being exposed to pesticides. Therefore, we constructed graphs that demonstrated that the most efficient definition of pesticide exposure, which discriminated (*a*) between incidental, bystander, and environmental exposure as compared with more intensive exposure and (*b*) between cases and controls, was a cumulative total of 10 h per year to any combination of pesticides. The screening questions in the postal questionnaire were used to trigger telephone interviews among those with cumulative exposure of ≥10 h/year to any combination of herbicides, insecticides, fungicides, fumigants, and/or algicides. The 68 cases and 103 controls who participated in the pilot study are not included in this report.

Pesticides. Pesticide is a generic term describing a variety of compounds of diverse chemical structures and biological modes of action. In this study, the term pesticide refers primarily to herbicides, insecticides, fungicides, and fumigants.

We conducted a validation pilot study of the modified questionnaires (21). Volunteer farmers ($n = 27$) completed the questionnaires and granted permission for us to access their records of purchases through their local agrochemical supplier. The concordance between the two sources was excellent and discordance was explainable by (*a*) the farmer paid in cash and the supplier discarded the record; (*b*) the farmer purchased the agrochemical in the United States, and, therefore, the local supplier did not have a record; (*c*) the farmer paid for professional ground or aerial spraying, and the account was listed in another name; or (*d*) the supplier had destroyed the records.

Questionnaires. The questionnaires were modified versions of the telephone interview questionnaire that was used in studies of pesticide exposure and rare tumors in Kansas (11) and Nebraska (13). With permission, we modified the questionnaire to create postal and telephone interview questionnaires. To control for the effects of other variables known or suspected to be associated with the development of NHL after conducting an extensive literature review, we used the postal questionnaire to capture demographic characteristics, antecedent medical history, family history of cancer, detailed lifetime job history, and occupational exposure history to selected substances, accidental pesticide spills, and use of protective equipment, as well as details of cigarette smoking history. The telephone questionnaire characterized exposure to individual pesticides. The pesticide data were collected at several levels beginning with the broadest categories (*e.g.*, minimal exposure, occupations with potential pesticide exposure) and progressing sequentially to major classes (*e.g.*, herbicides); to chemical groups (*e.g.*, phenoxy herbicides); and finally to individual compounds (*e.g.*, 2,4-D, MCPA, and 2,4,5-T).

In this report, we focus on lifetime exposure to individual pesticides classified by active ingredients and to major chemical classes of herbicides, insecticides, fungicides, and fumigants. We classified exposure by the number of herbicides, insecticides, fungicides, and fumigants reported by cases and controls as well as by the number of days per year of exposure to individual compounds.

Each subject who reported 10 h per year or more of exposure to pesticides (any combination of compounds) as defined by the screening questions, and a 15% random sample of the remainder was mailed a list of pesticides (both chemical and brand names) and an information letter. Each subject was subsequently telephoned to obtain details of pesticide use.

The listed pesticides were chosen for inclusion (22–25): (*a*) if the compound was ever registered for use in Canada and reviewed by the IARC; (*b*) if the pesticide was recently banned or restricted in Canada by the federal licensing agency; or (*c*) if the pesticide was commonly used in Canada for specific purposes.

To ensure consistency, we developed and distributed manuals for provincial study coordinators, interviewers, and data managers. Before commencing data collection, we held a 2-day workshop with provincial coordinators to review data collection procedures and policies, to practice interviewing skills, and to review SPSS-DE (Statistical Packages for the Social Sciences-Data Entry),[5] the custom data entry program that we used. On receipt of a postal questionnaire, the provincial coordinator reviewed it for internal consistency and completeness. Data were computer-entered and verified in the province of origin, transported to the coordinating center, and rechecked for completeness, after which statistical analyses were performed.

Copies of the questionnaires and additional information on pesticides that were not included in this report are available from the corresponding author.

Pathology Review. Pathologists in participating provinces were requested to send blocks or slides of tumor tissue removed at surgery to the reference pathologist. Ten subjects with Ka-

[5] SPSS-Data Entry II Statistical Package for the Social Sciences: Statistical Data Analysis. SPSS Inc., Chicago, Illinois, 1998.

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Table 1 Comparisons of demographic, antecedent personal medical, general pesticide exposures and cigarette smoking history between cases of NHL and control subjects based on the postal questionnaire

	NHL, $n = 517$		Controls, $n = 1506$		OR[a] (95% CI)
	n	%	*n*	%	
Age, yr					
<30	64	12.4	356	23.6	
30–39	87	16.8	255	16.9	
40–49	111	21.5	238	15.8	
50–59	143	27.7	370	25.6	
>60	112	21.7	287	19.0	
Mean ± SD	57.7 ± 14		55.0 ± 16		
Residence on a farm at any time					
Yes	235	45.5	673	44.7	
No (reference)	279	54.0	828	55.0	1.06 (0.86–1.20)
Missing	3	0.6	5	0.3	
Pesticide exposure (screening question)					
<10 h/yr (reference)	379	73.3	1142	75.8	
≥10 h/yr	138	26.7	364	24.2	1.22 (0.96–1.55)
Smoking History					
Nonsmoker (reference)	160	30.9	526	34.9	
Ex-smoker	254	49.1	648	43.0	1.10 (0.86–1.41)
Current smoker	91	17.6	298	19.8	0.98 (0.72–1.33)
Missing data	12	2.3	34	2.3	
Current or ex-smoker	345	66.7	946	62.8	1.06 (0.86–1.20)
Medical History[b]					
Measles (yes)	251	48.5	888	59.0	**0.64 (0.51–0.79)**
Mumps (yes)	194	37.5	588	39.0	**0.75 (0.60–0.93)**
Previous cancer (yes)	73	14.1	87	5.8	**2.43 (1.71–3.44)**
Skin-prick allergy test	34	6.6	196	13.0	**0.52 (0.34–0.76)**
Allergy desensitization shots (yes)	18	3.5	114	7.6	**0.49 (0.29–0.83)**
Family history of cancer any first-degree relative (yes)	219	42.4	497	33.0	**1.31 (1.05–1.62)**

[a] OR stratified by age and by province of residence.

[b] Also tested and found to be unassociated: acne; asthma; celiac disease; chickenpox; diabetes; hay fever; mononucleosis; rheumatic fever; rheumatoid arthritis; ringworm; shingles; syphilis; tuberculosis; urinary tract infections; whooping cough; allergies; drug treatment for overactive thyroid; treatment for head lice, body lice, or scabies; medical implants; drug treatment for epilepsy; tonsillectomy; positive allergy prick skin test, patch skin test, or positive patch skin test for allergy.

posi's sarcoma were omitted on the basis of the etiological association with HIV infection. Any other known HIV-positive subjects had been previously excluded. Eighty-four % (436 of 517) of the NHL tumors were validated. Because of a change midstudy in some hospitals' policies regarding supplying pathological material without charge, we were unable to obtain the remaining samples.

Statistical Analyses. Data from the postal and telephone interviews were merged by using the identification number. Of the individuals selected randomly for a telephone interview, most had used one or no chemical pesticides. We reviewed these data and decided to include them in the statistical analyses because they might be informative with respect to low levels of exposure to pesticides and their inclusion maximized our sample size with respect to other known or suspected risk factors for NHL. We conducted descriptive analyses of each variable, which included, where applicable, frequencies, ranges, means ± SD, and median values for cases and controls separately.

To evaluate putative risk factors for NHL, conditional logistic regression was used to compute ORs and 95% CIs, stratifying by age groups and province of residence.[6] ORs were calculated for categorical variables related to medical history that were selected based on previous studies (*e.g.*, measles, mumps, previous cancer, allergy desensitization treatment, skin prick allergy test); pesticide exposure (<10 and ≥10 h per year); and smoking history. Using conditional logistic regression, ORs were also calculated for (*a*) major chemical classes of herbicides, insecticides, fungicides, and fumigants; and (*b*) for individual active chemicals. The statistically significant ($P < 0.05$) medical variables were used to adjust the effect of exposure to pesticides classified by major chemical group and by individual active chemical. Given the study sample size and the case-control ratio, *a priori* power calculations indicated that we had sufficient statistical power to detect an OR of 2 when at least 1% of the controls was exposed to a specific pesticide or chemical class of pesticide. Conditional logistic analyses (26) were conducted that retained in the model, all covariates for which the P was ≤.05. The criterion for entry into models was a $P \leq 0.20$ in bivariate age and province stratified analyses.

We created dose-response levels based on days/year of personally mixing or applying selected herbicides, insecticides, fungicides, and fumigants. We reported ORs stratified by age and province of residence. We created exposure categories for exposures to multiple different herbicides, insecticides, fungicides, and fumigants. For these analyses, the unexposed category was specific to the class of pesticide. We also created exposure categories for exposures to combinations of herbicides, insecticides, fungicides, and fumigants for which the reference group did not report exposure to any of those classes of pesticides.

[6] EGRET Intuitive Software for DOS Micros Statistics and Epidemiology Research Corporation, 1993.

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Table 2 Herbicides: frequency of exposure to herbicides classified into major chemical classes and as individual compounds

The list includes only those reported by 1% or more of responders.

Major chemical classes	NHL $n = 517$		Controls $n = 1506$		OR[a] (95% CI)	OR_{adj}[b] (95% CI)
	n exposed	% exposed	n exposed	% exposed		
Phenoxyherbicides,[c] exposed	131	25.3	319	21.2	**1.46 (1.09–1.82)**	**1.38 (1.06–1.81)**
Individual phenoxyherbicides						
2,4-D	111	21.5	293	19.5	1.26 (0.97–1.64)	**1.32 (1.01–1.73)**
Mecoprop	53	10.2	81	5.4	**2.23 (1.38–3.07)**	**2.33 (1.58–3.44)**
MCPA	17	3.3	46	3.1	1.08 (0.59–1.94)	1.10 (0.60–2.00)
Diclofopmethyl	9	1.7	25	1.7	0.96 (0.42–2.20)	0.95 (0.41–2.22)
Phosphonic acid,[d] exposed	63	12.2	147	9.8	1.42 (0.95–1.90)	1.40 (0.94–1.89)
Individual phosphonic herbicides						
Glyphosate (Round-up)	51	9.9	133	8.8	1.26 (0.87–1.80)	1.20 (0.83–1.74)
Thiocarbamates,[e] exposed	21	4.1	49	3.3	1.41 (0.62–2.20)	1.46 (0.82–2.58)
Individual thiocarbamate herbicides						
Diallate (n exposed)	11	2.1	29	1.9	1.26 (0.59–2.67)	1.46 (0.68–3.14)
Phenols: Bromoxynil,[f] exposed	16	3.1	48	3.2	1.05 (0.41 1.69)	1.07 (0.58–1.99)
Dicamba,[g] exposed	73	14.1	131	8.7	**1.92 (1.39–2.66)**	**1.88 (1.32–2.68)**
Individual dicamba herbicides						
Dicamba (Banvel or Target)	26	5.0	50	3.3	1.59 (0.95–2.63)	**1.68 (1.00–2.81)**
Dinitroaniline,[h] exposed	11	2.1	31	2.1	1.17 (0.56–2.41)	1.20 (0.61–2.35)
Individual dinitroaniline herbicides						
Trifluralin	11	2.1	31	2.1	1.17 (0.56–2.41)	1.06 (0.50–2.22)

[a] ORs calculated with strata for the variables of age and province of residence.
[b] ORs adjusted for statistically significant medical variables (history of measles, mumps, cancer, allergy desensitization shots, and a positive family history of cancer in a first-degree relative), and with strata for the variables of age and province of residence.
[c] Phenoxyherbicides include the phenoxyacetic acids (*e.g.*, 2,4-D and MCPA), the phenoxy-2-propionic acids (*e.g.*, mecoprop); the phenoxybutanoic acids (*e.g.*, 2,4-DB) and other phenoxyalkanoic acids (*e.g.*, diclofopmethyl).
[d] Glyphosate is the only phosphonic acid herbicide reported by more than 1% of responders. Round-up, Touchdown, Victor, Wrangler, Laredo do not include dicamba, and Rustler is a mixture of dicamba and glyphosate.
[e] Thiocarbamate herbicides include diallate and triallate.
[f] Bromoxynil is the only phenol herbicide included.
[g] Dicamba as a major chemical class includes Banvel, and Target, and a mixture of dicamba and glyphosate (Rustler), or mixtures of dicamba, 2,4-D, and mecoprop (Dynel DS, Killex).
[h] Dinitroaniline herbicides include ethalfluralin and trifluralin.

Ethics. The protocol, letters of informed consent, questionnaires, and all other correspondence with potential subjects were approved by the relevant agencies in each province. All of the information that could be used to identify individuals remained within the province of origin under the control of the provincial principal investigators.

Results

Data from postal questionnaires based on responses from 517 NHL cases (67.1% of those contacted) and 1506 control subjects (48.0% of those contacted) were analyzed. Similar percentages of potential subjects resident in rural and urban areas responded. There were higher percentages of responders in the middle-age group than at either extreme among both cases and controls. Detailed information related to their pesticide exposure history was obtained by telephone interview from 119 NHL cases and 301 control subjects who indicated pesticide exposure of 10 h per year or more. A 15% random sample of cases and controls who indicated pesticide exposure of less than 10 h/year was also interviewed by telephone, resulting in detailed pesticide exposure information on 60 cases of NHL and on 155 controls. The total telephone interviewed sample consisted of 179 cases of NHL and 456 controls.

A summary of selected demographic, antecedent personal and familial medical history, general pesticide exposure as measured by the screening questions, and cigarette smoking history comparisons of NHL cases and population-based controls is shown in Table 1. Because all of the controls (age-matched for STS, MM, HD, and NHL) were used in the analysis, cases were older than controls. Cases and controls were similar in their smoking patterns. Cases were less likely to have a history of measles or mumps and more likely to have a personal history of a previous primary cancer. Cases were more likely than controls to have a positive family history of cancer, whereas more controls had undergone allergy desensitization injections. A slightly higher proportion of cases than controls indicated cumulative exposure to pesticides of $\geq$10 h per year.

Table 2 summarizes reported exposure to herbicides classified by major chemical classes (phenoxy, phosphonic acid, thiocarbamates, phenols, dicamba, and dinitroaniline) and by individual compounds for which at least 1% of responders reported exposure. ORs are also shown after adjustment for the statistically significant ($P < 0.05$) variables reviewed in Table 1, which included a history of measles, mumps, cancer, and allergy desensitization shots and a positive history of cancer in a first-degree relative. Cases experienced a significantly higher frequency of exposure to phenoxyherbicides, to dicamba or a mixture including dicamba, to 2,4-D, and to mecoprop.

Table 3 summarizes the insecticide exposure data. Exposure to two major chemical classes, carbamates and organophosphates, was statistically significantly associated with NHL, whereas exposure to organochlorines as a group was not.

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Table 3 Insecticides: frequency of exposure to insecticides classified into major chemical classes and as individual compounds

Major chemical classes	NHL n = 517		Controls n = 1506		OR[a] (95% CI)	OR_{adj}[b] (95% CI)
	n exposed	% exposed	n exposed	% exposed		
Carbamates,[c] exposed	37	7.2	60	4.0	**1.95 (1.25–3.05)**	**1.92 (1.22–3.04)**
Individual carbamate insecticides						
Carbaryl	25	4.8	34	2.3	**2.05 (1.18–3.55)**	**2.11 (1.21–3.69)**
Carbofuran	9	1.7	18	1.2	1.58 (0.68–3.67)	1.64 (0.70–3.85)
Methomyl	6	1.2	13	0.9	1.86 (0.67–5.17)	1.65 (0.54–5.03)
Organochlorine, (1)[d] exposed	50	9.7	134	8.9	1.16 (0.81–1.66)	1.27 (0.87–1.84)
Individual organochlorine (1) insecticides						
Chlordane	36	7.0	105	7.0	1.06 (0.71–1.59)	1.11 (0.74–1.69)
Lindane	15	2.9	23	1.5	**2.05 (1.01–4.16)**	**2.06 (1.01–4.22)**
Aldrin	10	1.9	6	0.4	**3.81 (1.34–10.79)**	**4.19 (1.48–11.96)**
Organochlorine (2) diphenylchlorides[e] exposed	86	16.6	233	15.5	1.24 (0.94–1.65)	1.21 (0.90–1.62)
Individual organochlorine (2) diphenylchlorides						
Methoxychlor	65	12.6	201	13.3	1.08 (0.79–1.47)	1.02 (0.74–1.41)
DDT	32	6.2	59	3.9	**1.63 (1.03–2.57)**	**1.73 (1.08–2.76)**
Organophosphorus,[f] exposed	90	17.4	167	11.1	**1.69 (1.26–2.27)**	**1.73 (1.27–2.36)**
Individual organophosphorus insecticides						
Malathion	72	13.9	127	8.4	**1.77 (1.28–2.46)**	**1.83 (1.31–2.55)**
Dimethoate	22	4.3	50	3.3	1.20 (0.71–2.03)	1.20 (0.70–2.06)
Diazinon	18	3.5	28	1.9	1.72 (0.92–3.19)	1.69 (0.88–3.24)

[a] ORs calculated with strata for the variables of age and province of residence.
[b] ORs adjusted for statistically significant medical variables (history of measles, mumps, cancer, allergy desensitization shots and a positive family history of cancer in a first-degree relative), and with strata for the variables of age and province of residence.
[c] Carbamate insecticides include carbaryl, carbofuran, and methomyl.
[d] Organochlorine insecticides class one includes aldrin; chlordane; dieldrin; endrin; heptachlor; lindane; and a mixture of lindane, carbathiin, and thiram (Vitavex).
[e] Organochlorine (2) diphenylchloride insecticides include DDT and methoxychlor.
[f] Organophosphorus insecticides include malathion, chlorpyrifos, diazinon, dimethoate, parathion, methidathion, and trichlorfon.

Table 4 Fungicides: frequency of exposure to fungicides classified into major chemical classes and as individual compounds

Major chemical classes	NHL n = 517		Controls n = 1506		OR[a] (95% CI)	OR_{adj}[b] (95% CI)
	n exposed	% exposed	n exposed	% exposed		
Amide,[c] exposed	30	5.8	58	3.9	**1.69 (1.05–2.73)**	**1.70 (1.04–2.78)**
Individual amide fungicides						
Captan	20	3.9	24	1.6	**2.48 (1.33–4.63)**	**2.51 (1.32–4.76)**
Vitavax	10	1.9	39	2.6	0.88 (0.42–1.85)	0.88 (0.41–1.87)
Aldehyde,[d] exposed	7	1.4	25	1.7	0.85 (0.35–2.07)	0.92 (0.37–2.29)
Individual aldehyde fungicides						
Formaldehyde	7	1.4	255	1.7	0.85 (0.35–2.07)	0.92 (0.37–2.29)
Mercury Containing,[e] exposed	18	3.5	48	3.2	1.09 (0.61–1.95)	1.28 (0.70–2.27)
Mercury-containing fungicides						
Mercury dust (n exposed)	15	2.9	39	2.6	1.08 (0.57–2.04)	1.23 (0.64–2.35)
Mercury liquid (n exposed)	8	1.5	22	1.5	1.15 (0.49–2.69)	1.40 (0.74–3.22)
Sulphur Compounds	17	3.3	21	1.4	**2.26 (1.16–4.40)**	**2.80 (1.41–5.57)**

[a] ORs calculated with strata for the variables of age and province of residence.
[b] ORs adjusted for statistically significant medical variables (history of measles, mumps, cancer, allergy desensitization shots, and a positive family history of cancer in a first-degree relative), and with strata for the variables of age and province of residence.
[c] Amide fungicides include captan and a mixture of carbathiin, thiram, and lindane (Vitavax).
[d] Aldehyde fungicides include formaldehyde and a mixture of formaldehyde and iprodione (Rovral Flo).
[e] Mercury-containing fungicides include mercury dusts (Ceresan, Reytosan, and Agrox) and mercury liquids (Panogen, Leytosol, and PMAS).

Among individual carbamate compounds, exposure to carbaryl was statistically significantly associated with NHL. Among organochlorines, exposure to lindane, to aldrin, and to DDT was significantly associated with NHL. Malathion was the only individual organophosphate exposure statistically significantly associated with NHL.

Exposure to fungicides is summarized in Table 4. The fungicides with an amide group (OR_{adj}, 1.70; 95% CI, 1.04–2.78) were associated with NHL, whereas aldehydes and those containing mercury were not. Among individual amide-containing compounds, exposure to captan (OR_{adj}, 2.51; 95% CI, 1.32–4.76) was associated with NHL.

Malathion used as a fumigant was not associated with NHL (Table 5). There were fewer users of malathion as a fumigant compared with its use on crops. Carbon tetrachloride fumigant exposure (OR_{adj}, 2.42; 95% CI, 1.19–5.14) was associated with NHL.

Table 6 shows the results of a conditional logistic regres-

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Table 5 Frequency of exposure to fumigants: individual compounds

Individual compounds+	NHL $n = 517$		Controls $n = 1506$		OR[a] (95% CI)	OR_{adj}[b] (95% CI)
	n exposed	% exposed	n exposed	% exposed		
Malathion[c]	12	2.3	23	1.5	1.49 (0.72–3.11)	1.54 (0.74–3.22)
Carbon tetrachloride[d]	13	2.5	18	1.2	**2.13 (1.02–4.47)**	**2.42 (1.19–5.14)**

[a] ORs calculated with strata for the variables age and province of residence.
[b] ORs adjusted for statistically significant medical variables (history of measles, mumps, cancer, allergy desensitization shots, and a positive family history of cancer in a first-degree relative) and with strata for the variables age and province of residence.
[c] Malathion is an organophosphorus insecticide which has been used indoors as a fumigant.
[d] Carbon tetrachloride was used as a grain fumigant.

Table 6 Most parsimonious model: conditional logistic regression analyses that contained major chemical classes of pesticides and important covariates ($P < 0.05$)

Phenoxyherbicides as a group, carbamate, and organophosphate insecticides, amide group containing fungicides, and carbon tetrachloride users/nonusers were included in the initial multivariate model and found not to contribute significantly to the risk of NHL.

Variable	Parameter Estimate ± SE	OR (95% CI)
Measles (yes)	−0.47 ± 0.11	**0.62 (0.50–0.78)**
Previous cancer (yes)	0.79 ± 0.18	**2.20 (1.54–3.15)**
First-degree relative with cancer (yes)	0.32 ± 0.11	**1.37 (1.10–1.71)**
Allergy desensitization shots (yes)	−0.65 ± 0.27	**0.52 (0.31–0.89)**
Dicamba mixtures (user)	0.67 ± 0.17	**1.96 (1.40–2.75)**

Table 7 Most parsimonious model: conditional logistic regression analyses that contained individual chemical pesticides and important covariates ($P < 0.05$)

Among individual pesticides, carbaryl, lindane, DDT, and malathion insecticides, and captan fungicide user/nonuser were included in the initial multivariate model and found not to contribute significantly to the risk of NHL.

Variable	Parameter estimate ± SE	OR (95% CI)
Measles (yes)	−0.48 ± 0.11	**0.50 (0.45–0.83)**
Previous cancer (yes)	0.80 ± 0.18	**2.23 (1.56–3.19)**
First-degree relative with cancer (yes)	0.32 ± 0.11	**1.38 (1.11–1.72)**
Allergy desensitization shots (yes)	−0.68 ± 0.27	**0.51 (0.30–0.87)**
Mecoprop (user)	0.80 ± 0.20	**2.22 (1.49–3.29)**
Aldrin (user)	1.23 ± 0.54	**3.42 (1.18–9.95)**

sion model that included major chemical classes of pesticides and all other covariates for which $P < 0.05$. The variables that remained statistically significantly associated with increased risk of NHL were a previous personal history of another malignancy, a history of cancer among first-degree relatives, and exposure to dicamba and mixtures containing dicamba. ORs for a personal history of measles or of allergy desensitization injections were significantly lower than those without this history. Table 7 summarizes a similar model that included individual pesticides and all of the other covariates for which $P < 0.05$ and in which mecoprop and aldrin exposure as well as the same covariates as in Table 6 were associated with NHL.

Table 8 shows the frequency of exposure to selected individual herbicides, insecticides, fungicides, and fumigants, stratified by the average number of days per year of exposure. In general, the results of these dose-response analyses are consistent with the exposed/nonexposed findings. Those compounds for which we found statistically significant case-control differences also have elevated ORs based on strata of the variable "days per year of exposure" (mecoprop, dicamba, malathion, DDT, captan, carbon tetrachloride, and sulfur). The exceptions were 2,4-D, for which there was no dose-response relationship, and glyphosate, which was not significant for exposure but for which we demonstrated a dose-response relationship.

Table 9 compares the frequencies of multiple herbicide, insecticide, fungicide, and fumigant use among cases and controls. Cases are significantly more likely to report exposure to between two and four herbicides or insecticides but not to five and more of either. An elevated OR was found for exposure to two or more fungicides. Table 9 also shows a dose-response relationship in comparisons of subjects who reported no pesticide exposure and those who reported using five or more pesticides.

Discussion

The hypothesis that farming (1–8), agricultural practices (9), and pesticide exposure (10–13, 22–25) are associated with NHL has been tested in a number of occupational studies. Not all of the studies confirm an association (27–29). Pesticides have diverse chemistry and biological modes of action. In addition to the active ingredients, there are emulsifiers, carriers, dispersants, and a variety of agents used to formulate liquids, granular and mists. The major chemical classes of *a priori* interest based on epidemiological studies (10–13, 22–25) were phenoxyherbicides, organophosphorus, organochlorines, aldehydes, and carbon tetrachloride. Occupational exposure to 2,4-D, 2,4,5-T, carbaryl, chlordane, DDT, diazinon, dichlorvos, lindane, malathion, nicotine, and toxaphene has been reported to be associated with NHL. In addition, our interest focused on pesticides classified as possibly or probably carcinogenic to humans based on evaluations by the IARC expert panels (Refs. 22–25; phenoxyherbicides including 2,4-D, MCPA, and 2,4,5-T as a group, atrazine, chlordane, DDT, dichlorvos, heptachlor, and pentachlorophenol). Our bivariate results for exposure to groups of phenoxyherbicides or dicamba-containing herbicides, for carbamates and organophosphorus insecticides, and for amide fungicides and for carbon tetrachloride were not attenuated when simultaneously adjusted for the important medical covariates (history of measles, mumps, cancer, allergy desensitization shots, and a positive history of cancer in a first-degree relative).

Among individual compounds, our results that related to exposure to 2,4-D, mecoprop, dicamba, malathion, DDT, carbaryl, lindane, aldrin, captan, and sulfur compounds were not attenuated after simultaneous adjustment for the same medical covariates. Clearly, we had few exposed men whose exposure was limited to one pesticide or to one class of pesticides. Our results show elevated risk for exposure to multiple herbicides, insecticides, and fungicides.

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Table 8 Frequency of exposure to selected herbicides, insecticides, fungicides, and fumigants stratified by the number of days per year of exposure

Models that included the time variable "days per year" and stratification for age and province of residence were also assessed for the individual herbicide compounds bromoxynil, 2,4-DB, diallate, MCPA, triallate, and treflan. No significant associations were found.

Individual compounds	Days/yr	NHL		Controls		OR[a] (95% CI)
		n	%	*n*	%	
Herbicides						
2,4-D	Unexposed	406	78.5	1213	80.5	1
	>0 and ≤2	55	10.6	160	10.6	1.17 (0.83–1.64)
	>2 and ≤5	36	7.0	82	5.4	1.39 (0.91–2.13)
	>5 and ≤7	9	1.7	20	1.3	1.38 (0.60–3.15)
	>7	11	2.1	31	2.1	1.22 (0.60–2.49)
Mecoprop	Unexposed	464	89.8	1425	94.6	1
	>0 and ≤2	31	6.0	48	3.2	**2.27 (1.40–3.68)**
	≥2	22	4.3	33	2.2	**2.06 (1.17–3.61)**
Phosphonic acid: glyphosate	Unexposed	466	90.1	1373	91.2	1
	>0 and ≤2	28	5.4	97	6.4	1.00 (0.63–1.57)
	>2	23	4.5	36	2.4	**2.12 (1.20–3.73)**
Dicamba	Unexposed	491	95.0	1456	96.7	1
	≥1	26	5.0	50	3.3	1.58 (0.96–2.62)
Insecticides						
Malathion	Unexposed	445	87.0	1379	91.6	1.00
	>0 and ≤2	50	9.7	88	5.8	**1.82 (1.25–2.68)**
	≥2	22	4.3	39	2.6	**1.75 (1.02–3.03)**
DDT	Unexposed	485	93.8	1447	96.1	1.00
	>0 and ≤2	18	3.5	32	2.1	1.75 (0.96–3.21)
	>2	14	2.7	27	1.8	1.50 (0.77–2.91)
Fungicides						
Captan	Unexposed	497	96.1	1482	98.4	1.00
	>0 and ≤2	11	2.1	12	0.8	**2.69 (1.17–6.19)**
	>2	9	1.7	12	0.8	**2.80 (1.13–6.90)**
Sulphur	Unexposed	500	96.7	1485	98.6	1.00
	Exposed ≥1	17	3.3	21	1.4	**2.26 (1.16–4.40)**
Fumigant						
Carbon tetrachloride	Unexposed	504	97.5	1488	98.8	1.00
	>0 and ≤2	13	2.5	18	1.2	**2.13 (1.02–4.47)**

[a] ORs calculated with strata for the variables age and province of residence.

The strength of our results is enhanced by their internal consistency as we applied the strategy of assessing risk by different analytic approaches progressing from exposure to: (*a*) major chemical classes of herbicides, insecticides, fungicides, and fumigants; (*b*) individual compounds within those major chemical classes; and (*c*) individual compounds stratified by days per year of exposure. We constructed models that included potential confounders (*e.g.*, positive history of cancer in a first-degree relative). Generally, the same individual compounds or class of compounds was associated with case status. The risk estimates based on exposure to major chemical classes or to individual compounds tended to be precise, as indicated by the 95% CIs.

Our results confirm previously reported associations of NHL and a personal history of cancer (30, 31), of NHL and a history of cancer among first-degree relatives (32, 33), and of NHL and exposure to selected pesticides (1, 3, 5, 9–13). We were unable to find a previous report suggesting a protective effect of allergy desensitization shots. Koepsell *et al.* reported little association of the number of allergy desensitization shots and MM (34). The relationship between allergy and cancer is complex with well-designed studies reporting opposite results (35–38). Cigarette smoking was not a risk factor overall, confirming one study (39) and contradicting others (40, 41), although certain subtypes (39, 40) of NHL may be associated with cigarette smoking.

The limitations of this study relate to those inherent in the case-control design, specifically the potential for recall bias and for misclassification of pesticide exposure. Hoar *et al.* and Zahm *et al.* (11, 13), as well as others (27–29, 42–45), have dealt extensively with these issues among farmers. We have included individuals in many different occupations as well as home and garden users. These are groups for whom we did not find extensive validation studies. Their inclusion may have biased our dose-response findings toward the null, although the yes/no responses to individual pesticides would be less affected. We reduced the number of surrogate responders by excluding deceased persons from our definition of eligible subjects. This strategy was useful in decreasing the potential for misclassification of exposure.

A second limitation is the less-than-optimal response rates. We continued to recruit subjects in each province until the target numbers were achieved. We compared respondents to nonrespondents using postal codes as an indicator of rural residence, and we did not find a rural bias among respondents.

We reported results for a number of chemical agents and exposures, not all of which were specified in the hypothesis. Therefore, the statistical analyses related to these unspecified agents should be considered exploratory. As a consequence of conducting multiple comparisons, a small number of statistically significant results may be attributable to chance.

The two-tiered study design permitted us to obtain detailed information related to factors other than pesticides that are known or suspected of being etiologically associated with NHL. The mailing of a list of pesticides with both trade and generic chemical names followed by a telephone interview

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Table 9 Distribution of numbers of exposures to multiple types of pesticides among cases and controls

	NHL		Controls		OR[a] (95% CI)
	n	%	*n*	%	
Multiple herbicide use					
Unexposed[b]	374	72.3	1148	76.2	1.00
Exposed 1	45	8.7	146	9.7	1.02 (0.70–1.47)
Exposed 2–4	73	14.1	151	10.0	**1.75 (1.27–2.42)**
Exposed ≥5	25	4.8	61	4.1	1.41 (0.84–2.35)
Multiple insecticide use					
Unexposed	370	71.6	1154	76.6	1.00
Exposed 1	44	8.5	127	8.4	1.24 (0.85–1.80)
Exposed 2–4	86	16.6	189	12.6	**1.58 (1.17–2.13)**
Exposed ≥5	17	3.3	36	2.4	1.46 (0.79–2.69)
Multiple fungicide use					
Unexposed	457	88.4	1361	90.4	1.00
Exposed 1	32	6.2	90	6.0	1.08 (0.70–1.67)
Exposed ≥2	28	5.4	55	3.7	1.61 (.99–2.63)
Multiple fumigant use					
Unexposed	487	94.2	1440	95.6	1.00
Exposed ≥1	30	5.8	66	4.4	1.45 (0.91–2.63)
Multiple pesticide use[c]					
Unexposed	357	69.1	1095	72.7	1.00
Exposed 1–4	77	14.9	230	15.3	1.09 (0.81–1.46)
Exposed ≥5	83	16.1	181	12.0	**1.57 (1.16–2.14)**

[a] ORs calculated with strata for the variables age and province of residence.
[b] With the exception of the variable multiple pesticide use, the "unexposed" referent category is specific to the class of pesticides.
[c] The unexposed referent category contains those who did not report exposure to herbicides, insecticides, fungicides, or fumigants.

allowed the collection of detailed information concerning pesticide exposure. The statistical power of our study was enhanced by the large number of cases and controls. In instances of rare exposures (<1% exposed), we had limited statistical power to detect associations. We restricted our analyses of individual pesticide compounds to those for which at least 1% of respondents indicated exposure.

The study was not restricted to pesticide exposure experienced by a specific occupational group. Occupational exposure was quite diverse; single *versus* multiple pesticides; indoor *versus* outdoor applications. For example, men who work in animal confinement buildings, grain elevators, and pesticide manufacturing have different exposure patterns in comparison with grain farmers and commercial applicators. Because this study encompassed a large geographical area of Canada, there was substantial diversity among agricultural enterprises and in the patterns and types of pesticide exposure.

Delineating the putative relationship between exposure to pesticides and NHL is complicated: (*a*) by the subject's exposure to a variety of different pesticides many of which are not mutagenic, teratogenic, or carcinogenic when tested as a single compound; (*b*) by the complexity of formulations of pesticides, the details of which are privileged proprietary information; (*c*) by the diversity of routes of possible exposure, which include ingestion, dermal, inhalation, and ocular; (*d*) by unexpected interactions among seemingly unrelated exposures, such as the increased permeability of rubber gloves to 2,4-D when exposed simultaneously to the insect repellent DEET and sunlight (46); and (*e*) by the role of differential genetic susceptibility.

Garry *et al.* (47) describe a potential mechanism to explain the relationship between exposure to specific pesticides and an increased risk of developing NHL. They have demonstrated specific chromosomal alterations in the peripheral lymphocytes of pesticide applicators exposed to a variety of pesticide classes. A higher frequency of chromosomal breaks involving band 18q21 was found in men who applied only herbicides compared with nonoccupationally exposed controls. Higher frequencies of rearrangements and breaks involving band 14q32 were found among men who applied herbicides, insecticides, and fumigants compared with controls. Reciprocal translocations between chromosomes 14q32 and 18q21 are frequently found in NHL patients.

Our results support previous findings of an association between NHL and specific pesticide exposures. Our strategy of assessing risk by several different approaches, beginning with general categories (*e.g.*, herbicides), proceeding through cumulative pesticide exposure to specific chemical classes, and proceeding further to specific chemicals, proved effective in delineating complex relationships. In our final models, NHL was associated with a personal history of cancer; a history of cancer in first-degree relatives; and exposure to dicamba-containing herbicides, to mecoprop, and to aldrin. A personal history of measles and of allergy desensitization treatments lowered risk.

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Exposure to Pesticides as Risk Factor for Non-Hodgkin's Lymphoma and Hairy Cell Leukemia: Pooled Analysis of Two Swedish Case-control Studies

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(In final form 30 October 2001)

Increased risk for non-Hodgkin's lymphoma (NHL) following exposure to certain pesticides has previously been reported. To further elucidate the importance of phenoxyacetic acids and other pesticides in the etiology of NHL a pooled analysis was performed on two case-control studies, one on NHL and another on hairy cell leukemia (HCL), a rare subtype of NHL. The studies were population based with cases identified from cancer registry and controls from population registry. Data assessment was ascertained by questionnaires supplemented over the telephone by specially trained interviewers. The pooled analysis of NHL and HCL was based on 515 cases and 1141 controls. Increased risks in univariate analysis were found for subjects exposed to herbicides (OR 1.75, CI 95% 1.26–2.42), insecticides (OR 1.43, CI 95% 1.08–1.87), fungicides (OR 3.11, CI 95% 1.56–6.27) and impregnating agents (OR 1.48, CI 95% 1.11–1.96). Among herbicides, significant associations were found for glyphosate (OR 3.04, CI 95% 1.08–8.52) and 4-chloro-2-methyl phenoxyacetic acid (MCPA) (OR 2.62, CI 95% 1.40–4.88). For several categories of pesticides the highest risk was found for exposure during the latest decades before diagnosis. However, in multivariate analyses the only significantly increased risk was for a heterogeneous category of other herbicides than above.

Keywords: Non-Hodgkin's lymphoma; Hairy cell leukemia; Pesticides; Phenoxyacetic acids; Glyphosate; Impregnating agents

INTRODUCTION

Non-Hodgkin's lymphoma (NHL) is one of the malignant diseases with the most rapidly increasing incidence in the western world [1]. In Sweden, the mean age-adjusted incidence increased yearly by 3.6% in men and 2.9% in women during the time period 1958–1992 [2]. Hairy cell leukemia (HCL) was first described in 1958 and is regarded as a rare subgroup of NHL. HCL is more common in men with 23 male and 9 female patients reported to the Swedish Cancer Register in 1999 for the whole country [3].

The etiology of NHL is regarded to be multifactorial with different environmental exposures being part of it. Certain immunodefective conditions are established risk factors such as immunosuppressive medication after organ transplantation [4,5] and HIV-infection [6]. Also viral genesis, especially regarding Epstein–Barr virus (EBV) and endemic African Burkitt lymphoma has been indicated [7].

Regarding chemicals, exposure to phenoxyacetic acids, chlorophenols and organic solvents were associated with increased risk for NHL in Swedish studies [8–10]. In subsequent studies exposure to phenoxyacetic acids, particularly 2,4-dichlorophenoxyacetic acid (2,4-D), was associated with an increased risk for NHL [11,12]. These associations have been reviewed by us giving reference also to other studies [13].

We have now performed one case-control study on NHL, which did not include HCL [14], and another on HCL, specifically [15]. Both these studies focused interest especially on exposure to pesticides. In the NHL study, we found increased risks for subjects exposed to herbicides or fungicides. Among herbicides, phenoxyacetic acids

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TABLE I Number of exposed cases and controls, odds ratio (OR) and 95% confidence interval (CI) for exposure to pesticides and organic solvents

Agent	Number of exposed cases/controls	OR	CI
Herbicides	77/103	1.75	1.26–2.42
Phenoxyacetic acids	64/90	1.65	1.16–2.34
MCPA	21/23	2.62	1.40–4.88
2,4-D + 2,4,5-T	48/70	1.48	0.99–2.20
Glyphosate	8/8	3.04	1.08–8.52
Other	15/13	2.90	1.34–6.37
Insecticides	112/184	1.43	1.08–1.87
DDT	77/138	1.27	0.92–1.73
Mercurial seed dressing	20/33	1.40	0.77–2.47
Pyrethrins	13/27	1.16	0.57–2.25
Fungicides	18/17	3.11	1.56–6.27
Impregnating agents	104/162	1.48	1.11–1.96
Chlorophenols	66/106	1.37	0.98–1.92
Pentachlorophenol	64/101	1.40	0.99–1.98
Arsenic	8/10	1.75	0.66–4.54
Creosote	22/35	1.54	0.87–2.66
Other	40/67	1.35	0.88–2.04
Organic solvents	250/492	1.16	0.93–1.44

dominated. One subclass of these, 4-chloro-2-methyl phenoxyacetic acid (MCPA), turned out to be significantly associated with NHL. For several categories of herbicides, we observed that only exposure during the latest decades before diagnosis of NHL was associated with an increased risk for NHL. In the HCL study, we found increased risk for exposure to different categories of pesticides [15]. However, due to comparatively low number of study subjects, it was not meaningful to make further analyses of the tumor induction period.

Thus, the risk patterns for NHL and HCL in these studies, performed by the same methodology, showed similarities with respect to pesticides. Since the NHL study included patients with many different variants of NHL, it seemed motivated also to include HCL, as nowadays being regarded as a NHL subgroup, in a pooled analysis regarding risks in relation to pesticide exposure. The purpose was to enlarge the study size thereby allowing more precise risk estimates.

MATERIALS AND METHODS

Cases

The NHL study encompassed male cases aged ≥25 years with NHL diagnosed during 1987–1990 and living in the four most northern counties of Sweden and three counties in mid-Sweden [14]. They were recruited from the regional cancer registries and only cases with histopathologically verified NHL were included, in total 442 cases. Of these cases 192 were deceased.

From the national Swedish Cancer Registry, 121 male patients with HCL diagnosed during 1987–1992 were identified from the whole country [15]. One case later turned out to have been diagnosed in 1993, but was included in the study. Only living cases were included.

Controls

For living NHL cases two male controls matched for age and county were recruited from the National Population Registry.

For each deceased case two deceased controls matched also for year of death were identified from the National Registry for Causes of Death. For deceased subjects interviews were performed with the next-of-kin.

Similarly, four male controls matched for age and county were drawn to each case of HCL from the National Population Registry.

Assessment of Exposure

In both studies a similar questionnaire was mailed to the study subjects or next-of-kin for deceased individuals. A complete working history was asked for as well as exposure to different chemicals. If the information was unclear a trained interviewer supplemented the answers over the phone, thereby using written instructions. Years and total number of days for exposure to various agents were assessed. Also names of different agents were carefully asked for. If necessary, the Swedish Chemical Inspectorate was contacted to obtain information on the chemical composition of different brands of pesticides and other agents. A minimum exposure of one working day (8 h) and a tumor induction period of at least one year were used in the coding of chemicals. Thus, total exposure less than one day as well as exposure within one year prior to diagnosis (corresponding time for the matched control) were disregarded. The questionnaires were blinded as to case or control status during the interviews and coding of data.

Statistical Analysis

Conditional logistic regression analysis for matched studies was performed with the SAS statistical program (SAS Institute, Cary, NC). Thereby odds ratios (OR) and

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TABLE II Exposure to different types of herbicides with dose-response calculations. High exposure is defined as > median number of days for exposed subjects. Range of exposure in days given within parenthesis

Agent	Total OR (CI)	Median number of days	OR (CI)	
			Low	High
Herbicides	1.75 (1.26–2.42)	33 (1–709)	1.74 (1.10–2.71)	1.79 (1.15–2.79)
Phenoxyacetic acids	1.65 (1.16–2.34)	33 (1–709)	1.65 (1.01–2.66)	1.67 (1.02–2.69)
MCPA	2.62 (1.40–4.88)	25 (1–491)	1.94 (0.79–4.55)	3.61 (1.49–9.05)
2,4-D + 2,4,5-T	1.48 (0.99–2.20)	30 (1–709)	1.87 (1.08–3.20)	1.20 (0.68–2.08)
Other	2.90 (1.34–6.37)	11 (1–220)	2.26 (0.76–6.77)	3.37 (1.08–11)

95% confidence intervals (CI) were obtained. Both univariate and multivariate analyses were done. In this pooled analysis adjustment was made for study, study area and vital status. When risk estimates for different pesticides were analyzed only subjects with no pesticide exposure were taken as unexposed, whereas subjects exposed to other pesticides were disregarded.

RESULTS

The questionnaire was answered by 404 cases (91%) and 741 controls (84%) in the NHL study. Regarding HCL 111 cases (91%) and 400 controls (83%) participated. In the following results are given for the pooled analysis containing 515 cases and 1141 controls.

An increased risk was found for exposure to herbicides, insecticides, fungicides and impregnating agents, Table I. Regarding specific agents OR was highest for glyphosate and MCPA.

For herbicides dose-response calculations were also performed by comparing high and low dose exposures divided by the median exposure time in days, Table II. Exposure to MCPA gave a dose-response effect. Also for the group constituting of other herbicides than phenoxyacetic acids the risk was highest in the group with high exposure.

For herbicides in total and phenoxyacetic acids as a group the highest risks were seen when first exposure occurred 10–20 years before diagnosis, Table III. This was also the case for insecticides and impregnating agents. Within the latter group, however, an induction period of 20–30 years gave the highest risk for both creosote and pentachlorophenol.

Time to diagnosis from last exposure to different agents was also used in the calculation of risk estimates, Table IV. For phenoxyacetic acids the OR was highest for exposure 1–10 years prior to diagnosis whereas no increased risk was seen for those with last exposure >20 years from the time of diagnosis.

TABLE III Exposure to phenoxyacetic acids, insecticides, impregnating agents and organic solvents. Calculations are made with exposure divided according to time span from first exposure to diagnosis (induction period)

Agent	Induction period, years			
	1–10 OR (CI)	>10–20 OR (CI)	>20–30 OR (CI)	>30 OR (CI)
Herbicides	1.00 (0.05–11)	2.32 (1.04–5.16)	1.63 (0.87–2.98)	1.70 (1.12–2.58)
Phenoxyacetic acids	–*	2.88 (1.11–7.72)	1.54 (0.85–2.76)	1.50 (0.94–2.37)
MCPA	–*	5.36 (1.57–21)	0.89 (0.20–3.03)	3.77 (1.49–9.99)
2,4-D + 2,4,5-T	–†	2.87 (0.81–11)	1.87 (0.98–3.53)	1.15 (0.67–1.93)
Insecticides	1.20 (0.25–4.70)	2.84 (0.95–8.54)	2.19 (1.14–4.17)	1.31 (0.96–1.77)
DDT	–†	2.64 (0.61–11)	1.63 (0.80–3.26)	1.17 (0.82–1.65)
Impregnating agents	1.20 (0.37–3.49)	2.27 (1.15–4.49)	1.89 (1.07–3.30)	1.23 (0.85–1.75)
Chlorophenols	–†	1.91 (0.82–4.44)	1.90 (0.98–3.65)	1.13 (0.73–1.71)
Pentachlorophenol	–†	1.91 (0.82–4.44)	2.13 (1.07–4.25)	1.13 (0.73–1.72)
Creosote	–*	0.88 (0.04–7.27)	5.33 (1.26–27)	1.34 (0.69–2.49)
Organic solvents	1.51 (0.65–3.37)	1.38 (0.84–2.24)	1.46 (1.00–2.12)	1.02 (0.79–1.30)

* No exposed cases, one exposed control.
† No exposed subjects.

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TABLE IV Exposure to phenoxyacetic acids, impregnating agents and organic solvents. Calculations are made with exposure divided according to time span from last exposure to diagnosis

	Time span, last exposure-diagnosis, years			
Agent	1–10 OR (CI)	>10–20 OR (CI)	>20–30 OR (CI)	>30 OR (CI)
Herbicides	2.53 (1.38–4.64)	1.68 (0.88–3.14)	1.22 (0.66–2.19)	1.84 (0.95–3.51)
Phenoxyacetic acids	3.22 (1.59–6.65)	2.06 (1.03–4.09)	1.01 (0.54–1.81)	1.26 (0.57–2.62)
MCPA	3.52 (1.58–7.99)	2.33 (0.56–9.09)	0.92 (0.13–4.39)	–*
2,4-D + 2,4,5-T	4.31 (1.12–21)	1.85 (0.90–3.78)	1.04 (0.54–1.94)	1.41 (0.65–2.92)
Insecticides	2.37 (1.40–4.02)	0.87 (0.48–1.53)	1.45 (0.85–2.41)	1.46 (0.94–2.24)
DDT	1.45 (0.65–3.10)	1.13 (0.62–1.97)	1.46 (0.83–2.50)	1.20 (0.69–2.02)
Impregnating agents	1.92 (1.30–2.82)	0.79 (0.40–1.46)	1.67 (0.88–3.11)	1.19 (0.61–2.21)
Chlorophenols	–†	1.52 (1.02–2.25)	1.36 (0.61–2.86)	0.84 (0.32–1.96)
Pentachlorophenol	–†	1.59 (1.06–2.37)	1.28 (0.58–2.67)	0.81 (0.29–2.01)
Creosote	2.56 (0.85–7.67)	0.93 (0.13–4.17)	1.17 (0.36–3.43)	1.54 (0.60–3.75)
Organic solvents	1.17 (0.91–1.50)	1.00 (0.66–1.50)	1.39 (0.84–2.25)	0.99 (0.56–1.69)

* one exposed case, one exposed control.
† No exposed case or control.

Furthermore, exposure to phenoxyacetic acids during different decades from the 1940s was analyzed. Increased risk was found during recent decades, Table V.

No statistically significant increased risk was found for the whole group of organic solvents in this pooled analysis, but when the solvents were subgrouped according to specific substances there were increased risks for vanolen (OR = 1.91, CI = 1.03–3.49; $n = 20$ cases) and aviation fuel (OR = 3.56, CI = 1.03–12; $n = 6$ cases).

Multivariate analysis of exposure to phenoxyacetic acids, insecticides, fungicides and impregnating agents is presented in Table VI. An increased risk persisted for exposure to herbicides, fungicides and impregnating agents, however not statistically significant.

A separate multivariate analysis was performed on exposure to herbicides. Lower risk estimates were obtained although all herbicides still constituted risk factors for NHL, Table VII.

DISCUSSION

The cases in this study were identified by using the Swedish Cancer Registry, which is composed by six regional registries. In Sweden, the reporting of malignant diseases to the Cancer Registry is compulsory, which makes it likely that most incident cases in the study area were identified. Controls were selected from the National Population Registry and, in order to minimize recall bias, deceased controls were used for deceased cases in one of the studies [14] which were the basis for this analysis. In the other only living cases were included [15]. Recall bias is always a matter of concern in a case-control study with self-reported exposures. Farmer as occupation did not increase the risk in this pooled analysis (OR = 1.19, CI = 0.95–1.49) which indicates that the risk increase for pesticides was not explained merely by misclassification of exposure. All interviews and coding of data were performed blinded as to case or control status in order to minimize observational bias.

TABLE V Exposure to phenoxyacetic acids during different decades. Note that one subject may occur during several decades

Decade	Cases/controls	OR	CI
1940s	4/6	1.46	0.37–5.23
1950s	35/53	1.44	0.91–2.26
1960s	43/58	1.68	1.10–2.55
1970s	32/33	2.37	1.42–3.95
1980s	16/33	3.25	1.53–7.07

TABLE VI Multivariate analysis of exposure to pesticides

	Univariate		Multivariate	
Agent	OR	CI	OR	CI
Herbicides	1.75	1.26–2.42	1.39	0.96–2.02
Insecticides	1.43	1.08–1.87	1.07	0.78–1.45
Fungicides	3.11	1.56–6.27	2.02	0.97–4.23
Impregnating agents	1.48	1.11–1.96	1.30	0.98–1.72

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TABLE VII Multivariate analysis of exposure to herbicides. Odds ratios (OR) and 95% confidence intervals (CI) are given

	Univariate		Multivariate	
Agent	OR	CI	OR	CI
MCPA	2.62	1.40–4.88	1.67	0.77–3.57
2,4-D + 2,4,5-T	1.48	0.99–2.20	1.32	0.88–1.96
Glyphosate	3.04	1.08–8.52	1.85	0.55–6.20
Other herbicides	2.90	1.34–6.37	2.28	1.02–5.15

This study was a pooled analysis of two case-control studies, one on NHL [14] and the other on HCL [15] to provide larger numbers, which would allow more detailed analyses regarding the timing of exposure and adjustment of multiple exposures. This method was justified since HCL is a type of NHL and similar methods and questionnaires were used in both studies. Also the findings regarding pesticide exposure were relatively homogenous for both studies. The smaller HCL study had a somewhat higher prevalence of exposure and therefore has in this pooled analysis more weight than one would expect.

Conditional logistic regression analysis was performed since both studies in this pooled analysis were matched. Heterogeneity in findings was averaged after stratification by study. Since the NHL study included also deceased cases and controls adjustment was made for vital status. Finally, in the HCL study the whole Sweden was included as study base whereas in the NHL study only parts of Sweden were included. Thus, adjustment was made for geographical area for cases and controls, i.e. county.

In the multivariate analysis exposure to herbicides, fungicides and impregnating agents increased the risk although OR was lower than in the univariate analysis. Significantly increased risk remained only for the heterogeneous group of "other herbicides". The results in multivariate analysis must be interpreted with caution since exposure to different types of pesticides correlate. Multivariate analysis is mainly useful to estimate the risk factors that seem to be most important.

Several previous studies have associated exposure to phenoxyacetic acids, primarily 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), with an increased risk for NHL [8–12,16–18]. Concerning MCPA data are sparse although in our first study on NHL, we found an increased risk [9,10].

In this pooled analysis, most subjects were regarding herbicides exposed to phenoxyacetic acids, mostly the combination of 2,4-D and 2,4,5-T. 2,4-D was withdrawn from the Swedish market in 1990 and 2,4,5-T was prohibited in 1977. Also MCPA, the phenoxy herbicide still commonly used in Sweden, increased the risk for NHL. Glyphosate is the herbicide now mostly used in Sweden. In this study, exposure to glyphosate was a risk factor for NHL. Thus, regarding herbicides lymphomagenesis seems not to be depending on contaminating dioxins, i.e. 2,3,7,8-TCDD in 2,4,5-T. A contributing effect of such exposure cannot be excluded, although not supported by mortality results in a cohort of workers exposed to 2,3,7,8-TCDD [19]. IARC classified recently 2,3,7,8-TCDD as a human carcinogen, Group I [20].

In the univariate analysis exposure to insecticides, mostly DDT, increased the risk for NHL. In the multivariate analysis no risk was found. This is in accordance with our previous results [9,10] and a pooled analysis of three case-control studies concluded that DDT is not a risk factor for NHL [21]. Furthermore, analysis of serum DDT/DDE has not given a clear association with NHL [22,24,25].

Regarding fungicides an increased risk for NHL has previously been reported from USA [11]. Our result with increased risk for NHL needs to be further studied since the finding was based on few subjects exposed to several types of fungicides.

Chlorophenols, which are chemically related to phenoxyacetic acids and have been used as e.g. wood preservatives, were banned in Sweden in 1978. An increased risk for NHL was found in this pooled analysis, but also for exposure to arsenic and creosote. Both chlorophenols and creosote have been associated with NHL [26,27].

An association between exposure to organic solvents and NHL has been described [9,10,28–30]. However, such an association was not confirmed now although an influence of tumor induction period can not be ruled out, *c.f.*, below. Another possibility might be that solvents used during later years are less toxic than previously, e.g. water based, and that they are more cautiously handled [31].

To further elucidate mechanisms in lymphomagenesis analysis of tumor-induction period (latency) and also time from last exposure to diagnosis was performed. Thereby the corresponding year for diagnosis was used for the matched control. For 2,4-D, 2,4,5-T and chlorophenols no subject had first exposure during 1–10 years prior to diagnosis due to restrictions in the use of these chemicals in Sweden during that time period. For fungicides such calculations were not meaningful due to low number of exposed subjects.

The highest risk for exposure to herbicides, insecticides and impregnating substances was found for last exposure 1–10 years prior to diagnosis. Correspondingly, in general the lowest risks were found for the longest tumor induction periods.

Do these results cast further light on the etiology of NHL? Certainly, exposure to some chemicals is of significance in lymphomagenesis. Furthermore, bearing in mind that several of these chemicals are immunotoxic, e.g. certain pesticides and chlorophenols [27,32,33] and immunosuppression is an established risk factor for NHL [34] such toxicity might be of importance for chemical agents.

Viruses have been associated with lymphomas in animals [35,36] and more specifically EBV for humans [7,37]. Virus proliferation in lymphocytes is held back by the immune system and immunosuppression may be followed by development of both B-cell and T-cell

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lymphoma in animals [38–39]. For renal transplant patients treated with immunosuppressive drugs the risk for NHL is highest during the first years after transplantation and then declines [40].

Timing of exposure in relation to risk of NHL, particularly in regard to higher risk for recent exposures, seemed to be an interesting result regarding lymphomagenesis. Several interpretations are possible such as chance finding, late stage in lymphomagenesis, type of exposure or interaction with other factors. Certainly immunmodulation by pesticides [32,33] is one hypothesis which should be more elaborated on, possibly with interaction with latent virus infection such as EBV. This might explain the short tumor induction period. In fact, results from the included HCL-study showed interaction between EBV-infection and exposure to such chemicals [41,42]. Additionally, polychlorinated biphenyls [22,24,25] and chlordanes [23,24], chemicals that are immunotoxic [43,44], have been associated with an increased risk for NHL.

The etiology of NHL is multifactorial and further studies should consider immunotoxic effects by the studied chemicals as well as tumor induction period and interaction with virus infection, e.g. EBV.

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Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men

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Background: An increased rate of non-Hodgkin's lymphoma (NHL) has been repeatedly observed among farmers, but identification of specific exposures that explain this observation has proven difficult.

Methods: During the 1980s, the National Cancer Institute conducted three case-control studies of NHL in the midwestern United States. These pooled data were used to examine pesticide exposures in farming as risk factors for NHL in men. The large sample size (n = 3417) allowed analysis of 47 pesticides simultaneously, controlling for potential confounding by other pesticides in the model, and adjusting the estimates based on a prespecified variance to make them more stable.

Results: Reported use of several individual pesticides was associated with increased NHL incidence, including organophosphate insecticides coumaphos, diazinon, and fonofos, insecticides chlordane, dieldrin, and copper acetoarsenite, and herbicides atrazine, glyphosate, and sodium chlorate. A subanalysis of these "potentially carcinogenic" pesticides suggested a positive trend of risk with exposure to increasing numbers.

Conclusion: Consideration of multiple exposures is important in accurately estimating specific effects and in evaluating realistic exposure scenarios.

Farming occupation has been associated with an increased risk of non-Hodgkin's lymphoma (NHL) in the United States and other countries.[1–4] Specific farming exposures contributing to the excess risk have not been clearly discerned, but pesticides have received considerable attention. Associations have been observed between NHL risk and exposure to phenoxyacetic acids, most notably 2,4-dichlorophenoxyacetic acid (2,4-D).[5–10] Organochlorine, organophosphate, carbamate, and triazine pesticides have also been implicated.[8 9 11–14]

There are several analytical challenges in studying health effects of pesticide exposures among farmers. Farmers are typically exposed to multiple pesticides during a lifetime, and pesticides are frequently used together or during the same growing season, posing a challenge for identifying specific risk factors. Although multiple and simultaneous exposures are common in epidemiology and the situation regarding pesticides is not unique, they do require large numbers to successfully identify risks from specific exposures. Many of the past studies of NHL and pesticides had limited power to adjust for potential confounding by associated pesticide exposures. Limited study power has also hindered investigation of the risk associated with common pesticide combinations.

In principle, multiple pesticide exposures should be modelled simultaneously to account for their probable correlation; however, modelling multiple pesticides can lead to imprecise estimates, particularly where exposures are infrequent. In addition, some estimates are expected to be very inaccurate, either due to chance or systematic error (such as recall bias). Hierarchical regression models, also known as multilevel or multistage models, allow the researcher to specify prior distributions for multiple effect parameters of interest (for example, pesticide effects), and to adjust the observed likelihood estimates towards these prior distributions with the objective of obtaining increased precision and accuracy for the ensemble of estimates.[15–17] Although the true prior distributions are rarely known, factors hypothesised to determine or explain the magnitude of the true effects of interest can be used to specify the form of the prior distributions, whose magnitudes are then estimated.[15]

During the 1980s, the National Cancer Institute conducted three population based case-control studies of NHL in Nebraska,[5] Iowa and Minnesota,[11] and Kansas.[7] Each of these studies focused on farming exposure to pesticides, and data from the three studies have been pooled. In the pooled data, certain organophosphate[12] and carbamate[13] insecticides were positively associated with the risk of NHL. Lindane use was associated with slightly increased incidence of NHL,[18] whereas DDT use was not.[19] There was also a slightly increased incidence associated with atrazine exposure.[20]

We used these pooled data to conduct an analysis of exposure to multiple pesticides in farming as risk factors for NHL among men. The larger sample size provided adequate numbers of exposed persons to analyse a set of pesticide exposures simultaneously, using hierarchical regression to adjust estimates based on prior distributions for the pesticide effects. In addition, effects of the number of pesticides used and of common pesticide combinations were explored to assess the risk associated with realistic scenarios of farmers' exposures to multiple pesticides.

METHODS

Study population

The three case-control studies had slightly different methods of subject recruitment. In Nebraska,[5] all cases of NHL diagnosed between July 1983 and June 1986 among white subjects 21 years of age and older, and living in one of the 66 counties of eastern Nebraska were identified through the Nebraska Lymphoma Study Group and area hospitals. In Iowa and Minnesota,[11] all newly diagnosed cases of NHL among

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; NHL, non-Hodgkin's lymphoma; OP, organophosphorus

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white men aged 30 years or older were ascertained from records of the Iowa State Health Registry from 1981 to 1983, and a special surveillance system of Minnesota hospitals and pathology laboratories from 1980 to 1982. In Kansas,[7] a random sample of cases diagnosed between 1979 and 1981 among white men age 21 years or older was selected from the statewide cancer registry run by the University of Kansas Cancer Data Service. Population based controls were randomly selected from the same geographical areas as the cases, frequency matched to cases by race, sex, age, and vital status at the time of interview. Potential controls were identified by random digit dialing and from Medicare records, and for deceased cases, from state mortality files.

Only one study included women; in this pooled analysis we excluded female cases and controls. Those who lived or worked on a farm when younger than 18 years of age, but not after age 18, were not asked about their pesticide use in the Nebraska study; persons with this history from any of the three studies were therefore excluded from analyses of the pooled data. Following exclusions, the study population included 870 cases and 2569 controls.

Interviews

Interviews were conducted with the subjects or their next of kin if the subjects were dead or incapacitated. In each study, detailed questions were asked about the use of agricultural pesticides as well as other known or suspected risk factors for NHL. In Nebraska, information was obtained through questioning about the use of any pesticide, followed by prompting for selected specific pesticides, with details on the total number of years of use and average number of days per year. In Iowa and Minnesota, use was assessed by a direct question about a selected list of specific pesticides. Pesticide users were also asked the first and last year each pesticide was used. In Kansas, use of pesticides was assessed by an open ended question without prompting for specific pesticides, and duration of use and days per year were obtained for groups of pesticides (herbicides, insecticides, and fungicides), but not for each pesticide individually.

Statistical analyses

Each pesticide for which there were data from all three studies, and to which 20 or more persons were exposed, was included in the pooled analysis. The set of pesticides examined included 47 insecticides and herbicides. Exposure to each pesticide was coded as an indicator variable for exposed (1) or not exposed (0). Because these analyses of multiple pesticides modelled the pesticides simultaneously, any subject with a missing or "don't know" response for any one of the 47 pesticides of interest was excluded from all analyses. Following exclusion of subjects with missing data, analyses of multiple pesticides included 650 cases (74.7%) and 1933 controls (75.2%). We employed two approaches to our analyses: standard logistic regression (maximum likelihood estimation) and hierarchical regression, calculating odds ratios to estimate the relative risk associated with each pesticide. All models included variables for age (coded as a quadratic spline variable with one knot at 50 years)[21] and indicator variables for study site. Other factors known or suspected to be associated with NHL, including first degree relative with haematopoietic cancer, education, and smoking, were evaluated and found not to be important confounders of the associations between NHL and pesticides. The standard logistic regression models did not assume any prior distribution of pesticide effects, in contrast to the hierarchical regression modelling.

Hierarchical regression of multiple pesticide exposures

In the first-level model of the hierarchical regression analysis, NHL disease status was regressed simultaneously on the 47 pesticide exposures, age, and study site. The maximum likelihood estimates for the 47 pesticides from the first-level model were regressed in a second-level linear regression model as a function of prespecified prior covariates for each of the pesticides. The second-level model should incorporate what is known about each true effect parameter prior to seeing the study data.[15 22] Information derived from the second-level model was used to adjust the beta coefficient for each pesticide exposure according to its "prior distribution"; the beta for each pesticide was adjusted in the direction of its prior mean, or expected value (from the second-level model), with the magnitude of shrinkage dependent on the precision of its likelihood estimate (from the first-level model) and a prespecified variance of the assumed normal distribution for that parameter. SAS Proc GLIMMIX was used to run the hierarchical models. This program can be adapted for the purpose of hierarchical modelling of multiple exposures, and uses a penalised likelihood function to fit the first- and second-level models by an iterative procedure.[23]

Information on pesticides that would give a priori reason to believe that the true effect parameters for certain specific pesticides would be more or less similar to each other was constructed into a matrix for use in the second level of the hierarchical regression analysis (table 1). The second-level, or prior covariates, were factors hypothesised to determine the magnitude of, or explain some of the variability between, the individual true effects. The covariates were indicators of pesticide class, structure, and toxicity, used to define categories of pesticide effects which would be regarded as "exchangeable", or as draws from a common prior distribution.[15 22] These "categories of exchangeability" included the groupings: insecticides (versus herbicides), organochlorines, organophosphates, carbamates, phenoxyacetic acids, triazines, amides, and benzoic acids (see table 1). In addition to categories of exchangeability, we defined a prior covariate incorporating prior evidence for carcinogenicity of the pesticide. Based on data from the United States Environmental Protection Agency's (US EPA) Integrated Risk Information System (http://www.epa.gov/iris/) and the International Agency for Research on Cancer's Program on the Evaluation of Cancer Risks to Humans (http://monographs.iarc.fr/), carcinogenic probability for any cancer (not limited to NHL), was defined as a continuous variable ranging between 0 and 1 (algorithm for variable definition is included as footnote to table 1).

Another component of each pesticide effect's prior distribution was a value for the residual variance, which captures effects above and beyond those accounted for by the "group" effects of the second-level covariates, and determines the degree of shrinkage of a likelihood estimate toward its prior mean.[15 22] This residual variance was defined as a value relating to a range of probable values for the true effect parameter. We assumed, with 95% certainty, that the rate ratio for each pesticide, after adjusting for the second-level covariates, would fall within a 10-fold range around its prior mean (for example, between 0.5 and 5.0), by defining the prior residual variance as 0.35 (note: for a 10-fold range, residual variance = $((\ln(10))/3.92)^2 \cong 0.35$), assuming normality).

Because our prior covariates were crudely defined, and because there is little information on factors that would be expected to affect the magnitude of the effect of pesticides on NHL incidence, we also performed a hierarchical regression analysis of multiple pesticides using an intercept-only model, in which all pesticide effects were assumed to arise from a common prior distribution, with a prior residual variance of 0.35. In other words, this modelling strategy assumed that there was no a priori reason to believe that any specific pesticide was more likely to be associated with NHL incidence than any other pesticide in the model.

Number of pesticides used

We conducted analyses to estimate NHL incidence associated with the number of pesticides used, out of the total number of

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Table 1 Second-level matrix for hierarchical regression analysis, showing values of "prior covariates" for each pesticide of interest*†

Pesticides	Insecticides	Organo-chlorines	Organo-phospates	Carbamates	Phenoxy-acetic acids	Triazines	Amides	Benzoic acids	Carcinogenic probability
Insecticides									
Aldrin	1	1	0	0	0	0	0	0	0.6
Bufencarb	1	0	0	1	0	0	0	0	0.3
Carbaryl	1	0	0	1	0	0	0	0	0.3
Carbofuran	1	0	0	1	0	0	0	0	0.3
Chlordane	1	1	0	0	0	0	0	0	0.8
Copper acetoarsenite*	1	0	0	0	0	0	0	0	1.0
Coumaphos	1	0	1	0	0	0	0	0	0.3
DDT	1	1	0	0	0	0	0	0	0.8
Diazinon	1	0	1	0	0	0	0	0	0.3
Dichlorvos	1	0	1	0	0	0	0	0	0.8
Dieldrin	1	1	0	0	0	0	0	0	0.6
Dimethoate	1	0	1	0	0	0	0	0	0.3
Ethoprop	1	0	1	0	0	0	0	0	0.3
Famphur	1	0	1	0	0	0	0	0	0.3
Fly, lice, tick spray	1	0	0	0	0	0	0	0	0.3
Fonofos	1	0	1	0	0	0	0	0	0.3
Heptachlor	1	1	0	0	0	0	0	0	0.8
Lead arsenate*	1	0	0	0	0	0	0	0	1.0
Lindane	1	1	0	0	0	0	0	0	0.3
Malathion	1	0	1	0	0	0	0	0	0.3
Methoxychlor	1	1	0	0	0	0	0	0	0.3
Nicotine	1	0	0	0	0	0	0	0	0.3
Phorate	1	0	1	0	0	0	0	0	0.3
Pyrethrins	1	0	0	0	0	0	0	0	0.3
Rotenone	1	0	0	0	0	0	0	0	0.3
Tetrachlorvinphas	1	0	1	0	0	0	0	0	0.3
Toxaphene	1	1	0	0	0	0	0	0	0.8
Terbufos	1	0	1	0	0	0	0	0	0.3
Herbicides									
Alachlor	0	0	0	0	0	0	1	0	0.3
Atrazine	0	0	0	0	0	1	0	0	0.3
Bentazon	0	0	0	0	0	0	0	0	0.1
Butylate	0	0	0	1	0	0	0	0	0.3
Chloramben	0	0	0	0	0	0	0	1	0.3
Cyanazine	0	0	0	0	0	1	0	0	0.3
2,4-D	0	0	0	0	1	0	0	0	0.5
Dicamba	0	0	0	0	0	0	0	1	0.3
EPTC	0	0	0	1	0	0	0	0	0.3
Glyphosate	0	0	0	0	0	0	0	0	0.3
Linuron	0	0	0	0	0	0	0	0	0.5
MCPA	0	0	0	0	1	0	0	0	0.3
Metolachlor	0	0	0	0	0	0	1	0	0.5
Metribuzin	0	0	0	0	0	0	0	0	0.3
Paraquat	0	0	0	0	0	0	0	0	0.5
Propachlor	0	0	0	0	0	0	1	0	0.3
Sodium chlorate	0	0	0	0	0	0	0	0	0.3
2,4,5-T	0	0	0	0	1	0	0	0	0.5
Trifluralin	0	0	0	0	0	0	0	0	0.5

*Carcinogenic probability value is created by combining the classifications from the IARC Monographs Programme on the Evaluation of Carcinogenic Risks to Humans and the US EPA Integrated Risk Information System. Assignment of carcinogenic probability by order of priority: 1.0 = classified as a human carcinogen on either assessment; 0.9 = probable human carcinogen in both assessments; 0.8 = probable human carcinogen in one assessment and possible human carcinogen in other assessment; 0.6 = probable human carcinogen in one assessment and unclassifiable in the other; 0.5 = possible human carcinogen in both assessments, or possible human carcinogen in one assessment and not assessed by the other group; 0.3 = not assessed by IARC or US EPA IRIS, or deemed unclassifiable in one or both assessments; 0.1 = evidence for non-carcinogenicity in either assessment.
†Used the IARC assessment for arsenic and arsenic compounds.

86 pesticides reported in all three of the pooled studies (many of these 86 pesticides were not included in the multivariable analysis of the set of 47 specific pesticides because of their infrequent use). The number of pesticides was coded using indicator variables (1 pesticide, 2–4 pesticides, 5 or more pesticides). Similar analyses were conducted for the number of insecticides and herbicides used. For those pesticides showing positive associations with NHL in the hierarchical regression analysis of 47 specific pesticides (nine pesticides total, see table 3), we conducted a similar analysis of the number of pesticides used, restricted to these "potentially carcinogenic" pesticides. In addition to logistic regression analyses, we evaluated the effect of the number of pesticides used by hierarchical regression with an intercept-only model, in which all pesticide effects (those indicating number of pesticides, as well as the 47 specific pesticides) were assumed to have been sampled from a common prior distribution with an unknown mean and a residual variance of 0.35.

Combined pesticide exposures

We explored the risk associated with combined pesticide exposures, defined as two pesticides used by the same person, but not necessarily at the same time. For any two pesticides for which more than 75 persons reported use of both (representing the 5% most common of all possible combinations of the 47 pesticides), and at least 20 persons reported use of each of the two individual pesticides not in combination, we evaluated potential superadditivity of pesticide effects on NHL (the appendix contains a list of the pesticide combinations evaluated). Individual and joint effects were first estimated

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Table 2 Characteristics of subjects in the study population* and those subjects included in analyses of multiple pesticides†

Characteristics	Pooled study			Included in analyses of multiple pesticides		
	Cases (n=870)	Controls (n=2569)	OR (95% CL)‡	Cases (n=650)	Controls (n=1933)	OR (95% CL)
Study site						
Iowa/Minnesota	520 (60.9%)	1039 (40.4%)	1.0	436 (67.1%)	895 (46.3%)	1.0
Kansas	153 (17.6%)	862 (33.6%)	0.3 (0.3 to 0.4)§	101 (15.5%)	596 (30.8%)	0.3 (0.3 to 0.4)
Nebraska	187 (21.5%)	668 (26.0%)	0.5 (0.4 to 0.7)§	113 (17.4%)	442 (22.9%)	0.5 (0.4 to 0.7)
Respondent status						
Self respondent	545 (62.6%)	1413 (55.0%)	1.0	449 (69.1%)	1166 (60.3%)	1.0
Proxy respondent	325 (37.4%)	1156 (45.0%)	0.7 (0.6 to 0.9)§	201 (30.9%)	767 (39.7%)	0.7 (0.6 to 0.8)
Age (years)						
<40	53 (6.1%)	280 (11.0%)	0.7 (0.5 to 1.0)§	40 (6.2%)	211 (10.9%)	0.7 (0.5 to 1.1)
40–59	196 (22.6%)	493 (19.3%)	1.5 (1.1 to 1.9)§	160 (24.6%)	388 (20.1%)	1.6 (1.2 to 2.1)
60–79	478 (55.1%)	1261 (49.4%)	1.4 (1.1 to 1.7)§	355 (54.6%)	969 (50.1%)	1.4 (1.1 to 1.8)
≥80	141 (16.2%)	521 (20.4%)	1.0	95 (14.6%)	365 (18.9%)	1.0
Educational level						
Less than high school graduation	387 (45.2%)	1126 (44.7%)	1.0	276 (43.0%)	806 (42.4%)	1.0
High school graduation or GED¶	226 (26.4%)	629 (25.0%)	1.0 (0.9 to 1.3)	171 (26.6%)	467 (24.6%)	1.1 (0.9 to 1.3)
Some college or vocational school	151 (17.6%)	457 (18.1%)	1.0 (0.8 to 1.2)	122 (19.0%)	368 (19.4%)	1.0 (0.8 to 1.2)
College graduate or more	93 (10.9%)	308 (12.2%)	1.0 (0.7 to 1.1)	73 (11.4%)	261 (13.7%)	0.8 (0.6 to 1.1)
Ever lived or worked on a farm as an adult						
No	243 (28.1%)	780 (30.4%)	1.0	243 (37.5%)	775 (40.1%)	1.0
Yes	621 (71.9%)	1780 (69.5%)	1.1 (0.9 to 1.3)	405 (62.5%)	1157 (59.9%)	1.1 (0.9 to 1.3)
First degree relative with haematopoietic cancer						
No	792 (92.5%)	2452 (96.8%)	1.0	594 (92.8%)	1863 (96.7%)	1.0
Yes	64 (7.5%)	80 (3.2%)	2.5 (1.8 to 3.5)	46 (7.2%)	63 (3.3%)	2.3 (1.5 to 3.4)
Histological subtype						
Follicular	243 (28.0%)			196 (30.1%)		
Diffuse	334 (38.5%)			233 (35.9%)		
Small lymphocytic	99 (11.4%)			77 (11.9%)		
Other	192 (22.1%)			144 (22.2%)		

*Pooled study population limited to males and following exclusions.
†Any observation with a missing value for any of the 47 multiple pesticides was not included in analyses.
‡Odds ratios (OR) and 95% confidence limits (CL).
§Odds ratios for the matching factors are not interpretable for their relation with NHL, but are presented for comparison to odds ratios for the subgroup included in analyses of multiple pesticides.
¶GED, General Equivalency Diploma.

using logistic regression in models including variables for the joint exposure and two individual exposures, the 45 other specific pesticides, age, and study site. Where the OR for the joint effect was 1.3 or higher, positive interaction on the additive scale was evaluated using the interaction contrast ratio ($ICR = OR_{joint\ exposure} - OR_{individual\ exposure\ \#1} - OR_{individual\ exposure\ \#2} + 1$).[24] ICR values above 0.5 were considered indicative of superadditivity, and these pesticide combinations were further analysed using hierarchical regression with an intercept-only model, in which all pesticide effects (those indicating joint and individual exposures to the two pesticides, as well as the other 45 specific pesticides) were assumed to have been sampled from a common prior distribution with an unknown mean and a residual variance of 0.35.

RESULTS

Table 2 shows characteristics of men in the pooled studies. In the control population, which was representative of this part of the midwestern United States, approximately 70% of the men had lived or worked on a farm as an adult. There was a 10% increased NHL incidence associated with living or working on a farm as an adult; this increase is similar in magnitude to meta-analyses of farming and NHL mortality and morbidity.[4 25] Cases were slightly more likely than controls to have been directly interviewed, to be between the ages of 40 and 79, and they were more than twice as likely to have a first degree relative with haematopoietic cancer. The subset of subjects included in analyses of multiple pesticides was less likely than those in the overall study population to be from the Kansas or Nebraska studies, to have lived or worked on a farm as an adult, or to have had a proxy respondent, and they were slightly more likely to be more highly educated; however, the relation of these factors with case status did not differ between the overall study and the subset included in the analyses of multiple pesticides.

Use of most specific pesticides was more frequent among cases than controls; however, most of the odds ratios were not increased in the multivariable models (table 3), primarily due to adjustment for study site, since both the frequency of pesticide use and case-to-control ratios differed by study site. The results of the hierarchical regression analysis of 47 pesticides were generally similar to, but had somewhat more narrow confidence intervals than results from the logistic regression model. Only a few pesticides were associated with a possible increased NHL incidence (judged by OR ≥1.3 and lower confidence limit ≥0.8), including the organophosphate (OP) insecticides coumaphos, fonofos, and diazinon, the organochlorine insecticides chlordane and dieldrin, the insecticide copper acetoarsenite, and the herbicides atrazine, glyphosate, and sodium chlorate. There was also a significantly decreased risk associated with aldrin exposure. These suggested effects occurred in both the logistic and hierarchical regression analyses. For pesticides that had wider confidence intervals in the logistic regression model, odds ratios from the hierarchical model were generally closer to the null value, based on a priori assumptions about the probable magnitudes of effect. For example, we assumed that the effect of sodium chlorate would be similar to that of other herbicides and other pesticides for which there was a low carcinogenic probability, and that after accounting for these prior covariates, the rate ratio would likely fall within a 10-fold range around its expected value. Based on these assumptions, a fourfold risk associated with the use of sodium chlorate in the logistic regression analysis was adjusted to a 1.8-fold risk using hierarchical regression. Although unstable estimates were adjusted, results of the

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Table 3 Effect estimates for use of specific pesticides and NHL incidence, adjusting for use of other pesticides*

Pesticides	Exposed [n (%)] Cases (n=650)	Controls (n=1933)	Logistic regression OR (95% CL)†	Hierarchical regression OR (95% CL)
Insecticides				
Aldrin	47 (7.2%)	115 (5.9%)	0.5 (0.3 to 0.9)	0.6 (0.4 to 1.0)
Bufencarb‡	6 (0.9%)	12 (0.6%)	1.1 (0.3 to 3.7)	1.0 (0.4 to 2.3)
Carbaryl	30 (4.6%)	57 (2.9%)	1.0 (0.5 to 1.9)	1.1 (0.6 to 1.9)
Carbofuran	41 (6.3%)	96 (5.0%)	0.9 (0.5 to 1.6)	1.0 (0.6 to 1.7)
Chlordane	39 (6.0%)	65 (3.4%)	1.5 (0.8 to 2.6)	1.3 (0.8 to 2.1)
Copper acetoarsenite	41 (6.3%)	68 (3.5%)	1.4 (0.9 to 2.3)	1.4 (0.9 to 2.1)
Coumaphos	15 (2.3%)	22 (1.1%)	2.4 (1.0 to 5.8)	1.7 (0.9 to 3.3)
DDT	98 (15.1%)	226 (11.7%)	1.0 (0.7 to 1.3)	1.0 (0.7 to 1.3)
Diazinon	40 (6.1%)	62 (3.2%)	1.9 (1.1 to 3.6)	1.7 (1.0 to 2.8)
Dichlorvos	16 (2.5%)	37 (1.9%)	0.9 (0.4 to 2.0)	0.9 (0.5 to 1.7)
Dieldrin	21 (3.2%)	39 (2.0%)	1.8 (0.8 to 3.9)	1.4 (0.8 to 2.6)
Dimethoate‡	5 (0.8%)	11 (0.6%)	1.2 (0.3 to 5.3)	1.2 (0.5 to 2.8)
Ethoprop‡	4 (0.6%)	14 (0.7%)	0.7 (0.2 to 2.9)	0.9 (0.4 to 2.1)
Famphur	12 (1.8%)	34 (1.8%)	0.7 (0.3 to 1.7)	0.8 (0.4 to 1.5)
Fly, lice, or tick spray	162 (24.9%)	408 (21.1%)	0.9 (0.7 to 1.1)	0.9 (0.7 to 1.1)
Fonofos	28 (4.3%)	44 (2.3%)	1.8 (0.9 to 3.5)	1.5 (0.9 to 2.7)
Heptachlor	28 (4.3%)	53 (2.7%)	1.1 (0.6 to 2.4)	1.1 (0.6 to 2.0)
Lead arsenate	9 (1.4%)	25 (1.3%)	0.5 (0.2 to 1.2)	0.6 (0.3 to 1.3)
Lindane	59 (9.1%)	109 (5.6%)	1.2 (0.7 to 2.0)	1.2 (0.8 to 1.9)
Malathion	53 (8.1%)	100 (5.2%)	1.1 (0.6 to 1.8)	1.1 (0.7 to 1.7)
Methoxychlor	9 (1.4%)	20 (1.0%)	0.8 (0.3 to 2.1)	0.9 (0.4 to 1.9)
Nicotine	24 (3.7%)	50 (2.6%)	0.9 (0.5 to 1.6)	1.0 (0.6 to 1.6)
Phorate	28 (4.3%)	67 (3.5%)	0.8 (0.4 to 1.6)	0.9 (0.5 to 1.5)
Pyrethrins‡	6 (0.9%)	12 (0.6%)	1.0 (0.3 to 3.2)	1.0 (0.4 to 2.3)
Rotenone	10 (1.5%)	26 (1.4%)	0.7 (0.3 to 1.7)	0.8 (0.4 to 1.5)
Tetrachlorvinphos‡	3 (0.5%)	11 (0.6%)	0.4 (0.1 to 1.8)	0.8 (0.3 to 1.9)
Toxaphene	17 (2.6%)	34 (1.8%)	1.1 (0.5 to 2.4)	1.1 (0.6 to 2.0)
Terbufos	21 (3.2%)	50 (2.6%)	0.8 (0.4 to 1.8)	0.8 (0.5 to 1.6)
Herbicides				
Alachlor	68 (10.5%)	152 (7.9%)	1.1 (0.7 to 1.8)	1.0 (0.6 to 1.6)
Atrazine	90 (13.8%)	185 (9.6%)	1.6 (1.1 to 2.5)	1.5 (1.0 to 2.2)
Bentazon	22 (3.4%)	58 (3.0%)	0.7 (0.3 to 1.5)	0.8 (0.4 to 1.4)
Butylate	28 (4.3%)	56 (2.9%)	1.2 (0.6 to 2.3)	1.2 (0.7 to 2.0)
Chloramben	34 (5.2%)	81 (4.2%)	0.9 (0.5 to 1.6)	0.9 (0.5 to 1.5)
Cyanazine	37 (5.7%)	96 (5.0%)	0.6 (0.3 to 1.0)	0.6 (0.4 to 1.1)
2,4-D	123 (18.9%)	314 (16.2%)	0.8 (0.6 to 1.1)	0.9 (0.6 to 1.2)
Dicamba	39 (6.0%)	79 (4.1%)	1.2 (0.6 to 2.3)	1.2 (0.7 to 2.1)
EPTC + protectant	13 (2.0%)	29 (1.5%)	1.2 (0.5 to 3.1)	1.1 (0.5 to 2.3)
Glyphosate	36 (5.5%)	61 (3.2%)	2.1 (1.1 to 4.0)	1.6 (0.9 to 2.8)
Linuron	5 (0.8%)	22 (1.1%)	0.3 (0.1 to 1.2)	0.5 (0.2 to 1.2)
MCPA	8 (1.2%)	16 (0.8%)	1.0 (0.4 to 2.6)	0.9 (0.4 to 2.0)
Metolachlor	13 (2.0%)	37 (1.9%)	0.7 (0.3 to 1.6)	0.7 (0.4 to 1.5)
Metribuzen	20 (3.1%)	53 (2.7%)	0.8 (0.4 to 1.7)	0.8 (0.4 to 1.5)
Paraquat‡	2 (0.3%)	15 (0.8%)	0.1 (0.02 to 0.7)	0.5 (0.2 to 1.2)
Propachlor	20 (3.1%)	50 (2.6%)	1.0 (0.5 to 2.0)	1.0 (0.6 to 1.9)
Sodium chlorate‡	8 (1.2%)	7 (0.4%)	4.1 (1.3 to 13.6)	1.8 (0.8 to 4.1)
2,4,5-T	25 (3.9%)	63 (3.3%)	1.0 (0.5 to 1.9)	0.9 (0.5 to 1.6)
Trifluralin	52 (8.0%)	120 (6.2%)	0.9 (0.5 to 1.6)	0.9 (0.5 to 1.4)

*Each estimate is adjusted for use of all other pesticides listed in table 3, age, and study site.
†Odds ratios (OR) and 95% confidence limits (CL).
‡Criteria for inclusion in the models was a pesticide use frequency of ≥20; however, some pesticide use frequencies are <20 in the multivariable models since observations with missing values were dropped.

hierarchical model including prior covariates and those from the hierarchical intercept-only model were virtually identical (results for intercept-only model not shown), indicating that the prior covariates representing pesticide category and carcinogenic probability were not important determinants of the variability between the observed effects, and that adjustment of estimates primarily occurred because of the a priori restriction on their variance. Indeed, a linear regression analysis of the 47 logistic regression beta coefficients for the pesticides regressed on the prior covariates found no statistically significant associations (at a significance level of $p < 0.05$; results not shown).

