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Review

The use of the lymphocyte cytokinesis-block micronucleus assay for monitoring pesticide-exposed populations

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ABSTRACT

Pesticides are widely used around the world, and hundreds of millions of people are exposed annually in occupational and environmental settings. Numerous studies have demonstrated relationships between pesticide exposure and increased risk of cancers, neurodegenerative and neurodevelopmental disorders, respiratory diseases and diabetes. Assessment of genotoxicity of pesticides and biomonitoring their effect in exposed populations is critical for a better regulation and protection, but it can be complicated because pesticides are often used as complex mixtures. The cytokinesis-block micronucleus assay in human lymphocytes (L-CBMN) is a validated method of assessment of DNA damage induced by clastogenic and aneuploidogenic mechanisms. The goal of this review is to provide an updated summary of publications on biomonitoring studies using this assay in people exposed to pesticides in different settings, and to identify gaps in knowledge, and future directions. A literature search was conducted through MedLine/PubMed and TOXLINE electronic databases up to December 2015. A total of 55 full-text articles, related to 49 studies, excluding reviews, were selected for in depth analysis, divided by the settings where exposures occurred, such as chemical plant workers, pesticide sprayers, floriculturists, agricultural workers and non-occupationally exposed groups. Majority of studies (36 out of 49) reported positive findings with L-CBMN assay. However, most of the studies of professional applicators that used single pesticide or few compounds in the framework of specific programs did not show significant increases in MN frequency. A decreased level of pesticide-induced genotoxicity was associated with the proper use of personal protection. In contrast, subjects working in greenhouses or during intensive spraying season and having acute exposure, showed consistent increases in MN frequency. Overall, this analysis confirmed that L-CBMN is an excellent tool for pesticide biomonitoring, and can validate the effects of educational and intervention programs on reducing exposure and genetic damage.

Contents

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Table 3 (*Continued*)

Country Exposure	Number of participants: exposed and controls (sex and age)	Exposure period Employment time (years)	Exposure measurement (type and value) Use of personal protection equipment (PPE)	Number of cells evaluated in L-CBMN assay	QS	Results (MN‰; Exposed vs Control)		FR	Reference
	from the same region 56 (19m+37f)	Leaf harvest 29.96 ± 15.47					↑ 17.35 vs 5.91***		
						Leave harvesting L-CBMN	↑ 7.75‰ vs 5.22‰	1.5	
						Comet Damage index	↑ 23.85 vs 5.91***	4.03	
Brazil Tobacco farmers	Exposed 30 (17m + 13f) (42.10 ± 10.15) Controls: employees in the same factory 10 (m + f) (40.17 ± 13.02)	Off-season Pesticide application Leave harvesting 29.23 ± 12.83 y	Exposure to complex mixtures BChE in blood: no difference between controls and exposed subjects Cotinine in plasma Leaf harvesters: ↑ 40 ng/ml vs 2 ng/ml	2000 BN cells Giemsa Comet 100 cells Damage index (DI)	14/ 27	Off-season L-CBMN	↑ 9.54‰ vs 6.19‰***	1.54	Da Silva. [47]
						Comet Damage index	↑ 14.53 vs 8.07*	1.80	
						Pesticide application L-CBMN	↔ 8.88‰ vs 6.19‰	1.43	
						Comet Damage index	↑ 15.24 vs 8.07*	2.18	
						Leave harvesting L-CBMN	↔ 5.76‰ vs 6.19 ‰		
						Comet Damage index	↑ 17.59 vs 8.07***	2.2	
Agriculture									
Italy Agricultural workers	Exposed 48m (46.5 ± 12.60) Controls: healthy volunteers living in the same area 50m (43.7 ± 9.86)	18.35 ± 12.42	Exposure to complex mixture of pesticides. Insecticides, fungicides and herbicides	1000 BN cells Giemsa staining	14/ 27	L-CBMN	↑ 15.98‰ vs 13.30‰*	1.2	Pasquini et al. [48]
						SCE (30 cells)	↔ 6.47/ cell vs 6.33/cell		
						No Age effect			
Chile Agricultural workers	Exposed 22m (34 ± 1.84) Controls: healthy donors 16m (31 ± 2.11)	7 y	Use of mixture of agrochemical formulations (23 listed) Mostly used: Bromadialone, captan, deltamethrin, diazinon, dichlorvos, linuron, methamidophos	1000 BN cells Giemsa	13/ 27	L-CBMN No age effect	↔ 12.73‰ vs 10.69‰		Venegas et al. [49]
Turkey Agricultural workers	Exposed 34m + 12f (46) (42.5 ± 1.5) from 5 villages Controls: healthy volunteers living in the same area 26m + 22f (48)	Spraying pesticides manually in open field	Different mixtures of agrochemical formulations Use of PPE: 17%	1000 BN cells Giemsa	10/ 27	L-CBMN	↑ 8.47‰ vs 4.1‰*	2	Coskun et al. [50]
Other pesticide application									
India Cotton crops	Exposed 76m (37.8 ± 9.5) Controls: volunteers from the same district without occupational exposure 76m (37.3 ± 12.2)	5.5 ± 1.74 h/ day for 4 months/year 16.3 ± 8.4 y	Exposure to complex mixture of pesticides : organophosphates and organochlorines Presence of benzene hexachloride isomers in serum	1000 BN cells Giemsa	19/ 27	L-CBMN	↔ 1.3‰ vs 1.2‰		Jonnalagadda et al. [51]
						CA (100 M)	↑ 2.8‰ vs 0.72‰*	3.8	
Brazil Sanitation surveillance	Exposed 30m (30.3 ± 1.48) Controls: healthy volunteers living in the same area 30m (28.2 ± 1.64)	40 h/week 5.28 ± 0.6 y	Spraying, sand mixed granulated, powder, pelleting bait, paraffin bait Use of mixture of insecticides and rodenticides: pyrethroids, organophosphates Hydroxycoumarin indandione Use of PPE except for sand- mixed granulated	1000 BN cells Giemsa	12/ 27	L-CBMN Age effect	↑ 15.10‰ vs 4.62‰	3.3	Kehdy et al. [52]

Abbreviations: Males abbreviated as "m", females as "f", years of age indicated as mean or mean ± SD; BChE, butylcholinesterase; QS, quality score based on the following parameters: (i) age-matching, (ii) gender-matching, (iii) smoking status-matching, (iv) alcohol intake-matching, (v) nutritional intake- matching, (vi) appropriate measurement of chemical exposure (see Table 1) 27 is the perfect score; BN, binucleated lymphocytes; CA, chromosomal aberrations; M, metaphases; SCE, sister chromatid exchange; PON, paraoxonase OGG1-8-oxoguanine glycosylase; ↑, significant increase; ↓, significant decrease (number following arrow in brackets indicates mean ratio); ↔, no effect; FR, Frequency Ratio (MN frequency in exposed subjects/MN frequency in Controls); *$p < 0.005$; **$p < 0.001$; ***$p < 0.0001$; NS, not statistically significant).

Table 4
L-CBMN and other genotoxicity assay studies in pesticide exposed subjects: floriculturists.

Country Exposure	Number of participants: exposed and controls (sex and age)	Exposure period Employment time (years)	Exposure measurement (type and value) Use of personal protection equipment (PPE)	Number of cells evaluated in L-CBMN assay	QS	Results (MN‰; Exposed vs Control)		FR	Reference
Italy Greenhouse and open field floriculturists	Exposed 68m + 3f (71) 46.0 Controls: healthy blood donors living in the same area 67m + 8f (75) 43.3	8 h/day 2–55 y	Mixture of fungicides (e.g. dithiocarbamates) insecticides (e.g. organophosphates chlororganics, pyrethroids) herbicides Continuous changes of agrochemical formulations	2000 BN cells Giemsa staining	16/27	L-CBMN	↑ 8.57‰ vs 6.67‰*	1.29	Bolognesi et al. [53,54]
						Duration of exposure			
						1–18	↔ 5.64‰ vs 6.67‰		
						19–30	↑ 8.72‰ vs 6.67‰*		
						>30	↑ 11.45‰ vs 6.67‰*	1.71	
Italy Greenhouse and open field floriculturists	Exposed 92m + 15f (107) 49.44 ± 13.69 Controls: healthy blood donors living in the same area 42m + 19f (61) (49.59 ± 13.62)	Constant use and application of pesticides 2–70 y	Exposure to a mixture of more than 50 different agrochemical formulations Organophosphates and carbamates represent >67% Use of PPE (84.1%)	2000 BN cells Giemsa	14/27	L-CBMN	↑ 4.41‰ vs 3.04‰*	1.45	Bolognesi et al. [55,56]
						Age and gender effect			
						Duration of exposure			
						1–20	↔ 3.36‰ vs 3.04‰		
						>20	↑ 4.93‰ vs 3.04‰*	1.62	
						Conditions of exposure			
						Greenhouse (N.19) vs openfield (N.49)	↑ 5.27‰ vs 3.04‰n.s.	1.73	
Italy Greenhouse floriculturists	Exposed 24m + 19f (43) (42.4 ± 9.6/ 36.7 ± 8.3) Controls: bank clerks 22m + 20f (42) (42.4 ± 9.5/ 37.0 ± 8.4)	1 month following extensive pesticide exposure	100 active ingredients Use of PPE	2000 BN cells Giemsa	14/27	High exposure		NS	Scarpato et al. [57]
						L-CBMN	↔ 4.05‰ vs 3.88‰		
						SCE (25 cells)	↔ 9.05/cell vs 8.35/cell		
						CA (100M)	↔ 2.24% vs 1.88%		
						low exposure			
						L-CBMN	↔ 3.84‰ vs 3.88‰		
						SCE (25 cells)	↔ 8.53/cell vs 8.35/cell		
						CA (100M)	↔ 2.14% vs 1.88%		
Italy Greenhouse and open field floriculturists	Exposed 13m + 10f (23) 42.0 ± 9.5/ 34.7 ± 8.0 Controls: bank clerks 10m+12f (22) 40.5 ± 9.9/ 37.2 ± 8.9	Cut-off lower quartile for treatments/y × he sprayed/y of pesticide exposure High and low exposure Two samplings: 1. high exposure season 2. low exposure season	Different formulations mostly belonging to benzimidazoles, carbamates, diphenylethanoles, dithiocarbamates, organophosphates, thiophthalimides Use of PPE	2000 BN cells Giemsa	14/27	High exposure		NS	Scarpato et al. [58]
						L-CBMN	1. ↔ 4.4‰ vs 4.1‰ 2. ↔ 6.6‰ vs 7.4‰		
						SCE (25 cells)	1. ↔ 9.2/cell vs 8.8/cell 2. ↔ 8.1/cell vs 7.8/cell		
						CA (100 M)	1. ↔ 2.4 % vs 2.3% 2. ↔ 1.7% vs 1.3%		
						Low exposure			
						L-CBMN	1. ↔ 3.9‰ vs 4.1‰ 2. ↔ 7.9‰ vs 7.4‰		
						SCE (25 cells)	1. ↔ 8.8/cell vs 8.8/cell		

Table 4 (Continued)

Country Exposure	Number of participants: exposed and controls (sex and age)	Exposure period Employment time (years)	Exposure measurement (type and value) Use of personal protection equipment (PPE)	Number of cells evaluated in L-CBMN assay	QS	Results (MN‰: Exposed vs Control)		FR	Reference
						CA (00 M)	2. ↔ 8.2/ cell vs 7.8/cell 1. ↔ 2.2% vs 2.3% 2. ↔ 1.6% vs 1.6%		
Italy Greenhouse and open field floriculturists	Exposed 20m + 14f (34) 39.8 ± 8.6 Controls: bank clerks 17m + 16f (33) 38.9 ± 9.1	Cut-off lower quartile for treatments/y × he sprayed/y of pesticide exposure High and low exposure Sampling during extensive pesticide application	Different formulations. Active ingredients mostly applied: acephate, captan, dichlorvos, dimethoate, metiram Use of PPE	Anti-BrdU technique	14/ 27	L-CBMN High exposure L-CBMN Low exposure L-CBMN	↔ 7.3‰ vs 6.8‰ ↑ 8.3‰ vs 6.8‰ ↔ 6.3‰ vs 6.8‰	1.66	Falck et al. [59]

Abbreviations: Males abbreviated as "m", females as "f", years of age indicated as mean or mean ± SD; Anti-BrdU, anti-bromodeoxyuridine; QS, quality score based on the following parameters: (i) age-matching, (ii) gender-matching, (iii) smoking status-matching, (iv) alcohol intake-matching, (v) nutritional intake- matching, (vi) appropriate measurement of chemical exposure (see Table 1) 27 is the perfect score; BN, binucleated lymphocytes; CA, chromosomal aberrations; M, metaphases; SCE, sister chromatid exchange; ↑, significant increase; ↓, significant decrease (number following arrow in brackets indicates mean ratio); ↔, no effect; *p < 0.005; **p < 0.001; ***p < 0.0001; NS, not statistically significant; FR, Frequency Ratio (MN frequency in exposed subjects/MN frequency in Controls).

employment were observed. The serum concentrations of HCB measured as a marker of exposure showed a correlation with years of employment, but not with the MN frequency, possibly due to the complex exposure of the workers at this plant to known and unknown compounds.

Two studies were carried out in Croatia in workers simultaneously exposed to a complex mixture of pesticide formulations containing 2,4-D, atrazine, alachlor, cyanazine and malathion during the process of production [29,30]. A significant increase in the MN frequency in exposed subjects (N = 20) compared with controls (N = 20) was observed after a 8-month period of high exposure (FR = 3.6) followed by a decrease (FR = 1.9) after 8 months with no exposure. A greater increase (FR = 7.1) was reported in a subgroup working in the unit of pesticide synthesis and in the production of concentrated emulsion, liquid and powder pesticides [30]. Parallel analyses of chromosomal aberrations (CA), sister chromatid exchanges (SCE), and DNA damage (using the Comet assay) corroborated the MN findings.

The L-CBMN assay applied to a group of workers (N = 29) at a pesticide company in Pakistan that was involved in a year round production of organophosphates and pyrethroids detected a significant increase (FR = 2.06) in MN in comparison with control group (N = 35). This increase was associated with elevated levels of the hepatic enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), and decreases in serum cholinesterase (SChE) [31].

The L-CBMN cytome approach associated with FISH analysis was applied in peripheral lymphocytes of a group of workers (N = 30) continuously exposed for at least 4 months to carbofuran in a production plant in Croatia [32]. An increased frequency of MN, nuclear buds, and nucleoplasmic bridges, and higher percentage of MN with centromeres was observed compared with a matched control population (N = 30), and it was associated with the duration of the exposure. However, a parallel analysis of DNA damage by the comet assay did not reveal any difference between exposed and control groups.

Overall, all five studies of chemical plant workers occupationally exposed to pesticides demonstrated a significantly elevated MN frequency in comparison to matched controls.

3.2. L-CBMN in pesticide sprayers

3.2.1. Application of a single compound or simple formulations of pesticides

Seven studies are available on the application of L-CBMN in sprayers using only one or a few pesticides (Table 2).

A biomonitoring study using the L-CBMN assay was carried out on 31 fumigators of commercial grain stores in Australia to evaluate the potential genotoxic risk associated with exposure to phosphine [33]. No significant urinary mutagenicity was observed consistent with likely low levels of pesticide exposure in this occupational setting.

Two studies were conducted in California (USA) with workers involved in the Mediterranean Fruit Fly Eradication Program. In 38 intermittently malathion-exposed sprayers, no increase in the frequency of MN in peripheral blood lymphocytes was detected in comparison with the control group (N = 16) [34]. In a second study, involving small groups of applicators (14 workers vs 4 controls) a non-significant increase in MN frequency was observed in workers exposed to malathion for more than 50 h during the previous 8 months, or with levels of malathion diacid over 100 ppb [35]. These results are consistent with the evidence from the *in vitro* studies in human lymphocytes that showed an elevated MN frequency at cytotoxic concentrations of malathion [34], and also with significant increases in the frequency of chromatid breaks and stable chromosome-type aberrations found in persons suffering from acute malathion toxicity [41].

A study carried out in the USA in male fumigators (N = 31) intermittently exposed to methyl bromide reported a 65% increase in the MN frequency in lymphocytes as well in oropharyngeal cells [36]. However, these differences were not statistically significant.

No increase in MN frequency was observed between a small group of sprayers from eastern Kansas, USA (N = 12) exposed to the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) and a control group (N = 9) before and after the spraying period. A parallel analysis revealed a statistically significant increase of the lymphocyte replicative index (RI) associated with the exposure to 2,4-D [37,38].