Among the farmers who used pesticides, the number of total pesticides ever used ranged between 1 and 32, but approximately 50% of farmers reported using only one or two pesticides. There was no association between NHL incidence and either the total number of pesticides or herbicides used (see table 4). There was a 40% increased incidence associated with the use of five or more insecticides; however, there was no apparent exposure-response trend. In an analysis of the number of "potentially carcinogenic" pesticides, NHL incidence increased by the number of pesticides used by the subject. Subjects who reported using any five or more "potentially carcinogenic" pesticides were twice as likely to be NHL cases than controls, compared to those using no pesticides. The results for "potentially carcinogenic" pesticides were highly sensitive to removal of certain pesticides from the count, including dieldrin, atrazine, or glyphosate. For example, removal of glyphosate from the count resulted in a lack of trend for increasing number of "potentially carcinogenic" pesticides (1 pesticide: OR = 1.2; 2–4 pesticides: OR = 1.2; ≥5 pesticides: OR = 1.1).

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Table 4 Effect of number of pesticides used on NHL incidence*

Number of pesticides used	Exposed [n (%)] Cases (n=650)	Controls (n=1933)	Logistic regression OR (95% CL)†	Hierarchical regression OR (95% CL)
Any pesticide				
0	370	1252	1.0	1.0
1	89 (13.7%)	230 (11.9%)	1.2 (0.8 to 1.8)	1.1 (0.9 to 1.7)
2–4	87 (13.4%)	221 (11.4%)	1.0 (0.6 to 1.6)	1.0 (0.7 to 1.5)
≥5	104 (16.0%)	230 (11.9%)	0.8 (0.4 to 1.9)	1.0 (0.5 to 1.8)
Any insecticide				
0	382	1292	1.0	1.0
1	114 (17.5%)	281 (14.5%)	1.3 (0.9 to 1.9)	1.2 (0.9 to 1.7)
2–4	86 (13.2%)	237 (12.3%)	1.0 (0.5 to 1.8)	0.9 (0.6 to 1.4)
≥5	68 (10.5%)	123 (6.4%)	1.9 (0.6 to 5.7)	1.4 (0.7 to 2.9)
Any herbicide				
0	489	1544	1.0	1.0
1	50 (7.7%)	132 (6.8%)	1.0 (0.6 to 1.9)	1.1 (0.7 to 1.7)
2–4	52 (8.0%)	132 (6.8%)	0.8 (0.4 to 1.9)	1.0 (0.6 to 1.6)
≥5	59 (9.1%)	125 (6.5%)	0.8 (0.2 to 3.3)	1.0 (0.5 to 2.2)
"Potentially carcinogenic" pesticides				
0	496	1632	1.0	1.0
1	74 (11.4%)	168 (8.7%)	1.6 (0.8 to 3.1)	1.1 (0.8 to 1.7)
2–4	68 (10.5%)	123 (6.4%)	2.7 (0.7 to 10.8)	1.3 (0.7 to 2.3)
≥5	12 (1.8%)	10 (0.5%)	25.9 (1.5 to 450.2)	2.0 (0.8 to 5.2)

*Each estimate is adjusted for use of all pesticides listed in table 3, age, and study site.
†Odds ratios (OR) and 95% confidence limits (CL).

The analysis of 48 pesticide combinations in relation to NHL incidence revealed few joint effects of 1.3 or higher that were indicative of superadditivity (table 5). Combined exposures to carbofuran and atrazine, diazinon and atrazine, and alachlor and atrazine had estimated joint effects that were more than additive (ICR ≥0.5), even following shrinkage in hierarchical regression analyses. Other joint pesticide effects which seemed indicative of superadditivity in results from logistic regression analyses, such as that for atrazine and dicamba, were probably misleading due to imprecision of estimates; these results did not hold up following shrinkage in hierarchical regression analyses, according to our prior distribution of complete exchangeability.

DISCUSSION

Incidence and mortality rates for NHL have been generally increasing in the United States and in most industrialised countries for several decades, with an 85–100% increase in

Table 5 Estimated individual and joint effects of pesticide combinations on NHL incidence*†

Individual and joint pesticide exposures	Exposed [n (%)] Cases (n=650)	Controls (n=1933)	Logistic regression OR (95% CL)‡	Hierarchical regression OR (95% CL)
Chlordane and DDT				
Neither	543	1687	1.0	1.0
Chlordane only	9 (1.4%)	20 (1.0%)	1.1 (0.4 to 2.7)	1.0 (0.5 to 1.9)
DDT only	68 (10.5%)	181 (9.4%)	0.9 (0.6 to 1.3)	0.9 (0.6 to 1.2)
Both	30 (4.6%)	45 (2.3%)	1.7 (0.7 to 3.2)	1.3 (0.8 to 2.3)
Carbofuran and atrazine				
Neither	557	1728	1.0	1.0
Carbofuran only	3 (0.5%)	20 (1.0%)	0.2 (0.1 to 1.1)	0.6 (0.3 to 1.3)
Atrazine only	52 (8.0%)	109 (5.6%)	1.4 (0.9 to 2.2)	1.3 (0.9 to 1.9)
Both	38 (5.9%)	76 (3.9%)	1.6 (0.8 to 3.3)	1.5 (0.9 to 2.7)
Diazinon and atrazine				
Neither	551	1730	1.0	1.0
Diazinon only	9 (1.4%)	18 (0.9%)	1.2 (0.5 to 3.1)	1.1 (0.5 to 2.3)
Atrazine only	59 (9.1%)	141 (7.3%)	1.5 (1.0 to 2.3)	1.3 (0.9 to 1.9)
Both	31 (4.8%)	44 (2.3%)	3.9 (1.7 to 8.8)	2.3 (1.2 to 4.2)
Alachlor and atrazine				
Neither	545	1695	1.0	1.0
Alachlor only	15 (2.3%)	53 (2.7%)	0.7 (0.3 to 1.3)	0.7 (0.4 to 1.3)
Atrazine only	37 (5.7%)	86 (4.5%)	1.3 (0.8 to 2.1)	1.2 (0.8 to 1.8)
Both	53 (8.2%)	99 (5.1%)	2.1 (1.1 to 3.9)	1.6 (1.0 to 2.7)
Atrazine and dicamba				
Neither	552	1729	1.0	1.0
Atrazine only	59 (9.1%)	125 (6.5%)	1.5 (1.0 to 2.4)	1.4 (0.9 to 2.0)
Dicamba only	8 (1.2%)	19 (1.0%)	0.9 (0.3 to 2.6)	1.0 (0.5 to 2.0)
Both	31 (4.8%)	60 (3.1%)	2.1 (1.0 to 4.7)	1.6 (0.9 to 2.9)

*Effects of combined pesticide exposures were estimated in models including terms for the joint exposure, two individual exposures, the use of each other pesticide listed in table 2, age, and study site.
†Pesticide combinations considered are listed in the appendix.
‡Odds ratios (OR) and 95% confidence limits (CL).

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mortality among whites and non-whites from the late 1940s to the late 1980s,[26] a time period relevant for this study. This increase may be partially attributed to improved diagnosis and in later years to AIDS related lymphomas, but cannot be completely explained by these factors.[27] Environmental factors such as pesticides could play a role in this persistent increase, since their use became more widespread during this time period.[28–30] Several aetiological mechanisms of pesticides in relation to NHL have been proposed, including genotoxicity and immunotoxicity,[31 32] increased cell proliferation,[33] and chromosomal aberrations.[34] In our analysis of multiple pesticides in farming, we found only a small number of the pesticides to be risk factors for NHL, with the highest increased risks among subjects exposed to five or more of these "potentially carcinogenic" pesticides, or those with certain combined pesticide exposures.

The large number of exposed subjects in this pooled analysis allowed adjustment for the use of other pesticides, and hierarchical regression modelling resulted in estimates that were in some instances more stable than those from logistic regression models. However, the effect estimates from the logistic and hierarchical analyses were quite similar overall, with a few standout exceptions. The hierarchical results are more conservative than those from the logistic regressions, given the uninformed nature of the prior distributions we specified, particularly in analyses of the number of pesticides used and combined pesticide exposures. For example, in the hierarchical regression analysis of the number of pesticides used, we assumed that the use of any five or more pesticides was no more likely to be associated with NHL than use of any one pesticide. A less conservative prior distribution could have been specified in which a higher probability would be placed on a positive association for the greater number of pesticides used. However, the uninformed nature of these priors seemed appropriate in a largely exploratory analysis of multiple exposures for which there is little prior knowledge about how pesticide exposures interact in relation to the risk of NHL. Both analyses showed increasing odds ratios with the number of "potentially carcinogenic" pesticides used, but the relative risks in the upper category were substantially different—25.9 for the logistic regression and 2.0 for the hierarchical analysis—probably indicating inappropriate use of logistic regression for these sparse data.

Adjustment for multiple pesticides suggested that there were few instances of substantial confounding of pesticide effects by other pesticides. Nevertheless, some previous findings in our data appear to be due to confounding by correlated pesticide exposures. In particular, a previously reported positive association for carbaryl[13] was not replicated in the adjusted analyses. Further analysis here revealed that carbaryl and diazinon use were highly associated ($p < 0.001$), and previously reported associations of different carbaryl measures with NHL were eliminated by adjustment for diazinon, including carbaryl use, personal handling of carbaryl, and use longer than 10 years. In the previous analysis, estimates were adjusted for groups of pesticides, including a group for organophosphate insecticides,[13] but adjustment for specific pesticides here gave different results. Similarly, previous observations of increased NHL risk associated with use of the OP insecticides dimethoate and tetrachlorvinphos[12] were negligible on inclusion of other OP insecticides in the model. These findings underscore the importance of considering correlated pesticide exposures.

Our observation of increased risk associated with the use of certain OP insecticides, including coumaphos, diazinon, and fonofos, is consistent with previous analyses of the pooled data,[12 20] and also corroborates findings of other studies.[8 34] OP insecticides are known to cause cytogenetic damage, and could thereby contribute to NHL aetiology.[35] There are data from in vitro, animal, and human studies that show effects of several OP insecticides on the immune system,[36–40] indicating another potential mechanism. OP compounds may impair immune function through pathways involving cholinergic stimulation,[41] or inhibition of serine esterases found in monocytes, natural killer cells, and cytotoxic T lymphocytes,[42] but it is unknown whether such immune effects might be chemical specific or related to general OP toxicity. Our data do not indicate an aetiological mechanism for NHL common to all OP insecticides, since increased NHL incidence was associated only with certain OPs evaluated.

We observed a possible effect of the organochlorine insecticides chlordane and dieldrin. There is some evidence that chlordane is immunotoxic, causing decreased lymphocyte function in vitro.[43] The concentration of chlordane in adipose tissue was higher among NHL cases than controls in a small case-control study in Sweden,[44] but a larger study in the United States found no such association.[45] Although these chemicals have been banned in the United States, their continued use in some developing countries, and bioaccumulation of their chemical residues in the food chain,[46] justify further research on health effects.

Use of the herbicide atrazine was associated with increased risk of NHL. Increased risk was observed in each of the three pooled studies separately, but a previous analysis of the Nebraska study data found that the risk was diminished on adjustment for use of OP insecticides and 2,4-D.[20] There have been few other epidemiological studies of atrazine in relation to NHL. In a cohort of triazine herbicide manufacturing workers, there was an excess number of deaths from NHL ($n = 3$) among a group of men with definite or probable exposure; however, some of the cases worked in triazine related jobs for short time periods, thus clouding interpretation.[47] A recent NHL study where cases were further distinguished by presence or absence of the t(14;18) chromosomal translocation found that the risk of NHL associated with atrazine use was solely observed among t(14;18) positive cases, suggesting a cytogenetic mechanism.[14] However, there is only very limited evidence for genotoxicity of atrazine, although there are no studies in humans.[48] A small number of studies of atrazine on immune function in rodents and in vitro suggest a decreased lymphocyte count and cytokine production following exposure; however, these effects were not always dose dependent or statistically significant.[37 48 49] In our data, there was an indication of superadditive effects of atrazine in combination with carbofuran, diazinon, or alachlor. This is a factor to consider in future studies of this widely used pesticide.

Glyphosate, commercially sold as Roundup, is a commonly used herbicide in the United States, both on crops and on non-cropland areas.[50] An association of glyphosate with NHL was observed in another case-control study, but the estimate was based on only four exposed cases.[51] A recent study across a large region of Canada found an increased risk of NHL associated with glyphosate use that increased by the number of days used per year.[8] These few suggestive findings provide some impetus for further investigation into the potential health effects of glyphosate, even though one review concluded that the active ingredient is non-carcinogenic and non-genotoxic.[50]

Much attention in NHL research has focused on the herbicide 2,4-D as a potential risk factor, and several studies have observed positive associations with 2,4-D exposure.[6 8 9] Whereas an indicated effect of 2,4-D exposure on NHL was reported in NCI's Nebraska and Kansas studies,[5 7] this analysis of the pooled data found no association with having ever used 2,4-D. The null association does not result from adjustment for other pesticides, missing data, or from the hierarchical regression modelling approach, but is rather due to pooling data from the Iowa and Minnesota study, in which no association of 2,4-D with NHL incidence was observed, with data from the Nebraska and Kansas studies. The literature on the relation between 2,4-D and NHL is not consistent.[32 52] Some recent studies have reported excess risk among

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manufacturers[53] and farmers,[8] while others have not.[51] The study in Nebraska,[5] however, observed that NHL risk increased by number of days per year of 2,4-D use, which we were unable to duplicate in the pooled analysis because of lack of such data from the other two studies. It is possible that a more refined metric incorporating frequency of use better captures relevant exposure. Some recent studies may shed light on potential mechanisms of 2,4-D in relation to NHL. A study of 10 farmers who applied 2,4-D and MCPA observed a significant reduction of several immune parameters, including CD4, CD8, natural killer cells, and activated CD8 cells (expressing the surface antigen HLA-DR), and a reduction in lymphoproliferative response.[54] Furthermore, a study of professional 2,4-D applicators in Kansas observed an increase in the lymphocyte replication index following application.[33]

This pooled study of multiple agricultural pesticides provided an opportunity to estimate the effect of each specific pesticide and certain pesticide combinations on NHL incidence, adjusted for the use of other pesticides. Overall, few pesticides and pesticide combinations were associated with increased NHL risk; this has several implications. First, it is consistent with results from bioassays where only a few of the pesticides tested have caused cancer in laboratory animals.[55] Although epidemiological data on cancer risks from exposure to specific pesticides are scant, it also suggests that while some pesticides may present a cancer risk to humans, many, maybe even most, pesticides do not. Second, the fact that there were few associations suggests that the positive results we observed are not likely to be due to a systematic recall bias for pesticide exposures, or selection bias for the subgroup included in the analyses of multiple pesticides. Third, although some of the positive results could be due to chance, the hierarchical regression analysis placed some restriction on the variance of estimates, theoretically decreasing the chances of obtaining false positive results. On the other hand, it is possible that the assumptions for the hierarchical regression are too restrictive and that this has increased the number of false negatives.

Certain limitations of our data hinder the inferences we can make regarding specific pesticides in their association with NHL. Our exposure metric of having ever used a pesticide is rather crude, offering no distinctions based on use by the number of years or the number of days per year. Further exploration of observed associations by more refined exposure metrics is warranted. In addition, this analysis provides no information on the timing of pesticide use in relation to disease onset or in conjunction with the timing of other pesticides used. This has particular relevance in our analysis of "combined pesticide exposures", in which two pesticides may or may not have been used at the same time or even during the same year. Lastly, if a study subject had a missing value for any one of the 47 pesticides evaluated, that person was excluded from analyses, resulting in analyses on a limited subset (about 75%) of the pooled study population. Although we have no way to evaluate potential bias due to missing data, some assurances are provided by the fact that cases and controls were equally likely to be included in analyses, and that there were similarities between the entire group of study subjects and subjects included our analyses, in terms of NHL status in relation to demographic factors (table 2). If simultaneous analysis of multiple exposures is to become standard, statistical techniques to impute values for subjects with "don't know" or missing responses should be further developed in order to prevent biased results.

Despite limitations of our study, certain inferences are possible. Our results indicate increased NHL incidence by number of pesticides used, only for the subgroup of "potentially carcinogenic" pesticides, suggesting that specific chemicals, not pesticides, insecticides, or herbicides, as groups, should be examined as potential risk factors for NHL. In addition, argument against an analysis approach focused on classes or groups of pesticides is provided by the fact that our prior covariates of pesticide classes and groups in the hierarchical regression model were not important predictors of the magnitude of observed pesticide effects. A chemical specific approach to evaluating pesticides as risk factors for NHL should facilitate interpretation of epidemiological studies for regulatory purposes. However, the importance of additionally considering multiple correlated exposures is clear.

APPENDIX

Table A1 shows the pesticide combinations considered in analyses of joint and individual exposures.

Table A1 Pesticide combinations considered in analyses of joint and individual exposures

Insecticides	Insecticide and herbicide	Herbicides
DDT and chlordane	Aldrin and alachlor	Alachlor and atrazine
DDT and lindane	Aldrin and atrazine	Alachlor and chloramben
DDT and malathion	Aldrin and 2,4-D	Alachlor and cyanazine
DDT and fly, lice, or tick spray	Aldrin and trifluralin	Alachlor and 2,4-D
DDT and aldrin	Carbofuran and alachlor	Alachlor and dicamba
Lindane and malathion	Carbofuran and atrazine	Alachlor and glyphosate
Lindane and aldrin	Carbofuran and 2,4-D	Alachlor and trifluralin
Malathion and aldrin	Chlordane and 2,4-D	Atrazine and cyanazine
	DDT and alachlor	Atrazine and 2,4-D
	DDT and atrazine	Atrazine and dicamba
	DDT and 2,4-D	Atrazine and glyphosate
	DDT and trifluralin	Atrazine and trifluralin
	Diazinon and atrazine	Chloramben and trifluralin
	Fly, lice, or tick spray and alachlor	Cyanazine and 2,4-D
	Fly, lice, or tick spray and atrazine	Cyanazine and trifluralin
	Fly, lice, or tick spray and 2,4-D	2,4-D and trifluralin
	Fly, lice, or tick spray and trifluralin	
	Lindane and alachlor	
	Lindane and atrazine	
	Lindane and 2,4-D	
	Lindane and trifluralin	
	Malathion and alachlor	
	Malathion and atrazine	
	Malathion and 2,4-D	

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Pesticides and Other Agricultural Risk Factors for Non-Hodgkin's Lympho among Men in Iowa and Minnesota

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ABSTRACT

Data from an in-person interview study of 622 white men with newly diagnosed non-Hodgkin's lymphoma and 1245 population-based controls in Iowa and Minnesota were used to measure the risk associated with farming occupation and specific agricultural exposures. Men who ever farmed were at slightly elevated risk of non-Hodgkin's lymphoma (odds ratio = 1.2, 95% confidence interval = 1.0–1.5) that was not linked to specific crops or particular animals. Elevated risks were found, with odds ratio generally 1.5-fold or greater, for personal handling, mixing, or application of several pesticide groups and for individual insecticides, including carbaryl, chlordane, dichlorodiphenyltrichloroethane, diazinon, dichlorvos, lindane, malathion, nicotine, and toxaphene. Associations were generally stronger for first use prior to 1965 than more recently, and when protective clothing or equipment was not used. Small risks were associated with the use of the phenoxyacetic acid herbicide 2,4-dichlorophenoxyacetic acid, but the risks did not increase with latency or failure to use protective equipment. Exposure to numerous pesticides poses problems of interpreting risk associated with a particular chemical, and multiple comparisons increase the chances of false-positive findings. In contrast, nondifferential exposure misclassification due to inaccurate recall can bias risk estimates toward the null and mask positive associations. In the face of these methodological and statistical issues, the consistency of several findings, both within this study and with observations of others, suggests an important role for several insecticides in the etiology of non-Hodgkin's lymphoma among farmers.

INTRODUCTION

While farmers generally have low rates of morbidity and mortality, they appear to be at excess risk of selected cancers, particularly some of the hematopoietic tumors (1). Some studies suggest that the elevated risk of NHL[2] and leukemia among farmers may be associated with exposure to pesticides and other agricultural chemicals (2). To further evaluate these associations, we conducted parallel population-based case-control interview studies of men newly diagnosed with non-Hodgkin's lymphoma and leukemia in the states of Minnesota and Iowa. Findings for leukemia are reported elsewhere (3).

METHODS

Case Selection. All newly diagnosed cases of non-Hodgkin's lymphoma among men aged 30 or older were ascertained from Iowa State Health Registry records and a special surveillance of Minnesota hospital and pathology laboratory records. In Iowa, the diagnosis period for eligibility was March 1981 to October 1983, and in Minnesota, October 1980 to September 1982. In Iowa, all cases who resided in the state were eligible. In Minnesota, eligibility was restricted to cases who resided in places other than the cities of Minneapolis, St. Paul, Duluth, or Rochester at the time of diagnosis.

Pathology Review. A review panel of 4 experienced regional pathologists confirmed diagnoses and classified NHL cases as to morphological type using the Working Formulation for classification of NHL (4). NHL subtype was designated when at least 3 panelists agreed on a specific diagnosis, either at the initial review or a supplementary review conducted for more difficult cases. The case was considered "unclassifiable" if the pathology panel could not come to consensus on NHL subtype, or if the tissue sample was not adequate to differentiate among subtypes. The NHL subtypes were collapsed into categories as follows: follicular (combining small cleaved cell, mixed cell, and large cell follicular cases); diffuse (combining small cleaved cell, mixed cell, and large cell diffuse cases); small lymphocytic; and "other NHL" (combining large cell immunoblastic, lymphoblastic, small noncleaved, other, and unclassified NHL cases). Additional details regarding histopathology review procedures are presented elsewhere (5, 6).

Control Selection. A population-based control group of white men without a hematopoietic or lymphatic cancer was randomly selected and frequency-matched to NHL and leukemia cases by 5-year age group, vital status at time of interview, and state of residence. The sources of controls were: (*a*) random digit dialing for living subjects under age 65 at diagnosis, using the Waksberg method (7, 8) (data from the 1980 United States Census report that 96 and 97% of Iowa and Minnesota households, respectively, had telephones); (*b*) a 1% random listing from Medicare files provided by the Health Care Financing Administration for living subjects aged 65 and older [United States citizens 65 years of age and older are eligible for Medicare insurance and over 98% have been estimated to be in the roster (9)]; and (*c*) state death certificate files for deceased subjects.

Data Collection. Interviews were conducted during the period of August 1981 to May 1984. A trained interviewer administered an in-person structured interview, taking 45–60 min, to the subject, or the spouse, other close relative, or friend of deceased or incompetent subjects. We asked about sociodemographic characteristics, medical history, smoking habit, occupational history, residential history, familial history of cancer, and other known and suspected risk factors. In addition, we requested a detailed farming and pesticide use history of all subjects who had worked on a farm at least 6 months since age 18. For each farm that the respondent had worked, we recorded the years of farming activity, the total acreage, the number and types of livestock, and the crops grown, with average acreage for each and the number of years they had been grown on that farm. We also asked for a detailed history of pesticide use. Pesticide lists for the questionnaire were developed with the assistance of local agricultural experts. We named 23 specific insecticides used on animals, 34 insecticides applied to crops, 38 herbicides, and 16 fungicides. For each pesticide, we asked if it had ever been used; the first and last year of use; the method of application (aerial, surface application, incorporated into soil, other); whether the respondent had personally applied, mixed, or handled it; and the use of protective equipment.

Response Rates. Seven hundred eighty presumptive NHL cases were ascertained, and 694 (89%) were interviewed. After pathology review of interviewed cases, 622 were confirmed as NHL (438 living cases with direct interviews, 184 deceased or incompetent cases with proxy interviews). Among the 72 cases that could not be confirmed, 26 were

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[2] The abbreviations used are: NHL, non-Hodgkin's lymphoma; DDT, dichlorodiphenyltrichloroethane; CLL, chronic lymphocytic leukemia; OR, odds ratio; CI, 95% confidence interval; 2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid.

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Table 1 *Characteristics of cases and controls from a study of non-Hodgkin's lymphoma in Iowa and Minnesota*[a]

	Cases		Controls	
	No.	(%)	No.	(%)
Type of NHL				
Follicular	195	(31)		
Diffuse	198	(32)		
Small lymphocytic	85	(14)		
Other	144	(23)		
Type of interview				
Direct	438	(70)	820	(66)
Surrogate	184	(30)	425	(34)
State of residence				
Iowa	293	(47)	603	(48)
Minnesota	329	(53)	642	(52)
Age				
<45	73	(12)	134	(11)
45–64	230	(37)	430	(35)
65+	319	(51)	681	(55)
Hair dye use (ever)?				
No	574	(92)	1194	(96)
Yes	48	(8)	51	(4)
Lymphopoietic cancer diagnosed in any first degree relative?				
No	557	(90)	1154	(93)
Yes	54	(9)	66	(5)
High risk occupation (ever)? [b]				
No	524	(84)	1174	(94)
Yes	98	(16)	71	(6)
Used high risk materials at least monthly for a year or more? [c]				
No	369	(59)	840	(67)
Yes	253	(41)	405	(33)
Cigarette smoking habit				
Never smoked	186	(30)	418	(34)
Past smoker	243	(40)	486	(39)
Current smoker	182	(30)	333	(27)

[a] Cases and controls numbered 622 and 1245, respectively. The number of respondents with missing values for selected characteristics is not explicitly listed.
[b] Persons ever employed at an occupation yielding an odds ratio of 1.5 or greater in Mantel-Haenzsel analyses adjusted for age (2 strata) and state of residence.
[c] Persons using one or more materials yielding an odds ratio of 1.5 or greater, from a list of 43 items that included paints, benzene, other organic solvents, resins, and others.

diagnosed as leukemia, and 46 with other conditions. Pathology review was not conducted on material of the persons who were not interviewed. Among random digit dialing controls, the household screening response rate was 87.5%, yielding 474 eligible persons, of whom 415 (87.6%) agreed to participate, for a net response rate of 76.7%. Among the 2 other control groups, 79% of the eligible controls selected from the Health Care Financing Administration rolls participated, and 77% of the eligible proxies for deceased controls provided complete interviews.

Statistical Analysis. The association between a variety of farm-related factors and risk of NHL was measured by the maximum likelihood estimate of the OR. ORs were adjusted for several known or suspected NHL risk factors, using unconditional logistic regression analysis with case-control status as the response variable (10, 11). OR for farmers who raised specific crops or animals, or were exposed to individual pesticides and families of pesticides, were calculated for all NHL and the NHL subtypes, comparing exposed persons to nonfarmers, except as noted. ORs for the histological subtypes of NHL were calculated using software for polychotomous logistic models developed by the Epidemiology and Biostatistics Program of the National Cancer Institute. Logistic models included the following potential confounding variables: vital status (alive, dead); state (Iowa, Minnesota); age (<45, 45–64, 65+); cigarette smoking habit (never, past, current); lymphopoietic cancer in a parent, sibling, or child (yes, no); nonfarming job related to NHL in this study (with OR of 1.5+); exposure to hair dyes (yes, no); and exposure to one or more other substances associated with NHL in this study [with OR of 1.5+, as calculated by standard methods with adjustment for age and state of residence (12)]. Tests for trend in the logistic analysis were obtained by categorizing the exposure variable and treating the scored variable as a continuous variable.

RESULTS

Study Population. Table 1 shows the distribution of the 622 cases and 1245 controls by type of NHL, type of interview, state of residence, age, hair dye use, having had a first degree relative with lymphopoietic cancer, employment in a high risk occupation (*a priori*), exposure to high risk materials (*a posteriori*), and cigarette smoking habit. Among the 622 respondent cases, the distribution of histological types was: 195 follicular (31.4%), 198 diffuse (31.8%), 85 small lymphocytic cell (13.7%), and 144 other and undefined lymphomas (23.2%).

We found elevated relative risks associated with certain occupational exposures and job classifications, hair dye use, as well as a history of familial cancer. These factors were entered as potential confounders in logistic regression models, as were variables for age, state of residence, and vital status of the study subject.

Farming. There was a small, but marginally significant increase in risk for all NHL (OR = 1.2, 95%, CI = 1.0–1.5) associated with ever living or working on a farm as an adult (Table 2). Fifty-seven % of the cases and 56% of controls reported some farm activity. When analyzed by NHL subtype, there was a small excess risk for each, but none was significant. Among subtypes, the highest observed risk for farming was found for small cell lymphocytic lymphoma (OR = 1.4, CI = 0.9–2.3).

No statistically significant trend by first and last year of farming activity, duration, or average yearly number of acres

Table 2 *OR and CI for non-Hodgkin's lymphoma according to ever having been a farmer, timing of farming occupation, and average size of farm (in acres)*[a]

	CO	CA	OR	CI
Nonfarmer	547	266	1.0	
Farmer	698	356	1.2	1.0, 1.5
First year farmed				
<1925	218	105	1.3	0.9, 1.8
1925–1934	200	92	1.1	0.8, 1.5
1935–1944	143	64	0.9	0.7, 1.3
1945+	136	94	1.4	1.0, 1.9
Missing	1	1		
Farmed until				
<1950	190	77	0.9	0.6, 1.3
1950–1969	190	113	1.4	1.1, 1.9
1970+	314	165	1.2	0.9, 1.6
Missing	4	1		
No. of years farmed				
<10	163	89	1.2	0.9, 1.6
10–39	289	153	1.2	0.9, 1.6
40+	239	112	1.2	0.9, 1.6
Missing	7	2		
Average no. of acres				
<120	129	62	1.1	0.8, 1.6
120–199	217	115	1.3	1.0, 1.7
200–319	183	96	1.2	0.9, 1.7
320+	140	72	1.1	0.8, 1.6
Missing	29	11		

[a] All OR relative to risk for subjects who were never farmers (266 cases, 547 controls). All ORs adjusted for vital status, age, state, cigarette smoking, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures in a logistic analysis.

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during farming years was observed for all NHL or any subtype (Table 2). However, we observed slightly higher risks among men who farmed after 1949 than those who stopped before 1950. Men who operated medium-size farms (120–199 acres or 200–319 acres) were at slightly higher risk for all NHL and for most NHL subtypes than men farming smaller or larger establishments.

There was no notable association of risk for all NHL associated with the cultivation of any major crop, nor with the husbandry of the major types of livestock (data not shown). The patterns of OR for the lymphoma histological subtypes, as related to particular crops and livestock, followed the overall pattern for farming in general, with elevated (mostly nonsignificant) OR for small lymphocytic lymphoma associated with corn (OR = 1.4, CI = 0.9–2.4; 52 cases), wheat (OR = 1.5, CI = 0.8–2.9; 21 cases), flax (OR = 2.3, CI = 1.0–5.0; 15 cases), barley (OR = 1.5, CI = 0.7–3.1; 15 cases), and hay (OR = 1.4, CI = 0.8–2.4; 31 cases). Associations of other NHL subtypes with specific crops and livestock were weaker, as were associations of small lymphocytic lymphoma with specific types of livestock.

Among the 356 cases and 698 controls who had lived and worked on one or more farms as an adult, 323 cases (90.7%) and 636 controls (91.4%) reported that they were farm operators on at least one farm. Operators usually plan and execute pest control activities, and are more likely than hired hands to have direct knowledge of the chemicals used.

Pesticide Use (Ever). Among farmers, 300 cases (84%) and 603 controls (86%) reported use of at least one pesticide (for all NHL, OR = 1.2, CI = 0.9–1.4, relative to nonfarmers). The OR for use of one or more insecticides on livestock was 1.1 (CI = 0.9–1.4); for crop insecticide use, 1.2 (CI = 0.9–1.5); for herbicide use, 1.3 (CI = 1.0–1.6); and for fungicide use, 1.3 (CI = 0.8–2.0).

Pesticide Families. Table 3 shows the numbers of cases and controls, OR, and CI for use of one or more members of the listed chemical families of pesticides, by broad grouping of livestock insecticides, crop insecticides, and herbicides. Classification of pesticides into chemical families was done by us. All OR shown are relative to nonfarmers, numbering 266 cases and 547 controls. Significant risk elevations were found for several livestock insecticide families: chlorinated hydrocarbons (OR = 1.3), in particular the cyclodienes (OR = 1.7); natural products (OR = 1.5); and organophosphates (OR = 1.5), in particular the halogenated aromatic organophosphates (OR = 2.0). Among insecticides used on crops, the chlorinated hydrocarbon family showed significant elevation in risk (OR = 1.4). Although based on small numbers, use of nonhalogenated organophosphates on crops was associated with a nonsignificant OR of 3.1. Use of insecticides on livestock or crops resulted in a significant increased risk of NHL associated with chlorinated hydrocarbons (OR = 1.3) and organophosphates (OR = 1.5). No single family of herbicides was significantly associated with overall NHL risk.

The use, handling, or application of pesticides in selected chemical families was associated with elevated risk for several of the NHL morphological subtypes. Significantly elevated OR were found for diffuse NHL and: organophosphates used on crops (OR = 2.3, CI = 1.4–3.8; 26 cases, 101 controls); nonhalogenated aliphatic organophosphates for crops (OR = 2.2, CI = 1.3–3.8; 24 cases, 95 controls); cyclodiene chlorinated hydrocarbons used on livestock (OR = 2.2, CI = 1.1–4.5; 11 cases, 42 controls); and triazine herbicides (OR = 1.6, CI = 1.0–2.6; 25 cases, 133 controls). Small lymphocytic NHL was significantly associated with natural product insecticides used for livestock application (OR = 2.4, CI = 1.1–5.2; 10 cases, 70 controls) and halogenated aromatic organophosphates for livestock (OR = 5.2, CI = 1.9–14.3; 6 cases, 23 controls). Other and unclassified forms of NHL were significantly linked to the chlorinated hydrocarbon insecticide family used for crops (OR = 1.8, CI = 1.1–3.0; 26 cases, 157 controls); the cyclodienes (OR = 2.1, CI = 1.0–4.7; 15 cases, 111 controls) for crops; and halogenated aliphatic organophosphates used on livestock (OR = 2.3, CI = 1.0–5.3; 8 cases, 41 controls). No significant associations with use, handling, or application of pesticide families were found for follicular NHL.

Table 3 *OR[a] and CI for the use of pesticide groups in which at least one pesticide was handled by the respondent[b]*

	Cases	Controls	OR	CI
Insecticides used on livestock				
Carbamates	6	15	0.8	0.3, 2.2
Chlorinated hydrocarbons	112	198	1.3	1.0, 1.7
Cyclodienes	34	42	1.7	1.0, 2.8
Natural products	46	70	1.5	1.0, 2.2
Organophosphates	68	101	1.5	1.0, 2.1
Halogenated aliphatics	20	41	1.2	0.7, 2.0
Nonhalogenated aliphatics	43	67	1.3	0.9, 2.1
Halogenated aromatics	21	23	2.0	1.1, 3.7
Nonhalogenated aromatics	12	16	1.7	0.8, 3.6
Insecticides used on crops				
Carbamates	41	80	1.2	0.8, 1.8
Chlorinated hydrocarbons	96	157	1.4	1.0, 1.9
Cyclodienes	57	111	1.2	0.8, 1.7
Arsenicals	43	75	1.3	0.8, 2.0
Organophosphates	60	101	1.3	0.9, 1.9
Nonhalogenated aliphatics	56	95	1.3	0.9, 1.9
Nonhalogenated aromatics	7	4	3.1	0.9, 11.0
Insecticides used on crops and/or livestock				
Carbamates	43	85	1.1	0.8, 1.7
Chlorinated hydrocarbons	150	262	1.3	1.0, 1.7
Cyclodienes	70	124	1.3	0.9, 1.8
Organophosphates	96	144	1.5	1.1, 2.0
Halogenated aliphatics	21	41	1.2	0.7, 2.1
Nonhalogenated aliphatics	78	119	1.4	1.0, 2.0
Nonhalogenated aromatics	17	20	1.8	0.9, 1.8
Herbicides				
Amides	59	114	1.2	0.8, 1.7
Benzoic acids	53	98	1.3	0.9, 1.9
Carbamates	24	50	1.1	0.7, 1.9
Dinitroaniline	46	88	1.2	0.8, 1.8
Heterocyclics	20	49	0.9	0.5, 1.6
Phenoxyacetic acids	118	231	1.2	0.9, 1.6
Triazines	64	133	1.1	0.8, 1.6
Ureas	5	18	0.6	0.2, 1.6

[a] OR relative to nonfarmers, numbering 266 cases and 547 controls. All ORs adjusted for vital status, age, state, cigarette smoking status, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures in a logistic analysis.

[b] Individual pesticides were categorized into chemical families by the authors.

Selected Pesticides. Tables 4–6 show the numbers of cases and controls, with OR and CI for all NHL, from analyses of farmers who ever personally handled, mixed, or applied specific pesticides, and for farmers who first handled them prior to 1965 (1965 was chosen because it was 15–18 years prior to diagnosis, a reasonable minimal period for latency). Among livestock insecticides (Table 4), there were significantly elevated risks for *ever* handled, mixed, or applied for chlordane and lindane. Most other livestock insecticides had OR greater than 1.0. In general, first use prior to 1965 was associated with higher risk than ever use, and was significant for early reported use of chlordane, lindane, malathion, and nicotine. Among subjects who *ever* personally handled, mixed, or applied specific

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Table 4 *Animal insecticides: ORs and CIs for ever having handled specific animal insecticides, and handled prior to 1965*

Insecticide	Ever handled				Handled prior to 1965			
	No. of cases	No. of controls	OR	CI	No. of cases	No. of controls	OR	CI
Chlordane	31	38	1.7	1.0, 2.9	22	22	2.2	1.2, 4.2
Coumaphos	13	18	1.6	0.8, 3.5	3	5	1.5	0.3, 6.3
DDT	79	149	1.2	0.9, 1.7	68	123	1.3	0.9, 1.8
Dichlorvos	20	38	1.2	0.7, 2.2	12	17	1.8	0.8, 3.9
Famphur	10	14	1.7	0.7, 4.0	1	1	2.4	0.1, 39
Lindane	55	90	1.4	1.0, 2.1	40	55	1.7	1.1, 2.7
Malathion	43	67	1.3	0.9, 2.1	25	30	1.8	1.0, 3.3
Methoxychlor	9	16	1.2	0.5, 2.7				
Nicotine	31	47	1.5	0.9, 2.5	28	36	1.8	1.0, 3.0
Rotenone	12	23	1.0	0.5, 2.2				
Toxaphene	8	19	0.8	0.3, 2.0				
Flyspray (NOS)	185	394	1.1	0.9, 1.4	173	368	1.1	0.9, 1.4

[a] OR relative to nonfarmers, numbering 266 cases and 547 controls. All ORs adjusted for vital status, age, state, cigarette smoking status, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures in a logistic analysis.

Table 5 *Crop insecticides: ORs and CIs for ever having handled specific insecticides, and handled prior to 1965*[a]

Insecticide	Ever handled				Handled prior to 1965			
	No. of cases	No. of controls	OR	CI	No. of cases	No. of controls	OR	CI
Aldrin	47	97	1.1	0.7, 1.7	34	59	1.3	0.8, 2.1
Carbofuran	29	65	1.0	0.6, 1.7	28	63	1.0	0.6, 1.7
Carbaryl	21	26	1.7	0.9, 3.1	7	4	3.8	1.1, 13.6
Chlordane	21	26	1.7	0.9, 3.2	12	16	1.6	0.7, 3.6
Copper acetoarsenate	36	63	1.3	0.8, 2.0	30	54	1.2	0.7, 2.0
DDT	57	75	1.7	1.2, 2.6	45	57	1.8	1.1, 2.7
Diazinon	27	39	1.5	0.9, 2.5	14	12	2.6	1.2, 5.9
Dieldrin	17	26	1.4	0.7, 2.8	10	13	1.9	0.8, 4.4
Fonofos[b]	15	30	1.1	0.6, 2.1				
Heptachlor	25	43	1.3	0.7, 2.2	14	25	1.3	0.6, 2.6
Lindane	21	23	2.0	1.0, 3.7	14	15	2.2	1.0, 4.7
Malathion	21	30	1.5	0.8, 2.7	11	9	2.9	1.1, 7.4
Phorate	21	48	1.0	0.6, 1.7	9	12	1.8	0.7, 4.5
Turbufos[b]	15	36	0.9	0.5, 1.7				
Toxaphene	10	13	1.5	0.6, 3.5	6	5	2.4	0.7, 8.2

[a] OR relative to nonfarmers, numbering 266 cases and 547 controls. All ORs adjusted for vital status, age, state, cigarette smoking status, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures in a logistic analysis.
[b] No reported use of fonofos or turbufos prior to 1965.

Table 6 *Herbicides: OR and CI for ever having handled specific herbicides, and handled prior to 1965*[a]

Herbicide	Ever handled				Handled prior to 1965			
	No. of cases	No. of controls	OR	CI	No. of cases	No. of controls	OR	CI
Alachlor	57	109	1.2	0.8, 1.7				
Atrazine	59	108	1.2	0.9, 1.8	19	32	1.3	0.7, 2.5
Bentazon	18	45	0.9	0.5, 1.6				
Butylate	22	44	1.2	0.7, 2.1	1	6	0.5	0.1, 4.3
Chloramben	39	70	1.3	0.8, 2.0	16	19	2.0	1.0, 4.0
Cyanazine	27	64	0.9	0.6, 1.5				
2,4-D	115	227	1.2	0.9, 1.6	86	153	1.3	0.9, 1.8
Dicamba	28	57	1.2	0.7, 2.0	7	7	2.8	0.96, 8.1
Glyphosate	26	49	1.1	0.7, 1.9				
Metribuzen	12	38	0.7	0.4, 1.4				
Popachlor	13	25	1.2	0.6, 2.5				
2,4,5-T	25	48	1.2	0.7, 1.9	13	18	1.7	0.8, 3.6
Trifluralin	45	87	1.2	0.8, 1.8	14	23	1.5	0.8, 3.1

[a] OR relative to nonfarmers, numbering 266 cases and 547 controls. All ORs adjusted for vital status, age, state, cigarette smoking status, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures in a logistic analysis.

insecticides for application on crops (Table 5), significant risk elevations were observed for DDT and lindane; and for use prior to 1965, carbaryl, DDT, diazinon, lindane, and malathion. We also calculated the OR for pre-1965 personal handling, mixing, or application of specific insecticides that could have been used on either animals or crops. Elevated risk was found for carbaryl (OR = 2.8, CI = 1.0–7.7; 9 cases), chlordane (OR = 1.8, CI = 1.1–3.1; 30 cases); DDT (OR = 1.4, CI = 1.0–1.8; 93 cases), dieldrin (OR = 2.2, CI = 1.0–4.9; 13 cases), lindane (OR = 1.7, CI = 1.1–2.7; 47 cases), and malathion (OR = 1.8, CI = 1.1–3.1; 31 cases). No significant risk elevations were observed for ever handling, mixing, or applying specific herbicides (Table 6). Among the herbicides marketed prior to 1965, use before 1965 of chloramben and dicamba was significantly associated with total NHL. The risk for *ever* having handled, mixed, or applied phenoxy acids was 1.2 for 2,4-D and for 2,4,5-T. For use and handling of these 2 chemicals prior to 1965, risks were 1.3 and 1.7, respectively. Analyses restricting the "exposed" group to farmers who reported that they had not used protective equipment in the handling of specific pesticides were conducted for pesticides showing associations with NHL in previous analyses, either for *ever* handling the pesticide, or

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Table 7 *Pesticides ever handled with and without protective clothing or equipment: OR and CI for selected pesticides*[a]

Pesticide	Ever handled[b]				Handled without protective equipment			
	No. of cases	No. of controls	OR	CI	No. of cases	No. of controls	OR	CI
Animal insecticides								
Chlordane	31	38	1.7	1.0, 2.9	24	30	2.2	1.2, 4.2
DDT	79	149	1.2	0.9, 1.7	72	127	1.3	0.9, 1.8
Lindane	55	90	1.4	1.0, 2.1	45	67	1.6	1.0, 2.4
Malathion	43	67	1.3	0.9, 2.1	33	52	1.4	0.8, 2.2
Nicotine	31	47	1.5	0.9, 2.5	24	41	1.4	0.8, 2.3
Crop insecticides								
Carbaryl	21	26	1.7	0.9, 3.1	22	22	2.2	1.2, 4.2
Chlordane	21	26	1.7	0.9, 3.2	17	18	2.1	1.1, 4.3
DDT	57	75	1.7	1.2, 2.6	48	54	2.0	1.3, 3.1
Diazinon	27	39	1.5	0.9, 2.5	17	22	1.7	0.9, 3.2
Lindane	21	23	2.0	1.0, 3.7	16	14	2.6	1.2, 5.5
Malathion	21	30	1.5	0.8, 2.7	14	16	1.9	0.9, 4.1
Herbicides								
Chloramben	39	70	1.3	0.8, 2.0	31	44	1.7	1.1, 2.8
2,4-D	115	227	1.2	0.9, 1.6	89	175	1.2	0.9, 1.7
Dicamba	28	57	1.2	0.7, 2.0	19	32	1.4	0.8, 2.5
2,4,5-T	25	48	1.2	0.7, 1.9	18	30	1.4	0.7, 2.5

[a] OR relative to nonfarmers, numbering 266 cases and 547 controls. All ORs adjusted for vital status, age, state, cigarette smoking status, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures in a logistic analysis.
[b] Results for ever having used or handled these pesticides (with or without protective clothing or equipment) are from Tables 4, 5, and 6.

Table 8 *Selected pesticides first used prior to 1965: OR and CI for residents of Iowa and Minnesota, respectively*[a]

Pesticide	Iowa				Minnesota			
	No. of cases	No. of controls	OR	CI	No. of cases	No. of controls	OR	CI
Animal insecticides								
Chlordane	15	15	2.2	1.0, 4.8	7	7	2.2	0.8, 6.6
DDT	27	67	0.9	0.5, 1.5	41	56	1.7	1.1, 2.7
Lindane	33	47	1.5	0.9, 2.5	7	8	1.9	0.6, 5.5
Malathion	16	21	1.5	0.7, 3.1	9	9	2.0	0.7, 5.3
Nicotine	15	16	2.1	1.0, 4.6	13	20	1.4	0.7, 2.9
Crop insecticides								
Carbaryl	5	3	3.5	0.8, 15.5	2	1	4.9	0.4, 56
Chlordane	8	13	1.3	0.5, 3.3	4	3	3.1	0.7, 14.7
DDT	28	40	1.5	0.9, 2.6	17	17	2.3	1.1, 4.8
Diazinon	10	10	2.4	0.9, 6.2	4	2	3.8	0.7, 22
Lindane	9	13	1.4	0.6, 3.5	5	2	6.5	1.2, 35
Malathion	6	6	2.1	0.6, 7.0	5	3	4.1	0.9, 18.6
Herbicides								
Chloramben	7	10	1.6	0.6, 4.4	9	9	2.6	1.0, 6.8
2,4-D	51	96	1.2	0.8, 1.9	35	57	1.4	0.9, 2.3
Dicamba	4	5	2.1	0.6, 8.1	3	2	3.9	0.6, 24
2,4,5-T	9	16	1.2	0.5, 2.9	4	2	4.7	0.8, 26.4

[a] OR relative to nonfarmers, numbering 120 cases and 255 controls in Iowa, and 146 cases and 292 controls in Minnesota. All ORs adjusted for vital status, age, cigarette smoking status, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures in logistic analyses.

handling it prior to 1965, as well as for the 2 most commonly used phenoxyacetic acid herbicides (Table 7). Among insecticides used on livestock, all except one (nicotine) showed a stronger association among those who did not use protective equipment than for the entire exposed group. All of the crop insecticides showed stronger risk among farmers who did not use protective gear, as did 3 of 4 herbicides (the OR for 2,4-D remained the same).

We also calculated odds ratios for pre-1965 use and handling of selected pesticides separately for respondents from Iowa and Minnesota (Table 8). The pesticides with OR greater than 1.5 in both states were: the insecticides chlordane, lindane, and malathion applied to livestock; the insecticides carbaryl, DDT, diazinon, and malathion applied to crops; and the herbicides chloramben and dicamba. Findings from analyses of pre-1965 use of specific pesticides that included only direct respondents resembled results of OR calculations that included both direct and proxy respondents.

There was minimal evidence for confounding of results for any single pesticide by exposure to pesticides belonging to other chemical families. This was indicated by little change in OR when a variable for exposure to any of several pesticide families was added to logistic regression models for individual pesticides (for use, handling, or applying prior to 1965) that had shown statistically significant results.

DISCUSSION

We conducted this population based case-control study of NHL in 2 states with intensive agricultural activity to investigate risk factors for NHL among farmers. As compared with nonfarmers, farmers were at slightly elevated risk of NHL (OR = 1.2), in agreement with some population surveys (13, 14) and other case-control studies of NHL or CLL (3, 15–25), based on mortality records or incident cases. Other population surveys have found no risk elevation for farmers (26–31); some case-

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control studies have observed elevated, though nonsignificant, risk elevations (32–36); and others, null or slightly lower risk for NHL (37–41). Among the studies that have found statistically significant positive associations for NHL or CLL among farmers, the risk ratios have generally been in the range of 1.2 to 1.9. In this study, the cell type with the strongest association with farming was small lymphocytic lymphoma (OR = 1.4), a NHL subtype morphologically similar to CLL. Farming occupation has been linked to CLL risk in several case-control studies, including the study parallel to this one (3) and others (21–23), with OR in the range of 1.4 to 1.8.

We found no striking differences or trends in NHL risk by several measures of the time or intensity of farming, including first year farmed, total duration of farming, or average number of acres farmed. However, the association among men who were farming after 1949 was slightly stronger than for those who stopped earlier. In addition, the NHL risk among farmers of midsized farms (average farm size of 120–199, or 200–319 acres) was slightly higher (OR of 1.3 and 1.2) than for men who farmed more acreage (OR of 1.1). This is consistent with findings from Saskatchewan, where NHL risk was higher among farmers of <300 acres than larger establishments (27). The findings that relate temporal period of farming and average farm size with NHL risk are consistent with associations with chemical pesticide use. There were increases in the use of agricultural chemicals after World War II (42, 43), and major usage occurred after 1950, increasing the opportunity for exposure among individuals who farmed more recently.

We observed no meaningful elevation or consistent trends in risk with average acreage of a number of major crops (including corn, wheat, and soybeans) or the average or maximum number of several types of livestock (including dairy cows, beef cattle, hogs, and chickens).

There were small elevations in risk for NHL among farmers who ever used pesticides, or who used pesticides belonging to very broad groups according to usage, including livestock insecticides, crop insecticides, herbicides, and fungicides. However, larger risks were observed when more specific definitions of pesticide exposure were used (*i.e.*, chemical classes or specific chemicals); when risk was measured by whether a farmer had personally handled, mixed, or applied the pesticides; and among farmers who did not use protective clothing or equipment. Among chemical classes of insecticides used on livestock, we found statistically elevated risk for the grouped chlorinated hydrocarbons, natural products, and organophosphates. Among the chlorinated hydrocarbons, larger OR occurred for the grouped cyclodienes (chlordane and dieldrin) and among the organophosphates, greater risks occurred for halogenated aromatics (chlorphyrifos, coumaphos, crufomate, ronnel, and tetrachlorvinphos). Among crop insecticide families that we evaluated, only the chlorinated hydrocarbons showed statistically elevated OR. No single family of herbicides was associated with NHL risk.

We found significantly elevated risks, with OR of 1.5 or more, for personal handling, mixing, or application of several individual insecticides, including carbaryl, chlordane, DDT, diazinon, lindane, malathion, and nicotine. Dieldrin, dichlorvos, famphur, and toxaphene also showed notable, though nonsignificant risk elevations. Patterns of risk from 3 other analyses were consistent with the hypothesis of an etiological role for these insecticides. Risk of NHL was greater for most chemicals among farmers who first used these chemicals before 1965 (15–18 years before diagnosis) and among those who did not use protective equipment, and there was notable consistency in the risk estimates from the 2 states. Associations with specific chemicals were not confounded by exposure to families of other pesticides. Other investigations of lymphopoietic cancer and pesticide exposure have also noted a rise in risk with increasing time since first exposure, suggesting the need for longer latency (3, 33, 39).

Three of the 4 chemicals that showed excesses, and are used both on crops and livestock, had larger OR associated with crops (DDT, lindane, and malathion), while for chlordane the OR was greater for use on animals. This contrasts with the parallel study of leukemia in Iowa and Minnesota, in which we generally found higher risks for chemicals used as animal insecticides (3).

Several insecticides associated with NHL in this study (chlordane, dieldrin, DDT, lindane, and toxaphene) are classified as having sufficient or limited evidence for carcinogenicity in animals by the International Agency for Research on Cancer (42). For some other insecticides associated here with NHL (carbaryl and malathion), information for evaluation is insufficient. With the exception of phenoxyacetic acid herbicides, the epidemiological literature regarding cancer risks from specific pesticide exposures is quite limited. Cancer risks have been assessed in cohort studies of insecticide manufacturing workers and applicators (44–55), but these are generally not useful in evaluating the risk of NHL associated with specific pesticides. In most cohort studies, the specific pesticide exposures experienced by individuals were not well documented, or the effects of multiple exposures could not be disentangled. In addition, most cohorts were too small or the follow-up period too brief to adequately assess risk of NHL. Hematopoietic and lymphopoietic cancers, however, have been elevated in some of these studies. In Northern Italy, incident lymphatic tissue cancers were in excess among agriculture and forestry workers licensed to use pesticides (Standardized Incidence Ratio = 1.4, CI = 1.0–1.9; 45 cases), especially among persons applying pesticides to only arable land (Standardized Incidence Ratio = 1.8, CI = 1.2–2.5; 31 cases) (47). Excess NHL risk was found in a cohort of United States grain industry workers (Standardized Mortality Ratio = 149), and within the cohort, a nested case-control study showed flour millers to be at especially high risk (OR = 4.2, CI = 1.2–14.2) (44). A variety of insecticides has been used in the grain industry, including DDT, hydrogen cyanide, ethylene dibromide, phosphine, and carbon tetrachloride. Among pesticide manufacturing workers exposed primarily to DDT (740 persons, 17,186.9 person-years of follow-up), no excess of all lymphopoietic and hematopoietic cancer was found (3 observed, 2.40 expected) (51).

Six case-control studies, 4 of NHL (19, 38, 39, 56) and 2 of CLL (3, 17), provide limited information on risk associated with exposure to specific insecticides or insecticide families. A third case-control study of CLL found a nonsignificant risk elevation among persons exposed to "pesticides," not further defined (57). Exposure to DDT was linked with CLL in 2 case-control studies (3, 17), and associated with NHL in 2 others (19, 56), with OR between 1.5 and 6.1. In the 2 other case-control studies, either DDT was not reported separately (39) or no association was found (0 exposed cases, 3 exposed controls) (38). In the current study, we found an association with ever handling, mixing, or applying DDT that was stronger for its use on crops than on livestock, and that was more pronounced for first exposure prior to 1965 than later. We found elevated

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risk for pre-1965 application of DDT to crops in both Iowa and Minnesota.

The grouped chlorinated hydrocarbon insecticides were associated with small (nonsignificant) risk elevations for NHL in a Nebraska study (58). Other than DDT, the only chlorinated hydrocarbons reported specifically in other case-control studies are chlordane and lindane. Chlordane was significantly associated with NHL risk in Nebraska (OR = 2.1), and nonsignificantly in Washington State (OR = 1.61) (19). Lindane, another organochlorine, was significantly associated here with NHL when used either on crops or animals, and risks were elevated in both Iowa and Minnesota. Lindane has also been associated with NHL in a study from Kansas (2).

Risks associated with organophosphate exposure, either collectively, or as individual chemicals, were reported for CLL in the parallel study of leukemia in Iowa and Minnesota (3) and for NHL in a study with similar methods from Eastern Nebraska (39, 58). In the Nebraska study, the OR for organophosphate exposure study was 1.9 (OR = 1.1–3.1), and risk increased with days/year of use to OR = 3.1 for 21+ days. In Nebraska, 2 organophosphates, diazinon and malathion, showed significant positive associations with NHL, similar to our findings. In the parallel leukemia study in Iowa and Minnesota (3), elevated risk was found for CLL among farmers exposed to dichlorvos as an animal insecticide (OR = 2.2, CI = 1.0–4.6). We found significant associations for the grouped organophosphate insecticides used on livestock (OR = 1.5), especially halogenated aromatic organophosphates (OR = 2.0, CI = 1.1–3.7). The ORs for grouped nonhalogenated aromatic organophosphates used on livestock and crops were also elevated, but not statistically significant. Regarding specific organophosphate insecticides, we observed significant associations of NHL with use of malathion prior to 1965 on both crops and animals, and OR were above 1.5 for both types of application in Iowa and in Minnesota. In addition, we found significant OR for pre-1965 use of diazinon on crops, with comparable risk elevations in the 2 study states. Use of other organophosphates before 1965, including coumaphos and dichlorvos on livestock, and phorate on crops, also were associated with increased risk of NHL, although the 95% confidence interval for each included 1.0.

In the study from Nebraska (58), the carbamate insecticide family was significantly associated with NHL (OR = 1.8). We did not find significant associations with carbamates as a group. However, use of carbaryl prior to 1965 was associated with NHL (OR = 3.8, CI = 1.1–13.6), and risk was elevated in both study areas. However, the number of exposed subjects was small (7 cases, 4 controls).

Phenoxyacetic acid herbicides have been linked to NHL risk in several (19, 33, 39, 56), but not all (38, 59), case-control studies. Excesses have also been noted in 2 phenoxyacetic acid manufacturing cohorts, although few deaths occurred (60, 61). In our data, the risk of NHL associated with *ever* handling, mixing, or applying members of the phenoxy acid herbicide family, or the specific herbicides 2,4-D or 2,4,5-T, was small and about the same as for farmers overall. However, when latency was considered, the association with 2,4,5-T was somewhat stronger. Although our findings are not entirely negative, the risk of NHL with 2,4-D use is considerably weaker than observed in studies of similar design from Kansas and Nebraska (33, 39). Risks here were considerably lower and did not increase with latency or failure to use protective equipment. The reasons for the inconsistencies are not obvious. Use patterns of 2,4-D in Iowa and Minnesota may differ from Kansas or Nebraska. In the latter states, the bulk of 2,4-D is for postemergent application on small grains, whereas in Iowa it may be more frequently used on corn. It is unclear whether this difference affects exposures to farmers. It is also possible that the inconsistencies between this and other studies of 2,4-D are simply due to chance, since random variation in risk estimates among studies is to be expected.

Additional comments on the limitations of this study are warranted. Some associations found here may have arisen due to chance or bias. Numerous comparisons were made, and results must be evaluated in this context and judged against epidemiological rules of causality. Bias in selecting cases or controls was absent since eligibility for the study was unrelated to current or previous status as a farmer or the exercise of particular agricultural practices. However, willingness to participate could have been related to farm residence or occupation as a farmer. The fairly high and similar response rates in cases and controls, however, diminishes the possibility of such bias.

Bias due to differential response or recollection of cases and controls regarding specific pesticide exposure is possible. Such bias is unlikely because at the time interviews were held, respondents and interviewers were not aware of hypotheses regarding specific pesticides. Moreover, we found no excess risk for many pesticides but rather some internal consistency for elevated risk with others, such as some of the chlorinated hydrocarbons and organophosphates.

Nondifferential misclassification of specific pesticide exposures is a more likely source of distortion of risk estimates. For dichotomous measures of exposure, however, this distortion would tend to bias risk estimates toward the null (62) and is unlikely to yield false-positive findings. The effect of nondifferential misclassification on polychotomous measures can be more complex (63). There are many ways in which exposure misclassification may occur in studies of this design (64). Most, however, would yield false-negative findings. More than 90% of the farmers in this study operated one or more farms, in contrast to working as hired help. Most farm operators plan their own pest control operations, personally purchase pesticides, and mix and apply the chemicals themselves. They are thus more likely to remember names of specific chemicals that they used than most other pesticide users. However, when many different chemicals were involved, when their use was several decades in the past, and when the use of particular chemicals was brief or episodic, accuracy in reporting chemical names and the timing of application undoubtedly suffers. Proxy respondents not directly involved in farming operations may have been more prone to inaccurate responses than directly interviewed subjects. Among farmers, proxies responded for 28.9% of cases and 34.2% of controls. Among controls who had farmed, 18.4% of proxies did not know whether crop insecticides had been used, and 17.2% did not know about herbicide use. In contrast, 3.3% of directly interviewed farmers didn't know about crop insecticide use, and 3.1% didn't know about herbicide use. Among the controls who reported insecticide use on crops, DDT use was reported as unknown by 11 of 86 proxies (13%) but only 8 of 233 alive subjects (3.4%), and crop application of malathion was unknown by 16 of 86 proxies (19%) and 7 of 233 living subjects (3.0%). Among controls who ever used herbicides, 2,4-D use was reported as unknown by 9 of 88 proxies (10.2%) and 5 of 256 direct respondents (2.0%). Differential effects on risk estimates due to proxy responses among cases and controls should not occur because we adjusted for

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type of respondent in the analysis.

This investigation supports findings from earlier studies that point to an elevated risk of non-Hodgkin's lymphoma among farmers, and our data strongly suggest a relationship with certain pesticide exposures. Interpretation of results regarding individual pesticides is fraught with difficulties, including the problems of interpreting risk of individual factors in the multiple exposure setting of modern agriculture as well as the chance occurrence of finding positive associations with multiple comparisons. Of equal concern is the possibility of missing important associations due to nondifferential exposure misclassification because of difficulties in accurate recall of past pesticide exposures. This would bias risk estimates toward the null. Despite these qualifications, the many internal consistencies of this study and concordance with observations of others support the notion that elevated NHL risk among farmers is associated with exposure to several insecticides, and support the use of protective equipment. The chemicals most strongly associated with risk of NHL were carbaryl, chlordane, DDT, diazinon, dichlorvos, lindane, malathion, nicotine, and toxaphene. Many of these insecticides are still in widespread use today, in the United States or elsewhere, and deserve further epidemiological evaluation.

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NON-HODGKIN'S LYMPHOMA AMONG ASTHMATICS EXPOSED TO PESTICIDES

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We conducted a pooled analysis of population-based case-control studies in Iowa, Minnesota and Nebraska to investigate whether asthma modifies risk of non-Hodgkin's lymphoma (NHL) associated with pesticide exposures. Cases (n = 872) diagnosed with NHL from 1980 to 1986 and frequency-matched controls (n = 2,381) randomly selected from the same geographic areas as the cases were included. Information on use of pesticides and history of asthma was based on interviews. Unconditional logistic regression was used to calculate ORs, adjusted for age, state and vital status. Of all subjects, 177 (45 cases, 132 controls) reported having been told by their doctor that they had asthma. Subjects with an asthma history had a nonsignificantly lower risk of NHL than nonasthmatics (OR = 0.6, 95% CI 0.3–1.4), and there was no main effect of pesticide exposure (OR = 1.0, 95% CI 0.8–1.2). However, asthmatics tended to have larger ORs associated with exposure to pesticides than nonasthmatics. The OR among asthmatics was 1.8 (95% CI 1.1–3.2) for ever-use of crop insecticides, 2.7 (95% CI 1.0–7.2) for chlordane, 2.4 (95% CI 1.0–5.7) for lindane and 3.7 (95% CI 1.3–10.9) for fonofos. Among nonasthmatics, ORs were 1.1 (0.9–1.3), 1.5 (1.1–2.2), 1.3 (0.97–1.8) and 1.6 (1.0–2.4), respectively. Although there is limited power for assessing interaction, our results suggest that the risk of NHL among asthmatics with pesticide exposure may be higher than among nonasthmatics with pesticide exposure.
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Key words: *asthma; insecticide; farmer; non-Hodgkin's lymphoma; pesticide exposure*

Incidence and mortality rates for non-Hodgkin's lymphoma (NHL) have been increasing worldwide over the past several decades.[1] Although the reasons for this increase are not fully understood, NHL is known to be associated with a compromised immune system, particularly acquired or genetic immunodeficiencies.[2,3] Medical conditions related to more subtle immune alteration, such as asthma and other allergic conditions, have also been studied as potential risk factors for NHL.[4–10] These reports have described a decreased risk for NHL among persons with a history of asthma or allergies,[4,5] no association[6–8] or an increase in risk.[9,10] Exposure to pesticides has also been suggested as a possible risk factor for NHL.[11–15] Pesticides may increase cancer risk by altering the immune system.[16–19] Because both asthma and pesticide exposure may change the risk of NHL by immunologic alterations, we investigated the relation between pesticide exposure, asthma and risk of NHL.

MATERIAL AND METHODS

Study population

We pooled data from 2 population-based case-control studies of NHL in 3 midwestern states in the United States, which have been described in detail previously.[20,21] In Iowa and Minnesota, all newly diagnosed cases of NHL among white men aged ≥30 were ascertained from records of the Iowa State Health Registry and a special surveillance system of Minnesota hospitals and pathology laboratories from 1980 to 1983 (n = 530). In Nebraska, all cases of NHL diagnosed between July 1983 and June 1986 among white men and women aged ≥21 in 45 eastern counties were identified through the Nebraska Lymphoma Study Group and area hospitals (n = 346). All cases were reviewed by pathologists, and only histologically confirmed cases were included in this analysis. Controls were randomly selected from the same geographic areas as cases with frequency matching by race, gender, age (5-year age group) and vital status at the time of interview. Control/case matching ratios were approximately 2:1 in Iowa and Minnesota and 4:1 in Nebraska. For living cases under the age of 65, controls were selected by 2-stage random digit dialing.[22] For living cases aged 65 and over, controls were selected from the records of the Health Care Financing Administration. Controls for deceased cases were selected from death records in each state, with additional matching for year of death. Persons whose underlying cause of death was NHL, Hodgkin's lymphoma, multiple myeloma, leukemia or malignancy of unknown sites were excluded as controls. A total of 2,357 controls (Nebraska 1,318, Iowa and Minnesota 1,039) were identified.

Interview

Interviews were conducted with subjects or their next-of-kin if subjects were dead or incapacitated. Interviews were held in person in Iowa and Minnesota and by telephone in Nebraska. Participation rates among cases were 89% in Iowa and Minnesota and 91% in Nebraska. Among controls, rates were 78% in Iowa and Minnesota and 85% in Nebraska. We used standardized and structured questionnaires to collect information on use of pesticides and other known or suspected risk factors for NHL. Questions included personal handling of groups of pesticides and individual pesticides used on crops or animals, with year of first and last use. We also asked whether subjects had ever been told by a doctor that they had asthma and, if so, their age at first diagnosis.

Statistical analysis

Subjects who did not have any information on asthma (n = 25) were excluded from the pooled data set, leaving 872 cases and 2,336 controls eligible for analysis. We used unconditional logistic regression to obtain odds ratios (ORs) and 95% confidence intervals (CIs) with Stata software (version 7.0).[23] The ORs for NHL among farmers exposed to pesticides with asthma were compared to those of nonfarmers without asthma (*i.e.,* individuals who had never lived or worked on a farm and did not have asthma) and to those of farmers without asthma. We estimated the risk of NHL by reported use of individual pesticides where sufficient numbers of exposed subjects were available. We present ORs for pesticides

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that were personally handled by at least 5 exposed cases. The logistic model included age (<60, 60–75, >75), state (Iowa, Minnesota, Nebraska) and vital status (alive, dead). Other variables, such as gender, smoking, having a first-degree relative with lymphohematopoietic cancer, ever having a job correlated with lymphohematopoietic cancers (*e.g.*, painting or welding) and use of protective equipment, were also evaluated as possible confounders. Adjustments of ORs for these variables had minimal impact on risk estimates of NHL, and the latter 2 variables have some missing cases. These variables were not included in the final model. To assess possible reporting bias, risks were estimated including and excluding proxy respondents. We also explored the risk of NHL by age at first diagnosis of asthma and duration of pesticide use.

RESULTS

Table I shows the distribution of the 872 cases and 2,336 controls by asthma history, age, gender, vital status, state of residence, having a first-degree relative with lymphohematopoietic cancer and type of NHL. Of the total subjects, 177 (5.5%) reported having been told by their doctor that they had asthma. Asthmatic NHL cases were more likely than asthmatic controls to be younger, male, alive at the time of interview and residing in Iowa. Nonasthmatic NHL cases were more likely than nonasthmatic controls to be male, to have family history of lymphohematopoietic cancer and to reside in Iowa/Minnesota.

We evaluated ORs for NHL by pesticide groups and asthma history (Table II). Among nonfarmers, subjects with asthma had a lower risk for NHL (not statistically significant) compared to nonfarmers without asthma (OR = 0.6, 95% CI 0.3–1.4). ORs for NHL among farmers without asthma were near 1.0 for all pesticide categories except chemical classes of insecticide. The risk of NHL was significantly increased for exposure to crop insecticides (OR = 1.8, 95% CI 1.1–3.2) and nonsignificantly increased for exposure to livestock insecticides (OR = 1.4, 95% CI 0.9–2.3), herbicides (OR = 1.5, 95% CI 0.9–2.5) and fungicides (OR = 1.4, 95% CI 0.5–4.3) among farmers with asthma. Only organophosphate insecticides had significant ORs among both asthmatics and nonasthmatics. The pattern was consistent by state of residence or interview type, although the results were limited by small numbers of cases (data not shown).

Table III presents ORs for NHL among farmers exposed to individual pesticides by asthma history. Among insecticides, risk of NHL was significantly elevated with exposure to chlordane (OR = 2.7, 95% CI 1.0–7.2), fonofos (OR = 3.7, 95% CI 1.3–10.9) and lindane (OR = 2.4, 95% CI 1.0–5.7) in asthmatics compared to nonfarmers without asthma. Many other insecticides (aldrin, carbaryl, carbofuran, diazinon, dieldrin, flyspray, heptachlor, malathion) also had larger ORs among farmers with a history of asthma than among those without asthma. However, none of these was significantly different from the risks in nonasthmatics. Among nonasthmatics, risk of NHL was also significantly elevated with exposure to chlordane, diazinon, fonofos and malathion; but the magnitude of risk was smaller than that among asthmatics. Use of individual herbicides was also associated with increased risk of NHL among asthmatics compared to nonasthmatics, but only cyanazine had a significant OR. No fungicide had 5 or more exposed cases and was significantly associated with NHL.

Analyses of pesticide exposure and asthma history among farmers only are presented in Table IV. The reference category was nonasthmatic farmers not exposed to each pesticide. Asthmatics with exposure to crop insecticides had significantly elevated risk of NHL (OR = 2.0, 95% CI 1.1–3.5), but the interaction risk for pesticide exposure and asthma was not statistically significant.

We explored the potential modifying effects of age at first diagnosis of asthma and duration of pesticide use on risk of NHL (Table V). Only asthmatic farmers exposed to pesticides were included in this analysis. Risks among subjects diagnosed with asthma after age 30 tended to be higher for all types of pesticide than those among subjects who had developed asthma relatively early. There was no clear pattern of ORs for NHL by duration of pesticide use and age at diagnosis of asthma. The results were limited due to the small number of asthmatic NHL cases, and further studies are needed to investigate these findings.

DISCUSSION

We found that farmers with potential exposure to pesticides and a history of asthma tended to have higher relative risks for NHL than pesticide-exposed farmers not reporting asthma. The excess risks among asthmatics with pesticide exposure were generally more pronounced when we analyzed by individual pesticides (*e.g.*,

TABLE I – CHARACTERISTICS OF CASES AND CONTROLS BY ASTHMA HISTORY

Characteristics	Nonasthmatics (n = 3,031)		Asthmatics (n = 177)	
	Cases (n = 827)	Controls (n = 2,204)	Cases (n = 45)	Controls (n = 132)
Age (years)				
<60	231 (27.9)[2]	585 (26.5)	18 (40.0)	24 (18.2)
60–75	348 (42.1)	875 (39.7)	17 (37.8)	51 (38.6)
>75	248 (30.0)	744 (33.8)	10 (22.2)	57 (43.2)
Gender				
Male	676 (81.7)	1,594 (72.3)	38 (84.4)	100 (75.8)
Female	151 (18.3)	610 (27.7)	7 (15.6)	32 (24.2)
Vital status				
Alive	572 (69.2)	1,486 (67.4)	34 (75.6)	71 (53.8)
Dead	255 (30.8)	718 (32.6)	11 (24.4)	61 (46.2)
State of residence				
Iowa	238 (28.8)	483 (21.9)	15 (33.3)	26 (19.7)
Minnesota	264 (31.9)	491 (22.3)	10 (22.2)	28 (21.2)
Nebraska	325 (39.3)	1,230 (55.8)	20 (44.5)	78 (59.1)
Family history of cancer[1]				
No	733 (90.7)	2,072 (95.4)	42 (93.3)	120 (92.3)
Yes	75 (9.3)	99 (4.6)	3 (6.7)	10 (7.7)
Histologic type				
Follicular	243 (29.5)	—	18 (40.9)	—
Diffuse	298 (36.1)	—	16 (36.4)	—
Small lymphocytic	90 (10.9)	—	4 (9.1)	—
Other	194 (23.5)	—	6 (13.6)	—

[1]Lymphohematopoietic cancers diagnosed in any first-degree relative.–[2]Percentage in parentheses.

TABLE II – RISKS OF NHL BY FARMING HISTORY, PESTICIDE USE AND ASTHMA HISTORY

	Nonasthmatics				Asthmatics			
	Cases	Controls	OR[1]	95% CI	Cases	Controls	OR	95% CI
Nonfarmers	259	684	1.0	Ref[2]	9	37	0.6	0.3–1.4
Farmers	560	1,510	1.0	0.8–1.2	36	95	1.1	0.7–1.6
No pesticide use	137	419	1.0	0.8–1.3	3	14	0.7	0.2–2.6
Pesticide use	423	1,091	1.0	0.8–1.2	33	81	1.1	0.7–1.7
Animal insecticides	363	900	1.0	0.8–1.2	28	52	1.4	0.9–2.3
Crop insecticides	239	572	1.1	0.9–1.3	23	32	1.8	1.1–3.2
Organochlorine	205	412	1.2	0.9–1.5	17	28	1.5	0.8–2.8
Organophosphate	149	269	1.4	1.1–1.7	14	17	2.0	1.0–4.2
Carbamate	79	154	1.3	0.9–1.7	8	9	2.2	0.8–5.9
Herbicides	260	639	1.0	0.8–1.3	23	43	1.5	0.9–2.5
Phenoxyacetic acid	176	409	1.0	0.8–1.3	17	33	1.3	0.7–2.4
Triazine	131	268	1.1	0.9–1.5	12	17	1.7	0.8–3.7
Amides	105	231	1.1	0.8–1.4	11	15	1.8	0.8–3.9
Fungicides	44	110	1.0	0.7–1.4	5	10	1.4	0.5–4.3

[1]OR adjusted for age, vital status and state.–[2]Ref, reference category was nonfarmers without asthma (259 cases, 684 controls) for all ORs.

TABLE III – RISKS OF NHL AMONG FARMERS EXPOSED TO INDIVIDUAL PESTICIDES[1] BY ASTHMA HISTORY

	Nonasthmatics				Asthmatics			
	Cases	Controls	OR[2]	95% CI	Cases	Controls	OR	95% CI
Nonfarmers	259	684	1.0	Ref[3]	9	37	0.6	0.3–1.4
Insecticides								
Aldrin	66	148	1.0	0.7–1.5	10	11	2.1	0.9–5.1
Carbaryl	42	77	1.4	0.9–2.0	6	6	2.4	0.8–7.6
Carbofuran	56	117	1.2	0.8–1.7	6	8	1.9	0.7–5.6
Chlordane	67	108	1.5	1.1–2.2	9	8	2.7	1.0–7.2
DDT	158	313	1.2	0.9–1.5	11	24	1.2	0.6–2.4
Diazinon	58	98	1.6	1.1–2.3	7	9	1.9	0.7–5.3
Dieldrin	30	63	1.2	0.7–1.9	5	3	4.2	0.98–18.2
Flyspray	189	442	0.9	0.7–1.1	14	27	1.1	0.6–2.2
Fonofos	41	69	1.6	1.0–2.4	8	6	3.7	1.3–10.9
Heptachlor	44	84	1.3	0.9–2.0	6	6	2.6	0.8–8.4
Lindane	84	146	1.3	0.97–1.8	11	11	2.4	1.0–5.7
Malathion	89	141	1.5	1.1–2.1	7	9	1.9	0.7–5.1
Herbicides								
2,4-D	172	402	1.0	0.8–1.3	17	33	1.3	0.7–2.5
2,4,5,-T	36	77	1.1	0.7–1.8	7	8	2.2	0.8–6.1
Alachlor	96	210	1.1	0.8–1.4	10	14	1.7	0.8–4.0
Atrazine	119	225	1.3	0.96–1.6	9	16	1.4	0.6–3.3
Butylate	38	75	1.1	0.7–1.7	5	6	2.0	0.6–6.9
Chloroamben	52	103	1.1	0.8–1.6	9	10	2.3	0.9–5.7
Cyanazine	53	131	0.9	0.6–1.3	8	7	2.8	1.0–8.1
Dicamba	49	106	1.0	0.7–1.5	6	7	2.0	0.6–6.0
Glyphosate	53	91	1.4	0.98–2.1	6	12	1.2	0.4–3.3
Trifluralin	73	168	1.0	0.7–1.3	8	10	1.9	0.7–4.8

[1]At least 5 cases handled each individual pesticide were included in this analysis.–[2]OR adjusted for age, vital status and state.–[3]Ref, reference category was nonfarmers without asthma (259 cases, 684 controls) for all ORs.

chlordane, fonofos, lindane, cyanazine) and occurred when either "nonfarmers" or "farmers" was used as the reference.

Although we had limited power for assessing effect modification, there might be synergism between asthma and pesticide exposure for developing NHL. One possible explanation is that there is immune deviation in asthma toward T-helper 2 (Th2) predominance, with elevated IL-4, IL-5 and IL-13, which might inhibit Th1 responses that could protect against cancer.[24,25] This skewing of the immune response toward the Th2 phenotype could exacerbate the effects of the pesticides, which may partly act as carcinogens, and may also inhibit the immune response, acting synergistically with the asthma. Some pesticides might also inhibit a different arm of the immune response, *e.g.*, cytotoxic T lymphocytes or natural killer (NK) cells,[26,27] so that the combination of asthma and pesticides exposure eliminates more than one mechanism of immunosurveillance. Moreover, IL-13, which is prominent in asthma, can also downregulate cytotoxic T lymphocyte–mediated tumor immunosurveillance,[28] reducing 2 arms of the immune response to cancer and specifically crippling immunosurveillance against cancer in a murine tumor model.

Various characteristics, such as history of allergy and serum IgE levels, between late-onset and early-onset asthma[29–31] might be related to higher risk of NHL among individuals diagnosed with asthma over age 30. Exposure to pesticides may influence the induction and aggravation of asthma through modification of autonomic control of airways.[32] Associations between asthma and use of cholinesterase-inhibiting pesticides were observed among Canadian farmers[33] and U.S. pesticide applicators.[34]

The strengths of our pooled study are a population-based design, high response rates and detailed information on pesticide use and potential etiologic factors for NHL. The relatively large sample size facilitated the simultaneous evaluation of asthma and pesticide use but was still not enough to carefully evaluate individual pesticides and asthma in relation to NHL.

We used self-reported information concerning prior asthma history. The sensitivity of ascertainment of physician-diagnosed asthma has been estimated at about 68% and the specificity at about 94% when validated against clinical diagnosis.[35] This type of misclassification is likely to cause underestimation of the asso-

TABLE IV – RISKS OF NHL AMONG FARMERS BY PESTICIDE EXPOSURE AND ASTHMA HISTORY[1]

	Nonasthmatics			Asthmatics			Interaction OR (95% CI)
	Cases	OR[2]	95% CI	Cases	OR	95% CI	
Any pesticide							
No	137	1.0	Ref[3]	3	0.7	0.2–2.5	
Yes	423	1.0	0.8–1.2	33	1.1	0.7–1.7	1.6 (0.4–6.2)
Crop insecticides							
No	252	1.0	Ref	12	0.9	0.5–1.8	
Yes	239	1.2	0.9–1.4	23	2.0	1.1–3.5	1.9 (0.8–4.6)
Animal insecticides							
No	143	1.0	Ref	6	0.8	0.3–2.1	
Yes	363	1.0	0.8–1.3	28	1.4	0.9–2.4	1.7 (0.6–4.9)
Herbicides							
No	232	1.0	Ref	12	1.0	0.5–1.9	
Yes	260	1.1	0.9–1.4	23	1.6	0.9–2.8	1.4 (0.6–3.4)
Fungicides							
No	433	1.0	Ref	28	1.2	0.8–1.9	
Yes	44	1.0	0.7–1.5	5	1.5	0.5–4.5	1.2 (0.4–4.2)

[1]Nonfarmers were excluded from this analysis.–[2]OR, adjusted for age, vital status and state.–[3]Ref, reference category was nonasthmatic farmers not exposed to each pesticide.

TABLE V – RISKS OF NHL AMONG ASTHMATIC FARMERS BY AGE AT FIRST DIAGNOSIS OF ASTHMA AND DURATION OF PESTICIDE USE[1]

Age at first diagnosis (years)	Duration of pesticide use					
	≤50th percentile			>50th percentile		
	Cases	OR[2]	95% CI	Cases	OR	95% CI
Any pesticide						
≤30	3	1.0	Ref[3]	8	4.5	0.7–27.3
>30	6	16.3	1.7–156.8	6	5.0	0.7–37.1
Crop insecticides						
≤30	4	1.0	Ref	6	2.5	0.3–19.6
>30	3	2.3	0.2–31.1	4	14.1	0.8–257.7
Animal insecticides						
≤30	3	1.0	Ref	6	2.8	0.4–19.5
>30	4	15.1	0.95–240.2	8	5.0	0.7–37.8
Herbicides						
≤30	2	1.0	Ref	6	1.7	0.1–29.4
>30	4	3.2	0.1–99.5	4	2.3	0.1–51.3

[1]Only asthmatic farmers exposed to pesticides were included in this analysis.–[2]OR adjusted for age, vital status and state.–[3]Ref, reference category was asthmatic farmers in the category of ≤30 years of age at first diagnosis of asthma and ≤50th percentile of each pesticide use.

ciation between asthma history and NHL risk. However, we think misclassification *per se* is unlikely to explain the observed effect of asthma because the reported prevalence of asthma in our study (5.5%) was consistent with that reported in other populations, ranging from 5% in the Agricultural Health Study in the United States[34] to 4–6% in rural Saskatchewan in Canada.[33,36] Asthma prevalence was also similar by self (5%) and proxy (6%) respondents.

Although farmers provide considerably accurate detail regarding past pesticide use,[37–39] misclassification of exposure is a concern. Use of proxy respondents may introduce nondifferential misclassification bias;[40] however, responses from proxies are reported to be adequate for epidemiologic studies of pesticides and cancer.[41] Our analyses based on direct interviews found the same pattern of results as seen for proxy respondents (data not shown). Based on a study of the quality of information on pesticide use provided by farmers or their proxy respondents,[42] the degree of misclassification was generally in the range observed for other factors obtained by interview in epidemiologic studies of such factors as diet and use of tobacco and alcohol. Therefore, it appears unlikely that misclassification of exposure could explain the observed increase in the risk of NHL among asthmatics exposed to pesticides.

Differential reporting bias is also a concern in case-control studies and could have resulted from an increased likelihood of cases to remember pesticide exposures compared to controls. However, comparison of reporting by cases and controls regarding pesticide use among our subjects provided no evidence of differential response bias.[37]

In summary, our findings suggest that the risk of NHL among asthmatics with pesticide exposure may be higher than that among nonasthmatics with pesticide exposure. Considering the widespread use of pesticides and the relatively high prevalence of asthma, further studies, particularly with carefully defined asthma diagnosis and biomarkers, such as cytokine levels and activity of different T and NK cells, are needed to confirm these findings and clarify the mechanisms involved.

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Cancer Incidence among Glyphosate-Exposed Pesticide Applicators in the Agricultural Health Study

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Glyphosate is a broad-spectrum herbicide that is one of the most frequently applied pesticides in the world. Although there has been little consistent evidence of genotoxicity or carcinogenicity from *in vitro* and animal studies, a few epidemiologic reports have indicated potential health effects of glyphosate. We evaluated associations between glyphosate exposure and cancer incidence in the Agricultural Health Study (AHS), a prospective cohort study of 57,311 licensed pesticide applicators in Iowa and North Carolina. Detailed information on pesticide use and other factors was obtained from a self-administered questionnaire completed at time of enrollment (1993–1997). Among private and commercial applicators, 75.5% reported having ever used glyphosate, of which > 97% were men. In this analysis, glyphosate exposure was defined as *a*) ever personally mixed or applied products containing glyphosate; *b*) cumulative lifetime days of use, or "cumulative exposure days" (years of use × days/year); and *c*) intensity-weighted cumulative exposure days (years of use × days/year × estimated intensity level). Poisson regression was used to estimate exposure–response relations between glyphosate and incidence of all cancers combined and 12 relatively common cancer subtypes. Glyphosate exposure was not associated with cancer incidence overall or with most of the cancer subtypes we studied. There was a suggested association with multiple myeloma incidence that should be followed up as more cases occur in the AHS. Given the widespread use of glyphosate, future analyses of the AHS will allow further examination of long-term health effects, including less common cancers. *Key words:* cancer, cohort study, farming, glyphosate, pesticide. *Environ Health Perspect* 113:49–54 (2005). doi:10.1289/ehp.7340 available via *http://dx.doi.org/* [Online 4 November 2004]

Glyphosate [*N*-(phosphonomethyl)glycine], commonly sold in the commercial formulation named Roundup (Monsanto Company, St. Louis, MO), has been a frequently used herbicide on both cropland and noncropland areas of the world since its introduction in the 1970s (Williams et al. 2000). Roundup is a combination of the active ingredient and other chemicals, including a surfactant (polyoxyethyleneamine) that enhances the spreading of spray droplets when they contact foliage. Glyphosate is a broad-spectrum herbicide of which the primary mechanism is inhibition of the enzyme 5-enolpyruvoylshikimate 3-phosphate synthase, which is essential for the formation of aromatic amino acids in plants (Steinrucken and Amrhein 1980). Because this specific biologic pathway operates only in plants and microorganisms, the mechanism is not considered to be a risk for humans. Nevertheless, genotoxic, hormonal, and enzymatic effects in mammals have been reported (Bolognesi et al. 1997; Daruich et al. 2001; El Demerdash et al. 2001; Hietanen et al. 1983; Lioi et al. 1998a, 1998b; Olorunsogo et al. 1979; Peluso et al. 1998; Walsh et al. 2000; Yousef et al. 1995).

Results from genotoxicity studies of glyphosate have been conflicting. Glyphosate did not show any genotoxic activity in a battery of assays (Garry et al. 1999; Grisolia 2002; Li and Long 1988; Wildeman and Nazar 1982). However, other studies observed that glyphosate treatment of human lymphocytes *in vitro* resulted in increased sister chromatid exchanges (Bolognesi et al. 1997), chromosomal aberrations (Lioi et al. 1998b), and indicators of oxidative stress (Lioi et al. 1998b). Some studies found slightly greater toxicity of the Roundup formulation compared with glyphosate, in terms of both acute toxicity (Folmar et al. 1979; Martinez et al. 1990; Mitchell et al. 1987) and genotoxicity (Bolognesi et al. 1997; Vigfusson and Vyse 1980). Roundup was associated with increased DNA adducts in mice (Peluso et al. 1998) and a weak mutagenic effect in the *Salmonella* assay (Kale et al. 1995; Moriya et al. 1983; Rank et al. 1993), whereas glyphosate alone did not show these effects. Chronic feeding studies of glyphosate have not provided evidence of a carcinogenic effect in mice or rats (Williams et al. 2000).

The U.S. Environmental Protection Agency (U.S. EPA 1993) and the World Health Organization (WHO 1994) reviewed the toxicology data on glyphosate and concluded that glyphosate is not mutagenic or carcinogenic. The U.S. EPA classified glyphosate as category E, indicating "evidence of noncarcinogenicity for humans" (U.S. EPA 1993). Despite this conclusion, three recent case–control studies suggested an association between reported glyphosate use and the risk of non-Hodgkin lymphoma (NHL) (De Roos et al. 2003b; Hardell and Eriksson 1999; Hardell et al. 2002; McDuffie et al. 2001). Considering the widespread and frequent use of glyphosate in both the United States and the rest of the world, ongoing risk assessment is of importance. We studied site-specific cancer incidence associated with glyphosate use among pesticide applicators in the Agricultural Health Study (AHS) cohort.

Materials and Methods

Cohort enrollment and follow-up. The AHS is a prospective cohort study in Iowa and North Carolina, which includes 57,311 private and commercial applicators who were licensed to apply restricted-use pesticides at the time of enrollment. Recruitment of the applicators occurred between 1993 and 1997 (Alavanja et al. 1996). Cohort members were matched to cancer registry files in Iowa and North Carolina for case identification and to the state death registries and the National Death Index (National Center for Health Statistics 1999) to ascertain vital status. Incident cancers were identified for the time period from the date of enrollment until 31 December 2001 and were coded according to the *International Classification of Diseases,* 9th Revision (WHO 1977). If cohort members had moved from the state, they were censored in the year they left. The median time of follow-up was 6.7 years.

Exposure assessment. Using a self-administered enrollment questionnaire, we collected comprehensive-use data on 22 pesticides, ever/never use information for 28 additional pesticides, and general information on pesticide application methods, personal protective equipment, pesticide mixing, and equipment repair. Data were also collected on basic demographic

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and lifestyle factors. Applicators who completed this questionnaire were given a self-administered take-home questionnaire, which contained additional questions on occupational exposures and lifestyle factors. The questionnaires are available from the AHS website (National Institutes of Health 2004).

We constructed three glyphosate exposure metrics for this analysis: *a*) ever personally mixed or applied products containing glyphosate (ever/never); *b*) cumulative lifetime days of use, or "cumulative exposure days" (years of use × days per year, categorized in tertiles among users: 1–20, 21–56, 57–2,678); and *c*) intensity-weighted cumulative exposure days (years of use × days per year × intensity level, categorized in tertiles: 0.1–79.5, 79.6–337.1, 337.2–18,241). Tertiles were chosen *a priori* as the cut points with which to categorize exposure data, to avoid sparse data for rare cancers in the high-exposure categories. Intensity levels were estimated using questionnaire data from enrollment and measurement data from the published pesticide exposure literature, as follows: intensity level = [(mixing status + application method + equipment repair status) × personal protective equipment use] (Dosemeci et al. 2002).

Data analysis. Persons whose first primary cancer occurred before the time of enrollment (n = 1,074) were excluded from analyses, as were subjects who were lost to follow-up or otherwise did not contribute any person-time (n = 298) and applicators who did not provide any information on age (n = 7) or whether they had ever used glyphosate (n = 1,678). After exclusions, 54,315 subjects were available for inclusion in the age-adjusted analyses of cancer incidence in relation to glyphosate use; however, other analyses contained fewer observations because of missing data for duration and frequency of glyphosate use or for covariates.

We compared certain baseline characteristics among three types of pesticide applicators: *a*) those applicators who never personally used glyphosate; *b*) applicators with the lowest glyphosate exposure, defined as being in the lowest tertile of cumulative exposure days; and *c*) those with higher glyphosate exposure, defined as being in the middle or highest tertile of cumulative exposure days. The purpose of the comparison was to identify potential confounders of glyphosate exposure–disease associations for the various analyses we conducted. Differences between the exposure groups were tested using the chi-square statistics and associated p-values.

Poisson regression analyses were carried out for all cancers combined and specific cancer sites to estimate rate ratios (RRs) and 95% confidence intervals (CIs) associated with glyphosate exposure metrics; the effect of each metric was evaluated in a separate model for each cancer. We analyzed tertile exposure variables in separate models using either the lowest-tertile–exposed or never-exposed subjects as the reference category. We investigated specific cancer sites for which there were at least 30 cases with sufficient information for inclusion in age-adjusted analyses. These cancers were then evaluated for all the exposure metrics and in adjusted analyses, despite smaller numbers of cases upon further adjustment. For each exposure metric, RRs were adjusted for demographic and lifestyle factors, including age at enrollment (continuous), education (dichotomous: ≤ high school graduate or GED/education beyond high school), pack-years of cigarette smoking [indicator variables: never, pack-years at or below the median (12 pack-years), pack-years above the median], alcohol consumption in the past year [indicator variables: none, frequency at or below the median (72 drinks), frequency above the median], family history of cancer in first-degree relatives (dichotomous: yes/no), and state of residence (dichotomous: Iowa/North Carolina). There was insufficient variability in sex or applicator type to adjust for these factors.

Potential confounding from exposure to other pesticides was explored by adjusting for the five pesticides for which cumulative-exposure-day variables were most highly associated with glyphosate cumulative exposure days [(2,4-dichlorophenoxy)acetic acid (2,4-D), alachlor, atrazine, metolachlor, trifluralin]; these pesticide exposures were coded as variables indicating never, low, and high, with the split between low and high as the median of their cumulative exposure days. Additionally, of the pesticides for which only ever/never use

Table 1. Selected characteristics of applicators in the AHS by glyphosate exposure, based on data from the enrollment questionnaire (1993–1997).[a]

Characteristic	Never exposed (n = 13,280) No. (%)	Lowest exposed (n = 15,911)[b] No. (%)	Higher exposed (n = 24,465)[c] No. (%)
State of residence			
Iowa	9,987 (75.2)	9,785 (61.5)	15,336 (62.7)
North Carolina	3,293 (24.8)	6,126 (38.5)	9,129 (37.3)
Age (years)			
< 40	2,279 (17.2)	2,226 (14.0)	4,190 (17.1)
40–49	3,420 (25.8)	4,279 (26.9)	7,899 (32.3)
50–59	2,989 (22.5)	3,931 (24.7)	6,035 (24.7)
60–69	2,715 (20.4)	3,266 (20.5)	3,997 (16.3)
70	1,877 (14.1)	2,209 (13.9)	2,344 (9.6)
Sex			
Male	12,778 (96.2)	15,505 (97.5)	23,924 (97.8)
Female	502 (3.8)	406 (2.6)	541 (2.2)
Applicator type[d]			
Private	12,067 (90.9)	15,008 (94.3)	21,938 (89.7)
Commercial	1,213 (9.1)	903 (5.7)	2,527 (10.3)
Education			
High school graduate or GED	8,898 (68.7)	8,997 (57.9)	11,975 (50.1)
Beyond high school	4,060 (31.3)	6,530 (42.1)	11,936 (49.9)
Smoking history			
Never	7,298 (57.3)	8,241 (53.2)	12,751 (53.7)
≤ 12 pack-years	2,866 (22.5)	3,597 (23.2)	5,572 (23.5)
> 12 pack-years	2,567 (20.2)	3,643 (23.5)	5,439 (22.9)
Alcohol consumption in past year			
None	4,087 (32.7)	5,352 (35.6)	7,023 (29.8)
≤ 6 drinks/month	4,461 (35.7)	5,291 (35.2)	8,149 (34.5)
> 6 drinks/month	3,936 (31.5)	4,387 (29.2)	8,422 (35.7)
Family history of cancer			
No	8,701 (65.5)	9,520 (59.8)	14,668 (60.0)
Yes	4,579 (34.5)	6,391 (40.2)	9,797 (40.0)
Use of other common pesticides			
2,4-D	7,030 (53.3)	11,879 (75.2)	20,699 (85.1)
Alachlor	4,896 (39.7)	7,321 (50.9)	13,790 (59.7)
Atrazine	7,707 (58.5)	10,533 (66.6)	18,237 (75.0)
Metolachlor	3,890 (31.6)	6,172 (43.1)	12,952 (56.2)
Trifluralin	4,239 (34.0)	7,109 (49.7)	14,675 (63.5)
Carbaryl	4,110 (33.7)	8,515 (58.1)	15,139 (64.8)
Benomyl	510 (4.3)	1,418 (9.9)	3,391 (14.8)
Maneb	492 (4.1)	1,412 (9.9)	2,929 (12.9)
Paraquat	1,067 (9.0)	3,021 (21.2)	8,031 (35.2)
Diazinon	1,906 (16.0)	4,615 (32.4)	9,107 (40.0)

[a]Includes observations for subjects included in age-adjusted Poisson regression models of cancer incidence (n = 54,315). [b]Lowest tertile of cumulative exposure days. [c]Highest two tertiles of cumulative exposure days; the sum of the three tertiles of cumulative exposure days (n = 40,376) does not equal the total number of subjects who reported having ever used glyphosate (n = 41,035) because of missing data on duration and frequency of use. [d]"Private" refers primarily to individual farmers, and "commercial" refers to professional pesticide applicators.

information was available, we adjusted for the five pesticides that were most highly associated with ever use of glyphosate (benomyl, maneb, paraquat, carbaryl, diazinon). Where inclusion of all 10 other pesticides in a model changed a glyphosate exposure estimate by at least 20% (compared with a model restricted to the same observations), these results were presented as the final results for that cancer; otherwise, estimates adjusted only for demographic and lifestyle factors are presented.

Tests for trend across tertiles were conducted by creating a continuous variable with assigned values equal to the median value of cumulative exposure days (or intensity-weighted exposure days) within each tertile; the p-value for the trend test was that from the Poisson model coefficient for this continuous variable. We considered p-values < 0.10 as indicative of a trend.

Additional analyses were conducted for cancers for which we observed elevated RRs, and for NHL because of its association with glyphosate in previous studies. These included analyses stratified by state and analyses across quartiles and quintiles (where numbers allowed) of exposure days metrics.

Results

Selected characteristics of the glyphosate-exposed and never-exposed applicators are presented in Table 1. Among 54,315 subjects included in age-adjusted analyses, 41,035 (75.5%) reported having ever personally mixed or applied products containing glyphosate, and 13,280 (24.5%) did not. The cohort, both exposed and never exposed, was composed of primarily of male, middle-aged, private applicators. This is a population with relatively low smoking prevalence; in both the exposed and never-exposed groups, more than half of the subjects reported that they had never smoked. Significant differences ($p < 0.05$) existed between never-exposed and lowest-exposed subjects for all of the characteristics in Table 1. Lowest- and higher-exposed subjects ($p < 0.05$) also differed on several factors, the most notable being that higher-exposed subjects were more likely to be commercial applicators, to have consumed greater amounts of alcohol in the past year, and to have used other specific pesticides. However, lowest- and higher-exposed subjects were similar to each other ($p \geq 0.05$) in characteristics including smoking and family history of cancer in a first-degree relative. In addition, lowest- and higher-exposed subjects were more similar to each other than to their never-exposed counterparts (by qualitative comparison of percentages only) in factors including North Carolina residence, education beyond high school, and use of other pesticides. Because of relative similarities between lowest- and higher-exposed in factors associated with socioeconomic status and other exposures, we decided to conduct some analyses using lowest-exposed rather than never-exposed applicators as the reference group, in order to avoid residual confounding by unmeasured covariates. However, we decided *a priori* that any association should be apparent regardless of which reference group was used.

RRs for the association of all cancers combined and specific cancers with having ever used glyphosate are presented in Table 2. RRs adjusted for age only are presented, as well as RRs adjusted for demographic and lifestyle factors and, in some cases, for other pesticides. The incidence of all cancers combined was not associated with glyphosate use, nor were most specific cancers. There was an 80% increased risk of melanoma associated with glyphosate use in the age-adjusted analysis, which diminished slightly upon further adjustment. Adjusted risk estimates for colon, rectum, kidney, and bladder cancers were elevated by 30–60%, but these estimates were not statistically significant. There was more than 2-fold increased risk of multiple myeloma associated with ever use of glyphosate in adjusted analyses, although this is based on a small number of cases. The association between myeloma incidence and glyphosate exposure was consistent in both states (ever used glyphosate, fully adjusted analyses: Iowa RR = 2.6; North Carolina RR = 2.7).

Results from analyses of tertiles of increasing glyphosate exposure level are presented in Table 3. A decreased risk of lung cancer was suggested for the highest tertile of both cumulative and intensity-weighted exposure days (p-value for trend = 0.02); however, a similar trend was not observed in analyses using never exposed as the referent (results not shown). There was a 40% increased risk of colon cancer for the highest tertile of intensity-weighted exposure; however, no clear monotonic trend was observed for either exposure metric. Elevated risks of leukemia and pancreas cancer were observed only for the middle tertiles of both cumulative and intensity-weighted exposure days, with no increased risk among those with the highest exposure. The associations we observed in the analysis of ever use of glyphosate (Table 2) for melanoma, rectum, kidney, and bladder cancers were not confirmed in analyses based on exposure-day metrics; similarly, no exposure–response patterns were observed in analyses using never exposed as the referent or in analyses across quintiles of exposure (results not shown). No association was observed between NHL and glyphosate exposure in any analysis, including an analysis comparing the highest with the lowest quintile of exposure (> 108 vs. > 0–9 cumulative exposure days: RR = 0.9; 95% CI, 0.4–2.1).

Elevated RRs were estimated for multiple myeloma, with an approximate 2-fold increased risk for the highest tertile of both cumulative and intensity-weighted exposure days (Table 3); however, small numbers precluded precise effect estimation ($n = 19$ in adjusted analyses of exposure-day metrics). The estimated intensity-level component of the intensity-weighted exposure-day metric was not associated with multiple myeloma (highest vs. lowest tertile: RR = 0.6; 95% CI, 0.2–1.8), and observed positive associations of the intensity-weighted exposure-day metric with myeloma relied solely

Table 2. Association of glyphosate exposure (ever/never used) with common cancers[a] among AHS applicators.

Cancer site	Total no. of cancers[c]	Ever used glyphosate (% of total)	RR (95% CI)[b] Effect estimates adjusted for age ($n = 54,315$)[d]	RR (95% CI)[b] Adjusted for age, demographic and lifestyle factors, and other pesticides[d]
All cancers	2,088	73.6	1.0 (0.9–1.1)	1.0 (0.9–1.2)
Lung	204	72.1	1.0 (0.7–1.3)	0.9 (0.6–1.3)
Oral cavity	59	76.3	1.1 (0.6–2.0)	1.0 (0.5–1.8)
Colon	174	75.3	1.1 (0.8–1.6)	1.4 (0.8–2.2)[e]
Rectum	76	77.6	1.2 (0.7–2.1)	1.3 (0.7–2.3)
Pancreas	38	76.3	1.2 (0.6–2.5)	0.7 (0.3–2.0)[e]
Kidney	63	73.0	1.0 (0.6–1.7)	1.6 (0.7–3.8)[e]
Bladder	79	76.0	1.2 (0.7–2.0)	1.5 (0.7–3.2)[e]
Prostate	825	72.5	1.0 (0.8–1.1)	1.1 (0.9–1.3)
Melanoma	75	84.0	1.8 (1.0–3.4)	1.6 (0.8–3.0)
All lymphohematopoietic cancers	190	75.3	1.1 (0.8–1.5)	1.1 (0.8–1.6)
NHL	92	77.2	1.2 (0.7–1.9)	1.1 (0.7–1.9)
Leukemia	57	75.4	1.1 (0.6–2.0)	1.0 (0.5–1.9)
Multiple myeloma	32	75.0	1.1 (0.5–2.4)	2.6 (0.7–9.4)[f]

[a]Cancers for which at least 30 subjects had sufficient information for inclusion in age-adjusted analyses. [b]RRs and 95% CIs from Poisson regression models. [c]Frequencies among subjects included in age-adjusted analyses. [d]Numbers of subjects in these analyses are lower than in age-adjusted analyses because of missing observations for some covariates (models adjusted for demographic and lifestyle factors include 49,211 subjects; models additionally adjusted for other pesticides include 40,719 subjects). [e]Estimates adjusted for other pesticides are shown because inclusion of other pesticide variables in the model changed the effect estimate for glyphosate by at least 20%. [f]The estimate for myeloma was not confounded by other pesticides according to our change-in-estimate rule of ≥ 20%; however, the fully adjusted estimate is shown for the purpose of comparison with state-specific estimates (in the text), which were confounded by other pesticides and required adjustment.

on the exposure-day component; therefore, only results for cumulative exposure days are shown further. When using never exposed as the referent, the association between glyphosate use and multiple myeloma was more pronounced, with more than 4-fold increased risk associated with the highest tertile of cumulative exposure days (tertile 1: RR = 2.3; 95% CI, 0.6–8.9; tertile 2: RR = 2.6; 95% CI, 0.6–11.5; tertile 3: RR = 4.4; 95% CI, 1.0–20.2; *p*-value for trend = 0.09). Although the myeloma cases were sparsely distributed in analyses of quartiles and quintiles, the highest increased risks were observed in the highest exposure categories (full set of results not shown: upper quartile vs. never exposed: RR = 6.6; 95% CI, 1.4–30.6; *p*-value for trend across quartiles = 0.01).

Discussion

There was no association between glyphosate exposure and all cancer incidence or most of the specific cancer subtypes we evaluated, including NHL, whether the exposure metric was ever used, cumulative exposure days, or intensity-weighted cumulative exposure days. The most consistent finding in our study was a suggested association between multiple myeloma and glyphosate exposure, based on a small number of cases.

Although our study relied on self-reported exposure information, farmers have been shown to provide reliable information regarding their personal pesticide use (Blair et al. 2002; Blair and Zahm 1993; Duell et al. 2001; Engel et al. 2001; Hoppin et al. 2002). Investigators have used pesticide supplier reports (Blair and Zahm 1993) and self-reported pesticide use information provided earlier (Engel et al. 2001) to assess the validity of retrospectively reported pesticide use data. Among farmers in the AHS, Blair et al. (2002) reported high reliability for reports of ever use of a particular pesticide (ranging from 70 to > 90%). Agreement for duration and frequency of use was lower but generally 50–60% for specific pesticides. Hoppin et al. (2002) have demonstrated that farmers provide plausible data regarding lifetime duration of use, with fewer than 5% reporting implausible values for specific chemicals.

There were rather few cases of NHL for inclusion in this analysis ($n = 92$); nevertheless,

Table 3. Association of glyphosate exposure (cumulative exposure days and intensity-weighted exposure days) with common cancers[a] among AHS applicators.

	Cumulative exposure days[b]				Intensity-weighted exposure days[c]			
Cancer site	Tertile cut points	No.	RR (95% CI)[d]	*p*-Trend	Tertile cut points	No.	RR (95% CI)[d]	*p*-Trend
All cancers	1–20	594	1.0		0.1–79.5	435	1.0	
	21–56	372	1.0 (0.9–1.1)		79.6–337.1	436	0.9 (0.8–1.0)	
	57–2,678	358	1.0 (0.9–1.1)	0.57	337.2–18,241	438	0.9 (0.8–1.1)	0.35
Lung	1–20	40	1.0		0.1–79.5	27	1.0	
	21–56	26	0.9 (0.5–1.5)[e]		79.6–337.1	38	1.1 (0.7–1.9)[e]	
	57–2,678	26	0.7 (0.4–1.2)[e]	0.21	337.2–18,241	27	0.6 (0.3–1.0)[e]	0.02
Oral cavity	1–20	18	1.0		0.1–79.5	11	1.0	
	21–56	10	0.8 (0.4–1.7)		79.6–337.1	14	1.1 (0.5–2.5)	
	57–2,678	10	0.8 (0.4–1.7)	0.66	337.2–18,241	13	1.0 (0.5–2.3)	0.95
Colon	1–20	32	1.0		0.1–79.5	25	1.0	
	21–56	28	1.4 (0.9–2.4)[e]		79.6–337.1	20	0.8 (0.5–1.5)[c]	
	57–2,678	15	0.9 (0.4–1.7)[e]	0.54	337.2–18,241	30	1.4 (0.8–2.5)[c]	0.10
Rectum	1–20	20	1.0		0.1–79.5	16	1.0	
	21–56	17	1.3 (0.7–2.5)		79.6–337.1	18	1.0 (0.5–2.0)	
	57–2,678	14	1.1 (0.6–2.3)	0.70	337.2–18,241	16	0.9 (0.5–1.9)	0.82
Pancreas	0–20	9	1.0		0–79.5	6	1.0	
	21–56	9	1.6 (0.6–4.1)		79.6–337.1	16	2.5 (1.0–6.3)	
	57–2,678	7	1.3 (0.5–3.6)	0.83	337.2–18,241	3	0.5 (0.1–1.9)	0.06
Kidney	1–20	20	1.0		0.1–79.5	20	1.0	
	21–56	8	0.6 (0.3–1.4)		79.6–337.1	7	0.3 (0.1–0.7)	
	57–2,678	9	0.7 (0.3–1.6)	0.34	337.2–18,241	10	0.5 (0.2–1.0)	0.15
Bladder	1–20	23	1.0		0.1–79.5	14	1.0	
	21–56	14	1.0 (0.5–1.9)		79.6–337.1	8	0.5 (0.2–1.3)	
	57–2,678	17	1.2 (0.6–2.2)	0.53	337.2–18,241	13	0.8 (0.3–1.8)	0.88
Prostate	1–20	239	1.0		0.1–79.5	167	1.0	
	21–56	132	0.9 (0.7–1.1)		79.6–337.1	169	1.0 (0.8–1.2)	
	57–2,678	145	1.1 (0.9–1.3)	0.69	337.2–18,241	174	1.1 (0.9–1.3)	0.60
Melanoma	1–20	23	1.0		0.1–79.5	24	1.0	
	21–56	20	1.2 (0.7–2.3)		79.6–337.1	16	0.6 (0.3–1.1)	
	57–2,678	14	0.9 (0.5–1.8)	0.77	337.2–18,241	17	0.7 (0.3–1.2)	0.44
All lymphohematopoietic cancers	1–20	48	1.0		0.1–79.5	38	1.0	
	21–56	38	1.2 (0.8–1.8)		79.6–337.1	40	1.0 (0.6–1.5)	
	57–2,678	36	1.2 (0.8–1.8)	0.69	337.2–18,241	43	1.0 (0.7–1.6)	0.90
NHL	1–20	29	1.0		0.1–79.5	24	1.0	
	21–56	15	0.7 (0.4–1.4)		79.6–337.1	15	0.6 (0.3–1.1)	
	57–2,678	17	0.9 (0.5–1.6)	0.73	337.2–18,241	22	0.8 (0.5–1.4)	0.99
Leukemia	1–20	9	1.0		0.1–79.5	7	1.0	
	21–56	14	1.9 (0.8–4.5)[e]		79.6–337.1	17	1.9 (0.8–4.7)[e]	
	57–2,678	9	1.0 (0.4–2.9)[e]	0.61	337.2–18,241	8	0.7 (0.2–2.1)[e]	0.11
Multiple myeloma	1–20	8	1.0		0–79.5	5	1.0	
	21–56	5	1.1 (0.4–3.5)[e]		79.6–337.1	6	1.2 (0.4–3.8)[e]	
	57–2,678	6	1.9 (0.6–6.3)[e]	0.27	337.2–18,241	8	2.1 (0.6–7.0)[e]	0.17

[a]Cancers for which at least 30 subjects had sufficient information for inclusion in age-adjusted analyses. [b]Numbers of subjects in analyses vary depending on missing observations for cumulative exposure days and some covariates (models adjusted for demographic and lifestyle factors include 36,823 subjects; models additionally adjusted for other pesticides include 30,699 subjects). [c]Numbers of subjects in analyses vary depending on missing observations for intensity-weighted cumulative exposure days and some covariates (models adjusted for demographic and lifestyle factors include 36,509 subjects; models additionally adjusted for other pesticides include 30,613 subjects). [d]Relative rate ratios and 95% CIs from Poisson regression analyses. [e]Estimates adjusted for other pesticides are shown because inclusion of other pesticide variables in the model changed the effect estimate for glyphosate by at least 20%.

the available data provided evidence of no association between glyphosate exposure and NHL incidence. This conclusion was consistent across analyses using the different exposure metrics and in analyses using either never exposed or low exposed as the referent. Furthermore, there was no apparent effect of glyphosate exposure on the risk of NHL in analyses stratified by state of residence or in analyses of highly exposed groups comparing the highest with the lowest quintile of exposure. These findings conflict with recent studies. The first report of an association of glyphosate with NHL was from a case–control study, but the estimate was based on only four exposed cases (Hardell and Eriksson 1999). A pooled analysis of this initial study with a study of hairy cell leukemia showed a relationship between glyphosate exposure and an increased risk of disease [unadjusted analysis: odds ratio (OR) = 3.0; 95% CI, 1.1–8.5] (Hardell et al. 2002). A more extensive study conducted across a large region of Canada found an elevated risk of NHL associated with glyphosate use more frequent than 2 days/year (OR = 2.1; 95% CI, 1.2–3.7) (McDuffie et al. 2001). Similarly, increased NHL risk in men was associated with having ever used glyphosate (OR = 2.1; 95% CI, 1.1–4.0) after adjustment for other commonly used pesticides in a pooled analysis of National Cancer Institute–sponsored case–control studies conducted in Nebraska, Kansas, Iowa, and Minnesota (De Roos et al. 2003b). These previous studies were retrospective in design and thereby potentially susceptible to recall bias of exposure reporting. Our analysis of the AHS cohort had a prospective design, which should largely eliminate the possibility of recall bias. Differences in recall bias could account for discrepant study results; however, evaluation of the potential for recall bias in case–control studies of pesticides among farmers has not uncovered evidence that it occurred (Blair and Zahm 1993).

Our finding of a suggested association of multiple myeloma incidence with glyphosate exposure has not been previously reported, although numerous studies have observed increased myeloma risk associated with farming occupation (Boffetta et al. 1989; Brownson et al. 1989; Cantor and Blair 1984; Cerhan et al. 1998; Cuzick and De Stavola 1988; Eriksson and Karlsson 1992; Figgs et al. 1994; Gallagher et al. 1983; La Vecchia et al. 1989; Nandakumar et al. 1986, 1988; Pasqualetti et al. 1990; Pearce et al. 1985; Pottern et al. 1992; Reif et al. 1989; Vagero and Persson 1986). A possible biologic mechanism of how glyphosate might act along the causal pathway of this plasma cell cancer has not been hypothesized, but myeloma has been associated with agents that cause either DNA damage or immunosuppression (De Roos et al. 2003a). The association we observed was with ever use of glyphosate and cumulative exposure days of use (a combination of duration and frequency), but not with intensity of exposure. Estimated intensity of glyphosate exposure was based on general work practices that were not glyphosate specific, including the percentage of time spent mixing and applying pesticides, application method, use of personal protective equipment, and repair of pesticide application equipment (Dosemeci et al. 2002). Information on work practices specific to glyphosate use would clarify whether intensity of exposure contributes to myeloma risk.

The number of myeloma cases in our study was small, and it is plausible that spurious associations arose by chance; however, several aspects of our results argue against a chance association. The findings were internally consistent, with increased risk observed in both states. Adding to the credibility of the association, there was some indication of a dose–response relationship, with risk estimates increasing across categories of increasing exposure and stronger associations observed when using never-exposed subjects as the referent (as opposed to low exposed). Another possible explanation for spurious associations is unadjusted confounding. Our risk estimates were adjusted for some demographic and lifestyle factors and other pesticides. Of the other pesticides included in the fully adjusted model, only diazinon and trifluralin were important confounders of the glyphosate–myeloma association. It is certainly possible that an unknown risk factor for myeloma could have confounded our results; however, any unknown confounder would have to be linked with glyphosate use. Finally, the increased myeloma risk associated with glyphosate use could be due to bias resulting from a selection of subjects in adjusted analyses that differed from subjects included in unadjusted analyses. Table 1 shows that 54,315 subjects were included in age-adjusted models, whereas because of missing data for covariates, only 40,719 subjects were included in fully adjusted analyses. The association of glyphosate with myeloma differed between the two groups, even without adjustment for any covariates, with no association among the full group and a positive association among the more restricted group. Subjects who answered all the questions and were thus included in adjusted analyses differed from those who dropped out of such analyses in that they were more likely to be from Iowa (71.8% in included group vs. 44.6% in dropped group), were younger (average age, 51.5 vs. 57.9 years), and were more highly educated (46.7% educated beyond high school graduate vs. 30.2%); however, the two groups were similar in their use of glyphosate (75.9% vs. 74.5%). The increased risk associated with glyphosate in adjusted analyses may be due to selection bias or could be due to a confounder or effect modifier that is more prevalent among this restricted subgroup and is unaccounted for in our analyses. Further follow-up of the cohort and reevaluation of the association between glyphosate exposure and myeloma incidence after a greater number of cases develop will allow more detailed examination of the potential biases underlying the association.

Certain limitations of our data hinder the inferences we can make regarding glyphosate and its association with specific cancer subtypes. Although the AHS cohort is large, and there were many participants reporting glyphosate use, the small numbers of specific cancers occurring during the follow-up period hindered precise effect estimation. In addition, most applicators were male, precluding our ability to assess the association between glyphosate exposure and cancer incidence among women, for both non-sex-specific cancers and sex-specific cancers (e.g., of the breast or ovary). Our analysis provides no information on the timing of pesticide use in relation to disease, limiting the ability to sufficiently explore latency periods or effects resulting from glyphosate exposure at different ages. Despite limitations of our study, certain inferences are possible. This prospective study of cancer incidence provided evidence of no association between glyphosate exposure and most of the cancers we studied, and a suggested association between glyphosate and the risk of multiple myeloma. Future analyses within the AHS will follow up on these findings and will examine associations between glyphosate exposure and incidence of less common cancers.

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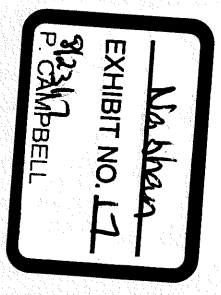
Original Contribution

Occupational Exposure to Pesticides and Risk of Non-Hodgkin's Lymphoma



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Pesticide exposure may be a risk factor for non-Hodgkin's lymphoma, but it is not certain which types of pesticides are involved. A population-based case-control study was undertaken in 2000–2001 using detailed methods of assessing occupational pesticide exposure. Cases with incident non-Hodgkin's lymphoma in two Australian states ($n = 694$) and controls ($n = 694$) were chosen from Australian electoral rolls. Logistic regression was used to estimate the risks of non-Hodgkin's lymphoma associated with exposure to subgroups of pesticides after adjustment for age, sex, ethnic origin, and residence. Approximately 10% of cases and controls had incurred pesticide exposure. Substantial exposure to any pesticide was associated with a trebling of the risk of non-Hodgkin's lymphoma (odds ratio = 3.09, 95% confidence interval: 1.42, 6.70). Subjects with substantial exposure to organochlorines, organophosphates, and "other pesticides" (all other pesticides excluding herbicides) and herbicides other than phenoxy herbicides had similarly increased risks, although the increase was statistically significant only for "other pesticides." None of the exposure metrics (probability, level, frequency, duration, or years of exposure) were associated with non-Hodgkin's lymphoma. Analyses of the major World Health Organization subtypes of non-Hodgkin's lymphoma suggested a stronger effect for follicular lymphoma. These increases in risk of non-Hodgkin's lymphoma with substantial occupational pesticide exposure are consistent with previous work.

case-control studies; herbicides; lymphoma, non-Hodgkin; occupational exposure; pesticides

Abbreviations: CI, confidence interval; DDT, dichlorodiphenyltrichloroethane; OR, odds ratio.

There has been considerable interest in the question of whether exposure to pesticides causes non-Hodgkin's lymphoma, with recent reviews highlighting pesticide exposure as one of the likely occupational risk factors for this cancer (1, 2). This hypothesis was originally derived from studies suggesting that farmers had increased rates of non-Hodgkin's lymphoma (3, 4). Although farmers are exposed to a number of potential carcinogens (diesel exhaust, animal viruses, etc.), researchers have concentrated on their occupational exposure to pesticides. There are hundreds of different types of pesticides in common use in developed countries, and many more have been banned or have had their use discontinued in the past 30 years.

Importantly, "pesticides" is a generic term that includes substances with a variety of different chemical structures and mechanisms of action. Only particular types of pesticides

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or specific chemicals might be related to non-Hodgkin's lymphoma. There has been interest recently in trying to determine which of the many pesticides in use may be responsible for the reported association with non-Hodgkin's lymphoma. Interest has focused on three groups of substances:

- phenoxy herbicides—general-use herbicides (chemicals that kill weeds) which include known animal carcinogens such as 2,4-dichlorophenoxyacetic acid;
- organophosphates—primarily insecticides which work by inhibiting acetylcholinesterase, resulting in neurotoxicity and paralysis (e.g., diazinon, parathion); and
- organochlorines—primarily insecticides and fungicides (e.g., chlordane, lindane) which include some substances known to persist for very long periods in the environment (e.g., dichlorodiphenyltrichloroethane (DDT)).

In addition, there is a wide range of other herbicides (e.g., triazines, dipyridyls (diquat, paraquat), chlorates) and pesticides (e.g., carbamates, pyrethroids) that are commonly used in farming.

In a case-control study of non-Hodgkin's lymphoma, we examined exposure to each of the above groups of pesticides using detailed methods of assessing pesticide exposure.

MATERIALS AND METHODS

Case and control recruitment

Details on case and control ascertainment for this study can be found in related articles (5, 6). Briefly, cases were persons with incident non-Hodgkin's lymphoma that was first diagnosed between January 1, 2000, and August 31, 2001, and reported to the Central Cancer Registry of New South Wales, Australia. Patients were 20–74 years of age and resident in New South Wales or the Australian Capital Territory. Ineligibility criteria included a history of organ transplantation or human immunodeficiency virus infection, poor English language skills, inability to complete a telephone interview, or a diagnosis of chronic lymphocytic leukemia, plasma cell myeloma, or B- or T-cell lymphoblastic leukemia. An anatomic pathologist reviewed all relevant pathology reports for all consenting patients. The pathologist reviewed diagnostic histopathology sections for all consenting patients judged to be less than 90 percent certain to have an eligible diagnosis of non-Hodgkin's lymphoma in the report review. The aim of this review was to assure the correct diagnosis and to obtain, where possible, a World Health Organization classification category (7) and the corresponding *International Classification of Diseases for Oncology*, Third Edition, code (8).

Controls were randomly selected from the New South Wales and Australian Capital Territory electoral rolls to approximately match the expected distributions of cases with regard to age, sex, and region of residence (New South Wales or Australian Capital Territory). Electoral registration is compulsory for Australian citizens aged 18 years or over. Similar eligibility criteria were used as for cases, except for human immunodeficiency virus infection, which was expected to be rare in the general population.

Cases and controls were mailed an introductory letter and an information leaflet, followed by a self-administered questionnaire to each consenting subject. The questionnaire included a diary with a detailed lifetime history of each job the subject had held for 1 year or more. Information obtained on each job included job title, employer, industry, start and finish years, number of hours worked per day, and number of days worked per week.

The final data set consisted of 694 cases (of 1,230 ascertained cases, 842 were apparently eligible and contactable) and 694 controls (of 1,687 controls selected, 1,136 were apparently eligible and contactable). Further details on response fractions are available in previous articles (5, 6). Twenty-three cases were excluded after the pathology reviews because the pathologist considered them not to have an eligible diagnosis. Ten of these cases were removed after review of the pathology sections; these 10 cases were included in an earlier report (5).

Exposure allocation

A total of 28 jobs and 16 industries were identified as being of particular interest because of the possibility of exposure to the substances evaluated in this study (6). For these 44 jobs and industries, detailed sets of questions (known as job-specific modules) were obtained from the US National Cancer Institute (9) and modified to suit this study. The resulting modules included 6–23 questions asking about specific tasks performed in that occupation. Respondents were asked how many weeks per year and how many hours per week they had spent in each task. Modules were allocated to subjects by an occupational hygienist according to whether or not the subjects had worked in one or more of the 44 jobs and industries. The questions in the relevant modules were asked in a customized computer-assisted telephone interview. The hygienist and the interviewers were blinded to the case or control status of subjects.

The same expert occupational hygienist (again blind to status) reviewed the occupational histories and the answers to the module questions and determined exposure to various substances, including organophosphates, organochlorines, phenoxy herbicides, other herbicides, and other pesticides. The hygienist allocated exposures occurring before 1985 and after 1985 separately, because use of organochlorines had been phased out around 1985 and use of other pesticides (mainly pyrethrins) had become widespread. A pesticide-crop matrix was developed for assistance with exposure assessment (10). The matrix included information on what kinds of pesticides were known to be used (or recommended by the Australian Department of Agriculture) for each combination of crop or animal raised and pest type (insect, weed, etc.). A table was also prepared for assistance with identification of chemical composition from trade names reported by the subjects. Former Department of Agriculture employees, environmental scientists, and pesticide manufacturers assisted with construction of the matrix.

The hygienist first allocated likelihood of exposure to each substance as probable, possible, or no exposure. He then allocated one of three levels of exposure using previous literature and his own professional knowledge, without

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regard to the probability of exposure. The reference levels were internationally recognized occupational safety guidelines (time-weighted average threshold limit values set by the American Conference of Governmental Industrial Hygienists (11)). Levels of exposure higher than the time-weighted average threshold limit values were considered high; those less than or equal to one 10th of the time-weighted average threshold limit values were considered low; and other exposures were considered medium. For the few people who reported wearing gloves and overalls while mixing and applying pesticides, the exposure level was dropped one level lower. Frequency of exposure was allocated as number of 8-hour days per year and was calculated using responses to the task questions. If no data on frequency of exposure were available ($n = 4$), subjects were assumed to have been exposed for 2 days per year.

Amount of exposure was calculated by combining data from all jobs held over the person's entire working life. Amount was classified as substantial if the subject was probably exposed to the substance at a medium or high level for more than five 8-hour days per year for a combined total of more than 5 years, and nonsubstantial if the dose involved any other combination of exposures.

Statistical analysis

The data were first examined by use of contingency tables and comparisons of mean values. Logistic regression was used to calculate odds ratios (as estimates of relative risk) for non-Hodgkin's lymphoma associated with exposure to any pesticide and exposure to each pesticide subtype in each amount category (substantial or nonsubstantial), with adjustment for age, sex, ethnic origin, and state of residence. In addition, logistic regression analyses were carried out for exposure to any pesticide after restricting the sample to males only and after excluding cases that were not on the electoral roll. We also repeated the analyses for each pesticide for B-cell non-Hodgkin's lymphomas only, for follicular lymphomas only, and for diffuse large B-cell lymphomas only. We also examined the odds of non-Hodgkin's lymphoma using the following metrics of exposure to any pesticide: maximum exposure level (low, medium, high); ever being exposed before 1985 (yes, no); maximum frequency of exposure (0, $\leq$4, or $>$4 days/year); and total number of years exposed (0, $\leq$5, or $>$5 years). For the latter two metrics, 4 days per year and 5 years were the median frequency and duration, respectively, in control subjects. All p values were two-sided.

Approval for this study was given by the human research ethics committee at each participating institution. Participants were sent detailed information sheets and were subsequently telephoned to obtain their consent.

RESULTS

Cases and controls were well-matched by sex and age, but controls were more likely to be of British or Irish ethnic origin (table 1), possibly because of a relative deficit of people of other origins on the electoral roll (12). There was no appreciable difference in socioeconomic status (based on the subjects' residential postcodes) between cases and controls. The subtypes of lymphoma evaluated comprised the following: diffuse large B-cell lymphoma (n = 231); follicular lymphoma (n = 227); extranodal marginal zone B-cell lymphoma (n = 37); chronic lymphocytic leukemia or small lymphocytic lymphoma (n = 27); lymphoplasmacytic lymphoma or Waldenstrom's macroglobulinemia (n = 26); mantle cell lymphoma (n = 22); other B-cell lymphoma subtypes (n = 39); combined B-cell lymphoma subtypes (n = 31); B-cell lymphoma, not otherwise classified (n = 25); T-cell lymphoma (n = 25); and non-Hodgkin's lymphoma, not otherwise classified (n = 4).

TABLE 1. Characteristics (%) of cases and controls in an Australian study of non-Hodgkin's lymphoma, 2000–2001

Characteristic	Controls (n = 694)	Cases (n = 694)
Sex		
Male	57.2	58.2
Female	42.8	41.8
Age group (years)		
20–29	3.0	2.9
30–39	6.6	6.2
40–49	16.4	17.1
50–59	28.1	29.4
60–69	30.1	30.4
70–74	15.7	14.0
Ethnic origin		
British/Irish	78.5	73.2
Asian	2.0	3.3
Mixed	9.4	9.5
Southern European	3.2	5.8
Other European	3.5	4.6
Other	3.5	3.6
State of residence		
New South Wales	95.2	96.0
Australian Capital Territory	4.8	4.0

Approximately 10 percent of cases and controls had been exposed to any pesticide at any level. Approximately 1 percent of controls (n = 9) and 4 percent of cases (n = 26) had incurred a substantial amount of exposure (table 2). Of those substantially exposed to any pesticide, the average total time of exposure was 675 8-hour days for cases and 494 days for controls. All but seven subjects substantially exposed to any pesticide (three controls and four cases) had been exposed for the total equivalent of 6 months or more.

Exposure to a substantial amount of any pesticide was associated with a trebling of the risk of non-Hodgkin's lymphoma (odds ratio (OR) = 3.09, 95 percent confidence interval (CI): 1.42, 6.70). Subjects with substantial exposure to each pesticide subgroup had increased risks of non-Hodgkin's lymphoma, although the lower bound of the 95 percent confidence interval was greater than 1.0 only for those with substantial exposure to "other pesticides."

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TABLE 2. Degree of exposure to pesticides and non-Hodgkin's lymphoma in an Australian case-control study, 2000–2001

Degree of exposure	Controls		Cases		All subjects	
	No.	%	No.	%	Odds ratio*	95% confidence interval
Any pesticide						
None	621	89.5	621	89.6	1.0	
Nonsubstantial	64	9.2	47	6.7	0.73	0.49, 1.09
Substantial	9	1.3	26	3.7	3.09	1.42, 6.70
Organophosphates						
None	660	95.1	662	95.4	1.0	
Nonsubstantial	28	4	20	2.8	0.71	0.39, 1.28
Substantial	6	0.9	12	1.7	2.11	0.78, 5.68
Organochlorines						
None	679	97.8	674	97.1	1.0	
Nonsubstantial	13	1.9	14	2	1.07	0.50, 2.32
Substantial	2	0.3	6	0.9	3.27	0.66, 16.4
Phenoxy herbicides						
None	677	97.6	679	97.9	1.0	
Nonsubstantial	14	2	10	1.4	0.73	0.32, 1.66
Substantial	3	0.4	5	0.7	1.75	0.42, 7.38
Other herbicides						
None	671	96.7	659	95	1.0	
Nonsubstantial	20	2.9	26	3.7	1.37	0.75, 2.49
Substantial	3	0.4	9	1.3	3.29	0.88, 12.3
Other pesticides						
None	640	92.2	639	92.2	1.0	
Nonsubstantial	51	7.3	43	6.1	0.86	0.56, 1.32
Substantial	3	0.4	12	1.7	4.24	1.18, 15.2

* Adjusted for sex, age, ethnicity, and region of residence.

Restricting the subjects to subgroups produced similar patterns, with statistically significant increases in risk for substantial exposure to any pesticide (table 3). The odds ratio for substantial exposure to any pesticide for males only was 3.7, and for persons on the electoral roll only, it was 2.9. The odds ratio for the 584 cases and 694 controls who were on the electoral roll was only 7 percent below the odds ratio for the entire group; this suggests that any bias which might have been due to the use of electoral rolls as a sampling frame for controls was largely controlled by adjustment for ethnic origin.

When we examined the individual exposure metrics separately (probability, level, frequency, duration, and years exposed), none of the individual effect estimates were statistically significant (table 3). When we used a continuous measure, number of years exposed to any pesticide, we found that risk of non-Hodgkin's lymphoma increased slightly with every year of exposure (OR = 1.01, 95 percent CI: 0.994, 1.027). Among those probably or definitely exposed to any pesticide, the mean number of years of exposure to any pesticide was 12.7 for controls and 16.6 for cases.

Restricting the case group to persons with B-cell lymphoma ($n = 665$) produced results similar to those for the entire sample (table 4). Restricting the cases to persons with diffuse large B-cell lymphoma ($n = 231$) resulted in generally lower effect measures, except that for "other pesticides" (OR = 4.96, 95 percent CI: 1.17, 21.1). When we used only cases with follicular lymphoma ($n = 227$), we found stronger associations, especially for exposures to any pesticide, organophosphates, and "other herbicides."

Of the 26 cases that entailed substantial exposure to pesticides, three (11.5 percent) involved T-cell subtypes as compared with 3.3 percent of the remaining 668 cases (Fisher's exact test: $p = 0.07$). Two were nasal natural killer T-cell lymphomas and one was an angioimmunoblastic T-cell lymphoma; all three contained Epstein-Barr virus early RNA upon in-situ hybridization.

DISCUSSION

We found that substantial exposure to any pesticide trebled the risk of non-Hodgkin's lymphoma. Although

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TABLE 3. Relations between exposure to any pesticides and risk of non-Hodgkin's lymphoma using different metrics and sample subgroups in an Australian case-control study, 2000–2001

Metric or subgroup and degree of exposure	Controls		Cases		All subjects	
	No.	%	No.	%	Odds ratio*	95% confidence interval
Males only						
None	335	84.4	343	84.9	1.0	
Nonsubstantial	55	13.9	36	8.9	0.64	0.41, 1.00
Substantial	7	1.8	25	6.2	3.67	1.56, 8.65
Persons on electoral roll only						
None	621	89.5	525	89.9	1.0	
Nonsubstantial	64	9.2	38	6.5	0.70	0.46, 1.07
Substantial	9	1.3	21	3.6	2.89	1.30, 6.41
Probability of exposure						
None	621	89.5	621	89.5	1.0	
Possible	5	0.7	5	0.7	0.96	0.27, 3.36
Probable	68	9.8	68	9.8	1.02	0.71, 1.47
Level of exposure						
None	621	89.2	621	89.5	1.0	
Low	35	5.3	30	4.3	0.81	0.49, 1.33
Medium	21	3.0	29	4.2	1.39	0.78, 2.49
High	17	2.4	14	2.0	0.86	0.42, 1.77
Frequency of exposure						
Never	621	89.5	621	89.9	1.0	
≤4 days/year	36	5.2	32	4.6	0.89	0.54, 1.46
>4 days/year	37	5.3	41	5.9	1.14	0.71, 1.81
Years of exposure						
None or <1	626	90.2	627	90.3	1.0	
1–5	34	4.9	19	2.7	0.57	0.32, 1.02
>5	34	4.9	48	6.9	1.42	0.89, 2.25
Exposed before 1985						
No	651	93.8	644	92.9	1.0	
Yes	43	6.2	49	7.1	1.18	0.77, 1.81

* Adjusted for sex, age, ethnicity, and region of residence.

many, but not all, previous studies have found increases in non-Hodgkin's lymphoma risk with exposure to pesticides (13–19), our finding is at the high end of the range of reported results. Our definition of substantial exposure was exposure that was at or above one 10th of the time-weighted average threshold limit values for more than five 8-hour days per year for a combined total of more than 5 years. Most people exposed had the equivalent of more than 6 months of use for 8 hours per day every day.

Exposure to pesticides is often seasonal, and spraying seasons may be only a few days to a few weeks in duration each year. Many previous studies have used any exposure (14, 16) or any exposure for more than 1 year (17–19). Case-control studies that have tried to isolate persons with higher levels of exposure have found results similar to ours. For example, in a US study, exposure to pesticides for more than 10 years increased the risk nearly threefold (OR = 2.72, 95 percent CI: 1.4, 5.4) (15), and in an Italian study, exposure to herbicides for more than 10 years increased the risk 5.2-fold (16). These definitions of high exposure take into account the length of exposure, which may be the important factor in determining risk. In our data, there was a weak relation between the number of years exposed to any pesticide and non-Hodgkin's lymphoma which was of borderline significance. There was little or no increase in risk with higher levels or frequencies of exposure; thus, from our data, it seems as though any risk of non-Hodgkin's lymphoma may be related to relatively high exposure to pesticides over a long period of time.

Substantial exposure to organophosphate pesticides approximately doubled the risk of non-Hodgkin's lymphoma in our study, although this finding was not statistically

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TABLE 4. Results from logistic regression analysis of the association between pesticide exposure and different subtypes of non-Hodgkin's lymphoma in an Australian case-control study, 2000–2001

Degree of exposure	B-cell lymphoma (n = 665)		Diffuse large B-cell lymphoma (n = 231)		Follicular lymphoma (n = 227)	
	OR*,†	95% CI*	OR†	95% CI	OR†	95% CI
Any pesticide						
None	1.0		1.0		1.0	
Nonsubstantial	0.75	0.50, 1.12	1.04	0.61, 1.76	0.56	0.29, 1.09
Substantial	2.88	1.31, 6.32	2.21	0.77, 6.35	4.3	1.73, 10.7
Organophosphates						
None	1.0		1.0		1.0	
Nonsubstantial	0.63	0.34, 1.17	0.63	0.26, 1.57	1.07	0.49, 2.33
Substantial	2.22	0.83, 5.97	2.14	0.60, 7.72	4.28	1.41, 13.0
Organochlorines						
None	1.0		1.0		1.0	
Nonsubstantial	1.13	0.52, 2.45	1.2	0.42, 3.44	1.84	0.72, 4.75
Substantial	3.46	0.69, 17.3	1.62	0.15, 18.1	3.46	0.48, 25.2
Phenoxy herbicides						
None	1.0		1.0		1.0	
Nonsubstantial	0.61	0.25, 1.47	0.45	0.10, 2.00	0.45	0.10, 2.01
Substantial	1.47	0.33, 6.64	2.16	0.36, 13.1	1.15	0.12, 11.2
Other herbicides						
None	1.0		1.0		1.0	
Nonsubstantial	1.38	0.75, 2.53	1.82	0.85, 3.91	0.64	0.21, 1.90
Substantial	3.1	0.81, 11.8	1.12	0.12, 10.9	4.83	1.06, 22.0
Other pesticides						
None	1.0		1.0		1.0	
Nonsubstantial	0.9	0.59, 1.38	1	0.55, 1.81	1.1	0.62, 1.96
Substantial	3.35	0.90, 12.5	3.18	0.63, 16.0	1.19	0.12, 11.6

* OR, odds ratio; CI, confidence interval.
† Adjusted for sex, age, ethnicity, and region of residence.

significant. A Canadian case-control study (20) found that exposure to any organophosphate insecticide was associated with a non-Hodgkin's lymphoma risk of 1.69 (95 percent CI: 1.28, 2.46), with statistically significant associations being found for malathion and diazinon. A US case-control study (21) that examined exposure to a large number of specific pesticides (adjusted for exposure to other pesticides) found significant associations with coumaphos (OR = 2.4, 95 percent CI: 1.0, 5.8) and diazinon (OR = 1.9, 95 percent CI: 1.1, 3.6) but not with malathion. However, another large US study of pesticide exposure did not find any association between organophosphate use and non-Hodgkin's lymphoma (19). It may be that different organophosphate pesticides have different effects, but we had insufficient numbers of subjects to analyze specific types of organophosphates.

Few people had substantial exposure to organochlorine pesticides in our study, so although the point estimate was quite high, the confidence intervals were wide. Previous studies have attempted to examine individual organochlorine pesticides, such as DDT, and have also found suggestive increases but wide confidence intervals (19, 21–24). Prediagnostic serum levels of various organochlorines were not associated with non-Hodgkin's lymphoma in a nested case-control study (25).

Phenoxy herbicides were not strongly associated with non-Hodgkin's lymphoma in our study. The literature on phenoxy herbicides is inconsistent. Several case-control studies have found increased risks of non-Hodgkin's lymphoma (20, 22, 23, 26, 27), while others have found no association (19, 28, 29). Cohort studies of pesticide users and manufacturers have found risks ranging from 1.0 to 2.4, not all of which were statistically significant (30–32). In general, the literature seems to show that case-control studies with more sophisticated exposure assessment (such as ours) tend to find smaller risks than those based on self-reports, which are liable to recall bias (Neil Pearce, Centre for Public Health Research, Massey University (Palmerston North, New Zealand), personal communication, 2004). In addition, studies carried out in Sweden tend to find higher risks than studies conducted elsewhere, and it is possible that conditions of use in Australia are more similar to those in New Zealand (where no increase in risk was found by

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Pearce (29)) than to those in Sweden. A German study of pesticide manufacturing workers found higher risks of non-Hodgkin's lymphoma in plants where dioxin contamination of the phenoxy herbicides had occurred (33) and suggested that the risk arises from dioxin, not the herbicide itself. However, Pearce argues that this explanation does not fit the available data and that there is more likely to be a small but real increase in risk due to exposure to phenoxy herbicides (Neil Pearce, Centre for Public Health Research, Massey University, personal communication, 2004).

We found increases in the risk of non-Hodgkin's lymphoma for persons exposed to "other herbicides" (mainly glyphosate and carbamates) and "other pesticides" (mainly phosphine, arsenicals, and pyrethrins). Past and present use of phosphine as a fumigant for grain crop storage was commonly reported by subjects in our study. Arsenicals were used in Australia until the 1970s, and their use was reported by subjects only in jobs held prior to 1985. The pyrethrins were introduced in the 1980s, and reported exposures occurred mainly in the 1990s. The herbicide glyphosate has been found to be associated with non-Hodgkin's lymphoma in three case-control studies (20–22), although in the last of these studies (22) the confidence intervals included unity. Several other studies have examined exposure to carbamates and have found risks ranging from 0.9 to 1.5, mostly not statistically significant (19–22, 28, 34).

Overall, our study was limited by the relatively small numbers of subjects exposed at a substantial level. This resulted in quite wide confidence intervals, especially in the analysis of subgroups. Still, the findings were reasonably consistent in showing a statistically significant trebling of risk with high exposure to pesticides.

There was some suggestion of a stronger link between organophosphates and "other pesticides" with follicular non-Hodgkin's lymphomas as compared with diffuse large B-cell subtypes. Findings from studies that used earlier classifications of lymphoma (such as the Working Formulation (35)) are difficult to extrapolate to the new classifications of non-Hodgkin's lymphoma. In addition, these studies had conflicting results. One found the effect estimates for pesticides to be slightly higher for follicular lymphomas than for large-cell diffuse lymphomas (19), while another found the effect estimates to be higher for small lymphocytic non-Hodgkin's lymphoma (36). One possible mechanism is a translocation involving the immunoglobulin heavy chain t(14;18). This translocation is found in farmers with heavy exposure to pesticides (37, 38), and it is most common in follicular and diffuse large (B)-cell lymphomas in the Revised European-American Lymphoma classification of histologic subtypes (37).

Of the cases that involved substantial exposure to pesticides, more than expected were T-cell subtypes, and all of them were positive for Epstein-Barr virus early RNA. An association of nasal natural killer T-cell lymphoma with pesticide use has been reported in a father and son (39), and elevated Epstein-Barr virus antibodies have been reported in several studies of non-Hodgkin's lymphoma that included measures of pesticide exposure (24, 40), suggesting a possible interaction. One subtype of T-cell non-Hodgkin's lymphoma that has been examined is mycosis fungoides, a very rare form of T-cell non-Hodgkin's lymphoma; it does not appear to be linked with pesticide exposure (41, 42).

Small numbers of subjects in each subgroup limit the conclusions that can be made regarding associations between pesticides and histologic subtypes of non-Hodgkin's lymphoma in a single study. Collaborative studies with pooling of rare subtypes and multifactorial analyses are needed. One factor moderating the effect of pesticides is the use of personal protective equipment, such as masks and respirators, when preparing and spraying chemicals (43). We found that use of personal protective equipment was low overall and only appeared at all common in jobs held from the mid-1980s onwards. In assessing the level of exposure, the hygienist considered the use of personal protective equipment where it was used.

Exposure assessment in this study was very detailed and used the best methods available for assessing exposure to pesticides (9). A complete job history was taken from each subject, and then additional questions were asked about specific jobs, including farming, pest control, gardening, crop dusting, and janitorial work (44). The job-specific module for farmers and pesticide users was highly detailed and elicited information from subjects regarding the types of crops and animals which the hygienist found appropriate. The pesticide exposure matrix developed for the study (10) was found to be very useful for identifying the likely pesticides used. We did not rely on the subjects' recall of exactly which pesticide(s) they had used, unlike previous studies that have used self-reports for assessment of pesticide exposure. A recent study found that self-reports of pesticide exposure 20 years prior to the study were reasonable when compared with self-reports recorded 20 years earlier (45). Another study compared self-reports from licensed pesticide applicators with known dates of introduction and use of specific pesticides and found that most responses were "plausible" (46). In our study, approximately 10 percent of farmers answered "unable to recall" when asked for specific product details. A study that compared matrix-derived exposures and self-reports of pesticide use found different odds ratios for non-Hodgkin's lymphoma with use of the two measures—1.16 for matrix-derived data and 0.76 for self-reports—but offered no evidence on which of the measures better classified exposure (13).

Other studies, even recent ones, have simply used job titles as a surrogate for exposure (47–50). Problems with this method include the facts that not all people with a particular job title will be exposed to the same pesticides and that people exposed to pesticides often have a number of different job titles, resulting in small numbers for any given title.

The major limitation of the exposure assessment method we used was its cost. Review of job histories, administration of telephone interviews, and review of responses to the assigned occupational modules are highly labor-intensive. In addition, lengthy consultation with experts in agriculture, farming, and pesticide exposure monitoring was required to construct the pesticide exposure matrix. Use of an existing job exposure matrix would have been less intensive but possibly subject to significant nondifferential misclassification.

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In this study, we had a reasonably large sample size and used an intensive exposure assessment process. We found increases in risk of non-Hodgkin's lymphoma with high levels of pesticide exposure and no evidence of risk with lower levels of exposure. This study strengthens the existing evidence that occupational exposure to pesticides increases risk of non-Hodgkin's lymphoma.

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Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis

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We report a population based case–control study of exposure to pesticides as risk factor for non-Hodgkin lymphoma (NHL). Male and female subjects aged 18–74 years living in Sweden were included during December 1, 1999, to April 30, 2002. Controls were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total 910 (91%) cases and 1016 (92%) controls participated. Exposure to herbicides gave odds ratio (OR) 1.72, 95% confidence interval (CI) 1.18–2.51. Regarding phenoxyacetic acids highest risk was calculated for MCPA; OR 2.81, 95% CI 1.27–6.22, all these cases had a latency period >10 years. Exposure to glyphosate gave OR 2.02, 95% CI 1.10–3.71 and with >10 years latency period OR 2.26, 95% CI 1.16–4.40. Insecticides overall gave OR 1.28, 95% CI 0.96–1.72 and impregnating agents OR 1.57, 95% CI 1.07–2.30. Results are also presented for different entities of NHL. In conclusion our study confirmed an association between exposure to phenoxyacetic acids and NHL and the association with glyphosate was considerably strengthened.
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Key words: phenoxyacetic acids; MCPA; glyphosate; insecticides; impreganting agents; non-Hodgkin lymphoma

Non-Hodgkin lymphoma (NHL) is a heterogeneous group of lymphoid malignancies, where new classification systems based on immunohistochemistry, cytogenetics and evolving knowledge in clinical presentation and course has lead to modern classification systems.[1] Today, it is therefore more adequate to discuss NHL as many different diseases, which share some features but also differ in several aspects.

Interest in the etiology of NHL has been strengthened by an observed substantial increase in the incidence of the disease from the 1960's to the 1980's as reported from most countries with reliable cancer registries. However, this increase has clearly leveled off in many countries since the early 1990's, *i.e.*, in Sweden, Denmark and the USA.[2] The established risk factors for development of NHL include different immunosuppressive states, *e.g.*, human immunodeficiency virus (HIV), autoimmune diseases as Sjögren's syndrome and systemic lupus erythematosus (SLE), immunodepressants used after organ transplantation and some inherited conditions, for review see *e.g.*, Ref. 3. However, these causes may only explain a minority of cases, with a possible exception for HIV-related increases among younger persons in certain areas.[4]

It has been shown that Epstein-Barr virus (EBV) plays an essential role in the pathogenesis of lymphomas after organ transplantation.[5] A relation between lymphoma and elevated EBV-titers has been reported in a cohort.[6] Normally, EBV-production is held back by active cellular and humoral immune mechanisms. In immunodeficiency states this balance is disrupted and EBV-infected B-cells begin to proliferate.[7]

During the last decades, research on the etiology of NHL has been directed towards other potential causes such as pesticides, which may explain the impressive increase in the incidence. Today, it is also reasonable to consider the leveling off in incidence as a probable consequence of a reduced carcinogenic influence related to NHL. Furthermore, our emerging knowledge concerning the spectrum of NHL subgroups makes it reasonable to investigate causative agents for these different types of disease.

In 1981, we published results from a case–control study from Sweden, indicating statistically significant increased odds ratios for NHL and Hodgkin lymphoma (HL) in persons who had been exposed to phenoxyacetic herbicides or impregnating chlorophenols.[8] Our study was initiated by a case report.[9] Some of these chemicals were contaminated by dioxins, of which 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) has been recognised as a complete carcinogen by IARC.[10] Furthermore, these and several other related chemicals are immunotoxic.[11–15] Our results have been confirmed in some other studies, regarding phenoxyacetic herbicides from *e.g.*, Kansas[16] and Nebraska.[17]

Furthermore, in 1999 we reported a new case–control study performed to evaluate more recent exposure to pesticides and other chemicals, and we could thereby confirm our earlier findings regarding a relation with phenoxyacetic herbicides that was related to latency period.[18]

In that study, however, some newer compounds that are widely used today, such as the herbicide glyphosate, were still not very common. During the 1970's certain chemicals, *e.g.*, the phenoxy herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), chlorophenols, and the insecticide dichlorodiphenyltrichloroethane (DDT), were prohibited due to health concerns. Later also the phenoxy herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) was banned in Sweden. Reporting of these agents is therefore nowadays much less likely. It is also probable that the risk pattern has been influenced by protective measures during the last decades.

To further evaluate the relation between exposure to pesticides and other chemicals, focusing also on newer types of compounds, we have performed a new case–control study in Sweden. In our study we have also evaluated exposures in relation to different histopathological subtypes according to the most recent classification.[1]

Material and methods

The study covered 4 out of 7 health service regions in Sweden, associated with the University Hospitals in Lund, Linköping, Örebro and Umeå, and was approved by the ethics committees. Data were collected during December 1, 1999, to April 30, 2002, which was the time period for diagnosis of the cases. Regarding recruitment of cases and controls collaboration was established with another research group, which at the same time performed a parallel study on NHL in Sweden and Denmark.

Cases

All consecutive patients aged 18–74 years with newly diagnosed NHL, identified through physicians treating lymphoma and through pathologists diagnosing the disease, were approached if their physician did not judge this as less appropriate by ethical rea-

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sons. This was done regardless of whether the person had accepted to participate in the parallel study with which we collaborated in the recruitment procedure. If they accepted to participate they were included as potential cases, and went through the data assessment procedure described below. No cases were excluded because of specific conditions potentially associated with NHL, but no cases with *e.g.*, HIV or postransplantation NHL occurred. All the diagnostic pathological specimens were scrutinised by 1 out of 5 Swedish expert lymphoma reference pathologists, if they had not been initially judged by one of these 5. About 70% of all included cases were reviewed, whereas the remaining had been previously classified by one of the reference pathologists. If there was a disagreement from the original report the sample was reviewed by a panel of these pathologists. Therefore, some potential cases could later be excluded if a NHL diagnosis was not verified, and in those occasions all collected exposure information was disregarded. The pathologists also subdivided all NHL cases according to the WHO classification,[1] to enable etiological analyses also for the different diagnostic NHL entities. Since all lymphoma treating clinics and all lymphoma pathologists in the involved regions were covered by the study, it may well be regarded as population based, although the possibility of some individuals not reported through the case ascertainment system used.

Controls

From the population registry covering whole Sweden, randomly chosen controls living in the same health service regions as the cases were recruited during several occasions within the study period. The controls were frequency-matched in 10 years age and sex groups to mirror the age and sex distribution of the included cases, and to increase efficacy in the adjusted analyses. If they accepted to participate, they were included as controls.

Assessment of exposure

All subjects who accepted to participate received a comprehensive questionnaire, which was sent out shortly after the subjects had been telephone interviewed by the other research group we had collaboration with as stated earlier. Their interview, however, did not focus on work environment or chemical exposure, but rather dealt with other life style factors and diseases. Our questionnaire included a total work history with in depth questions regarding exposure to pesticides, organic solvents and several other chemicals. For all pesticides not only numbers of years and numbers of days per year, but also approximate length of exposure per day were questioned. Since most work with pesticides was performed in an individualized manner, no job-exposure matrix was judged to be applicable. Furthermore, the questionnaire also included questions on *e.g.*, smoking habits, medications, leisure time activities and proximity from home to certain industrial installations, but data on these factors are not included in this article.

Specially trained interviewers scrutinized the answers and collected additional exposure information by phone if important data were lacking, incomplete or unclear. These interviewers were blinded with regard to case/control status. All exposures during the same calendar year as the diagnosis and the year before were disregarded in the cases. Correspondingly, the year of enrolment and the year before were disregarded for the controls. As in our previous lymphoma studies we used a minimum criterion of one full day exposure to be categorized as exposed.[8,18]

Statistical methods

Unconditional logistic regression analysis (Stata/SE 8.2 for Windows; StataCorp, College Station, TX) was used to calculate odds ratios (OR) and 95% confidence intervals (CI). Adjustment was made for age, sex and year of diagnosis (cases) or enrolment (controls). In the univariate analysis, different pesticides were analyzed separately and the unexposed category consisted of subjects that were unexposed to all included pesticides. When analyzing subgroups of NHL all controls were used in the separate analyses. In the dose-response calculations made for agents with at least 20 exposed subjects, median number of days of exposure among controls was used as cut-off. Latency period calculations and multivariate analyses included agents with statistically significant increased OR, or with an OR > 1.50 and at least 10 exposed subjects.

TABLE I – NON-HODGKIN LYMPHOMA CASES DIVIDED ON HISTOPATHOLOGICAL SUBTYPES ACCORDING TO WHO CLASSIFICATION.

WHO diagnosis	Number of cases
B-cell lymphomas, total	819
Lymphocytic lymphoma/B-CLL (SLL/CLL)	195
Follicular, grade I–III (FL)	165
Diffuse large B-cell lymphoma (DLBCL)	239
Other specified B-cell lymphoma	131
Unspecified B-cell lymphoma	89
T-cell lymphomas	53
Unspecified non-Hodgkin lymphoma	38
Total	910

Results

In total, 1,163 cases were reported from the participating clinics. Of these, 46 could not participate because of medical conditions, 88 died before they could be interviewed. Since these were primarily excluded by the reporting physicians we had no information on *e.g.*, final WHO categories on these cases. Three NHL cases were not diagnosed during the study period, 1 lived outside the study area and 30 were excluded not being NHL (HL 20, acute lymphoblastic leukaemia 1, other malignancy 7 and unclear diagnosis 2). Of the finally included 995 cases with NHL, 910 (91%) accepted to participate and answered the questionnaire. Of these, 819 were B-cell, 53 T-cell and 38 unspecified lymphomas, Table I.

Among the 1,108 initially enrolled controls 92 did not respond to the mail questionnaire, resulting in 1,016 (92%) controls to be included in the analyses.

The medium and median age in cases was 60 and 62 years, and in controls it was 58 and 60 years, respectively. Of the cases, 534 were males and 376 females, and of the controls the corresponding numbers were 592 and 424.

This report presents exposure data regarding different types of pesticides.

Herbicides

Exposure to herbicides gave for all NHL OR 1.72 (95% CI 1.18–2.51), Table II. Exposure to phenoxyacetic acids yielded OR 2.04 (95% CI 1.24–3.36). This group was further subdivided in 3 categories; (*i*) 4-chloro-2-methyl phenoxyacetic acid (MCPA), which is still on the market and not known to be contaminated by dioxins; (*ii*) 2,4,5-T and/or 2,4-D which often were used together and were potentially contaminated with different dioxin isomers; (*iii*) other types. MCPA seemed to give the most pronounced increase in OR. Exposure to other herbicides, regardless if they also had been exposed to phenoxyacetic acids or not, also gave a statistically significant OR 1.82 (95% CI 1.08–3.06). In this category the dominating agent was glyphosate, which was reported by 29 cases and 18 controls, which produced OR 2.02 (95% CI 1.10–3.71). If both phenoxyacetic acids and glyphosate were excluded, exposure to other herbicides (37 different agents reported, but no one by more than 6 subjects at most) gave a nonsignificant OR of 1.22 (95 % CI 0.63–2.39).

Dose-response analyses regarding herbicides in total and glyphosate yielded an increased OR in the higher exposed group, Table II. For phenoxyacetic acids, however, no such association was demonstrated.

Regarding phenoxy herbicides and glyphosate an analysis was made taken the latency period for exposure into account. For the

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latency period 1–10 years no exposed cases were found for MCPA and 2,4,5-T and/or 2,4-D. Regarding glyphosate OR 1.11 (95% CI 0.24–5.08) was obtained. Latency period >10 years yielded for MCPA OR 2.81 (95% CI 1.27–6.22), for 2,4,5-T and/or 2,4,-D OR 1.72 (95% CI 0.98–3.19), and for glyphosate OR 2.26 (95% CI 1.16–4.40).

When different NHL entities were analysed separately, the OR for the subtype small lymphocytic lymphoma/chronic lymphocytic leukaemia (SLL/CLL) was increased for both phenoxy herbicides and, especially, glyphosate, Table III. The entity diffuse large B-cell lymphoma (DLBCL) was significantly associated with exposure to phenoxyacetic acids, but not to other herbicides. On the other hand, the group follicular lymphoma was not clearly associated with phenoxyacetic acids, and only nonsignificantly with glyphosate. The category "other specified B-cell lymphoma" (*e.g.*, mantle cell lymphoma, marginal zone lymphoma) was significantly associated with exposure to phenoxyacetic acids, and an increased risk was also indicated for glyphosate. T-cell lymphomas seemed to be associated with all types of herbicides, but no statistically significant ORs were found due to relatively few exposed subjects. The least numerous categories ("unspecified NHL") yielded high and statistically significant ORs for phenoxy herbicides and glyphosate.

TABLE II – EXPOSURE TO VARIOUS HERBICIDES

Agents	Cases/controls	OR	CI
Herbicides, total	74/51	1.72	1.18–2.51
≤20 days	36/27	1.58	0.95–2.65
>20 days	38/24	1.87	1.10–3.18
Phenoxyacetic acids	47/26	2.04	1.24–3.36
≤45 days	32/13	2.83	1.47–5.47
>45 days	15/13	1.27	0.59–2.70
MCPA	21/9	2.81	1.27–6.22
≤32 days	15/5	3.76	1.35–10.5
>32 days	6/4	1.66	0.46–5.96
2,4,5-T and/or 2,4-D	33/21	1.61	0.87–2.97
≤29 days	21/11	2.08	0.99–4.38
>29 days	12/10	1.33	0.57–3.13
Other	7/7	1.21	0.42–3.48
Herbicides except phenoxyacetic acids	38/26	1.82	1.08–3.06
≤24 days	20/13	1.91	0.93–3.89
>24 days	18/13	1.73	0.84–3.60
Glyphosate	29/18	2.02	1.10–3.71
≤10 days	12/9	1.69	0.70–4.07
>10 days	17/9	2.36	1.04–5.37
Other herbicides	18/18	1.22	0.63–2.39
≤32 days	12/9	1.64	0.68–3.96
>32 days	6/9	0.80	0.28–2.29

Number of exposed cases/controls, odds ratios (OR) and 95% confidence intervals (CI). Agents with more than 20 exposed subjects were also divided in two groups based on median number of days among exposed controls. Adjustment was made for age, sex and year of diagnosis or enrolment.

Insecticides

In our study no overall increased OR was demonstrated for exposure to insecticides, OR 1.28 (95% CI 0.96–1.72), Table IV. The most reported insecticide DDT yielded OR 1.46 (95% CI 0.94–2.28). Increased risk was shown for mercurial seed dressing, OR 2.03 (95% CI 0.97–4.28).

In the dose-response analysis, OR 1.47 (95% CI 0.99–2.16) was found for the high category of insecticide exposure, Table IV. Similar trends were found for DDT and mercurial seed dressing.

Different NHL entities were analysed separately, Table V. Hereby, certain exposures seemed to be associated with subtypes of NHL. Thus, the group follicular lymphoma was associated with DDT, OR 2.14 (95% CI 1.05–4.40) and mercurial seed dressing, OR 3.61 (95% CI 1.20–10.9). Furthermore, exposure to DDT increased the risk also for T-cell lymphoma, OR 2.88 (95% CI 1.05–7.95).

Fungicides and rodenticides

Exposure to fungicides was not a risk factor in our study, neither in total, OR 1.11 (95% CI 0.56–2.23), Table IV, nor for different subtypes of NHL, Table VI. Furthermore, there were no single substances among 24 reported that significantly differed between cases and controls. Also for rodenticides no increased risk was found, Table IV.

Impregnating agents

Exposure to impregnating agents yielded a statistically significant OR 1.57 (95% CI 1.07–2.30), Table IV. In a dose-response calculation OR increased further in the high exposure group. Creosote showed a statistically significant OR for high exposure, OR 3.33 (95% CI 1.20–9.27).

Table VI presents results for different NHL entities. An increased risk for SLL/CLL was associated with exposure to impregnating agents in total, and most pronounced for creosote,

TABLE III – EXPOSURE TO VARIOUS HERBICIDES DIVIDED ACCORDING TO DIFFERENT LYMPHOMA ENTITIES

Lymphoma entities	Herbicides, total	Phenoxyacetic acids (ph)	MCPA	2,4,5-T and/or 2,4-D	Herbicides except ph	Glyphosate	Other
B-cell lymphomas, total (n = 819)	1.68 1.14–2.48	1.99 1.20–3.32	2.59 1.14–5.91	1.69 0.94–3.01	1.72 1.003–2.94	1.87 0.998–3.51	1.14 0.57–2.31
Lymphocytic lymphoma/B-CLL (n = 195) (SLL/CLL)	2.27 1.28–4.01	2.11 0.995–4.47	2.57 0.74–8.97	1.93 0.85–4.41	2.56 1.17–5.60	3.35 1.42–7.89	1.39 0.45–4.31
Follicular, grade I–III (n = 165) (FL)	1.78 0.88–3.59	1.26 0.42–3.75	–[1]	1.21 0.35–4.22	2.32 0.96–5.60	1.89 0.62–5.79	1.48 0.42–5.23
Diffuse large B-cell lymphoma (n = 239) (DLBCL)	1.44 0.81–2.59	2.16 1.08–4.33	3.94 1.48–10.5	1.65 0.71–3.82	1.20 0.51–2.83	1.22 0.44–3.35	1.00 0.33–3.03
Other specified B-cell lymphoma (n = 131)	1.62 0.82–3.19	2.60 1.20–5.64	3.20 0.95–10.7	2.21 0.90–5.44	1.38 0.51–3.73	1.63 0.53–4.96	1.15 0.33–4.03
Unspecified B-cell lymphoma (n = 89)	1.09 0.41–2.89	1.14 0.33–3.95	1.35 0.16–11.2	0.88 0.20–3.92	1.52 0.44–5.27	1.47 0.33–6.61	0.71 0.09–5.53
T-cell lymphomas (n = 53)	1.64 0.55–4.90	1.62 0.36–7.25	2.40 0.29–20.0	1.02 0.13–7.95	1.57 0.35–6.99	2.29 0.51–10.4	2.24 0.49–10.3
Unspecified non-Hodgkin lymphoma (n = 38)	2.86 1.001–8.18	3.75 1.16–12.1	9.31 2.11–41.2	3.21 0.85–12.1	5.29 1.60–17.5	5.63 1.44–22.0	1.88 0.23–15.4

Odds ratios (OR) and 95% confidence intervals (CI). Adjustment was made for age, sex and year of diagnosis or enrolment.
[1]No exposed cases

OR 2.91 (95% CI 1.01–8.33). Regarding follicular lymphomas and DLBCL, increased risks were also noted after creosote exposure, and for the latter subtype this was also the case for all impregnating agents together. T-cell lymphomas were also associated with impregnating agents, and it seemed to be specifically chlorophenols. In the group of patients whose lymphomas were not possible to classify histopathologically, increased risks were indicated for all types of impregnating agents.

TABLE IV – EXPOSURE TO VARIOUS OTHER PESTICIDES

Agents	Cases/controls	OR	CI
Insecticides, total	112/101	1.28	0.96–1.72
≤40 days	44/51	1.03	0.68–1.57
>40 days	65/50	1.47	0.99–2.16
DDT	50/37	1.46	0.94–2.28
≤37 days	20/19	1.17	0.62–2.22
>37 days	30/18	1.76	0.97–3.20
Mercurial seed dressing	21/11	2.03	0.97–4.28
≤12 days	7/6	1.27	0.42–3.83
>12 days	14/5	2.93	1.04–8.25
Pyretrine	15/10	1.74	0.78–3.91
≤25 days	8/5	1.86	0.60–5.75
>25 days	6/5	1.36	0.41–4.51
Permetrine	9/9	1.23	0.48–3.14
Other insecticides	28/26	1.25	0.72–2.16
≤33 days	9/14	0.79	0.34–1.85
>33 days	18/12	1.67	0.79–3.51
Fungicides	16/18	1.11	0.56–2.23
≤37 days	9/9	1.29	0.51–3.31
>37 days	7/9	0.94	0.35–2.57
Impregnating agents	70/51	1.57	1.07–2.30
≤45 days	27/25	1.23	0.71–2.16
>45 days	43/24	2.04	1.21–3.42
Chlorophenols	40/36	1.24	0.77–1.98
≤33 days	23/18	1.46	0.78–2.74
>33 days	17/17	1.08	0.54–2.15
Arsenic	7/5	1.63	0.51–5.20
Creosote	19/10	2.10	0.96–4.58
≤39 days	4/5	0.87	0.23–3.29
>39 days	15/5	3.33	1.20–9.27
Tar	8/5	1.84	0.59–5.69
Other impregnating agents	27/20	1.55	0.85–2.81
≤7 days	4/10	0.44	0.14–1.42
>7 days	22/10	2.55	1.19–5.47
Rodenticides	5/4	1.67	0.44–6.29

Number of exposed cases/controls, odds ratios (OR) and 95% confidence intervals (CI). Agents with more than 20 exposed subjects were also divided in two groups based on median number of days among exposed controls. In some subjects, number of days was not known (excluded in dose-response calculations). Adjustment was made for age, sex and year of diagnosis or enrolment.

Multivariate analysis

Since mixed exposure to several pesticides was more a rule than an exception, and all single agents were analyzed without adjusting for other exposure, a multivariate analysis was made to elucidate the relative importance of different pesticides. Criteria for agents to be included in this analysis are defined in Statistical Methods above. As seen in Table VII increased ORs were found but in general lower than in the univariate analysis.

Discussion

This was a population based case–control study on NHL, which is a strength of the investigation. Only living cases and controls were included, which was of advantage in comparison with interviewing next-of-kins. The study covered all new cases of NHL during a specified time. Pathologists in Sweden that were experts in lymphoma diagnosis confirmed all diagnoses. Thus, a main advantage compared with the earlier studies was the possibility to study the different NHL entities, classified according to the recently developed WHO classification system. The histopathological subgroups may well be regarded as separate in etiology and pathogenesis, as well as they are known to be different regarding course, prognosis and best treatment.

The frequency matching on age groups, gender and health service regions increased the efficacy of the study and ensured exposure conditions for the controls representative for the population in the included geographical areas. We achieved a high response rate among cases and controls, which is another advantage. A motivating introduction letter that was sent out with the questionnaire and with reminders if needed may explain this.

Exposures were assessed by questionnaires with information supplemented over the phone. Thereby use of different pesticides could be checked by information in *e.g.*, receipts and bookkeeping. However, no registries exist in Sweden on such individual use, which is a weakness in the assessment of exposure. Exposure to pesticides may be difficult to assess, and some misclassification regarding quantity of exposure has probably occurred, but such misclassification would most probably be nondependent of case/control status, and therefore only weaken any true risks. Use of protective equipment was not asked for which might have been a disadvantage of the study. However, such use would dilute the exposure and thus bias the result towards unity.

We have earlier published the results from 2 Swedish case–control studies on lymphomas, the first one on NHL and HL[8,19] and later on NHL.[18] These studies showed an increased risk for lymphomas as a result of exposure to herbicides belonging to the class phenoxyacetic acids. In the first study we also found correlation with chlorophenols and organic solvents. Several other studies,

TABLE V – EXPOSURE TO VARIOUS INSECTICIDES DIVIDED ACCORDING TO DIFFERENT LYMPHOMA ENTITIES

Lymphoma entities	Insecticides, total	DDT	Mercurial seed dressing	Pyretrine	Other
B-cell lymphomas, total (n = 819)	1.19	1.32	1.81	1.68	1.08
	0.88–1.61	0.83–2.10	0.84–3.93	0.73–3.86	0.60–1.94
Lymphocytic lymphoma/B-CLL (n = 195) (SLL/CLL)	1.46	1.39	0.75	2.40	1.57
	0.91–2.35	0.69–2.83	0.16–3.47	0.73–7.89	0.66–3.75
Follicular, grade I–III (n = 165) (FL)	1.37	2.14	3.61	2.60	0.28
	0.79–2.38	1.05–4.40	1.20–10.9	0.79–8.51	0.04–2.11
Diffuse large B-cell lymphoma (n = 239) (DLBCL)	1.23	1.24	2.20	1.25	1.31
	0.78–1.93	0.61–2.49	0.79-6.12	0.34–4.61	0.58–2.97
Other specified B-cell lymphoma (n = 131)	1.32	1.33	2.39	1.49	1.42
	0.77–2.27	0.57–3.10	0.73–7.81	0.32–6.94	0.53–3.80
Unspecified B-cell lymphoma (n = 89)	0.42	0.23	–[1]	–[1]	0.42
	0.15–1.18	0.03–1.75			0.06–3.18
T-cell lymphomas (n = 53)	1.61	2.88	2.08	2.20	1.59
	0.72–3.60	1.05–7.95	0.25–17.1	0.27–17.8	0.36–7.02
Unspecified non-Hodgkin lymphoma (n = 38)	1.91	2.39	5.43	3.14	4.70
	0.79–4.62	0.77–7.42	1.34–22.0	0.37–26.3	1.48–14.9

Odds ratios (OR) and 95% confidence intervals (CI). Adjustment was made for age, sex and year of diagnosis or enrolment.
[1]No exposed cases.

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TABLE VI – EXPOSURE TO FUNGICIDES AND IMPREGNATING AGENTS DIVIDED ACCORDING TO DIFFERENT LYMPHOMA ENTITIES

Lymphoma entities	Fungicides	Impregnating agents, total	Chlorophenols	Creosote	Other
B-cell lymphomas, total (n = 819)	1.01	1.41	1.12	2.09	1.51
	0.48–2.09	0.95–2.11	0.69–1.84	0.94–4.64	0.82–2.78
Lymphocytic lymphoma/B-CLL (n = 195)	1.33	1.71	1.35	2.91	2.23
	0.43–4.12	0.94–3.11	0.64–2.85	1.01–8.33	0.97–5.13
Follicular, grade I–III (n = 165)	–[1]	1.49	0.91	2.56	1.80
		0.70–3.19	0.31–2.66	0.68–9.68	0.59–5.48
Diffuse large B-cell lymphoma (n = 239)	1.26	1.70	1.40	1.75	1.51
	0.45–3.47	0.97–2.96	0.70–2.78	0.54–5.74	0.62–3.67
Other specified B-cell lymphoma (n = 131)	1.56	1.24	0.95	2.58	1.09
	0.51–4.76	0.58–2.63	0.36–2.51	0.78–8.55	0.31–3.78
Unspecified B-cell lymphoma (n = 89)	–[1]	0.41	0.54	–[1]	0.54
		0.10–1.75	0.12–2.32		0.07–4.19
T-cell lymphomas (n = 53)	1.10	3.26	2.39	–[1]	2.07
	0.14–8.70	1.39–7.63	0.78–7.28		0.45–9.53
Unspecified non-Hodgkin lymphoma (n = 38)	3.73	2.52	2.02	4.94	1.40
	0.77–18.0	0.88–7.19	0.56–7.31	0.97–25.2	0.17–11.2

Odds ratios (OR) and 95% confidence intervals (CI). Adjustment was made for age, sex, and year of diagnosis or enrolment.
[1]No exposed cases.