A small study on 11 sprayers in tobacco fields in western Greece who used metalaxyl as fungicide and imidacloprid as insecticide,

Table 5
L-CBMN and other genotoxicity assay studies in pesticide exposed subjects: agricultural workers.

Country Exposure	Number of participants: exposed and controls (sex and age)	Exposure period Employment time (years)	Exposure measurement (type and value) Use of personal protection equipment (PPE)	Number of cells evaluated in L-CBMN assay	QS	Results (MN‰; Exposed vs Control)		FR	Reference
Italy Horticulture Greenhouse and open field	Exposed 46m + 17f (62) 47.6 y Controls: 21m + 8f (29) 43.6 y	Constant use of pesticides 2–52 y	Mixture of fungicides (e.g. dithiocarbamates), insecticides (e.g. organophosphates chlororganics, pyrethroids) and herbicides	2000 BN cells Giemsa staining	15/27	L-CBMN	↔ 5.45‰ vs 4.49‰	NS	Bolognesi et al. [60]
Spain Greenhouse agriculture	Exposed 64m (32.83 ± 1.14 y) Controls: healthy subjects from the same area 50m (38.56 ± 1.37)	Constant use of pesticides 9.82 ± 1.01 y	Exposure to pesticide mixtures: insecticides, fungicides (26 formulations listed) Mostly applied: abamectin, endosulfan, imidacloprid, metamidophos, methomyl Use of PPE: 80%	1000 BN cells Giemsa	15/27	L-CBMN BCMN Polymorphisms No effect of GSTT1 and GSTM1 genotype	↔ 8.72‰ vs 7.32‰ ↔ 1.83‰ vs 1.77‰	NS	Lucero et al. [61]
Greece Cultivation of ornamental plants and vegetables in greenhouses	Exposed 30m + 20f (50) (42.98 ± 1.60) Controls: clerical workers 41m + 25f (66) (43.94 ± 1.11)	Last pesticide application: 7.29 ± 1.05 days ago Average pesticide application: 2.45hrs/week 8.62 ± 1.13 y	Exposure to pesticide mixtures: insecticides, fungicides (21 formulations listed) Mostly applied: endosulfan, imidacloprid, metamidophos, methomyl, mancozeb Use of PPE: 62%	1000 BN cells Giemsa	15/27	L-CBMN BCMN Effect of age and gender	↔ 11.12‰ vs 14.42‰ ↔ 1.45‰ vs 1.73‰	NS	Pastor et al. [62]
Poland Cultivation of ornamental plants and vegetables in greenhouses and open field	Exposed 50m (39.14 ± 1.4) Controls: healthy subjects from the same area 49m (39.53 ± 1.56)	Last pesticide application: 24days Average pesticide application: 0.88 h/week 16.28 ± 1.1 y	Exposure to pesticide mixtures: insecticides, fungicides (21 formularions listed) Mostly applied: deltamethrin dimethoate, methomyl, carbosulfan Exposed also to dust, paints, solvents Use of PPE: 78%	1000 BN cells Giemsa	13/27	L-CBMN BCMN	↔ 18.28 ‰ vs 17.67‰ ↔ 1.59‰ vs 1.92‰	NS	Pastor et al. [63]
Spain Agriculture exclusively in greenhouses	Exposed 39m (31 ± 1.37) Controls: healthy subjects from the same area 22m (37.55 ± 2.18)	Constant use of pesticides 8.31 ± 1.12 y	Exposure to pesticide mixtures: insecticides, fungicides (26 formulations listed) Mostly applied: abamectin, endosulfan, imidacloprid, metamidophos, methomyl	1000 BN cells Giemsa	13/27	Sample A: high exposure (March-April) L-CBMN Sample B. low exposure (Nov–Dec) L-CBMN	↔ 8.49‰ vs 8.45‰ ↔ 7.69‰ vs 11.59‰	NS	Pastor et al. [64]
Hungary Cultivation of vegetables in greenhouses and open field	Exposed 58m + 26f (84) (41.98 ± 0.73) Controls: healthy subjects from the same area 53m 12f (65) (45.05 ± 0.96)	Constant use of pesticides 18.75 ± 0.89 y	Exposure to complex pesticide mixtures, including insecticides, fungicides and herbicides Highly exposed group (more than 4 symptoms and depressed cholinesterase) 19m Moderately exposed group: 31m ± 26f (57) Use of PPE: 85%	1000 BN cells Giemsa	14/27	L-CBMN BCMN Highly exposed vs moderately exposed L-CBMN BCMN Effect of age	↔ 9.30‰ vs 9.15‰ ↔ 1.62‰ vs 1.98‰ ↔ 10.58‰ vs 8.88‰ ↔ 1.94‰ vs 1.52‰	NS	Pastor et al. [65]
USA Farm workers	Exposed 12m+3f (15) (57 ± 2.9) Controls: urban participants 6m + 4f (10) (46 ± 1.6)	During the growing season (Jun–Nov) 18.2 ± 1.3 y	Complex mixture (endosulfan, chlorpyriphos, dimethoate, diazinon, maleic hydrazide) Use of PPE: 18%	1000 BNcells Giemsa	9/27	L-CBMN	↑ 8.1‰ vs 4.6‰	1.7	Tope et al. [66]
Mexico Agricultural workers	Exposed 15m + 22f (37) Controls (21) 9m (26 ± 5.2) 12f (29.8 ± 10.3)	Agriculture	Exposure to complex mixture of pesticides and solvents	2000 BN cells Giemsa	11/27	L-CBMN Age and BMI effect Polymorphisms GSTT1− vs GSTT1+ GSTM1− vs GSTM1+	↑ 1.91‰ vs 0.97‰* ↑ 2.62‰ vs 1.64‰ ↔ 1.87‰ vs 1.65‰	1.9 1.6	Montero et al. [67]
Portugal Pesticide applicators in open field and	Exposed 33 (17m + 16f) (43 ± 10) Controls	15.0 ± 13.0 y (0.5–48 y)	Exposure to complex mixture of pesticides 33 compounds listed	1000 BN cells Giemsa	15/27	L-CBMN SCE (50 cells)	↑ 9.03‰ vs 3.27‰*	2.8 1.20	Costa et al. [68,69]

Table 5 (*Continued*)

Country Exposure	Number of participants: exposed and controls (sex and age)	Exposure period Employment time (years)	Exposure measurement (type and value) Use of personal protection equipment (PPE)	Number of cells evaluated in L-CBMN assay	QS	Results (MN‰; Exposed vs Control)		FR	Reference
greenhouse workers	33 (17m+16f) (41 ± 9)		(fungicides, insecticides, rodenticides and herbicides)				↑ 5.19/cell vs 4.33/ cell*		
						CA (100 M)	↔ 2.52% vs 2.90%		
						Greenhouse vs open field			
						L-CBMN	↑ 9.03‰ vs 3.27‰ *		
						Use of gloves Yes/no			
						L-CBMN	↔ 14.00‰ vs 7.5‰	1.9	
						Polymorphisms			
						No effect of GSTM1, GSTP1, CYP2E1,GSTT1		2.7	
						mEH activity low vs high			
						L-CBMN	↑ 11‰ vs 4‰*		
Portugal Pesticide applicators in open field and greenhouse workers	Exposed 84 (42m+42f) 40 ± 12 Controls: administrative officers 93 (39m + 54f) 39 ± 13	4 months pesticide exposure 23.0 ± 16.1 y	No data on the exposure 26 compounds listed (fungicides, insecticides, aphicides and herbicides)	1000 BN cells Giemsa	16/ 27	L-CBMN	↑ 6.76‰ vs 2.66‰*	2.5	Costa et al. [70]
						MN-RET(%)	↑ 1.14% vs 0.47%*	2.4	
						Higher MN frequency in spring-summer vs autumn-winter			
						no effect of age, gender and smoke			
Argentina Agriculture	Exposed 20m (36.25 ± 12.25) Controls: healthy subjects from the same area 10m (37.0 ± 12.86)	66.5% of workers applied pesticides during the summer season (Sept-March) 9.93 ± 11.64	Exposure to complex pesticide mixtures, including insecticides, and herbicides. (10 formulations listed) Mostly applied: cypermethrin, clorpyriphos, endosulfan, glyphosate 58.3% reported some poisonings Use of PPE: 75%	1000 BN cells Giemsa	11/ 27	L-CBMN	↑ 15.15‰ vs 7.20‰***	2.1	Gentile et al. [71]
Portugal Greenhouse and open field agriculture	Pesticide exposed workers 43m + 42f (85) (40.0 ± 12.2) Organic farmers 17m ± 19f (36) 39.6 ± 14.5 Controls: healthy subjects from the same area 26m + 35f (61) 39.5 ± 12.3	Involved in pesticide preparation or application for at least 4 months Pesticide exposed workers 22.7 ± 16.2 y Organic farmers 9.5 ± 12.3 y	Exposure to complex and different pesticide mixtures, including pyrethroids, carbamates, organophosphates Use of PPE:64.7% Urinary PYR: no difference OP/CRB: pesticide exposed vs organic farmers vs controls 2.23/1.86/1.54	1000 BN cells Giemsa	15/ 27	Pesticide exposed workers			Costa et al. [72]
						L-CBMN	↑ 6.69‰ vs 2.33‰***	2.8	
						MN ret	↑ 1.14‰ vs 0.51‰***	1.89	
						CA (100 M)	↑ 1.56% vs 0.92%*	2.19	
						Comet (% t)	↑ 15.05% vs 8.03%*	1.71	
						Organic farmers			
						L-CBMN	↑ 3.45‰ vs 2.33‰*	1.48	
						MN ret	↔ a		
						CA (100 M)	↔ a		
						Comet (% t)	↔ a		
						a. data not reported			
Turkey Agriculture	Exposed 51m + 7f (58) (39.8–59) Controls: 42m +16f (22–44.3)	Involved in activities such as cultivating and harvesting mixing and application of pesticide 17.19 ± 1.08 y	Exposure to 40 chemicals, including organophosphates, pyrethroid insecticides, fungicides, and carbamates Use of PPE: 22.4%	2000 BNcells Giemsa	15/ 57	L-CBMN	↑ 8.97‰ vs 2.64‰***	3.39	Tumer et al. [73]
						Polymorphisms			
						GSTT1− vs GSTT1+	↔ 9.93 ‰ vs 8.66 ‰		
						GSTM1− vs GSTM1+	↑ 10.10‰ vs 7.75‰*	1.3	
						GSTP1− vs GSTP1+			
						Ile–Ile vs Ile-Val/Val–Val	↔ 8.86‰ vs 9.14‰		
						Concomitant			
						GSTT1− vs GSTT1+			
						GSTM1− vs GSTM1+	↑ 11.38‰ vs 7.68‰*	1.48	

Abbreviations: Males abbreviated as "m", females as "f", years of age indicated as mean or mean ± SD; PYR, pyrethroids; OP, organophosphates; CRB, carbammates; QS, quality score based on the following parameters: (i) age-matching, (ii) gender-matching, (iii) smoking status-matching, (iv) alcohol intake-matching, (v) nutritional intake-

matching, (vi) appropriate measurement of chemical exposure (see Table 1) 27 is the perfect score; BN, binucleated lymphocytes; BCMN, MN in buccal cells; CA, chromosomal aberrations; M, metaphases; SCE, sister chromatid exchange; MN ret, MN frequency in reticulocytes; BMI, body mass index; GSTT1, glutathione-S-transferase T1; GSTM1, glutathione-S-transferase M1; GSTP1, glutathione-S-transferase P1; CYP2E1, cytochrome P450 monooxygenase E1; mEH, epoxide hydrolase; ↑, significant increase; ↓, significant decrease (number following arrow in brackets indicates mean ratio); ↔, no effect; *$p < 0.005$; **$p < 0.001$; ***$p < 0.0001$; NS, not statistically significant; FR, Frequency Ratio (MN frequency in exposed subjects/MN frequency in Controls).

did not show statistically significant difference in the frequency of MN when compared with the control group (N = 11) [39].

The L-CBMN assay was used in a biomonitoring study carried out in Colombia in two different regions (Narino and Putumajo) where glyphosate formulations were used for control of illicit crops, and in an area for weed control in sugar cane fields (Valle del Cauca). Three samplings were performed in 30 pairs of exposed and control subjects – before, 5 days, and 4 months after spraying. Higher baseline MN frequencies were observed in these three exposed populations compared with age matched control groups (FR = 1.9, 2.4, 3.1, in subjects from Narino, Putumajo, and Valle del Cauca, respectively). A significant increase in the MN frequency associated with exposure was detected (FR = 2.5–4.7 for three areas), while a decrease 4 months after spraying was evident only in one area (Valle del Cauca). The study suggested a potential genotoxic risk associated with the aerial spraying of glyphosate formulations, although clear conclusions could not be drawn due to the lack of more specific data on the exposure [40].

Overall, the L-CBMN assay in professional applicators that used single pesticide or few compounds in the framework of specific programs did not show statistically significant increases in MN. This may be explained by relatively low levels or length of exposure, and by the use of personal protection devices.

3.3. Application of pesticide mixtures

Nine studies are available on pesticide sprayers involved in cultivation of different crops and applying different complex mixtures of compounds (Table 3 and Fig. 2).

Three studies were conducted with the groups of vineyard workers from Serbia, Brazil and Greece employed in spraying complex mixtures of agrochemical formulations [42–45].

Significant increases in the MN frequency and chromosomal aberrations were detected in the Serbian study one month after the start of the spraying season, followed by a further increase at the end of the spraying season in MN frequency (up to 7- fold) but not CA or SCE frequencies, in comparison with the controls [42]. It involved a group of subjects (N = 27) heavily exposed to mixture of insecticides, fungicides and herbicides, the most commonly used being diazinon and dithiocarbamates. An increase in the MN

Table 6
L-CBMN and other genotoxicity assay studies in pesticide exposed subjects: without direct spraying.

Country Exposure	Number of participants: exposed and controls (sex and age) Exposure period Employment time (years) Exposure measurement (type and value) Use of personal protection equipment (PPE)	Number of cells evaluated in L-CBMN assay	QS	Results (MN‰; Exposed vs Control)		FR	Reference
Canada Seasonal workers Berry pickers	Exposed South-Asian 18f (57.9 ± 6.4) Controls: healthy volunteers from the same community 21f (54.1 ± 12) Fruit picking season Working for at least 6 weeks Exposure to pesticide residues in fruit or foliage	2000 BN cells DAPI	15/27	L-CBMN	↔ 21.76‰ vs 19.20‰	NS	Davies et al. [75]
Costa Rica Banana farmers	Exposed 32f (35.7) Controls: unexposed subjects from the same region 37f (32.9) Subjects worked > 4 consecutive months in selecting, spraying and branding of bananas Exposed to (malzalil, thiabendazole, chlorpyriphos	800–1100 BN cells Giemsa	14/27	L-CBMN	↔ 4.9‰ vs 5.0‰	NS	Ramirez and Cuenca [76]
Chile Greenhouse and plant nurseries Thinning and pruning fruit trees, harvesting and packaging fruits	Exposed 64f (36.6 ± 11.4) Controls: people from the same region 30f (40.5 ± 7.8) Subjects rotating among the different tasks during the season 8 ± 4.8y Exposure to pesticide mixtures: insecticides, fungicides, herbicides (22 formulations listed) No use of PPE	1000 BN cells Giemsa	16/27	L-CBMN Age effect	↑ 36.9‰ vs 9.9‰*	3.7	Marquez et al. [77]
Pakistan Female cotton pickers	Exposed 69f (37.55 ± 12.75) Controls 69f (37.52 ± 13.47) C:healthy subjects recruited in the same area not exposed to pesticides 10.26 ± 6.14y (4–27 y) Exposure to complex mixtures: carbamates, organophosphates pyrethroids No protection devices ALT, AST, ALP (↑) Serum cholinesterase: ↓ 7428.1 vs 8520.8	1000 BN cells Giemsa	14/27	L-CBMN Exposure: >15 y vs 1–5 y	↑ 12.72‰ vs 4.35‰ *** ↑ 14.83‰ vs 11.39‰***	2.92 1.3	Ali et al. [78]
Colombia Pesticide mixture	Exposed 31m + 31f (62) (29.1 ± 8.8) Controls: healthy volunteers from an area where organic coffee is grown without the use of pesticides 30m + 30f (60) 27 ± 5.6 Manual eradication of illicit crops Exposure to complex mixture of pesticides and solvents	2000 BN cells Giemsa	18/27	L-CBMN Age and gender effect	↑ 5.64‰ vs 1.83‰*	3	Bolognesi et al. [40]

Abbreviations: Males abbreviated as "m", females as "f", years of age indicated as mean or mean ± SD; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; BN, binucleated lymphocytes; QS, quality score based on the following parameters: (i) age-matching, (ii) gender-matching, (iii) smoking status-matching, (iv) alcohol intake-matching, (v) nutritional intake- matching, (vi) appropriate measurement of chemical exposure (see Table 1) 27 is the perfect score; ↑, significant increase; ↓, significant decrease (number following arrow in brackets indicates mean ratio); ↔, no effect; *$p < 0.005$; **$p < 0.001$; ***$p < 0.0001$; NS, not statistically significant; FR, Frequency Ratio (MN frequency in exposed subjects/MN frequency in Controls).