TABLE VII – MULTIVARIATE ANALYSES INCLUDING AGENTS ACCORDING TO SPECIFIED CRITERIA, SEE TEXT

Agents	Univariate		Multivariate	
	OR	CI	OR	CI
MCPA	2.81	1.27–6.22	1.88	0.77–4.63
2,4,5-T and/or 2,4-D	1.61	0.87–2.97	1.24	0.68–2.26
Glyphosate	2.02	1.10–3.71	1.51	0.77–2.94
Mercurial seed dressing	2.03	0.97–4.28	1.58	0.74–3.40
Arsenic	1.63	0.51–5.20	1.17	0.34–4.02
Creosote	2.10	0.96–4.58	1.70	0.73–3.98
Tar	1.84	0.59–5.69	1.39	0.43–4.48

Odds ratios (OR) and 95% confidence intervals (CI). Adjustment was made for age, sex and year of diagnosis or enrolment.

but not all, from different research groups have supported our results, as reviewed,[20] and also confirmed later, *e.g.*, Ref. 21.

Furthermore, other groups have demonstrated associations between NHL and other classes of pesticides, especially different types of insecticides, *e.g.*, organophosphates,[22] carbamate,[23] lindane[24] and chlordane,[25] but also other groups of herbicides as atrazine.[26] Some case–control studies have found associations between several classes of pesticides, *e.g.*, Ref. 27 or merged groups of pesticides as in one recent study,[28] which demonstrate a significantly increased risk for NHL associated with exposure to "nonarsenic pesticides." These authors discuss the fact that several pesticides are chemically related and may exert their effects on humans through a similar mechanism of action, which may explain the wide range of pesticides that have been related to NHL over time in different countries and with different exposure conditions.

Several factors urged for a third Swedish study on the relation between pesticides, other chemicals and NHL, and the present study also used a somewhat changed methodology, which also may be of interest.

Thus, the use of phenoxyacetic herbicides, which earlier were dominating both as weed killers in agriculture and against hard wood in forestry, have substantially decreased during the last decades. 2,4,5-T, which was contaminated by TCDD, was prohibited in Sweden 1977, and 2,4-D was withdrawn from the market in 1990. MCPA, even if still used, has been largely substituted by other agents, among which glyphosate has been clearly dominating. This change of herbicide practice along with successively strengthened protection instructions has prompted our new study, reflecting also later years of exposure.

Furthermore, the changing trend of the incidence of NHL in many countries with reliable cancer registries, *e.g.*, Sweden, with a substantial and steady increase during the 1960's through 1980's but a leveling off or even slight decrease after that, makes it important to find etiological factors contributing to this shift in trend. Chlorinated compounds in the environment, which have been regulated during the 1970's and 1980's, may at least partly explain this trend, as discussed by us.[2] Phenoxyacetic herbicides with potential contaminating dioxins are examples of such substances. However, the prohibition of common environmental pollutants as polychlorinated biphenyls (PCB) and the following decline in the environment is probably more important to explain the leveling off of the incidence.[2]

In contrast to our 2 former case–control studies on NHL, this study included both genders and only consecutive living cases and living controls. In our earlier studies we have only studied male lymphoma cases, making the results of this study more representative for the whole population. To facilitate comparisons with our earlier results we also made additional analyses of herbicide exposure by gender. Only few women were exposed and separate analyses for both sexes still yielded an increased risk for NHL. Thus, in the total material herbicide exposure gave OR = 1.72, 95% CI 1.18–2.51 (n = 74 cases, 51 controls), whereas for men only OR = 1.71, 95% CI = 1.15–2.55 (n = 68 cases, 47 controls) and for women only OR = 1.82, 95% CI = 0.51–6.53 (n = 6 cases, 4 controls) were calculated.

In our study lymphocytic lymphoma/B-CLL was significantly associated with herbicides with highest OR for glyphosate but also creosote. Follicular lymphoma was significantly associated with DDT and mercurial seed dressing, diffuse large B-cell lymphoma with MCPA, and T-cell lymphoma with DDT and impregnating agents overall. Unspecified NHL was significantly associated with MCPA, glyphosate and mercurial seed dressing. It should be noted that several ORs were increased for herbicides; insecticides and impregnating agents but the calculations were hampered by low numbers of exposed cases and controls.

Our earlier results of exposure to phenoxyacetic herbicides as a risk factor for NHL were confirmed in our study. As in our previous lymphoma studies exposure to MCPA seemed to yield the highest OR among the different phenoxyacetic acids. This is of interest because MCPA is known not to be contaminated by dioxins, as 2,4-D and 2,4,5-T. At the same time MCPA is the only phenoxyacetic acid still in wider use in Sweden and many other countries.

Glyphosate is a broad-spectrum herbicide, which inhibits the formation of amino acids in plants.[29] The US Environmental Protection Agency[30] and the World Health Organization[31] have concluded that glyphosate is not mutagenic or carcinogenic. Since then, however, some experimental studies indicate genotoxic, hormonal and enzymatic effect in mammals, as reviewed.[32] Of particular interest is that glyphosate treatment of human lymphocytes *in vitro* resulted in increased sister chromatid exchanges,[33] chromosomal aberrations and oxidative stress.[34,35]

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Glyphosate was associated with a statistically significant increased OR for lymphoma in our study, and the result was strengthened by a tendency to dose-response effect as shown in Table II. In our former study[18] very few subjects were exposed to glyphosate, but a nonsignificant OR of 2.3 was found. Furthermore, a meta-analysis combining that study with an investigation on hairy-cell leukaemia, a rare NHL variant, showed an OR for glyphosate of 3.04 (95% CI 1.08–8.52).[36] Recent findings from other groups also associate glyphosate with different B-cell malignancies such as lymphomas and myeloma.[32,37,38]

Glyphosate has succeeded MCPA as one of the most used herbicides in agriculture, and many individuals that used MCPA earlier are now also exposed to glyphosate. This probably explains why the multivariate analysis does not show any significant ORs for these compounds.

Exposure to insecticides was associated with a slightly increased OR, Table IV. In some other studies on the relation between pesticides and NHL, insecticides seem to be of some importance as causative agents.[27,37,38] Especially, different organophosphates were indicated as risk factors in those studies, with a Canadian study[37] showing statistical significant ORs for malathion and diazinon. In our study, only few subjects were exposed to different organophosphates, but we found a nonsignificant OR of 2.81 (95% CI 0.54–14.7) for malathion based on 5 exposed cases and 2 controls, not shown in Table.

The organochlorine DDT has shown suggestive but rarely significant association with NHL in some studies.[8,19,38–40] Our study showed a moderately but not significant increased OR for exposure to DDT.

Fungicides were not associated with the risk for NHL in our study, but few subjects were exposed to a wide range of different agents. In some earlier studies increased risks have also been noted for this group of pesticides.[16,18]

Exposure to impregnating agents produced a significant OR with a dose-response relation, Table IV. The highest risk was found for high exposure to creosote, which gave a significant OR. This finding was in contrast to our previous results on NHL,[18] but another Swedish study also found an association between creosote and NHL.[41] Chlorophenols have been the most common group of impregnating agents in Sweden, but were banned in 1977. In our first NHL study, reflecting exposures mainly during the time these substances were used, we found a strong association with NHL. As in the present study, however, no association was found in our second study on NHL.[18]

In conclusion, this study, which mirrors pesticide exposure during later years than in our previous studies, confirmed results of an association between exposure to phenoxyacetic herbicides and NHL. Furthermore, our earlier indication of an association between glyphosate and NHL has been considerably strengthened.

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Nabhan
EXHIBIT NO. 19
8/23/17
P. CAMPBELL

Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study

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ABSTRACT

Objectives: Investigating the relationship between occupational exposure to pesticides and the risk of lymphoid neoplasms (LNs) in men.

Methods: A hospital-based case-control study was conducted in six centres in France between 2000 and 2004. The cases were incident cases with a diagnosis of LN aged 18–75 years. During the same period, controls of the same age and sex as the cases were recruited in the same hospital, mainly in the orthopaedic and rheumatological departments. Exposures to pesticides were evaluated through specific interviews and case-by-case expert reviews. Four hundred and ninety-one cases (244 cases of non-Hodgkin's lymphoma (NHL), 87 of Hodgkin's lymphoma (HL), 104 of lymphoproliferative syndromes (LPSs) and 56 of multiple myeloma (MM) cases) and 456 controls were included in the analyses. The odds ratios (ORs) and 95% CI were estimated using unconditional logistic regressions.

Results: Positive associations between HL and occupational exposure to triazole fungicides and urea herbicides were observed (OR = 8.4 (2.2 to 32.4), 10.8 (2.4 to 48.1), respectively). Exposure to insecticides, fungicides and herbicides were linked to a threefold increase in MM risk (OR = 2.8 (1.2 to 6.5), 3.2 (1.4 to 7.2), 2.9 (1.3 to 6.5)). For LPS subtypes, associations restricted to hairy-cell leukaemia (HCL) were evidenced for exposure to organochlorine insecticides, phenoxy herbicides and triazine herbicides (OR = 4.9 (1.1 to 21.2), 4.1 (1.1 to 15.5), 5.1 (1.4 to 19.3)), although based on small numbers. Lastly, despite the increased ORs for organochlorine and organophosphate insecticides, carbamate fungicides and triazine herbicides, no significant associations were evidenced for NHL.

Conclusions: The results, based on case-by-case expert review of occupation-specific questionnaires, support the hypothesis that occupational pesticide exposures may be involved in HL, MM and HCL and do not rule out a role in NHL. The analyses identified specific pesticides that deserve further investigation and the findings were consistent with those of previous studies.

Lymphoid neoplasms (LNs) are the most frequent cancers in France after smoking-related cancers, with around 17 000 new cases diagnosed each year.[1] The incidence of non-Hodgkin's lymphoma (NHL) increased in France over the 1980–2005 period, at an annual rate of 3% on average (2.7% in men), but the rate has levelled off over the last 5 years.[1] The increase probably cannot be entirely explained by changes in registration. One hypothesis is that pesticide use may explain the increase. Although the prevalence of farming has decreased, the use and variety of pesticides increased until 2000, particularly among farmers.[2] Numerous studies have investigated the association between farming and the main types of LN. Meta-analyses have shown that farming was positively, but weakly, associated with NHL,[3] Hodgkin's lymphoma (HL)[4] and multiple myeloma (MM),[5] and that the associations were more marked in the USA. Regarding occupational exposure to pesticides, case-control studies conducted in the USA,[6–11] Canada,[12 13] Australia[14] and Europe[15–22] have shown associations between LN, especially NHL and MM, and various pesticide classes or chemical sub-families. Lymphoproliferative syndrome (LPS) has been less documented, but a French[23] and a Swedish case-control study[24] have shown positive relationships with hairy-cell leukaemia (HCL), a rare LPS subtype. The reports on the prospective Agricultural Health Study also evidenced increased NHL risks with the highest exposure to the herbicide, atrazine[25] and organochlorine insecticide, lindane,[26] and increased MM risks with the highest exposure to the herbicides, alachlor[27] and glyphosate.[28] The roles of farming, crop growing and pesticide exposure in the main categories of LN (HL, NHL, LPS and MM), were investigated in a multicentre case-control study. In the present study, the role of occupational exposures to pesticides was evaluated through specific interviews and case-by-case expert reviews.

MATERIAL AND METHODS

Subjects

A hospital-based case-control study was carried out in the main hospitals of the French cities of Brest, Caen, Nantes, Lille, Toulouse and Bordeaux between September 2000 and December 2004. Pursuant to the French regulations at the time the study was conducted, the hospital-based design of the study was chosen to address the need for case and control blood samples. Eligible cases were subjects of either sex, aged 20–75 years, residing in the hospital's catchment area and recently diagnosed with any lymphoid neoplasm except acute lymphoid leukaemia. The diagnoses were classified using the WHO ICD-O-3 codes. All the diagnoses were cytologically or histologically confirmed and reviewed by a panel of pathologists and haematologists. Patients with a history of immunosuppression or taking

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immunosuppressant drugs were not eligible. The present analysis only includes men, whose occupational pesticide use was nearly three times more prevalent than that for women. Of the 513 male eligible cases during the recruitment period, 22 (4.3%) refused to be interviewed. Thus, the study sample comprised 491 incident cases of LN, classified using the ICD-O-3 codes (table 1), and further divided into four broad categories: HL (n = 87), NHL (n = 244), MM (n = 56) and LPS (n = 104).

Except for the LPS cases, most of the cases (88.1%) were recruited within 3 months of diagnosis (median: 34 days). Inclusion of LPS cases was allowed up to 18 months post-diagnosis due to their excellent survival and the usual uncertainty with respect to the actual date of disease onset

The controls were patients with no prior history of LN, recruited in the same hospitals as the cases, mainly in orthopaedic and rheumatological departments and residing in the hospital's catchment area (i.e., in the hospital *département* or in the immediately neighbouring *départements*). In order to avoid overestimation of factors of interest, patients admitted for cancer or a disease directly related to occupation, smoking or alcohol abuse were not eligible as controls, but a history of such diseases did not prevent control selection. The controls were individually matched with the cases by centre, age (±3 years) and sex. The aim of the matching was to ensure that at least one control would be available for each case. Out of the 501 eligible male controls ascertained during the recruitment period, 44 (8.8%) refused to participate. A further control was excluded since his interview was incomplete. Thus, 456 men were included as controls in the analysis. The reasons for hospitalisation were most often orthopaedic or rheumatological (fractures (21.3%), wounds (1.3%), other non-occupational injuries (12.5%), osteoarthritis (23.0%), back diseases (15.6%), polyarticular diseases (2.9%), infectious diseases of the bones and joints (2.6%), minor musculoskeletal malformations (2.0%), other diseases of the bones and joints (6.8%), peripheral nervous disorders (1.3%)), gastrointestinal or genitourinary tract diseases (4.8%), cardiovascular diseases (1.1%), skin and subcutaneous tissue diseases (1.8%) and infections (3.0%).

Data collection

Both the patients and interviewers were blind to the study hypotheses. Data collection was conducted in two stages. The case and control patients first completed a standardised self-administered questionnaire on their socioeconomic characteristics, familial medical history, and lifelong residential and occupational histories. For each job held for at least 6 months, the subjects were asked to report the job title, company name and business (if appropriate), the start and end dates of the job, and a description of the specific tasks and products personally handled (open-ended question).

The patients then underwent a face-to-face interview (average duration: 80 minutes) by trained staff using a structured standardised questionnaire eliciting personal and familial medical histories, lifestyle characteristics (smoking and alcohol, tea and coffee consumption) and outdoor leisure activities. Non-occupational exposure to pesticides was sought through questions about gardening (use of insecticides, fungicides and herbicides, pesticide targets and periods of use) and use of insecticides in the home (with questions on insect target and period of use). At the end of the interview, the self-administered questionnaire was reviewed with the interviewer. Finally, a specific agricultural occupational questionnaire was systematically administered to each patient who had worked as a farmer or gardener for at least 6 months during any period of his life. This questionnaire was designed to allow standardised case-by-case pesticide exposure assessment by experts. First, all the farms where the patient had worked for at least 6 months were listed with location, period of occupation and area, and with the farmer's status (owner, worker, helper) at that time. A farm was considered to become a different farm if its size changed. Second, for each farm, the crops and animal husbandry were listed with their mean sizes. Then, all the pesticides used

Table 1 Distribution of cases by ICD-O-3 classification

Diagnosis	ICD-O-3 codes	No
Hodgkin's lymphoma	**9650–9667/3**	**87**
non-Hodgkin's lymphoma		**244**
B-cell lymphoma		204
Diffuse large B-cell lymphoma	9679–9680/3	107
Follicular lymphoma	9690/3	50
Waldenstrom macroglobulinaemia	9671/3	16
Marginal zone B-cell lymphoma (MALT type)	9699/3	9
Splenic marginal zone B-cell lymphoma	9689/3	1
Mantle-cell lymphoma	9673/3	21
T-cell lymphoma		21
Mature T-cell lymphoma, NOS	9702/3	3
Angioimmunoblastic T-cell lymphoma	9705/3	2
Cutaneous T-cell lymphoma, NOS	9709/3	1
Anaplastic large cell, T-cell and Null cell type lymphoma	9714/3	8
Intestinal T-cell lymphoma	9717/3	1
Precursor T-cell lymphoblastic lymphoma	9729/3	6
Other lymphoma		19
non-Hodgkin's lymphoma, NOS	9591/3	12
Burkitt-like lymphoma	9687/3	7
Multiple myeloma	**9732/3**	**56**
Lymphoproliferative syndrome		**104**
Chronic lymphocytic leukaemia	9823/3, 9670/3	77
Hairy-cell leukaemia	9940/3	27

Values in bold refer to the number of the main groups of lymphoid neoplasm under study. MALT, mucosa-associated lymphatic tissue; NOS, not otherwise specified.

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on each crop during a given period were reported. The subjects were asked whether they had personally prepared the pesticide mixture and whether they had personally sprayed it. They were also asked to state the chemical used and, if possible, its brand name, main use, type of spraying equipment used, and the annual number and duration of applications. The questionnaire also elicited the use of pesticides in farm buildings for animals, grain, hay or straw, or to clear lanes and yards. The interviewers underwent a short specific training course on farming given by occupational hygienists, and were asked to systematically request consent to possible repeat interviews.

Blood samples were obtained from the cases and controls after consent form signature and the biological specimens (sera, constitutional DNA, tumour tissue) were placed on storage. The study protocol complied with the French regulations relating to databases and ethics and the pertinent approvals (CNIL No. 90003 and DGS No. 2000/0107, respectively) were obtained.

Case-by-case pesticide exposure assessment

Two persons, one occupational hygienist (LD) trained on retrospective evaluation of farming exposures for epidemiology and an agronomist specialised in the technical aspects of pesticide handling (PD), individually reviewed each self-administered questionnaire and specific questionnaire. Most of the 168 subjects who were administered the specific agricultural occupational questionnaire had to be re-interviewed by telephone because the information was insufficient. Repeat interviews of 95 subjects (56.8%) were conducted, but not of 35 others (20.8%), who refused (n = 15), had died, were in poor health (n = 10), or could not be contacted (n = 15). The whole process was blind to case-control status and the proportion of patients re-interviewed was the same for the cases and controls. The experts reviewed the consistency of the subjects' statements with respect to product availability dates, type and size of the crops, geographic location of the farm and frequency of treatment, and coded the chemical using a three-digit ad hoc code (first digit: pesticide category: insecticides, fungicide, herbicide; second digit: chemical family (e.g., organochlorine insecticide, carbamate fungicide, etc); third digit: chemical subfamily (e.g., DDT, Lindane, etc). A database was constructed using the annual directories of phytochemicals published by the Association de Coordination Technique Agricole and used to facilitate the process. The directories include the recommendations for use of the products, which are identified by their chemical and brand names, by crop and pest.

When information on pesticides was missing or unreliable, the experts were asked to allocate a list of chemicals that may have been used, based on the crops treated, method of spraying, period and frequency of treatment and pests targeted. They also provided the likelihood of each suggested exposure.

Variables analysed

Jobs were coded using the 1968 edition of the International Labour Office (ILO) classification. Socioeconomic categories were generated from the last job held and encoded at the two-digit level. For all exposure variables, the subjects never exposed to the specific crop, animal or pesticide were taken as the reference category. Dichotomous variables were generated for exposure to crops, animal husbandry, each pesticide category (insecticide, fungicide, herbicide) and chemical family. Two-exposure definitions were used. The wider definition, *possible or definite exposure*, included any declared exposures and those assessed by the experts for missing values. The narrower definition, *definite exposure*, was restricted to the exposures that were considered certain by the experts, and those that had been assigned to missing values with a probability of at least 70%. The duration of exposure to each crop, animal, pesticide category or chemical family was obtained by summing all the periods in which the specific crop, animal, pesticide or chemical family was present. The resulting variables were classified with respect to the median durations of exposure among the exposed controls as: never exposed; duration <median; duration ≥median. The intensities of exposure as a function of the type of spraying equipment and annual number of passages could not be quantified because there were too many missing values.

Statistical analysis

All the analyses were performed using SAS software V.9.1. The initial pairmatching used as a basis for the recruitment was broken to allow the use of the whole control group for the analysis of all LN types, with stratification by age (5-year age groups) and centre. The controls that belonged to strata without a case of the subgroup LPS, HL, NHL or MM under study were excluded from the corresponding unconditional analyses. Odds ratios (ORs) and their 95% CI were estimated using unconditional logistic regression models including the stratification variables, age and centre, as categorical variables. Tests for trend of duration were conducted by fitting models using a quantitative variable equal to the median value of the exposure classes (0, duration <overall median, duration ≥overall median). Analyses were conducted separately for the LN subgroups (HL, NHL, LPS and MM) and for all LNs taken together. Additional analyses by NHL subtype (diffuse large B-cell lymphoma (DLCL), follicular lymphoma (FL) or other NHL) and LPS subtype (chronic lymphocytic leukaemia (CLL) or hairy-cell leukaemia (HCL)) were conducted by polytomous logistic regression with a nominal non-ordered response variable in which the comparator group was the specific LN subgroup's control set.

In order to check the robustness of the results, conditional logistic regressions restricted to the pair-matched case-control samples were also conducted and sensitivity analyses were performed by excluding the subjects in each centre and the controls sharing the same broad reason-for-admission category, in turn, from the analyses.

In an attempt to disentangle multiple pesticide exposures, all the combinations of pesticide families associated with the LN subtype considered and with a p value of at least 10%, were included, two by two, in the logistic models.

All p values were two sided and considered significant at the 0.05 level.

Study power

For NHL, with a power of 80% and a two-sided alpha error of 5%, the size of the study sample was sufficient to evidence OR ranging from 1.7 to 3.3 for exposures with prevalences ranging from 2 to 20%. For the other types of LN (HL, LPS, MM), OR between 2.0 and 6.0 could be evidenced for the same exposure prevalences.

RESULTS

Distribution of cases and controls by stratification and socioeconomic variable

The use of the whole control group assigned more than two controls per case in most strata, except for the youngest

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categories, in which HL predominated. This led to significant age difference between the cases and controls. Significant differences between the centres were also observed, mainly because Caen hospital recruited a higher proportion of LPS than the other centres. With regard to socioeconomic characteristics, the cases and controls were well balanced with respect to socioeconomic category, urban or rural residential status and educational level, except for the HL cases, who were less often factory workers than the controls (supplementary table A).

Farming practices

Agricultural questionnaires were administered to the 168 subjects who reported having worked on a farm (97 cases, 71 controls) and 257 different farms were thus described. Mixed farming predominated since only 16 farms (6.2%) were specialised in a specific crop with no animal husbandry and only three (1.2%) in specific animal husbandry with no crop growing. Cereal growing and cattle farming were the main prevalent practices, despite the fact that their weight in farming practices decreased over time. Most of the farms were of medium size, and the overall size had increased with time over the three last decades in all regions. The largest farms were located in the south-west of France while large cattle farms were located in the west and north. As expected, vineyards were more prevalent in the south-west (supplementary table B).

Farming, crop growing and animal husbandry

The associations between farming (crops and animal husbandry) and the main categories of LN are shown in table 2. Employment for at least 6 months in an agriculture-related job was significantly associated with all LNs except LPS. The associations were more marked for farm owners and for agricultural workers employed for more than 20 years.

Cereal growing and corn growing were significantly and positively associated with both NHL and HL and marginally with MM. Beet and colza growing were also associated with NHL. Positive associations between vines and MM (OR (95% CI) 4.6 (1.4 to 14.9)) and between forage and HL (3.0 (1.1 to 8.5)) were observed. No crop was related to LPS considered overall, but subtype analyses revealed estimates near 1.0 for CLL. The associations for HCL were greater and significant for cereal, corn and vine growing (3.5 (1.1 to 11.3), 7.6 (2.1 to 28.1) and 8.5 (1.6 to 44.6), respectively). No difference between the DLCL and FL NHL subtypes was observed, irrespective of crop. With regard to animal husbandry, pig breeding was related to HL (3.8 (1.3 to 11.1)) and sheep breeding to the FL (5.6 (1.7 to 18.6)) subtype. No other significant association was observed.

Occupational exposure to pesticides

Table 3 shows the associations between pesticides and the main LN subgroups. Overall, the ORs associated with exposure to pesticides were 1.5, 2.1 and 3.5 for NHL, HL and MM, respectively. The association was significant for MM. Only two cases and no controls were exposed to insecticides only; nine cases and two controls were exposed to fungicides only; three cases and three controls were exposed to herbicides only. Most of the subjects (40 cases and 26 controls) were exposed to all three pesticide categories. Overall, significant associations between MM and the use of insecticides, fungicides and herbicides and between HL and the use of fungicides and insecticides (borderline significance) were observed. Detailed analyses showed significant associations between MM and fungicides (benzene, amide, and morpholine derivatives) and herbicides (picoline and urea derivatives) but not with particular insecticides. For HL, positive associations with all organic insecticides were evidenced, although the association was only marginally significant for organophosphates. HL was also significantly associated with carbamate and triazole fungicides and with the herbicide groups: carbamates, phenoxy and picoline derivatives, amides and urea derivatives. In an attempt to disentangle multiple pesticide exposures, all the combinations of pesticide families associated with the LN subtype considered with a p value of at least 10% were included, two by

Table 2 Association between farming, exposure to crops and animal husbandry and lymphoid neoplasm (LN)

	NHL (244 Ca/436 Co)		HL (87 Ca/265 Co)		LPS (104 Ca/305 Co)		MM (56 Ca/313 Co)		All LN (491 Ca/456 Co)	
	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)
Job title										
Farmers, agricultural or forestry workers (ILO 6)	59/92	1.5 (1.0 to 2.3)	15/56	1.5 (0.7 to 3.2)	33/77	1.4 (0.8 to 2.4)	19/71	2.2 (1.1 to 4.6)	126/94	1.6 (1.1 to 2.2)
Farm owners (ILO 6.1)	19/25	1.9 (1.0 to 3.7)	7/13	5.3 (1.6 to 17.2)	9/23	1.3 (0.5 to 3.1)	10/23	4.1 (1.6 to 10.5)	45/25	2.3 (1.3 to 3.9)
Agricultural workers (ILO 6.2)	51/73	1.7 (1.1 to 2.7)	11/41	1.5 (0.6 to 3.3)	30/62	1.5 (0.8 to 2.7)	14/55	1.9 (0.8 to 4.2)	106/75	1.7 (1.1 to 2.4)
Crops										
Cereals	33/42	1.9 (1.1 to 3.2)	9/18	4.0 (1.5 to 10.7)	22/41	1.5 (0.8 to 2.9)	11/35	2.2 (1.0 to 5.2)	75/43	2.0 (1.3 to 3.1)
Corn	21/23	1.9 (1.0 to 3.7)	6/11	3.2 (1.0 to 10.3)	10/18	1.8 (0.8 to 4.4)	7/19	2.7 (1.0 to 7.2)	44/23	2.0 (1.2 to 3.5)
Beet	24/26	2.3 (1.2 to 4.3)	3/9	2.2 (0.5 to 10.0)	17/24	1.9 (0.9 to 4.0)	5/21	1.4 (0.4 to 4.3)	49/26	2.1 (1.2 to 3.5)
Grape vines	12/13	1.8 (0.8 to 4.2)	1/8	0.8 (0.1 to 7.3)	6/11	2.3 (0.7 to 7.2)	6/11	4.6 (1.4 to 14.9)	25/13	2.0 (1.0 to 4.2)
Potatoes	19/27	1.5 (0.8 to 3.0)	4/8	3.6 (0.9 to 14.3)	13/25	1.3 (0.6 to 2.9)	6/22	1.5 (0.5 to 4.4)	42/27	1.6 (0.9 to 2.7)
Vegetables	14/26	1.0 (0.5 to 2.1)	3/13	0.9 (0.2 to 3.8)	12/21	1.9 (0.8 to 4.2)	5/19	1.6 (0.5 to 4.9)	34/26	1.3 (0.8 to 2.3)
Forage	25/37	1.5 (0.8 to 2.7)	7/17	3.0 (1.1 to 8.5)	15/38	0.9 (0.4 to 1.8)	9/34	1.6 (0.7 to 3.9)	56/38	1.6 (1.0 to 2.5)
Animal husbandry										
Cattle	37/54	1.5 (0.9 to 2.5)	7/24	1.6 (0.6 to 4.4)	24/49	1.4 (0.7 to 2.6)	10/46	1.3 (0.6 to 3.1)	78/55	1.5 (1.0 to 2.3)
Sheep	10/17	1.3 (0.6 to 3.1)	2/6	1.6 (0.3 to 9.4)	4/16	0.5 (0.2 to 1.7)	1/15	0.3 (0.0 to 2.8)	17/17	1.0 (0.5 to 2.0)
Pigs	19/34	1.1 (0.6 to 2.1)	7/13	3.8 (1.3 to 11.1)	17/31	1.6 (0.8 to 3.2)	5/28	1.0 (0.3 to 2.9)	48/34	1.4 (0.9 to 2.4)
Horses	21/33	1.4 (0.7 to 2.6)	3/14	1.3 (0.3 to 5.3)	19/32	1.8 (0.9 to 3.6)	3/28	0.5 (0.1 to 1.8)	46/34	1.4 (0.9 to 2.3)
Rabbits	11/27	0.9 (0.4 to 1.9)	4/11	2.4 (0.6 to 8.6)	16/26	1.6 (0.8 to 3.4)	5/24	1.3 (0.4 to 3.8)	36/27	1.4 (0.8 to 2.4)
Poultry	23/39	1.3 (0.7 to 2.3)	6/15	2.2 (0.7 to 6.6)	20/35	1.6 (0.8 to 3.1)	6/32	1.0 (0.4 to 2.8)	55/39	1.5 (0.9 to 2.3)

ORs (95% CI) were estimated by unconditional logistic regression including the stratification variables, age, centre and socioeconomic category (white collar/blue collar).
Ca, number of cases; Co, number of controls; NHL, non-Hodgkin's lymphoma; HL, Hodgkin's lymphoma; ILO, International Labour Office classification; LPS, lymphoproliferative syndrome; MM, multiple myeloma; OR, odds ratio.

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two, in the logistic models. Some exposures were highly correlated and could not be separated. HL was significantly associated with triazole fungicides and urea herbicides. However, the exposures could not be distinguished since eight of the nine subjects who had used urea herbicides had also used triazole fungicides, both pesticides being applied to cereals, corn and sunflowers. Similarly, all the subjects who had used carbamate insecticides had also used pyrethrin insecticides. No significant association was evidenced for NHL or LPS. The analyses by NHL and LPS subtypes showed similar estimates for the DLCL and FL subtypes (table 4). In contrast, the associations with LPS subtypes differed, with lower OR for CLL and higher OR for HCL, for most exposures. Although the numbers were very small, HCL was significantly associated with organochlorine insecticides, phenoxyacetic herbicides and triazine herbicides (4.9 (1.1 to 21.2), 4.1 (1.1 to 15.5), 5.1 (1.4 to 19.3), respectively).

Non-occupational exposure to pesticides

The use of pesticides taken together and the uses of insecticides, fungicides or herbicides for gardening were not associated with LN or any LN subtype (table 3). The domestic use of insecticides was more frequent among HL cases than among controls.

Stability of the results

Similar patterns were observed when different lag times were considered, that is, when the 10, 20, 30 or 40 years prior to diagnosis or interview were considered unexposed. However, for HL, it was impossible to investigate long latency periods (supplementary table C). The patterns were also similar when the exposure was restricted to time windows of 0–10, 10–20, 20–30, 30–40 years before diagnosis or interview.

With a view to limiting exposure to definite exposure, the subjects possibly exposed were pooled with the unexposed subjects. The analyses based on that definition gave higher estimates for all the significant associations evidenced for HL, while for MM the OR became lower and non-significant. Lastly, the results were the same when the category "never used any pesticides" was taken as reference. It is noteworthy that the relationship with agricultural jobs was restricted to pesticides users (1.7 (1.1 to 2.7) and 1.3 (0.8 to 2.1) for users and non-users, respectively).

The results remained stable after adjustment for the rural/urban status of the place of residence, type of housing (flat/house), educational level and factors related to LN in previous analyses (history of mononucleosis, history of influenza immunization, familial history of cancer, skin characteristics, smoking status, and alcohol drinking status). The results were also unchanged when conditional analyses were performed, or when the missing values were all considered "never used" or "ever used". Lastly, the estimates did not change when each centre and each group of controls sharing a similar reason for admission were excluded, in turn, from the analysis.

DISCUSSION

In the present study, MM was significantly associated with insecticides, fungicides and herbicides and HL, with all organic insecticides, carbamate and triazole fungicides and urea derivatives. Significant associations between HCL and organochlorine insecticides, and phenoxyacetic and triazine herbicides were observed. Despite increased OR with organochlorine and organophosphate insecticides, carbamate fungicides and triazine herbicides, no significant associations were evidenced for NHL. A clear relationship between HL and domestic use of

Table 3 Association between pesticides exposure and lymphoid neoplasm (LN)

	NHL (244 Ca/436 Co)		HL (87 Ca/265 Co)		LPS (104 Ca/305 Co)		MM (56 Ca/313 Co)		All LN (491 Ca/456 Co)	
	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)
Occupational pesticide use	32/47	1.5 (0.9 to 2.5)	9/24	2.1 (0.8 to 5.2)	15/39	1.2 (0.6 to 2.4)	15/37	3.5 (1.6 to 7.7)	71/47	1.7 (1.1 to 2.5)
Insecticides	26/37	1.5 (0.8 to 2.6)	8/19	2.3 (0.9 to 6.0)	11/30	1.1 (0.5 to 2.5)	11/29	2.8 (1.2 to 6.5)	56/37	1.6 (1.0 to 2.5)
Organochlorine	15/17	1.8 (0.9 to 3.8)	4/6	4.7 (1.1 to 20.8)	8/15	1.7 (0.7 to 4.4)	4/16	1.4 (0.4 to 4.8)	31/17	1.9 (1.0 to 3.5)
Organophosphate	20/24	1.7 (0.9 to 3.3)	6/12	3.0 (1.0 to 9.4)	5/20	0.8 (0.3 to 2.2)	6/20	2.2 (0.8 to 6.2)	37/24	1.6 (0.9 to 2.8)
Pyrethrin	10/17	1.3 (0.5 to 2.9)	7/11	3.6 (1.2 to 11.2)	1/14	0.2 (0.0 to 1.8)	5/14	3.1 (1.0 to 10.0)	23/17	1.4 (0.7 to 2.7)
Fungicides	26/35	1.6 (0.9 to 2.8)	9/17	4.5 (1.6 to 12.2)	11/34	1.0 (0.5 to 2.2)	13/32	3.2 (1.4 to 7.2)	59/35	1.8 (1.2 to 2.9)
Carbamates	15/17	1.8 (0.9 to 3.7)	5/9	5.1 (1.4 to 18.4)	4/16	0.8 (0.3 to 2.7)	6/15	2.9 (1.0 to 8.6)	30/17	1.9 (1.0 to 3.5)
Imide	6/10	1.1 (0.4 to 3.2)	3/4	5.2 (1.0 to 27.8)	2/10	0.6 (0.1 to 2.9)	5/10	3.3 (1.0 to 11.0)	16/10	1.6 (0.7 to 3.6)
Triazole	8/9	1.9 (0.7 to 5.3)	6/6	8.4 (2.2 to 32.4)	1/9	0.4 (0.0 to 3.1)	3/8	3.4 (0.8 to 14.6)	18/9	2.2 (0.9 to 4.9)
Herbicides	25/42	1.3 (0.7 to 2.2)	7/22	1.5 (0.6 to 4.1)	9/34	0.7 (0.3 to 1.7)	12/32	2.9 (1.3 to 6.5)	53/42	1.3 (0.8 to 2.0)
Phenoline	13/17	1.7 (0.8 to 3.7)	4/8	4.3 (1.1 to 17.2)	5/15	0.8 (0.3 to 2.4)	5/16	2.0 (0.6 to 6.1)	27/17	1.7 (0.9 to 3.2)
Phenoxy	11/25	0.9 (0.4 to 1.9)	6/14	2.5 (0.8 to 7.7)	7/20	1.0 (0.4 to 2.5)	7/20	2.6 (0.9 to 7.0)	31/25	1.3 (0.7 to 2.2)
Picoline	5/10	1.0 (0.3 to 3.2)	5/4	9.4 (2.0 to 43.1)	0/8	.	4/8	3.9 (1.0 to 14.7)	14/10	1.5 (0.6 to 3.4)
Triazine	17/20	1.9 (0.9 to 3.8)	5/10	3.2 (0.9 to 10.9)	8/17	1.6 (0.6 to 4.0)	4/17	1.7 (0.5 to 5.9)	34/20	1.8 (1.0 to 3.3)
Amide	5/12	0.9 (0.3 to 2.8)	6/8	3.8 (1.1 to 12.7)	2/8	0.6 (0.1 to 3.2)	1/8	0.8 (0.1 to 7.0)	14/12	1.2 (0.5 to 2.7)
Urea	5/7	1.8 (0.5 to 6.0)	5/4	10.8 (2.4 to 48.1)	4/7	1.7 (0.4 to 6.4)	5/6	7.2 (1.8 to 28.4)	19/7	2.9 (1.2 to 7.0)
Quaternary ammonium	4/12	0.7 (0.2 to 2.3)	2/7	1.3 (0.2 to 7.3)	5/11	1.5 (0.5 to 4.9)	2/9	1.6 (0.3 to 8.2)	13/12	1.1 (0.5 to 2.5)
Glyphosate	12/24	1.0 (0.5 to 2.2)	6/15	1.7 (0.6 to 5.0)	4/18	0.6 (0.2 to 2.1)	5/18	2.4 (0.8 to 7.3)	27/24	1.2 (0.6 to 2.1)
Garden pesticide use	123/194	1.4 (1.0 to 2.0)	23/103	0.9 (0.5 to 1.6)	61/164	0.9 (0.6 to 1.6)	26/146	0.9 (0.5 to 1.7)	233/201	1.1 (0.9 to 1.5)
Insecticides	81/133	1.2 (0.8 to 1.7)	11/68	0.6 (0.3 to 1.3)	39/112	0.9 (0.5 to 1.5)	13/97	0.6 (0.3 to 1.2)	144/138	0.9 (0.7 to 1.2)
Fungicides	38/60	1.2 (0.7 to 1.9)	4/31	0.6 (0.2 to 1.7)	23/49	1.7 (0.9 to 3.0)	9/50	1.1 (0.5 to 2.4)	74/61	1.2 (0.8 to 1.7)
Herbicides	86/155	1.0 (0.7 to 1.5)	19/84	0.8 (0.4 to 1.6)	49/130	1.0 (0.6 to 1.6)	22/115	1.0 (0.6 to 2.0)	176/161	1.0 (0.7 to 1.3)
Domestic insecticide use	74/142	1.1 (0.7 to 1.5)	38/74	2.9 (1.6 to 5.4)	46/114	0.9 (0.5 to 1.6)	16/108	0.6 (0.3 to 1.3)	174/150	1.2 (0.9 to 1.6)

ORs (95% CI) were estimated by unconditional logistic regression including the stratification variables, age, centre and socioeconomic category (white collar/blue collar).
Ca, number of cases; Co, number of controls; NHL, non-Hodgkin's lymphoma; HL, Hodgkin's lymphoma; LPS, lymphoproliferative syndrome; MM, multiple myeloma; OR, odds ratio.

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Table 4 Occupational pesticide use by non-Hodgkin's lymphoma (NHL) and lymphoproliferative syndrome (LPS) subtype

	NHL				LPS			
	Diffuse large cell lymphoma (n = 107)		Follicular lymphoma (n = 50)		Chronic lymphocytic leukaemia (n = 77)		Hairy-cell leukaemia (n = 27)	
	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)
Pesticides	16/47	1.7 (0.9 to 3.4)	6/47	1.3 (0.5 to 3.5)	10/39	0.9 (0.4 to 2.0)	5/39	3.0 (0.9 to 10.2)
Insecticides	13/37	1.8 (0.9 to 3.7)	6/37	1.7 (0.6 to 4.5)	7/30	0.8 (0.3 to 2.1)	4/30	2.8 (0.8 to 10.1)
Organochlorine	7/17	2.0 (0.8 to 5.2)	4/17	2.5 (0.7 to 8.3)	5/15	1.2 (0.4 to 3.7)	3/15	4.9 (1.1 to 21.2)
Organophosphate	8/24	1.5 (0.6 to 3.7)	6/24	2.7 (1.0 to 7.7)	4/20	0.7 (0.2 to 2.4)	1/20	0.9 (0.1 to 7.6)
Pyrethrin	3/17	0.8 (0.2 to 3.0)	4/17	3.0 (0.9 to 10.4)	0/14	–	1/14	1.1 (0.1 to 10.4)
Fungicides	12/35	1.7 (0.8 to 3.5)	6/35	1.9 (0.7 to 5.3)	7/34	0.7 (0.3 to 1.8)	4/34	2.7 (0.7 to 9.6)
Carbamate	5/17	1.3 (0.5 to 3.7)	5/17	3.5 (1.1 to 10.7)	1/16	0.2 (0.0 to 1.9)	3/16	3.7 (0.9 to 15.6)
Imide	2/10	1.0 (0.2 to 4.8)	1/10	0.8 (0.1 to 6.8)	0/10	–	2/10	3.5 (0.6 to 19.6)
Triazole	3/9	1.8 (0.5 to 7.1)	3/9	4.1 (1.0 to 17.7)	0/9	–	1/9	1.4 (0.2 to 12.9)
Herbicides	12/42	1.5 (0.7 to 3.0)	5/42	1.2 (0.4 to 3.4)	5/34	0.5 (0.2 to 1.3)	4/34	2.4 (0.7 to 8.6)
Phenoline	7/17	2.3 (0.9 to 6.1)	3/17	1.9 (0.5 to 7.3)	2/15	0.3 (0.1 to 1.6)	3/15	3.7 (0.9 to 16.1)
Phenoxy	5/25	1.0 (0.4 to 2.8)	2/25	0.8 (0.2 to 3.6)	3/20	0.4 (0.1 to 1.7)	4/20	4.1 (1.1 to 15.5)
Picoline	3/10	1.3 (0.3 to 5.0)	1/10	1.1 (0.1 to 9.7)	0/8	–	0/8	–
Triazine	8/20	2.1 (0.8 to 5.0)	4/20	2.3 (0.7 to 7.7)	4/17	0.9 (0.3 to 3.0)	4/17	5.1 (1.4 to 19.3)
Amide	1/12	0.4 (0.0 to 3.0)	2/12	1.8 (0.4 to 9.3)	0/8	–	2/8	3.8 (0.6 to 23.0)
Urea	3/7	2.7 (0.6 to 11.5)	2/7	4.7 (0.8 to 28.6)	2/7	0.9 (0.2 to 4.8)	2/7	5.7 (0.9 to 34.6)
Glyphosate	5/24	1.0 (0.3 to 2.7)	3/24	1.4 (0.4 to 5.2)	2/18	0.4 (0.1 to 1.8)	2/18	1.8 (0.3 to 9.3)

ORs (95% CI) were estimated by polytomous logistic regression including the stratification variables, age, centre and socioeconomic category (white collar/blue collar).
Ca, number of cases; Co, number of controls; OR, odds ratio.

insecticides, in line with the relationship observed with pyrethrins, was also observed.

The hospital-based design, required for the blood samples, was carefully implemented in order to recruit the cases and controls from the same population. Recruitment was restricted to cases and controls residing in the hospital catchment areas and the cases were recruited from the main hospital and not from private clinics. Recruitment from clinics might have attracted a specific population that might have been better informed and/or receiving better care. All the cases diagnosed in the hospitals during the recruitment period were systematically contacted and the refusal rate was low (4.3%). Thus, there is no obvious reason for preferential selection of cases more exposed to specific occupational exposures, particularly pesticides. Moreover, the results were shown to be robust in the sensitivity analyses in which each centre was excluded in turn. This suggests that the results are not explained by local selection. Selection by survival could have occurred if exposure to pesticides is related to the seriousness of the disease or the response to treatment, which is unlikely. In addition, only incident cases were recruited and inclusion took place within 6 months of diagnosis and within 3 months for most subjects (88.1%), which minimises the possibility of a survival bias.

The controls were mainly recruited in rheumatological and orthopaedic departments and were residents of the hospital catchment areas. The controls had not been admitted for diseases related to smoking or drinking in order to avoid over-representation of those habits, which are known to be less frequent among farmers. Inclusion of those diseases could thus have led to under-representation of farmers among the controls. The controls were not admitted for occupational diseases or injuries. Overall, the control diseases are not known to be related to farming or exposure to pesticides. Control non-eligibility was based on the reason for hospital admission and not on the subject's medical history. Controls who had had smoking- or alcohol-related or occupational diseases/injuries in the past were eligible. Furthermore, the results are unlikely to be due to a particular control subgroup since the sensitivity analyses, in which each group of controls sharing similar reasons for admission was excluded in turn, did not generate different results. Similarly, conditional analyses, in which independent control groups were used for each LN case subgroup, yielded estimates similar to those of the unconditional regression analyses. Smoking and drinking were as prevalent in the control group as in the national survey, "Enquête Décennale Santé", for the same geographical areas and age groups.[29] Lastly, the controls reported the specific regional farming practices expected on the basis of the surveys by the French Ministry of Agriculture.[30]

In order to limit differential misclassifications, the information was collected from the cases and controls, under very similar conditions, in hospital and by the same interviewer, using standardised structured questionnaires. The patients and interviewers were informed that the study was related to "the environment and health", but were unaware of any specific hypotheses connected to a particular practice or product. Occupational pesticide exposure was elicited using a standardised structured ad hoc occupational questionnaire and case-by-case exposure was assessed blind to case/control status. The occupational hygienist reviewed each questionnaire blind to case/control status; the additional telephone interviews that were made to obtain more precise data or correct inconsistencies were administrated as frequently for cases as for controls. Lastly, the same proportions of missing values were observed for cases and controls. Because of those measures, differential misclassification is unlikely to explain the results.

Non-differential misclassification is probably more important since exposure was based on the patient's recall, but it is expected to be more marked for pesticide reports than for crop or husbandry reports.[31–34] Non-differential misclassification may have reduced power, particularly for specific pesticides and/or for products used long before inclusion or for a short period. The use of a sensitive definition of exposure ("possible exposure") may also have contributed to non-differential misclassification; use of a more specific definition (definite exposure) yielded similar or stronger results.

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Main messages

- The case-control study incorporated careful expert case-by-case review of occupational exposure to pesticides.
- Occupational exposures to several pesticides were significantly associated with multiple myeloma, Hodgkin's lymphoma and hairy-cell leukaemia. Non-significant positive associations with non-Hodgkin's lymphoma were also observed.

Policy implications

- The results strengthen the hypothesis that occupational pesticide exposures may be involved in the aetiology of lymphoid neoplasms.

All the analyses were adjusted for age and centre for all LNs, and for socioeconomic category also for HL. Additional analyses in which education, housing and socioeconomic category were included as potential confounders were performed even though the cases and controls did not differ with respect to those characteristics. The results were unchanged. In addition, factors previously evidenced in other reports on the study, such as influenza immunisation, previous history of mononucleosis,[35] skin type[36] and smoking and alcohol drinking status,[37] were also accounted for. There was no substantial change in the results. Confounding by other pesticide exposures could not be controlled since all pesticide uses were closely related to each other.

The number of exposures and LN categories led to multiple comparisons. Therefore, some of the results, particularly those for HL and MM, may have been observed by chance. Conversely, true associations may have remained undetected due to lack of power. That could be the case for NHL, which was positively, but not significantly, related to several pesticides. For NHL, the size of the study allowed minimum detectable OR ranging from 1.9 to 4.6, for an alpha error of 5%, a power of 80%, and a risk factor prevalence ranging from 1 to 10%.

Nevertheless, the non-significant associations between NHL and organochlorine (1.8 (0.9 to 3.8)) and organophosphate (1.7 (0.9–3.3)) insecticides observed are consistent with those previously reported in the literature and have similar orders of magnitude. Among the LNs, NHL has been most investigated with respect to occupational exposure to pesticides, although the definition of NHL varies across the studies. Case-control studies carried out in the USA,[38 39] Australia[14] and Canada[40] have shown positive associations with occupational exposure to organochlorine insecticides. Exposure to organophosphate insecticides was also related to NHL in the pooled analysis of the three case-control studies conducted by the National Cancer Institute.[41] It is noteworthy that, in the present study, almost all the subjects who reported having used organophosphate also reported having used organochlorine insecticides. The two exposures cannot therefore really be distinguished. The association between NHL and exposure to triazine herbicides (OR = 1.9 (0.9 to 3.8)) observed in the present study is consistent with the association with atrazine reported by the agricultural health study[25] and in the pooled analysis of the NCI case-control studies.[10] Phenoxy herbicides (including 2,4-D, 2,4,5-T, 2-methly-4-chlorophenoxyacetic acid) were associated with NHL in some,[40 42 43] but not all[10 19] studies. In the present study, there was no indication of any association with phenoxy herbicides (OR = 0.9 (0.4 to 1.9)).

Several studies have also reported associations between MM and pesticide exposure.[16] Phenoxy herbicides were positively related to MM in case-control studies carried out in Sweden[16] and Canada.[18] Recently, the Agricultural Health Study also evidenced increased risks of MM for the highest category of exposure to the herbicides Alachlor[27] and Atrazine.[25]

The relationships between LPS and pesticide exposure remain poorly documented. Nevertheless, the associations with HCL observed in the present study, although based on very small numbers, are consistent with the findings reported by Clavel *et al*[28] and Nordström *et al*.[24]

Even fewer studies have investigated the relationship between HL and occupational pesticide exposure. Franceschi *et al*[44] reported increased OR for the longest occupational and non-occupational exposures to pesticides and herbicides, while a recent Canadian case-control study[18] failed to reveal any association with exposure to phenoxy herbicides.

CONCLUSION

The results of this study, based on case-by-case expert review of occupation-specific questionnaires, support the hypothesis that occupational pesticide exposures may be involved in HL, MM and HCL and do not rule out a role in NHL. Consistently with previous publications, the analyses identified specific pesticides that deserve further investigation.

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ORIGINAL ARTICLE

Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study

Nahrstedt EXHIBIT NO. 20 8/23/17 P. CAMPBELL

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ABSTRACT

Objectives We investigated the role of occupational exposure to specific groups of agrochemicals in the aetiology of lymphoma overall, B cell lymphoma and its most prevalent subtypes.

Methods In 1998–2003, 2348 incident lymphoma cases and 2462 controls were recruited to the EPILYMPH case-control study in six European countries. A detailed occupational history was collected in cases and controls. Job modules were applied for farm work including specific questions on type of crop, farm size, pests being treated, type and schedule of pesticide use. In each study centre, industrial hygienists and occupational experts assessed exposure to specific groups of pesticides and individual compounds with the aid of agronomists. We calculated the OR and its 95% CI associated with lymphoma and the most prevalent lymphoma subtypes with unconditional logistic regression, adjusting for age, gender, education and centre.

Results Risk of lymphoma overall, and B cell lymphoma was not elevated, and risk of chronic lymphocytic leukaemia (CLL) was elevated amongst those ever exposed to inorganic (OR=1.6, 95% CI 1.0 to 2.5) and organic pesticides (OR=1.5, 95% CI 1.0 to 2.1). CLL risk was highest amongst those ever exposed to organophosphates (OR=2.7, 95% CI 1.2 to 6.0). Restricting the analysis to subjects most likely exposed, no association was observed between pesticide use and risk of B cell lymphoma.

Conclusions Our results provide limited support to the hypothesis of an increase in risk of specific lymphoma subtypes associated with exposure to pesticides.

What this paper adds

- Inconsistent opinions exist about the evidence linking occupational exposure to pesticides with lymphoma risk.
- The complex array of chemicals comprised in the pesticide definition and the heterogeneity of the pathological diagnoses included in the lymphoma or non-Hodgkin's lymphoma definitions might contribute to the controversy.
- We used the WHO classification of lymphoma to identify specific lymphoma entities, and state of the art retrospective exposure assessment for occupational exposure to chemical classes of pesticides and specific agrochemicals in a population-based case-control study.
- Our results provide limited evidence of an increase in risk of chronic lymphocytic leukaemia associated with exposure to organophosphates, and no association for other lymphoma subtypes.

INTRODUCTION

Among hundreds of agents and groups of agents examined in 35 years of International Agency for Research on Cancer (IARC) Monographs (volumes 1–99)[1] pesticides account for two dozens; only a few of those are still in use worldwide, some are obsolete but still in use in developing countries, and most have been banned or abandoned for some decades. Only arsenic and arsenical pesticides are group 1 human carcinogens, while occupational exposure in the spraying and application of non-arsenical insecticides overall is included in group 2A, because of limited evidence from epidemiological studies. Group 2A also includes two active ingredients, namely the fungicide captafol, which uses have been restricted in the USA and most world countries from 1999,[2] and ethylene dibromide, which is used as a grain fumigant. As for the rest, the insufficient evidence from human studies is coupled with the sufficient, limited or unavailable evidence from experimental animal studies. Nowadays, thousands of chemicals are available to farmers to treat plant diseases and protect their crops; their use changes year by year, across countries and within each country, and by type of crop and type of disease being treated: the difficulty of conducting epidemiological studies of the long term effects of agrochemicals is reflected in the poor information on their human carcinogenicity and the absence of evaluation by international scientific and regulatory agencies.

Reviews of the scientific literature reported inconsistent opinions about the association between occupational exposure to pesticides and non-Hodgkin's lymphoma (NHL).[3 4] In fact, while

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several meta-analyses have come to positive conclusions on NHL risk,[5–10] particularly for prolonged exposures,[11–13] or for exposure in the years relatively close to the diagnosis,[14] risk has been shown to vary by gender,[15] or specific jobs,[16] and by specific chemicals.[17] Besides, the causal link is not always recognised,[18] and negative studies have also been published.[19–24] In some instances, interpretation of findings is limited by imprecise definition of either exposure or disease entity[22] or a small study size.[23] Geographical variation in NHL mortality has also been reported in relation to the prevalent type of crop, and therefore the pesticide used:[25–28] for instance, NHL mortality in the female population was elevated in an area of Minnesota where wheat, corn and soy crops were prevalent.[28]

METHODS

The EPILYMPH study, a multicentre case-control study on environmental exposures and lymphoid neoplasms, was conducted in Czech Republic, France, Germany, Italy, Ireland and Spain from 1998 to 2004. Details about the study have been described elsewhere.[29] Briefly, cases were all consecutive adult patients first diagnosed with lymphoma during the study period, resident in the referral area of the participating centres. The diagnosis was classified according to the 2001 WHO classification of lymphoma,[30] and slides of about 20% of cases from each centre were reviewed centrally by a panel of pathologists, coordinated by MM. Controls from Germany and Italy were randomly selected by sampling from the general population, matched to cases on gender, 5-year age-group, and residence area. The rest of the centres used matched hospital controls, with eligibility criteria limited to diagnoses other than cancer, infectious diseases and immunodeficient diseases. Approval by the relevant Ethics Committees was obtained in all centres. Informed consent was obtained for the 2348 lymphoma cases and 2462 controls who participated to the study. Overall, the participation rate was 88% in cases, 81% in hospital controls and 52% in population controls.

Trained interviewers conducted in person interviews with cases and controls, using the same structured questionnaire translated into the local language. Questions sought information on sociodemographic factors, lifestyle, health history and a list of all full time jobs held for 1 year or longer. Industrial hygienists in each participating centre coded the occupations and industries using the 5-digit 1968 International Labour Office International Standard Classification of Occupations[31] and the 4-digit codes of the 1996 European Statistical Classification of Economic Activities, revision 1 (NACE, rev. 1).[32] Study subjects who reported having worked in agriculture were given a job-specific module inquiring in detail into the following: detailed description of the tasks; kind of the crops and size of the cultivated area; type of pests being treated; pesticides used, and procedures of crop treatment; use of personal protective equipment; re-entry after treatment; frequency of the treatment in days/year.

Occupational exposure assessment

With the support of a local agronomist, and the support of a crop-exposure matrix, created by LM, to supplement the available information, industrial hygienists and occupational experts in each participating centre reviewed the general questionnaires and job modules to assess exposure to pesticides classified into inorganic (mainly sulphur and arsenic salts) and organic (carbamates, organophosphates, organochlorines, triazines and triazoles, phenoxyacids, and chlorophenols). Exposure was classified according to the following exposure metrics:

confidence, representing the industrial hygienist's degree of certainty that the worker had been truly exposed to the agent, based upon two criteria: 1. a summary evaluation of the probability of the given exposure (1= possible, but not probable; 2=probable; and 3=certain); and 2. the proportion of workers exposed in the given job (1≤40%; 2=40–90%; 3≥90%);

intensity of exposure, expressed in relation to the circumstances of use (personal preparation of the pesticide mixture, use of hand pump or tractor, size of the area being treated, re-entry after treatment) and use of personal protective equipment. Semiquantitative estimates of exposure were derived from the publicly available EUROPOEM programme,[33] and then categorised on a 4-point scale (0=unexposed; 1=low; 2=medium; 3=high);

frequency of exposure, expressed in annual days of pesticide use reported in the questionnaire or estimated based on the type of plant disease and the size of the crop or the livestock being treated (low≤50 days/year; medium 51–100 days/year; high≥101 days/year).

A cumulative exposure score was calculated for each pesticide group as follows: $C_i = S(y_j \times f_j/3)^{xj}$ where C is the cumulative exposure score; i the study subject; j the jth job in the work history of study subject i; y the duration of exposure (in years); x the exposure intensity level f the exposure frequency level.

Cumulative exposure scores for each pesticide group were then categorised by tertiles of their distribution among the exposed (cases and controls combined).

Consistency in the occupational coding and exposure assessments was optimised through several meetings of the industrial hygienists.

Statistical methods

We assessed risk of B cell lymphoma, and its most prevalent subtypes, diffuse large B cell lymphoma (DLBCL) and chronic lymphocytic leukaemia (CLL), associated with ever exposure to inorganic and organic pesticides (all types), and the organic pesticide groups listed in table 1. The analysis was led by PC, supported by GS, SD, MP and TN, both on all exposed subjects, and after restriction to subjects whose exposure was assessed with high confidence. Linear trends in all exposure metrics were also estimated. The OR was calculated using unconditional logistic regression, adjusted for age, gender, education and centre. Two-tailed 95% CI for the OR were estimated using the Wald statistics ($e^{\beta} \pm (z_{\alpha/2} \times se_{\beta})$). Subjects unexposed to any pesticide comprised the reference category used for all the analyses. Trends in the ORs were assessed using the Wald test for trend.

Role of the funding sources

The private and public institutions that sponsored this study did not influence or intervene in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

RESULTS

Details on the study size, number of cases and controls by participating centre, and their frequency distribution by selected variables of interest in the occupational analyses were reported elsewhere.[34] Table 1 shows the frequency distribution of exposure to pesticide groups for which exposure was assessed in the EPILYMPH study, by country. In a footnote, the active ingredients within each group are reported, selected among those reported by study subjects or suggested by the collaborating agronomists. Overall, the prevalence of exposure to pesticides in

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Table 1 Prevalence of exposed to the individual pesticide groups by country in the EPILYMPH study

Pesticide groups*	Spain N=1222	France N=574	Germany N=1413	Italy N=598	Ireland N=409	Czech republic N=594	Total N=4810
Inorganic pesticides	88 (7.2)	14 (2.4)	28 (2.0)	31 (5.2)	14 (3.4)	6 (1.0)	181 (3.8)
Arsenicals	30 (2.4)	0 (0.0)	9 (0.6)	0 (0.0)	3 (0.7)	0 (0.0)	42 (0.9)
Organic pesticides	127 (10.4)	38 (6.7)	86 (6.1)	48 (8.2)	38 (9.4)	5 (0.8)	342 (7.1)
Carbamates	3 (0.2)	4 (0.7)	11 (0.8)	15 (2.5)	2 (0.5)	0 (0.0)	35 (0.7)
Organophosphates	7 (0.6)	4 (0.7)	11 (0.8)	16 (2.7)	14 (3.4)	1 (0.2)	53 (1.1)
Organochlorines	22 (1.8)	8 (1.4)	22 (1.6)	13 (2.2)	5 (1.0)	0 (0.0)	70 (1.5)
Triazines and triazoles	0 (0.0)	3 (0.6)	2 (0.1)	13 (2.2)	0 (0.0)	2 (0.3)	20 (0.4)
Phenoxyacids	2 (0.2)	4 (0.7)	10 (0.7)	5 (0.8)	4 (1.0)	0 (0.0)	25 (0.5)
Chlorophenols	46 (3.7)	9 (0.9)	31 (2.0)	19 (3.0)	10 (2.0)	0 (0.0)	115 (2.4)

For each country, the total number of participants is provided upon which the percentage of exposed (in brackets) is calculated.
*Note: Inorganic pesticides include: sulphur, arsenic, fluorine, zinc, mercury derivatives and other; arsenicals include: ammonium, calcium, sodium and potassium arsenate; organic pesticides include carbamates (aldicarb, carbaryl, mancozeb, methomyl, propoxur and other), organophosphates (acephate, diazinon, dimethoate, glyphosate, malathion, parathion and other), organochlorines (aldrin, DDT, chlordane, endrin, lindane, metoxychlor, endosulfan and other), triazines and triazoles (atrazine, propazine, terbutryn and other), phenoxyacids (2,4 dichlorophenoxyacetic acid, 2,4,5 T, methylchloro-phenoxyacetic acid, mecoprop and other), chlorophenols (2 chlorophenol, pentachlorofenol and other).

our study was low, with only 3.7% of participants exposed to inorganic pesticides and 6.4% exposed to organic pesticides, and it was lowest for triazines and triazoles and phenoxy acids. The prevalence of exposed was highest in Spain and lowest in the Czech Republic. The prevalence of exposure to the specific groups of pesticides varied by country. Use of inorganic pesticides was widespread, but it mainly consisted of copper sulphide or other sulphur compounds as reported by study subjects, indicated by the agronomist or by the crop-exposure matrix. Arsenicals were mainly used in Spain and, to a smaller extent, in Ireland. Among organic pesticides, chlorophenols were most frequently represented, and their prevalence was highest in Spain, Italy and Germany. Organophosphates were the most prevalent group of organic pesticides in Ireland. The most variegate pattern of pesticide use was described in Italy.

Table 2 shows risk of lymphoma overall, B cell lymphoma, DLBCL and CLL, amongst those ever exposed to each type of pesticide considered in this study. No excess risk of lymphoma

Table 2 Risk of lymphoma and major subtypes associated with ever exposure to pesticide groups in the Epilymph study

	Lymphoma (all types)			B cell lymphoma			Diffuse large B cell lymphoma			Chronic lymphocytic leukaemia		
Pesticide group	Cas/cont	OR	95% CI	Cai/cont	OR	95% CI	Cas/cont	OR	95% CI	Cas/cont	OR	95% CI
Inorganic pesticides												
Any confidence level	100/81	1.3	0.9 to 1.7	81/81	1.2	0.8 to 1.6	13/81	0.7	0.4 to 1.3	28/81	1.6	1.0 to 2.5
High confidence	57/46	1.3	0.9 to 2.0	42/46	1.1	0.7 to 1.8	7/46	0.7	0.3 to 1.6	15/46	1.6	0.8 to 2.9
Arsenicals												
Any confidence level	18/24	0.8	0.4 to 1.4	14/24	0.7	0.4 to 1.3	2/24	0.4	0.1 to 1.6	6/24	1.1	0.4 to 2.7
High confidence	4/5	0.8	0.2 to 3.1	2/5	0.5	0.1 to 2.6	0/5	0.0	–	0/5	0.0	–
Organic pesticides												
Any confidence level	180/162	1.2	0.9 to 1.4	148/162	1.2	0.9 to 1.5	28/162	0.8	0.5 to 1.2	45/162	1.5	1.0 to 2.1
High confidence	101/91	1.1	0.8 to 1.5	79/91	1.1	0.8 to 1.5	13/91	0.7	0.4 to 1.2	23/91	1.4	0.8 to 2.2
Carbamates and thiocarbamates												
Any confidence level	16/19	0.9	0.5 to 1.7	9/19	0.7	0.3 to 1.5	1/19	0.2	0.0 to 1.8	3/19	1.1	0.3 to 3.8
High confidence	4/8	0.5	0.2 to 1.7	3/8	0.5	0.1 to 2.0	0/8	0.0	–	1/8	0.9	0.1 to 7.2
Organophosphates												
Any confidence level	32/21	1.6	0.9 to 2.8	23/21	1.4	0.8 to 2.6	5/21	1.1	0.4 to 2.9	9/21	2.7	1.2 to 6.0
High confidence	11/7	1.6	0.6 to 4.2	7/7	1.4	0.5 to 3.9	1/7	0.6	0.1 to 5.3	1/7	0.9	0.1 to 7.7
Organochlorines												
Any confidence level	33/37	0.9	0.6 to 1.5	27/37	0.9	0.5 to 1.4	5/37	0.6	0.2 to 1.6	10/37	1.2	0.6 to 2.5
High confidence	12/12	1.0	0.5 to 2.3	11/12	1.1	0.5 to 2.6	2/12	0.7	0.2 to 3.3	5/12	1.9	0.6 to 5.6
Triazines and triazoles												
Any confidence level	8/12	0.7	0.3 to 1.7	6/12	0.7	0.2 to 1.7	2/12	0.8	0.2 to 3.4	2/12	0.9	0.2 to 4.1
High confidence	5/6	0.9	0.3 to 2.8	3/6	0.6	0.2 to 2.5	1/6	0.8	0.1 to 6.4	1/6	0.8	0.1 to 6.9
Phenoxy acids												
Any confidence level	14/11	1.3	0.6 to 2.9	12/11	1.4	0.6 to 3.1	4/11	1.7	0.5 to 5.2	2/11	0.9	0.2 to 4.1
High confidence	5/5	1.0	0.3 to 3.6	4/5	1.1	0.3 to 4.1	2/5	1.9	0.4 to 9.9	0/5	0.0	–
Chlorophenols												
Any confidence level	59/56	1.1	0.8 to 1.6	49/56	1.1	0.7 to 1.6	13/56	1.1	0.6 to 2.0	13/56	1.1	0.6 to 2.2
High confidence	32/27	1.2	0.7 to 2.1	25/27	1.1	0.6 to 2.0	6/27	1.0	0.4 to 2.5	5/27	1.0	0.4 to 2.6

Results are presented for all confidence levels combined and limited to study subjects with high confidence of exposure.

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Table 3 Chronic lymphocytic leukaemia risk and intensity of exposure to pesticide groups (all levels of confidence)

	Unexposed			Low			Medium			High		
Pesticide groups	Ca/co	OR	95% CI	Ca/co	OR	95% CI	Ca/co	OR	95% CI	Ca/co	OR	95% CI
Inorganic pesticides	362/2262	1.0	–	14/33	2.2	1.1 to 4.2	10/34	1.2	0.6 to 2.5	3/11	1.2	0.3 to 4.3
Organic pesticides	362/2262	1.0	–	21/81	1.4	0.8 to 2.3	18/55	1.6	0.9 to 2.8	6/26	1.2	0.5 to 3.0
Carbamates*	362/2262	1.0	–	0/10	–	–	3/9	1.8	0.5 to 6.9			
Organophosphates*	362/2262	1.0	–	5/13	2.7	0.9 to 7.8	4/8	2.6	0.7 to 9.2			
Organochlorines*	362/2262	1.0	–	5/15	1.8	0.6 to 5.0	5/20	1.0	0.4 to 2.8			
Phenoxy acids*	362/2262	1.0	–	0/7	–	–	2/4	2.4	0.4 to 13.8			
Chlorophenols	362/2262	1.0	–	7/27	1.2	0.5 to 2.6	5/18	1.4	0.4 to 3.9	1/11	0.5	0.1 to 4.2

*Medium and high intensity categories combined.

(all types), B cell lymphoma and DLBCL was observed in association with ever exposure to inorganic or organic pesticide, nor to any of the organic pesticide groups assessed in this study. Risk of CLL was significantly associated with ever exposure to organic pesticides and particularly to organophosphates (OR=2.6, 95% CI 1.2 to 6.0). An elevated risk of CLL was also associated with ever exposure to inorganic pesticides (OR=1.6, 95% CI 1.0 to 2.5), but not with arsenical pesticides. Because of the a priori hypothesis of an association, we cite the moderate excess risk of B cell lymphoma associated with ever exposure to phenoxyacids (OR=1.4, 95% CI 0.6 to 3.1). No excess risk was observed in association with ever exposure to the other pesticide groups. The results did not change when exploring risk for NHL, thus excluding CLL and multiple myeloma, but including T-cell lymphomas. Further adjustment for ever exposure to solvents or contact with livestock did not virtually change the risk estimates.

Table 3 shows risk of CLL by intensity of exposure. The excess risk associated with ever exposure to inorganic pesticides was limited to the lowest category of intensity of exposure. Risk for medium-high intensity of exposure to organophosphates showed a 2.6-fold excess (CI 95% 0.7 to 9.2), matching that observed for the low intensity category; however, the CI was wide at either level, and there was no trend in risk (Wald test for trend=0.14; p=0.44).

Risk of B cell lymphoma associated with ever exposure to organophosphates did not vary according to whether exposure started before 1980 or from 1980 onwards and it did not increase by cumulative exposure tertiles (Wald test for trend p=0.09). Instead, CLL risk was highest when exposure started from 1980 onwards (OR=4.0, 95% CI 0.9 to 16.6), and it increased significantly by increasing cumulative exposure tertile (Wald test for trend p=0.02).

Only a few individual agrochemicals were represented by a sizable number of study subjects, and the exposed cases of DLBCL, CLL and even B cell lymphoma overall were too few for any meaningful inference to be drawn. Exposure to the three most frequently identified individual organophosphate pesticides, namely dimethoate and parathion, among the most commonly used agricultural insecticides, and glyphosate, an organophosphorous herbicide, was more prevalent among B cell lymphoma cases, while exposure to 2,4 dichlorophenoxyacetic acid (2,4 D) was not (table 4). Four cases and no controls had been exposed to methylchloro-phenoxyacetic acid (MCPA) (p=0.13); these were one case of diffuse large B cell lymphoma, one case of follicular lymphoma and two cases of unspecified non-Hodgkin's lymphoma. Three cases and one control to other phenoxy herbicides (p=0.28) not shown in the tables.

When limiting the analysis to the only study subjects whose exposure was assessed with a high degree of confidence, numbers became smaller and CIs wider. The excess risk of CLL associated with exposure to organophosphates and that of B cell lymphoma associated with exposure to phenoxyacids were no longer observed. Risk was not significantly elevated for the B cell lymphomas overall among study subjects with high confidence of exposure to organophosphates, and CLL risk was moderately increased among study subjects with high confidence of exposure to organochlorines . Overall, these results were not interpretable because of the small number of cases and the rarity of the exposed.

Table 4 Risk of B cell lymphoma and occupational exposure to selected specific active ingredients of pesticides

	B cell Lymphoma		
Pesticide	Ca/Co	OR	95% CI
Mancozeb	2/4	0.6	0.1 to 3.5
Methomyl	0/4	–	
Dimethoate	3/2	1.8	0.3 to 10.6
Glyphosate	4/2	3.1	0.6 to 17.1
DDT	3/3	1.2	0.2 to 5.9
Endosulfan	0/4	–	
2,4-dicholorophenol	2/4	0.6	0.1 to 3.5
Methylchloro phenoxyacetic acid	4/0	∞	

DDT, dichloro-diphenyl-trichloro-ethane.

DISCUSSION

Our results provide limited support to the hypothesis of an association between occupational exposure to organophosphorous pesticides and risk of CLL. We did not find evidence of an association with lymphoma overall, B cell lymphoma as a group of different subtype entities and DLBCL. The low prevalence of exposed in our community based study did not allow to explore the association with other less prevalent lymphoma subgroups, nor to detect unquestionable associations with specific agrochemicals. Also, we were unable to confirm the repeatedly reported association between exposure to phenoxyacids and lymphoma. It is worth reporting, however, that while we did not observe any indication of a higher prevalence of exposure to 2,4 D among cases in respect to controls, four B cell lymphoma cases and no controls were identified as exposed to MCPA.

Organophosphate insecticides were introduced for agricultural use in Europe mainly in the early 1970s, when insect resistance to organochlorines became manifest. Their use was

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associated with an almost twofold increase in risk of NHL in a Nebraska case-control study;[35] women appeared to be at greater risk.[15] Similar findings were reported in Italy and China.[36 37] An increase in NHL risk was also reported in Australia for exposures defined as substantial, although no increasing trend in risk was observed with frequency, intensity level, probability, duration and period of exposure.[38] Specific organophosphates were investigated in several studies. Malathion, one the most frequently used organophosphorous insecticide, showed an association in a Canadian case-control study,[39] and in another study conducted in Iowa and Minnesota.[40] Diazinon and dichlorvos also showed an association in the Minnesota study.[40] The positive association with exposure to diazinon was confirmed, but limited to lymphocytic lymphoma, in one study,[41] while results of the US Agricultural Health Study were negative for malathion.[42] Studies were negative for phorate,[43] and positive for terbufos, scoring the fourth in the US sales of organophosphates, although again no trend was observed by exposure metrics.[44] Selected lymphoma subtypes, such as multiple myeloma[45] and hairy cell leukaemia[46] were reported in association with exposure to glyphosate. The four B cell lymphoma cases exposed to glyphosate in our study included one case each of DLBCL, CLL, multiple myeloma and unspecified B cell lymphoma.

2,4 D is the best known phenoxy acid. Its association with NHL risk was first reported in Sweden[47] and thereafter confirmed in the US,[48] with a sixfold increase among farmers using it for more than 20 days/year, and significant increases in risk for direct use and lack of use of personal protective equipment. Further positive results were reported in case-control studies,[35 49 50] while a Danish follow-up study[51] was negative for an association, and a multicentre mortality study of 19 000 European workers exposed to phenoxy acids and chlorophenols[52–54] confirmed the association in presence of concurrent exposure to 2,2,4,4 tetrachlorodibenzodioxin, a frequent contaminant of phenoxy herbicides and chlorophenols, but not when tetrachlorodibenzodioxin exposure was excluded. Recent updates of historical cohorts of phenoxyacids manufacturers and trichlorophenol manufacturers provided negative or conflicting findings.[55–57] On the other hand, a dose-related increase in NHL mortality by semiquantitative indicators of exposure to pentachlorophenol was observed in a cohort of woodworkers in British Columbia, Canada[58] and two out of three NHL cases in a small cohort of Swedish woodworkers exposed to phenoxyacid herbicides belonged to the highest exposure subgroup.[59] Although the most recent case-control studies, with a more accurate exposure assessment, tend to confirm the association of NHL risk with phenoxy herbicides, and particularly 2,4D, 4-chlor-2-MCPA, and 4-chlor-2-methyl phenoxypropionic acid (MCPP or meclorpop),[14 39 44–46 60 61] reviews still underline the uncertainties and inconsistency in the results.[62] A specific effect of 2,4D on the haemolymphopoietic tissue was supported by the observation of an increase of the lymphocyte proliferation index in workers exposed to the herbicide.[63]

Our results did not find an association with phenoxyacid herbicides and chlorophenols, and provide only limited support to an increase in risk associated with exposure to organophosphate insecticides and herbicides. We did not observe an association with exposure to carbamates and thiocarbamates, organochlorines, and triazines and triazoles.

Use of carbamates in general, and carbaryl in particular, was associated with an increase in NHL risk in Italy,[36] Canada[39] and the USA[40] and the association was inconsistent by exposure metric in the US Agricultural Health Study,[64] while, in this large survey, NHL risk showed an increasing trend with increasing exposure to butilate, a thiocarbamate,[65] and sevin was the only carbamate associated with a dose-related increase in NHL risk in a Chinese study.[66] We did not find an association between exposure to carbamates and risk of lymphoma or its most prevalent subtypes.

A first suggestion of an increasing NHL risk among exposed to organochlorine insecticides, namely chlordane, toxafene, aldrin, lindane and DDT, came from several international case-control studies, where concurrent exposure to numerous other pesticides also occurred.[36 39 40] Multiple myeloma,[67] CLL[38] and hairy cell leukaemia[68] were more frequently associated. However, detailed analyses of US case-control studies found out that the positive association with exposure to DDT or lindane disappeared after adjusting for exposure to organophosphates and phenoxyacids.[69 70] Positive findings have been more recently published on lindane,[71 72] but they keep being inconclusive for the entire class of organochlorines.[38]

Suggestions of a positive association between triazine herbicides and NHL risk provided limited evidence because of multiple concurrent exposures and the small study size;[68 73] besides, the pooled analysis of three case-control studies[74] and the analysis of the repeatedly cited US Agricultural Health Study[75 76] did not support the hypothesis of a role of atrazine and cyanazine. Less relevant in this regard seems to be the increased risk associated with exposure to metribuzin in one study, as all lymphopoietic malignancies were considered altogether and the CI included unity.[77]

Our study presented the advantage of a very detailed exposure assessment, coupled with an up-to-date pathological definition of disease entities. Such conditions represent substantial improvements in the assessment of occupational exposures and in lessening exposure and disease misclassification in population-based studies, which would help in revealing true associations. While this differentiates our effort from most previous population-based case-control studies, loss of statistical power is the unavoidable consequence of the gain in specificity. In fact, the overall prevalence of exposed was small, with 3.8% participants exposed to inorganic pesticides and 7.1% exposed to organic pesticides, and when restricting the analysis to study subject with high confidence exposure to pesticides in general, or when investigating individual chemicals, numbers were further reduced and the CI of the risk estimates widened. For the same reason, no analysis on the effects of specific combined pesticide exposures was conducted. On the other hand, since biological effects are likely to vary by individual chemicals in a chemical class or functional class, when exploring associations with such classes results may be diluted, thereby missing true effects related to individual chemicals.

The use of hospital controls in several centres contributing to this multinational effort, and the low response rate in the two centres where controls were population-based, may have introduced selection bias, further limiting the interpretation of our study results.

We included age, gender, education, and centre as covariates in our regression models to adjust our risk estimates, as a set of core factors potentially relevant in subgroup analyses. We selected education as a surrogate for lifestyle factors potentially acting as confounders of the association between pesticide exposure and risk of B cell lymphoma. In turn, lifestyle might surrogate exposure to endotoxin, a component of the outer membrane of Gram-negative bacteria, a common contaminant associated with poverty, crowding, pets, household cleanliness and the rural environment.[78 79] However, the endotoxin role in

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lymphomagenesis remains to be investigated. Other potential confounders would include occupational exposure to solvents and livestock, and household use of insecticides. We did not observe any change in the risk estimates for B cell lymphoma and CLL associated with ever exposure to organophosphates after adjusting by ever exposure to solvents or contact with livestock. Information on household insecticide use was self-reported by the study subjects; however, the low prevalence of occupationally exposed in our population based study, and the poor ability of study subjects to identify the chemical class of the household insecticides, did not allow the use of such information. We cannot exclude that bias might have resulted, although it seems unlikely that it would have acted only on the specific association between occupational exposure to organophosphates and CLL.

Caution is therefore required in interpreting our findings. Among pesticides considered in the IARC Monographs for their potential human carcinogenicity,[1] subjects in our study mentioned having used arsenicals, DDT, chlorophenols and phenoxyacids. In most instances, the use of these chemicals date to early periods in the work histories of study subjects, while the limited evidence of an association with CLL risk was related to still popular organophosphorous insecticides and phenoxy herbicides that did not undergo specific IARC evaluations thus far. The lack of consistent dose response trends with all the exposure metrics might support chance as the explanation for the observed associations, or it might imply some mechanism different from a direct intervention in the carcinogenic process. For instance, dimethoate was shown to have the lowest cytotoxic and genotoxic potential in cultured cells, compared to other three organophosphates and the organochlorine endosulfan;[80] however, its administration in experimental female mice caused a decrease in total immunoglobulins and IgM and in the number of plaque forming cells;[81] the same effects were observed over three generations following repeated administration of low doses dimethoate in outbred Wistar rats.[82] Functional activity of Th1 lymphocytes, immune reactions associated with these cells, and interferon-γ production were impaired after subacute malathion intoxication in albino rats,[83] while thymic atrophy and reduction in splenic germinal centres followed methylparathion administration in rabbits.[84] Such immunosuppressive effects do not seem related to acetylcholinesterase inhibition, the typical toxicological mechanism of organophosphate poisoning, and cover a large number of pesticides, including organochlorines, organophosphates, carbamates and pyrethroids.[85 86] It is unclear whether the typically toxicological criterion of dose-response in establishing causal association would apply also in mechanisms involving the immune system.

In conclusion, our analysis of a large European data set provides no support to a role of occupational exposure to several specific agrochemicals in the aetiology of B cell lymphoma, and limited support in the aetiology of CLL. Further multicentre studies in international settings coupling state of the art exposure assessment in farm work and availability of detailed pathological diagnoses with a larger study size might provide the proper setting to further test the hypothesis.

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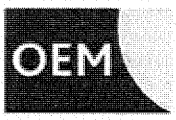

Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study

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Pesticide Product Use and Risk of Non-Hodgkin Lymphoma in Women

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A population-based, incidence case–control study was conducted among women in upstate New York to determine whether pesticide exposure is associated with an increase in risk of non-Hodgkin lymphoma (NHL) among women. The study involved 376 cases of NHL identified through the State Cancer Registry and 463 controls selected from the Medicare beneficiary files and state driver's license records. Information about history of farm work, history of other jobs associated with pesticide exposure, use of common household pesticide products, and potential confounding variables was obtained by telephone interview. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using an unconditional logistic regression model. The risk of NHL was doubled (OR = 2.12; 95% CI, 1.21–3.71) among women who worked for at least 10 years at a farm where pesticides were reportedly used. When both farming and other types of jobs associated with pesticide exposure were combined, there was a progressive increase in risk of NHL with increasing duration of such work (p = 0.005). Overall cumulative frequency of use of household pesticide products was positively associated with risk of NHL (p = 0.004), which was most pronounced when they were applied by subjects themselves. When exposure was analyzed by type of products used, a significant association was observed for mothballs. The associations with both occupational and household pesticides were particularly elevated if exposure started in 1950–1969 and for high-grade NHL. Although the results of this case–control study suggest that exposure to pesticide products may be associated with an increased risk of NHL among women, methodologic limitations related to selection and recall bias suggest caution in inferring causation. *Key words:* case–control study, mothballs, NHL, pesticides. *Environ Health Perspect* 112:1275–1281 (2004). doi:10.1289/ehp.7070 available via *http://dx.doi.org/* [Online 3 June 2004]

The U.S. Environmental Protection Agency (EPA) estimates that approximately 1.2 billion pounds of pesticides were used in the United States in 1999 (Donaldson et al. 2002), which was equivalent to 4.4 pounds per capita in the U.S. population. Of these pesticides, 76% were used in agriculture, 11% in other industries/governments, and 13% in homes and gardens; also, they were used by 77% of U.S. households and 1.2 million certified professional applicators (Donaldson et al. 2002). Despite a recent decline in overall usage after a marked increase in the 1950s and 1960s, and despite the fact that registrations of some pesticides found to have unacceptable toxicity have been canceled, there has been a concern about their long-term effects on human health, because some pesticides persist in human tissues, soil, foods, and the home environment (Muller 2000).

One of the major health concerns is carcinogenicity. More than 30 pesticides or groups of pesticides have been identified as possible carcinogens to humans by several national and international institutions [International Agency for Research on Cancer (IARC) 1987, 1991; U.S. EPA 2004]. Pesticides may increase the risk of cancer through various mechanisms. Some are known to be genotoxic (mutagenic) or tumor promotive, whereas others possess hormonal, immunotoxic, or hematotoxic properties (Acquavella et al. 2003; Dich et al. 1997). Furthermore, it has been reported that exposure to certain pesticides synergistically increases the mutagenicity of diet-derived heterocyclic amines (Wagner et al. 2003). Higher frequencies of chromosome aberrations, sister chromatid exchanges, and micronuclei have been observed in peripheral lymphocytes of pesticide applicators and certain groups of farmers (Bolognesi 2003; Maroni and Fait 1993). Because of these chromosome abnormalities, cancers in the hematolymphoid tissues [e.g., non-Hodgkin lymphoma (NHL), Hodgkin lymphoma, multiple myeloma, and leukemia] have been a central issue in the evaluation for potential health consequences of pesticide exposure. Particularly, NHL has received research attention because the recent rapid increase in its incidence parallels an exponential growth in pesticide use with a few decades of lag (Ries et al. 2003).

There have been extensive reviews (Acquavella et al. 1998; Dich et al. 1997; Maroni and Fait 1993; Morrison et al. 1992; Zahm and Ward 1998) on cancer risk associated with farming and pesticide exposure as well as a number of more recent articles on specific types of cancer and specific classes of pesticides (Blair et al. 1998; Buckley et al. 2000; Cantor et al. 2003; Hardell et al. 2002; Kogevinas et al. 1995; McDuffie et al. 2001; Meinert et al. 2000; Nanni et al. 1996; Schroeder et al. 2001; Waddell et al. 2001; Woods et al. 1987; Zahm et al. 1990; Zheng et al. 2001). However, the vast majority of those studies have focused only on occupational exposures, except for some childhood cancer studies in which parental exposures in and around the home were assessed (Buckley et al. 2000; Meinert et al. 2000; Zahm and Ward 1998). Because of the widespread use of these chemicals in and around the home and because of the longer time spent at home than at work, especially among women, information about pesticide use around the home is critical to obtain a better picture of the overall effects of pesticides in the general population. In this population-based case–control study in upstate New York, we attempted to address whether pesticide product use at home as well as at work is associated with increased risk of NHL among women.

Materials and Methods

Study population. This study was designed as a population-based case–control study of incident NHL in the upstate counties of New York State (NYS; i.e., excluding New York City and surrounding counties) to examine the associations with several environmental exposures. The study population base consisted of women 20–79 years of age who lived in the defined area of NYS at any time during the case-ascertainment period. Males were excluded because a primary focus of the study was on hair dyes, which will be reported separately.

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Women with a prior history of any type of hematologic cancer were also excluded from the study population.