Table 7
L-CBMN and other genotoxicity assay studies in populations environmentally exposed to pesticides.

Country Exposure	Number of participants: exposed and controls (sex[a] and age)	Exposure period Employment time (years)	Exposure measurement (type and value) Use of personal protection equipment (PPE)	Number of cells evaluated in L-CBMN assay	QS[b]	Results (MN‰; Exposed vs Control)[c]		FR[d]	Reference
Spain Exposed to simazine in drinking water	Exposed 34 m (45 ± 2.50) Controls: non-exposed subjects 26m (40.84 ± 1.78) Controls; 28 m (42.96 ± 1.98).	Continuous exposure	10–30 ppm MAL (Maximun Admissible Limit) 0.0001 ppm	1000 BN cells Giemsa	16/27	L-CBMN SCE (50 cells)	↔ 9.12‰ vs 9.85‰ ↔ 5.85/cell vs 5.95/cell	NS	Suarez et al. [79]
Mexico Agrarian community	mother/newborn pairs (umbilical cord blood) Exposed 16 pairs from agricultural area Controls: 21 pairs from urban area	Exposure to pesticides (organophosphates) during summer and autumn	AChE in serum: no inhibition	1000 BN cells Wright stain	13/27	L-CBMN Mothers Newborn	↔ 4.5‰ vs 3.7‰ ↔ 2‰ vs 1‰	NS	Levario-Carillo et al. [80]
Mexico Agrarian community	50f (23.4 ± 4.9) Maternal blood and umbilical cord	Exposure to organochlorine pesticide mixtures in agricultural non malaria zone	Median concentrations of pesticides in plasma (ng/g lipid) pp-DDT 3/2 pp-DDE 2/8 a-HCH 13/3 b-HCH 18/12 g-HCH 7/3 HCB 0.9/0.4	2000 BN cells Giemsa	NA	L-CBMN	maternal ↔ 1.1‰ vs UC 0.78‰	NS	Alvarado-Hernandez et al. [81]

Abbreviations: Males abbreviated as "m", females as "f", years of age indicated as mean or mean ± SD; AChE, Acetylcholinesterase; QS quality score based on the following parameters: (i) age-matching, (ii) gender-matching, (iii) smoking status-matching, (iv) alcohol intake-matching, (v) nutritional intake- matching, (vi) appropriate measurement of chemical exposure (see Table 1) 27 is the perfect score. BN, binucleated lymphocytes; SCE, sister chromatid exchange; ↑, significant increase, ↓, significant decrease, number following arrow in brackets indicates mean ratio, ↔, no effect *$p < 0.005$; **$p < 0.001$; ***$p < 0.0001$; FR, Frequency Ratio (MN frequency in exposed subjects/MN frequency in Controls).

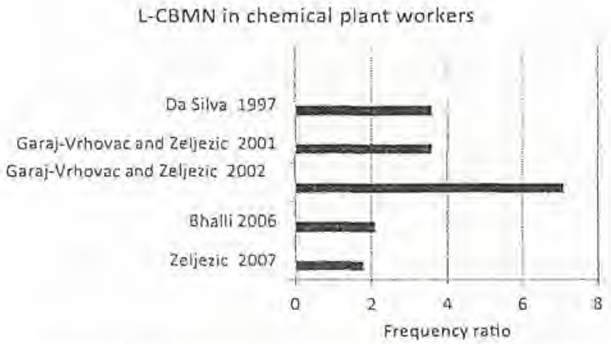

Fig. 1. Increase of MN frequency in peripheral blood lymphocytes of pesticide exposed subjects compared with the controls. Chemical plant workers.

frequency was also observed in control subjects ($N = 15$) living in the vine-growing area with respect to controls recruited outside the area ($N = 20$), showing a possible contamination of the environment.

Positive results were also obtained in a study carried out in Crete in a small group of vineyard workers ($N = 11$) who were also involved in cultivating olive trees [43]. They were exposed throughout the year to mixtures of insecticides, fungicides and herbicides; the most common ones being parathion, cypermetrin and glyphosate.

A genotoxic risk associated with prolonged exposure to complex mixtures of pesticides was detected in vineyard workers in Brazil, based on elevated MN frequencies (FR = 1.7) and also increased DNA damage evaluated by the comet assay [44,45]. A large variability among the subjects was observed. Inter-individual variation in expression of polymorphic genes involved in the metabolism of organophosphates, such as the paraoxonase (*PON*) and in DNA repair (*OGG1*) is likely contributing to observed variability.

Tobacco farming is associated with the use of large quantities of pesticides, including insecticides, herbicides, fungicides and plant regulators, and with different types of work including harvesting leaves and plants by sickle, and separation of leaves. Two studies were carried out in the state of Rio Grande do Sul, Southern Brazil where tobacco production has great economic relevance [46,47]. The first study involved 111 tobacco farmers and 56 matched controls sampled two times, during pesticide application and during leaf harvesting. Significant increases in MN frequency and DNA damage were detected in exposed subjects compared with controls, and in leaf harvesters compared with pesticide application group, indicating a genotoxic risk associated with a potential exposure to pesticide residues during the harvesting. No association was found between markers of individual susceptibility, such as *PON1*, *CYP2A6*, *GSTM1*, *GSTP1*, *OGG1*, *XRCC1*, *XRCC4* and *RAD51*, and MN frequency in the pesticide-exposed subjects [46]. These results were not confirmed in a second study carried out on 60 tobacco farmers exposed to complex mixture of pesticides, and nicotine present in tobacco leaves [47]. This study group had a higher MN frequency with respect to a matched control group only at the first sampling (off season) (FR = 1.54). A parallel analysis of DNA damage using the comet assay showed a progressive increase associated with the different seasonal activities with high level during pesticide application, in comparison to off season or harvest.

Three studies were available in pesticide sprayers involved in cultivation of different crops, in Italy, Chile and Turkey. A study carried out in the last decade of the 20th century in Central Italy in

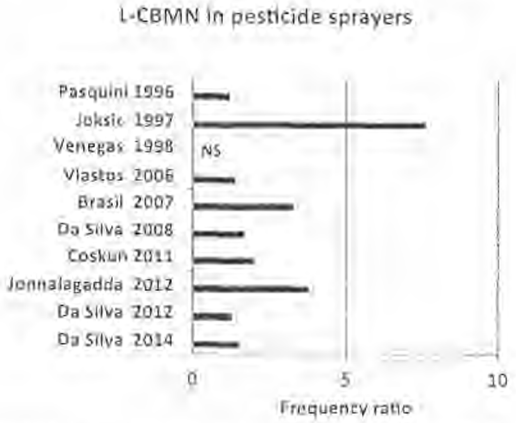

Fig. 2. Increase of MN frequency in peripheral blood lymphocytes of pesticide exposed subjects compared with the controls. Pesticide sprayers.

a group of farmers growing cereals, fruits and vegetables and exposed to mixtures of different insecticides, fungicides and herbicides (N = 48), reported an increased MN frequency associated with the duration of exposure [48]. Participants were exposed during mixing and spraying of pesticides with little or no personal protection. Parallel analyses of SCE in the same group did not reveal any change.

A study of workers in Chile (N = 22) spraying a variety of pesticides, mainly deltamethrin and diclorvos, showed no increase in the MN frequency [49]. The goal of this study was to test a protocol designed to reduce the pesticide exposure by introducing specific safety procedures.

A study of agricultural workers in Turkey (N = 50) involved in manual spraying of unreported pesticide mixture in open field without adequate personal protection shows a significantly increased MN frequency (FR = 2.0) [49].

A recent study carried out in the South of India in a group of 152 farmers engaged in cultivation of cotton and using mainly a mixture of organophosphorus and organochlorine pesticides, showed no increase of MN, but higher frequency of CA was associated with the presence of benzene hexachloride in serum [51].

An increased MN frequency (FR = 3.3) was reported in a group of sanitation workers from Belo Horizonte (Brazil) (N = 30) involved in the application of insecticides, mainly cypermetrin and malathion, and rodenticides in different form (spray, powder, sand mixed granulated) with or without personal protection [52].

Overall, in the studies on sprayers using complex mixtures of pesticides in different occupational settings, the difference in working environment, formulation of pesticide mixtures, treatment schedule, exposure conditions, and the use of personal protection makes it difficult to compare these studies, and to analyze the factors that may contribute to the discordant findings.

3.4. Floriculturists

The L-CBMN assay was used to evaluate the genotoxic damage associated with the flower growing industry which involves heavy use of multiple agrochemical formulations belonging to different chemical classes, mainly in greenhouses.

A number of studies were carried out in the region of Liguria (Northwest Italy). A first study in 1993 on 71 workers exposed to mixtures of different agrochemical ingredients, mainly dithiocabamates, organophosphates and chloro-organics reported a significant increase in MN frequency, showing a dose-response relationship with duration of exposure. There was a maximum increase of 71% in subjects exposed for over 30 years [53,54].

A further study in the same area of 107 workers growing ornamental plants and vegetables confirmed the previous results showing an increase in the MN frequency with the duration of exposure (FR = 1.62 in subjects exposed for ≥ 20 years) mainly in workers not using protective measures during high exposure activities. A 28% higher occurrence of MN was observed in greenhouse workers when compared with subjects working only in open field. The difference is not significant probably due to the small number of subjects working exclusively in greenhouses (N = 19). The majority of pesticides used in these occupational groups have mutagenic properties as assessed by different genetic endpoints [55]. The use of the L-CBMN assay associated with fluorescence *in situ* hybridization with a pan-centromeric probe demonstrated a higher percentage of C+ micronuclei (66.52% vs. 63.78%) in a subgroup of subjects from the same population that used benzimidazolic compounds, compared with the floriculturist population exposed to a complex pesticide mixture not including benzimidazolics [56].

No increase in MN frequency was detected in studies carried out in Tuscany (Central Italy) involving floriculturists engaged in mixing and spraying of pesticides and in re-entry activities (cutting and harvesting flowers) (N = 43) [57]. No association was observed between the MN frequency and genetic polymorphisms in the *GSTM1*, *GSTT1* and *NAT2* genes [58]. Nevertheless, a subsequent study in subgroups of workers from the same population showed that *GSTM1* positive and *NAT2* fast individuals appear to have higher MN levels [59].

In summary, flower production is consistently associated with an increase in genotoxicity due to heavy and frequent use of multiple pesticides in an enclosed environment (greenhouses) which would tend to increase the individual exposures. It depends on the length and type of employment, and on incorporation of especially potent chemicals in the formulations used.

3.5. Agricultural workers

Multiple studies were conducted to assess the risk of pesticide exposure in different categories of agricultural work ranging from growing vegetables in greenhouses and open field workers (Table 5 and Fig. 3).

A study carried out in the region of Liguria (Norwest of Italy) involving 48 workers growing vegetables in greenhouses and in open field did not find any increase in MN frequency in exposed subjects in comparison to the control group [60].

Two studies (N = 64 and N = 39) conducted in the South-eastern region of Spain also failed to detect MN induction in peripheral lymphocytes or buccal exfoliated cells from agricultural workers exposed to complex mixtures of pesticides in greenhouses and the open field. No statistically significant differences in MN frequencies were found between high exposure (spring-summer) and lower exposure (autumn-winter) sampling periods [61,64]. No effect of *GSTT1* or *GSTM1* genotypes on the levels of cytogenetic damage was evident [61].

Three different studies carried out by the same research group in Poland (N = 50), Greece (N = 50), and Hungary (N = 58) with workers growing ornamental plants and vegetables, both in open field and greenhouses, and exposed to different mixtures of pesticides, did not find any increase in the MN frequency in either blood lymphocytes or buccal cells [62,63,65,74]. Carbamates, organophosphates and pyrethroids were the most commonly used chemical classes of pesticides. A characteristic similar for these three studies is the large percent of workers (almost 80%) reported

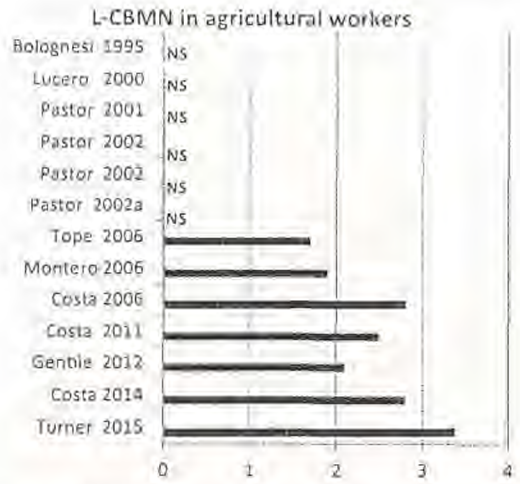

Fig. 3. Increase of MN frequency in peripheral blood lymphocytes of pesticide exposed subjects compared with the controls. Agricultural workers.

the use of personal protection devices during the preparation and application of pesticides.

A repeated assessment by the L-CBMN assay was performed among a small group of farmers (N = 15) in USA during the growing season over a period of six months [66]. They were occupationally exposed to pesticide mixtures including mainly endosulfan, chlorpyriphos, dimethoate, diazinon and maleic hydrazide. The results show a significant increase in the MN frequency in exposed workers compared with the controls (FR = 1.7). This increase was associated with a decrease in erythrocyte superoxide dismutase (SOD) activity involved in the antioxidant defense.

An increased MN frequency was detected in a group of agricultural workers (N = 37) from the region of the Atoyac River (state of Tlaxcala, Mexico) exposed to pesticides mixtures and solvents. The presence of the *GSTT1* null polymorphism significantly correlated with the increased MN frequency [67].

A study carried out in a group of 20 agricultural workers mainly involved in crop spraying with different pesticide formulations (mostly used: cypermethrin, clorpyriphos, endosulfan, glyphosate) in the province of Cordoba (Argentina) compared with 10 controls reported a significantly increased MN frequency associated with occurrence of poisoning, specific health conditions, or symptoms related to pesticide exposures. Incorrect or insufficient use of protective devices was also associated with the increase [71].

A study performed in the area of Oporto (Portugal) reported a significant increase of the MN frequency (FR = 2.8) in a group of farmers (N = 33) involved in different agricultural practices associated with the exposure to a complex mixtures of pesticides (33 pesticides were listed). Higher MN frequencies were detected in greenhouse workers, but no differences were observed between pesticide applicators and non-applicators. Parallel analyses revealed increase of SCE (FR = 1.2), but not of CA in the same group of subjects. No association was found between MN levels and duration of pesticide exposure. No effect of polymorphisms of *GSTM1*, *CYP2E1* and *EPHX1* on the MN frequency was observed, while low mEH (microsomal epoxide hydrolase) activity as well as the *GSTT1* positive genotype were associated with increased cytogenetic damage [68,69].

Another study conducted in a larger group of subjects (N = 84) regularly exposed to pesticides from the same population confirmed the increase of cytogenetic damage in peripheral lymphocytes, as well as in reticulocytes. Working environment, time of exposure, and direct involvement in the application of pesticide did not affect the MN frequency, but higher levels were observed in samples collected in spring-summer when the pesticide application was more frequent, as compared to the autumn-winter period [70].

A study from the same research group compared the genotoxic risks in organic and traditional farming. A higher MN frequency was found in lymphocytes of pesticide-exposed workers (N = 85) and organic farmers (N = 36) in comparison to the controls (N = 61) (FR = 2.80 and 1.48, respectively). Increased levels of CA (FR = 2.19) and DNA damage (FR = 1.71) evaluated by comet assay were detected only in the pesticide-exposed workers. Higher urinary levels of organophosphates and carbamates were observed in workers recently exposed to pesticides and in samples collected during the summer season [72].

A study carried out in Turkey with agricultural workers exposed to complex mixtures of pesticides (more than 40 different chemicals) during pesticide mixing and application, cultivating and harvesting reports a substantial increase (FR = 3.39) of MN frequency. A significant increase (FR = 1.5) in MN frequency was also found in workers with null genotypes of *GSTM1* and *GSTT1* compared with those with positive genotypes for both *GSTs* [73].