Cases were newly diagnosed with NHL during the 3-year period between 1 October 1995 and 30 September 1998 and were identified through a rapid case-ascertainment system coordinated with the NYS Cancer Registry. The completeness of case ascertainment was verified by linkages with the whole state cancer registry database and with state death certificates. From 722 initially identified eligible cases, we excluded 3.4% because their physician's consent could not be obtained and an additional 4.2% because we could not find a valid contact address of the patients. Population-based controls were frequency matched to the projected age distribution of the cases and were selected from an age-stratified random sample from the NYS Department of Motor Vehicles (DMV) driver's license files for those < 65 years of age, or from the Health Care Financing Administration (HCFA) beneficiary files for those ≥ 65 years of age. However, the frequency matching was only partially successful because of age-related differences in response rates. To increase comparability between cases and controls, we excluded cases < 65 years of age without a valid NYS driver's license. No monetary incentives were offered for participation. Among those with valid address information who met all other eligibility criteria, the final participation rate in the study was 56% (n = 376, with a median age at diagnosis of 65 years) among the cases, 30% (n = 248) among the DMV controls, and 67% (n = 215) among the HCFA controls. The participation rate of cases and DMV controls was low in part because of a requirement by the NYS Department of Health institutional review board that they first be sent a study solicitation letter by the NYS Cancer Registry; only if they returned a signed consent form could we contact them for an interview. Verbal consent to participate in the study was approved by the New York University (NYU) School of Medicine institutional review board for the HCFA controls.

Demographic characteristics of the participants have been published elsewhere (Kato et al. 2002). Briefly, both the case and control participants were primarily white (98%), born in NYS (77%), and married (59%). Mean age at the index date (defined below) was 60.5 years for the cases and 54.6 years for the controls. More controls had a college education (61%) than did cases (45%). The proportion of smokers was similar in the two groups (22% in cases and 19% in controls). Family history of hematologic cancer was more common in cases (11%) than in controls (6%).

Data collection. Cases and controls were interviewed over the telephone by an interviewer at NYU who was not aware of the case–control status of the participants. The structured questionnaire was developed specifically for this study. Next of kin were interviewed for the cases (20.5%) and controls (3.2%) who were found to be deceased or medically incapable of participating in an interview. The most common surrogates were children (47%), followed by husbands (27%). In advance of the interview, each participant was mailed a package containing a letter outlining the study and a booklet displaying lists of product/chemical names to be discussed in the interview. The median time between NHL diagnosis and the telephone interview was 1.2 years, ranging from 2 months to 3.3 years. Information was collected on the lifetime history of living or working on a farm, exposures to pesticides from other types of jobs, and the lifetime history of pesticide product use in and around the home. For the subjects who worked on a farm, we asked whether pesticides were used on the farm and whether the pesticides were applied by the subject herself. When the subject applied or handled pesticides herself, details about pesticides (name and duration) were elicited. We asked about other occupational exposures in three separate categories: insecticides, herbicides, and wood preservatives. For each category, the number of hours exposed per day, week, month, or year and total duration of employment were elicited. We asked about pesticide product use in and around the home in 12 separate categories principally based on the purposes of use: to control ants, cockroaches/silverfish, bees, flies/mosquitoes, moths (mothballs), or termites; to treat indoor plants, trees/shrubs, plants in the garden/outdoor pots, or lawns; to control head lice; and use of an indoor/outdoor fogger. For each group of pesticide products, information on application methods (indoor/outdoor and by self/others), year or age first used, year or age last used, and average frequency of use in a year/season was elicited. Based on the average frequency and total duration of use, we calculated the cumulative number of uses for each product or group of products as well as for each mode of application.

Classification of NHL. Copies of medical records of the cases were obtained and reviewed to confirm their diagnosis and eligibility. In addition, to allow for a uniform classification of NHL, pathology slides were obtained and reviewed by an expert hematopathologist at NYU (G.F.). It was possible to complete the review for 268 cases (71%). Approximately 26% of these slides were sent to a second expert hematopathologist consultant (J.S.B.) to resolve discrepancies between the original diagnoses and the review diagnoses at NYU. In our review, NHL was classified according to both the REAL (Revised European-American Classification of Lymphoid Neoplasms) system (Harris et al. 1994) and the Working Formulation (Weisenburger 1992). Classification by immunophenotype was based on the final REAL categories from our pathologic review whenever available, otherwise on the immunophenotype obtained at the original institution. If neither was available (9.8%), follicular lymphomas by histology were considered B-cell in type, and the others were left unclassified. As a result, 322 were considered B-cell, 25 T-cell, and 29 unclassified. Lymphomas were also grouped by grade based on the Working Formulation: 54 low grade, 189 intermediate grade, 25 high grade, and 8 unclassified.

Statistical analysis. In order to eliminate reported exposures that occurred after diagnosis of NHL and to allow a minimum latency (lag) period of 1 year from exposure to diagnosis for each case, we set an index date, after which any exposures should be excluded from the analysis. The index date was defined as the date 1 year before diagnosis. To ensure comparable exposure assessment periods between cases and controls, within 5-year age strata we randomly assigned lag periods (i.e., index dates) to controls corresponding to the frequency distribution of lags among the cases of comparable age. Any exposures and events reported after their index dates were discounted for both cases and controls. The average lag time between the index date and the date of interview was 2.5 years for both cases and controls.

The odds ratios (ORs) and 95% confidence intervals (95% CIs) for NHL according to various indices for pesticide exposure were calculated using the unconditional logistic regression model (Breslow and Day 1980), adjusted for selected covariates: four continuous variables (age at index date, year of interview, and frequencies of use of pain-relieving drugs and of cortisone injections) and five indicator variables (college education, surrogate interview, family history of hematologic cancer, and personal history of eczema/hives and of antihistamine use). These covariates were selected according to the following three criteria: *a*) known risk factors for NHL (age and family history of hematologic cancer); *b*) factors that influence data quality (education, surrogate status, and year of interview); and *c*) potential risk factors associated with pesticide product use/farm work (frequencies of use of pain-relieving drugs and of cortisone injections and personal history of eczema/hives and of antihistamine use) (Holly et al. 1999; Kato et al. 2002; McWhorter 1988). Whenever possible, the ORs were calculated for ordered categories (in quartiles, tertiles, or halves) of cumulative number of uses or total duration of exposure, compared with nonusers or no-exposure groups. Tests for linear trend in the logit of risk with increasing frequency or duration of

exposure were performed using natural-log–transformed continuous values. Selected analyses were repeated for subtypes of lymphoma. All statistical analyses were conducted using SAS software (SAS Institute, Cary, NC).

Results

First, we examined the associations with potential exposure to pesticides at work (Table 1). There was a marginal trend in risk of NHL with the number of years worked on a farm ($p = 0.053$). This trend became more significant ($p = 0.03$) when only farm work involving pesticide use was considered. The OR associated with such farm work of ≥ 10 years was 2.12 (95% CI, 1.21–3.71). Applying or handling pesticides by the women themselves was not associated with appreciably increased risk. Furthermore, < 50% of the women who applied/handled pesticides could recall the product names; thus, evaluation by chemical class of pesticides was not feasible. When types of crops handled by the study subjects were considered, the OR appeared to be higher for vegetables, grain, and other crops than for fruits and flowers, although none of them was statistically significant. Exposure to pesticides was also reported under various types jobs other than farming ($n = 61$). About half of these jobs ($n = 32$) involved a passive low level of exposure to periodic building/lawn treatment with pesticides. Common jobs in this category were restaurant jobs, office work, and miscellaneous other jobs. The second category of jobs ($n = 9$) represented a possible intermediate level of exposure, for example, retail jobs handling pesticides, crop-processing factory work, or working in an office adjacent to a farm or florist. The final category of jobs represented occupations that may have entailed direct exposure to pesticides through application ($n = 20$). This consisted of structure maintenance or environmental control jobs, horticultural work, veterinary jobs, and wood-handling factory jobs. The number of hours of actual exposure was reported to be much shorter for the low-exposure job category (median, 12 hr/year), compared with those in the intermediate- and high-exposure job categories (medians, 192 hr/year and 55 hr/year, respectively). With increasing cumulative number of hours exposed to pesticides at these jobs other than farming, there was a marginal increasing trend in risk of NHL ($p = 0.08$). When farming and other jobs associated with pesticide exposure were combined, the total duration at any of these jobs was significantly positively associated with the risk of NHL ($p = 0.005$). This increase in risk of NHL was more pronounced when exposure started in 1950–1969 than when it first occurred before or after this period.

The ORs and 95% CIs associated with pesticide use in and around the home are presented in Table 2. We grouped products based on the target pest. As a result, insecticides were categorized into those for crawling insects (ants, cockroaches/silverfish, and termites), for flying insects [bees, flies/mosquitoes, and moths (except mothballs) and indoor/outdoor fogger], mothballs, and antilice products. Products to treat indoor plants, trees/shrubs, or plants in garden/outdoor pots were combined into one group, that is, fungicides/plant pesticides. Products to treat lawns were considered herbicides/lawn pesticides. Products to control moths were assumed to be mothballs if they were used exclusively indoors; otherwise, they were counted in the categories for the flying insects. Correlations among these groups of home pesticide products ranged from –0.07 to 0.27. For all products combined, there was a linear increase in risk of NHL with increasing cumulative number of uses ($p = 0.004$). The positive trend was observed for most of the products groups, except for the herbicide and fungicide groups. Logistic regression for individual product groups with simultaneous adjustment for use of all other products revealed a significant positive association of NHL with mothballs ($p = 0.03$) and a marginally significant association with insecticides for flying insects/foggers ($p = 0.07$). When no-exposure groups were excluded from the trend analyses, the regression coefficient for mothballs approached zero, whereas those for the others changed minimally. When time of first use was analyzed for all household pesticide products combined, the association with NHL was significant only for pesticide use started during 1950–1969 (OR = 2.42; 95% CI, 1.16–5.02), whereas weaker associations were found for pesticide use started before 1950 or after 1969 (OR = 1.42 and 1.25, respectively; data not shown).

For pesticides for flying and crawling insects and for all pesticide products combined, we calculated the ORs for NHL according to application methods that were separated into three groups based on presumed exposure intensity, namely, pesticides applied by the respondent, applied indoors by others, or applied outdoors by others (Table 3). For individual groups of pesticide products, we also adjusted for other pesticide use via the same application method in these analyses. The positive linear trend with cumulative number

Table 1. ORs and 95% CIs for NHL associated with occupational pesticide exposures.

Type of exposure	No. of cases/controls	OR[a]	95% CI
Worked on a farm (years)			
0	258/352	1.00	—
0.1–4	26/35	1.03	0.56–1.90
4.1–8	25/28	1.33	0.71–2.48
8.1–15	32/19	2.16	1.09–4.26
≥ 15.1	27/28	1.40	0.74–2.63
		$p^b = 0.053$	
Worked on a farm using pesticides (years)[c]			
< 10	30/35	1.09	0.61–1.95
≥ 10	43/32	2.12	1.21–3.71
		$p = 0.020$	
Applied pesticides on a farm[c]			
Yes	25/24	1.18	0.59–2.38
Crops handled[c]			
Fruit	30/35	1.18	0.65–2.13
Vegetables	62/55	1.50	0.96–2.35
Grain	40/33	1.53	0.87–2.69
Other	18/17	1.74	0.79–3.82
Other occupations with pesticide exposure (cumulative hours)			
0	346/432	1.00	—
< 180	13/18	1.11	0.50–2.49
≥ 180	17/13	2.21	0.94–5.17
		$p = 0.077$	
Any occupations with pesticide exposure (years)			
0	277/371	1.00	—
0.1–4.9	16/26	1.01	0.48–2.11
5.0–9.9	22/25	1.13	0.58–2.20
10–17.9	29/20	2.72	1.37–5.40
≥ 18.0	28/20	1.80	0.93–3.48
		$p = 0.005$	
Year of starting job with pesticide exposure			
None	277/371	1.00	—
≤ 1949	39/35	1.24	0.71–2.16
1950–1969	32/21	2.86	1.50–5.45
1970–index date	23/35	1.19	0.63–2.26

[a]Adjusted for age at index date, family history of hematologic cancer, college education, surrogate status and year of interview, frequencies of use of pain-relieving drugs and of cortisone injections, history of eczema/hives, and history of antihistamine use. [b]*p*-Values for trend based on natural-log–transformed continuous values. [c]Compared with subjects who never worked on a farm.

of uses was most evident when pesticides were applied by women themselves for all products combined ($p = 0.01$), but the risk associated with insecticides for flying insects was only significant when they were applied outdoors by others. The association with mothballs was virtually the same when exposure occurred through self use or use by others, although a limited number of subjects were exposed through use by others (data not shown).

We also examined combined and separate effects of occupational and home pesticide exposure. To study combination effects, we divided exposures into two levels using the medians: 10 years for duration of jobs associated with pesticide exposure and 70 times for cumulative number of uses of any household pesticide products. The OR was 2.33 (95% CI, 0.93–5.85) for the subjects with higher exposures for both ($n = 54$), 1.46 (95% CI, 0.72–2.98) for those with higher exposure only at home or only at job ($n = 381$), and 1.00 (95% CI, 0.49–2.04) for those who had lower exposure for both or combinations of no exposure and lower exposure at home and job ($n = 354$), compared with the subjects with neither exposure ($n = 48$), and this trend was statistically significant ($p = 0.005$). When the subjects were limited to those without any occupational exposure to pesticides ($n = 648$), the association with cumulative number of uses of any type of home pesticide products remained highly statistically significant ($p = 0.005$). The number of women who were not exposed to any home pesticide products was too small ($n = 54$) to analyze the effects of occupational exposure separately. However, simultaneous adjustment for home pesticide use did not affect the association with occupational pesticide exposure ($p = 0.01$).

Table 4 presents the results of analysis by subtype of NHL according to levels of total pesticide exposure from work and around the home. There were no clear differences in trends in the ORs between B-cell and T-cell subtypes, but the increasing risk of NHL with the number of years worked in pesticide-related jobs and with the cumulative number of any pesticide product uses around the home was most pronounced for high-grade lymphoma ($p < 0.001$ and $p = 0.002$, respectively).

Discussion

The results of this case–control study suggest that exposure to pesticide products may lead to an increased risk of NHL among women. This finding was supported by the dose–response relationship observed with length of exposure, cumulative number of uses, and potential intensity of exposure.

Compared with studies using biologic or environmental samples at single time points, a questionnaire-based study has an advantage in the assessment of long-term exposure by reconstructing the whole personal history. However, it also has limitations. First, there may be bias in recall: cases with serious disease may be likely to report hypothesized exposures more completely than controls in good health. This especially may occur when there is enhanced public health concern about an exposure (Infante-Rivard and Jacques 2000; Weinstock et al. 1991), as may be the case for pesticides.

Obtaining information on specific chemicals over a long period of time is challenging, given the large number of products on the market, but is crucial when exposure effects may be cumulative. For nonoccupational exposure, Teitelbaum (2002) has suggested that asking about treatments for specific pest problems may be an effective way to help subject recall, a practice we implemented in designing our questionnaires. Notably, reasonable correlations have been observed between self-reported household chemical use and measurements of pesticides and their metabolites in urine of household members (Kieszak et al. 2002) and in indoor air (Van Winkel and Scheff 2001). Therefore, this type of questionnaire design seems useful in the assessment of household pesticides, at least for recent exposure. One shortcoming of our assessment of nonoccupational pesticide exposure is that we did not include dietary exposure, which may contribute a substantial fraction of pesticide exposures (Whitmore et al. 1994; Yess et al. 1991). However, misclassification of exposure due to the omission of dietary sources is most likely to be nondifferential because many foods are known to contain pesticide residues (Yess et al. 1991).

It has been suggested that self-reported occupational pesticide exposure tends to overestimate exposure (Daniels et al. 2001; Meinert et al. 2000) because people often do not know

Table 2. ORs and 95% CIs for NHL associated with home pesticide use.

Type of home pesticides	Cumulative no. of uses	No. of cases/controls	OR[a]	95% CI
Insecticides for flying bugs or foggers	0	117/161	1.00	—
	1–3	54/95	0.90	0.56–1.45
	4–16	53/78	1.07	0.66–1.75
	17–86	75/66	1.69	1.04–2.75
	≥ 87	77/63	1.31	0.80–2.15
			$p^b = 0.070$	
Insecticides for crawling bugs	0	124/171	1.00	—
	1–3	63/81	1.16	0.73–1.83
	4–15	51/77	0.76	0.46–1.24
	16–46	71/65	1.40	0.86–2.28
	≥ 47	67/69	1.18	0.73–1.92
			$p = 0.227$	
Anti-lice products	0	229/307	1.00	—
	1	56/71	1.20	0.76–1.89
	2–3	45/37	1.48	0.87–2.52
	≥ 4	36/37	1.23	0.69–2.18
			$p = 0.224$	
Mothballs	0	217/354	1.00	—
	1–10	39/24	2.19	1.21–3.97
	11–25	34/32	1.36	0.77–2.42
	26–44	38/27	1.82	1.01–3.29
	≥ 45	39/25	1.33	0.70–2.52
			$p = 0.025$	
Herbicides/lawn pesticides	0	231/287	1.00	—
	1–4	33/44	0.88	0.50–1.53
	5–17	30/47	0.74	0.42–1.32
	18–39	27/41	0.98	0.56–1.71
	≥ 40	40/37	0.89	0.51–1.54
			$p = 0.658$	
Fungicides/plant pesticides	0	201/263	1.00	—
	1–7	35/58	1.01	0.60–1.71
	8–27	36/58	0.80	0.48–1.34
	28–79	52/42	1.42	0.85–2.39
	≥ 80	51/42	1.07	0.63–1.84
			$p = 0.596$	
Any type	0	23/33	1.00	—
	1–20	60/135	0.81	0.40–1.68
	21–69	91/105	1.62	0.80–3.31
	70–184	94/102	1.38	0.67–2.82
	≥ 185	108/88	1.62	0.79–3.32
			$p = 0.004$	

[a]Adjusted for age at index date, family history of hematologic cancer, college education, surrogate status and year of interview, frequencies of use of pain-relieving drugs and of cortisone injections, history of eczema/hives, and history of antihistamine use; use of each type of pesticide was adjusted for use of other types of pesticides combined. [b]*p*-Values for trend based on natural-log–transformed continuous values.

for sure about actual chemical contents used at their work places. Farmers may be an exception (Blair and Zahm 1990), but indeed fewer than half of the women who applied pesticide themselves in this study could recall at least one of the product names they used. This proportion appears to be lower than in farmer studies (Dosemeci et al. 2002; Zahm et al. 1993) but may be because most of the farm work was in the distant past (median interval between last farm work and interview was 37 years, and median duration of farm work was only 8 years). Poor recall may also account for our failure to detect an excess risk among women who applied or handled pesticides. However, reentry to areas that were recently treated with pesticides for harvesting may result in greater cumulative exposure to pesticide residues than application itself (Garcia 2003); Coronado et al. (2004) recently reported that detectable levels of pesticide metabolite were not higher among workers who were engaged in mixing, loading, or applying pesticide formulations than among those who did not perform these tasks, contrary to expectation. Some investigators have found that including information from surrogates biases the results (Blair and Zahm 1990; Waddell et al. 2001), but when we limited our analysis to the subjects themselves, the strength of the associations remained almost the same as those observed in the entire sample.

Finally, the relatively low overall participation rate in this study raises issues of selection bias and of generalizability of the results. The probable reasons for the lower response rates among the DMV controls and the cases have been discussed elsewhere (Kato et al. 2002). Cases in this study were similar in age distribution to all the cases diagnosed in NYS during the same time period, but white and married women were overrepresented in both the case and control groups. Although we do not have external data to estimate the magnitude of selection bias, the results of hypothetical sensitivity analyses based on a selection bias factor defined by Rothman and Greenland (1998) suggest that the ORs obtained in this study are more likely to have been underestimated than overestimated. This relies on an assumption that exposed controls were more likely to respond to this survey than were nonexposed controls because both the study invitation letter and the study packet (product list) indicated that pesticides were one of our major research interests, whereas this selection should play a minor role among the

Table 3. ORs and 95% CIs for NHL associated with selected home pesticides by application type.

		Applied by self			Indoor application by others			Outdoor application by others		
Pesticide type	Quartile[a]	No. of cases/controls	OR[b]	95% CI	No. of cases/controls	OR	95% CI	No. of cases/controls	OR	95% CI
Insecticides for flying bugs or foggers	1	42/48	1.60	0.92–2.77	28/40	1.10	0.59–2.02	27/51	0.68	0.37–1.28
	2	39/52	1.09	0.62–1.91	13/26	0.95	0.42–2.12	37/54	1.07	0.62–1.84
	3	48/43	1.73	0.97–3.03	29/29	1.76	0.90–3.42	43/42	1.56	0.88–2.78
	4	46/44	0.97	0.55–1.71	28/27	1.28	0.65–2.52	53/32	2.37	1.32–4.24
			$p^c = 0.653$			$p = 0.149$				$p = 0.005$
Insecticides for crawling bugs	1	35/51	0.92	0.53–1.61	33/42	1.06	0.59–1.90	25/27	1.16	0.60–2.26
	2	36/50	0.82	0.46–1.44	32/44	1.16	0.65–2.08	25/27	1.12	0.57–2.20
	3	48/40	1.65	0.94–2.88	38/39	1.14	0.62–2.07	25/27	0.87	0.42–1.78
	4	42/44	1.27	0.72–2.24	38/36	1.52	0.84–2.75	31/21	1.69	0.85–3.38
			$p = 0.098$			$p = 0.327$				$p = 0.205$
Any type	1	55/114	0.88	0.42–1.83	44/59	1.26	0.58–2.76	54/86	1.13	0.54–2.38
	2	76/93	1.36	0.66–2.80	45/71	1.35	0.63–2.90	66/74	1.58	0.75–3.34
	3	84/86	1.51	0.73–3.12	54/56	1.53	0.70–3.32	69/72	1.44	0.69–3.04
	4	92/77	1.64	0.79–3.40	59/51	1.68	0.77–3.67	77/63	1.58	0.75–3.32
			$p = 0.012$			$p = 0.141$				$p = 0.177$

[a]Quartile cutoff points for self, indoor by others, and outdoor by others are, respectively, 1–3, 4–15, 16–75, ≥ 76; 1, 2–3, 4–21, ≥ 22; and 1–2, 3–9, 10–48, ≥ 49 for insecticides for flying bugs/foggers; 1–3, 4–13, 14–41, ≥ 42; 1, 2–8, 9–24, ≥ 25; and 1, 2–8, 9–20, ≥ 21 for insecticides for crawling bugs; and 1–8, 9–36, 37–100, ≥ 101; 1–2, 3–9, 10–35, ≥ 36; and 1–6, 7–27, 28–80, ≥ 81 for any type. [b]Adjusted for age at index date, family history of hematologic cancer, college education, surrogate status and year of interview, frequencies of use of pain-relieving drugs and of cortisone injections, history of eczema/hives, and history of antihistamine use, in comparison with a common reference group of subjects with no exposure to a given pesticide group through any application methods. Use of each type of pesticide was adjusted for use of other types of pesticides combined. [c]*p*-Values for trend based on natural-log–transformed continuous values including level 0 (reference group with no exposure through any application methods).

Table 4. ORs[a] and 95% CIs for NHL associated with occupational and home pesticide exposure by type of NHL.

	Level of pesticide exposure										
	0	1			2			3			
Exposure type, NHL cell type, and grade	No. of cases	No. of cases	OR	95% CI	No. of cases	OR	95% CI	No. of cases	OR	95% CI	*p*-Value for trend[b]
At job[c]											
B-cell	238	32	1.06	0.61–1.82	24	2.48	1.21–5.08	24	1.77	0.90–3.48	0.014
T-cell	17	3	2.94	0.61–14.03	3	18.20	3.47–95.44	2	1.79	0.25–12.77	0.005
Low	107	16	1.03	0.52–2.04	17	3.99	1.80–8.80	12	1.80	0.79–4.11	0.007
Intermediate	151	16	1.04	0.53–2.05	10	1.64	0.67–4.04	10	1.34	0.56–3.20	0.276
High	14	4	3.07	0.79–11.98	2	7.27	1.31–40.40	5	6.11	1.46–25.57	< 0.001
No. of controls	371	51			20			20			
At home[d]											
B-cell	73	80	1.82	1.16–2.86	79	1.52	0.96–2.40	90	1.76	1.11–2.81	0.014
T-cell	5	5	4.22	0.88–20.25	3	1.56	0.27–8.93	12	3.58	0.83–15.42	0.077
Low	35	34	1.53	0.86–2.73	41	1.44	0.82–2.54	44	1.49	0.83–2.66	0.143
Intermediate	45	49	2.03	1.16–3.55	44	1.64	0.92–2.93	51	1.98	1.11–3.52	0.026
High	2	6	9.90	1.49–65.77	5	6.25	0.92–42.72	12	15.02	2.47–91.29	0.002
No. of controls	168	105			102			88			

[a]Adjusted for age at index date, family history of hematologic cancer, college education, surrogate status and year of interview, frequencies of use of pain-relieving drugs and of cortisone injections, history of eczema/hives, and history of antihistamine use. [b]*p*-Values for trend based on natural-log–transformed continuous values. [c]Total number of years at job with pesticide exposure, defined as follows: 0, none; 1, < 10 years; 2, 10–17.9 years; 3, ≥ 18 years. [d]Cumulative number of uses of any home pesticides, defined as follows: 0, 0–20; 1, 21–69; 2, 70–184; 3, ≥ 185.

cases who were already motivated because of their diagnosed disease. In addition, the DMV controls, who were < 65 years of age and had a lower overall participation rate than cases, may have been more motivated to participate in research related to environmental issues and therefore may have had better recall of pesticide exposure. This would tend to counterbalance the hypothesized biased recall among cases discussed above, unless such motivated people tend to live in better housing conditions that require less use of pesticides.

It is possible that pesticide use is a marker for other possible causative factors for NHL. For instance, occupational exposure to pesticides is often accompanied by exposure to other possible hazardous substances, such as solvents, fuels, and dusts (Maroni and Fait 1993; Morrison et al. 1992), that have been associated with increased NHL risk (Mao et al. 2000; Rego 1998). Similarly, people who use pesticides in and around the home may tend to use other household chemicals more often than those who do not. Another possibility is that pesticide use is an indicator of exposure to insects that may act as vectors to transmit viruses and bacteria. Certain types of viruses and bacteria have been identified as etiologic factors for NHL (Pagano 2002; Persing and Prendergast 1999).

Some earlier studies have pointed to associations between specific types of pesticide or pesticide groups and NHL risk (Dich et al. 1997). Three groups of pesticides have received special research attention: phenoxy herbicides and organochlorine and organophosphate insecticides. However, the results have been inconclusive because initial positive findings that were usually based on small numbers of subjects have often not been confirmed in larger studies or in multivariate analyses taking other pesticides into consideration (Cantor et al. 2003; Hardell et al. 2002; Morrison et al. 1992). In this study, we were not able to analyze any specific classes of chemicals because the women had limited recall of the particular chemicals used. Yet, the finding that pesticide use starting in 1950–1969 was associated with the most pronounced risk of NHL suggests a potential role of organochlorine insecticides that became widely available during this period. Alternatively, it may be a chance finding or simply indicate that a 25–45 year latency period is typical of pesticide-induced NHL.

A finding that is relatively unique in this study is the increased risk of NHL associated with mothball use, although a dose response was not clearly demonstrated among users. In the United States, major chemical constituents of mothballs are naphthalene or *para*-dichlorobenzene (*p*-DCB). These chemicals are also constituents of other common household products, such as air fresheners and solid toilet bowl deodorizers, which were not included in our questionnaire. Vapors from mothballs can be absorbed not only by inhalation but also by direct skin contact. Both of these chemicals are known to have hematotoxicity, including reports of hemolytic anemia (Hallowell 1959; Santucci and Shah 2000) and aplastic anemia (Harden and Baetjer 1978). In addition, *in vitro* and *in vivo* studies have demonstrated cytotoxicity and genotoxicity of these chemicals and their metabolites (Bagchi et al. 1998; Brusick 1986; Carbonell et al. 1991; Tingle et al. 1993), and carcinogenicity has been shown in animal models (Preuss et al. 2003; Umemura et al. 1992). Importantly, both naphthalene and *p*-DCB are among the most ubiquitously detected hazardous household chemicals in indoor air (Van Winkel and Scheff 2001), and concentrations in indoor air samples and urine samples of residents are correlated with reported mothball use (Kieszak et al. 2002; Van Winkel and Scheff 2001). This suggests that the association between mothball use and NHL merits further investigation.

We found that the association with pesticide exposure was most pronounced for high-grade lymphoma. The results for subtypes of NHL, however, should be interpreted cautiously because of small numbers of cases by subtype and because of the multiple comparisons involved. Data have been limited and inconsistent in earlier studies concerning types of lymphoma associated with pesticide exposure. There have been reports of relatively stronger associations of various types of agricultural insecticides with low-grade lymphoma (Nanni et al. 1996), carbamate insecticides with small lymphocytic lymphoma (Zheng et al. 2001), organophosphate pesticides and phenoxy herbicides with intermediate grade lymphoma (Waddell et al. 2001; Zahm et al. 1990), and phenoxy herbicides with B-cell lymphoma (Zahm et al. 1990). Schroeder et al. (2001) reported that a type of B-cell lymphoma that carries a specific chromosomal translocation was associated with occupational exposure to several types of pesticides. Finally, a case–control study of NHL among children revealed that the associations with parental occupational and household exposure to pesticides were more clear for higher grade lymphomas, whereas there were no differences between B- and T-cell types (Buckley et al. 2000). Although mechanistic bases for possible carcinogenic actions by pesticides are largely unknown, Schroeder et al. (2001) speculate that they are different from those for NHL linked to immunosuppression, based on their observation of a specific genetic change associated with pesticide exposure.

In conclusion, the results of our case–control study suggest an association of pesticide exposures with NHL. However, methodologic limitations related to selection and recall bias suggest caution in inferring causation. In order to draw more definitive conclusions and to make public recommendations, more research is needed, integrating various types of studies, such as surveillance for personal pesticide product use, development and application of new biomarkers for pesticide exposure, and assessment of genetic polymorphisms related to pesticide metabolism.

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Review

Non-Hodgkin Lymphoma and Occupational Exposure to Agricultural Pesticide Chemical Groups and Active Ingredients: A Systematic Review and Meta-Analysis

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Abstract: This paper describes results from a systematic review and a series of meta-analyses of nearly three decades worth of epidemiologic research on the relationship between non-Hodgkin lymphoma (NHL) and occupational exposure to agricultural pesticide active ingredients and chemical groups. Estimates of associations of NHL with 21 pesticide chemical groups and 80 active ingredients were extracted from 44 papers, all of which reported results from analyses of studies conducted in high-income countries. Random effects meta-analyses showed that phenoxy herbicides, carbamate insecticides, organophosphorus insecticides and the active ingredient lindane, an organochlorine insecticide, were positively associated with NHL. In a handful of papers, associations between pesticides and NHL subtypes were reported; B cell lymphoma was positively associated with phenoxy herbicides and the organophosphorus herbicide glyphosate. Diffuse large B-cell lymphoma was positively associated with phenoxy herbicide exposure. Despite compelling evidence that NHL is associated with certain chemicals, this review indicates the need for investigations of a larger variety of pesticides in more geographic areas, especially in low- and middle-income countries, which, despite producing a large portion of the world's agriculture, were missing in the literature that were reviewed.

Keywords: pesticides; insecticides; herbicides; fungicides; lymphoma; non-Hodgkin; occupational; agricultural

1. Introduction

Striking increases in the incidence of non-Hodgkin lymphoma (NHL) cancer have occurred in the last 30 years [1,2], and interest in identifying environmental and occupational exposures associated with this cancer has accompanied this trend. Several environmental exposures have been proposed and investigated as potentially important—pesticides, dioxins, solvents, oils, and viruses, among others [3,4]. Farmers experience low overall mortality but high rates of some cancers; this suggests that some or several agricultural exposures may be key determinants [5,6]. Indeed, positive associations between NHL and farm related exposures, including pesticides, fertilizers, chemicals, animals, viruses, and endotoxin, have been observed previously [3,5,7]. However, the wide variety of chemical and microbial exposures that occur simultaneously in agricultural production makes disentangling the effects of these factors challenging. Of the many exposures experienced in farm settings, pesticides have drawn particular attention, especially since the increased incidence of NHL in the mid- to late-1900s followed widespread use of synthetic organic pesticides [4]. Also, several epidemiologic studies have reported positive associations between NHL and pesticide exposure in occupational manufacturing settings [8,9].

The United States Environmental Protection Agency defines pesticides as substances intended to prevent, destroy, repel, or mitigate a pest [10]. Within this broad category, pesticides are often grouped according to the type of pests that they control; for example, fungicides are used to kill fungi, insecticides to kill insects, and herbicides to kill weeds and plants. In addition to function, pesticides vary in terms of structure, and they are sometimes grouped according to chemical relationships. Furthermore, applicators often use a variety of pesticides simultaneously. These characteristics make designing and conducting epidemiologic studies of their health effects both challenging and expensive.

Because pesticides are thought to have different toxicologic and immunologic effects, identifying the chemicals and chemical groups that are most dangerous to humans and non-target living organisms is important [11]. From a research perspective, the decision about what chemicals to investigate has implications for disease prevention, and it impacts the information that is available to policy makers and the public.

These challenges and needs motivated us to systematically review the published epidemiologic literature of relationships of NHL with occupational exposures to agricultural pesticide chemical groups and active ingredients. The primary objectives of this paper were to investigate the depth of the literature on the relationship between specific pesticide chemicals and NHL, to identify gaps in this area of research, and to elucidate pesticide chemical groups and active ingredients that have shown particularly strong relationships with NHL. To help us to achieve these objectives, we conducted a series of meta-analyses of associations of individual pesticide chemicals with NHL.

2. Methods

2.1. Article Identification

We performed a search of literature on associations between occupational pesticide exposure and NHL. We restricted our search to articles published since 1980. This time period is consistent with that used in previous meta-analyses of farming exposures [5], and it captured the epidemiologic literature

that has not been reviewed by early IARC monograph evaluations of pesticides [12]. The search used combinations of the following words: occupational exposure, pesticides, insecticides, herbicides, fungicides, neoplasms, cancer, lymphomas, non-Hodgkin lymphoma, cancer mortality, agricultural workers' diseases/chemically induced, and humans. We entered combinations of these terms into PubMed and Web of Science. Details of the search are given in Supplementary file S1.

2.2. Article Selection

To identify eligible studies, we reviewed the titles and abstracts of papers. When it was unclear from the abstract and title whether the paper fit these criteria, the full text of the paper was reviewed. We included estimates from papers with the following characteristics:

(1) Written and published in English;
(2) Reported results of analyses of case control or cohort epidemiology studies;
(3) Reported results of studies that used interviews, questionnaires, and/or exposure matrices to assess exposure;
(4) Reported associations of NHL with occupational, agricultural pesticide exposures;
(5) Reported quantitative associations of NHL overall and/or NHL subtypes with specific individual active ingredients or chemical groups.

We excluded papers with the following characteristics:

(1) Written in a language other than English;
(2) Did not report on associations with NHL;
(3) Were a commentary, letter to the editor, or monograph;
(4) Did not report associations with individual pesticide active ingredients or chemical groups; we excluded papers that reported associations with only the broadly defined categories of pesticide, insecticide, herbicide, or fungicide;
(5) Reported results of analyses of ecologic studies;
(6) Reported results of analyses of data from studies that were not case control or cohort in design;
(7) The exposure definition/classification was ambiguous;
(8) The exposure route was not occupational;
(9) The exposure route was not agricultural;
(10) Reported only associations within unique subpopulations (e.g., HIV positive patients);
(11) Reported analyses of manufacturing cohorts;
(12) Reported associations with NHL as a second primary;
(13) Reported results of studies in which exposure was assessed using biological markers.

2.3. Data Extraction

We extracted the following information from the full text of each eligible paper:

- author;
- publication year;
- study location;

- study design (case-control or cohort);
- source population for the controls in case-control studies;
- whether case-control studies were matched, and if so, the matching factors;
- diagnosis period if a case-control study or cancer follow-up period if a cohort study;
- number of cohort participants or number of cases and controls;
- cancer definition or ICD codes used to identify the cancers;
- method of assessing exposure;
- exposure metrics and definitions;
- referent categories used in the analysis;
- active ingredient(s) and/or chemical group(s) studied;
- covariates entered into the model to adjust for confounding;
- type of effect estimate reported;
- number of exposed participants;
- effect estimates and confidence interval limits; and
- gender restrictions, if any.

We also identified papers that were related to each other (e.g., pooled analyses that used data that were analyzed and reported on previously, papers that reported on different analyses from the same study, studies that were follow up analyses of the same population). In cases of related papers, we used a specific set of rules to decide which effect estimate to report and use in the meta-analyses; this rule is described in Section 2.5.

2.4. Chemical Group Classification

We reported results for all chemical groups for which there was information from the available literature. We did not consider exposures to chlorophenols in this paper, since much of the exposure to this chemical group comes from non-agricultural settings. We classified pesticide active ingredients into chemical groups based on Alan Wood's classification system [13].

2.5. Reporting of Results for the Systematic Review

From every relevant paper, we extracted an effect estimate for each active ingredient and/or chemical group. We extracted results for associations with NHL, and when available, for associations with subtypes of NHL.

We used the following algorithm to determine which effect estimates to use:

(1) For related papers that examined the same exposure/outcome association, we used the results from the most complete and updated analysis with the greatest number of participants;

(2) If more than one exposure definition was considered and reported, we used the definition that best represented agricultural exposures (e.g., we selected results for farmers who worked with phenoxy herbicides, instead of results for herbicide applicators, gardeners, or landscapers);

(3) The various papers used different confounder adjustment sets, which were selected based on different criteria. In an effort to use the most unbiased estimate, we extracted the most adjusted effect estimate;

(4) Most papers defined exposure dichotomously. Papers that reported results according to more than two categories used a variety of definitions for the exposure metrics, including duration of use, days/year of use, time since first exposure, and cumulative days of exposure. Because the definitions and metrics used to define categories varied, it was not possible to combine estimates based on multiple categories of exposure in formal meta-analyses. Therefore, for the meta-analyses, we used the result for the dichotomously defined exposure with the greatest number of exposed cases. To assess dose-response relationships, we qualitatively examined results in association with multiple categories;

(5) Some papers only reported results in association with multiple categories of exposure. We extracted these results for the systematic review, since they can be used to qualitatively evaluate trends in association of NHL with active ingredient or chemical group and are important for identifying dose-response relationships;

(6) Some studies only reported estimates of association between pesticide exposures and subtypes of NHL. We abstracted these estimates for presentation and analysis of association of pesticide exposures with NHL subtypes.

We present results from the systematic review sorted by chemical group and, within chemical group, by active ingredient.

2.6. Meta Analysis

2.6.1. Grouping

When possible, we conducted separate meta-analyses for each chemical group and active ingredient. We conducted meta-analyses for associations of these pesticides with NHL and NHL subtypes. Although we abstracted results according to dichotomous exposure and multiple levels of exposure, we conducted formal meta-analyses for dichotomously categorized exposures only.

2.6.2. Analytic Methods

Because we identified a variety of sources of heterogeneity between papers, we decided a priori to calculate meta- risk ratio (RR) estimates and 95% confidence intervals (CIs) using random effect models, allowing between study heterogeneity to contribute to the variance [14,15]. We report I^2 values, which represent the percentage of the total variance explained by study heterogeneity and measure inconsistency in results. Larger I^2 values indicate greater inconsistency [15]. We did not perform formal heterogeneity tests; Cochran's Q statistic has been shown to have low power to detect true heterogeneity across studies, especially in meta-analyses that include a small number of papers [15]. Following recommendations for meta-analyses of observational studies, we also identified possible sources of heterogeneity and used sensitivity analyses to evaluate these, as described in Section 2.6.3 [16]. We evaluate the meta- estimates of association based on the magnitude of the point estimate and interpret the associated 95% CIs as indicators of precision. To aid this interpretation, we have calculated and reported confidence limit ratios (CLRs), which are the ratio of the upper to the lower CI limit [17]. We also present forest plots for meta-analyses to which five or more papers contributed.

2.6.3. Sensitivity Analysis

We conducted sensitivity analyses to evaluate robustness of our results to the following sources of heterogeneity: study design (case-control versus cohort), gender (male only versus both genders), geographic area, decade of cancer diagnosis, and source of the controls in case-control studies (population-based versus hospital).

One paper presented results of analyses of women only [18]. Thus, we were not able to conduct a sensitivity analysis for analyses of women; we were able to conduct sensitivity analyses using papers that reported results for men and for men and women. Only two papers reported estimates of association from studies in which controls were drawn from hospitals, and these two studies reported associations of NHL with different pesticides. Therefore, our sensitivity analysis of the control source in case-control studies was restricted to controls drawn from the population. Data from only one cohort study contributed to our meta-analyses. Therefore, we could not restrict meta-analyses to cohort studies only.

The geographic areas that we investigated separately in sensitivity analyses were North America, the United States, Europe, and Sweden. We selected these because there was more than one study within each area that investigated associations of NHL with a particular pesticide. In addition to maintaining Sweden and the United States in sensitivity analyses of Europe and North America, respectively, we analyzed results from Sweden separately from the rest of Europe, and results from the United States separately from Canada. We conducted these separate analyses because more than one paper reported effect estimates of association with a pesticide from each of these countries, and because we believed effects might be different when separated from the rest of the continent. Although we identified papers from Australia and New Zealand we were not able to analyze these separately because there was not more than one effect estimate of association with an individual pesticide from either country.

We investigated the following diagnosis periods: 1975–1989, 1990–1999, and year 2000 and later. If any part of the diagnosis period overlapped these periods, we included the estimate from the paper in the sensitivity analysis. We selected these periods based on the periods that appeared in the papers that we reviewed and on the different editions of the ICD coding systems [1].

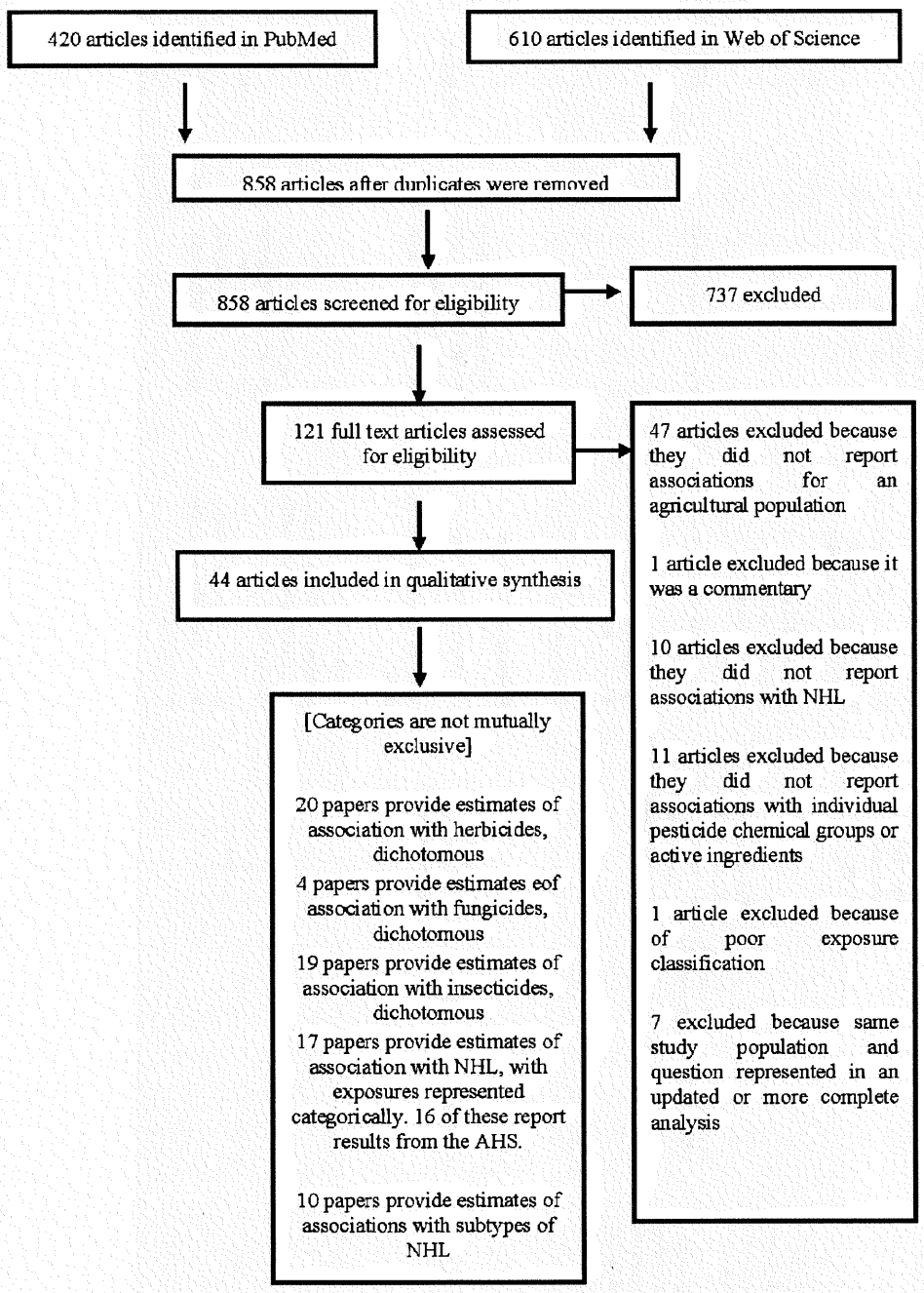
After performing meta-analyses for each active ingredient or chemical group, we repeated analyses, removing studies that differed from the others based on the above-described characteristics. In cases when results from individual studies were also represented in papers that analyzed these data pooled with data from other studies, we performed sensitivity analyses by replacing the results from the pooled analyses with the individual studies, or the individual studies with the results from the pooled analyses.

3. Results

3.1. Systematic Review

The PubMed and Web of Science searches yielded 858 unique articles (Figure 1). After screening the abstracts and titles, we excluded 737 articles. Of the remaining 121 articles, 47 were excluded because they reported results within a non-agricultural population. We decided to exclude non-agricultural populations because the nature of exposure they receive is different compared to agricultural groups. Because of contamination and production of multiple chemicals simultaneously, it is difficult to determine the exact chemical to which manufacturing cohort participants have been exposed.

Figure 1. Flow chart showing the articles that were included and excluded in the systematic review, with reasons for the exclusions.



After excluding 27 additional articles because they did not meet one or more of the inclusion criteria described in the methods section, we included 44 papers in our qualitative synthesis. Of these, 20 papers provided estimates of association with herbicide chemical groups or active ingredients, four papers provided estimates of association with fungicides, and 17 with insecticides.

3.2. Summary of Studies from Which Estimates were Extracted

A summary of the 44 papers from which effect estimates were abstracted is presented in Table 1.

Table 1. Summary of papers from which effect estimates were extracted.

Author, year, location	Design	Source for controls	Matching	Diagnosis or follow-up period (cancer)	No. Participants	Exposure assessment	Referent category for exposure, exposure definition(s)/metric	Men only	Adjustment set	Pesticides	Reported results by subtype
Barry 2012 [19] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2007	53,588	Self-administered questionnaire completed during enrollment and interviewer administered follow-up questionnaire	Referent: No exposure Intensity weighted lifetime exposure days, 15 year lag Intensity weighted lifetime exposure days, no lag	No	Age, gender, race, state of residence, applicator type, enrollment year, cigarette smoking, alcohol consumption, education, family history of cancer, 5 most correlated pesticides	Methyl bromide	No
Baris 1998 [20] Iowa, Kansas, Minnesota, Nebraska, USA	Pooled analysis of 3 CC studies	Population	Matched by race, gender, age, and vital status at the time of interview, year of death for controls matched to deceased cases	Dx period [1]: 1979–1983	993 cases/2,918 controls	Telephone interviews (Kansas and Nebraska, USA), In-person interviews (Iowa and Minnesota)	Referent: Non-farmers Used *vs.* did not use on crops and animals , Used *vs.* did not use on crops, Used *vs* did not use on animals Duration of use, in years (1-4, 5-9, ≥10) Days/year of use (≤5, >5)	Yes	Age, state of residence	DDT	Yes
Beane Freeman 2005 [21] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2007	23,106	Two self-administered questionnaires	Referent: No exposure Lifetime exposure days Intensity weighted exposure days	Yes	Age, smoking, education, family history of cancer, state of residence, total days of any pesticide application	Diazinon	No
Beane Freeman 2011 [22] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2007	36,357	A self-administered questionnaire	Referent 1: No exposure Referent 2: Lowest quartile of exposure Lifetime days of exposure Intensity weighted lifetime days of exposure	No	Age, state, license type, gender, smoking status, alcohol consumption, education, use of most highly correlated pesticides	Atrazine	Yes

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Table 1. *Cont.*

Author, year, location	Design	Source for controls	Matching	Diagnosis or follow-up period (cancer)	No. Participants	Exposure assessment	Referent category for exposure, exposure definition(s)/metric	Men only	Adjustment set	Pesticides	Reported results by subtype
Blair 1998 [23] Iowa, Kansas, Minnesota, Nebraska, USA	CC	Population	Matched by race, gender, age, vital status at the time of interview	Dx period [1]: 1979–1983	987 cases/2,895 controls	Telephone interviews (Kansas and Nebraska, USA), In-person interviews (Iowa and Minnesota)	Referent: nonfarmer Farmers who ever used Days/year of use (≤4 days, ≥5 days) First lindane use (≥20 years ago, <20 years ago)	Yes	Age, proxy/direct interview, state of residence	Lindane	Yes
Bonner 2010 [24] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2005	44,624	Self-administered questionnaires	Referent 1: Nonexposed Referent 2: Lowest tertile of exposure Intensity weighted lifetime exposure days	No	Age, gender, education, family history of cancer, smoking, alcohol, year of enrollment, state of residence, correlated pesticides	Terbufos	No
Bonner 2005 [25] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2001	49,877	Self-administered questionnaire	Referent 1: Nonexposed Referent 2: Lowest tertile of exposure Lifetime exposure days Intensity weighted lifetime exposure days	No	Age, gender, education, family history of cancer, smoking, alcohol, year of enrollment, state of residence, exposure to correlated pesticides	Carbofuran	No
Bonner 2007 [26] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2002	19,717	Self-administered questionnaire	Referent 1: Nonexposed Referent 2: Lowest tertile of exposure Lifetime exposure days, Intensity weighted lifetime exposure days	No	Age, gender, smoking, alcohol, education, family history of cancer, year of enrollment, state of residence	Malathion	No

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Table 1. *Cont.*

Author, year, location	Design	Source for controls	Matching	Diagnosis or follow-up period (cancer)	No. Participants	Exposure assessment	Referent category for exposure, exposure definition(s)/metric	Men only	Adjustment set	Pesticides	Reported results by subtype
Cantor 1992 [27] Iowa and Minnesota, USA	CC	Population	Matched by 5-year age group, vital status at time of interview, and state of residence	Dx period [1]: 1979–1983	622 cases/1,245 controls	In-person interviews	Referent: Non-farmers Ever handled, Handled prior to 1965 Handled without protective equipment	Yes	Age, vital status, state, cigarette smoking, family history of lymphohematapoeitic cancer, high risk occupations, high risk exposures	2,4-D, 2,4,5-T, alachlor, atrazine, aldrin, bentazon, butylate, carbofuran, carbaryl, chlordane, chloramben, copper acetoarsenate, cyanazine, coumaphos, diazinon, dicamba, dichlorvos, DDT, famphur, Flyspray, fonofos, glyphosate, heptachlor, lindane, malathion, methoxychlor, metribuzen, nicotine, phorate, popachlor, rotenone, toxaphene, trifluralin, turbufos,	No
Cocco 2013 [28] Multicentre: Czech Republic, France, Germany, Italy, Ireland, Spain	CC	Population (German and Italian centers), Hospital (Czech Republic, French, Irish, Spanish centers)	Matched by gender, 5-year age group, and residence area	1998–2004	2,348 cases/2,462 controls	Structured in-person interviews conducted by trained interviewers, jobs were coded by industrial hygienists; industrial hygienists and occupational experts reviewed the questionnaires and job modules to assess exposures to pesticides (with the help of a crop exposure matrix)	Referent: Never exposed Ever exposed, by level of industrial hygienists's degree of confidence that the participant was truly exposed to the agent: Any level of confidence High confidence	No	Age, gender, education, study center	Carbamates, OPs, OC, Triazines and triazoles, phenoxy acids, chlorophenols, mancozeb, methomyl, dimethoate, glyphosate, DDT, endosulfan, 2,4-D, MCPA	Only reported for subtypes
Delancey 2009 [29] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2004	23,072	Two self-administered questionnaires	Referent: Lowest tertile of exposure Lifetime exposure days Intensity weighted lifetime exposure days	Yes	Age, smoking, alcohol consumption, education, family history of cancer, state of residence, exposure to all pesticides	Metribuzin	No

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Table 1. *Cont.*

Author, year, location	Design	Source for controls	Matching	Diagnosis or follow-up period (years)	No. Participants	Exposure assessment	Referent category for exposure, exposure definition(s)/metric	Men only	Adjustment set	Pesticides	Reported results by subtype
de Roos 2003 [30] Iowa, Kansas, Minnesota, Nebraska, USA	CC	Population	Matched by race, gender, age, vital status at the time of interview	Dx period [1]: 1979–1983	650 cases/1,933 controls	Telephone interviews (Kansas and Nebraska, USA). In-person interviews (Iowa and Minnesota)	Referent: Not exposed Exposed	Yes	Age, study site, use of all other pesticides	Aldrin, bufencarb, carbaryl, carbofuran, chlordane, copper acetoarsenite, coumaphos, DDT, diazinon, dichlorvos, dieldrin, dimethoate, ethoprop, famphur, fly/tick/lice spray, fonofos, heptachlor, lead arsenate, lindane, malathion, methoxychlor, mevinphos, phorate, pyrethrins, rotenone, tetrachlorvinphos, toxaphene, terbufos, alachlor, atrazine, bentazon, butylate, chloramben, cyanazine, 2,4-D, dicamba, EPTC, glyphosate, linuron, MCPA, metolachlor, metribuzin, paraquat, propachlor, sodium chlorate, 2,4,5-T, trifluralin	No
de Roos 2005 [31] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2001	54,315	Self-administered questionnaire	Referent 1: Never used Referent 2: Lowest tertile of exposure Ever used Cumulative exposure days Intensity weighted exposure days	No	Age at enrollment, education, cigarette smoking, alcohol consumption, family history of cancer, state of residence, other pesticides	Glyphosate	No
Eriksson 2008 [32] Sweden	CC	Population	Matched in 10 year age and gender groups to mirror the age and gender distribution of the cases	Dx period: 1999–2002	1,163 cases/1,016 controls	Telephone interview on life style factors and diseases; Self-administered questionnaire on work history and chemical exposures; follow up telephone interviews to collect incomplete data	Referent: Never exposed Ever exposed, Days of exposure (categorized at the median of the exposure distribution),	No	Age, gender, year of Dx/enrollment	Phenoxyacetic acids, MCPA, 2,4,5-T and/or 2,4-D, glyphosate	Yes

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Table 1. *Cont.*

Author, year, location	Design	Source for controls	Matching	Diagnosis or follow-up period (cancer)	No. Participants	Exposure assessment	Referent category for exposure, exposure definition(s)/metric	Men only	Adjustment set	Pesticides	Reported results by subtype
Hardell 2002 [33] Sweden	CC, pooled analysis of two studies, one of hairy cell lymphoma and one of NHL	Population	Matched by age and county	Dx period: 1987–1990 (NHL); 1987–1992 (hair cell lymphoma)	515 cases/1,141 controls	Self-administered questionnai-re supplement-ed by telephone interviews by a trained interviewer when information was unclear	Referent 1: Not exposed Ever exposure, High exposure (>median number of days for exposed participants) Low exposure (<=median number of days for exposed participants) Years between first exposure and diagnosis: Referent 2: 1-10years, >10-20 years, >20-30 years, >30 years Years between last exposure and diagnosis: Referent 3: 1-10 years, >10-20 years, >20-30 years, >30 years Decade of exposure	Yes	Study, study area, vital status, age	Phenoxy acids, MCPA, 2,4-D + 2,4,5-T, glyphosate, DDT, mercurial seed dressing, pyrethrins, arsenic	No
Hoar 1986 [34] Kansas, USA	CC	Population	Matched by age and vital status	Dx period: 1976–1982	170 cases of NHL/948 controls (no. included in NHL analysis unclear)	Telephone interviews, with questions on years living/ working on a farm, and herbicides/insecticides used.	Referent: Non-farmers Ever use, Duration of use (years), Frequency of use (days/year), First year of use	Yes	Age	Phenoxyacetic acids, Triazine herbicides, Amide herbicides, Benzoic herbicides, Carbamate herbicides, Trifluralin herbicides, Uracil herbicides	No

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Table 1. *Cont.*

Author, year, location	Design	Source for controls	Matching	Diagnosis or follow-up period (cancer)	No. Participants	Exposure assessment	Referent category for exposure, exposure definition(s)/metric	Men only	Adjustment set	Pesticides	Reported results by subtype
Kang 2008 [35] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2002	50,127	Self-administered questionnai-res completed during enrollment and interviewer administered follow-up questionnaires	Referent 1: Nonexposed Referent 2: Lowest tertile of exposure Lifetime exposure days, Intensity weighted lifetime exposure days	No	Age at enrollment, education, cigarette smoking, alcohol consumption, family history of cancer, state of residence, top five most highly correlated pesticides	Trifluralin	No
Koutros 2009 [36] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2004	49,398	Self-administered questionnaire	Referent: Nonexposed Intensity weighted lifetime exposure days	No	Age, year of enrollment, race	Imazethapyr	No
Koutros 2008 [37] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2004	49,762	Self-administered questionnaire	NA	No	Not applicable, since an adjusted effect estimate for an association with NHL was not reported	Dichlorvos	No
Lee 2004 [38] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2001	54,383	Self-administered questionnaire	Referent 1: Nonexposed Lifetime exposure days, Intensity weighted lifetime exposure days	No	Age, gender, alcohol consumption, smoking history, educational level, family history of cancer, year of enrollment, state of residence, use of 4 correlated pesticides	Chlorpyrifos	No
Lee 2004 [39] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2000	49,980	Self-administered questionnaire	Referent 1: Nonexposed Exposed, Referent 2: Lowest quartile of exposure Lifetime exposure days, Intensity weighted lifetime exposure days	No	Age, sex, alcohol, smoking, education, family history of cancer, enrollment year, state of residence, 5 correlated pesticides	Alachlor	No

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Table 1. *Cont.*

Author, year, location	Design	Source for controls	Matching	Diagnosis or follow-up period (cancer)	No. Participants	Exposure assessment	Referent category for exposure, exposure definition(s)/metric	Men only	Adjustment set	Pesticides	Reported results by subtype
Lynch 2009 [40] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2004	19,655	Self-administered questionnaire	Referent 1: Nonexposed Referent 2: Lowest tertile of exposure Lifetime exposure days, Intensity weighted lifetime exposure days	No	Age at enrollment, gender, race, smoking status, education, family history of cancer, atrazine, 5 most correlated pesticides	Butylate	No
Lynch 2006 [41] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2002	50,800	Self-administered questionnaire	Referent: Lowest tertile of exposure[1] Lifetime exposure days, Intensity weighted lifetime exposure days	No	Age, race, gender, alcohol consumption, smoking status, education level, family history of cancer, state of residence, 5 most correlated pesticides	Cyanazine	No
Mahajan 2007 [42] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2003	21,416	Self-administered questionnai-re	Referent 1: Nonexposed Referent 2: Lowest tertile of exposure Lifetime exposure days, Intensity weighted lifetime exposure days	No	Age, smoking, gender, state of residence, use of 4 correlated pesticides	Carbaryl	No
McDuffie 2001 [43] Six Canadian provinces	CC	Population	Frequency matched by age and province of residence	Dx period: 1991–1994	517 cases/1,506 controls	Self-administered postal questionnai-re followed by telephone interview with participants who had 10 or more hours of pesticide use in lifetime plus a 15% random sample of those with fewer than 10 hours pesticide use	Referent: Not exposed Exposed, Frequency of exposure (days/year)	Yes	Age, providence of residence	2,4-D, mecoprop, MCPA, DiclofopmethylGlyphosate, phosphonic acids, phenoxy herbicides, thiocarbamates,diallate, dicamba, dinitroaniline, trifluralin, carbaryl, carbofuran, methomyl, carbamate insecticides, organochlorine insecticides, chlordane, lindane, aldrin, methoxychlor, DDT, Captan, vitavax, aldehyde, formaldehyde, mercury dust, mercury liquid, malathion, carbon tetrachloride	

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Table 1. *Cont.*

Author, year, location	Design	Source for controls	Matching	Diagnosis or follow-up period (cancer)	No. Participants	Exposure assessment	Referent category for exposure, exposure definition(s)/metric	Men only	Adjustment set	Pesticides	Reported results by subtype
Miligi 2006 [44] Italy	CC	Population	Stratified by gender and 5-year age groups	Dx period: 1991–1993	1,145 cases/1,232 controls	In-person interviews, including questions on crops grown and whether pesticides were used combined with exposure matrix	Reference: Those who never worked in agriculture Overall exposure, Probability of use >low and lack of protective equipment	No	Age, gender, area	Phenoxy herbicides, 2,4-D, MCPA	No
Mills 2005 [45] California	CC	Same source as the cases (United Farm Workers of America cohort)	Matched by gender, hispanic ethnicity and +/− one year of birth	Dx period: 1988–2001	60 cases/300 controls	Work histories combined with exposure matrix	Reference: Low use High use	No	Age, gender, length of union affiliation, date of first union affiliation	Methyl bromide, diazinon, malathion, dichloro-propane, captan, simazine, chlorthalonil, mancozeb, methyl parathion, nitrofen, propyzamide, toxaphene, trifluralin, 2,4-D, maneb	No
Orsi 2009 [46] France	CC	Hospital	Matched by center, age +/− 3 year, gender	2000–2004	244 cases/436 controls	Self-administered questionnaires, followed by face to face interviews with trained staff, and review of interviews by experts to verify logical consistency with pesticide product availability, geographic location, *etc.*	Reference: Nonexposed Exposed	No	Age, center, socioeconomic characteristic (white collar vs blue collar)	Organochlorine insecticides, organophosphorus insecticides, pyrethrin, carbamate fungicides, imide fungicides, triazole fungicides, phenoline herbicides, phenoxy herbicides, picoline herbicides, triazine herbicides, amide herbicides, urea herbicides, quaternary ammonium herbicides, glyphosate	Yes
Pahwa M 2012 [47] Six Canadian provinces	CC	Population	Frequency matched by age and province of residence	Dx period: 1991–1994	513 cases/ 506 controls	Self-administered postal questionnaire followed by telephone interview with participants who had 10 or more hours of pesticide use in lifetime plus a 15% random sample of those with fewer than 10 hours pesticide use	Reference: No use Use	Yes	Age, province of residence, respondent type (self or proxy), diesel oil exposure	OC insecticides, DDT, OP insecticides, malathion, phenoxy herbicides, MCPA, mecoprop, 2,4-D	No

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Table 1. *Cont.*

Author, year, location	Design	Source for controls	Matching	Diagnosis or follow-up period (cancer)	No. Participants	Exposure assessment	Referent category for exposure, exposure definition(s)/metric	Men only	Adjustment set	Pesticides	Reported results by subtype
Pearce 1987 [48] New Zealand	CC	Cancer registry	Matched by year of cancer registration and age (± 2 years)	Dx period: 1977–1981	183 cases/338 controls	Telephone interviews	Reference: Nonexposed Used any agricultural chemical spray in a farming setting	Yes	Decade of birth, type of interview respondent (self or relative)	Phenoxy herbicides	No
Persson 1989 [49] Sweden	CC	Population	Unmatched	Dx period: 1964–1986	106 cases/275 controls	Self-administered questionnaire	Reference: Not exposed Exposed	No	Age, date of Dx, gender, farming, exposure to fresh wood, other exposures associated with at least a doubled risk for hodgkins disease or NHL	Phenoxy herbicides, DDT	No
Persson 1993 [50] Sweden	CC	Population	Unmatched	Dx period: 1975–1984	93 cases/204 controls	Self-administered questionnaires	Reference: Not exposed Exposed	No	Age, other exposures investigated with OR ≥2 or significantly below unity and with at least 5 exposed subjects	Phenoxy herbicides, DDT	No
Purdue 2007 [51] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2002	51,011	Self-administered questionnaire	Reference 1: Never use/unexposed Ever use Lifetime days of exposure Intensity weighted lifetime days of exposure	No	Age, sate, gender, education level, smoking status, alcohol use, family history of cancer, lifetime days of total pesticide application	OC insecticides, aldrin, chlordane, DDT, dieldrin, heptachlor, lindane, toxaphene	No
Rafnsson 2006 [52] Iceland	CC	Non-cases from cohort of sheep owners	Unmatched	Dx period: 1966–2003	45 cases/221 controls	Records of sheep owned, used as a proxy measure for dermal exposure from sheep dipping; sheep dipping used as a proxy for exposure to hexa-chlorocyclohexane, which is a mixture of different isomers containing around 15% lindane. <100 sheep owned was used to indicate unexposed	Referent: <100 sheep ≥100 sheep , Categories of number of sheep owned: 100-199 sheep, 200-683 sheep	Yes	Age	Hexachlorocyc-lohexane	No

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Table 1. *Cont.*

Author, year, location	Design	Source for controls	Matching	Diagnosis or follow-up period (cancer)	No. Participants	Exposure assessment	Referent category for exposure, exposure definition(s)/metric	Men only	Adjustment set	Pesticides	Reported results by subtype
Rusiecki 2009 [53] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2004	49,093	Self-administered questionnaire	Referent: Nonexposed Lifetime days of exposure Intensity weighted	No	Age, gender, race, family history of cancer, cigarette smoking, state of residence, enrollment year	Permethrin	No
Rusiecki 2006 [54] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2002	50,193	Self-administered questionnaire	Referent: Lowest tertile of exposure Lifetime days of exposure Intensity weighted lifetime days of exposure	No	Age, gender, race, smoking, alcohol, applicator status, family history of cancer, sate of residence, most highly correlated pesticides	Metolachlor	No
van Bemmel 2008 [55] Iowa and North Carolina, USA	C (AHS)	NA	NA	1993–2004	48,378	Self-administered questionnaire	Referent: No exposure Lifetime days of exposure Intensity weighted lifetime days of exposure	Yes	Age, race, smoking, alcohol use, applicator type, family history of cancer, state of residence, total days of pesticide use	EPTC	No
Waddell 2001 [56] Iowa, Kansas, Minnesota, Nebraska, USA	Pooled analysis of 3 CC studies	Population	Matched by race, gender, age, and vital status at the time of interview, year of death for controls matched to deceased cases	Dx period 1: 1979–1983	748 cases/2,236 controls	Telephone interviews (Kansas and Nebraska, USA); In-person interviews (Iowa and Minnesota)	Referent: Non-farmers Ever Used First used Years used Days/year of use Protective gear	Yes	Age, state of residence, respondent type (proxy or direct)	OP insecticides, dichlorvos, trichlorfon, dimethoate, diazinon, disulfoton, ethoprop, malathion, phorate, terbufos, chlorpyrifos, coumaphos, crufomate, fonnel, tetrachlorvinphos, fensulfothion, famphur, fonofos, parathion	Yes

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Table 1. *Cont.*

Author, year, location	Design	Source for controls	Matching	Diagnosis or follow-up period (cancer)	No. Participants	Exposure assessment	Referent category for exposure, exposure definition(s)/metric	Men only	Adjustment set	Pesticides	Reported results by subtype
Woods 1987 [57] Washington state, USA	CC	Population	Matched by vital status and 5-year age group	Dx period: 1981–1984	746 cases/910 controls	In-person interviews about occupational history and self-reported chemical exposure	Referent: No exposure Farming exposures to phenoxy herbicides Any exposure to DDT Any exposure to chlordane Estimated intensity of occupational exposure to phenoxy herbicides: Low/medium/high	Yes	Age	Phenoxy herbicides, DDT, Chlordane	No
Zahm 1990 [58] Nebraska, USA	CC	Population	Matched by race, gender, vital status, age	Dx period: 1983–1986	201 cases/725 controls	In-person interviews about agricultural pesticide use	Referent: Never lived or worked on a farm Mixed or applied Days/year mixing or applying Years used on a farm First year of use	Yes	Age	2,4-D	No
Zahm 1993 [59] Iowa, Kansas, Minnesota, Nebraska, USA	Pooled analysis of 3 CC studies	Population	Matched by race, gender, age, vital status at the time of interview	Dx period [1]: 1979–1983	993 cases/2,918 controls	Telephone interviews (Kansas and Nebraska, USA), In-person interviews (Iowa and Minnesota)	Referent: Non-farmers Used atrazine [1] Personally handled Used but did not handle Duration of use (years) Days/year handled Year of first use	Yes	Age, state	Atrazine	Yes

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Table 1. *Cont.*

Author, year, location	Design	Source for controls	Matching	Diagnosis or follow-up period (veneer)	No. Participants	Exposure assessment	Referent category for exposure, exposure definition(s)/metric	Men only	Adjustment set	Pesticides	Reported results by subtype
Zahm 1993 [18] Nebraska, USA	CC	Population	Matched by race, gender, vital status, and age (5 year age groups)	Dx period: 1983–1986	119 cases/471 controls	In-person interviews about agricultural pesticide use	Referent: women who never lived or worked on a farm Used on farm	No (women only)	Age	Phenoxy herbicides, triazine herbicides, amide herbicides, benzoic acid herbicides, carbamate herbicides, trifluralin herbicides, chlorinated hydrocarbons, carbamate insecticides, OP insecticides	No
Zheng 2001 [60] Nebraska, USA, Iowa and Minnesota, Kansas	Pooled analysis of 3 CC studies	Population	Matched by gender, age, race, vital status, state of residence	Dx period [1]: 1979–1983	985 cases/2,895 controls	In-person interviews about agricultural pesticide use	Referent: Non-farmers Used Personally handled Year since first use Years of use Days/year of use	Yes	Age, type of respondent (proxy or direct), state of residence, first-degree family history of cancer, use of hair dye, use of private wells, tobacco smoking	Carbaryl, carbamate herbicides, carbamate insecticides	Yes

Notes: 2,4-D, 2,4-Dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-Trichlorophenoxyacetic acid; AHS, Agricultural Health Study; C, cohort study; CC, case-control study; DDT, dichlorodiphenyltrichloroethane; DX, Diagnosis; EPTC, s-ethyl dipropylthiocarbamate; MCPA, 2-methyl-4-chlorophenoxyacetic acid; OC, organochlorine; OP, organophosphorus; [1] Diagnosis period varied by state: July 1983–June 1986 (Nebraska, USA), October 1980–September 1982 (Minnesota), March 1981-October 1983 (Iowa), 1979–1981 (Kansas).

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3.2.1. Studies Conducted in the United States

Nineteen papers [19,21,22,24–26,29,31,35,36,38–42,51,53–55] report results from analyses of data from the Agricultural Health Study, which is a prospective cohort study of licensed pesticide applicators and their spouses living in Iowa and North Carolina, USA. Enrollment began in 1993 and the study is still ongoing [61]. The number of participants included in the analyses varied due to exclusions and completeness of exposure data. The last year of follow-up was defined by the last date on which the incident cancers were identified. At enrollment, participants completed a questionnaire in which they provided historical data on exposure to pesticides. They were also given a take home questionnaire to complete. Most analyses of the Agricultural Health Study data classified active ingredient exposures using two metrics: (1) lifetime exposure days, defined as number of years of use x number of days used per year; and (2) intensity-weighted lifetime exposure days, which was defined as years of use x number of days used per year x personal protective equipment use x intensity level, which incorporates factors that modify pesticide exposure, such as mixing status, application method, equipment repair status. Four papers [31,38,39,51] also reported associations using ever/never use categories; we used these estimates in the meta-analyses.

Six papers reported results from pooled analyses of three case-control studies that were conducted by the USA National Cancer Institute [20,23,30,56,59,60], in Iowa and Minnesota, Kansas, and Nebraska. Diagnosis periods for NHL ranged from 1979 to 1986, depending upon the study. In all studies, population based controls were frequency matched to cases by race, sex, age, and vital status at the time of the interview, and lifetime exposure to pesticides was assessed via telephone interviews. Using these pooled data, De Roos *et al.* [30] examined associations of NHL with 47 active ingredients. The authors investigated pesticides for which there was exposure data from all three studies and to which at least 20 participants were exposed. They used standard logistic regression to model the association of NHL with the multiple pesticides, simultaneously. These analyses were restricted to participants with complete information on all of the pesticides. Other papers reported results from analyses of these pooled data. Baris *et al.* [20] examined associations with dichlorodiphenyltrichloroethane (DDT), Blair *et al.* [23] with lindane, Zahm *et al.* [59] with atrazine, Waddell *et al.* [56] with organophosphates, and Zheng *et al.* [60] with carbamates. We also extracted results from analyses of the individual studies. Using data from the study in Iowa and Minnesota, Cantor *et al.* [27] examined associations with multiple pesticides. In Kansas, Hoar *et al.* [34] examined associations with exposures to various herbicides. In Nebraska, Zahm *et al.* [58] examined associations with 2,4-Dichlorophenoxyacetic acid (2,4-D).

In a population based case-control study in western Washington State, USA Woods *et al.* [57], examined associations between phenoxy exposure and NHL. Controls were group matched to cases diagnosed 1981–1984, based on vital status and age. Lifetime occupational histories and self-reported pesticide chemical exposures were ascertained using in-person interviews. The authors reported exposure to phenoxy herbicides by occupational type. We extracted the result for farming exposure to phenoxy herbicides. Exposures to DDT and chlordane were reported as ever/never, but they were not stratified by occupation.

We also extracted results from a USA based case-control study nested in a cohort of primarily Hispanic members of the California farm worker labor union [45]. Cases were diagnosed 1988–2001.

Controls were selected from the same cohort as cases and matched on the basis of gender, Hispanic ethnicity, and year of birth. Pesticide exposure was defined as low *versus* high use, with the category cut-points based on the distribution of use of the top 15 pesticides. To estimate exposure, union job history data that described crops farmed in a given month/year and county was combined with data collected by the California Pesticide Databank that describes pesticides used on a crop in a given county and time period.

3.2.2. Canadian Studies

Two papers reported results from the Cross-Canada Study of Pesticides and Health, which was a case control study conducted in six Canadian provinces [43,47]. Population based controls were frequency matched to NHL cases, diagnosed 1991–1994, based on age and province of residence. Detailed information on specific pesticide use was ascertained by telephone interviews. The questionnaires used for this study were based on the one used in the USA National Cancer Institute led case-control studies [20,23,30,56,59,60] in Nebraska [18,58] and Kansas [34]. McDuffie *et al.* [43] and Pahwa *et al.* [47] present results from some of the same analyses with the same population. When the same analysis was reported in both papers we selected the effect estimate from the paper by Pahwa *et al.* [47] because the authors excluded four NHL cases based on pathology review that occurred subsequent to the analyses reported in McDuffie *et al.* [43].

3.2.3. European Studies

Four papers [32,33,49,50] reported results from distinct case-control studies conducted in Sweden. The papers by Eriksson *et al.* [32] and Hardell *et al.* [33] reported analyses from population based case-control studies; case diagnosis periods were 1999–2002 and 1987–1992, respectively. A complete lifetime occupational and chemical exposure history was ascertained using self-administered questionnaires followed by telephone interviews when clarification was needed. The two studies by Persson *et al.* [49,50] report results from unmatched population based case-control studies; the results reported from the paper published in 1993 [50] were performed in an adjacent region of Sweden to the area represented in the earlier paper [49]. They examined the association of NHL with various occupational exposures, including phenoxy herbicides and DDT. Case diagnosis periods were 1964–1986 and 1975–1984, respectively.

We extracted results from papers that report results from analyses of data collected in France [46], Italy [44], Iceland [52], and multiple European centers that form parts of the EPILYMPH study [28]. All of these studies were case-control in design. In France [46], cases (diagnosed 2000–2004) and controls were recruited in the same hospitals. Exposure was assessed using self-administered questionnaires, followed by face-to-face interviews in which participants reported information about farms on which they worked for a minimum of six months; they reported information about location, period, crops and animals farmed, name of pesticides mixed or sprayed, duration and number of pesticide applications. Pesticide exposure was classified as possible or definite; the referent category included people never exposed to the pesticide. In the Italian study [44], cases were diagnosed from 1991 to 1993. Participants were interviewed about agricultural work, crop diseases, pesticides used to treat diseases, frequency of pesticide treatments, period of treatment, protective equipment used,

means of application, and re-entry tasks. Exposure was classified into low, medium, and high probabilities of use. The Icelandic case-control study [52] was nested in a cohort of male sheep owners. The authors included cases diagnosed 1966–2003. Paper records on sheep dipping in hexachlorocyclohexane, an organochlorine insecticide that contains lindane, were used as a proxy measure for exposure; records were available for the period 1962–1980. Number of sheep owned was used as a surrogate measure for exposure. In the EPILYMPH study [28], in-person interviews were conducted to ascertain detailed job histories, including information about farm size, crops farmed, pests treated, types and frequency of pesticides used, protective equipment, and re-entry tasks. Industrial hygienists classified pesticide exposure as possible, probable, or certain. In analysis, contrasts were made between high confidence of ever lifetime exposure *versus* never exposure, and any level of confidence of ever exposure *versus* never exposure.

3.2.4. Studies from Australia and New Zealand

Only two papers reported results from analyses of studies conducted outside of North America and Europe. Pearce *et al.* [48] reported analyses of data from a New Zealand based case-control study of agricultural exposures. Cases were diagnosed 1977–1981. Telephone interviews were used to ascertain lifetime occupational history and work with chemicals (phenoxy herbicides). In analysis, Pearce *et al.* [48] stratified phenoxy herbicide exposure by occupation (farming, forestry, railway work, *etc.*). We extracted the estimate of association with any phenoxy herbicide exposure in farming occupations. In Australia [62], Fritschi *et al.* enrolled incident NHL cases diagnosed between 2000 and 2001. They matched controls to cases based on age, gender, and region of residence. In structured telephone interviews, participants provided occupational histories. Occupational hygienists reviewed the responses to these questions and, with the help of a pesticide crop matrix, assigned likelihood of exposure to pesticides (probable, possible, no exposure), level of exposure, and frequency of exposure. These assignments were combined to classify participants' lifetime amount of exposure (substantial, meaning the person was probably exposed to the substance at a medium or high level for more than five 8-h days per year for a combined total of five years, nonsubstantial, or none).

3.2.5. Gender

Nineteen of the papers reported results from analyses that were restricted to men only [20,21,23,27–30,33,34,43,47,48,52,55–60]. One paper reported results from an analysis that was restricted to women [18]. The other papers reported results from analyses of study populations with men and women; in the analytic models reported in these papers, gender was treated as a confounder.

3.2.6. Covariates

In all papers, age was included in models to adjust for potential confounding. Location (state of residence, study center) was also a common adjustment factor. Other variables that were included in models as covariates were race, smoking status, alcohol consumption, correlated pesticides, education level, year of study enrollment, family history of cancer (all cancers or lympho-hematopoetic),

other environmental risk factors for NHL (e.g., gasoline exposure), and type of respondent to the interview used for exposure assessment (direct or proxy).

3.2.7. Reference Groups

In the majority of papers reviewed, the reference group contained farmers and non-farmers who were not exposed to the pesticide. However, there were exceptions to this, either because of study design or analytic decisions.

By design, all participants in the Agricultural Health Study were either pesticide applicators or spouses of applicators. Most of the analyses from this cohort contrasted exposed participants with two different referent groups: (1) participants with no exposure to the pesticide; and (2) participants in the lowest category of exposure. Similarly, all of the participants in the California based study reported in Mills *et al.* [45] were farm workers. The referent group in this analysis consisted of those with estimated low use of the pesticide being analyzed. Both cases and controls in the Icelandic study on which Rafnsson *et al.* [52] reported were sheep owners; people who owned <100 sheep made up the reference group.

By contrast, in some papers, the authors defined the reference group as those who neither worked nor lived on a farm. Miligi *et al.* [44] defined the referent group as participants who never worked in agriculture. Similarly, in papers reporting analyses of the case-control studies in Iowa, Minnesota, Nebraska, and Kansas, the referent group was defined as participants who never worked or lived on a farm. The exception to this was the paper by De Roos *et al.* [30]; the authors used pooled data from these case-control studies but defined the referent group as farmers and non-farmers who never used the pesticide being considered.

3.2.8. Exposure Period and Definition

Pesticide exposure periods and definitions varied, also. For the most part, papers investigated associations of NHL with ever lifetime pesticide exposure. However, some were more specific in their definition, and not all papers used the ever lifetime exposure metric.

In the cohort of California based union farm workers, Mills *et al.* [45] assessed pesticide exposure in the two to three year decade period prior to cancer diagnosis or enrollment. In Canada, McDuffie *et al.* [43] and Pahwa *et al.* [47] defined pesticide exposure as ever *versus* never lifetime use of pesticides for at least 10 h. In Sweden, Eriksson *et al.* [32] and Hardell *et al.* [33] required participants to have worked with the pesticide for a minimum of eight hours in a day, and the pesticide exposure was required to have occurred at least one year prior to the time of diagnosis or enrollment. Persson *et al.* [49,50] only classified as exposed those participants who were exposed to the chemical for at least one year, five to 45 years prior to case diagnosis. In the Italian study described by Miligi *et al.* [44], an agricultural pesticide questionnaire was only administered to participants who had worked on a farm for at least six months; presumably, therefore, anyone who had worked with pesticides but worked on a farm for less than six months was excluded from the exposed group. In the Icelandic study that Rafnsson *et al.* [52] described, records on sheep ownership, which were used to estimate lindane exposure, were available for the period 1962–1980; however, the cancer diagnosis period was 1966–2003.

3.3. Individual Effect Estimates and Dose Response Relationships

Table 2 presents effect estimates from studies in which chemical exposures were represented using multiple categories. Strong dose response relationships were generally absent; most analyses that examined associations with multiple categories of exposure derived imprecise estimates with wide confidence intervals. McDuffie *et al.* [43] and Eriksson *et al.* [32] observed increased odds of NHL in association with a greater number of days/year of glyphosate exposure. De Roos *et al.* [31] did not observe a similar relationship in analyses of Agricultural Health Study data. McDuffie *et al.* [43] observed elevated effect estimates in association with exposure to 2,4-D; however, they did not observe a dose-response relationship with days/year exposed. In analyses of Agricultural Health Study data, Lynch *et al.* [40] observed a nearly three-fold increase in the rate of NHL among those with ≥26 lifetime- and intensity-weighted exposure days to butylate, although the rate ratio comparing those with one to 25 lifetime exposure days to non-exposed applicators was close to the null.

Table 2. Effect estimates from studies that investigated associations between non-Hodgkin lymphoma and herbicides, insecticide, and fungicide exposures classified using multiple categories.