3.6. Agricultural workers without direct pesticide exposure

Table 6 and *Fig. 4* summarizes the studies on mixed populations involved in different types of agricultural work such as picking fruit or cotton, thinning and pruning fruit trees, harvesting and packaging fruits but not directly responsible for pesticide preparation or application.

A study carried out in seasonal berry pickers (N = 18) in British Columbia (Canada) compared with 21 age-matched controls did not show an increase in the MN frequency. However, the highest frequency values were found in the subjects with the longest history of employment as farmworkers [75].

Negative results were also obtained in a study in Costa Rica in 32 women potentially exposed to imalzalile, thiabendazole and chlorpyriphos working in selecting, branding and packaging of bananas for four consecutive months, in comparison to the controls (N = 37). However a subgroup of women in this study with a high frequency of abortions had higher MN levels (Odd Ratio = 1.45 $P < 0.005$) [76].

A substantial increases in MN frequency (FR = 3.7) was detected in a group of 64 women working in thinning and pruning fruit trees, harvesting and packaging fruits during the spring and summer season without any use of protective devices in Bio–Bio Region (Chile) where the use of pesticides is intensive [77].

An increased MN frequency (FR = 2.9), associated with higher level of hepatic enzymes and decreased serum cholinesterase, was reported in a biomonitoring study of 69 females involved in cotton picking activity without protection compared to 69 controls in the

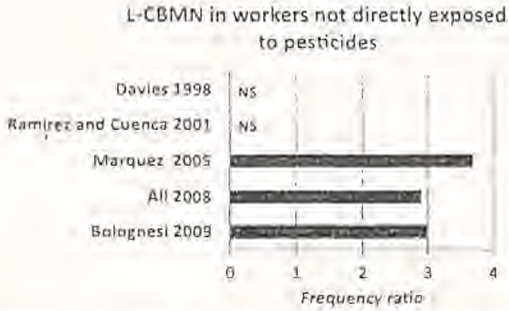

Fig. 4. Increase of MN frequency in peripheral blood lymphocytes of pesticide exposed subjects compared with the controls. Workers not directly exposed to pesticides.

Bahawalpur area (Pakistan) where carbamates, organophosphates and pyrethroids were mostly applied [78].

The manual eradication of illicit plants in areas where a large number of persistent pesticides were applied in Colombia was associated with an increase of cytogenetic damage, measured as the MN frequency (FR = 3.0) in 31 couples of reproductive age compared to 30 control couples [40].

3.7. Non-occupationally exposed populations

Environmental exposure to pesticides can occur via contamination of drinking water or food plants, or by inhalation or dermal exposure from living in an area where pesticide applications are common.

The continuous exposure to simazine at a concentration of 10–30 ppm in drinking water (3–10× higher with respect to the maximum contamination limit) in a group of 34 males in the community of Estremadura (Spain) did not induce an increase in cytogenetic damage, measured as MN frequency and SCE [79].

A study of newborns was performed using their umbilical cord blood. It compared the MN levels in 16 newborns from an agricultural area in the North of Mexico where pesticide mixtures (mainly organophosphates) were applied during the summer and autumn spraying cycles, to 21 controls from urban area. Although more babies with higher MN frequencies were found within the pesticide exposed group, the difference from controls was not statistically significant [80].

The effects of exposure to organochlorine pesticides were studied in a group of mother/infant pairs (N = 50) in a rural area in Mexico where pesticides were applied via airplanes. Significantly higher pesticide levels were found in umbilical cord blood than in the mother's plasma. No increase in MN frequency associated with pesticide exposure was observed, but DNA damage, evaluated by comet assay, was significantly higher in umbilical cord blood of newborns than in mothers [81].

3.8. L-CBMN assay and other DNA damage biomarkers

The majority of the available studies (28 out of 49) report data on MN frequency in peripheral blood lymphocytes only. Five studies [36,61–63,65] describe the application of MN assay in parallel in peripheral lymphocytes and buccal cells. One of these studies [36], carried out in a small group of fumigators exposed to methyl bromide, revealed a suggestive increase of MN frequencies in both cell types without reaching the statistical significance. Other four studies [61–63,65] related to the biomonitoring of agricultural workers in different European countries, were characterized by the common use of personal protection equipment, and do not show any increase of MN frequencies in either peripheral lymphocytes nor buccal cells. Consistent negative results with L-CBMN, CA and SCE assays, were also reported in two studies on floriculturists [57,58] and in a study of subjects exposed to simazine in drinking water [79] respectively. A further study on farmers involved in cultivation of cotton in India [51] reports negative results with MN assay and an increase of CA (FR = 3.8).

Fig. 5 shows the comparison of the frequency ratio for MN and other DNA biomarkers(CA, SCE and Comet assays) related to 10 studies where they were applied in parallel.

Only one study [29] describes the application of the four bioassays, MN, CA, SCE and Comet, showing a good agreement between the biomarkers with highest increase with CA assay. Two studies report on MN, CA and comet data showing an agreement between the assays. Significant increase of MN frequency and negative results with SCE are shown in two other studies [42,48]. In one study [32] positive effect with MN assay is not associated with

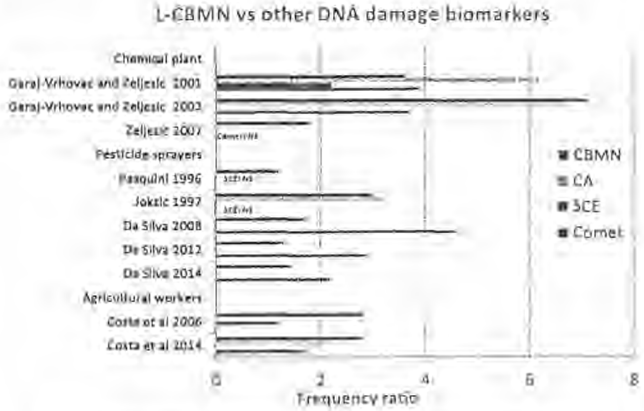

Fig. 5. Comparison of frequency ratio (FR) from different biomarkers.

an increase of DNA damage detected with comet assay. Three studies applying MN and Comet assay [47,51,52] show an agreement between the results with higher trend with Comet assay. The difference in the extent of effects observed with MN frequency and with Comet assay reflects that different parameters of DNA damage were measured. The MN test evaluates the expression of the chromosomal damage as an index of a time integrated exposure, while the Comet assay detects the DNA single strand breaks as a result of a recent exposure.

3.9. L-CBMN as a biomarker of risk for cancer and degenerative diseases

A growing number of epidemiological studies provide substantial evidence that some classes of pesticides are associated with excess cancer risk [8–11]. Chronic exposure to pesticides has also been associated with a variety of neurological disorders [12–15], as well as respiratory [16], endocrine and reproductive adverse outcomes [21–23].

Table 8 shows the main compounds or chemical classes of pesticides reported to be used in the biomonitoring studies analyzed in the present review and classified as carcinogens by the International Agencies [7,82] or reported to be associated with degenerative diseases. This list is far from being exhaustive because many studies describe only the pesticide categories, the chemical classes or a partial list of the active ingredients applied. In addition, the classification for some compounds was not recently updated. For example, methyl bromide, a genotoxic compound still widely used and recently associated with an increased risk for prostate cancer [83], was not included.

The most represented chemical class is the organophosphates including a number of insecticides and an herbicide classified as probable or possible human carcinogens [7,82]. Organochlorine class is represented by some fumigants and fungicides no longer applied. Two fungicides, metiram and mancozeb belonging to the class of ethylene bis-dithio-carbamates, are classified as possible human carcinogens on the basis of their metabolite, ethylene thiourea, that was shown to cause thyroid tumors in rodents [7]. 2,4-D widely used to control weeds in agriculture, forestry, as well as in urban and residential settings, was recently classified as possibly carcinogenic to humans by IARC [7] on the basis of limited evidence in epidemiological and experimental studies. Other compounds, such as benomyl and carbendazim (both belong to the class of benzimidazoles), and cyanazine are classified as possible carcinogens by EPA [82].

The exposure to organophosphates,carbamates, organochlorines, pyrethroids and paraquat [90,94,95,98,99], which interfere

Table 8
List of pesticides reported to be used in the evaluated studies that are classified as carcinogens or associated with degenerative chronic diseases.