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
HERBICIDES				
Amide herbicides				
Lee 2004 [39]	Alachlor	Lifetime exposure days [1]		Rate ratio, 95% CI:
		Quartile 1, 0.1–19.9	5	1.0, Referent
		Quartile 2, 20.0–56.0	4	0.6, 0.1–2.5
		Quartile 3, 56.1–116.0	8	1.5, 0.4–5.4
		Quartile 4, ≥116.1	10	1.1, 0.3–4.4
				P for trend: 0.5
		Intensity weighted exposure days [1]		
		Quartile 1, 0.1–101.9	5	1.0, Referent
		Quartile 2, 102.0–253.1	3	0.6, 0.1–3.4
		Quartile 3, 253.2–710.4	10	2.4, 0.7–8.8
		Quartile 4, ≥710.5	9	1.4, 0.3–6.1
				P for trend: 0.4
Rusiecki 2006 [54]	Metolachlor	Lifetime exposure days [1]		Rate ratio, 95% CI:
		Tertile 1, ≤20	14	1.0, Referent
		Tertile 2, 21–56	11	0.8, 0.3–1.7
		Tertile 3, >56	11	0.7, 0.3–1.7
				P for trend: 0.5
		Intensity-weighted lifetime exposure days [1]		
		Tertile 1, ≤20	13	1.0,Referent
		Tertile 2, 21–56	10	0.7, 0.3–1.7
		Tertile 3, >56	13	1.0, 0.4–2.7
				P for trend: 0.7

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
Dinitroaniline herbicides				
Kang 2008 [35]	Trifluralin	Lifetime days of exposure [2]		Rate ratio, 95% CI
		Non-exposed	53	1.0, referent
		Tertile1, 1-24.4	17	0.9, 0.5–1.5
		Tertile 2, 24.5-108.4	23	1.0, 0.6–1.8
		Tertile 3, Lower half, 108.5-224.75	6	0.6, 0.2–1.4
		Tertile 3, Upper half, >224.75	4	0.6, 0.2–1.7
				P for trend: 0.2
		Intensity weighted lifetime days [2]		
		Tertile 1, 0-162.1	15	0.7, 0.4–1.4
		Tertile 2, 162.2-593	20	1.1, 0.8–2.9
		Tertile 3, Lower half, 593.1-1176.0	9	0.9, 0.4–2.0
		Tertile 3, Upper half, >1176.0	4	0.4, 0.1–1.1
				P for trend: 0.1
Glyphosate				
McDuffie 2001 [43] [3]	Glyphosate	Days/year of exposure		OR, 95% CI:
		Unexposed	466 cases/1,373 controls	1.0, Referent
		>0–≤2	28 cases/97 controls	1.0, 0.6–1.6
		>2	23 cases/36 controls	2.1, 1.2–3.7
De Roos 2005 [31] [3]	Glyphosate			
		Lifetime days of exposure [2]		Rate ratio, 95% CI:
		Tertile 1, 1–20	29	1.0, Referent
		Tertile 2, 21–56	15	0.7, 0.4–1.4
		Tertile 3, 57–2678	17	0.9, 0.5–1.6
				P for trend: 0.7
		Intensity weighted exposure days [2]		
		Tertile 1, 0.1–79.5	24	1.0, Referent
		Tertile 2, 79.6–337.1	15	0.6, 0.3–1.1

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
		Tertile 3, 337.2–1,824.1	22	0.8, 0.5–1.4
				P for trend: 1.0
Eriksson 2008 [32] [3]	Glyphosate			OR, 95% CI:
		Days of exposure [4]		
		>0–≤10 days	12 cases/9 controls	1.7, 0.7–4.1
		>10 days	17 cases/9 controls	2.4, 1.0–5.4
Imidazolinone herbicides				
Koutros 2009 [36]	Imazethapyr	Intensity weighted exposure days [5]		Rate ratio, 95% CI:
		No exposure:	80	1.0, Referent
		Tertile 1, <54.1	15	1.0, 0.6–1.7
		Tertile 2, 54.1–<152.9	13	0.9, 0.5, 1.6
		Tertile 3, lower half, 152.9–<311.9	7	0.8, 0.3–1.8
		Tertile 3, upper half, ≥311.9	11	1.4, 0.8–2.7
				P for trend: 0.4
Phenoxy herbicides				
Fritschi 2005 [62]	Phenoxy herbicides, group	Degree of pesticide exposure [6]		OR, 95% CI:
		None	679 cases/677 controls	1.0, Referent
		Nonsubstantial	10 cases/14 controls	0.7, 0.3–1.7
		Substantial	5 cases/3 controls	1.8, 0.4–7.4
Eriksson 2008 [32] [3]	Phenoxy herbicides, group	Days of exposure [4]		
		>0–≤45 days	32 cases/13 controls	2.8, 1.5–5.5
		>45 days	15 cases/13 controls	1.3, 0.6–2.7
Hardell 2002 [33] [3]	Phenoxy herbicides, group			OR, 95% CI:
		Number of days of exposure		
		Not exposed	NR	1.0, Referent
		Low	NR	1.7, 1.0–2.7
		High	NR	1.7, 1.0–2.7

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
		Years between first exposure and diagnosis		
		1–10	NR	-
		>10–20	NR	2.9, 1.1–7.7
		>20–30	NR	1.5, 0.9–2.8
		>30	NR	1.5, 0.9–2.4
		Years between last exposure and diagnosis		
		1–10	NR	3.2, 1.6–6.7
		>10–20	NR	2.1, 1.0–4.1
		>20–30	NR	1.0, 0.5–1.8
		>30	NR	1.3, 0.6–2.6
		Decade of exposure		
		1940s	4 cases/6 controls	1.5, 0.4–5.2
		1950s	35 cases/53 controls	1.4, 0.9–2.3
		1960s	43 cases/58 controls	1.7, 1.1–2.6
		1970s	32 cases/33 controls	2.4, 1.4–4.0
		1980s	16 cases/33 controls	3.3, 1.5–7.1
Eriksson 2008 [32] [3]	MCPA	Days exposed [4]		
		≤32	15 cases/5 controls	3.8, 1.4–10.5
		>32	6 cases/4 controls	1.7, 0.5–6.0
Hardell 2002 [33] [3]	MCPA			OR, 95% CI:
		Number of days of exposure		
		Not exposed	NR	1.0, Referent
		Low	NR	1.9, 0.8–4.6
		High	NR	3.6, 1.5–9.1
		Years between first exposure and diagnosis		
		1–10	NR	-
		>10–20	NR	5.4, 1.6–21

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
		>20–30	NR	0.9, 0.2–3.0
		>30	NR	3.8, 1.5–10.0
		Years between last exposure and diagnosis		
		1–10	NR	3.5, 1.6–8.0
		>10–20	NR	2.3, 0.6–9.1
		>20–30	NR	0.9, 0.1–4.4
		>30	NR	-
McDuffie 2001 [43] [3]	Mecoprop	Days/year exposed		
		Unexposed	464 cases/1,425 controls	1.0, Referent
		>0–≤2	31 cases/48 controls	2.3, 1.4–3.7
		≥2	22 cases/33 controls	2.1, 1.2–3.6
Hardell 2002 [33] [3]	2,4-D + 2,4,5-T			OR, 95% CI:
		Number of days of exposure		
		Low	NR	1.9, 1.1–3.2
		High	NR	1.2, 0.7–2.1
		Years between first exposure and diagnosis		
		1–10	NR	-
		>10–20	NR	2.9, 0.8–11.0
		>20–30	NR	1.9, 1.0–3.5
		>30	NR	1.2, 0.7–1.9
		Years between last exposure and diagnosis		
		1–10	NR	4.3, 1.1–21.0
		>10–20	NR	1.9, 0.9–3.8
		>20–30	NR	0.9, 0.1–4.4
		>30	NR	1.4, 0.7–2.9
Eriksson 2008 [32] [3]	2,4,5-T and/or 2,4-D	Days exposed [4]		OR, 95% CI:
		Non-exposed		1.0, Referent

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
		≤29	21 cases/11 controls	2.1, 1.0–4.4
		>29	12 cases/10 controls	1.3, 0.6–3.1
Zahm 1990 [58] [3]	2,4-D			OR, 95% CI:
		Never lived/worked on a farm	54 cases/184 controls	1.0, Referent
		Days/year mixing or applying		
		1–5	16 cases/44 controls	1.2, 0.6–2.4
		6–20	12 cases/25 controls	1.6, 0.7–3.6
		21+	3 cases/4 controls	3.3, 0.5–22.1
		Unknown	12 cases/25 controls	-
				P for trend: 0.1
		Years used on farm		
		1–5	3 cases/12 controls	0.9, 0.2–3.6
		6–15	11 cases/15 controls	2.8, 1.1–7.1
		16–20	3 cases/18 controls	0.6, 0.1–2.1
		21+	13 cases/33 controls	1.3, 0.6–2.7
		Unknown	15 cases/29 controls	-
				P for trend: 0.3
		First year of use		
		Prior to 1945	8 cases/21 controls	1.4, 0.5–3.5
		1946–1955	13 cases/39 controls	1.1, 0.5–2.3
		1956–1965	5 cases/8 controls	2.1, 0.6–7.7
		1965–1986	4 cases/12 controls	1.3, 0.3–4.9
		Unknown year	13 cases/18 controls	-
				P for trend: 0.2
McDuffie 2001 [43] [3]	2,4-D	Days/yr exposed		OR, 95% CI:
		Unexposed	406 cases/1,213 controls	1.0, Referent
		>0–≤2	55 cases/160 controls	1.2, 0.8–1.6
		>2–≤5	36 cases/82 controls	1.4, 0.9–2.1

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
		>5–≤7	9 cases/20 controls	1.4, 0.6–3.2
		>7	11 cases/31 controls	1.2, 0.6–2.5
Thiocarbamate herbicides				
Zheng 2001 [60] [3]	Butylate			OR, 95% CI
		Non-farmers	243 cases/775 controls	1.0, Referent
		Years since first use		
		<20	34 cases/56 controls	1.5, 0.9–2.4
		≥20	4 cases/10 controls	1.1, 0.3–3.7
		Years of use		
		<7	21 cases/35 controls	1.5, 0.9–2.8
		≥7	20 cases/37 controls	1.5, 0.8–2.7
		Days/year of use		
		<5	3 cases/5 controls	2.6, 0.6–11.1
		≥5	2 cases/2 controls	4.7, 0.6–34.5
Lynch 2009 [40]	Butylate	Lifetime exposure days [5]		Rate ratio, 95% CI:
		No exposure	27	1.0, Referent
		Low exposure, 1–25	6	0.9, 0.4–2.0
		High exposure, ≥26	12	2.9, 1.5–5.8
				P for trend: 0.0
		Intensity weighted exposure days [5]		
		No exposure	27	1.0, Referent
		Low exposure, 1–157	5	0.8, 0.3–2.0
		High exposure, ≥158	13	2.9, 1.5–5.5
				P for trend: 0.0
Van Bemmel 2008 [55]	EPTC	Lifetime exposure days [5]		Rate ratio, 95% CI:
		No exposure	83	1.0, Referent
		Tertile 1, 1–9	10	1.2, 0.6–2.3
		Tertile 2, 10–49	7	1.5, 0.7–3.2

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
		Tertile 3, 50+	5	0.8, 0.3–2.0
				P for trend:0.7
		Intensity-weighted lifetime exposure days [5]		Rate ratio, 95% CI:
		No exposure	83	1.0, Referent
		Tertile 1, 1–47	8	1.4, 0.7–2.8
		Tertile 2, 48–111	4	0.9, 0.3–2.5
		Tertile 3, 112+	10	1.1, 0.6–2.1
				P for trend:0.9
Zheng 2001 [60] [3]	EPTC + Protectant			OR, 95% CI:
		Non-farmers:		1.0, Referent
		Years since first use		
		<20	19 cases/34 controls	1.7, 0.9–3.1
		≥20	0 cases/1 control	-
		Years of use		
		<7	15 cases/20 controls	2.2, 1.1–4.4
		≥7	7 cases/26 controls	1.0. 0.4–2.4
		Days/year of use		
		<5	7 cases/12 controls	2.2, 0.8–5.8
		≥5	1 case/5 controls	0.9, 0.1–7.7
Triazine herbicides				
Lynch 2006 [41]	Cyanazine	Lifetime exposure days [5]		Rate ratio, 95% CI
		Tertile 1, 1–16	9	1.0, Referent
		Tertile 2, 17–56	18	1.6, 0.7–3.5
		Tertile 3, ≥57	9	1.3, 0.5–3.4
				P for trend: 1.0
		Intensity-weighted exposure days [5]		
		Tertile 1, 1–83	10	1.0, Referent
		Tertile 2, 84–314.35	12	1.3, 0.6–3.0

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
		Tertile 3, ≥315.35	13	1.4, 0.6–3.4
				P for trend: 0.5
Zahm 1993 [59] [3]	Atrazine			OR (95% CI not presented)
		No use	445 cases/1507 controls	1.0, Referent
		Years of use		
		1–5	4 cases/14 controls	0.4
		6–15	5 cases/20 controls	0.5
		16–20	5 cases/8 controls	0.6
		≥21	7 cases/11 controls	0.8
		Days/year personally handled		
		1–5	7 cases/20 controls	0.6
		6–20	8 cases/17 controls	0.7
		≥21	1 cases/1 control	1.4
		Year of first use		
		1965 or prior	10 cases/35 controls	0.4
		1966 or later	10 cases/18 controls	1.0
Beane Freeman 2011 [22]	Atrazine	Lifetime days of exposure [5]		Rate ratio, 95% CI
		Quartile 1, >0–20	41	1.0, Referent
		Quartile 2, 21–56	41	1.1, 0.7–1.7
		Quartile 3, >56–178.5	38	0.9, 0.6–1.5
		Quartile 4, >178.5	32	1.0, 0.6–1.6
				P for trend: 0.7
		Intensity weighted lifetime days of exposure [5]		
		Quartile 1, >0–20	38	1.0, Referent
		Quartile 2, 21–56	45	1.2, 0.8–1.9
		Quartile 3, >56–178.5	34	0.9, 0.6–1.5
		Quartile 4, >178.5	34	0.9, 0.6–1.5
				P for trend: 0.5

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
Triazinone herbicides				
Delancey 2009 [29]	Metribuzin	Lifetime days of exposure [5]		Rate ratio, 95% CI
		No exposure	39	
		Tertile 1, >0–8.75	5	1.0, Referent
		Tertile 2, 8.76–24.5	6	2.3, 0.6–8.6
		Tertile 3, >24.5	8	2.6, 0.8–9.1
				P for trend: 0.1
		Intensity weighted lifetime exposure days [5]		
		No exposure: 0	39	
		Tertile 1, >0–58.3	4	1.0, Referent
		Tertile 2, >58.3–174.4	6	1.4, 0.3–5.8
		Tertile 3, >174.5	9	2.5, 0.7–9.6
				P for trend: 0.1
FUNGICIDES				
McDuffie 2001 [43] [3]	Captan	Days/year exposed		OR, 95% CI:
		Unexposed	497 cases/1,482 controls	1.0, Referent
		>0–≤2	11 cases/12 controls	2.7, 1.2–6.2
		>2	9 cases/12 controls	2.8, 1.1–6.9
INSECTICIDES				
Carbamate insecticides				
Zheng 2001 [60] [3]	Carbaryl			OR, 95% CI:
		Non-farmers	243 cases/775 controls	1.0, Referent
		Years since first use		
		<20	19 cases/44 controls	1.1, 0.6–2.0
		≥20	14 cases/21 controls	1.8, 0.9–3.7
		Years of use		
		<7	16 cases/36 controls	1.1, 0.6–2.1
		≥7	15 cases/26 controls	1.5, 0.8–3.0

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
		Days/year of use		
		<5	9 cases/14 controls	2.4, 1.0–5.9
		≥5	2 cases/4 controls	1.8, 0.3–10.0
Mahajan 2007 [42]	Carbaryl	Lifetime days of exposure [2]		Rate ratio, 95% CI
		No exposure	23	1.0, Referent
		Tertile 1, 1–9	5	0.7, 0.2–1.8
		Tertile 2, 10–56	8	1.2, 0.5–3.0
		Tertile 3, >56	10	1.7, 0.6–4.5
				P for trend: 0.3
Zheng 2001 [60] [3]	Carbofuran			OR, 95% CI:
		Nonfarmers	243 cases/775 controls	1.0, Referent
		Years since first use		
		<20	32 cases/63 controls	1.3, 0.8–2.1
		≥20	15 cases/30 controls	1.6, 0.8–3.1
		Years of use		
		<7	30 cases/48 controls	1.7, 1.0–2.9
		≥7	24 cases/47 controls	1.4, 0.8–2.4
		Days/year of use		
		<5	9 cases/15 controls	2.7, 1.1–6.4
		≥5	12 cases/16 controls	3.1, 1.4–6.8
Bonner 2005 [25]	Carbofuran	Lifetime days of exposure [2]		Rate ratio, 95% CI
		No exposure	44	1.0, Referent
		Tertile 1, 1–9	6	0.8, 0.3–1.9
		Tertile 2, 10–56	7	1.3, 0.6–2.9
		Tertile 3, >56	7	1.4, 0.6–3.3
				P for trend: 0.4

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
Organophosphorus insecticides				
Fritschi 2005 [62]	Organophosphorus insecticides, group	Degree of exposure [6]		OR, 95% CI:
		No exposure	662 cases/660 controls	1.0, Referent
		Nonsubstantial exposure	20 cases/28 controls	0.7, 0.4–1.3
		Substantial exposure	12 cases/6 controls	2.1, 0.8–5.7
Waddell 2001 [56] [3]	Organophosphorus pesticides, group			OR, 95% CI:
		Non-farmers	243 cases/775 controls	1.0, Referent
		Years since first use		
		<20	44 cases/94 controls	1.0, 0.7–1.5
		≥20	79 cases/188 controls	1.6, 1.1–2.2
		Years used		
		<10	34 cases/69 controls	1.1, 0.7–1.7
		10–19	44 cases/71 controls	1.4, 0.9–2.1
		20+	39 cases/59 controls	1.5, 1.0–2.4
Lee 2004 [38]	Chlorpyrifos	Lifetime days of exposure [1]		Rate ratio, 95% CI:
		Nonexposed	53	1.0, Referent
		Quartile 1, 0.1–8.8	10	0.6, 0.2–1.5
		Quartile 2, 8.9–24.5	13	1.8, 0.9–3.5
		Quartile 3, 24.6–56.0	5	0.9, 0.4–2.4
		Quartile 4, ≥56.1	9	1.0, 0.4–2.4
				P for trend: 0.7
		Intensity-weighted lifetime days of exposure [1]		
		Nonexposed	53	1.0, Referent
		Quartile 1, 0.1–48.9	6	0.9, 0.3–2.2
		Quartile 2, 49.0–135.9	6	0.6, 0.2–1.8
		Quartile 3, 136.0–417.6	10	1.2, 0.6–2.7
		Quartile 4, ≥417.7	10	1.6, 0.7–3.5
				P for trend: 0.4

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
Beane Freeman 2005 [21]	Diazinon			Rate ratio, 95% CI:
		No exposure	26	1.0, Referent
		Lifetime days of exposure [2]		
		Tertile 1, <20	6	1.8, 0.7–4.4
		Tertile 2, 20–38.8	3	1.4, 0.4–4.6
		Tertile 3, >38.8	2	0.9, 0.2–4.1
				P for trend: 1.0
		Intensity-weighted lifetime days of exposure [2]		
		Tertile 1	5	1.9, 0.7–5.1
		Tertile 2	2	0.7, 0.2–3.1
		Tertile 3	4	1.7, 0.6–5.2
				P for trend: 0.4
Waddell 2001 [56] [3]	Diazinon			OR, 95% CI:
		Non-farmers	243 cases/775 controls	1.0, Referent
		Years since first use		
		<20	20 cases/34 controls	1.1, 0.6–2.0
		≥20	16 cases/24 controls	1.4, 0.7–2.7
		Years used		
		<10	20 cases/40 controls	0.9, 0.5–1.7
		10–19	10 cases/11 controls	1.8, 0.7–4.4
		20+	1 cases/1 controls	1.9, 0.1–31.6
		Days/year of use		
		<5	6 cases/11 controls	1.3, 0.5–3.9
		≥5	6 cases/6 controls	2.4, 0.7–8.0

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
Waddell 2001 [56] [3]	Fonofos			OR, 95% CI:
		Non-farmers	243 cases/775 controls	1.0, Referent
		Years since first use		
		<20	20 cases/36 controls	1.0, 0.6–1.9
		≥20	5 cases/6 controls	1.6, 0.5–5.5
		Years used		
		<10	16 cases/25 controls	1.2, 0.6–2.4
		10–19	7 cases/9 controls	1.5, 0.5–4.1
		20+	2 cases/1 control	4.2, 0.4–47.2
		Days/year of use		
		<5	2 cases/6 controls	0.7, 0.1–3.8
		≥5	9 cases/6 controls	3.4, 1.1–10.3
McDuffie 2001 [43] [3]	Malathion	Days/year of exposure		OR, 95% CI:
		Unexposed	445 cases/1,379 controls	1.0, Referent
		>0–≤2	50 cases/88 controls	1.8, 1.3–2.7
		≥2	22 cases/39 controls	1.8, 1.0–3.0
Waddell 2001 [56] [3]	Malathion			OR, 95% CI:
		Non-farmers	243 cases/775 controls	1.0, Referent
		Years since first use		
		<20	22 cases/46 controls	0.9, 0.5–1.6
		≥20	35 cases/39 controls	1.7, 1.1–2.9
		Years used		
		<10	22 cases/39 controls	1.1, 0.6–1.9
		10–19	23 cases/23 controls	1.9, 1.0–3.5
		20+	10 cases/18 controls	1.1, 0.5–2.4
		Days/year of use		
		<5	7 cases/8 controls	2.1, 0.7–6.1
		≥5	5 cases/7 controls	1.5, 0.5–5.2

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
Bonner 2007 [26]	Malathion	Lifetime days of exposure [5]		Rate ratio, 95% CI
		No exposure	1.0 Referent	
		Tertile 1, >0–9	0.6, 0.2–1.6	
		Tertile 2, 10–39	0.7, 0.3–1.8	
		Tertile 3, >39	0.8, 0.3–2.0	
		Intensity-weighted lifetime days of exposure [2]		Rate ratio, 95% CI
		No exposure	14	1.0, Referent
		Tertile 1, >0–58	5	0.5, 0.2–1.5
		Tertile 2, 59–245	9	0.7, 0.3–1.8
		Tertile 3, >245	9	0.8, 0.3–2.0
				P for trend: 0.9
Waddell 2001 [56] [3]	Phorate			OR, 95% CI:
		Non-farmers	243 cases/775 controls	1.0, Referent
		Years since first use		
		<20	19 cases/43 controls	0.8, 0.4–1.5
		≥20	14 cases/23 controls	1.3, 0.6–2.6
		Years used		
		<10	20 cases/33 controls	1.2, 0.6–2.1
		10–19	9 cases/19 controls	0.9, 0.4–2.1
		20+	4 cases/5 controls	1.5, 0.4–5.9
		Days/year of use		
		<5 days	5 cases/9 controls	1.3, 0.4–4.0
		≥5 days	7 cases/8 controls	2.0, 0.7–5.9
Waddell 2001 [56] [3]	Terbufos			OR, 95% CI:
		Non-farmers	243 cases/775 controls	1.0, Referent
		Years since first use		
		<20	23 cases/51 controls	0.9, 0.5-1.5
		≥20	0 cases/1 control	-

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
		Years used		
		<10	13 cases/38 controls	0.6, 0.3–1.3
		10–19	6 cases/8 controls	1.5, 0.5–4.4
		20+	0 cases/1 control	-
		<20	23 cases/51 controls	0.9, 0.5–1.5
		≥20	0 cases/1 control	-
		Days/year of use		
		<5	3 cases/8 controls	0.8, 0.2–3.4
		≥5	7 cases/4 controls	4.0, 1.1–14.5
Bonner 2010 [24]	Terbufos	Intensity weighted lifetime exposure days [5]		Hazard ratio, 95% CI
		No exposure	69	1.0, referent
		Tertile 1, >0–107	17	1.3, 0.7–2.3
		Tertile 2, >107–352	24	1.9, 1.2–3.2
		Tertile 3, >352	15	1.2, 0.7–2.2
				P for trend: 0.6
Organochlorine insecticides				
Fritschi 2005 [62]	Organochlorines, group	Degree of exposure [6]		OR, 95% CI:
		None	674 cases/679 controls	1.0, Referent
		Nonsubstantial	14 cases/13 controls	1.1, 0.5–2.3
		Substantial	6 cases/2 controls	3.3, 0.7–16.4
Purdue 2007 [51] [3]	Organochlorines, group	Lifetime days of exposure [5]		Rate ratio, 95% CI:
		Unexposed	16	1.0, Referent
		Tertile 1, 1–110	8	1.2, 0.5–2.8
		Tertile 2, 111–450	10	1.5, 0.6–3.5
		Tertile 3, >450	11	1.5, 0.6–3.8
				P for trend: 0.3

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
		Intensity weighted lifetime days of exposure [5]		
		Unexposed	16	1.0, Referent
		Tertile 1, 1–110	9	1.3, 0.6–3.1
		Tertile 2, 111–450	7	1.1, 0.4–2.9
		Tertile 3, >450	13	1.7, 0.7–4.2
				P for trend: 0.3
Purdue 2007 [51] [3]	Aldrin	Lifetime days of exposure [5]		Rate ratio, 95% CI:
		Unexposed	38	1.0, Referent
		1–20	5	0.8, 0.3–2.1
		>20	4	0.4, 0.1–1.5
				P for trend: 0.2
		Intensity weighted lifetime days of exposure [5]		
		Unexposed	38	1.0, Referent
		1–20	4	0.6, 0.2–1.9
		>20	5	0.6, 0.2–1.8
				P for trend: 0.4
Purdue 2007 [51] [3]	Chlordane	Lifetime days of exposure [5]		Rate ratio, 95% CI:
		Unexposed	32	1.0, Referent
		1–9	9	1.6, 0.8–3.6
		>9	6	1.8, 0.7–4.6
				P for trend: 0.2
		Intensity weighted lifetime days of exposure [5]		
		Unexposed	32	1.0, Referent
		1–9	8	1.8, 0.8–4.0
		>9	7	1.6, 0.7–3.9
				P for trend: 0.3
Baris 1998 [20] [3]	DDT			OR, 95% CI:
		Non-farmers	243 cases/775 controls	1.0, Referent

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
		Days/year of use		
		≤5	12 cases/35 controls	1.0, 0.5–2.1
		>5	11 cases/15 controls	2.1, 0.9–4.9
		Duration of use in years		
		1–4	36 cases/79 controls	1.0, 0.7–1.6
		5–9	31 cases/53 controls	1.4, 0.8–2.2
		≥15	39 cases/64 controls	1.5, 0.9–2.3
McDuffie 2001 [43] [3]	DDT	Days/year of exposure		OR, 95% CI:
		Unexposed	485 cases/1,447 controls	1.0, Referent
		>0–≤2	18 cases/32 controls	1.8, 1.0–3.2
		≥2	14 cases/27 controls	1.5, 0.8–2.9
Hardell 2002 [33] [3]	DDT			OR, 95% CI:
		Never exposed	NR	1.0, Referent
		Years between first exposure and diagnosis		
		1–10	NR	-
		>10–20	NR	2.6, 0.6–11.0
		>20–30	NR	1.6, 0.8–3.3
		>30	NR	1.2, 0.8–1.7
		Years between last exposure and diagnosis		
		1–10	NR	1.5, 0.7–3.1
		>10–20	NR	1.1, 0.6–2.0
		>20–30	NR	1.5, 0.8–2.5
		>30	NR	1.2, 0.7–2.0

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
Purdue 2007 [51] [3]	DDT			
		Lifetime days of exposure [5]		Rate ratio, 95% CI:
		Unexposed	32	1.0, Referent
		1–20	5	0.7, 0.3–1.9
		>20	9	1.2, 0.5–2.8
				P for trend: 0.6
		Intensity weighted lifetime days of exposure [5]		
		Unexposed	32	1.0, Referent
		1–20	6	0.9, 0.3–2.2
		>20	8	1.0, 0.4–2.5
				P for trend: 0.9
Eriksson 2008 [32] [3]	DDT	Days of exposure [4]		
		≤37 days	20 cases/19 controls	1.2, 0.6–2.2
		>37 days	30 cases/18 controls	1.8, 1.0–3.2
Purdue 2007 [51] [3]	Dieldrin			
		Lifetime days of exposure [5]		Rate ratio, 95% CI:
		Unexposed	46	1.0, Referent
		1–20	1	0.6, 0.1–4.2
		>20	1	0.9, 0.1–6.9
				P for trend: 0.8
		Intensity weighted lifetime days of exposure [5]		
		Unexposed	46	1.0, Referent
		1–20	1	0.7, 0.1–5.0
		>20	1	0.7, 0.1–5.5
				P for trend: 0.7

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
Purdue 2007 [51] [3]	Heptachlor	Lifetime days of exposure		Rate ratio, 95% CI:
		Unexposed	38	1.0, Referent
		1–9	6	1.5, 0.6–4.1
		>9	4	1.1, 0.4–3.2
				P for trend: 0.8
		Intensity weighted lifetime days of exposure		
		Unexposed	38	1.0, Referent
		1–9	4	1.2, 0.4–3.6
		>9	6	1.4, 0.5–3.7
				P for trend:0.6
Blair 1998 [23] [3]	Lindane			OR, 95% CI:
		Nonfarmer	243 cases/775 controls	1.0, Referent
		Years since first use		
		≥20	59 cases/83 controls	1.7, 1.1–2.5
		<20	18 cases/30 controls	1.3, 0.7–2.4
		Days/ year of use		
		≤4	8 cases/16 controls	1.6, 0.6–4.0
		≥5	5 cases/8 controls	2.0, 0.6–6.4
Rafnsson 2006 [52] [3]	Lindane	Number of sheep owned		OR, 95% CI:
		100–199 sheep	22 cases/71 controls	3.8, 1.6–9.3
		200–683 sheep	15 cases/62 controls	3.4, 1.3–9.0
Purdue 2007 [51] [3]	Lindane	Lifetime days exposed [5]		Rate ratio, 95% CI:
		Unexposed	34	1.0, Referent
		1–22 days	6	1.9, 0.8–4.7
		>22 days	7	2.1, 0.8–5.5
				P for trend: 0.1

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
		Intensity-weighted lifetime days of exposure [5]		
		Unexposed	34	1.0, Referent
		1–22	5	0.9, 0.3–3.0
		>22	8	2.6, 1.1–6.4
				P for trend: 0.04
Purdue 2007 [51] [3]	Toxaphene	Lifetime days exposed [5]		Rate ratio, 95% CI:
		Unexposed	35	1.0, Referent
		1–25 days	10	2.3, 1.1–5.0
		>25 days	2	1.3, 0.3–5.5
				P for trend: 0.8
		Intensity-weighted lifetime days of exposure [5]		
		Unexposed	35	1.0, Referent
		1–25	7	2.3, 1.0–5.4
		>25	5	1.6, 0.5–4.8
				P for trend:0.4
Pyrethroid insecticides				
Rusiecki 2009 [53]	Permethrin	Lifetime days of exposure [5]		Relative rate, 95% CI:
		Nonexposed	94	1.0, Referent
		Tertile 1, ≤8.74	8	0.8, 0.4–1.7
		Tertile 2, 8.75–50.75	5	0.6, 0.3–1.7
		Tertile 3, >50.75	5	0.7, 0.3–1.7
				P for trend: 0.2
		Intensity-weighted lifetime days of exposure [5]		
		Nonexposed	94	1.0, Referent
		Tertile 1, ≤8.74	7	0.8, 0.4–1.8
		Tertile 2, 8.75–50.75	7	0.9, 0.4–2.0
		Tertile 3, >50.75	4	0.5, 0.2–1.3
				P for trend: 0.2

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Table 2. *Cont.*

Author, date	Pesticide	Category of exposure	N exposed	Effect estimate, 95% CI
Other insecticides				
Eriksson 2008 [32] [3]	Pyretrine	Days of exposure [4]		OR, 95% CI:
		≤25	8 cases/5 controls	1.9, 0.6–5.8
		>25	6 cases/5 controls	1.4, 0.4–4.5
	Mercurial seed dressing	Days of exposure [4]		
		≤12	7 cases/6 controls	1.3, 0.4–3.8
		>12	14 cases/5 controls	2.9, 1.0–8.3
	Fumigant fungicides			
Barry 2012 [19]	Methyl Bromide	Intensity weighted lifetime days of exposure [5]		Rate ratio, 95% CI:
		Nonexposed	166	1.0, Referent
		Tertile 1, >0–310	21	2.3, 1.4–3.9
		Tertile 2, 311–1519	8	0.7, 0.3–1.6
		Tertile 3, >1519	6	0.6, 0.3–1.5
				P for trend: 0.1
		Intensity weighted lifetime days of exposure, 15 year lag [5]		
		Nonexposed	174	1.0, Referent
		Tertile 1, >0–310	13	1.7, 0.9–3.1
		Tertile 2, 311–1519	6	0.6, 0.3–1.5
		Tertile 3, >1519	8	1.0, 0.5–2.1
				P for trend: 0.7

Notes: CI, confidence interval; EPTC, s-ethyl dipropylthiocarbamate; MCPA, 2-methyl-4-chlorophenoxyacetic acid; NHL, non-Hodgkin lymphoma; OR, odds ratio; CI, confidence interval; [1] Categories based on mid-points of the questionnaire category; [2] Categories based on distribution among users; [3] Effect estimates in association with dichotomous exposure were also reported; [4] Categories based on the number of days of exposure among controls; [5] Categories based on the distribution of exposure among cancer cases; [6] Substantial indicates the person was exposed to the substance at a medium or high level for more than five 8-hour days per year for a combined total of more than 5 years. Non-substantial indicates any other combination of exposures; estimates derive from a case-control study.

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Table 3. Associations of subtypes of non-Hodgkin lymphoma with herbicides and insecticides.

	Chemical	Number of exposed cases	Risk ratio, 95% CI
B cell lymphoma			
HERBICIDES			
Organophosphorus herbicides			
Eriksson 2008 [32]	Glyphosate (OP herbicide)	NR	1.9, 1.0–3.5
Phenoxy herbicides			
Cocco 2012 [28]	Phenoxy herbicides	12 cases	1.4, 0.6–3.1
Eriksson 2008 [32]	Phenoxy herbicides	NR	2.0, 1.2–3.3
Fritschi 2005 [62] [1]	Phenoxy herbicides	NR	No exposure: 1.0, Referent
			Non-substantial exposure: 0.6, 0.3–1.5
			Substantial exposure: 1.5, 0.3–6.6
Eriksson 2008 [32]	2,4,5-T and/or 2,4-D (Phenoxy herbicide)	NR	1.7, 0.9–3.0
Cocco 2012 [28]	2,4-D (Phenoxy herbicide)	2 cases	0.6, 0.1–3.5
Cocco 2012 [28]	MCPA (Phenoxy herbicide)	4 cases	Infinity (zero exposed controls)
Eriksson 2008 [32]	MCPA (Phenoxy herbicide)	NR	2.6, 1.1–5.9
Thiocarbamate herbicides			
Zheng 2001 [60]	Butylate (Thiocarbamate herbicides)	4 cases (small lymphocytic)	1.1, 0.3–3.4
Zheng 2001 [60]	EPTC + Protectant (Thiocarbamate herbicides)	2 cases (small lymphocytic)	1.5, 0.3–7.1
Cocco 2012 [28]	Triazines and triazoles	6 cases	0.7, 0.2–1.7
INSECTICIDES			
Carbamate insecticides			
Zheng 2001 [60]	Carbaryl (Carbamate insecticide)	9 cases(small lymphocytic)	2.9, 1.2–7.0
Zheng 2001 [60]	Carbofuran (Carbamate insecticide)	7 cases (small lymphocytic)	1.5, 0.6–3.8
Cocco 2012 [28]	Methomyl (Carbamate insecticide)	0 cases	NR(zero exposed cases)
Cocco 2012 [28]	Mancozeb (Dithiocarbate fungicide)	2 cases	0.6, 0.1–3.5
Cocco 2012 [28]	Glyphosate (OP herbicide)	4 cases	3.1, 0.6–17.1

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Table 3. *Cont.*

Author, date	Chemical	Number of exposed cases	Risk ratio, 95% CI
Organochlorine (OC) insecticides			
Cocco 2012 [28]	Organochlorines	27 cases	0.9, 0.5–1.4
Fritschi 2005 [62] [1]	Organochlorines	NR	No exposure: 1.0, Referent
			Nonsubstantial: 1.1, 0.5–2.5
			Substantial: 3.5, 0.7–17.3
Baris 1998 [20] [2]	DDT (OC insecticides)	22 cases	1.6, 0.8–2.9
Eriksson 2008 [32]	DDT (OC insecticide)	NR	1.3, 0.8–2.1
Cocco 2012 [28]	DDT (OC insecticide)	3 cases	1.2, 0.2–5.9
Cocco 2012 [28]	Endosulfan (OC insecticide)	0 cases	NR, zero exposed cases
Organophosphorus insecticides			
Cocco 2012 [28]	Organophosphates	23 cases	1.4, 0.8, 2.6
Zheng 2001 [60] [2]	Organophosphates	18 cases	1.6, 0.8–3.2
Fritschi 2005 [62] [1]	Organophosphates	NR	No exposure [1]: 1.0, Referent
			Non-substantial: 0.6, 0.3–1.2
			Substantial: 2.1, 0.8–5.7
Cocco 2012 [28]	Dimethoate (OP insecticide)	3 cases	1.8, 0.3–10.6
Waddell 2001 [56] [2]	Fonofos (OP insecticide)	5 cases	2.6, 0.8–8.5
Waddell 2001 [56] [2]	Malathion (OP insecticide)	10 cases	1.9, 0.8–4.7
Waddell 2001 [56] [2]	Diazinon (OP insecticide)	9 cases	2.8, 1.1–7.3
Waddell 2001 [56] [2]	Phorate (OP insecticides)	8 cases	2.3, 0.9–6.0
Waddell 2001 [56] [2]	Terbufos (OP insecticides)	5 cases	2.2, 0.7–7.4
Other insecticides			
Eriksson 2008 [32]	Pyrethrine (Botanical insecticide)	NR	1.7, 0.7–3.9
Eriksson 2008 [32]	Mercurial seed dressing	NR	1.8, 0.8–3.9

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Table 3. *Cont.*

Author, date	Chemical	Number of exposed cases	Risk ratio, 95% CI
Mature B cell lymphoma			
Beane Freeman 2011 [22]	Atrazine (Triazine herbicide)	Lifetime days of exposure:	
		Quartile 1, >0–20: 36	1.0, Referent
		Quartile 2, 21–56: 34	1.0, 0.7–1.7
		Quartile 3, >56–178.5: 31	0.9, 0.5–1.4
		Quartile 4, >178.5: 28	0.9, 0.6–1.6
			P for trend: 0.8
		Intensity weighted lifetime days of exposure:	
		Quartile 1, >0–20: 34	1.0, Referent
		Quartile 2, 21–56: 38	1.1, 0.7–1.8
		Quartile 3, >56–178.5: 25	0.8, 0.5–1.3
		Quartile 4, >178.5: 31	0.9, 0.6, 1.5
			P for trend: 0.7
Diffuse large B cell lymphoma			
HERBICIDES			
Organophosphorus herbicides			
Eriksson 2008 [32]	Glyphosate (OP herbicides)	NR	1.2, 0.4–3.4
Phenoxy herbicides			
Cocco 2012 [28]	Phenoxy herbicides	4 cases	1.7, 0.5–5.2
Eriksson 2008 [32]	Phenoxy herbicides	NR	2.2, 1.1–4.3
Fritschi 2005 [62] [1]	Phenoxy herbicides	NR	No exposure [1]: 1.0, Referent
			Nonsubstantial exposure: 0.5, 0.1–2.0
			Substantial exposure: 2.2, 0.4–13.1
Eriksson 2008 [32]	MCPA (Phenoxy herbicide)	NR	3.9, 1.5–10.5
Eriksson 2008 [32]	2,4,5-T and/or 2,4-D (Phenoxy herbicide)	NR	1.7, 0.7–3.8

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Table 3. *Cont.*

Author, date	Chemical	Number of exposed cases	Risk ratio, 95% CI
Thiocarbamate herbicides			
Zheng 2001 [60]	Butylate (Thiocarbamate herbicides)	15 cases	1.6, 0.9–3.1
Zheng 2001 [60]	EPTC + Protectant (Thiocarbamate herbicides)	10 cases	1.8, 0.8–3.7
Triazine herbicides			
Cocco 2012 [28]	Triazines and triazoles	2 cases	0.8, 0.2–3.4
Beane Freeman 2011 [22]	Atrazine (Triazine herbicides)	Lifetime exposure days:	
		Quartile 1, >0–20: 20	1.0, Referent
		Quartile 2, 21–56: 14	0.8, 0.4–1.6
		Quartile 3, >56–178.5: 14	0.7, 0.4–1.5
		Quartile 4, >178.5: 11	0.7, 0.3–1.6
			p for trend:0.5
		Intensity-weighted lifetime exposure days:	
		Quartile 1, >0–20: 15	1.0, Referent
		Quartile 2, 21–56: 18	1.2, 0.6–2.5
		Quartile 3, >56–178.5: 11	0.8, 0.4–1.7
		Quartile 4, >178.5: 15	1.1, 0.5–2.3
			p for trend:0.96
Zahm 1993 [59]	Atrazine (Triazine herbicides)	66 cases	1.6, 1.1–2.2
INSECTICIDES			
Carbamate insecticides			
Zheng 2001 [60]	Carbaryl (Carbamate insecticides)	15 cases	1.5, 0.8–2.8
Zheng 2001 [60]	Carbofuran (Carbamate insecticides)	24 cases	1.6, 1.0–2.7

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Table 3. *Cont.*

Author, date	Chemical	Number of exposed cases	Risk ratio, 95% CI
Organochlorine insecticides			
Cocco 2012 [28]	Organochlorines	5 cases	0.6, 0.2–1.6
Fritschi 2005 [62] [1]	Organochlorines	NR	No exposure [1]: 1.0, Referent
			Non-substantial exposure: 1.2, 0.4–3.4
			Substantial exposure: 1.6, 0.2–18.1
Eriksson 2008 [32]	DDT (OC insecticide)	NR	1.2, 0.6–2.5
Baris 1998 [20]	DDT (OC insecticide)	53 cases	1.2, 0.8–1.7
Organophosphorus insecticides			
Cocco 2012 [28]	Organophosphates	5 cases	1.1, 0.4–2.9
Waddell 2001 [56]	Organophosphates	63 cases	1.8, 1.2–2.6
Fritschi 2005 [62]	Organophosphates	NR	No exposure [1]: 1.0, Referent
			Non-substantial exposure: 0.6, 0.3–1.6
			Substantial exposure: 2.1, 0.6–7.7
Waddell 2001 [56]	Fonofos (OP insecticide)	10 cases	1.3, 0.6–2.7
Waddell 2001 [56]	Malathion (OP insecticide)	19 cases	1.1, 0.6–1.9
Waddell 2001 [56]	Diazinon (OP insecticide)	13 cases	1.2, 0.6–2.4
Waddell 2001 [56]	Phorate (OP insecticide)	10 cases	0.8, 0.4–1.8
Waddell 2001 [56]	Terbufos (OP insecticide)	7 cases	0.8, 0.4–2.0
Other insecticides			
Cocco 2012 [28]	Arsenicals	2 cases	0.4, 0.1–1.6
Eriksson 2008 [32]	Pyrethrine (Botanical insecticide)	NR	1.3, 0.3–4.6
Eriksson 2008 [32]	Mercurial seed dressing	NR	2.2, 0.8–6.1
Chronic lymphocytic leukemia			
HERBICIDES			
Cocco 2012 [28]	Phenoxy acids	Ever *vs.* never exposed	
		2 cases ever exposed	0.9, 0.2–4.1
		Intensity of exposure	
		Unexposed: 362 cases	1.0, Referent

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Table 3. *Cont.*

Author, date	Chemical	Number of exposed cases	Risk ratio, 95% CI
		Low: 0 cases	
		Medium/high: 2 cases	2.4, 0.4–13.8
Cocco 2012 [28]	Triazines and triazoles	2 cases	0.9, 0.2–4.1
INSECTICIDES			
Cocco 2012 [28]	Arsenicals	15 cases	1.6, 0.8–2.9
Cocco 2012 [28]	Carbamates		
Cocco 2012 [28]	Organochlorines	Ever *vs* never exposed	
		10 cases ever exposed	1.2, 0.6–2.5
		Intensity of exposure	
		Unexposed: 362 cases	1.0, Referent
		Low: 5 cases	1.8, 0.6–5.0
		Medium/high: 5 cases	1.0, 0.4–2.8
Cocco 2012 [28]	Organophosphates	Ever *vs* never exposed	
		9 cases ever exposed	2.7, 1.2–6.0
		Intensity of exposure	
		Unexposed: 362 cases	1.0, Referent
		Low: 5 cases	2.7, 0.9–7.8
		Medium/high: 4 cases	2.6, 0.7–9.2
Lymphocytic lymphoma			
HERBICIDES			
Organophosphorus herbicides			
Eriksson 2008 [32]	Glyphosate (OP herbicide)	NR	3.4, 1.4–7.9
Phenoxy herbicides			
Eriksson 2008 [32]	Phenoxy herbicides	NR	2.1, 1.0–4.5
Cocco 2013 [28]	Phenoxy herbicides	NR	0.9, 0.2–4.1

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Table 3. *Cont.*

Author, date	Chemical	Number of exposed cases	Risk ratio, 95% CI
Eriksson 2008 [32]	2,4,5-T and/or 2,4-D (Phenoxy herbicides)	NR	1.9, 0.9–4.4
Eriksson 2008 [32]	MCPA (Phenoxy herbicides)	NR	2.6, 0.7–9.0
INSECTICIDES			
Organochlorine insecticides			
Eriksson 2008 [32]	DDT (OC insecticides)	NR	1.4, 0.7–2.8
Organophosphorus insecticides			
Fritschi 2005 [62] [1]	Organophosphates	NR	No exposure: 1.0, Referent Non-substantial exposure: 1.1, 0.5–2.3 Substantial exposure: 4.3, 1.4–13.0
Other insecticides			
Eriksson 2008 [32]	Pyrethrine (Botanical insecticide)	NR	2.4, 0.7–7.9
Eriksson 2008 [32]	Mercurial seed dressing	NR	2.9, 1.0–8.3
Follicular lymphoma			
HERBICIDES			
Organophosphorus herbicides			
Eriksson 2008 [32]	Glyphosate (OP herbicide)	NR	1.9, 0.6–5.8
Phenoxy herbicides			
Eriksson 2008 [32]	Phenoxy herbicides	NR	1.3, 0.4–3.8
Fritschi 2005 [62] [1]	Phenoxy herbicides	NR	No exposure: 1.0, Referent Non-substantial exposure: 0.5, 0.1–2.0 Substantial exposure: 1.2, 0.1–11.2
Eriksson 2008 [32]	2,4,5-T and/or 2,4-D (Phenoxy herbicide)	NR	1.2, 0.4–4.2
Eriksson 2008 [32]	MCPA (Phenoxy herbicide)	NR	No exposed cases
Thiocarbamate herbicides			
Zheng 2001 [60]	Butylate (Thiocarbamate herbicides)	17 cases	1.5, 0.8–2.8
Zheng 2001 [60]	EPTC + Protectant use (Thiocarbamate herbicides)	10 cases	1.7, 0.8–3.8

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Table 3. *Cont.*

Author, date	Chemical	Number of exposed cases	Risk ratio, 95% CI
Triazine herbicides			
Zahm 1993 [59]	Atrazine (Triazine herbicide)	40 cases	1.3, 0.9–1.9
Beane Freeman 2011 [22]	Atrazine (Triazine herbicide)	Lifetime exposure days, by quartile:	
		Quartile 1, >0–20: 10	1.0, Referent
		Quartile 2, 21–56: 8	0.9, 0.3–2.2
		Quartile 3, >56–178.5: 6	0.6, 0.2–1.7
		Quartile 4, >178.5: 8	1.0, 0.4–2.6
			p for trend: 0.9
		Intensity-weighted exposure days:	
		Quartile 1, >0–20: 10	1.0, Referent
		Quartile 2, 21–56: 10	1.0, 0.4–2.4
		Quartile 3, >56–178.5: 8	0.8, 0.3–2.1
		Quartile 4, >178.5: 4	0.4, 0.1–1.3
			p for trend: 0.07
INSECTICIDES			
Carbamate insecticides			
Zheng 2001 [60]	Carbaryl (Carbamate insecticides)	14 cases	1.3, 0.6–2.4
Zheng 2001 [60]	Carbofuran (Carbamate insecticides)	22 cases	1.4, 0.8–2.4
Organochlorine insecticides			
Fritschi 2005 [62] [1]	Organochlorines	NR	No exposure: 1.0, Referent
			Non-substantial exposure: 1.8, 0.7–4.8
			Substantial exposure: 3.5, 0.5–25.2
Eriksson 2008 [32]	DDT (OC insecticide)	NR	2.1, 1.1–4.4
Baris 1998 [20]	DDT (OC insecticide)	47 cases	1.3, 0.8–1.9
Organophosphorus insecticides			
Waddell 2001 [56]	OP pesticides, group	50 cases	1.3, 0.9–2.0
Waddell 2001 [56]	Fonofos (OP insecticide)	14 cases	1.2, 0.6–2.4
Waddell 2001 [56]	Malathion (OP insecticide)	29 cases	1.3, 0.8–2.2

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Table 3. *Cont.*

Author, date	Chemical	Number of exposed cases	Risk ratio, 95% CI
Waddell 2001 [56]	Diazinon (OP insecticide)	17 cases	1.3, 0.7–2.3
Waddell 2001 [56]	Phorate (OP insecticide)	10 cases	0.7, 0.3–1.4
Waddell 2001 [56]	Terbufos (OP insecticide)	9 cases	0.7, 0.3–1.6
Eriksson 2008 [32]	Mercurial seed dressing	NR	3.6, 1.2–10.9
Eriksson 2008 [32]	Pyrethrine (Botanical insecticide)	NR	2.6, 0.8–8.5
T cell lymphoma			
HERBICIDES			
Organophosphorus herbicides			
Eriksson 2008 [32]	Glyphosate (OP insecticide)	NR	2.3, 0.5–10.4
Phenoxy herbicides			
Eriksson 2008 [32]	Phenoxy herbicides	NR	1.6, 0.4–7.3
Eriksson 2008 [32]	2,4,5-T and/or 2,4-D (Phenoxy herbicides)	NR	1.0, 0.1–8.0
Eriksson 2008 [32]	MCPA (Phenoxy herbicides)	NR	2.4, 0.3–20.0
INSECTICIDES			
Eriksson 2008 [32]	DDT (OC insecticide)	NR	2.9, 1.1–8.0
Eriksson 2008 [32]	Mercurial seed dressing	NR	2.1, 0.3–17.1
Eriksson 2008 [32]	Pyrethrine (Botanical insecticide)	NR	2.2, 0.3–17.8
Unspecified NHL			
HERBICIDES			
Organophosphorus herbicides			
Eriksson 2008 [32]	Glyphosate (OP insecticide)	NR	5.6, 1.4–22.0
Phenoxy herbicides			
Eriksson 2009 [32]	Phenoxy herbicides	NR	3.8, 1.2–12.1
Eriksson 2008 [32]	2,4,5-T and/or 2,4-D (Phenoxy herbicide)	NR	3.2, 0.9–12.1
Eriksson 2008 [32]	MCPA (Phenoxy herbicide)	NR	9.3, 2.1–41.2
INSECTICIDES			
Eriksson 2008 [32]	DDT (OC insecticide)	NR	2.4, 0.8–7.4
Eriksson 2008 [32]	Mercurial seed dressing	NR	5.4, 1.3–22.0

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Table 3. *Cont.*

Author, date	Chemical	Number of exposed cases	Risk ratio, 95% CI
Eriksson 2008 [32]	Pyrethrine (Botanical insecticide)	NR	3.1, 0.4–26.3
Other specified B cell lymphoma			
HERBICIDES			
Organophosphorus herbicides			
Eriksson 2008 [32]	Glyphosate (OP herbicide)	NR	1.6, 0.5–5.0
Phenoxy herbicides			
Eriksson 2008 [32]	Phenoxy herbicides	NR	2.6, 1.2–5.6
Eriksson 2008 [32]	2,4,5-T and/or 2,4-D (Phenoxy herbicide)	NR	2.2, 0.9–5.4
Eriksson 2008 [32]	MCPA (Phenoxy herbicide)	NR	3.2, 1.0–10.7
INSECTICIDES			
Eriksson 2008 [32]	DDT (OC insecticide)	NR	1.3, 0.6–3.1
Eriksson 2008 [32]	Mercurial seed dressing	NR	2.4, 0.7–7.8
Eriksson 2008 [32]	Pyretrine	NR	1.5, 0.3–6.9
Unspecified B cell lymphoma			
HERBICIDES			
Organophosphorus herbicides			
Eriksson 2008 [32]	Glyphosate (OP herbicide)	NR	1.5, 0.3–6.6
Phenoxy herbicides			
Eriksson 2008 [32]	Phenoxy herbicides	NR	1.1, 0.3–4.0
Eriksson 2008 [32]	2,4,5-T and/or 2,4-D (Phenoxy herbicide)	NR	0.9, 0.2–3.9
Eriksson 2008 [32]	MCPA (Phenoxy herbicide)	NR	1.4, 0.2–11.2
INSECTICIDES			
Eriksson 2008 [32]	DDT (OC insecticide)	NR	0.2, 0.0–1.8
Eriksson 2008 [32]	Mercurial seed dressing	NR	No exposed cases

Notes: 2,4-D, 2,4-Dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-Trichlorophenoxyacetic acid; DDT, dichlorodiphenyltrichloroethane; EPTC, s-ethyl dipropylthiocarbamate; MCPA, 2-methyl-4-chlorophenoxyacetic acid; NHL, non-Hodgkin lymphoma; NR, Not reported; OC, Organochlorine; OP, Organophosphorus; [1] Substantial indicates the person was exposed to the substance at a medium or high level for more than five 8-hour days per year for a combined total of more than 5 years. Nonsubstantial indicates any other combination of exposures; estimates derive from a case-control study; [2] NHL subtype is labeled small lymphocytic in the paper.

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In the Agricultural Health Study, Delancey *et al.* [29] observed a fairly strong dose response relationship between exposure to metribuzin, a triazinone herbicide, and NHL (P for trend: 0.13). Waddell *et al.* [56] observed a dose-response relationship between years of use of the organophosphorus insecticide fonofos and NHL. These authors also observed a strong positive relationship between days/year of exposure to another organophosphorus insecticide, terbufos, and NHL (OR, 95% CI for ≥5 days *vs.* non-farmers: 4.0, 1.1–14.5).

Table 3 shows estimates of association between subtypes of NHL and chemical groups or active ingredients. Table 4 shows the individual effect estimates of associations with herbicides, fungicides, and insecticides, coded dichotomously.

Table 4. Effect estimates from papers that investigated associations between non-Hodgkin lymphoma and herbicide, fungicide, and insecticide exposures, categorized dichotomously.

Author, date	N exposed	Risk ratio, 95% CI
HERBICIDES		
Amide herbicides		
Amide herbicides, group		
Hoar 1986 [34]	8 cases/22 controls	2.9, 1.1–7.6
Cantor 1992 [27]	58 cases/114 controls	1.2, 0.8–1.7
Zahm 1993 [18] [1]	8 cases/34 controls	0.9, 0.4–2.2
Orsi 2009 [46]	5 cases/12 controls	0.9, 0.3–2.8
Alachlor		
De Roos 2003 [30]	68 cases/152 controls	1.1, 0.7–1.7
Lee 2004 [39] [2]	29 cases	0.7, 0.5–1.1
Metolachlor		
De Roos 2003 [30]	13 cases/37 controls	0.7, 0.3–1.6
Propachlor		
De Roos 2003 [30]	20 cases/50 controls	1.0, 0.5–2.0
Propyzamide		
Mills 2005 [45]	NR	0.7, 0.3–1.4
Organophosphorus herbicides		
Glyphosate		
McDuffie 2001 [43]	51 cases/133 controls	1.2, 0.8–1.7
Hardell 2002 [33]	8 cases/8 controls	3.0, 1.1–8.5
De Roos 2003 [30]	36 cases/61 controls	2.1, 1.1–4.0
De Roos 2005 [31] [2]	71 cases	1.1, 0.7–1.9
Eriksson 2008 [32]	29 cases/18 controls	2.0, 1.1–3.7
Orsi 2009 [46]	12 cases/24controls	1.0, 0.5–2.2
Phosphonic acid		
McDuffie 2001 [43]	63 cases/147 controls	1.4, 0.9–1.9
Phenoxy herbicides		
Phenoxy herbicides, group		
Hoar 1986 [34]	24 cases/78 controls	2.2, 1.2–4.1
Pearce 1987 [48]	81 cases/143 controls	1.0, 0.8–1.4
Woods 1987 [57]	NR	1.3, 0.9–2.0
Persson 1989 [49]	6 cases/6 controls	4.9, 1.0–23.5

Table 4. *Cont.*

Author, date	N exposed	Risk ratio, 95% CI
Cantor 1992 [27]	118 cases/231 controls	1.2, 0.9–1.6
Persson 1993 [50]	10 cases/14 controls	2.3, 0.2–2.8
Zahm 1993 [18] [1]	14 cases/63 controls	0.9, 0.4–1.7
Hardell 2002 [33]	64 cases/90 controls	1.7, 1.2–2.3
Miligi 2006 [44]	32 cases/28 controls	1.1, 0.6–1.8
Eriksson 2008 [32]	47 cases/26 controls	2.0, 1.2–3.4
Orsi 2009 [46]	11 cases/25 controls	0.9, 0.4–1.9
Pahwa 2012 [47]	129 cases/138 controls	1.5, 1.1–1.9
2,4-D		
Zahm 1990 [58]	43 cases/98 controls	1.5, 0.9–2.5
Cantor 1992 [27]	Ever handled: 115 cases/227 controls	1.2, 0.9–1.6
Cantor 1992 [27] [3]	Handled prior to 1965: 86 cases/153 controls	1.3, 0.9–1.8
Mills 2005 [45]	NR	3.8, 1.9–7.8
Miligi 2006 [44]	17 cases/18 controls	0.9, 0.5–1.8
Pahwa 2012 [47]	110 cases/293 controls	1.3, 1.0–1.7
2,4,5-T		
De Roos 2003 [30]	Ever handled: 25 cases/63 controls	1.0, 0.5–1.9
Cantor 1992 [27] [3]	Handled prior to 1965: 13 cases/18 controls	1.7, 0.8–3.6
2,4,5-T and/or 2,4-D		
Eriksson 2008 [32]	33 cases/21 controls	1.6, 0.9–3.0
Diclofop-methyl		
McDuffie 2001 [43]	9 cases/25 controls	1.0, 0.4–2.2
MCPA		
Hardell 2002 [33]	21 cases/23 controls	2.6, 1.4–4.9
De Roos 2003 [30] [1]	8 cases/16 controls	1.0, 0.4–2.6
Miligi 2006 [44]	18 cases/19 controls	0.9, 0.4–1.8
Eriksson 2008 [32]	21 cases/9 controls	2.8, 1.3–6.2
Pahwa 2012 [47]	17 cases/46 controls	1.1, 0.6–2.0
Carbamate/Thiocarbamate herbicides		
Carbamate/Thiocarbamate herbicides, group		
Zahm 1993 [18] [1]	2 cases/14 controls	0.6, 0.1–2.8
McDuffie 2001 [43]	21 cases/49 controls	1.5, 0.8–2.6
Zheng 2001 [60]	60 cases/108 controls	1.5, 1.1–2.3
Butylate		
Cantor 1992 [27] [3]	Handled prior to 1965: 1 case/6 controls	0.5, 0.1–4.3
Zheng 2001 [60]	45 cases/76 controls	1.6, 1.0–2.4
Diallate		
McDuffie 2001 [43]	11 cases/29 controls	1.5, 0.7–3.1
EPTC + Protectant		
Zheng 2001 [60]	23 cases/49 controls	1.6, 0.9–2.7

Table 4. *Cont.*

Author, date	N exposed	Risk ratio, 95% CI
Aromatic acid herbicides		
Benzoic acid herbicides		
Hoar 1986 [34]	1 case/2 controls	4.0, 0.1–62.6
Cantor 1992 [27]	53 cases/98 controls	1.3, 0.9–1.9
Zahm 1993 [18] [1]	4 cases/12 controls	1.2, 0.3–4.4
Chloramben		
Cantor 1992 [27] [3]	Handled prior to 1965: 16 cases/19 controls	2.0, 1.0–4.0
De Roos 2003 [30]	34 cases/81 controls	0.9, 0.5–1.6
Dicamba		
Cantor 1992 [27] [3]	Handled prior to 1965: 7 cases/7 controls	2.8, 1.0–8.1
McDuffie 2001 [43]	26 cases/50 controls	1.6, 1.0–2.6
De Roos 2003 [30]	39 cases/79 controls	1.2, 0.6–2.3
Dinitroaniline herbicides		
Dinitroanilines, group		
Cantor 1992 [27]	46 cases/88 controls	1.2, 0.8–1.8
McDuffie 2001 [43]	11 cases/31 controls	1.2, 0.6–2.4
Trifluralin		
Cantor 1992 [27] [3]	Handled prior to 1965: 14 cases/23 controls	1.5, 0.8–3.1
Zahm 1993 [18] [1]	3 cases/24 controls	0.5, 0.1–1.7
McDuffie 2001 [43]	11 cases/31 controls	1.1, 0.5–2.2
De Roos 2003 [30]	52 cases/120 controls	0.9, 0.5–1.6
Mills 2005 [43,45]	NR	0.9, 0.4–1.8
Triazine herbicides		
Triazine herbicides, group		
Hoar 1986 [34]	14 cases/43 controls	2.5, 1.2–5.4
Cantor 1992 [27]	64 cases/133 controls	1.1, 0.8–1.6
Zahm 1993 [18] [1]	12 cases/38 controls	1.2, 0.6–2.6
Orsi 2009 [46]	17 cases /20 controls	1.9, 0.9–3.8
Atrazine		
Zahm 1993 [59]	130 cases/249 controls	1.4, 1.1–1.8
Cyanazine		
De Roos 2003 [30]	37 cases/96 controls	0.6, 0.3–1.0
Metribuzin		
De Roos 2003 [30]	20 cases/53 controls	0.8, 0.4–1.7
Simazine		
Mills 2005 [45]	NR	1.7, 0.9–3.0
Urea herbicides		
Urea herbicides		
Cantor 1992 [27]	5 cases/18 controls	0.6, 0.2–1.6
Orsi 2009 [46]	5 cases/7 controls	1.8, 0.5–6.0
Linuron		
De Roos 2003 [30]	5 cases/22 controls	0.3, 0.1–1.2

Table 4. *Cont.*

Author, date	N exposed	Risk ratio, 95% CI
Other herbicides		
Bentazon		
Cantor 1992 [27]	22 cases/58 controls	0.7, 0.3–1.5
Nitrofen		
Mills 2005 [45]	NR	1.2, 0.6–2.5
Paraquat		
De Roos 2003 [30]	2 cases/15 controls	0.1, 0.2–0.7
Quaternary ammonium compounds, group		
Orsi 2009 [46]	4 cases/12 controls	0.7, 0.2–2.3
Sodium chlorate		
De Roos 2003 [30]	8 cases/7 controls	4.1, 1.3–13.6
Uracil herbicides		
Hoar 1986 [34]	19 cases/114 controls	1.3, 0.7–2.5
FUNGICIDES		
Aldehyde fungicides		
Aldehyde fungicides, group		
McDuffie 2001 [43]	7 cases/25 controls	0.9, 0.4–2.3
Formaldehyde		
McDuffie 2001 [43]	7 cases/25 controls	0.9, 0.4–2.3
Amide fungicides		
Amide fungicides, group		
McDuffie 2001 [43]	30 cases/58 controls	1.7, 1.0–2.8
Captan		
McDuffie 2001 [43]	20 cases/24 controls	2.5, 1.3–4.8
Mills 2005 [45]	NR	0.9, 0.5–1.6
Vitavax		
McDuffie 2001 [43]	10 cases/39 controls	0.8, 0.4–1.9
Carbamate and dithiocarbamate fungicides		
Carbamate fungicides		
Orsi 2009 [46]	15 cases/17 controls	1.8, 0.9–3.7
Maneb		
Mills 2005 [45]	NR	1.1, 0.6–2.1
Mancozeb		
Mills 2005 [45]	NR	0.9, 0.5–1.9
Triazole fungicides		
Triazole fungicides, group		
Orsi 2009 [46]	8 cases/9 controls	1.9, 0.7–5.3
Mecoprop		
Pahwa 2012 [47]	51 cases/81 controls	2.3, 1.5–3.3
Mercury containing fungicides		
Mercury fungicides, group		
McDuffie 2001 [43]	18 cases/48 controls	1.3, 0.7–2.3
Mercury dust		
McDuffie 2001 [43]	15 cases/39 controls	1.2, 0.6–2.4

Table 4. *Cont.*

Author, date	N exposed	Risk ratio, 95% CI
Mercury liquid		
McDuffie 2001 [43]	8 cases/22 controls	1.4, 0.7–3.2
Fumigant fungicides		
Methyl bromide		
Mills 2005 [45]	NR	1.5, 0.8–2.7
Dichloro-propane		
Mills 2005 [45]	NR	0.9, 0.5–1.7
Other fungicides		
Chlorothalonil		
Mills 2005 [45]	NR	1.2, 0.6–2.2
Sulfur compounds		
McDuffie 2001 [43]	17 cases/21 controls	2.8, 1.4–5.6
INSECTICIDES		
Arsenicals		
Acetoarcenate		
De Roos 2003 [30]	41 cases/68 controls	1.4, 0.9–2.3
Arsenic		
Hardell 2002 [33]	8 cases/10 controls	1.8, 0.7–4.5
Eriksson 2008 [32]	7 cases/5 controls	1.6, 0.5–5.2
Lead arsenate		
De Roos 2003 [30]	9 cases/25 controls	0.5, 0.2–1.2
Botanical insecticides		
Nicotine		
Cantor 1992 [27]	31 cases/47 controls	1.5, 0.9–2.5
Cantor 1992 [27] [3]	Handled prior to 1965: 28 cases/36 controls	1.8, 1.0–3.0
Pyrethrine		
De Roos 2003 [30]	6 cases/12 controls	1.0, 0.3–3.2
Eriksson 2008 [32]	15 cases/10 controls	1.7, 0.8–3.9
Rotenone		
Cantor 1992 [27]	12 cases/23 controls	0.5, 2.2–1.0
Carbamate insecticides		
Carbamate insecticides, group		
McDuffie 2001 [43]	37 cases/60 controls	1.9, 1.2–3.0
Zahm 1993 [18] [1]	7 cases/17 controls	1.6, 0.6–4.4
Zheng 2001 [60]	89 cases/172 controls	1.6, 1.0–2.4
Bufencarb		
De Roos 2003 [30]	6 cases/12 controls	1.1, 0.3–3.7
Carbaryl		
Cantor 1992 [27] [3]	Handled prior to 1965: 7 cases/4 controls	3.8, 1.1–13.6
De Roos 2003 [30]	30 cases/57 controls	1.0, 0.5–1.9
McDuffie 2001 [43]	25 cases/34 controls	2.1, 1.2–3.7
Carbofuran		
Cantor 1992 [27] [3]	Handled prior to 1965: 28 cases/63 controls	1.0, 0.6–1.7
McDuffie 2001 [43]	9 cases/18 controls	1.6, 0.7–3.9
Zheng 2001 [60]	66 cases/131 controls	1.6, 1.1–2.3

Table 4. *Cont.*

Author, date	N exposed	Risk ratio, 95% CI
Methomyl		
McDuffie 2001 [43]	37 cases/60 controls	2.1, 1.2–3.7
Fly spray		
Cantor 1992 [27]	185 cases/394 controls	1.1, 0.9–1.4
Cantor 1992 [27] [3]	Handled prior to 1965: 173 cases/368 controls	1.1, 0.9–1.4
Organochlorine insecticides		
Organochlorine insecticides, group		
Cantor 1992 [27]	150 cases/162 controls	1.3, 1.0–1.7
Zahm 1993 [18] [1]	20 cases/46 controls	1.6, 0.8–3.1
Orsi 2009 [46]	15 cases/17 controls	1.8, 0.9–3.8
Purdue 2007 [51]	58 cases/44 non cases	0.8, 0.5–1.3
Pahwa 2012 [47]	106 cases/276 controls	1.3, 1.0–1.7
Aldrin		
Cantor 1992 [27] [3]	Handled prior to 1965: 34 cases/59 controls	1.3, 0.8–2.1
McDuffie 2001 [43]	10 cases/6 controls	4.2, 1.5–12.0
De Roos 2003 [30]	47 cases/97 controls	1.1, 0.7–1.7
Purdue 2007 [51]	21 cases/79 non-cases	0.6, 0.3–1.0
Chlordane		
Woods 1987 [57]	NR	1.6, 0.7–3.8
Cantor 1992 [27] [3]	Handled prior to 1965: 22 cases/22 controls	2.2, 1.2–4.2
McDuffie 2001 [43]	36 cases/105 controls	1.1, 0.7–1.7
De Roos 2003 [30]	21 cases/26 controls	1.7, 0.9–3.2
Purdue 2007 [51]	27 cases/73 non-cases	0.7, 0.4–1.2
DDT		
Woods 1987 [57]	Not reported	1.8, 1.0–3.2
Cantor 1992 [27] [3]	Handled prior to 1965: 68 cases/123 controls	1.3, 0.9–1.8
Persson 1993 [50]	4 case/3 controls	2.0, 0.2–18.9
Baris 1998 [20]	161 cases/340 controls	1.2, 1.0–1.6
Hardell 2002 [33]	77 cases/138controls	1.2, 0.9–1.7
De Roos 2003 [30]	98 cases/226 controls	1.0, 0.7–1.3
Purdue 2007 [51]	37 cases/63 noncases	0.9, 0.6–1.5
Eriksson 2008 [32]	50 cases/37 controls	1.5, 0.9–2.3
Pahwa 2012 [47]	33 cases/59 controls	1.7, 1.1–2.7
Dieldrin		
Cantor 1992 [27] [3]	Handled prior to 1965: 10 cases/13 controls	1.9, 0.8–4.4
De Roos 2003 [30]	21 cases/39 controls	1.8, 0.8–3.9
Purdue 2007 [51]	7 cases/92 controls	0.6, 0.2–1.3
Heptachlor		
Cantor 1992 [27] [3]	Handled prior to 1965: 14 cases/25 controls	1.3, 0.6–2.6
De Roos 2003 [30]	25 cases/43 controls	1.3, 0.7–2.2
Purdue 2007 [51] [2]	18 cases/82 noncases	0.8, 0.4–1.4
Lindane		
Cantor 1992 [27] [3]	Handled prior to 1965: 14 cases/25 controls	2.2, 1.0–4.7
Blair 1998 [23]	93 cases/151 controls	1.5, 1.1–2.0

Table 4. *Cont.*

Author, date	N exposed	Risk ratio, 95% CI
McDuffie 2001 [43]	15 cases/23 controls	2.1, 1.0–4.2
Rafnsson 2006 [52]	37 cases/133 controls	3.5, 1.4–9.0
Purdue 2007 [51] [2]	24 cases/76 controls	1.3, 0.8–2.1
Methoxychlor		
McDuffie 2001 [43]	65 cases/201 controls	1.0, 0.7–1.4
De Roos 2003 [30]	9 cases/16 controls	1.2, 0.5–2.7
Toxaphene		
Cantor 1992 [27] [3]	Handled prior to 1965: 6 cases/5 controls	2.4, 0.7–8.2
De Roos 2003 [30]	10 cases/13 controls	1.5, 0.6–3.5
Purdue 2007 [51] [2]	24 cases/75 controls	1.5, 0.9–2.5
Organophosphorus insecticides		
Organophosphorus insecticides		
Zahm 1993 [18] [1]	14 cases/43 controls	1.2, 0.6–2.5
Waddell 2001 [56]	158 cases/279 controls	1.5, 1.2–1.9
Orsi 2009 [46]	20 cases/24 controls	1.7, 0.9–3.3
Pahwa 2012 [47]	92 cases/169 controls	1.9, 1.4–2.6
Chlorpyrifos		
Waddell 2001 [56]	7 cases/8 controls	3.2, 1.1–9.2
Lee 2004 [38] [2]	37 participants	1.0, 0.6–1.7
Coumaphos		
Cantor 1992 [27] [3]	Handled prior to 1965: 3 cases/5 controls	1.5, 0.3–6.3
Waddell 2001 [56]	23 cases/37 controls	1.7, 1.0–2.9
Crufomate		
Waddell 2001 [56]	5 cases/8 controls	1.6, 0.5–4.9
Diazinon		
Cantor 1992 [27] [3]	Handled prior to 1965: 14 cases/12 controls	2.6, 1.2–5.9
McDuffie 2001 [43]	18 cases/28 controls	1.7, 0.9–3.2
Waddell 2001 [56]	60 cases/93 controls	1.7, 1.2–2.5
Mills 2005 [45]	NR	1.4, 0.8–2.5
Dichlorvos		
Cantor 1992 [27] [3]	Handled prior to 1965: 12 cases/17 controls	1.8, 0.8–3.9
Waddell 2001 [56]	23 cases/51 controls	1.0, 0.6–1.7
Koutros 2008 [37] [2]	6 exposed cases	NR
Dimethoate		
McDuffie 2001 [43]	22 cases/50 controls	1.2, 0.7–2.1
Waddell 2001 [56]	12 cases/22 controls	1.8, 0.9–3.8
Disulfoton		
Waddell 2001 [56]	7 cases/13 controls	2.0, 0.8–5.3
Ethoprop		
Waddell 2001 [56]	7 cases/17 controls	0.9, 0.4–2.3
Famphur		
Waddell 2001[56]	18 cases/47 controls	1.0, 0.5–1.8
Fensulfothion		
Waddell 2001 [56]	4 cases/4 controls	2.0, 0.5–8.2

Table 4. *Cont.*

Author, date	N exposed	Risk ratio, 95% CI
Fonofos		
Waddell 2001 [56]	43 cases/67 controls	1.7, 1.1–2.6
Malathion		
Cantor 1992 [27] [3]	Handled prior to 1965: 11 cases/9 controls	2.9, 1.1–7.4
Waddell 2001 [56]	91 cases/147 controls	1.6, 1.2–2.2
Mills 2005 [45]	NR	1.8, 1.0–3.2
Pahwa 2012 [47]	72 cases/127 controls	2.0, 1.4–2.7
Methyl parathion		
Mills 2005 [45]	NR	0.6, 0.3–1.2
Parathion		
Waddell 2001 [56]	5 cases/8 controls	2.9, 0.9–9.7
Phorate		
Cantor 1992 [27] [3]	Handled prior to 1965: 9 cases/12 controls	1.8, 0.7–4.5
Waddell 2001 [56]	44 cases/97 controls	1.1, 0.8–1.7
Ronnel		
Waddell 2001 [56]	6 cases/11 controls	1.3, 0.5–3.6
Terbufos		
Waddell 2001 [56]	32 cases/97 controls	1.1, 0.7–1.8
Tetrachlorvinphos		
Waddell 2001 [56]	9 cases/17 controls	1.8, 0.7–4.7
Toxaphene		
Mills 2005 [45]	NR	0.9, 0.5–1.9
Trichlorfon		
Cantor 1992 [27] [3]	Handled prior to 1965: 6 cases/5 controls	2.4, 0.7–8.2
Waddell 2001 [56]	7 cases/11 controls	1.8, 0.7–4.7

Notes: 2,4-D, 2,4-Dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-Trichlorophenoxyacetic acid; EPTC, S-Ethyl dipropylthiocarbamate; MCPA, 2-methyl-4-chlorophenoxyacetic acid; DDT, dichlorodiphenyltrichloroethane; NHL, non-Hodgkin lymphoma; NR, Not reported; [1] Only women included in analysis; [2] Cohort study; [3] Effect estimate not included in the meta-analysis; another estimate from the same paper with a larger number of exposed cases was used..

3.4. Meta Analyses

When there was more than one effect estimate for a chemical group or active ingredient, the estimates shown in Tables 3 and 4 were combined to produce meta-analytic summary estimates and 95% CIs (Table 5).

The strongest meta RR estimates were associated with subtypes of NHL. There was a positive association between exposure to organophosphorus herbicide, glyphosate, and B cell lymphoma (2.0, 95% CI: 1.1–3.6, CLR: 3.2). Phenoxy herbicide exposures were associated with B cell lymphoma (1.8, 95% CI: 1.2–2.8, CLR: 2.4), lymphocytic lymphoma (1.8, 95% CI: 0.9–3.5, CLR: 3.8), and diffuse large B-cell lymphoma (DLBCL; 2.0, 95% CI: 1.1–3.7, CLR: 3.3). All these effect estimates were relatively precise, with CLRs < 4.

Table 5. Meta analytic summary estimates of association between herbicides and insecticides with non-Hodgkin lymphoma.

Chemical group or active ingredient	Meta Risk Ratio estimate, 95% CI	I^2	Papers contributing
HERBICIDES			
Amide herbicides			
Amide herbicides	1.3, 0.8–1.9	22.2%	[18,27,34,46]
Alachlor	0.9, 0.6–1.3	43.0%	[30,39]
Aromatic acid herbicides			
Benzoic acid herbicides	1.3, 0.9–1.9	0.0%	[18,27,34,46]
Dicamba	1.4, 1.0-2.1	0.0%	[30,43]
Carbamate/thiocarbamate herbicides			
Carbamate/thiocarbamate herbicides	1.4, 1.1–2.0	0.0%	[18,43,60]
Dinitroanilines			
Dinitroanilines	1.2, 0.8–1.7	0.0%	[27,43]
Trifluralin	0.9, 0.6–1.3	0.0%	[18,30,43,45]
Organophosphorus herbicides			
Glyphosate	1.5, 1.1–2.0	32.7%	[30–33,43,46]
Glyphosate-association with B cell lymphoma	2.0, 1.1–3.6	0.0%	[32,63]
Phenoxy herbicides			
Phenoxy herbicides	1.4, 1.2–1.6	37.7%	[27,32–34,44, 46–50,57,59]
Phenoxy herbicides, association with B cell lymphoma	1.8, 1.2–2.8	0.0%	[32,63]
Phenoxy herbicides, association with DLBCL	2.0, 1.1–3.7	0.0%	[32,63]
Phenoxy herbicides, association with lymphocytic lymphoma	1.8, 0.9–3.5	0.0%	[32,63]
2,4-D	1.4, 1.0–1.9	61.5%	[27,44,45,47,58]
MCPA	1.5, 0.9–2.5	54.4%	[30,32,33,44,47]
Triazine herbicides			
Triazine herbicides	1.5, 1.0, 2.1	38.5%	[18,27,34,46]
Urea herbicides			
Urea herbicides, group	1.0, 0.3–2.9	43.4%	[27,46]
INSECTICIDES			
Arsenicals			
Arsenic	1.7, 0.8–3.6	0.0%	[32,33]
Botanical insecticides			
Pyrethrine	1.4, 0.8–2.8	0.0%	[30,32]
Carbamate insecticides			
Carbamate insecticides, group	1.7, 1.3–2.3	0.0%	[18,43,60]
Carbaryl	1.7, 1.3–2.3	0.0%	[43,60]
Carbofuran	1.6, 1.2–2.3	0.0%	[43,60]
Organophosphorus insecticides			
Organophosphorus insecticides, group	1.6, 1.4–1.9	0.0%	[18,46,47,56]
Chlorpyrifos	1.6, 0.6–4.9	72.0%	[38,56]
Diazinon	1.6, 1.2–2.2	0.0%	[43,45,56]
Dimethoate	1.4, 0.9–2.1	0.0%	[43,56]
Malathion	1.8, 1.4–2.2	0.0%	[45,47,56]

Table 5. *Cont.*

Chemical group or active ingredient	Meta Risk Ratio estimate, 95% CI	I^2	Papers contributing
Organochlorine insecticides			
Organochlorine insecticides, group	1.3, 1.0–1.5	19.6%	[18,27,46,47,51]
DDT	1.3, 1.1–1.5	0.0%	[20,32,33,47,50,51,57]
DDT-association with B cell lymphoma	1.4, 1.0–2.0	0.0%	[20,32,63]
DDT-association with DLBCL	1.2, 0.9–1.7	0.0%	[20,32]
DDT-association with follicular lymphoma	1.5, 1.0–2.4	26.6%	[20,32]
Methoxychlor	1.0, 0.7–1.4	0.0%	[30,43]
Aldrin	1.0, 0.4–2.7	84.6%	[30,43,51]
Chlordane	1.1, 0.8–1.6	32.5%	[30,43,51,57]
Dieldrin	1.1, 0.4–3.1	67.6%	[30,51]
Heptachlor	0.9, 0.6–1.5	0.0%	[30,51]
Lindane	1.6, 1.2–2.2	26.0%	[23,43,51,52]
Toxaphene	1.4, 0.9–2.1	0.0%	[30,45,51]
Amide fungicides			
Captan	1.5, 0.5–4.2	82.5%	[43,45]

Notes: 2,4-D, 2,4-Dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-Trichlorophenoxyacetic acid; DDT, dichlorodiphenyltrichloroethane; EPTC, s-ethyl dipropylthiocarbamate; MCPA, 2-methyl-4-chlorophenoxyacetic acid; NHL, non-Hodgkin lymphoma; DLBCL, diffuse large B cell lymphoma; OC, Organochlorine; OP; Organophosphorus.

The meta RR estimates (95% CI) of association between phenoxy herbicide exposure and NHL subtypes were more positive than those for NHL overall, although the estimate of association with NHL overall was more precise (meta RR, 95% CI: 1.4, 1.2–1.6, CLR: 1.4). Only two papers contributed to each of the estimates of association between phenoxy herbicide exposures and NHL subtypes, and 12 papers contributed to the meta RR estimates for the relationship between phenoxy herbicide exposure and NHL overall.

There was a positive and relatively precise association between NHL and the phenoxy herbicide 2-methyl-4-chlorophenoxyacetic acid (MCPA) (meta RR, 95% CI: 1.5, 0.9–2.5, CLR: 2.6). Five estimates contributed to this summary estimate; an I^2 value of 54.4% indicates some inconsistency in the effect estimates. The forest plot for the meta-analysis of MCPA, along with plots for meta-analyses of phenoxy herbicicides as a group, the phenoxy herbicide 2,4-D, glyphosate, organochlorine insecticides as a group, and the organochlorine insecticide DDT, are presented in Supplementary Figure S1.

In addition to assessing the association of ever exposure to MCPA with NHL, Hardell *et al.* [33] investigated dose-response relationships between number of days of exposure; they observed increasing odds in association with increased number of days of MCPA exposure (Table 2). In similar analyses, Eriksson *et al.* [32] and Mcduffie *et al.* [43] did not observe dose-response relationship between days/year of MCPA exposure and NHL.

There was a positive but less precise estimate of association between arsenic and NHL (meta RR, 95% CI: 1.7, 0.8–3.6, CLR: 4.4). Meta estimates of association between NHL and carbamate insecticides and carbaryl, a carbamate insecticide, were nearly identical (meta RR, 95% CI: 1.7, 1.3–2.3,

CLR: 1.8) and both were positive and precise. Estimates from three papers contributed to the meta analysis of carbamate insecticides. The I^2 value was 0%, indicating consistency in effect estimates. Carbofuran, another carabamate insecticide, was positively associated with NHL (meta RR, 95% CI: 1.6, 1.2–2.3, CLR: 2.0). However, in two investigations from the Agricultural Health Study that reported estimates of association with tertiles of lifetime days of exposure to carbofuran [25] and carbaryl [42], the relationships were imprecise and there was a lack of a dose-response relationship (Table 2).

There were positive and precise estimates of association between NHL and organophosphorus insecticides (meta RR, 95% CI: 1.6, 1.4–1.9, CLR: 1.4), and the organophosphorus insecticides diazinon (meta RR, 95% CI: 1.6, 1.2–2.2, CLR: 1.8), and malathion (meta RR, 95% CI: 1.8, 1.4–2.2, CLR: 1.5). Although Fritschi *et al.* [62] studied the relationship between organophosphorus insecticides and NHL, we did not include the estimate from their paper in the meta analysis because they investigated the association with exposure in three categories (no exposure, non-substantial exposure, substantial exposure). Fritschi *et al.* [62] reported a positive but imprecise estimate for substantial exposure *versus* no exposure (odds ratio, 95% CI: 2.1, 0.8–5.7, CLR: 7.3). The meta RR estimate of association between NHL and the organophosphorus insecticide chlorpyrifos was positive but imprecise (meta RR, 95% CI: 1.6, 0.6–4.9, CLR: 8.9). There was a positive and precise association with lindane, an organochlorine insecticide (meta RR, 95% CI: 1.6, 1.2–2.2, CLR: 1.8); estimates of association with other organochlorine insecticides were closer to the null.

3.5. Sensitivity Analyses

We conducted sensitivity analyses to examine the effect of gender (Supplementary Table S1), study design (Supplementary Table S2), diagnosis period (Supplementary Table S3), geographic region (Supplementary Table S4), source for controls in case-control studies (Supplementary Table S5) and/or the effect of using alternative papers that represent the same study population (Supplementary Table S6). For the most part, meta-estimates were robust.

3.5.1. Gender

When we subset the analyses of associations between NHL and amide herbicides to the two studies that included men only, the association became more positive but less precise (meta RR, 95% CI: moved from 1.3, 0.8–1.9, CLR: 2.3 to 1.7, 0.7–3.8, CLR: 5.3). Restricting to all male studies moved the summary estimate of the relationship with aldrin up and across the null; however, the estimate in the sensitivity analysis was too unstable to interpret (meta RR, 95% CI: moved from 1.0, 0.4–2.7, CLR: 7.8 to 1.4, 0.2–11.1, CLR: 65.0). Restricting the analysis to studies that included men and women caused the meta RR estimate of association between NHL and 2,4-D to become more positive but less precise; it moved from 1.4, 1.0–1.9, CLR: 1.9 to 1.8, 0.5–7.5, CLR: 16.7. We were not able to conduct sensitivity analyses for female only studies, since only one paper reported results for women only [18].

3.5.2. Study Design

Nearly all of the studies that contribute to the meta estimates were case control in design. The only cohort study was the Agricultural Health Study. In nearly all of the analyses of data from the

Agricultural Health Study, exposure was defined using multiple categories. However, in the papers on glyphosate [31], chlorpyrifos [38], organochlorine insecticides, aldrin, chlordane, dieldrin, lindane, and toxaphene [51], the association with ever/never use of exposure was analyzed. For the most part, restricting analyses to case control studies did not cause the meta estimate to change substantially (Supplementary Table S2). However, the magnitude of the meta RR for aldrin moved up and away from the null, but became more imprecise (it moved from 1.0, 0.4–2.7, CLR: 6.8 to 1.4, 0.2–11.1, CLR: 55.5). For lindane it changed from 1.6, 1.2–2.2, CLR: 1.8 to 1.9, 1.2–2.9, CLR: 2.4.

3.5.3. Diagnosis Period

We also investigated the sensitivity of the meta-analytic estimates to decade of cancer diagnosis (Supplementary Table S3). For the most part, estimates were robust. However, when we subset the meta-analysis of glyphosate to the two papers in which cases were diagnosed from 1975–1989, the meta RR, 95% CI changed from 1.5, 1.1–2.0, CLR: 1.8 to 2.3, 1.4–4.0, CLR: 3.0. Similarly, for the phenoxy herbicide 2,4-D, when we included estimates from the three papers with diagnosis periods from 1975 to 1989, the summary estimate was more positive but less precise (meta RR, 95% CI: 1.8, 1.0–3.1, CLR: 3.2) compared to the full meta-analysis estimate (1.4, 95% CI: 1.0–1.9; CLR: 1.9).

3.5.4. Geographic Area

We investigated the impact of geographic area on the meta-analytic RR estimates (Supplementary Table S4). For glyphosate exposure, including estimates from papers that reported results from Swedish studies caused the estimate to become more positive; it moved from 1.5, 95% CI: 1.1–2.0, CLR: 1.8 to 2.2, 95% CI: 1.3–3.8, CLR: 2.9. Similarly, restricting estimates of the relationship between NHL and phenoxy herbicide exposure to Sweden caused the estimate to become more positive; it changed from 1.4, 95% CI: 1.2–1.6, CLR: 1.4 to 1.9, 1.4–2.4, CLR: 1.7. When we restricted estimates of association with MCPA to those that came from North American studies, the meta RR moved towards the null, from 1.5, 0.9–2.5, CLR: 2.6 to 1.1, 0.7–1.8, CLR: 2.7. In contrast, restricting to European and Swedish studies caused the estimate of association with MCPA to become more positive (meta RR, 95% CI: 1.9, 0.9–3.8, CLR: 4.1 and 2.7, 1.6–4.4, CLR: 2.7 respectively). When we included estimates of association with aldrin that came from studies conducted in the USA, the estimate became more precise but moved down and away from the null (meta RR, 95% CI: 1.0, 95% CI: 0.4–2.7, CLR: 7.8 changed to 0.5, 95% CI: 0.4–0.8, CLR: 2.3).

3.5.5. Source of Controls in Case Control Studies

Only two papers reported results from case control studies in which controls were selected from the hospital [46,48]. The meta-analytic RR estimates remained robust when we restricted the estimates to those resulting from population-based case-control studies (Supplementary Table S5).

3.5.6. Alternative Papers

In several cases, analyses of the same study populations were represented in multiple papers. For the meta-analyses, we included the result(s) that represented the largest number of participants.

In some cases, we selected the result from a pooled analysis instead of the individual, original studies. In other cases, use of effect estimates from the individual studies was preferable because it represented more people. We performed sensitivity analyses to evaluate the impact of replacing results from pooled analyses of multiple studies [23,30,59,60] with the original ones [27,34,58], or the original ones with the pooled analyses (Supplementary Table S6).

When we replaced the estimate of a relationship between carbofuran exposure and NHL reported in Zheng *et al.* [60] by that reported in Cantor *et al.* [27] the relationship became weaker and less precise; the meta RR and 95% CI changed from 1.6, 1.2–2.3, CLR: 2.0 to 1.1, 0.7–1.8, CLR: 2.4. Using the estimate reported in De Roos *et al.* [30] yielded a similar result (meta RR, 95% CI changed to 1.1, 0.6–2.0, CLR: 3.1). For the relationship between aldrin and NHL, we replaced the estimate reported in De Roos *et al.* [30] by that reported by Cantor *et al.* [27]; the estimate moved from a null relationship to a positive one (meta RR, 95% CI changed from 1.0, 0.4–2.7 to 1.3, 0.5–2.9).

4. Discussion

This systematic review and series of meta-analyses show that there is consistent evidence of positive associations between NHL and carbamate insecticides, organophosphorus insecticides, lindane, an organochlorine insecticide, and MCPA, a phenoxy herbicide. Our results represent an important contribution to a growing body of literature on agricultural exposures associated with cancer. Past review papers and meta-analyses have identified positive associations between NHL and farming related exposures, including fertilizers, chemicals, and animals [5], and occupational exposures to pesticides [6].

We extracted estimates of association of NHL with individual pesticide chemical groups or active ingredients from 44 papers that reported analyses of results from 17 independent studies. The studies represented data collected in 12 countries, the majority of which were located in either Europe or North America. Several of the papers that we identified were related to one another; many used data from the same cohort study, the Agricultural Health Study, and several others pooled the same data from individual studies. Thus, although this review identified 44 papers, it also highlights the need for additional epidemiologic studies in a larger variety of geographic locations.

In the papers from which we extracted information, estimates of associations with NHL were reported with 13 herbicide chemical groups and 28 herbicide active ingredients, five fungicide groups and 12 fungicide active ingredients, and three insecticide groups and 40 insecticide active ingredients. More than 1,700 active ingredients are listed in Alan Wood's compendium of pesticide common names, although not all of these are necessarily used in agriculture or currently registered for use in any or all countries [13]. Many chemicals remain for consideration in future epidemiologic analyses of associations between NHL and pesticides. It would be useful to identify pesticides to investigate by ranking, by country, the most commonly used chemicals.

The positive and precise estimate of associations of NHL with carbamate insecticides, organophosphorus insecticides, and lindane were robust to sensitivity analyses of gender, geographic area, and cancer diagnosis period. The positive association between MCPA and NHL was robust to a sensitivity analysis of diagnosis period, but when we restricted the meta-analysis to estimates from studies conducted in North America, the estimate moved to the null.

Consistent with the results from the meta-analysis of lindane exposure, analyses of data from the American cohort, the Agricultural Health Study, revealed a positive dose-response relationship between NHL and intensity weighted lifetime days of lindane exposure, where the referent group consisted of applicators never exposed to pesticide products containing the active ingredient [51]. In this same paper, however, the estimate of association with dichotomously coded exposure to lindane was close to the null and imprecise. This difference in results within the Agricultural Health Study suggests that dichotomous classification of exposure might be too crude; the binary categories could lead to exposure misclassification and attenuated effect estimates. Because of variability in definitions and cut-points across papers, we were unable to conduct formal meta-analyses of exposures classified using multiple categories. When they were available, we reviewed estimates of dose-response relationships from the individual papers. We found that, in most of the papers in which dose-response relationships were investigated, effect estimates were imprecise due to small numbers of exposed cases within categories.

There were positive meta RR estimates of association of NHL with two carbamate insecticides, carbaryl and carbofuran, and the organophosphorus insecticide active ingredients diazinon and malathion. However, results from analyses of Agricultural Health Study data, which were not included in the meta-analyses, did not show dose response relationships between NHL and lifetime days of exposure to carbofuran [25], carbaryl [42], diazinon [29] or malathion [21,26].

Some discrepancies in findings from the Agricultural Health Study compared to the other studies could be due to differences in design (cohort *versus* case-control). Differences could also be the result of different referent category compositions. All participants of the Agricultural Health Study were pesticide applicators; therefore, the referent group generally consisted of applicators who were not exposed to the pesticide active ingredient of interest. In contrast, in the papers contributing to the meta-RR estimate for carbaryl [30,43] and carbofuran [43,60], the referent groups consisted of farmers and non-farmers [30,43], or only of non-farmers [60]. In the papers contributing to the meta-analyses of malathion and diazinon, the referent categories consisted of non-farmers [56], farmers and non-farmers [43,47], and only farm-workers [45]. It is possible that, in studies that included non-farmers in the referent group, confounding by other agricultural exposures, not adjusted for in analysis, caused estimates of association to be higher than results from Agricultural Health Study analyses.

Only a handful of papers reported associations of pesticides with NHL subtypes; this is probably due to small sample sizes. Our meta-analyses of these relationships suggested the need for further studies of this kind, especially since some of the strongest relationships were seen with the most common subtype of NHL, B cell lymphoma and, more specifically, with DLBCL. NHL are a heterogeneous group of malignancies that include multiple subtypes with varied characteristics and possibly diverse etiologies [4]. Consequently, the overall group of neoplasms represented by NHL might be too diverse as a study endpoint to adequately detect associations with pesticide exposures in epidemiologic analyses. Some but not all specific subtypes of lymphoma might be associated with pesticides, and these relationships would only be revealed by analyses of the subtypes. Pooling projects that include cases of the NHL subtypes that have been classified according to the more recent and etiologically specific definitions (B-cell, T-cell, and within these, more refined subtypes of T- and B-cell neoplasms) [65] present the opportunity to perform more sensitive

epidemiologic analyses and identify important relationships that may have been undetected if the cancer outcome was defined broadly as NHL overall. Such projects are particularly attractive for studying rarer subtypes (*i.e.*, T-cell). To this end, a pooling project within the AGRICOH consortium [64] is currently underway to investigate these associations.

There are various sources of heterogeneity across the studies that contributed to these meta-analyses; these include gender, region, cancer diagnosis period, exposure assessment methods, exposure definitions, referent groups, study populations, and/or analysis adjustment sets. Different activity patterns, which might cause differences in exposure, combined with different biological mechanisms, could result in between-gender differences in chemical exposure and disease risk associations. Pesticide use, application, and handling patterns, regulations and legislation, demographics and genetics differ by region, which could contribute to area-specific differences in associations. In the papers that contributed to the meta analyses, a variety of exposure assessment methods were used; these included self-reported chemical exposures, exposure matrices, and approximations based on number of animals raised. Differences in exposure assessment methods could influence the magnitude of effects observed, especially since some methods might be superior to others in terms of reducing the potential for exposure misclassification. Study design (case-control *versus* cohort) and source of controls in case-control studies (hospital *versus* population) could also influence the magnitude of the exposure estimates observed. In case-control studies, exposure is assessed retrospectively, which could lead to recall bias. In contrast, in the Agricultural Health Study, the only cohort included in this review, exposure was assessed when participants were cancer-free. Finally, NHL classification systems have changed over time, reflecting changes in disease definitions [1]. Recently (after year 2000), the definition of NHL has become more comprehensive. The definition now includes disease entities that were excluded from earlier definitions, such as plasma cell neoplasms (*i.e.*, multiple myeloma) and chronic lymphocytic leukemia. These malignancies are also among the most frequently reported sub-types within NHL [65]. Thus, estimates of association between pesticides and overall NHL from studies conducted in earlier periods may not be entirely comparable to estimates from research conducted since the year 2000 that used the updated NHL definition.

We did not conduct a formal test of publication bias; it is unclear if asymmetry tests with funnel plots are useful in meta-analyses of observational studies, and it has been recommended that these tests not be used when fewer than 10 studies contribute to a meta-analysis [66]. For the most part, we believe that our review was systematic and comprehensive.

Nevertheless, we did not identify papers that published results of studies conducted in middle- and low-income countries. It is possible that, in such regions where cancer-follow and exposure ascertainment may be particularly challenging, no studies have investigated the relationship of NHL with pesticide exposures. Restricting our literature search to articles published in English could be another reason that we did not identify studies in lower-income countries. A lack of studies in these areas is potentially alarming, since these regions are responsible for much of the world's agricultural production [67]. Also, lympho-hematopoetic malignancies represent a substantial proportion of cancers in low- and middle-income countries. For example, based on estimates from the World Health Organization's GLOBOCAN 2012, NHL accounted for 37.7% of the estimated prevalent cancer cases diagnosed in the past 5 years, among adults in less-developed regions (Africa, Asia excluding Japan, Latin America and the Caribbean, Melanesia, Micronesia, and Polynesia) [68]. Nevertheless, research results

from higher-income countries could be transferable and have important implications for pesticide regulation and legislation world-wide, especially in low-income countries where protective equipment may be less available and/or used.

There are several mechanisms by which pesticide exposure might be associated with NHL. First, pesticides might cause chromosomal aberrations and genetic mutations. An often studied chromosomal abnormality is the t(14;18) translocation, which is particularly common among cases of follicular lymphoma and diffuse large B-cell lymphoma [69]. In a paper that used data from the Iowa/Minnesota case-control study that contributed to several of the pooled and individual analyses that we reviewed [23,30,59], Schroeder *et al.* [70] investigated the relationship between pesticide exposures and the t(14;18) translocation. Compared with controls, t(14;18) positive NHL cases but not t(14;18) negative cases had a higher odds of exposure to dieldrin, toxaphene, lindane, and atrazine. Chiu *et al.* [69,71] performed a similar analysis using data from the Nebraska-based case-control study and reported positive associations between t(14;18) positive NHL and dieldrin, toxapehen, and lindane. A second mechanism by which pesticide exposure may cause NHL is by altering cell mediated immune function. Indeed, immunological changes have been observed following short-term exposure to phenoxy herbicides (2,4-D and MCPA) among farmers [72].

The IARC Monographs have evaluated the carcinogenicity of a handful of pesticides. Of these, only arsenic and inorganic arsenic compounds have been given a Group 1 rating (carcinogenic to humans) [73]. The fumigant insecticide ethylene dibromide was classified as a group 2A carcinogen based on inadequate evidence for carcinogenicity in humans but sufficient evidence in experimental animals; the overall evaluation was upgraded to 2A (probably carcinogenic to humans) with supporting evidence from other relevant data [74]. In Volume 53 (1991) [75], the fungicide captafol was also classified as a group 2A carcinogen based on sufficient evidence in experimental animals but no available data from human studies. In this same volume, several other pesticides were classified as either group 2B (possibly carcinogenic to humans) or group 3 carcinogens (not classifiable as to its carcinogenicity)—aldicarb chlordane/heptachlor, DDT, deltamethrin, dichlorvos, fenvalerate, permethrin, thiram, ziram, atrazine, monuron, picloram, simazine, and trifluralin. The IARC monographs have classified other pesticides, including heptachlor, chlordane, and toxaphene [76], as group 2B carcinogens; in each of these cases, the 2B classification was based on inadequate evidence in humans but sufficient evidence in experimental animals. Chlorophenoxy herbicides were classified as group 2B carcinogens based on limited evidence for carcinogenicity in humans, and inadequate evidence for carcinogenicity of 2,4-D and 2,4,5-T in animals [77].Similarly, hexachlorocyclohexanes were evaluated as group 2B carcinogens due to inadequate evidence for carcinogenicity to humans, sufficient evidence for carcinogenicity to animals for the technical-grade and the alpha isomers but limited for the beta and gamma (lindane) isomers [77]. Several other pesticides, including malathion and maneb [77] have been classified as group 3 carcinogens. These evaluations took place several decades ago and there is now more epidemiologic literature that can provide information. There also remains a need for further epidemiologic research of certain chemicals, which could help to inform future evaluations. In the current systematic review, we did not observe entirely consistent trends in association for all of the active ingredients within chemical groups. Furthermore, classification of active ingredients into groups is subjective and there is not a consistent and established scheme for doing so. Therefore, evaluations of individual active ingredients rather than chemical groups might be more useful.

Limitations and Strengths

Because of variability in definitions and metrics that were used in published papers, we were not able to consider additional exposure definitions, such as exposure lags, duration of exposure (e.g., number of days/year exposed), or routes of exposure (e.g., application *versus* mixing of pesticides). In an effort to use similar exposure definitions from the various papers, we only included dichotomous definitions in the meta-analyses. Since dose-response relationships could not be summarized, this restricted the strength of our conclusions from an etiologic perspective. Furthermore, we were not able to conduct analyses of certain active ingredients or chemical groups due to a lack of published literature. In other cases, very few papers contributed to the meta-analyses. The largest number of papers contributing to any meta-analysis was 12 for phenoxy herbicides, followed by eight for DDT. Most meta-analyses included estimates from only two to three studies. In most papers, associations with NHL overall, rather than with subtypes of NHL, were investigated. Thus, most of our meta-analyses were of associations with NHL rather than with its subtypes, which are probably more homogeneous disease entities for assessing the relationship with pesticides. It is possible that this led to a dilution of effects, since the various NHL subtypes have diverse etiologies and some might be more strongly associated with certain pesticides than others.

Nevertheless, this systematic review represents a novel contribution to the literature on NHL and pesticide exposure. We identified trends in the relationship of NHL and NHL subtypes with chemical groups and active ingredient groups. To our knowledge, this is the most comprehensive systematic review and meta-analysis to investigate associations with specific agricultural pesticide active ingredients. We observed fairly consistent results for certain pesticide groups and active ingredients. We evaluated the robustness of our meta-analyses by examining the sensitivity of the estimates to gender, study design, region, diagnosis period, control source in case-control studies, and paper that provided the effect estimate.

5. Conclusions

We systematically reviewed more than 25 years' worth of epidemiologic literature on the relationship between pesticide chemical groups and active ingredients with NHL. This review indicated positive associations between NHL and carbamate insecticides, OP insecticides, the phenoxy herbicide MCPA, and lindane. Few papers reported associations with subtypes of NHL; however, based on the few that did, there were strong associations between certain chemicals and B cell lymphomas. Our results show that there is consistent evidence that pesticide exposures experienced in occupational agricultural settings may be important determinants of NHL. This review also revealed clear research needs, including further investigation of some already studied pesticide active ingredients, of additional pesticides that have not yet been investigated in epidemiologic analyses, of the strength of association of pesticide exposures with subtypes of NHL, and of the relationship between NHL and pesticides in middle- and low- income areas.

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Author Contributions

Leah Schinasi conducted the literature search, screened the papers from the search, abstracted data from the papers, conducted the meta-analyses, and led the writing of the manuscript. Maria E. Leon co-defined the scope of the review, the search strategy for the literature search and contributed to the writing of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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DRAFT-

Lymphoma risk and pesticide use in the Agricultural Health Study

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P. CAMPBELL

ABBREVIATIONS

Agricultural Health Study (AHS)

Rate ratios (RR)

95% confidence intervals (CI)

Organochlorine insecticides (OC)

Organophosphate insecticides (OP)

United States Environmental Protection Agency (U.S. EPA)

International Agency for Research on Cancer (IARC)

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Running Title: Pesticides and Non-Hodgkin Lymphoma

Abstract: 247 words: 250 word limit for EHP.

Manuscript, references and tables 1-5: 8,162 including title page etc.. [narrative (abstract & main manuscript 3,717, references 1,411, tables 2942] 7000 word limit for EHP.

Comment [a1]: If we have the message and analyses right we have to cut 1,200 words for EHP. We may want to go to another journal.

Comment [AB2]: I suggest go to another journal.

ABSTRACT

Background: Farming and eExposure to pesticides hayes been linked to non-Hodgkin lymphoma (NHL) in a number of previous studies. **Objective**: To evaluate specific pesticides for associations with NHL and NHL subtypes in a prospective cohort of farmers and commercial pesticide applicators~~registered pesticide applicators~~. **Methods**: We examined NHL incidence in a prospective cohort of 57,310 licensed pesticide applicators in Iowa and North Carolina from 1993- 2008. Information on pesticide and other agricultural eExposure, ~~information~~ lifestyle and medical history~~health histories~~ wasere obtained from a self-administered questionnaires administered at enrollment (1993-1997) and in a telephone follow-up questionnaire administered approximately five years later (1998-2004). Poisson regression modeling was used to evaluate the association between use of specific pesticides and the rate ratios of NHL and NHL subtypes while adjusting for age and other potential confounding variables. **Results**: A statistically significant monotonic increase in the risk of overall NHL with increasing life-time exposure-days for lindane (organochlorine insecticide) was observed and a significant positive non-monotonic trend was observed for butylate (thiocarbamate herbicide), among 50 pesticides evaluated. Significantly increasing risk of specific NHL subtypes with increasing life-time exposure-days of use were observed for lindane, butylate, dicamba, terbufos, alachlor, EPTC, imazethapyr and trifluralin. The total number of different pesticides used was not associated with NHL risk overall, but the number of different triazine/triazone herbicides was significantly associated NHL. Chlorinated and organophosphate insecticide and triazine/triazone herbicides used, was related to risk in specific NHL subtypes. **Conclusions**: A wide variety of chemically-distinct herbicides and insecticides were significantly associated with different NHL subtypes. Most pesticides are associated with only one NHL subtype.

Comment [AB3]: Need to indicate which subtypes were associated with which pesticides.

Comment [AB4]: Mention the chemical class – subtype associations before the specific pesticide associations. Go from the general to the specific.

Comment [AB5]: I am not sure we want to deliver this message. As written it says we believe we found a number of meaningful pesticide – subtype links and that the links were specific. This implies we believe these findings are probably "real." I think the message should be – this is one of the few studies (and the only prospective study I think) that has looked at specific pesticide – subtype associations. Since different subtypes may have different etiologies these findings provide leads for future evaluations.

Keywords: Cohort Study, Farming, Pesticide Exposure, Non-Hodgkin Lymphoma.

INTRODUCTION

Non-Hodgkin lymphomas (NHLs) are a heterogeneous group of over ~~20~~ different B and T-cell neoplasms affecting the immune system/ lymphatic system arising primarily in the lymph nodes (Swerlow et al. 2008; Shankland et al., 2012). M~~Numerous~~ eta-analyses (Blair et al., 1985; Blair et al., 1993; Beane Freeman, 2009) studies relate lymphohaematopoietic cancers with farming (~~Blair A et al., 1993; Blair and~~ Beane Freeman, 2009), with exposure to pesticides being a hypothesized etiologic agent. Since the 1980s a number of studies have been conducted to evaluate possible links between specific pesticides and NHL. A meta-analysis of 13 case-control studies published between1993-2005 observed an overall significant meta-odds ratio between occupational exposure to pesticides and NHL (OR=1.35; 95% CI: 1.2-1.5). When observations were limited to those that had more than 10 years of exposure the risk increased (OR=1.65; 95% CI: 1.08-1.95) (Merhi M, et al., 2007). While the meta-analysis supports the hypothesis that pesticides are associated with NHL, it did not~~they lack sufficient detail about~~ evaluate exposure to specific pesticide exposure and other information on risk factors for hematopoietic cancers to identify specific causes (Merhi M, et al., 2007). In individual studies of NHL have reported links a number of specific pesticides including phenoxy acid herbicides (Dich et al 1997; Hardell L et al., 1981; Hoar SK et al., 1986; Zahm et al, 1990, Miligi et al, 2006, McDuffie et al, 2001Eriksson M et al., 2008; Burns et al., 2011; ~~8~~), ~~and~~ chlorinated pesticides (McDuffie et al, 2001, Colt et al., 2006; Spinelli JJ et al 2007, Purdue et al, 2007, Brauner EV, et al., 2012; Quintana et al., 2004; Coco et al., 2004), organophosphates (Waddell et al., 2001; Hohenadel et al., 2011)dicamba (McDuffie et al., 2001; nitro-derivaties (Miligi et al., 2003); and triazole fungicides and urea herbicides (Orsi et al., 2009)~~have been suggested as causes of NHL, ,~~ but the evidence has been inconsistent. Little evidence of an association between phenoxy acid herbicides and NHL was observed in New Zealand (Pearce NE et al 1987), Washington state (USA) (Woods JS, et al 1987), or Minnesota and Iowa (USA) (Cantor KP et al, 1992) and little evidence for chlorinated pesticides was observed in a European study that measure pesticide metabolites in plasma samples (Cocco P et al, 2008). A variety of other pesticides have also been associated with NHL but the evidence available to date does not conclusively link a specific pesticide to NHL (Alavanja M et al., 2012; Cocco P et al., 2013). In a study from the six Canadian provinces case-control study, the risk of NHL increased with the number of different pesticides used (Hohenadel K et al., 2011).(I think the flow of this first

Comment [AB6]: References are numbered in the reference list, but not in the test.

Comment [AB7]: Is the Beane Freeman article cited here Laura's livestock article? It is the only one in the references.

Comment [a8]: Moved the Merhi study up to mention the general association first and later the pesticide class specific-Done

Comment [a9]: Added reference

Comment [a10]: Added reference

Comment [a11]: Added reference

Comment [a12]: Added Purdue

Comment [a13]: Sentence added in reference to Laura's comment to mention other chemical associations by way of citing a review article.-Done We are >8,100 words, EHP limit 7,000

Comment [a14]: Cindy suggests cutting down the introduction. --Done

paragraph can be modified to make it clearer. Start with farming, then list pesticides that have been linked to NHL in some studies. This should cover the different pesticides that have been linked to NHL. Then list your review and Cocco (2013) to indicate that the evidence is not conclusive for any pesticide).

In the Agricultural Health Study (AHS) we had the opportunity to evaluate the risk of NHL overall and by cell type by ~~both the association of~~ lifetime use of individual pesticides obtained from enrollment and follow-up questionnaires ~~and the number of different pesticides used and NHL incidence overall and by cell type~~ in a prospective cohort study of licensed pesticide applicators in Iowa and North Carolina.

~~We evaluated potential confounders including a previous history of malignant disease (Wang et al., 2007), different immunosuppressive states (Simard JF, et al., 2012), and body mass index (BMI) (Patel et al., 2013) and other factors observed to be associated with NHL in the AHS cohort.~~

MATERIALS & METHODS

Study Population

The AHS is a prospective cohort study of 52,394 licensed private pesticide applicators in Iowa and North Carolina and 4,916 licensed commercial applicators from Iowa. The cohort has been described in detail (Alavanja et al., 1996). Briefly, the cohort included individuals seeking licenses for restricted use pesticides from December 1993 through December 1997 (82% of the target population enrolled). The protocol was approved by relevant institutional review boards. We obtained cancer incidence information by regular linkage to cancer registry files in Iowa and North Carolina. In addition, we matched cohort members to state residential mortality registries and the National Death Index to identify vital status, and to address records of the Internal Revenue Service, motor vehicle registration files, and pesticide license registries of state

Comment [a15]: Infor about cancer registries deleted as suggested by Laura.

agricultural departments to determine residence in Iowa or North Carolina. The current analysis included all incident primary non-Hodgkin lymphomas (*n*=333) diagnosed from enrollment (1993-1997) through December 31, 2008. We censored follow-up at diagnosis of NHL or any other cancer, date of death, movement out of state, or December 31, 2008, whichever was earlier. Person-years of follow-up summed to 714,770.

Tumor Characteristics

Information on tumor characteristics was obtained from state cancer registries. Cases were classified into 5 groups of cell types according to the Surveillance Epidemiology and END Result (SEER) coding scheme (http://seer.cancer.gov/lymphomarecode) SEER recodes of cell type are listed in appendix 1.- The first group (n=117) includes chronic B-cell lymphocytic lymphomas (CLL) /small B-cell lympocytic lymphomas (SLL) [n=101], and mantle-cell lymphomas (MCL) (n=16). The second group includes 94 diffuse large B-cell lymphomas; the third group includes 53 follicular lymphomas. There were 34 'other B-cell lymphomas' consisting of a diverse set of B-cell lymphomas including precursor acute lymphoblastic leukemia/lymphoma (n=4), Waldenstrom macro globulinemia (n=2), lymphoplasmacytic lymphoma (n=2), hairy-cell leukemia (n=6), B-cell non-Hodgkin lymphoma not otherwise specified(n=6), Burkitt lymphoma/leukemia (n=1), and extra-nodal Marginal Zone Lymphomas (MZL)/ MALT type/ Nodal MZL(n=13). The fifth grouping included 35 cases consisting of T-cell lymphomas (n=12) and non-Hodgkin lymphoma of unknown lineage (n=23). The fifth grouping was excluded from cell type-specific analyses because of small numbers of cases with identified cell types. Although multiple myeloma (MM) (n=77) and plasmacytomas (n=6) are

Comment [lbf16]: Did you remove prevalent cancers? Does this mean that you also included second cancers if they were NHL? Eg. If someone had an incident prostate cancer and then was diagnosed with an NHL, do you consider them to be an NHL case? Or, did you censor them at their diagnosis of prostate cancer? I would remove all prevalent cancers (n=1,074) and only include first primary NHL diagnoses, censoring at diagnosis of any cancer.

Comment [a17]: Yes, we removed all prevalent cancers and included only primary NHL cases.- clarification made in sentence.-no other change necessary.

Comment [a18]: Cindy would like the 5 groups to be named. They do not have names so it is may be inappropriate to give them non-standard names. I gave the SEER recode number in the table as a means of identification.

Comment [lbf19]: Since you present them in the appendix, I would suggest taking them out of the text here—it'shard to read with all these numbers. You could also add them to the relevant tables under the specific sub-types.

Comment [a20]: SEER recodes deleted as recommended by Laura.

now classified as a type of non-Hodgkin lymphoma (Morton LM et al., 2007), the pesticide literature prior to 2008 (including the AHS) examined multiple myeloma (and plasmacytomas) separately. (AB - I wonder if the decision not to include myeloma might seem inconsistent with our decision to go with the new definition of NHL. We say we are changing the cancers we characterize as NHL to fit the new definition, but then we promptly say we are not going to follow the new definition for all of the new inclusions, i.e., myeloma will not be included. It is inconsistent and seems gerrymandered. The reason given also does not seem adequate (myeloma has been analyzed separately for pesticides) because there have also been studies that looked a pesticides and chronic lymphocytic leukemia, yet it is included as NHL here. Not sure what to do but the whole thing just seems messy. We need to talk about this on an EC call.) We continue to examine MM separately to facilitate comparisons to the previous literature. We provide supplemental table 7 which shows NHL risk (previous definition, ICD-O-3) and lifetime use of individual pesticides (AB - I think to make clear the possible the impact, or lack of it, of changing the NHL definition, Table 7 needs to include ORs from both definitions of NHL for the same length of follow up. This would make it clear that any difference regarding specific pesticides would be due to differences in disease classification.) A comparison of cell types in the previous (ICD-O-3) and recent Inter Lymph hierarchical classification of NHL is provided in appendix 2.

Comment [a21]: We added the phrase 'prior to 2008" to avoid a large increase in citations which would contribute an additional 90 words or more (approximately).

Comment [lbf22]: You will need to cite these papers in the discussion.

Exposure Assessment

Information on lifetime use of 50 pesticides was captured in two self-administered questionnaires (http://aghealth.org/questionnaires.html) completed during cohort enrollment (Phase 1). All 57,310 applicators completed the first enrollment questionnaire, which inquired about ever/never use of the 50 pesticides, as well as duration (years) and frequency (average days/year) of use for a subset of 22 pesticides. In addition, 25,291 (44.1%) of the applicators returned the second (take-home) questionnaire, which inquired about duration and frequency of use for the remaining 28 pesticides.

A follow-up questionnaire, which ascertained pesticide use since enrollment, was administered about five~~5~~ years after enrollment (1998-2003, Phase 2) and completed by 36,342 (63%) of the original participants. For participants who did not complete a Phase 2 questionnaire (20,968 applicators~~, 37%~~), a data-driven multiple imputation procedure based on logistic regression and stratified sampling was employed to impute likely use of specific pesticides in Phase 2 (Heltshe et al.,2012) ~~which used logistic regression and stratified sampling to impute the use of specific pesticides in phase 2.~~

Comment [a23]: Description of imputation procedure shortened considerable per suggestion.- Done

Information on pesticide use obtained from Phase 1 and Phase 2 interviews was used to construct two individual pesticide exposure metrics~~We used 2 exposure metrics to assess cumulative exposure to each pesticide~~: (i) lifetime days of pesticide use, i.e. the product of years of use of a specific pesticide and the number of days used per year; and (ii) intensity-weighted lifetime days of use, i.e. the product of lifetime days of use and a measure of exposure intensity. Intensity of exposure was derived from an algorithm using questionnaire data on mixing status, application method, equipment repair and use of personal protective equipment (Coble et al. 2011).

Comment [a24]: Dropped Dosemeci as suggested. Dosemeci is referenced in Coble et al. No additional changes made to this section.

We analyzed total NHL risk and specific cell type NHL by pesticide classes, individual pesticides ~~use~~, and by the number of different pesticides used within a chemical/functional class and the total number of different pesticides used in a working lifetime.

Comment [a25]: Analysis requested by Aaron.

Statistical Analyses

We used Poisson regression to calculate rate ratios (RR) and 95% confidence intervals (95% CI) for overall NHL and four NHL subtypes in relation to pesticide use. Data were obtained from AHS data release versions P1REL201005.00 (for Phase 1) and P2REL201007.00 (for Phase 2). We evaluated pesticides with 15 or more exposed cases of total NHL, thereby excluding aldicarb, aluminum phosphide, carbon tetrachloride/carbon disulfide, dieldrin, (Might look specifically at dieldrin even though it is below your cutpoint because it has been linked to NHL in the past.) ethylene dibromide, maneb, parathion, 2,4,5-TP, trichlorofon, and ziram (This list is different than that provided in the first draft. Why the change?). For each pesticide analyzed, we categorized exposure into non-exposed and tertiles of exposure based on the distribution of exposed cases. A first set of rate ratios were adjusted for age and a second set of rate ratios were adjusted for age and other statistically significant (α=0.05) predictors of NHL in the AHS. We evaluated several lifestyle and demographic measures and identified the following as potential confounding variables: age at enrollment (<40, 40-49, 50-59, 60-70, ≥70), race (White, Black, other, missing), state (Iowa, North Carolina), family history of lymphoma in first-degree relatives (yes, no, missing), body mass index (BMI <25, 25-<30, ≥30), cigarette smoking history (never, former, current, missing), alcohol consumption per week (none, < once per week, ≥ once

Comment [a26]: Correction suggested by Cindy.

Comment [a27]: We analyzed BMI and it was not a confounder. We added to table 1.

We examined available pack-years and there was no confounding.

per week) and several occupational exposures (i.e., number of livestock, poultry, acres planted, welding, diesel use, number of different pesticides used, and pesticides shown to be associated with NHL in the current analysis)(So all of these factors all significantly associated with risk of NHL here? From Table 1 it looked like most of the other adjustment factors were not significantly associated with NHL.). Tests for trend used the midpoint value of each exposure category, and the Likelihood Ratio tests were used to assess differences between strata (p-interaction). All tests were two-sided and conducted at the α=0.05 level. (I do not quite understand the rationale for the tables. The above indicates ORs were adjusted for several factors. The first set of tables say they are "age adjusted." The supplemental tables have more extensive adjustment. If it is important to adjust for factors other than age, why are these analyses in supplemental tables. If they are not important, why are they done at all. In any case I am not sure you need two tables. Often you see age adjusted and more extensively adjusted ORs in the same table. That would be better because it allows the reader to see if the additional adjusment made any difference in the ORs.)

We also conducted various sensitivity analyses. We analyzed Phase 1 data alone to assess the impact of the additional information collected or imputed from Phase 2. We also explored the effect of lagging exposure data 5 and 15 years since ~~recent these~~ recent exposures may not have had an impact on the development of cancer. Reported results show un-lagged exposure data from Phase 1 and Phase 2 combined for cumulative intensity-weighted and un-weighted days of use. (AB - I think we should start doing some analyses by type of protective equipment used. I know it is supposedly taken into account in the intensity score, but it would be informative if there were differences in OR by different protective approaches. It could be used with number

Comment [AB28]: Probably need to add you chose to show these data because the other analyses had not impact.

of days of pesticide use where it has not been taken into account. It provides information that is useful to farmers and extension agents.)

RESULTS

The risk of NHL increased significantly and in a near monotonic fashion with age in the AHS cohort (Table 1). The age-adjusted risk of NHL is significantly lower in NC compared to IA and among current smokers compared to nonsmokers. Other demographic factors including gender, license type, educational level, alcohol consumption, BMI, and a family history of lymphomas were not significant risk factors of NHL in this cohort. We evaluated whether other occupational factors were associated with NHL. Of those evaluated, the number of livestock on the farm and whether cohort members drove farm equipment with diesel engines significantly increased risk of NHL.

The age-adjusted risk of NHL and NHL subtypes from possible exposure to~~associated with~~ 16 insecticides and herbicides ~~associated with NHL or NHL subtypes or~~ previously associated with NHL are listed in Table 2 (age-adjusted risk of NHL for all other evaluated pesticides in the AHS may be found in supplemental table 1 and fully-adjusted risk of NHL in supplemental table 2). Lindane, an organochlorine insecticide, is the only pesticide showing a monotonic rise in overall NHL risk with increasing life-time days of use (p trend=0.003) and intensity-weighted lifetime days of use (p trend=0.05). Butylate, a thiocarbamate herbicide, showed a significant increasing trend in life-time days of use (p trend=0.004) and intensity-weighted lifetime days of

Comment [lbf29]: I think that you can cut down on reporting the results that are presented in the tables, but I would like to see some more results in the text that aren't in the tables. E.g., what happens when you put both lindane and butylate in the model? What is frequency of use of chemicals, etc.?

Comment [a30]: Narrative now mentions that there is no apparent confounding between lindane and butylate. Only pesticides with 15 or more exposed cases are listed in the tables for analysis. Space limits more extensive discussion of frequency of pesticide use in the AHS, although this can be ascertained from use in controls.

Comment [AB31]: The Methods says they were significant risk factors.

Comment [a32]: Previous table 2 deleted and discussion of potential confounding variables shortened as suggested by Laura.

Comment [t33]: It's not clear why you are showing these 22 pesticides

Comment [AB34]: I think it would help the reader if you presented ever/never results for all pesticides analyzed. This would set the stage for the exposure response analyses. You would largely include only those pesticides with some excess in the ever category in the trend analyses. Now it is not clear why some are listed and others are not. As of now the Results just sort of jump into detailed exposure-response analyses.

Comment [t35]: If there's not a big difference between age and fully adjusted models I would delete fully adjusted

use (p trend=0.04) but the associations were not monotonic. Some other pesticides had individual point estimates that were significant but did not show a significant pattern of increasing risk with increasing exposure. Lindane and butylate did not ~~show~~ confound~~ing~~ ~~with~~ each other when they were put in the same model. The significant increasing trend of NHL risk with exposure to lindane and butylate was also not changed with the adjustment days of all other pesticide use, nor with adjustment for days of use of organophosphate insecticides, carbamate insecticides, other insecticides, triazine/triazone herbicides, other herbicides, fungicides, or fumigants. The results from fully adjusted risk of NHL (i.e., Age [<45,45-49,50-54,55-59,60-64,65-69,≥70], smoking status(current, former, never), number of livestock (0,,<100,100-999,>999), drove diesel tractor (<weekly,≥weekly, state (NC, IA) [data not shown were comparable to the age-adjusted risk]. Also, these unlagged results were comparable (not shown) to 5 year and 15 year lagged exposures, therefore we present RRs for unlagged exposure only.

Comment [lbf36]: I find these lists of RR and 95% CI throughout to be a bit hard to read, plus they take up a lot of words. I think it would be better to provide more information in the text about results that aren't presented in the tables. E.g., for lindane,, how many people reported using it in Phase 1 vs. Phase 2 as it was approaching phase out. This will help to set the stage for putting the results in context later in the discussion.

Comment [a37]: Point estimates deleted to reduce word count as recommended.

Comment [a38]: Need to define the pesticides included in each group appendix 2-done

Comment [AB39]: Supplement Table 2 does show the fully adjusted model, right?

We also analyzed Phase 1 data only to assess the impact of the additional information collected or imputed from Phase 2, although there was an increase in precession including phase 2 estimates, no meaningful change was observed in the risk estimates. ,

Comment [lbf40]: I don't think you mention this in the results.

Comment [lbf41]: How did you choose the 22 pesticides in this table? Why not 28 as in table 2? Regardless, need to explain rationale/criteria for presenting some and not others

The risk of the four major categories of B cell lymphomas by number of days of use of individual pesticide is shown in Table 3. For the CLL/SLL/MCL group of lymphomas, dicamba, a carbamate herbicide (p trend=0.03) and butylate, a thiocarbamate herbicide (p trend=0.04), and

lindane, a chlorinated insecticide, (p trend=0.005) were observed to have a significant increased trend of risk with increasing lifetime-days of use. Metribuzin, a triazone herbicide, (p trend=0.06) had a near significant relationship with this group of lymphomas. Carbaryl, a carbamate insecticide, was observed to have a significant inverse relationship (p trend=0.007).

Comment [a42]: Metribuzin, is a triazone herbicide not a triazine herbicide.-corrected

A significant increase in the risk of Other B-cell Lymphomas was associated with the number of life-time days of use of six herbicides and one insecticide: alachlor (p trend=0.02); butylate, (p trend=0.0499); dicamba (p trend=0.02); EPTC use (p trend=0.01): imazethapyr (p trend=0.03); trifluralin use (p trend=0.01); and terbufos (p trend=0.01) (Table 3). Risk of other B-cell lymphomas was also associated with a non-significant elevated risk for the low and medium exposure categories and was significantly associated with the highest category of exposure for atrazine use (RR=3.6 [95% CI: 1.2-10.8]; p trend=0.06).

Comment [AB43]: Since insecticides come before the herbicides in the table discuss terbufos before the herbicides here in the text.

No pesticide had a significant exposure response pattern with either diffuse large B-cell lymphomas or follicular B-cell lymphomas, although significant point estimates of risk were identified for butylate, terbufos, and methyl bromide.

Comment [AB44]: Glyphosate had a significant trend for diffuse and chlordane and malathion were borderline. EPTC and butylate had borderline trends for follicular.

The number of different triazine/triazone herbicides used, adjusted for age and lifetime days of use of triazine/triazone herbicides was associated with a significant increasing trend with total NHL risk (p trend=0.04) (Table 4). No other chemical/functional class showed a significant pattern of NHL risk. The association between the age-adjusted risk of the four NHL B-cell sub-types and the total number of different pesticides by chemical class used is presented in Table 5. For the CLL/SLL/MCL group of lymphomas, the number of different chlorinated insecticides (p

Comment [AB45]: Not sure what is meant here. Triazine/triazones adjusted for triazine/triazone?

trend=0.02) and the number of different organophosphate insecticides (p trend= 0.03) showed a significant trend of increase risk with increasing number of insecticides from these chemical/functional classes. Similar trends were observed for the number of different triazine/triazone herbicides (p trend=0.07), other herbicides (p trend=0.06) and fungicides (p trend=0.11) but the trends were not statistically significant.

Comment [a46]: Typo corrected as suggested.

For either diffuse large B-cell lymphomas or follicular B-cell lymphomas, no pesticide class had a significant pattern of increasing risk with number of pesticides used, although a significant decreased risk with increasing number of pesticides used was observed for chlorinated pesticides (p trend=0.05) and other insecticides (p trend= 0.04) with the diffuse large B-cell lymphoma group.

For the other B-cell lymphoma group, the number of different triazine/triazone herbicides (p trend=0.006) and the number of different acetamide herbicides (p trend= 0.009) both were observed to have a significant trend of increasing risk with increasing days of use. Similar trends were observed for the number of different carbamate herbicides (p trend=0.11) and 'other herbicides' (p trend=0.06) but these trends were not statistically significant.

Comment [a47]: These will be adjusted for total number of exposure days to chemicals in this class.- Done

DISCUSSION

Comment [lbf48]: Throughout , you need to reference the previous analyses of AHS data and specific chemicals. You reference Mark Purdue's paper in the intro, but no others

Comment [a49]: See changes made throughout to address these points.

AB – I think we need to start with the big picture comparisons first. I suggest the order for the discussion should be: (1) Ever/never comparisons for NHL overall, (2) Then move to trends for NHL overall, (3) Then trends for subtypes. (4) Next have a discussion of how the change in

Comment [lbf50]: *This paper just came out and used the most recent definitions of NHL. Actually supportive of these AHS findings.*
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doi:10.1136/oemed-2012-100845

Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study

NHL definition might affect comparison of our results with those from the literature. (5) Comparison of these results with literature pesticide by pesticide (or pesticide group). (6) Strengths and limitations. (7) Conclusions.

In this analysis, we observed a significant increase in the risk of overall NHL with two pesticides, lindane an organochlorine insecticide no longer registered for use in the U.S and butylate a thio-carbamate herbicide widely used in the United States and other countries. Our findings for total NHL are inconsistent with a number of other studies which found increased risks with a variety of chlorinated and organophosphate insecticides and triazine and phenoxy acid herbicides (Dich et al 1997; Hardell L et al., 1981; Hoar SK et al., 1986; Zahm et al, 1990). However, we did find significantly increasing risk of specific NHL subtypes with increasing life-time exposure days of individual pesticides use. Butylate and dicamba, carbamate herbicides, and lindane, a chlorinated insecticide, were observed to have a significant increasing risk of the CLL/SLL/ MCL lymphomas sub-types with increasing lifetime-days of use. (This first paragraph just sort of jumps into the subtype/specific pesticide links. I think a smoother opening paragraph would be to comment on ever/never for specific pesticides, then exposure trends by specific pesticide, and finally exposure trends by NHL subtypes. This summary of the findings should then be followed by a discussion of the effects, or lack of them, from the change in the definition of NHL. Then the findings from this analysis can be compared to the previous literature.)

Comment [lbf51]: What was percentage of use in P1 vs. P2? If people aren't still using, but we still have excess then we need to explore this further. Do we see stronger effects in earlier time periods? Do we expect this to not be aproblem since lindane is no longer on the market? Or, is this going to be a persistent problem? We also need to say something about when lindane was taken off the market.

Comment [AB52]: There is a bit of an inconsistency here. Says there is an excess for lindane, but these findings differ from earlier work that saw excesses for a variety of chlorinated insecticides. Lindane is a chlorinated insecticide.

Comment [lbf53]: This sounds like all the other studies are positive, which isn't actually true. I think that you need to have a more in-depth discussion of specific pesticides and findings.

Comment [AB54]: I do not think we can make this statement of differences with past studies without immediately including a discussion of the difference in disease definition and whether or not this might account for the differences/or similarities with past research. Probably need to start the discussion with comparison of results of analyses for the two different definitions to orient the reader regarding what changes occurred simply because of the change in definition. Then this should be followed with a discussion of findings from an ever/never comparison. Then you go to trends.

Other B-cell lymphomas are a varied group including 8 different cell types of lymphomas. Excess risks of other B-cell lymphomas were observed for several widely-used pesticides including: the organophosphorous insecticide terbufos, for alachlor, an acetanilide-herbicide, imazethapyr, an imidazoline-herbicides, and trifluralin, a dinitroaniline-herbicide, and for

butylate, dicamba, and, EPTC which all belong to the family of carbamate herbicides. The triazine herbicides atrazine and cyanazine had specific point estimates that were elevated but the trends of risk were neither significant nor monotonic. ~~Metribuzin, a triazone herbicide. had too few other B-cell lymphomas to evaluate.~~ The wide array of functional groups and chemical classes that are associated with an increased risk of Other B-cell lymphomas does not suggest a single known mechanism of action. Multiple pathways seem to be involved.

Comment [AB55]: I am not sure you want to talk about pathways. This assumes that the links observed here are real. Perhaps the wide array of function groups and chemical classes is just noise. You might try to dissect the individual histologies in this "Other B-cell" to see if any one stands out with a particular pesticide.

In a Swedish case-control study a significant excess risk of NHL was associated with the phenoxy herbicide MCPA and glyphosate (Ericksson et al., 2008). 2,4-D and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) have been banned from Sweden and could not be evaluated (Eriksson M et al.,2008). In our study we could not evaluate MCPA but found no excess risk of NHL or its subtypes with the use of glyphospate, 2,4-D or 2,4,5-T.

Comment [AB56]: Check to make sure 2,4-D was banned during the time of pesticide use by people in Eriksson's study. My impression is that it just was not used much in Scandinavia, but was not banned until later.

In a population-based case-control study conducted in six Canadian provinces increased risk to NHL was associated with a positive family history of cancer both with and without pesticide exposure [OR=1.72 (95% CI 1.21-2.45) and OR=1.43 (95% CI: 1.12-1.83), respectively] (McDuffie HH, et.al, 2009). In this same case-control study six pesticides/pesticide analytes also showed a significant association with NHL [beta-hexachlorocyclohexane, *p, p'*- dichloro-diphenyl-dichloroethylene (DDE), hexachlorobenzene, mirex, oxychlordane and trans-nonachlor] (Spinelli et al., 2007). The strongest association was found for oxychlordane, a metabolite of the pesticide chlordane (highest vs. lowest quartile OR=2.68, 95% CI 1.69-4.2). These finding were not confirmed in a recent analysis of plasma samples from 174 NHL cases and 203 controls from France, Germany and Spain. The risk of NHL did not increase with

Comment [AB57]: Not sure we need this sentence. Certainly should not lead with it because family history was not evaluate our NHL study.

plasma levels of hexachlorobenzene, beta-hexachlorobenzene or DDE (Cocco P et al., 2008). In our study NHL was associated with lindane but no excess risk was observed for chlordane and no excess risk was observed among those with a family history of lymphoma. ~~The other chemicals evaluated in the Canadian six province study were not evaluated in the AHS cohort.~~

New evidence linking NHL with chlorinated pesticide use (Brauner EV, et al., 2012) and a study linking the number of different pesticides used with NHL (Hohenadel K et al., 2011) are somewhat supported by our findings in the AHS cohort. While the number of different pesticides used overall was not associated with NHL risk in the AHS, a significant increase in the CLL/SLL/MCL sub-group of NHL was observed with the number of different chlorinated pesticides used and the number of different organophosphate chemicals used. A similar pattern of increase risk was observed in the other B-cell lymphoma subgroup of NHL with an increasing number of triazine/triazone pesticides used.

Comment [lbf58]: Expand to discuss what these actually show—similar to ours? Not similar to ours?

Comment [a59]: Modified sentence in response to comment.

Comment [AB60]: I have a hard time following the discussion. I wonder if it might not be clearing if the link to previous literature is done pesticide by pesticide. Then you could indicate what is found here and follow that with findings for that pesticide in the literature. This means previous studies could be cited numerous times, but it would be easier to see the relationship between our findings and those from other studies for individual pesticides.

A strength of this investigation is that a relatively large population of licensed pesticide applicators provided reliable information regarding their pesticide application history (Blair et al. 2002; Coble et al. 2011, should cite Jane's paper on reliability also). In the AHS, a priori derived algorithm scores that incorporated several exposure determinants were found to be able to ~~used to~~ predict urinary pesticide levels (Thomas et al., Coble 2011). Few? studies of pesticide use with a prospective design have been large enough or had sufficiently detailed exposure information, to evaluate the potential link between NHL, NHL subtypes and specific pesticide exposures (Are there any other prospective studies that could look at specific pesticides?). Also, because occupational pesticide users are seldom exposed to a single agent, we controlled for the total pesticide exposure days and total pesticide exposure days by chemical/functional class and found

no meaningful change in the associations. Additionally, potential confounding of pesticides by other occupational exposures was reported to be minimal in the AHS (Coble et al., 2002) and adjustment for various agricultural exposures did not fundamentally change calculated RR for NHL from various pesticide exposures. ~~–~~ (Mention ability to control of possible non-occupational confounders. use of incidence rather than mortality)

Comment [AB61]: I have a real problem with this approach and the interpretation of the findings from it. Is total pesticide exposure days associated with NHL? If not, then it clearly does not control from individual pesticides because some individual pesticides are associated with NHL. This would work if most pesticides were associated with NHL, but most are not Thus, this total pesticide scale is so water down that it cannot control for anything. This said, I doubt that there is confounding among the pesticides, but we cannot us this approach as evidence for no confounding. The most straightforward, and usual approach, is to adjust the RR for one pesticide by each individual pesticide thought to be a potential confounder.

Although this is a large prospective study, there are limitations~~limitations should be acknowledged~~. Cell-type information in the AHS was obtained from the cancer registry database and did not involve pathologic re-review of diagnostic slides. Other limitations including a small number of exposed cases for certain chemical of interest.

Comment [AB62]: I do not think I would list this. These are data that are used to establish cancer patterns by the NCI. I think the reliability/validity of the diagnosis from tumor registries is well accepted.

Need to add a paragraph of exposure assessment. Discuss the information on our exposure scale in relation to the monitoring work. Discuss the likely magnitude of misclassification and its likely impact on the estimates of RR. Might also want to say something about multiple exposures. Cannot look only at a single exposure. This is an issue raised by critics. Just as well address it here.

AB – This next paragraph seems part of the conclusions. I would try to merge it with the conclusions paragraph.

In our study no pesticide had a significant exposure response pattern with either diffuse large B-cell lymphoma or follicular B-cell lymphoma, although significant relative~~point estimates of~~ risks were identified for butylate (a carbamate herbicide), terbufos (an organophosphate insecticide), and methyl bromide (an organic halide)(Not clear what you are trying to say here – No exposure-response pattern, but significant RRs.). Previously, NHL subtypes with t (14;18) translocations were associated with the chlorinated insecticides dieldrin, lindane, and toxaphene

Comment [AB63]: But there were borderline trends for these subtypes.

and the triazine herbicide atrazine (chiu BCH et al., 2006 and Chiu BCH and Blair A 2009). We were unable to evaluate translocations in this analysis. Although it is possible that t (14;18) translocations are an initiating event of a causative cascade leading to an NHL subtype, follicular lymphoma (FL), much more work needs to be done to establish this etiologic pathway. (Not sure mentioning t(14;18) is worthwhile here. This study sheds no light on this issue. This point might be combined in a paragraph that discusses future research, but it does not fit by itself)—.

Conclusion:

(I do not think you should start the conclusion with comments about subtypes. Start with NHL overall. In summary, our results suggest that there is subtype specificity in associations between NHL and pesticides exposures. The varying etiology of NHL sub-types may have masked real associations between pesticides and NHL in previous studies where NHL sub-type information was not available (Not sure how varying etiology by subtype would mask associations with NHL overall. If each study had all the subtypes then either the subtype links power through to overall NHL or they do not. The reverse is true. Looking only at NHL overall would hide associations with specific subtypes.). Although the epidemiological evidence for associations between specific pesticides and specific cell types is growing (probably should cite the other papers that have information on specific pesticides and subtypes), the observation that pesticides of different chemical and functional classes and different known toxicological properties are associated with the same cell type (Is it know that different pesticides are associated with the same cell type?) indicates that relatively little is known about the biological/toxicological mechanisms by which these compounds may be contributing to this disease. Cautious interpretation of these results is advised since the number of exposed-cases for

each subgroup of NHL in the AHS is still relatively small. (Overall I think the conclusion is too strong. It seems to say that the links between specific pesticides and certain NHL subtypes observed in this study are real and this is why we do not understand the mechanisms for pesticides causing cancer. The findings here are interesting, but they are leads to be confirmed. I do not think they are strong enough to be making statements about what this says about mechanisms. I think the tone should be – few studies have been able to look at specific pesticides and NHL subtypes. What we found is interesting. Need to see if other studies will have similar findings. I may be in a minority about this, but I would like to have a discussion about this on an EC call.)

Acknowledgements

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Comment [AB64]: This affiliation does not cover ally coauthors. Don't we usually put some comment of appreciation to the participants in the AHS in the acknowledgements?

Comment [a65]: Get correct contract numbers here.

The authors have no conflicts of interest in connection with this manuscript.

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Table 1. Baseline characteristics of AHS study participants in the NHL incidence analysis from 1993 through 2008				
	All NHL cases	Cohort Person-years.	RR[1]	95% CI
Age at Enrollment				
<45	51	368,766.80	1.0 (ref)	
45-49	34	88,648.48	2.8	1.8-4.3
50-54	51	75,781.37	4.9	3.3-7.2
55-59	59	67,981.37	6.3	4.3-9.1
60-64	46	53,346.73	6.2	4.2-9.3
65-69	46	34,532.71	9.6	6.5-14.4
≥70	46	25,713.12	12.9	8.7-19.3
Gender				
Male	328 (ref)	695,190.90	1.0 (ref)	
Female	5	19,579.34	0.5	0.2-1.3
State				
IA	213 (ref)	461,697.24	1.0 (ref)	
NC	120	253,072.27	0.8	0.6-0.97
License type				
Private	318	652,562.25	1.0 (ref)	
Commercial	15	62,207.89	0.9	0.5-1.5
Education				
<12 yrs.	57	61,656.39	1.0 (ref)	
HS/GED	143	326,344.92	0.8	0.6-1.1
>12 yrs.	121	297,437.85	1.0	0.7-1.4
Smoking Status				

Never	165	371,929.66	1.0 (ref)	
Former	127	203,445.28	0.93	0.7-1.2
Current	29	116,254.87	0.6	0.4-0.9
Body Mass Index (BMI)				
<25	58		1.0 (ref)	
25-<30	138		1.1	0.8-1.5
≥30	61		0.94	0.7-1.4
Alcohol consumption per week				
None	128	212,928.70	1.0 (ref)	
<once a week	89	217,015.35	1.0	0.8-1.4
≥once a week	89	240,745.51	1.0	0.8-1.4
First degree relative with lymphoma				
No	291	639,748.82	1 (ref)	
Yes	7	12,606.85	1.1	0.5-2.4

[1] All variables except age are age adjusted (<45,45-49,50-54,55-59,60-64,65-69,≥70)

[2] Numbers do not sum to totals (333 cases, 714,770 person-years) due to missing data.

Table 2. Pesticide exposure (Lifetime Days [LD] & intensity weighted Lifetime Days [IWLD]) and the age-adjusted risk of NHL incidence (1993 through 2008)

Insecticides				
Pesticide (chemical-functional class) [median days of lifetime exposure for each category]	NHL Cases	RR[1] (95%) by Total Days of Exposure	NHL Cases	RR[1] (95% CI) Intensity-weighted days of exposure
Carbaryl **(carbamate-insecticide)**				
None	81	1.0 (ref)	81	1.0 (ref)
Low [8.75]	31	0.9 (0.5-1.5)	27	0.9 (0.5-1.5)
Medium [56]	23	0.7 (0.4-1.1)	26	0.8 (0.5-1.4)
High [124.5]	25	0.9 (0.6-1.5)	26	0.8 (0.5-1.3)
		P trend=0.86		P trend=0.47
Malathion **(organophosphorous-insecticide)**				
None	55	1.0 (ref)	55	1.0 (ref)
Low [8.75]	46	1.0 (0.7-1.5)	37	1.0 (0.7-1.6)
Medium [42.75]	28	0.7 (0.4-1.2)	38	0.8 (0.5-1.3)
High [103.75]	36	1.0 (0.7-1.6)	35	0.91 (0.6-1.4)
		P trend=0.74		P trend=0.71
Terbufos **(organophosphorous-insecticide)**				
None	157	1.0 (ref)	157	1.0 (ref)
Low [24.5]	58	1.4 (1.1-1.9)	43	1.3 (0.92-1.8)
Medium [56]	38	2.0 (1.4-2.8)	43	2.0 (1.4-2.8)
High [116]	34	1.2 (0.8-1.7)	42	1.2 (0.9-1.8)

		P trend=0.23		P trend=0.19
Chlorinated Insecticide				
Chlordane (Chlorinated Insecticide)				
None	223	1.0 (ref)	223	1.0 (ref)
Low [8.75]	23	0.9 (0.6-1.4)	13	1.1 (0.7-2.0)
Medium [20]	6	1.7 (0.8-3.8)	13	0.9 (0.5-1.6)
High [38.75]	9	0.8 (0.4-1.6)	12	0.9 (0.5-1.6)
		P trend=0.89		P trend=0.77
DDT (Chlorinated Insecticide)				
None	194	1.0 (ref)	194	1.0 (ref)
Low [8.75]	20	0.8 (0.5-1.3)	19	0.9 (0.6-1.5)
Medium [56]	18	0.9 (0.6-1.6)	18	0.8 (0.5-1.4)
High [116]	17	1.5 (0.9-2.5)	18	1.4 (0.8-2.2)
		P trend=0.14		P trend=0.28
Lindane (Chlorinated Insecticide)				
None	209	1.0 (ref)	209	1.0 (ref)
Low [17.75]	11	1.0(0.5-2.0)	10	1.1(0.6-2.0)
Medium [56]	10	1.2(0.6-2.3)	11	1.4(0.7-2.6)
High [116]	10	2.7(1.4-5.1)	9	1.9(0.95-3.7)
		P trend=0.003		P trend=0.04
Herbicides				
Alachlor (acetamide-herbicide)				
None	138	1.0 (ref)	138	1.0 (ref)

Comment [lbf66]: I like this heading—suggest using them throughout the tables and then deleting the chemical class in parentheses

Low [24.5]	65	1.0 (0.7-1.3)	53	1.0 (0.7-1.3)
Medium [116]	49	0.9(0.6-1.2)	50	0.9 (0.6-1.2)
High [224.75]	43	1.3(0.9-1.9)	51	1.2 (0.9-1.7)
		P trend=0.12		P trend=0.19
Atrazine (triazine-herbicide)				
None	85	1.0 (ref)	85	1.0 (ref)
Low [38.75]	88	1.2(0.8-1.7)	79	1.1(0.8-1.6)
Medium [114.5]	72	1.3(0.96-1.9)	78	1.4(1.0-2.0)
High [224.75]	77	1.2(0.9-1.6)	78	1.2(0.8-1.6)
		P trend=0.56		P trend=0.68
Butylate (thiocarbamate-herbicide)				
None	107	1.0 (ref)	107	1.0 (ref)
Low [24.5]	22	1.0(0.6-1.5)	16	0.9(0.5-1.5)
Medium [56]	18	2.8(1.7-4.7)	16	2.1(1.2-3.5)
High [56]	7	1.1(0.5-2.4)	15	1.5(0.9-2.6)
		P trend=0.004		P trend=0.04
Dicamba (benzoic-herbicide)				
None	121	1.0 (ref)	121	1.0 (ref)
Low [20]	66	1.3(0.94-1.8)	56	1.2(0.9-1.8)
Medium [56]	52	1.5(1.1-2.1)	54	1.5(1.1-2.1)
High [128.5]	47	1.2(0.9-1.7)	55	1.3(0.9-1.8)
		P trend=0.38	P trend=0.23	
2,4-D (phenoxy-herbicide)				

None	71	1.0 (ref)	71	1.0 (ref)
Low [46.75]	83	1.0(0.7-1.4)	82	1.0(0.7-1.4)
Medium [133.35]	83	1.2(0.8-1.6)	83	1.1(0.8-1.6)
High [371.75]	82	1.0(0.7-1.4)	81	1.0(0.7-1.4)
		P trend=0.96		P trend=0.94
EPTC (thiocarbamate-herbicide)				
None	229	1.0 (ref)	229	1.0 (ref)
Low [8.75]	28	1.3(0.9-2.0)	20	1.3(0.8-2.1)
Medium [50.75]	14	1.0(0.6-1.7)	20	1.2(0.7-1.8)
High [108.5]	18	1.3(0.8-2.0)	19	1.1(0.7-1.8)
		P trend=0.35		P trend=0.54
Glyphosate (phosphinic acid-herbicide)				
None	70	1.0 (ref)	70	1.0 (ref)
Low [20]	89	0.8(0.6-1.2)	83	0.9(0.6-1.3)
Medium [65.75]	78	0.8(0.6-1.2)	84	0.8(0.5-1.1)
High [173.25]	83	1.0(0.7-1.4)	82	1.0(0.7-1.3)
		P trend=0.58		P trend=0.81
Imazethapyr (imidazolinone-herbicide)				
None	181	1.0 (ref)	181	1.0 (ref)
Low [8.75]	39	0.9(0.6-1.3)	36	1.0(0.7-1.4)
Medium [28.75]	34	0.9(0.6-1.4)	37	0.9(0.6-1.3)
High [56]	35	1.2(0.8-1.7)	35	1.2(0.8-1.7)
		P trend=0.54		P trend=0.55
Metribuzin				

(triazine-herbicide)				
None	94	1.0 (ref)	94	1.0 (ref)
Low [8.75]	28	1.0 (0.7-1.7)	21	1.2(0.7-2.0)
Medium [50.75]	15	0.9(0.5-1.6)	23	1.1(0.7-1.7)
High [56]	20	1.7(1.0-2.7)	19	1.3(0.8-2.2)
		P trend=0.06		P trend=0.28
Trifluralin (dinitroaniline-herbicide)				
None	140	1.0 (ref)	140	1.0 (ref)
Low [25]	51	1.0 (0.7-1.4)	50	1.0(0.7-1.4)
Medium [108.5]	58	1.1(0.8-1.5)	52	1.1(0.8-1.5)
High [224.75]	43	1.0(0.7-1.3)	48	0.9(0.7-1.3)
		P trend=0.81		P trend=0.65

[1]Age adjusted (<45,45-49,50-54,55-59,60-64,65-69,≥70)

[2] Numbers do not sum to total number of NHL cases (n=333) due to missing data.

Table 3. Pesticides exposure (Lifetime-days and the age-adjusted risk of NHL by cell type (1993-2008).

	CLL, SLL, MCL		Diffuse Large B-cell		Follicular B-cell		Other B-cell types	
Insecticides, fungicide and fumigant								
	RR[1] (95% CI)	**n**	**RR[1] (95% CI)**	**n**	**RR[1] (95% CI)**	**n**	**RR[1] (95% CI)**	N
Carbaryl								
None	1.0 (ref)	32	1.0 (ref)	23	1.0 (ref)	9	1.0 (ref)	9
Low	1.1(0.5-2.2)	15	0.7(0.3-1.5)	10	1.1(0.3-4.0)	5	Xxx	6
Medium	1.0(0.2-4.2)	2	1.3(0.6-3.0)	8	1.8(0.6-5.9)	4	Xxx	0
High	0.4(0.2-0.8)	8	1.5(0.7-3.5)	8	1.3(0.4-4.1)	4	xxx-	1
	P trend=0.007		P trend=0.19		P trend=0.66		P trend=xxx	
Malathion								
None	1.0 (ref)	21	1.0 (ref)	16	1.0 (ref)	5	1.0 (ref)	6
Low	0.94(0.5-1.8)	17	0.8(0.4-1.7)	16	1.0(0.3-3.6)	6	xxx-	8
Medium	0.8(0.4-1.7)	11	0.9(0.4-2.1)	8	1.2(0.3-4.3)	5	-xxx	0
High	0.8(0.4-1.7)	11	1.7(0.8-3.8)	11	1.5(0.4-4.9)	5	-xxx	3
	P trend=0.52		P trend=0.07		P trend=0.48		P trend=xxx	
Terbufos								
None	1.0 (ref)	53	1.0 (ref)	47	1.0 (ref)	26	1.0 (ref)	10
Low	1.8(1.0-3.1)	17	0.9(0.4-1.7)	12	2.5(1.1-5.4)	8	2.3 (0.8-6.6)	6
Medium	2.2(1.3-3.6)	21	2.2(1.2-4.2)	12	1.8(0.7-4.3)	7	3.1(1.1-9.2)	5
High	1.4(0.8-2.6)	13	1.1(0.5-2.3)	10	0.7(0.3-1.8)	6	4.1(1.4-11.9)	5
	P trend=0.16		P trend=0.34		P trend=0.54		P trend=0.01	
Chlorinated pesticides								
Chlordane								
None	1.0 (ref)	74	1.0 (ref)	68	1.0 (ref)	35	1.0 (ref)	21

Comment [lbf67]: Insert the codes here and then you can remove them from the text.

Comment [lbf68]: Would suggest using the headings as suggest in Table 2 to orient people to chemical class.

Low	1.4 (0.7-2.7)	10	0.8 (0.4-2.0)	6	1.6 (0.4-6.9)	2	Xxx	1
Medium	2.8 (0.9-9.0)	3	1.8 (0.6-5.1)	4	0.8 (0.2-3.4)	2	Xxx	2
High	0.8 (0.3-2.7)	3	1.0 (0.2-4.1)	2	0.7 (0.1-5.1)	1	Xxx	0
	P trend=0.56		P trend=0.09		P trend=0.92		P trend=xxx	
DDT								
None	1.0 (ref)	62	1.0 (ref)	53	1.0 (ref)	36	1.0 (ref)	22
Low	0.91 (0.4-2.0)	8	1.1 (0.5-2.6)	7	1.1 (0.4-3.4)	4	0.4 (0.1-1.9)	2
Medium	1.1 (0.5-2.4)	8	2.3 (1.0-5.4)	7	0.3 (0.1-2.6)	1	1.4 (0.3-6.2)	2
High	2.3 (1.0-5.3)	7	1.2 (0.5-2.9)	6	0.7 (0.1-5.0)	1	0.9 (0.1-6.7)	1
	P trend=0.45		P trend=0.31		P trend=0.72		P trend=0.77	
Lindane								
None	1.0 (ref)	41	1.0 (ref)	39	1.0 (ref)	14	1.0 (ref)	14
Low	1.6(0.7-3.6)	8	0.7(0.2-3.0)	9	2.7(0.8-9.4)	3	Xxx	1
Medium	1.1(0.3-4.8)	3	1.1(0.3-3.7)	6	3.6(0.8-15.9)	2	Xxx	0
High	3.8(1.5-9.6)	5	1.3(0.2-9.7)	5	2.4(0.5-10.4)	2	Xxx	0
	P trend=0.005		P trend=0.25		P trend=0.25		P trend=xxx	
Herbicides								
Alachlor (acetanilide)								
None	1.0 (ref)	53	1.0 (ref)	42	1.0 (ref)	22	1.0 (ref)	9
Low	0.9(0.6-1.5)	23	0.9(0.5-1.6)	13	1.3(0.6-2.6)	10	1.6 (0.6-4.4)	7
Medium	0.8(0.5-1.4)	18	0.7(0.4-1.3)	14	0.8(0.3-1.6)	9	2.1 (0.8-5.3)	10
High	1.1(0.6-2.1)	14	0.8(0.4-1.6)	10	1.1(0.4-2.7)	6	4.0 (1.2-13.0)	4
	P =0.67		P trend=0.52		P trend=0.99		P trend=0.02	
Atrazine (triazine)								
None	1.0 (ref)	34	1.0 (ref)	26	1.0 (ref)	12	1.0 (ref)	5

Low	1.0 (0.6-1.7)	29	1.1(0.6-2.0)	21	1.7(0.7-3.9)	17	2.4 (0.9-6.8)	13
Medium	1.2 (0.7-2.0)	25	1.1(0.6-2.2)	23	1.3(0.5-3.4)	10	1.7(0.5-5.9)	6
High	1.0 (0.6-1.7)	26	0.9(0.5-1.7)	19	1.4(0.6-3.4)	13	3.6 (1.2-10.8)	9
	P trend=0.90		P trend=0.62		P trend=0.83		P trend=0.06	
Butylate (thio-carbamate-)								
None	1.0 (ref)	40	1.0 (ref)	33	1.0 (ref)	14	1.0 (ref)	8
Low	0.8(0.4-1.9)	7	1.1(0.4-3.0)	4	0.8(0.2-2.9)	3	3.0 (0.8-11.3)	3
Medium	3.5(1.6-7.6)	8	1.2(0.4-3.5)	4	6.3(2.1-19.3)	4	4.0(1.2-13.7)	4
High	1.3(0.4-4.3)	3	0.8(0.2-2.5)	3	1.0(0.1-7.9)	1	2.4 (0.3-19.7)	1
	P trend=0.04		P trend=0.69		P trend=0.07		P trend=0.0499	
2,4-D (Chlorinated Phenoxy)								
None	1.0 (ref)	25	1.0 (ref)	23	1.0 (ref)	9	1.0 (ref)	5
Low	0.90(0.5-1.5)	31	0.9(0.5-1.7)	23	1.8(0.8-4.4)	14	1.9 (0.6-6.2)	10
Medium	1.2(0.7-2.0)	29	1.0(0.6-1.9)	21	1.0(0.4-2.4)	14	1.7 (0.5-5.6)	9
High	1.3(0.7-2.2)	29	0.7(0.4-1.3)	21	1.4(0.6-3.4)	12	2.2 (0.7-7.2)	9
	P trend=0.20		P trend=0.23		P trend=0.84		P trend=0.35	
Dicamba (benzoic acid)								
None	1.0 (ref)	39	1.0 (ref)	40	1.0 (ref)	22	1.0 (ref)	6
Low	1.5 (0.9-2.6)	23	1.1 (0.6-2.1)	12	1.5(0.7-3.4)	9	3.2 (1.0-9.9)	8
Medium	1.5 (0.9-3.4)	20	1.1 (0.6-2.1)	13	1.8(0.90-4.0)	10	5.2(1.6-16.6)	7
High	2.0 (1.1-3.4)	20	0.7 (0.4-1.4)	11	0.7(0.3-1.5)	8	5.1(1.6-16.1)	7
	P trend=0.03		P trend=0.26		P trend=0.32		P trend=0.02	

EPTC (thio-carbamate)								
None	1.0 (ref)	86	1.0 (ref)	62	1.0 (ref)	40	1.0 (ref)	19
Low	1,2(0.6-2.3)	9	1.2(0.6-2.7)	7	xxx	3	2.1 (0.7-6.0)	4
Medium	1.2(0.6-2.5)	8	1.7(0.7-4.2)	5	xxx	0	2.1 (0.6-7.1)	3
High	1.4(0.6-3.4)	5	0.8(0.3-2.3)	4	xxx	1	4.9 (1.4-16.7)	3
	P trend= 0.41		P trend=0.98		P trend=0.10		P trend=0.01	
Glyphosate (isopropyl-amine)								
None	1.0 (ref)	25	1.0 (ref)	19	1.0 (ref)	13	1.0 (ref)	10
Low	0.6(0.4-1.1)	32	1.3(0.7-2.6)	23	0.7(0.3-1.7)	15	0.4 (0.1-1.2)	9
Medium	1.1(0.6-1.9)	29	1.1(0.5-2.1)	23	0.6(0.2-1.4)	11	0.6 (0.2-1.6)	7
High	1.1(0.6-1.8)	29	0.7(0.4-1.3)	22	0.7(0.3-1.8)	12	0.6 (0.2-1.8)	7
	P trend=0.21		P trend=0.05		P trend=0.66		P trend=0.98	
Imazethapyr (imid-azolinone)								
None	1.0 (ref)	68	1.0 (ref)	57	1.0 (ref)	29	1.0 (ref)	12
Low	1.0(0.6-1.8)	16	0.7(0.3-1.4)	10	0.7(0.3-1.7)	6	1.6 (0.6-3.8)	8
Medium	0.8(0.4-1.6)	11	0.6(0.3-1.4)	6	1.1(0.3-3.5)	6	5.2 (1.6-16.6)	4
High	1.2(0.6-2.2)	12	0.5(0.2-1.2)	5	1.0(0.4-2.8)	5	3.2 (1.0-10.0)	4
	P trend=0.71		P trend=0.16		P trend=0.90		P trend=0.03	
Metribuzin (Triazone)								
None	1.0 (ref)	30	1.0 (ref)	35	1.0 (ref)	13	1.0 (ref)	9
Low	1.5(0.7-2.9)	11	0.5(0.2-1.4)	5	1.4(0.5-3.9)	5	1.0 (0.2-4.9)	3

Medium	2.1(1.1-4.0)	13	0.5(0.1-2.0)	3	0.8(0.2-2.9)	3	2.8 (0.9-8.9)	5
High	1.8(0.6-5.2)	4	0.4(0.1-1.6)	2	1.3(0.2-9.8)	1	-	0
	P trend=0.06		P trend=0.13		P trend=0.88		P trend=0.60	
Trifluralin (dinitro-aniline)								
None	1.0 (ref)	45	1.0 (ref)	43	1.0 (ref)	25	1.0 (ref)	10
Low	1.1(0.7-1.9)	23	0.9(0.5-1.7)	14	0.9(0.4-1.9)	8	1.2 (0.4-3.2)	7
Medium	1.6(0.9-2.6)	21	0.8(0.4-1.7)	11	0.8(0.4-1.8)	8	2.7 (1.0-7.0)	7
High	1.1(0.6-1.9)	15	0.6(0.3-1.2)	11	0.8(0.3-1.9)	7	3.3 (1.2-9.1)	6
	P trend= 0.81		P trend=0.13		P trend=0.62		P trend=0.01	

[1] Age adjusted (<45,45-49,50-54,55-59,60-64,65-69,≥70)
[2] Numbers do not sum to NHL subtype totals due to missing data.

Table 4: The number of different pesticides in a pesticide class used and the risk of NHL (95% CI)

Number pesticides in a pesticide class	All NHL Cases[1]	Cohort Person-Years	RR[2]	95% CI
All pesticide				
0-4	36	46,624	1.0 (ref)	
5-8	58	62,304	1.2	(0.8-1.9)
9-11	50	56,373	1.2	(0.8-2.0)
12-16	65	93,714	0.9	(0.5-1.4)
17-20	48	57,874	1.1	(0.7-1.8)
>20	75	71,281	1.1	(0.7-1.8)
			P trend=0.53	
Chlorinated Insecticides				
0	111	344,026	1.0 (ref)	
1	63	131,439	1.1	(0.6-1.9)
2	42	77,989	1.1	(0.6-2.0)
≥3	89	122,276	0.9	(0.5-1.7)
			P trend=0.45	
Organophosphate insecticides				
0	38	90,621	1.0 (ref)	
1	59	128,694	1.2	(0.7-1.8)
2	69	146,183	1.3	(0.8-2.0)
3	56	133,273	1.1	(0.6-1.8)
≥4	107	208,634	1.2	(0.7-2.1)
			P trend=0.59	
Carbamate insecticide				
0	104	231,849	1 (ref)	
1	126	294,727	0.7	(0.5-1.0)
≥2	89	163,706	0.9	(0.6-1.4)
			P trend=0.64	
Other insecticides				
0	251	532,835	1.0 (ref)	
>1	43	112,489	1.1	(0.6-1.8)
			P trend=0.36	
Triazine herbicides				
0	67	161,040	1.0	
1	92	187,057	1.2	(0.6-2.4)
2	78	185,777	1.0	(0.5-2.1)
3	92	173,920	1.4	(0.7-3.0)
			P trend=0.04	
Acetamide herbicides				
0	90	206,537	1.0	
1	115	236,407	1.6	(0.8-3.4)
2	102	219,200	1.7	(0.7-3.7)

			P trend=0.10	
Carbamate herbicides				
0	193	414,729	1.0 (ref)	
1	79	179,871	0.8	(0.5-1.2)
2	40	84,589	0.8	0.8 (0.4-1.4)
			P trend=0.80	
Other herbicides				
0	13	25,880	1.0 (ref)	
1-2	67	131,595	1.1	(0.5-2.7)
3-4	76	162,359	1.0	(0.4-2.4)
5-6	78	185,337	1.0	(0.4-2.5)
≥7	97	205,915	1.1	(0.4-2.6)
			P trend=0.19	
Fungicides				
0	203	442,307	1.0 (ref)	
1	73	152,882	1.1	(0.8-1.5)
≥2	52	110,590	1.5	(0.99-2.3)
			P trend=0.31	
Fumigants				
0	240	538,867	1.0 (ref)	
1	73	123,473	1.4	(0.9-2.1)
≥2	15	42,165	0.9	(0.4-1.9)
			P trend=0.24	

[1] Numbers do not sum to totals (333 cases, 714,770 person-years) due to missing data
[2] NHL risks are age adjusted (<45,45-49,50-54,55-59,60-64,65-69,≥70)and adjusted for lifetime days of use of pesticides in the specific pesticide class

Table 5. Number of different pesticides used by pesticide type (in the NHL incidence analysis from 1993 through 2008) for B cell sub-types.[1,2]

	CLL, SLL, PLL, MCL		Diffuse Large B-cell		Follicular B-cell		Other B-cell types	
	RR [1] (95% CI)	n	RR[1] (95% CI)	n	RR[1] (95% CI)	n	RR[1] (95% CI)	n
Insecticides								
Carbamate insecticides[3]								
0	1.0 (ref)	34	1.0(ref)	33	1.0(ref)	12	1.0 (ref)	13
1	0.8 (0.5-1.3)	45	0.7(0.4-1.2)	36	1.5(0.8-3.0)	26	0.3 (0.1-0.8)	7
2-3	1.1 (0.7-1.7)	32	0.7(0.4-1.2)	20	1.2(0.5-2.7)	12	1.2 (0.5-2.5)	13
	P trend= 0.82		P trend=0.21		P trend=0.63		P trend= 0.75	
Chlorinated insecticides[4]								
None	1.0 (ref)	8	1.0(ref)	16	1.0(ref)	3	1.0 (ref0	6
1	1.6 (0.7-3.8)	17	0.9 (04-1.7)	18	4.1(1.2-14.1)	15	0.9 (0.3-2.7)	7
2	2.2 (0.95-5.0)	19	0.6(0.3-1.3)	10	2.5(0.6-9.6)	7	0.5 (0.1-1.9)	3
3	2.4 (1.2-5.2)	41	0.5(0.3-1.0)	17	1.7(0.5-6.5)	9	0.8 (0.3-2.3)	10
	P trend=0.02		P trend=0.05		P trend=0.73		P trend= 0.48	
Organophosphate Insecticides[5]								
0	1.0 (ref)	13	1.0 (ref)	14	1.0(ref)	5	1.0	5
1	0.93(0.4-2.0)	15	1.2(0.6-2.4)	21	1.3(0.4-3.9)	8	0.8 (0.2-2.8)	5
2	1.4 (0.7-2.7)	25	1.0(0.5-2.0)	20	1.7(0.6-4.7)	12	1.3 (0.4-4.0)	9
3	1.3 (0.6-2.5)	20	0.8(0.4-1.7)	14	1.4(0.5-4.1)	9	0.5 (0.1-2.1)	3
≥4	1.7 (0.92-3.2)	42	0.8(0.4-1.6)	23	1.6(0.6-4.4)	17	1.3 (0.5-3.7)	12

Comment [lbf69]: Interesting results

	P trend =0.03		P trend= 0.28		P trend=0.38		P trend=0.67	
Other Insecticides[6]								
0	1.0 (ref)	86	1.0 (ref)	71	1.0(ref)	35	1.0 (ref)	22
1	0.94 (0.6-1.6)	19	0.5(0.2-1.0)	9	1.3(0.6-2.4)	12	1.1 (0.5-2.8)	6
	P trend=0.78		P trend= .04		P trend=0.49	6	P trend=0.82	
Herbicides								
Acetamide Herbicide[7]								
0	1.0 (ref)	37	1.0(ref)	32	1.0(ref)	14	1.0	6
1	0.97 (0.6-1.5)	35	1.0(0.6-1.6)	32	1.3(0.7-2.6)	19	1.4 (0.5-4.0)	8
2	1.2 (0.8-2.0)	39	0.6(0.4-1.1)	18	1.2(0.6-2.4)	15	3.9 (1.2-8.2)	16
	P trend=0.35		P trend=0.16		P trend=0.72		P trend= 0.009	
Carbamate Herbicide[8]								
0	1.0 (ref)	67	1.0(ref)	58	1.0(ref)	27	1.0	16
1	0.98 (0.6-1.5)	27	0.7(0.4-1.2)	17	1.3(0.7-2.5)	16	1.5 (0.7-3.4)	10
2	1.5 (0.9-2.5)	17	0.9(0.4-1.7)	9	0.6(0.2-1.8)	3	2.2 (0.9-5.7)	6
	P trend=0.29		P trend=0.33		P trend=0.71		P trend=0.11	
Other herbicides[9]								
0	1.0 (ref)	6	1.0(ref)	6	1.0(ref)	1	1.0	2
1-2	1.2(0.5-2.8)	25	1.0(0.4-2.5)	22	3.2(0.5-27.0)	13	0.6 (0.1-3.1)	4
2-4	0.9 (0.4-2.2)	20	1.4(0.6-3.4)	33	2.5(0.3-19.2)	10	0.94(0.2-4.6)	7
5-6	1.2 (0.5-2.8)	26	0.7(0.3-1.7)	16	4.0(0.5-29.8)	17	1.2(0.3-5.7)	9
≥7	1.7 (0.7-4.1)	38	0.7(0.3-1.7)	16	2.5(0.3-19.3)	11	1.7(0.4-7.6)	12
	P trend=0.06		P trend=0.08		P trend=0.84		P trend= 0.06	
Triazine/Triazone herbicides[10]								
0	1.0	29	1.0 (ref)	22	1.0(ref)	6	1.0 (ref)	4
1	0.8 (0.5-1.4)	24	1.5(0.9-2.6)	34	3.2(1.3-8.0)	20	2.0 (0.6-6.6)	8

Comment [lbf70]: Interesting results

2	1.0(0.6-1.7)	27	0.8(0.4-1.5)	17	2.1(0.8-6.7)	13	2.5 (0.8-8.3)	9
3	1.5 (0.91-2.5)	35	1.1(0.6-2.0)	20	2.3(0.9-6.1)	13	4.2 (1.4-13.1)	13
	P trend=0.07		P trend=0.64		P trend=0.30		P trend=.006	
Fungicides and Fumigants								
Fungicides[11]								
0	1.0 (ref)	4	1.0 (ref)	6	1.0(ref)	3	1.0	2
1	1.3 (0.4-3.6)	29	0.7(0.3-1.8)	28	1.1(0.3-3.6)	23	1.2 (0.3-5.6)	14
2	1.7 (0.6-4.6)	81	0.8(0.3-1.8)	58	0.6(0.2-2.1)	26	0.8 (0.2-3.4)	18
	P trend=0.11		P trend=0.75		P trend=0.10		P trend=0.29	
Fumigants[12]								
0	1.0 (ref)	43	1.0 (ref)	30	1.0(ref)	25	1.0	9
1	1.0 (0.6-1.9)	13	2.0(1.1-3.7)	17	0.6(0.2-1.7)	4	2.8 (1.0-7.4)	7
≥2	0.95(0.6-1.4)	58	1.1(0.7-1.8)	45	0.7(0.4-1.2)	22	1.5(0.7-3.3)	18
	P trend=0.81		P trend=0.75		Ptrend=0.20		P trend=0.43	

[1]Age adjusted (<45,45-49,50-54,55-59,60-64,65-69,≥70 [2] Numbers do not sum to NHL subtype totals due to missing data [3]Carbamate insecticides: carbofuran, aldicarb, carbaryl [4]Chlorinated insecticides: aldrin, chlordane, dieldrin, DDT, heptachlor, lindane, toxaphene [5]Organophosphate insecticides: Chlorpyrifos, coumaphos, diazinon, dichlorvos, fonofos, malathion, parathion, phorate, terbufos, [6]Other insecticides: permethrin [7]Acetamide: metolachlor, alachlor [8]Carbamate herbicide: Butylate: EPTC [9]Other herbicides: Glyphosate, imazethapyr, herbicide oil, paraquat, chlorimuron ethyl, dicamba, pendimethalin, trifluralin, 2,4-D, 2,4,5-T, 2,4-TP[10]Triazine herbicides: Atrazine, cyanazine, metribuzin [11]Fungicides: Benomyl, chlorthalonil, captan, maneb/macozeb, metalaxyl, ziram [12]Fumigants: methyl bromide, aluminum phosphate, ethylene dibromide, carbon tetra chloride/carbon disulfide

Supplemental Table 1 Other pesticide exposures (lifetime days [LD} and intensity weighted total days) and age-adjusted risk of NHL incidence (1993 through 2008).				
Pesticide (chemical-functional class) [median days of lifetime exposure for each category]	NHL Cases	RR (95%) by Lifetime- Days of Exposure	NHL Cases	RR (95% CI) Intensity weighted Lifetime-Days of exposure
Benomyl **(carbamate-fungicide)**				
None	134	1.0 (ref)	134	1.0 (ref)
Low [0.5]	6	5.6 (2.4-12.6)	6	4.1(1.8-9.3)
Medium [12.25]	5	1.0 (0.4-2.6)	5	1.0 (0.4-2.6)
High [108.5]	5	0.8 (0.3-1.9)	5	0.8 (0.3-1.9)
		P for trend=0.50		P for trend=0.57
Captan **(dicarboximide-fungicide)**				
None	258	1.0 (ref)	258	1.0 (ref)
Low [4]	8	0.6 (0.3-1.3)	8	0.7 (0.4-1.5)
Medium [12.25]	8	1.6 (0.6-4.1)	7	1.2 (0.5-2.9)
High [124]	7	0.6 (0.3-1.5)	7	0.5 (0.2-1.3)
		P for trend=0.33		P for trend=0.20
Carbofuran **(carbamate-insecticide)**				
None	199	1.0 (ref)	199	1.0 (ref)
Low [8.75]	35	1.1 (0.8-1.6)	29	1.2 (0.8-1.8)
Medium [38.75]	25	1.0 (0.7-1.6)	29	0.9 (0.6-1.3)
High [56]	28	1.0 (0.7-1.5)	28	1.1 (0.8-1.7)

Comment [lbf71]: I think that you need to put number of days for each pesticide. Low/Med/High is not the same for each pesticide under study and this leaves the impression that they are.

Comment [a72]: Lifetime days added as suggested.

		P trend=0.81		P trend=0.74
Chlorpyrifos (organophosphate-insecticide)				
None	189	1.0 (ref)	189	1.0 (ref)
Low [14.75]	44	1.1 (0.7-1.5)	40	1.1 (0.8-1.5)
Medium [38.75]	45	1.3(0.9-1.8)	41	1.0 (0.7-1.5)
High [116]	43	0.9 (0.7-1.3)	39	1.1 (0.8-1.5)
		P trend=0.57		P trend=0.67
Chlorthalonil (thalonitrile-fungicide)				
None	301	1.0 (ref)	301	1.0 (ref)
Low [8]	7	1.3 (0.6-2.7)	7	1.1 (0.5-2.4)
Medium [54.25]	6	0.6 (0.2-1.6)	6	0.6 (0.2-1.5)
High [79]	6	0.6 (0.2-1.2)	6	0.7 (0.3-1.5)
		P for trend=0.12		P for trend=0.23
Coumaphos (0rganophosphate-insecticide)				
None	258	1.0(ref)	258	1.0 (ref)
Low [8.75]	12	1.2 (0.7-2.2)	10	1.6 (0.8-2.9)
Medium [38.75]	10	1.4 (0.8-2.7)	11	1.2 (0.6-2.1)
High [63.75]	8	1.2 (0.6-2.4)	9	1.2 (0.6-2.3)
		P for trend=0.41		P for trend=0.55
DDVP (dimethyl phosphate-insecticide)				
None	261	1.0 (ref)	261	1.0 (ref)

Low [8.75]	10	1.2 (0.6-2.2)	10	1.2 (0.7-2.3)
Medium [108.5]	11	1.1 (0.6-2.0)	9	0.8 (0.4-1.6)
High [457.25]	7	0.7 (0.3-1.5)	9	1.0 (0.5-1.9)
		P for trend=0.42		P for trend=0.95
Diazinon (organophosphosphorous-insecticide)				
None	113	1.0 (ref)	113	1.0 (ref)
Low [8.75]	19	1.2 (0.7-2.0)	14	1.3 (0.7-2.2)
Medium [30]	10	0.7 (0.3-1.7)	15	0.9 (0.5-1.7)
High [56]	13	1.1 (0.6-2.1)	13	1.1 (0.6-1.9)
		P trend=0.73		P trend=0.92
Fonofos (phosphonothioate-insecticide)				
None	220	1.0 (ref)	220	1.0 (ref)
Low [20]	28	1.3 (0.9-1.9)	23	1.2 (0.8-1.9)
Medium [50.75]	19	1.2 (0.8-2.0)	23	1.4 (0.93-2.2)
High [108.5]	22	1.1 (0.7-1.7)	22	1.0 (0.6-1.5)
		P for trend=0.67		P for trend=0.98
Matalaxyl (analine methyl ester-fungicide)				
None	126	1.0 (ref)	126	1.0 (ref)
Low [3.5]	10	1.2 (0.6-2.2)	10	1.8 (0.95-3.4)
Medium [24.5]	11	0.9 (0.5-1.7)	11	0.7 (0.4-1.4)
High [50]	9	0.8 (0.4-1.5)	9	0.8 (0.4-1.5)

		P for trend=0.43		P for trend=028
Methyl bromide (methyl halide-fumigant)				
None	268	1.0 (ref)	268	1.0 (ref)
Low [8]	25	1.9 (1.2-2.8)	17	1.9 (1.2-3.1)
Medium [15.5]	9	0.9 (0.4-1.7)	16	1.3 (0.8-2.1)
High [28]	16	0.6 (0.3-0.9)	16	0.5 (0.3-0.9)
		P for trend=0.03		P for trend=0.02
Permethrin Animals (pyrethroid-insecticide)				
None	263	1.0 (ref)	263	1.0 (ref)
Low [8.75]	15	1.3 (0.8-2.3)	10	1.3 (0.7-2.5)
Medium [24]	5	0.8 (0.3-2.5)	10	0.8 (0.4-1.7)
High [56]	9	0.6 (0.3-1.2)	9	0.8 (0.4-1.5)
		P trend= 0.18		P trend=0.43
Permethrin Crops (pyrethroid-insecticide)				
None	249	1.0 (ref)	249	1.0 (ref)
Low [8.75]]	17	1.0 (0.6-1.7)	12	1.1 (0.5-2.2)
Medium [24.5]	9	1.1 (0.5-2.3)	12	1.2 (0.7-2.2)
High [59]	10	0.7 (0.4-1.4)	11	0.6 (0.3-1.1)
		P for trend=0.36		P for trend=0.15
Phorate (organophosphate-insecticide)				
None	102	1.0 (ref)	102	1.0 (ref)
Low [20]	20	1. (0.6-1.6)	17	0.9(0.5-1.5)

Comment [lbf73]: Do you show permethrin on crops anywhere?

Medium [24.5]	20	2.2 (1.4-3.5)	17	1.9 (1.1-3.1)
High [56]	10	0.7 (0.4-1.3)	16	1.0(0.6-1.7)
		P for trend=0.80		P for trend=0.67
Herbicide exposures				
	Life-time days of Exposure		Intensity weighted days of exposure*	
	NHL Cases	RR (95%)	NHL Cases	RR (95% CI)
Chlorimuron-ethyl (benzoic acid ester-herbicide)				
None	105	1.0 (ref)	105	1.0 (ref)
Low [8.75]	28	1.2(0.9-1.8)	18	1.1(0.6-1.9)
Medium [24.5]	18	1.9(1.2-3.2)	18	1.5(0.9-2.5)
High [24.5]	7	0.7(0.3-1.5)	17	1.1(0.7-1.9)
		P for trend=0.83		P for trend=0.60
Cyanazine (triazine-herbicide)				
None	162	1.0 (ref)	162	1.0 (ref)
Low [20]	58	1.4(0.9-1.9)	45	1.3(0.8-1.7)
Medium [56]	43	1.2(0.8-1.7)	45	1.4(1.0-1.9)
High [116]	35	1.1(0.8-1.6)	44	1.1(0.8-1.5)
		P for trend=0.81		P for trend=0.67
Herbicide Oil (Petroleum oils-herbicide)				
None	120	1.0 (ref)	120	1.0 (ref)
Low [20]	14	1.0(0.6-1.9)	13	1.3(0.7-2.3)
Medium [56]	13	1.8(1.0-1.1)	12	1.1(0.6-1.9)

High [173.25]	10	1.0(0.5-2.0)	12	1.3(0.7-2.4)
		P for trend=0.84		P for trend=0.36
Metolachlor (acetamide-herbicide)				
None	145	1.0 (ref)	145	1.0 (ref)
Low [20]	50	1.2(0.9-1.7)	49	1.2(0.8-1.6)
Medium [56]	54	1.3(0.94-1.5)	49	1.4(1.0-2.0)
High [116]	44	1.1(0.8-1.5)	48	1.1(0.8-1.5)
		P for trend=0.67		P for trend=0.28
Paraquat				
None	127	1.0 (ref)	127	1.0 (ref)
Low [7]	10	1.5(0.8-2.8)	10	1.9(1.0-3.7)
Medium [24.5]	10	0.8(0.4-1.5)	9	0.5(0.3-1.1)
High [116]	8	1.0(0.5-2.0)	9	1.5(0.8-3.0)
		P for trend= 0.88		P for trend=0.26
Pendimethalin				
None	96	1.0 (ref)	96	1.0 (ref)
Low [8.75]	32	1.1(0.7-1.6)	25	1.1(0.6-1.8)
Medium [24.5]	23	1.2(0.7-2.0)	26	1.0(0.7-1.6)
High [56]	20	1.0(0.6-1.6)	24	1.2(0.7-1.8)
		P for trend=0.87		P for trend=0.52
2,4,5 T (phenoxyacetic acid)				
None	71	1.0 (ref)	71	1.0 (ref)
Low [8.75]	30	1.7(1.1-2.5)	17	1.6(0.9-2.8)
Medium [8.75]	4	1.2(0.4-3.3)	16	1.9(1.1-3.2)
High [20]	15	1.2(0.7-2.2)	16	1.0(0.6-1.7)

		P for trend=0.52		P for trend=0.51

[1]Age adjusted (<45,45-49,50-54,55-59,60-64,65-69,≥70)

Supplemental Table 2. Pesticide exposures (total days and intensity weight total days) fully adjusted risks of NHL incidence (1993 through 2008).				
	NHL Cases	RR (95%) by Total Days of Exposure	NHL Cases	RR (95% CI) Intensity weighted days of exposure
Benomyl				
none	134	1.0 (ref)	134	1.0 (ref)
Low	6	6.1(2.7-13.8)	6	4.6 (2.0-10.6)
medium	5	1.0(0.4-2.6)	5	1.4 (0.6-3.5)
High	5	1.0(0.4-2.6)	5	1.1 (0.4-2.8)
		P trend (full)=0.98		P trend (full)=0.94
Captan				
none	258	1.0 (ref)	258	1.0 (ref)
Low	8	0.6(0.3-1.2)	8	0.7 (0.3-1.4)
medium	8	1.7(0.7-4.3)	7	1.2 (0.5-2.0)
High	7	0.7(0.3-1.6)	7	0.6 (0.2-1.4)
		P trend (full)=0.45		P trend (full)=0.28
Carbaryl				
none	81	1.0(ref)	81	1.0 (ref)
Low	31	0.96(0.6-1.6)	27	0.91 (0.6-1.5)
medium	23	0.8(0.5-1.4)	26	0.99 (0.6-1.6)
High	25	1.3(0.8-2.2)	26	1.1 (0.7-1.9)
		P trend (full)=0.26		P trend (full)=0.54
Carbofuran				
none	199	1.0 (ref)	199	1.0 (ref)
Low	35	1.0(0.7-1.5)	29	1.1(0.8-1.6)
medium	25	0.97(0.6-1.5)	29	0.8(0.5-1.2)
High	28	0.96(0.6-1.4)	28	1.1(0.7-1.6)

		P trend (full)=0.83		P trend (full)=0.95
Chlorthalonil				
none	301	1.0 (ref)	301	1.0 (ref)
Low	7	1.4(0.7-3.0)	7	1.2 (0.6-2.6)
Medium	6	0.7(0.3-1.8)	6	0.6 (0.2-1.9)
High	6	0.6 (0.3-1.4)	6	0.7 (0.3-1.6)
		P trend (full)=0.21		P trend (full)=0.37
Chlorpyrifos				
None	189	1.0 (ref)	189	1.0 (ref)
Low	44	1.0(0.7-1.5)	40	1.0 (0.7-1.5)
Medium	45	1.2(0.9-1.7)	41	0.94 (0.7-1.3)
High	43	0.8(0.6-1.2)	39	1.0 (0.7-1.4)
		P trend (full)=0.31		P trend (full)=0.99
Coumaphos				
none	258	1.0 (ref)	258	1.0 (ref)
Low	12	1.1(0.6-2.0)	10	1.4 (0.8-2.7)
medium	10	1.3 (0.7-2.5)	11	1.1 (0.6-2.0)
High	8	1.1(0.5-2.2)	9	1.1 (0.6-2.1)
		P trend (full)=0.62		P trend (full)=0.75
Diazinon				
None	113	1.0 (ref)	113	1.0 (ref)
Low	19	1.3(0.8-2.1)	14	1.3 (0.7-2.2)
medium	10	0.8(0.3-1.8)	15	0.9 (0.5-1.7)
High	13	1.3(0.7-2.5)	13	1.3 (0.7-2.3)
		P trend (full)=0.41		P trend (full)=0.50

DDVP				
none	261	1.0 (ref)	261	1.0 (ref)
Low	10	1.0 (0.5-1.9)	10	1.1 (0.6-2.1)
medium	11	0.92 (0.5-1.7)	9	0.7 (0.4-1.4)
High	7	0.6 (0.3-1.3)	9	0.9 (0.4-1.7)
		P trend (full)=0.22		P trend (full)=0.61
Fonofos				
None	220	1.0 (ref)	220	1.0 (ref)
Low	28	1.2(0.8-1.7)	23	1.1(0.7-1.7)
medium	19	1.1(0.7-1.7)	23	1.2(0.8-1.9)
High	22	0.9 (0.6-1.5)	22	0.9(0.5-1.3)
		P trend (full)=0.76		P trend (full)=0.51
Lindane				
None	122	1.0 (ref)	122	1.0 (ref)
Low	11	0.9(0.5-1.8)	10	1.0(0.5-1.8)
medium	10	1.0(0.5-2.0)	11	1.2(0.6-2.3)
High	10	2.3(1.2-4.5)	9	1.7(0.9-3.3)
		P trend (full)=0.01		P trend (full)=0.12
Malathion				
none	55	1.0 (ref)	55	1.0 (ref)
Low	46	0.9(0.6-1.3)	37	0.9 (0.6-1.4)
medium	28	0.7(0.4-1.1)	38	0.8 (0.5-1.1)
High	36	1.0(0.7-1.5)	35	0.9 (0.6-1.4)
		P trend (full)=0.68		P trend (full)=0.91
Metalaxyl				
none	126	1.0 (ref)	126	1.0 (ref)
Low	10	1.2(0.6-2.4)	10	1.7 (0.9-3.4)

medium	11	1.1(0.6-2.2)	11	0.9 (0.4-1.7)
High	9	1.1(0.5-2.3)	9	1.0 (0.5-2.2)
		P trend (full)=0.89		P trend (full)=0.93
Methyl bromide				
none	268	1.0 (ref)	268	1.0 (ref)
Low	25	2.2 (1.4-3.4)	17	2.3 (1.4-3.8)
medium	9	1.1 (0.5-2.1)	16	1.5 (0.9-2.6)
High	16	0.7 (0.4-1.2)	16	0.7 (0.4-1.1)
		P trend (full)=0.13		P trend (full)=0.07
Permethrin Animals				
None	263	1.0 (ref)	263	1.0 (ref)
Low	15	1.1(0.7-1.9)	10	1.1(0.6-2.1)
medium	5	0.7(0.2-2.1)	10	0.7(0.3-1.4)
High	9	0.5(0.3-1.0)	9	0.6(0.3-1.2)
		P trend (full)=0.055		P trend (full)=0.15
Permethrin Crops				
None	249	1.0 (ref)	249	1.0 (ref)
Low	17	0.9(0.5-1.6)	12	1.0(0.5-2.0)
medium	9	1.1(0.5-2.2)	12	1.2(0.7-2.2)
High	10	0.8(0.4-1.5)	11	0.6(0.3-1.2)
		P trend (full)=0.44		P trend (full)=0.18
Phorate				
none	102	1.0 (ref)	102	1.0 (ref)
Low	20	0.8(0.5-1.3)	17	0.7 (0.4-1.2)
medium	20	1.7(1.0-2.8)	17	1.5 (0.9-2.5)
High	10	0.6(0.3-1.0)	16	0.8 (0.5-1.4)
		P trend (full)=0.26		P trend (full)=0.70

Terbufos				
None	157	1.0 (ref)	157	1.0 (ref)
Low	58	1.3(0.9-1.8)	43	1.2(0.8-1.7)
medium	38	1.7(1.2-2.5)	43	1.7(1.2-2.4)
High	34	1.0(0.7-1.5)	42	1.1(0.8-1.6)
		P trend (full)=0.78		P trend (full)=0.65
Herbicide exposures				
	Life-time days of Exposure		Intensity weighted days of exposure*	
	NHL Cases	RR (95%)	NHL Cases	RR (95% CI)
Alachlor				
None	138	1.0 (ref)	138	1.0 (ref)
Low	65	0.9 (0.7-1.2)	53	0.9(0.7-1.2)
medium	49	0.8((0.6-1.1)	50	0.8 (0.6-1.1)
High	43	1.2((0.9-1.8)	51	1.2 (0.8-1.6)
		P trend (full)=0.20		P trend (full)=0.27
Atrazine				
None	85	1.0 (ref)	85	1.0 (ref)
Low	88	1.1(0.8-1.5)	79	1.0(0.7-1.4)
medium	72	1.2 (0.8-1.6)	78	1.2(0.9-1.7)
High	77	1.0 (0.7-1.4)	78	0.98(0.7-1.4)
		P trend (full)= 0.72		P trend (full)=0.73
Butylate				
None	107	1.0 (ref)	107	1.0 (ref)
Low	22	0.9(0.5-1.4)	16	0.8 (0.5-1.3)
medium	18	2.4(1.4-4.0)	16	1.8 (1.0-3.0)
High	7	1.0(0.4-2.1)	15	1.3 (0.8-2.3)

		P trend (full)=0.03		P trend (full)=0.14
Chlorimuron-ethyl				
None	105	1.0 (ref)	105	1.0 (ref)
Low	28	1.1 (0.7-1.7)	18	1.0 (0.6-1.7)
medium	18	1.7 (1.0-2.9)	18	1.3(0.8-2.2)
High	7	0.7 (0.3-1.5)	17	1.1(0.6-1.8)
		P trend (full)=0.69		P trend (full)=0.68
Cyanazine				
None	162	1.0 (ref)	162	1.0 (ref)
Low	58	1.3(0.94-1.8)	45	1.2(0.8-1.7)
medium	43	1.1(0.8-1.6)	45	1.3(0.9-1.8)
High	35	1.0(0.7-1.4)	44	1.0(0.7-1.4)
		P trend (full)=0.65		P trend (full)=0.76
Dicamba				
None	121	1.0 (ref)	121	1.0 (ref)
Low	66	1.2 (0.8-1.7)	24	1.1(0.7-1.6)
medium	52	1.3 (0.9-1.9)	54	1.3(0.9-1.9)
High	47	1.1 (0.7-1.6)	55	1.1(0.8-1.6)
		P trend (full)=0.99		P trend (full)=0.76
2,4-D				
None	71	1.0 (ref)	71	1.0 (ref)
Low	83	0.9(0.6-1.3)	82	0.9 (0.6-1.2)
medium	83	1.0(0.7-1.4)	83	0.97 (0.7-1.4)
High	82	0.8(0.6-1.2)	81	0.9 (0.6-1.2)
		P trend (full)=0.35		P trend (full)=0.46
EPTC				
None	229	1.0 (ref)	229	1.0 (ref)

Low	28	1.2(0.8-1.8)	20	1.2 (0.8-2.0)
medium	14	0.9(0.7-1.9)	20	1.1 (0.7-1.7)
High	18	1.2(0.7-1.9)	19	1.0 (0.6-1.7)
		P trend (full)=0.56		P trend (full)=0.85
Glyphosate				
None	70	1.0 (ref)	70	1.0 (ref)
Low	89	0.8(0.6-1.2)	83	0.91 (0.6-1.3)
medium	78	0.8(0.6-1.2)	84	0.8 (0.5-1.1)
High	83	1.0(0.7-1.4)	82	0.97 (0.7-1.4)
		P trend (full)=0.63		P trend (full)=0.69
Herbicide Oil				
None	120	1.0 (ref)	120	1.0 (ref)
Low	14	1.0(0.6-1.7)	13	1.2 (0.6-2.1)
medium	13	1.7(0.93-2.9)	12	1.0 (0.5-1.8)
High	10	0.9((0.5-1.8)	12	1.2 (0.7-2.2)
		P for trend (full)=0.88		P for trend (full)=0.56
Imazethapyr				
None	181	1.0 (ref)	181	1.0 (ref)
Low	39	0.8(0.5-1.2)	36	0.8 (0.6-1.2)
medium	34	0.8(0.5-1.2)	37	0.7 (0.5-1.1)
High	35	1.0(0.7-1.5)	35	0.99 (0.7-1.5)
		P trend (full)=0.90		P trend (full)=0.92
Metolachlor				
None	145	1.0 (ref)	145	1.0 (ref)
Low	50	1.2 (0.8-1.6)	49	1.1(0.8-1.5)
medium	54	1.2 (0.8-1.7)	49	1.3(0.9-1.9)
High	44	1.0 (0.7-1.4)	48	0.98(0.7-1.4)

		P trend (full)=0.90		P trend (full)=0.81
Metribuzin				
None	94	1.0 (ref)	94	1.0 (ref)
Low	28	1.0(0.6-1.5)	21	1.0 (0.6-1.7)
medium	15	0.8(0.4-1.3)	23	0.91 (0.6-1.5)
High	20	1.4(0.8-2.3)	19	1.1 (0.7-1.9)
		P trend (full)=0.29		P trend (full)=0.66
Paraquat				
None	127	1.0 (ref)	127	1.0 (ref)
Low	10	1.6(0.8-3.0)	10	2.0 (1.0-3.7)
medium	10	0.9(0.5-1.7)	9	0.6 (0.3-1.3)
High	8	1.2(0.6-2.5)	9	1.9 (0.9-3.9)
		P trend (full)=0.72		P trend (full)=0.08
Pendimethalin				
None	96	1.0 (ref)	96	1.0 (ref)
Low	32	1.0(0.6-1.5)	25	0.9 (0.5-1.6)
medium	23	1.0(0.6-1.8)	26	0.9 (0.6-1.4)
High	20	1.0(0.6-1.5)	24	1.1 (0.7-1.8)
		P trend (full)=0.72		P trend (full)=0.60
Trifluralin				
None	140	1.0 (ref)	140	1.0 (ref)
Low	51	0.9(0.7-1.3)	50	0.9 (0.6-1.2)
medium	58	1.0(0.7-1.3)	52	1.0 (0.7-1.4)
High	43	0.8(0.6-1.2)	48	0.8 (0.6-1.1)
		P trend (full)=0.41		P trend (full)=0.30
2,4,5 T				
None	71	1.0 (ref)	71	1.0 (ref)

Low	30	1.6(1.0-2.4)	17	1.6 (0.9-2.6)
medium	4	1.1(0.4-3.0)	16	1.7 (1.0-2.9)
High	15	1.1(0.7-2.0)	16	1.0 (0.6-1.7)
		P trend (full)=0.78		P trend (full)=0.23

[1]Age adjusted (<45,45-49,50-54,55-59,60-64,65-69,≥70), smoking status(current, former, never), number of livestock (0,<100,100-999,>999), drove diesel tractor(<weekly,≥weekly), state (NC, IA)

Supplemental Table 1A. Chlorinated Insecticide exposure (in total days and intensity weighted days) and NHL age-adjusted relative risk(1993 through 2008).				
	Total exposure days		Intensity weight exposure days	
	NHL cases	RR (95% CI)[1]	NHL cases	RR (95% CI)
Aldrin (Chlorinated Insecticide)				
None	232	1.0 (ref)	232	1.0 (ref)
Low [8.75]	14	0.8 (0.5-1.6)	12	0.9(0.5-1.6)
Medium [56]	14	0.8(0.5-1.4)	12	0.8(0.4-1.4)
High [116]	7	1.6(0.7-3.4)	11	1.0(0.6-1.9)
		P trend=0.70		P trend=0.86
Aldrin				
None	232	1.0 (ref)	232	1.0 (ref)
Low	14	0.8 (0.5-1.4)	12	0.9 (0.5-1.6)
medium	14	1.6 (0.8-3.4)	12	1.0 (0.6-1.9)
high	7	0.9 (0.7-1.2)	11	0.9 (0.7-1.2)
		P for trend=0.42		P for trend=0.95
		P for trend (full)=0.34		P for trend (full)=0.60
Heptachlor (Chlorinated Insecticide)				
None	240	1.0 (ref)	240	1.0 (ref)
Low [8.75]	11	2.1 (1.3-3.6)	10	2.8 (1.5-5.3)
Medium [24.5]	15	0.9 (0.3-2.1)	10	1.0 (0.5-1.9)
High [24.5]	5	1.0 (0.7-1.3)	10	1.0 (0.7-1.30
		P trend=0.26		P trend=0.42

Heptachlor				
None	240	1.0 (ref)	240	1.0 (ref)
Low	11	0.9 (0.5-1.6)	11	0.9 (0.5-1.7)
medium	15	2.1 (1.3-3.6)	10	2.8 (1.5-5.3)
high	5	0.9 (0.4-2.1)	10	1.0 (0.5-1.9)
		P for trend=0.11		P for trend=0.41
		P for trend (full)=0.19		P for trend (full)=0.16
2,4,5 TP				
None	276	1.0 (ref)	276	1.0 (ref)
Low	8	1.8 (0.9-3.7)	4	1.6 (0.6-4.3)
medium	0	0.6 (0.2-1.9)	4	1.4 (0.5-3.8)
high	3	0.9 (0.6-1.2)	3	0.8 (0.2-2.4)
		P for trend=0.40		P for trend=0.75
		P for trend (full)=0.27		P for trend (full)=0.74
Toxaphene (Chlorinated Insecticide)				
None	250	1 .0 (ref)	250	1.0 (ref)
Low [8.75]	10	3.4(1.4-8.3)	7	0.8(0.4-1.6)
Medium [20]	5	0.6(0.3-1.3)	8	0.7(0.3-1.6)
High [50.75]	6	1.0(0.7-1.3)	6	1.0(0.7-1.3)
	P trend=0.66		P trend=0.83	
Toxaphene				
None	250	1.0 (ref)	250	1.0 (ref)
Low	10	3.4 (1.4-8.3)	7	1.6 (0.8-3.5)
medium	5	0.6 (0.3-1.3)	8	0.8 (0.4-1.6)
high	6	1.0 (0.7-1.3)	6	0.7 (0.3-1.6)

	P for trend=0.33		P for trend=0.31
	P for trend (full)= 0.12		P for trend (full)=0.69

[1]Age adjusted (<45,45-49,50-54,55-59,60-64,65-69,≥70)

Supplemental Table 2A. Chlorinated Insecticide exposure (in total days and intensity weighted days) and NHL fully adjusted relative risk (1993 through 2008).

	Life-time exposure days		Intensity weight exposure days	
	NHL cases	RR (95% CI)[1]	NHL cases	RR (95% CI)
Aldrin				
None	232	1.0 (ref)	232	1.0 (ref)
Low	14	0.7 (0.4-1.3)	12	0.8 (0.5-1.5)
medium	14	0.7 (0.4-1.2)	12	0.7 (0.4-1.3)
high	7	1.4 (0.7)	11	0.9 (0.5-1.7)
		P for trend (full)=0.34		P for trend (full)=0.60
Chlordane				
None	223	1.0 (ref)	223	1.0 (ref)
Low	23	1.0 (0.6-1.6)	13	1.2 (0.7-2.2)
medium	6	1.8 (0.8-4.2)	13	0.9 (0.5-1.7)
high	9	0.4 (0.4-1.7)	12	1.0 (0.6-1.8)
		P for trend (full)=0.63		P for trend (full)=0.90
DDT				
None	194	1.0 (ref)	194	1.0 (ref)
Low	20	0.8 (0.5-1.3)	19	0.9 (0.6-1.5)

medium	18	1.0 (0.6-1.6)	18	0.9 (0.5-1.4)
high	17	1.5 (0.9-2.5)	18	1.4 (0.9-2.4)
		P for trend (full)=0.48		P for trend (full)=0.61
Heptachlor				
None	240	1.0 (ref)	240	1.0 (ref)
Low	11	0.8 (0.4-1.5)	11	0.8 (0.5-1.6)
medium	15	1.9 (1.1-3.3)	10	2.4 (1.3-4.7)
high	5	0.8 (0.3-1.9)	10	0.9 (0.5-1.8)
		P for trend (full)=0.19		P for trend (full)=0.16
Lindane				
None	122	1.0 (ref)	122	1.0 (ref)
Low	11	0.9 (0.5-1.8)	10	1.0(0.5-1.8)
medium	10	1.0 (0.5-2.0)	11	1.2(0.6-2.3)
high	10	2.4 (1.2-4.5)	9	1.7(0.9-3.3)
		P for trend (full)=0.01		P for trend (full)=0.12
Toxaphene				
None	250	1.0 (ref)	250	1.0 (ref)
Low	10	0.91 (0.5-1.7)	7	1.6 (0.7-3.3)
medium	5	3.4 (1.4-8.3)	8	0.8 (0.4-1.6)
high	6	0.6 (0.3-1.3)	6	0.7 (0.3-1.7)
		P for trend (full)= 0.12		P for trend (full)=0.69

Supplemental Table 3. Herbicide exposures (Life-time days) and age-adjusted NHL risk by cell type (1993 through 2008).

Pesticide (chemical class)	CLL, SLL, PLL, MCL		Diffuse Large B-cell		Follicular B-cell		Other B-cell types	
	RR (95% CI)	n	RR (95% CI)	n	RR (95% CI)	n	RR (95% CI)	n
Alachlor (acetanilide)								
None	1.0 (ref)	53	1.0 (ref)	43	1.0 (ref)	22	1.0 (ref)	9
low	0.9(0.6-1.5)	23	0.9(0.5-1.6)	13	1.3(0.6-2.6)	10	1.6 (0.6-4.4)	7
medium	0.8(0.5-1.4)	18	0.7(0.4-1.3)	14	0.8(0.3-1.6)	9	2.1 (0.8-5.3)	10
high	1.1(0.6-2.1)	14	0.8(0.4-1.6)	10	1.1(0.4-2.7)	6	4.0 (1.2-13.0)	4
	LD P =0.67		LD P trend=0.52		LD P trend=0.99		LD P trend=0.02	
	IWLD P=0.49		IWLD P trend=0.092		IWLD P trend=0.97		IWLD P trend= 0.20	
Atrazine (triazine)								
None	1.0 (ref)	34	1.0 (ref)	26	1.0 (ref)	12	1.0 (ref)	5
low	1.0 (0.6-1.7)	29	1.1(0.6-2.0)	21	1.7(0.7-3.9)	17	2.4 (0.9-6.8)	13
medium	1.2 (0.7-2.0)	25	1.1(0.6-2.2)	23	1.3(0.5-3.4)	10	1.7(0.5-5.9)	6
high	1.0 (0.6-1.7)	26	0.9(0.5-1.7)	19	1.4(0.6-3.4)	13	3.6 (1.2-10.8)	9
	LD P trend=0.90		LD P trend=0.62		LD P trend=0.83		LD P trend=0.06	
	IWLD P trend=0.75		IWLD P trend=0.87		IWLD P trend=0.76		IWLD P trend=0.22	

Butylate (thio-carbamate-)								
None	1.0 (ref)	40	1.0 (ref)	33	1.0 (ref)	14	1.0 (ref)	8
low	0.8(0.4-1.9)	7	1.1(0.4-3.0)	4	0.8(0.2-2.9)	3	3.0 (0.8-11.3)	3
medium	3.5(1.6-7.6)	8	1.2(0.4-3.5)	4	6.3(2.1-19.3)	4	4.0(1.2-13.7)	4
high	1.3(0.4-4.3)	3	0.8(0.2-2.5)	3	1.0(0.1-7.9)	1	2.4 (0.3-19.7)	1
	LD P trend=0.04		LD P trend=0.69		LD P trend=0.07		LD P trend=0.05	
	IWLD P trend=0.19		IWLD P trend=0.89		IWLD P trend=0.12		IWLD P trend=0.13	
Chlorimuron-ethyl (Sulfonylurea)								
None	1.0 (ref)	38	1.0 (ref)	29	1.0 (ref)	14	1.0 (ref)	14
low	1.3(0.7-2.6)	11	1.4(0.7-3.0)	9	0.9(0.3-3.1)	3	-	1
medium	2.9(1.4-6.6)	9	1.2(0.4-4.0)	3	2.8(0.9-8.7)	4	-	1
high	0.3(0.1-2.5)	1	1.4(0.5-3.9)	4	0.7(0.9-5.1)	1	-	0
	LD P for trend=0.91		LD P trend=0.21		LD P trend=0.56		LD P for trend=xx	
	IWLD P trend=0.56		IWLD P trend=0.92		IWLD P trend=0.62		IWLD P trend=	
Cyanazine (triazine)								
None	1.0 (ref)	65	1.0 (ref)	46	1.0 (ref)	24	1.0 (ref)	10
low	1.2 (0.7-2.2)	15	1.4 (0.8-2.4)	16	1.9(0.9-3.8)	12	3.7(1.4-9.7)	7
medium	0.9 (0.5-1.6)	16	0.8 (0.4-1.8)	8	1.7(0.8-3.6)	9	2.9 (1.5-7.5)	8
high	1.1(0.6-2.0)	14	1.0 (0.5-2.1)	8	0.8(0.3-2.2)	4	2.6(0.9-7.5)	5
	LD P trend=0.93		LD P trend=0.93		LD P trend=0.87		LD P trend=0.17	

	IWLD P trend=0.35		IWLD P trend=0.47		IWLD P trend=0.68		IWLD P trend=0.15	
2,4-D (Chlorinated Phenoxy)								
None	1.0 (ref)	25	1.0 (ref)	23	1.0 (ref)	9	1.0 (ref)	5
low	0.90(0.5-1.5)	31	0.9(0.5-1.7)	23	1.8(0.8-4.4)	14	1.9 (0.6-6.2)	10
medium	1.2(0.7-2.0)	29	1.0(0.6-1.9)	21	1.0(0.4-2.4)	14	1.7 (0.5-5.6)	9
high	1.3(0.7-2.2)	29	0.7(0.4-1.3)	21	1.4(0.6-3.4)	12	2.2 (0.7-7.2)	9
	LD P trend=0.20		LD P trend=0.23		LD P trend=0.84		LD P trend=0.35	
	IWLD P trend=0.83		IWLD P trend=0.41		IWLD P trend=0.22		IWLD P trend=0.75	
Dicamba (benzoic acid)								
None	1.0 (ref)	39	1.0 (ref)	40	1.0 (ref)	22	1.0 (ref)	6
low	1.5 (0.9-2.6)	23	1.1 (0.6-2.1)	12	1.5(0.7-3.4)	9	3.2 (1.0-9.9)	8
medium	1.5 (0.9-3.4)	20	1.1 (0.6-2.1)	13	1.8(0.90-4.0)	10	5.2(1.6-16.6)	7
high	2.0 (1.1-3.4)	20	0.7 (0.4-1.4)	11	0.7(0.3-1.5)	8	5.1(1.6-16.1)	7
	LD P trend=0.03		LD P trend=0.26		LD P trend=0.32		LD P trend=0.02	
	IWLD P trend=0.04		IWLD P trend=0.35		IWLD P trend=0.22		IWLD P trend=0.02	
EPTC (thio-carbamate)								
None	1.0 (ref)	86	1.0 (ref)	62	1.0 (ref)	40	1.0 (ref)	19
low	1,2(0.6-2.3)	9	1.2(0.6-2.7)	7	-	3	2.1 (0.7-6.0)	4
medium	1.2(0.6-2.5)	8	1.7(0.7-4.2)	5	-	0	2.1 (0.6-7.1)	3
high	1.4(0.6-3.4)	5	0.8(0.3-2.3)	4	-	1	4.9 (1.4-16.7)	3
	LD P trend= 0.41		LD P trend=0.98		LD P trend=0.10		LD P trend=0.01	
	IWLD P trend=0.43		IWLD P trend=0.59		IWLD P trend=0.14		IWLD P trend=0.15	

Glyphosate (isopropyl-amine)								
None	1.0 (ref)	25	1.0 (ref)	19	1.0 (ref)	13	1.0 (ref)	10
low	0.6(0.4-1.1)	32	1.3(0.7-2.6)	23	0.7(0.3-1.7)	15	0.4 (0.1-1.2)	9
medium	1.1(0.6-1.9)	29	1.1(0.5-2.1)	23	0.6(0.2-1.4)	11	0.6 (0.2-1.6)	7
high	1.1(0.6-1.8)	29	0.7(0.4-1.3)	22	0.7(0.3-1.8)	12	0.6 (0.2-1.8)	7
	LD P trend=0.21		LD P trend=0.05		LD P trend=0.66		LD P trend=0.98	
	IWLD P trend=0.18		IWLD P trend=0.19		IWLD P trend=0.83		IWLD P trend=0.75	
Herbicide Oil (petroleum oil)								
None	1.0 (ref)	42	1.0 (ref)	35	1.0 (ref)	17	1.0 (ref)	14
low	1.8(0.8-4.3)	7	1.0(0.4-2.5)	6	1.4(0.3-5.9)	2	-	1
medium	2.6(1.0-6.7)	5	2.8(0.7-11.9)	2	1.1(0.1-8.4)	1	-	1
high	1.0(0.4-2.6)	5	1.4(0.4-4.5)	3	0.5(0.1-3.6)	1	0	0
	LD P trend=0.76		LD P trend=0.55		LD P trend=0.46		LD P trend=xxx	
	IWLD P trend=0.88		IWLD P trend=0.16		IWLD P trend=0.40		IWLD P trend=xxx	
Imazethapyr (imid-azolinone)								
None	1.0 (ref)	68	1.0 (ref)	57	1.0 (ref)	29	1.0 (ref)	12
low	1.0(0.6-1.8)	16	0.7(0.3-1.4)	10	0.7(0.3-1.7)	6	1.6 (0.6-3.8)	8
medium	0.8(0.4-1.6)	11	0.6(0.3-1.4)	6	1.1(0.3-3.5)	6	5.2 (1.6-16.6)	4
high	1.2(0.6-2.2)	12	0.5(0.2-1.2)	3	1.0(0.4-2.8)	5	3.2 (1.0-10.0)	4
	LD P trend=0.71		Ld P trend=0.16		LD P trend=0.90		LD P trend=0.03	
	IWLD P trend=0.95		IWLD P trend=0.34		IWLD P trend=0.83		IWLD P trend=0.03	

Metolachlor (chlor-acetanilide)								
None	1.0 (ref)	52	1.0 (ref)	48	1.0 (ref)	20	1.0 (ref)	10
low	1.2(0.7-2.0)	23	0.9(0.4-2.1)	11	1.4(0.6-3.2)	9	2.7 (1.0-7.0)	9
medium	1.7(0.95-3.2)	17	1.3(0.7-2.4)	12	1.4(0.6-3.7)	9	2.1 (0.6-7.7)	4
high	1.3(0.8-2.3)	18	0.4(0.2-0.9)	9	1.5(0.7-3.6)	8	2.6 (0.9-7.2)	6
	LD P trend=0.19		LD P trend=0.07		LD P trend=0.43		LD P trend=0.19	
	IWLD P trend=0.20		IWLD P trend=0.23		IWLD P trend=0.33		IWLD P trend=0.64	
Metribuzin (Triazinone)								
None	1.0 (ref)	30	1.0 (ref)	35	1.0 (ref)	13	1.0 (ref)	9
low	1.5(0.7-2.9)	11	0.5(0.2-1.4)	5	1.4(0.5-3.9)	5	1.0 (0.2-4.9)	3
medium	2.1(1.1-4.0)	13	0.5(0.1-2.0)	3	0.8(0.2-2.9)	3	2.8 (0.9-8.9)	5
high	1.8(0.6-5.2)	4	0.4(0.1-1.6)	2	1.3(0.2-9.8)	1	-	0
	LD P trend=0.06		LD P trend=0.13		LD P trend=0.88		LD P trend=0.60	
	IWLD P trend=0.03		IWLD P trend=0.21		IWLD P trend=0.10		IWLD P trend=0.43	
Paraquat (bi-pyridylium)								
None	1.0 (ref)	48	1.0 (ref)	37	1.0 (ref)	15	1.0 (ref)	14
low	1.0(0.4-2.4)	5	2.4(0.9-6.7)	4	2.9(0.7-12.7)	2	-	1
medium	1.0(0.2-4.0)	2	0.7-0.2-2.3)	3	1.2(0.3-5.3)	2	-	1
high	1.0(0.3-3.2)	3	0.8(0.2-3.4)	2	1.0(0.1-7.6)	1	-	0
	Ld P trend=0.99		LD P trend=0.23		LD P trend=0.94		LD P trend=xxx	
	IWLD P trend=0.44		IWLD P trend=0.78		IWLD P trend=0.75		IWLD P trend=xxx	

Pendi-methalin (dinitro-aniline)								
None	1.0 (ref)	38	1.0 (ref)	28	1.0 (ref)	11	1.0 (ref)	8
low	1.2(0.6-2.2)	12	1.0(0.4-2.2)	9	1.4(0.5-4.2)	6	1.8 (0.5-6.2)	5
medium	1.2(0.6-2.7)	8	0.92(0.3-2.6)	6	1.5(0.4-5.4)	4	2.3 (0.6-8.9)	4
high	0.8(0.3-1.9)	6	0.8(0.3-2.1)	5	1.4(0.5-4.5)	4	1.8 (0.5-6.9)	3
	LD P trend=0.66		LD P trend=0.66		LD P trend=0.57		LD P trend=0.42	
	IWLD P trend=0.44		IWLD P trend= 0.88		IWLD P trend=0.49		IWLD P trend=0.70	
Trifluralin (dinitro-aniline)								
None	1.0 (ref)	45	1.0 (ref)	43	1.0 (ref)	25	1.0 (ref)	10
low	1.1(0.7-1.9)	23	0.9(0.5-1.7)	14	0.9(0.4-1.9)	8	1.2 (0.4-3.2)	7
medium	1.6(0.9-2.6)	21	0.8(0.4-1.7)	11	0.8(0.4-1.8)	8	2.7 (1.0-7.0)	7
high	1.1(0.6-1.9)	15	0.6(0.3-1.2)	11	0.8(0.3-1.9)	7	3.3 (1.2-9.1)	6
	LD P trend= 0.08		LD P trend=0.13		LD P trend=0.62		LD P trend=0.01	
	IWLD P trend=0.80		IWLD P trend=0.11		IWLD P trend=0.65		IWLD P trend=0.08	
2,4,5 T								
None	1.0 (ref)	37	1.0 (ref)	33	1.0 (ref)	14	1.0 (ref)	12
low	2.1(1.1-3.9)	14	1.3(0.6-3.0)	7	4.6(1.3-16.1)	3	-	3
medium	2.4(0.7-7.00	3	0.9(0.2-3.7)	2	2.1(0.6-7.2)	3	-	0
high	1.1(0.4-2.8)	5	1.3(0.4-4.3)	3	1.1(0.2-4.8)	2	-	1
	LD P trend= 0.33		LD P trend=0.71		LD P trend=0.73		LD P trend=xxx	
	IWLD P trend=0.83		IWLD P trend=0.90		IWLD P trend=0.80		IWLD P trend=0.97	

[1]Age adjusted (<45,45-49,50-54,55-59,60-64,65-69,≥70)

[2] Numbers do not sum to NHL subtype totals due to missing data

Supplemental Table 4. Insecticides, fungicide and fumigant exposure (life-time days) and age-adjusted risk of NHL by cell type (1993 through 2008).

	CLL, SLL, PLL, MCL		Diffuse Large B-cell		Follicular B-cell		Other B-cell types	
	RR (95% CI)	n	RR (95% CI)	n	RR (95% CI)	n	RR (95% CI)	n
Aldicarb								
None	1.0 (ref)	51	1.0 (ref)	40	1.0 (ref)	19	1.0 (ref)	15
low	1.9(0.3-13.4)	1	1.7(0.4-7.2)	2	6.1(0.8-45.7)	1	-	1
medium	0.95(0.1-6.9))	1	4.8(1.2-19.8)	2	1.2(0.2-9.4)	2	-	1
high	-	0	0.5(0.1-4.1)	1	-	0	-	0
	LD P trend=0.15		LD P trend=0.72		LD P trend=0.63		LD P trend=xxx	
	IWLD P trend=0.14		IWLD P trend=0.89		IWLD P trend=0.64		IWLD P trend=xxx	
Carbaryl								
None	1.0 (ref)	32	1.0 (ref)	23	1.0 (ref)	9	1.0 (ref)	9
low	1.1(0.5-2.2)	15	0.7(0.3-1.5)	10	1.1(0.3-4.0)	5	xxx-	6
medium	1.0(0.2-4.2)	2	1.3(0.6-3.0)	8	1.8(0.6-5.9)	4	xxx-	0
high	0.4(0.2-0.8)	8	1.5(0.7-3.5)	8	1.3(0.4-4.1)	4	xxx-	1
	LD P trend=0.007		LD P trend=0.19		LD P trend=0.66		LD P trend=xxx	
	IWLD P trend=0.02		IWLD P trend=0.27		IWLD P trend=0.81		IWLD P trend=xxx	
Carbofuran								
None	1.0 (ref)	67	1.0 (ref)	58	1.0 (ref)	33	1.0 (ref)	19
low	1.4(0.8-2.5)	15	0.9(0.4-1.9)	8	0.96(0.4-2.5)	5	1.0 (0.4-2.7)	5

Comment [lbf74]: It looks like in the main tables you have restricted presenting results when there aren't 5 cases in a cell. You should use the same rules in the supplemtnal tables.

medium	1.2(0.6-2.4)	10	0.9(0.4-1.8)	9	1.6(0.7-3.9)	6	1.4(0.2-10.7)	1
high	1.3(0.7-2.4)	12	1.1(0.5-2.9)	5	0.6(0.2-2.0)	3	0.94(0.2-4.1)	2
	LD P trend=0.36		LD P trend=0.81		LD P trend=0.79		LD P trend=0.99	
	IWLD P trend=0.79		IWLD P trend=0.71		IWLD P trend=0.72		IWLD P trend=xxx	
Chlorpyrifos								
None	1.0 (ref)	69	1.0 (ref)	55	1.0 (ref)	26	1.0 (ref)	18
low	0.9(0.5-1.7)	15	1.2(0.6-2.1)	13	1.4(0.7-3.1)	10	0.9(0.3-2.6)	5
medium	1.1(0.7-2.0)	16	1.0(0.5-1.7)	15	1.2(0.5-2.9)	7	4.2(1.7-10.6)	6
high	1.0(0.5-1.7)	14	0.9(0.6-4.0)	7	1.4(0.6-3.4)	6	0.8(0.3-2.3)	4
	LD P trend=0.99		LD P trend=0.66		LD P trend=0.56		LD P trend=0.97	
	IWLD P trend=0.88		IWLD P trend=0.67		IWLD P trend=0.22		IWLD P trend=	
Chlorthalonil								
None	1.0 (ref)	107	1.0 (ref)	84	1.0 (ref)	45	1.0 (ref)	32
low	0.9(0.3-2.9)	3	1.6(0.4-6.6)	2	3.1(0.7-12.6)	2	-	1
medium	0.7(0.2-2.7)	2	1.4(0.3-5.6)	2	1.2(0.3-4.8)	2	-	0
high	0.7(0.2-2.7)	2	0.2(0.1-1.4)	1	0.6(0.1-4.4)	1	-	0
	LD P trend=0.46		LD P trend=0.11		LD P trend=0.61		LD P trend=xxx	
	IWLD P trend=0.96		IWLD P trend=0.17		IWLD P trend=0.41		IWLD P trend=xxx	
Coumaphos								
None	1.0 (ref)	92	1.0 (ref)	72	1.0 (ref)	42	1.0 (ref)	22
low	1.1(0.4-3.1)	4	0.7(0.2-2.3)	3	1.9(0.6-6.0)	3	xxx-	4
medium	2.0(0.8-4.9)	5	2.1(0.5-8.5)	2	0.5(0.1-4.0)	1	xxx-	0

high	1.3(0.4-4.0)	3	1.5(0.4-5.9)	2	2.2(0.3-16.3)	1	-	1
	LD P trend=0.36		LD P trend=0.47		LD P trend=0.43		LD P trend=xxx	
	IWLD P trend=0.53		IWLD P trend=0.74		IWLD P trend=0.82		IWLD P trend=xxx	
Diazinon								
None	1.0 (ref)	40	1.0 (ref)	33	1.0 (ref)	13	1.0 (ref)	12
low	1.5(0.7-3.1)	9	1.2(0.4-3.1)	5	1.6(0.4-5.5)	3	xxx-	2
medium	1.2(0.4-3.6)	5	0.9(0.3-2.8)	4	1.6(0.4-7.4)	3	xxx-	1
high	1.2(0.5-3.0)	5	1.2(0.4-3.8)	3	2.0(0.4-10.0)	2	xxx-	0
	LD P trend=0.72		LD P trend=0.84		LD P trend=0.35		LD P trend=xxx	
	IWLD P trend=0.60		IWLD P trend=0.84		IWLD P trend=0.53		IWLD P trend=xxx	
DDVP								
None	1.0 (ref)	95	1.0 (ref)	74	1.0 (ref)	43	1.0 (ref)	24
low	1.3(0.5-3.5)	4	4.1(1.0-16.9)	2	0.7(0.2-3.1)	2	xxx-	1
medium	1.4(0.6-3.4)	5	0.5(0.1-1.9)	2	2.2(0.3-16.1)	1	xxx-	2
high	0.3(0.1-2.1)	3	0.3(0.1-2.2)	1	0.5(0.1-3.9)	1	-xxx	0
	LD P trend=0.46		LD P trend=0.25		LD P trend=0.54		LD P trend=xxx	
	IWLD P trend=0.85		IWLD P trend=0.54		IWLD P trend=0.53		IWLD P trend=xxx	
Fonofos								
None	1.0 (ref)	79	1.0 (ref)	61	1.0 (ref)	40	1.0 (ref)	17
low	1.6(.8-2.9)	12	1.5(0.8-3.1)	9	-	5	2.2(0.8-5.9)	5
medium	1.2(0.5-2.9)	5	1.0(0.4-2.3)	6	-	0	2.0(0.6-6.7)	3
high	0.9(0.5-2.0)	8	1.3(0.5-3.2)	5	-	2	2.3(0.3-17.0)	1
	LD P trend=0.88		LD P trend=0.62		LD P trend=0.20		LD P trend=0.19	

	IWLD P trend=0.94		IWLD P trend=0.77		IWLD P trend=0.18		IWLD P trend=xxx	
Lindane								
None	1.0 (ref)	41	1.0 (ref)	39	1.0 (ref)	14	1.0 (ref)	14
low	1.6(0.7-3.6)	8	0.7(0.2-3.0)	9	2.7(0.8-9.4)	3	xxx-	1
medium	1.1(0.3-4.8)	3	1.1(0.3-3.7)	6	3.6(0.8-15.9)	2	xxx-	0
high	3.8(1.5-9.6)	5	1.3(0.2-9.7)	5	2.4(0.5-10.4)	2	xxx-	0
	LD P trend=0.005		LD P trend=0.25		LD P trend=0.25		LD P trend=xxx	
	IWLD P trend=0.04		IWLD P trend=0.29		IWLD P trend=0.18		IWLD P trend=xxx	
Malathion								
None	1.0 (ref)	21	1.0 (ref)	16	1.0 (ref)	5	1.0 (ref)	6
low	0.94(0.5-1.8)	17	0.8(0.4-1.7)	16	1.0(0.3-3.6)	6	-xxx	8
medium	0.8(0.4-1.7)	11	0.9(0.4-2.1)	8	1.2(0.3-4.3)	5	-xxx	0
high	0.8(0.4-1.7)	11	1.7(0.8-3.8)	11	1.5(0.4-4.9)	5	-xxx	3
	LD P trend=0.52		LD P trend=0.07		LD P trend=0.48		LD P trend=xxx	
	IWLD P trend=0.24		IWLD P trend=0.33		IWLD P trend=0.56		IWLD P trend=xxx	
Maneb								
None	1.0 (ref)	52	1.0 (ref)	37	1.0 (ref)	19	1.0 (ref)	16
low	2.9(0.9-9.4)	3	2.6(0.6-10.9)	2	2.6(0.4-19.8)	1	-xxx	0
medium	1.6(0.4-6.6)	2	1.3(0.4-4.2)	3	1.1(0.1-8.0)	1	-xxx	0
high	0.3(0.1-2.4)	1	3.5(0.5-25.4)	1	-	0	-xxx	0
	LD P trend=0.43		LD P trend=0.19		LD P trend=0.55		LD P trend=xxx	
	IWLD P trend=0.49		IWLD P trend=0.17		IWLD P trend=0.66		IWLD P trend=xxx	

Metalaxyl								
None	1.0 (ref)	46	1.0 (ref)	34	1.0 (ref)	18	1.0 (ref)	
Low	3.9(1.7-9.3)	6	1.1(0.3-3.6)	4	0.8(0.2-3.4)	2	-xxx	
medium	1.3(0.3-5.4)	2	1.4(0.5-3.9)	5	2.1(0.5-9.2)	2	-xxx	
high	0.4(0.1-1.2)	3	0.9(0.2-4.0)	2	0.9(0.1-6.4)	1	-xxx	
	LD P trend=0.08		LD P trend=0.92		LD P trend=0.81		LD P trend=xxx	
	IWLD P trend=0.04		IWLD P trend=0.85		IWLD P trend=0.83		IWLD P trend=xxx	
Methylbromide								
None	1.0 (ref)	101	1.0 (ref)	65	1.0 (ref)	45	1.0 (ref)	14
low	0.8(0.3-2.1)	4	4.8(2.5-9.3)	10	1.4(0.3-5.8)	2	-xxx	1
medium	0.7(0.3-1.6)	5	1.3(0.6-3.1)	6	1.2(0.4-4.0)	3	-xxx	1
high	0.4(0.1-1.3)	3	1.2(0.5-2.6)	7	-	0	-xxx	0
	LD P trend=0.09		LD P trend=0.71		LD P trend=0.08		LD P trend=xxx	
	IWLD P trend=0.02		IWLD P trend=0.57		IWLD P trend=0.09		IWLD P trend=xxx	
Permethrin animals								
None	1.0 (ref)	95	1.0 (ref)	78	1.0 (ref)	38	1.0 (ref)	25
low	1.3(0.5-3.3)	5	0.2(0.1-1.3)	1	2.8(1.1-7.0)	5	-xxx	1
medium	0.9(0.2-3.7)	3	0.5(0.1-3.4)	1	2.9(0.7-12.0)	2	-xxx	2
high	0.8(0.3-2.5)	3	-	0	0.8(0.2-3.5)	2	-xxx	0
	LD P trend=0.75		LD P trend=0.19		LD P trend=0.93		LD P trend=0.87	
	IWLD P trend=0.70		IWLD P trend=0.29		IWLD P trend=0.73		IWLD P trend=xxx	
Permethrin crops								

None	1.0 (ref)	86	1.0 (ref)	72	1.0 (ref)	39	1.0 (ref)	23
low	1.9(0.6-5.4)	6	0.6(0.1-2.2)	3	1.1(0.3-3.5)	3	-xxx	4
medium	0.8(0.4-1.9)	6	2.7(0.7-10.6)	2	1.5(0.4-6.4)	2	-xxx	0
high	1.2(0.4-4.0)	4	0.4(0.1-1.8)	2	0.5(0.1-3.9)	2	-xxx	0
	LD P trend=0.76		LD P trend=0.28		LD P trend=0.57		LD P trend=0.37	
	IWLD P trend=0.70		IWLD P trend=0.33		IWLD P trend=0.45		IWLD P trend=xxx	
Phorate								
None	1.0 (ref)	36	1.0 (ref)	29	1.0 (ref)	15	1.0 (ref)	10
low	1.4(0.7-3.0)	9	1.0(0.4-2.6)	5	0.6(0.1-2.7)	2	1.4 (0.4-4.6)	4
medium	1.4(0.6-3.2)	6	2.0(0.9-4.7)	7	2.9(0.96-8.7)	4	1.5 (0.2-11.6)	1
high	0.94(0.4-2.4)	5	0.7(0.2-2.4)	3	-	0	1.4 (0.2-11.2)	1
	LD P trend=0.90		LD P trend=0.92		LD P trend=0.82		LD P trend=XXX	
	IWLD P trend=0.53		IWLD P trend=0.98		IWLD P trend=0.33		IWLD P trend=xxx	
Terbufos								
None	1.0 (ref)	53	1.0 (ref)	47	1.0 (ref)	26	1.0 (ref)	10
low	1.8(1.0-3.1)	17	0.9(0.4-1.7)	12	2.5(1.1-5.4)	8	2.3 (0.8-6.6)	6
medium	2.2(1.3-3.6)	21	2.2(1.2-4.2)	12	1.8(0.7-4.3)	7	3.1(1.1-9.2)	5
high	1.4(0.8-2.6)	13	1.1(0.5-2.3)	10	0.7(0.3-1.8)	6	4.1(1.4-11.9)	5
	LD P trend=0.16		LD P trend=0.34		LD P trend=0.54		LD P trend=0.01	
	IWLD P trend=0.14		IWLD P trend=0.40		IWLD P trend=0.18		IWLD P trend=xxx	

[1]Age adjusted (<45,45-49,50-54,55-59,60-64,65-69,≥70)

Supplemental Table 5. Estimated individual and joint effects of pesticide combinations and age-adjusted risk of NHL		
Individual and joint pesticide exposures	**Exposed cases**	**Poisson Regression RR (95% CI)[1]**
Chlordane and DDT		
--Neither	174	1.0 (reference)
--Chlordane only	19	0.6 (0.4-1.0)
--DDT only	49	0.8(0.6-1.2)
--Both	56	0.9 (0.7-1.3)
Chlordane and Lindane		
--Neither	200	1.0 (reference)
--Chlordane only	47	0.8(0.6-1.2)
--Lindane only	23	1.0(0.6-1.5)
--both	28	1.0(0.7-1.6)
Lindane and dicamba		
--Neither	113	1.0 (reference)
--Lindane only	15	1.0 (0.6-1.7)
--dicamba only	120	1.3 (0.98-1.6)
--both	32	1.2 (0.8-1.8)
Atrazine and Chlordane		
--Neither	58	1.0 (reference)
--atrazine only	162	1.3(0.97-1.8)
--Chlordane only	19	1.0(0.6-1.7)
--Both	57	1.1(0.8-1.6)
2,4,5 t and Lindane		
--Neither	190	1.0 (reference)
--2,4,5-t only	57	1.1(0.9-1.6)

Comment [a75]: Need to delete. No really interesting findings, no space. Timing of pesticides not possible.

--Lindane only	27	1.1(0.7-1.6)
--Both	25	1.2 (0.8-1.8)
Atrazine and Lindane		
--Neither	73	1.0 (reference)
--Atrazine only	173	1.1 (0.9-1.5)
--Lindane only	4	0.5 (0.2-1.3)
--both	47	1.3 (0.9-1.9)
Atrazine and Dicamba		
--Neither	61	1.0 (reference)
--Atrazine only	72	1.0 (0.7-1.4)
--Dicamba only	17	1.0 (0.6-1.7)
--both	140	1.3 (0.97-1.8)
Atrazine and Carbofuran		
--Neither	68	1.0 (reference)
--Atrazine only	132	1.1 (0.9-1.5)
--Carbofuran only	9	0.9 (0.4-1.8)
--Both	81	1.2 (0.9-1.6)
Atrazine and Diazinon		
--Neither	58	1.0 (reference)
--atrazine only	163	1.2 (0.9-1.7)
--Diazinon only	20	0.9 (0.5-1.5)
--Both	59	1.1 (0.8-1.6)
Atrazine and alachlor		
--Neither	65	1.0 (reference)
--atrazine only	73	1.1 (0.8-1.5)

--alachlor only	16	0.8 (0.5-1.4)
--Both	146	1.1 (0.8-1.5)
2,4, 5 t and dicamba		
--Neither	94	1.0 (reference)
--2,4,5-t only	32	1.3 (0.9-1.9)
--dicamba only	107	1.4 (1.0-1.8)
--Both	45	1.3 (0.9-1.8)
2,4-D and Chlordane		
--Neither	55	1.0 (reference)
--2,4-D only	164	1.1(0.8-1.5)
--Chlordane only	7	0.7(0.3-1.5)
--Both	70	1.0 (0.7-1.5)
Glyphosate and atrazine		
--Neither	30	1.0 (reference)
--Glyphosate only	60	0.96(0.6-1.5)
--atrazine only	63	1.4(0.9-2.1)
--Both	171	1.1(0.7-1.6)
Glyphosate and 2,4-D		
--Neither	32	1.0 (reference)
--Glyphosate only	44	1.1(0.7-1.7)
--2,4-D only	61	1.4(0.9-2.1)
--Both	188	1.1(0.7-1.5)
Glyphosate and Chlordane		
--Neither	72	1.0 (reference)
--Glyphosate only	147	0.9 (0.7-1.2)

--chlordane only	13	1.0 (0.5-1.7)
--Both	64	0.8 (0.6-1.1)
2,4-D and Lindane		
---Neither	60	1.0 (reference)
---only 2,4-D	180	1.1(0.8-1.4)
---only lindane	3	0.6(0.2-1.8)
---both	48	1.2(0.8-1.7)
2,4-D and atrazine		
---Neither	41	1.0 (reference)
---only 2,4-D	49	1.0(0.7-1.5)
---only atrazine	35	1.2(0.8-1.9)
---both	199	1.2(0.8-1.7)
2,4-D and dicamba		
---Neither	51	1.0 (reference)
---only 2,4-D	81	0.9(0.6-1.3)
---only dicamba	13	1.2(0.7-2.2)
---both	144	1.2(0.9-1.7)
2,4-D and cyanazine		
---Neither	58	1.0 (reference)
---only 2,4-D	104	0.9(0.6-1.2)
---only cyanazine	11	0.9(0.5-1.7)
---both	130	1.2(0.9-1.6)
2,4-D and terbufos		
---Neither	48	1.0 (reference)
---only 2,4-D	113	1.0(0.7-1.5)

---only terbufos	16	1.7(0.97-3.0)
---both	115	1.5(1.0-2.0)
Cyanazine and atrazine		
---Neither	72	1.0 (reference)
---only cyanazine	11	1.3(0.7-2.4)
---only atrazine	90	1.0(0.8-1.4)
---both	130	1.3(0.97-1.7)

[1]Age adjusted (<45,45-49,50-54,55-59,60-64,65-69,≥70)

Appendix 1.
Frequency of NHL in Agricultural Health Study applying New (InterLymp hierarchial classification of lymphoid neoplasms) and Older Definitions (ICD-O-3)

Lymphoma category and type (ICD-O-3 codes)[1]	Number NHL cases, new definition (InterLymph hierarchical classification)[1]	Number cases NHL, older definition (ICD-O-3)[2]	SEER Recode[1]
CLL/SLL/PLL/MCL (Mature NHL, B-cell)			
Small lymphocytic lymphoma (9670)	27	27	08
Chronic lymphocytic leukemia/small lymphocytic lymphoma (9823)	74	0	08
Mantle -cell lymphoma (9673)	16	16	10
Diffuse Large B-cell Lymphoma (Mature NHL, B-cell)			
DLBCL (9680)	94	94	13
Follicular Lymphoma (Mature NHL, B-cell)			
Follicular lymphoma (9690, 9691,9695,9698)	53	53	21
Other B-cell Types			
Precursor acute lymphoblastic leukemia/lymphoma (9835(B), 9836)	4	0	07
Waldenstrom macroglobulinemia (9761)	2	0	12
Lymphoplasmacytic lymphoma (9671)	2	2	11
Hairy-cell leukemia (9940)	6	0	22
NHL, NOS (9591(B), 9675(B))	6	6	26
Burkitt lymphoma/leukemia (9687)	1	1	17
Extranodal marginal zone lymphoma (MZL), Malt type & Nodal MZL (9699)	13	13	19, 20
Plasma cell neoplasms			
Plasmacytoma (9734, 9731)	6	0	23
Multiple myeloma (9732)	77	0	24
Other NHL Types			
Precursor acute lymphoblastic leukemia/lymphoma (9835(T), 9837)	1	0	27
Mycosis fungoides (9700)	6	6	28
Peripheral T-cell lymphoma, NOS (9702)	2	2	30
Anaplastic large cell lymphoma, T or null cell (9714)	2	2	33
Enteropathy type T-cell lymphoma (9717)	1	1	35
Primary cutaneous anaplastic large cell lymphoma (9718)	1	1	37
T-cell lymph, nasal-type/aggressive NK leukemia (9719)	1	1	39
NHL, NOS (9591(T))	1	1	42
Lymphoid leukemia, NOS (9820(U))	1	0	
Precursor acute lymphoblastic leukemia/lymphoma (9727(U), 9835(U))	3	1	43
NHL, NOS (9591(U), 9675(U))	6	6	45
Lymphoid neoplasm, NOS (9590(U))	10	10	47
Total	416	243	

Lineage: B=B-cell, T=T-cell, U=Unknown
[1] http://seer.cancer.gov/lymphomarecode based on Morton LM et al. Blood, 2007;110:695-708.
[2] Percy C. et al., Lyon, France: IARC Press: 2001.

Comment [CL76]: This was originally coded as 9713, which is an ICD-O-2 code, which becomes 9719 in ICD-O-3. Since we are presenting ICD-O-3 codes in this table, I have changed this code to 9719.

Comment [CL77]: Since IA and NC cancer registries are not yet using 2008 WHO codes, the reference for this table should be the Morton LM et al. publication noted here. This reference should also be noted in the text. Reference to the 2010 blood paper should not be noted in regard to the NHL classification used in this paper.

Appendix 2. Pesticide Classification by Chemical/Functional Class	
Chemical/functional class	**Pesticide**
Acetamide herbicide	Metolachlor, alachlor
Carbamate herbicde	Butlylate, EPTC
Other herbicides	Chloromuron ethyl, 2,4-D, dicamba, glyphosate, herbicide oil, imazethapyr. Paraquat, pendimethalin, 2,4,5-T, 2,4,5TP, trifluralin
Triazine/triazinone herbicides	Atrazine, cyanazine, metribuzin
Carbamate insecticides	Carbofuran, aldicarb, carbaryl
Chlorinated insecticides	Aldrin, chlordane, DDT, dieldrin, heptachlor, lindane, toxaphine
Organophosphate insecticides	Chlorpyrifos, coumaphos, diazinon, dichlorvos, fonofos, malathion, parathion, phorate, terbufos
Other insecticides	Permethrin (crops & animals), trichlorfon
Fungicides	Benomyl, chlorthalonil, captan, maneb/mancozeb, methylaxyl, ziram
Fumigants	Methyl bromide, aluminum phosphate, ethylene dibromide, carbon tetra chloride/carbondisulfide

Supplemental table 7: Pesticide exposures (total days and intensity weight total days) age- adjusted risks of NHL incidence (1993 through 2008)[old nhl definition; n=243].

	NHL Cases	RR[1] (95%) by Total Days of Exposure	NHL Cases	RR[1] (95% CI) Intensity-weighted days of exposure
Insecticides, Fungicides and Fumigants				
		P trend=		
Carbaryl (carbamate-insecticide)				
None	56	1.0 (ref)	56	1.0 (ref)
Low	19	0.8 (0.5-1.3)	19	0.9(0.6-1.6)
Medium	20	0.9(0.5-1.5)	20	0.7(0.4-1.2)
High	18	1.1(0.6-1.8)	18	1.2(0.7-2.0)
		P trend=0.64		P trend=0.42
Carbofuran (carbamate-insecticide)				
None	140	1.0 (ref)	140	1.0 (ref)

Low	26	1.2(0.8-1.8)	22	1.0(0.7-1.7)
Medium	18	1.1 (0.7-1.7)	21	1.0 (0.6-1.6)
High	21	1.1(0.7-1.7)	21	1.3(0.8-2.0)
		P trend=0.70		P trend=0.37
Chlorpyrifos (organophosphate-insecticide)				
None	134	1.0 (ref)	134	1.0 (ref)
Low	33	1.2(0.8-1.8)	30	1.2(0.8-1.8)
Medium	33	1.2(0.8-1.8)	30	0.9 (0.6-1.3)
High	32	0.9(0.6-1.3)	29	1.2 (0.8-1.7)
		P trend=0.50		P trend=0.56
Coumaphos				
None	186	1.0(ref)	186	1.0 (ref)
Low	9	1.3(0.7-2.5)	7	1.6(0.7-3.3)
Medium	7	1.1(0.5-2.3)	8	1.1(0.5-2.2)
High	5	1.4(0.6-3.4)	6	1.2(0.5-2.7)
		P trend=0.45		P trend=0.65
Diazinon (organophosphosphorous-insecticide)				
None	80	1.0 (ref)	80	1.0 (ref)
Low	12	1.0(0.6-1.9)	10	1.0(0.5-2.0)
Medium	8	0.9(0.4-1.9)	10	1.1(0.6-2.1)
High	9	1.2(0.6-2.4)	9	1.1(0.5-2.1)
		P trend=0.66		P trend=0.82
DDVP				
None	190	1.0(ref)	190	1.0 (ref)
Low	6	1.0(0.4-2.1)	6	1.1 (0.5-2.5)
Medium	6	0.9(0.4-2.0)	6	0.6(0.3-1.3)

High	5	0.6(0.3-1.6)	5	1.0(0.4-2.4)
		P trend=0.30		P trend=0.99
Fonofos				
None	163	1.0(ref)	163	1.0 (ref)
Low	18	1.1(0.7-1.8)	15	1.3(0.8-2.2)
Medium	13	1.1(0.6-2.0)	15	1.3(0.8-2.2)
Low	13	0.9(0.5-1.5)	14	0.7(0.4-1.2)
		P trend=0.		P trend=0.19
Malathion (organophosphorous-insecticide)				
None	39	1.0 (ref)	39	1.0 (ref)
Low	32	1.0(0.6-1.6)	26	1.1(0.7-1.8)
Medium	23	0.8(0.5-1.3)	27	0.7(0.4-1.2)
High	23	1.0 (0.6-1.7)	25	1.0(0.6-1.7)
		P trend=0.70		P trend=0.79
Metalaxyl				
None	91	1.0 (ref)	91	1.0 (ref)
Low	12	1.0 (0.5-1.8)	7	0.8(0.4-1.7)
Medium	3	0.7 (0.2-2.1)	7	1.1(0.5-2.4)
High	5	0.8 (0.3-2.0)	6	0.8(0.3-1.7)
		P trend=0.56		P trend=0.62
Methylbromide				
None	189	1.0 (ref)	189	1.0 (ref)
Low	16	2.7(1.6-4.5)	15	2.6 (1.6-4.5)
Medium	13	1.3(0.7-2.2)	13	1.5(0.8-2.6)
High	13	0.7(0.4-1.2)	13	0.6(0.4-1.1)
		P trend=0.24		P trend=0.07
Permethrin Animals				

(pyrethroid-insecticide)				
None	189	1.0 (ref)	189	1.0 (ref)
Low	9	1.1(0.6-2.2)	7	1.3(0.6-2.8)
Medium	5	0.9(0.4-2.1)	7	0.7(0.3-1.6)
High	6	0.7(0.3-1.5)	6	0.7(0.3-1.7)
		P trend= 0.27		P trend=0.04
Phorate (organophosphate-insecticide)				
None	72	1.0 (ref)	72	1.0 (ref)
low	15	1.0(0.6-1.8)	12	1.3(0.7-2.5)
medium	15	2.3(1.3-4.1)	12	1.2(0.7-2.3)
high	5	0.5(0.2-1.2)	11	0.9(0.5-1.6)
		P for trend=0.53		P for trend=00.86.
Terbufos (organophosphorous-insecticide)				
None	114	1.0 (ref)	114	1.0 (ref)
Low	40	1.4(0.94-1.9)	31-	1.3(0.9-1.9)
Medium	26	1.9(1.2-2.8)	31	1.7(1.2-2.6)
High	26	1.2(0.8-1.9)	30	1.3(0.9-2.0)
		P trend=0.24		P trend=0.16
Chlorinated insecticides				
Aldrin				
None	86	1.0 (ref)	86	1.0 (ref)
Low	9	0.8(0.4-1.6)	9	1.0(0.5-1.9)
Medium	8	0.7(0.4-1.5)	7	0.7(0.3-1.5)
High	6	2.4(1.0-5.4)	7	1.3(0.6-2.9)
		P trend=0.21		P trend=0.86
Chlordane				

None	78	1.0 (ref)	78	1.0 (ref)
Low	10	1.2(0.7-2.0)	10	1.5(0.8-2.9)
Medium	8	1.3(0.7-2.4)	9	1.0(0.4-2.3)
High	10	1.0(0.9-1.1)	9	1.1(0.6-2.1)
		P trend=0.89		P trend=0.77
DDT				
None	71	1.0 (ref)	71	1.0 (ref)
Low	14	0.9(0.5-1.7)	13	1.1(0.6-2.2)
Medium	12	1.4(0.7-2.6)	12	1.0(0.5-1.8)
High	11	1.1(0.6-2.2)	12	1.3(0.7-2.4)
		P trend=0.61		P trend=0.47
Dieldrin				
None	101	1.0 (ref)	101	1.0 (ref)
Low	3	0.9(0.3-2.9)	3	1.9(0.6-5.9)
Medium	3	2.9(0.9-9.2)	2	1.3(0.3-5.2)
High	1	1.1(0.1-7.7)	2	0.9(0.2-3.8)
		P trend=0.47		P trend=0.97
Heptachlor				
None	88	1.0 (ref)	88	1.0 (ref)
Low	8	0.9(0.7-2.6)	7	1.2(0.6-2.4)
Medium	8	1.4(0.7-2.6)	8	1.7(0.7-3.8)
High	5	1.1(0.6-2.2)	6	1.4(0.6-3.3)
		P trend=0.26		P trend=0.42
Lindane				
None	86	1.0 (ref)	86	1.0 (ref)
Low	7	1.0(0.5-2.1)	7	1.1(0.5-2.3)
Medium	8	1.2(0.6-2.4)	7	1.0(0.5-2.2)
High	6	3.7(1.6-8.4)	6	2.8(1.2-6.4)

		P trend=0.0.01		P trend=0.04
Toxaphene				
None	90	1.0 (ref)	90	1.0 (ref)
Low	8	1.2(0.6-2.5)	6	1.6(0.7-3.5)
Medium	4	4.4(1.6-12.1	7	1.3(0.6-3.0)
High	6	0.9(0.4-2.0)	5	0.9(0.4-2.3)
		P trend=0.66		P trend=0.83
Herbicides				
Alachlor (acetamide-herbicide)				
None	96	1.0 (ref)	96	1.0 (ref)
Low	39	1.1(0.8-1.6)	38	1.1(0.7-1.6)
Medium	45	0.9(0.6-1.2)	40	0.8 (0.6-1.2)
High	31	1.4(0.9-2.0)	36	1.4(0.96-2.1)
		P trend=0.22		P trend=0.09
Atrazine (triazine-herbicide)				
None	59	1.0 (ref)	59	1.0 (ref)
Low	64	1.1(0.8-1.6)	58	1.1(0.8-1.6)
Medium	56	1.3(0.9-1.9)	59	1.2(0.9-1.8)
High	55	1.2(0.8-1.7)	57	1.3(0.9-1.8)
		P trend=0.52		P trend=0.27
Butylate (thiocarbamate-herbicide)				
None	75	1.0 (ref)	75	1.0 (ref)
Low	14	0.9 (0.5-1.6)	12	0.9(0.5-1.6)
Medium	15	3.4(1.9-5.9)	11	2.7(1.4-5.0)
High	5	1.1(0.4-2.7)	11	1.6(0.9-3.0)

		P trend=0.005		P trend=0.049
Chlorimuron-ethyl (benzoic acid ester-herbicide)				
None	75	1.0 (ref)	75	1.0 (ref)
low	20	1.1(0.7-1.9)	13	1.1(0.6-2.0)
medium	11	1.5(0.8-2.9)	12	1.3(0.7-2.4))
high	6	0.7(0.3-1.7)	12	1.0(0.5-1.9)
		P for trend=0.73		P for trend=0.94
Cyanazine (triazine-herbicide)				
None	114	1.0 (ref)	114	1.0 (ref)
Low	41	1.4(0.95-1.9))	33	1.2(0.8-1.7)
Medium	32	1.3(0.9-1.9)	32	1.3(0.9-1.9)
High	25	1.1(0.7-1.6)	32	1.2(0.8-1.8)
		P for trend=0.0.89		P for trend=0.34
Dicamba (benzoic-herbicide)				
None	92	1.0 (ref)	92	1.0 (ref)
Low	39	1.5(1.0-2.2)	38	1.2(0.8-1.8)
Medium	38	1.2(0.8-1.8)	39	1.4(0.9-2.0)
High	38	1.0(0.7-1.5)	37	1.0(0.7-1.5)
		P trend=0.64		P trend=0.95
2,4-D (phenoxy-herbicide)				
None	53	1.0 (ref)	53	1.0 (ref)
Low	60	0.9(0.6-1.3)	59	0.9(0.6-1.4)
Medium	59	1.0(0.7-1.5)	60	1.0(0.7-1.4)
High	59	0.9(0.6-1.3)	58	0.9(0.6-1.3)

		P trend=0.61		P trend=0.69
EPTC (thiocarbamate-herbicide)				
None	164	1.0 (ref)	164	1.0 (ref)
Low	21	1.3(0.9-2.1)	15	1.4(0.8-2.4)
Medium	9	1.1(0.6-2.2)	12	1.1(0.6-2.0)
High	10	0.8(0.4-1.5)	13	0.8(0.5-1.5)
		P trend=0.39		P trend=0.61
Glyphosate (phosphinic acid-herbicide)				
None	48	1.0 (ref)	48	1.0 (ref)
Low	72	1.0(0.7-1.4)	61	1.1(0.7-1.6)
Medium	51	0.7(0.5-1.0)	61	0.7(0.5-1.0)
High	60	1.0(0.7-1.4)	60	0.9(0.6-1.4)
		P trend=0.79		P trend=0.0.99
Herbicide Oil				
None	84	1.0 (ref)	84	1.0 (ref)
Low	9	1.0(0.5-1.9)	9	1.2(0.6-2.4)
Medium	10	1.8(0.95-3.6)	10	1.1(0.6-2.1)
High	8	1.1(0.6-2.6)	8	1.5(0.7-3.1)
		P trend=0.62		P trend=0.29
Imazethapyr (imidazolinone-herbicide)				
None	132	1.0 (ref)	132	1.0 (ref)
Low	30	0.9(0.6-1.3)	25	1.0(0.6-1.5)
Medium	20	0.8(0.5-1.2)	25	0.8(0.5-1.3)
High	24	0.9(0.6-1.4)	24	0.8(0.5-1.2)
		P trend=0.50		P trend=0.64

Metolachlor				
None	101	1.0 (ref)	101	1.0(ref)
Low	36	1.2(0.8-1.8)	35	1.1(0.8-1.7)
Medium	36	1.3(0.9-1.9)	36	1.4(0.9-2.0)
High	34	1.1(0.7-1.6)	34	1.1(0.8-1.6)
		P trend=0.73		P trend=0.71
Metribuzin (triazine-herbicide)				
None	70	1.0 (ref)	70	1.0 (ref)
Low	15	0.8 (0.5-1.5)	14	0.9(0.5-1.6)
Medium	20	1.2(0.7-2.0)	14	1.1(0.6-2.0)
High	6	1.1 (0.5-2.5)	13	1.2(0.6-2.1)
		P trend=0.0.59		P trend=0.55
Paraquat				
None	88	1.0 (ref)	88	1.0(ref)
Low	8	2.1(1.0-4.3)	8	4.8(2.3-9.9)
Medium	8	0.8(0.4-1.7)	7	0.7(0.3-1.5)
High	6	1.0(0.4-2.3)	7	0.9(0.4-2.0)
		P trend=0.91		P trend=0.73
Pendimethalin				
None	63	1.0 (ref)	63	1.0(ref)
Low	22	1.3(0.8-2.0)	19	1.5(0.9-2.5)
Medium	17	1.3(0.8-2.3)	19	1.0(0.6-1.7)
High	17	1.1(0.6-1.9)	18	1.3(0.8-2.2)
		P trend=0.68		Ptrend=0.43
Permethrin (Crop)				
None	179	1.0 (ref)	179	1.0 (ref)
Low	12	1.0(0.6-1.9)	9	1.4(0.7-2.7)

Medium	6	2.2(1.0-5.1)	9	1.2(0.6-2.4)
High	8	0.6(0.3-1.2)	8	0.6(0.3-1.2)
		P trend=0.18		P trend=0.15
Trifluralin (dinitroaniline-herbicide)				
None	104	1.0 (ref)	104	1.0 (ref)
Low	39	1.0 (0.7-1.5)	37	1.0(0.7-1.4)
Medium	40	1.0(0.7-1.4)	36	1.0(0.7-1.4)
High	29	0.8(0.6-1.3)	34	0.9(0.6-1.3)
		P trend=0.0.36		P trend=0.44
2,4,5 T (phenoxyacetic acid)				
None	73	1.0 (ref)	73	1.0 (ref)
low	22	1.9(1.2-3.1)	13	2.0(1.1-3.6)
medium	3	1.3(0.4-4.3)	12	1.8(0.99-3.4)
high	12	1.5(0.8-4.3)	12	1.4(0.7-2.5)
		P for trend=0.0.27		P for trend=0.94

Carbofuran								
None	1.0(ref)	67	1.0(ref)	58	1.0(ref)	33	1.0(ref)	19
Low	1.4 (0.8-2.5)	15	0.9 (0.4-1.9)	8	0.96(0.4-2.5)	5	1.0(04-2.7)	5
Medium	1.2 (0.6-2.4)	10	0.9 (0.4-1.8)	9	1.6(0.7-3.9)	6	1.4(0.2-10.7)	1
High	1.3 (0.7-2.4)	12	1.1 (0.5-2.9)	5	0.6(0.2-2.0)	3	0.94(0.2-4.1)	2
	P trend=0.36		P trend=0.81		P trend=0.79		P trend=0.99	
Chlorpyrifos								
None	1.0 (ref)	69	1.0 (ref)	55	1.0 (ref)	26	1.0 (ref)	18
Low	0.9(0.5-1.7)	15	1.2(0.6-2.1)	13	1.4(0.7-3.1)	10	0.9(0.3-2.6)	5
Medium	1.1(0.7-2.0)	16	1.0(0.5-1.7)	15	1.2(0.5-2.9)	7	4.2(1.7-10.6)	6
High	1.0(0.5-1.7)	14	0.9(0.6-4.0)	7	1.4(0.6-3.4)	6	0.8(0.3-2.3)	4
	P trend=0.99		P trend=0.66		P trend=0.56		P trend=0.97	
Diazinon								
None	1.0 (ref)	40	1.0 (ref)	33	1.0 (ref)	13	1.0 (ref)	12
Low	1.5(0.7-3.1)	9	1.2(0.4-3.1)	5	1.6(0.4-5.5)	3	xxx	2
Medium	1.2(0.4-3.6)	5	0.9(0.3-2.8)	4	1.6(0.4-7.4)	3	xxx-	1
High	1.2(0.5-3.0)	5	1.2(0.4-3.8)	3	2.0(0.4-10.0)	2	xxx	0
	P trend=0.72		P trend=0.84		P trend=0.35		P trend=xxx	
Permethrin animals								
None	1.0 (ref)	95	1.0 (ref)	78	1.0 (ref)	38	1.0 (ref)	25
Low	1.3(0.5-3.3)	5	Xxx	1	2.8(1.1-7.0)	5	xxx-	1
Medium	0.9(0.2-3.7)	3	xxx	1	2.9(0.7-12.0)	2	-xxx	2
High	0.8(0.3-2.5)	3	-xxx	0	0.8(0.2-3.5)	2	-xxx	0
	P trend=0.75		P trend=xxx		P trend=0.93		P trend=xxx	
Cyanazine								

(triazine)								
None	1.0 (ref)	65	1.0 (ref)	46	1.0 (ref)	24	1.0 (ref)	10
Low	1.2 (0.7-2.2)	15	1.4 (0.8-2.4)	16	1.9(0.9-3.8)	12	3.7(1.4-9.7)	7
Medium	0.9 (0.5-1.6)	16	0.8 (0.4-1.8)	8	1.7(0.8-3.6)	9	2.9 (1.5-7.5)	8
High	1.1(0.6-2.0)	14	1.0 (0.5-2.1)	8	0.8(0.3-2.2)	4	2.6(0.9-7.5)	5
	P trend=0.93		P trend=0.93		P trend=0.87		P trend=0.17	

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Alavanja MCR, Mage D, Rothman N, Rowland A, Sandler D, Blair A. A quantitative approach for estimating exposure to pesticides in the Agricultural Health Study. The Annals of occupational Hygiene 2002; 46:245-260.

42. Turner JJ, Morton LM, Linet MS, Clarke CA, Kadin ME, Vajdic CM, Monnereau A, Maynadie M, Chiu B C,H, Marcos-Gragera R, Constantini AS, Cerhan JR, Weisenberger DD. InterLymph hierarchical classification of lymphoid neoplasms for epidemiologic research based on the WHO classification (2008): update and future directions. Blood, 18 November 2010;116(20):e90-e98.

Monsanto billing: Q1-2017

1/10/2017: 2 hours reviewing papers

1/14/2017: 2 hours reviewing human papers

1/21/2017: 2 hours review data of animal studies

1/25/2017: 1 hour rodent data

2/5/2017: 6 hours reviewing data and starting to draft the written report

2/6/2017: 2 hours reviewing Pottier, IARC, and BfR

2/7/2017: 1 hour

2/8/2017: 1 hour

2/9/2017: 1 hour

2/13/2017: 3 hours

2/15/2017: 3 hours

2/16/2017: 2 hours

2/19/2017: 3 hours

3/1/2017: 1 hour literature search

3/2/2017: 2 hours reviewing depositions: Dr. Saltmiras

3/5/2017: 4 hours: completing the deposition

3/8/2016: 1 hour

3/12/2017: 3 hours: reviewing depositions plus the oxidative stress and working on the written report

3/13/2017: 1 hour: reviewing oxidative stress relevant data

3/18/2017: 4 hours: reviewing Dr. Heydens deposition

3/22/2017: 1 hour: reviewing data on glyphosate absorption and amending the report

Total: **46 hours** (January, February, and March)

Total due: **$25,300.00**

Please make check payable to:

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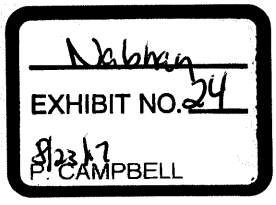


Timothy Litzenburg

From: Nabhan, Chadi <[redacted]
Sent: Saturday, July 1, 2017 10:43 PM
To: Timothy Litzenburg
Subject: Invoice for Q2 2017 Monsanto

Hi Tim,

Below is my invoice for the work done regarding the Monsanto case for Q2-2017 (April, May, and June 2017):

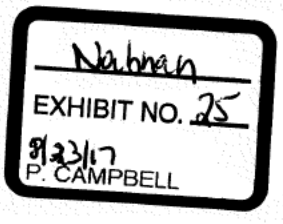
Sunday 4/2/2017:	1.0 hour:	Review depositions
Saturday 4/8/2017:	2.0 hours	Review depositions
Sunday 4/9/2017:	7.0 hours:	Initiating the draft report and continuing to review depositions
Monday 4/17/2017:	2.0 hours:	Literature review
Tuesday 4/18/2017:	0.5 hour:	Phone call with Tim Litzenburg
Wednesday 4/19/2017:	3.0 hours:	Working on the draft report
Thursday 4/20/2017:	3.0 hours:	Reviewing depositions and oxidative stress literature data
Friday 4/21/2017:	2.0 hours:	Reviewing genotoxicity data
Sunday 4/23/2017:	6.0 hours:	Editing and drafting the expert witness report
Friday 4/28/2017:	2.0 hours	Continuing to work on the report
Saturday 4/29/2017:	1.0 hour	Review data
Monday 5/15/2017:	1.0 hour:	Review additional information provided
Wednesday 5/17/2017:	5.0 hours	Finalizing the draft report of the expert witness
Sunday 5/28/2017:	5.0 hours:	Review the records and case of Nicholas Sharp (patient) as well as literature pertaining to this case
Sunday 6/18/2017:	5.0 hours:	Review the records of Dewayne (Lee) Johnson as well as literature pertaining to his case
Friday 6/23/2017:	2.0 hours:	Review additional records, draft from attorneys, revise, and sign the document for Mr. Lee Johnson; review data and literature specific to CTCL and large cell transformation
Wednesday 6/28/2017:	2.0 hours:	In-person meeting with Tim Litzenburg, Esq.

Total Hours spent:	49.5 hours
Rate:	$550.0/hour
Total due:	$27,225.00

Please send check to:

974 Bristol Drive, Deerfield, IL 60015

Thanks again and looking forward to continuing the work.
Chadi



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