Chemical class Compound	CAS	Use	Carcinogenicity classification	Other diseases or health effects	Genotoxicity	Mechanism of action
Benzimidazoles		fungicides		Reproductive effects [84]	equivocal results for gene mutation	Alteration of microtubule-kinetochore attachment and chromosome alignment at the metaphase plate [87]
Benomyl	17804-35-2		Group C EPA	Parkinson's disease [86]	Positive for DNA and chromosomal damage in vitro and in vivo [25,85]	Aldehyde dehydrogenase inhibition [86]
Carbendazim	10605-21-7		Group C EPA			Induction of aromatase activity [88]
Bypiridylics				Neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis [89]	Negative for gene mutation	Oxidative stress
Paraquat	1919-42-5	fungicide	Not classified		DNA and chromosomal damage [25,90]	Epigenetic mechanism (Histone acetylation) [91]
Chlorophenoxy compounds 2,4-D (2,4-diclorofenossiacetico)	9475-7	Herbicides	2B IARC	Birth defects and developmental toxicity [92]	Negative [7,25] DNA damage in vitro [93]	Oxidative stress Immunosuppression Endocrine disruption
Ethylene bisdithiocarbamates		fungicides	Group B EPA based on metabolite and environmental degradate ethylene thiourea	Neurotoxicity [94,95] development and reproduction deficiencies [84]		Generation of Reactive Oxygen Species (ROS) generation [96,97] Endocrine disruption Alteration of thyroid hormone homeostasis
Metiram	9006-42-2				Negative for gene mutation [25]	
Mancozeb	8018-01-7				Positive for Chromosomal damage [7,25]	
Organochlorines				Neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis [98] Diabetes [99]		Oxidative stress, Mitochondrial dysfunction, Epigenetic changes [100,101]
Carbon tetrachloride	56-23-5	fumigant	2B IARC	Hepatotoxic and nephrotoxic	Negative Positive for aneuploidy in some in vitro studies (CA and MN) [7]	Damage to nuclear protein and to DNA damage as a secondary effect to general toxicity
Hexachlorobenzene	118-74-1	fungicide	2B IARC	Reproductive effects [102]	Negative [7] Positive in MN test in human and rat primary hepatocytes in vitro [7]	DNA damage Endocrine disruption
Perchloroethylene	127-18-4	Intermediate in pesticide production	2A IARC	Neurological effects [103]	Positive after metabolic activation [7]	Genotoxic and non genotoxic (peroxisome proliferation, immunological effects)
Organophosphates				Neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis [99] Diabetes [99]		Oxidative stress; Mitochondrial dysfunction [100,101] Lipid peroxidation and decreased activity of antioxidant enzymes
Dichlorvos	62-73-7	insecticide	2B IARC	Acute neurotoxic	Gene mutation and chromosomal damage in vitro Negative in vivo [7]	DNA damage and immunosuppression Cholinesterase inhibition
Diazinon	333-41-5	insecticide	2A IARC	Reproductive effects	Positive [7]	Oxidative stress
Dimethoate	60-51-5	insecticide	Group C EPA	Effects on reproduction	DNA and chromosomal damage in vitro [25]	DNA damage Cholinesterase inhibitor
Glyphosate	1071-83-6	herbicide	2A IARC	No data	Negative for gene mutation Positive for DNA and chromosomal damage in vitro and in vivo [7]	Genotoxicity Oxidative stress
Malathion	121-75-5	insecticide	2A IARC	Aplastic anemia	Positive [7]	DNA damage, Hormone-mediated effects, Oxidative stress Cell proliferation
Parathion	56-38-2	insecticide	2B IARC	Neurotoxicity	Negative for gene mutation Positive for DNA	DNA damage, Oxidative stress

Table 8 (Continued)

Chemical class Compound	CAS	Use	Carcinogenicity classification	Other diseases or health effects	Genotoxicity	Mechanism of action
					and chromosomal damage in vitro [7]	
Tetrachlorvinphos	22248–79-9	insecticide	2B IARC	Neurotoxicity	Negative for gene mutation Positive for chromosomal damage in vitro [7]	Oxidative damage Cell proliferation
Triazines						
Cyanazine	21725–46-2	herbicide	Group C EPA	Reproductive effects	Equivocal Gene mutation in mammalian cells [104]	Prolactin mechanism Endocrine disruption [105]
Pyrethroids				Neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis [99]		Oxidative stress Mitochondrial dysfunction [101,102]

Abbreviations: IARC classification, Group 2A probably carcinogenic to humans; Group 2B possibly carcinogenic to humans [7]. EPA classification, Group B: Possible human carcinogen; Group C: probable human carcinogen [82].

with neurotransmission and function of ion channels in the nervous system, has been associated with increased risk for neurodegenerative diseases such as Parkinson's disease (PD), Alzheimer's disease and amyotrophic lateral sclerosis.

Reproductive effects have also been described for benzimidazoles [84], 2,4D [92], ethylene-bisdithiocarbamates [84], hexachlorobenzene [102], diazinon, dimethoate and cyanazine.

The listed pesticides were tested in a wide variety of mutagenicity assays that cover gene mutations, chromosomal alterations, and DNA damage. The majority of the compounds were classified as positive for genotoxicity, mainly inducing chromosomal damage. Direct and indirect DNA damage was considered as a main mechanism associated with their carcinogenicity.

Multiple mechanisms are likely involved in pesticide-mediated etiology of neurodegenerative disorders [100,101]. Most of the published literature points toward DNA and protein damage mediated by the oxidative stress. Exposure to pesticides may cause mitochondrial disfunction and the production of reactive oxygen species (ROS) when antioxidant defense mechanisms are overwhelmed. The fungicidal action of benomyl resulting from microtubule assembly impairment [87], has been also implicated in PD pathogenesis.

Overall, main mechanisms likely to be involved in the etiology of cancer and other degenerative diseases associated with pesticide exposure are likely an induction of DNA alterations that could be expressed as chromosomal damage easily detectable by the MN assay.

4. Discussion

Pesticides, due to their multiple uses in agricultural and public health, are one of the most investigated categories of chemical compounds. A growing number of epidemiological and experimental studies indicate that occupational and environmental pesticide exposure is a contributing risk factor for cancer and different degenerative diseases. However the rapid change of chemical formulations in the market due to the pest resistance, the lack of reliable data on exposure, and the large number of confounding factors, including the typical exposure to more than one class of pesticide, prevent the estimation of a clear cause-effect relationship, and the identification of specific compounds or classes of compounds responsible for the association with cancer.

Genotoxicity biomarkers, including genetic endpoints, such as DNA damage, chromosomal damage (aberrations and MN), and gene mutations as early indicators of risk for cancer and other chronic diseases were extensively studied in human populations exposed to pesticides. A large number of studies are available in the scientific literature, with the majority reporting positive results. Previous reviews addressed the analysis of these studies [106–108] in order to estimate the potential genotoxic risk of pesticide exposure and to identify the predictive roles of the different genotoxicity biomarkers. The conclusions of these reviews were that exposure to pesticides was genotoxic, but with many limitations and uncertainty and a need for further studies. A recent meta-analysis [109] of pooled data from 22 studies on L-CBMN assay in different populations exposed to single pesticides or to mixtures of compounds belonging to different chemical classes, confirmed the utility of the L-CBMN assay in peripheral lymphocytes for pesticide biomonitoring.

The focus of this review is on the L-CBMN assay as biomarker with consideration of different types of applications and work conditions associated with pesticide manufacture and use in occupational setting and via environmental routes of exposure. More than 1000 references identified in the initial search were considered. The quality of many studies was low due to the small size of the exposed and control groups, poor characterization of exposure, and other confounding factors. Therefore, based on the quality and relevance, 49 studies were selected for in depth analysis. The large majority of these studies were carried out in Europe (25 studies) and South American (14 studies), followed by the USA (6 studies), Asia (3 studies) and Australia (1).

A high percentage of the studies (75%; 36 out of 49) reported positive results for MN associated with pesticide exposure. The range of the increases in the MN frequencies (FR = 1.2–7.6) was related to the extent, duration, and types of exposure. All the studies available on chemical plant workers involved in the production of different compounds and mixtures, mainly organochloride, organophosphate and pyrethroid pesticides, showed an increase in the MN frequency [28–32]. Eight [42,43,45–48,50,52] out of 10 studies of pesticide sprayers from various geographical areas using complex mixture of pesticides in different settings and working conditions reported positive results with a wide range of FR. Significant increases in MN frequency were also detected in large studies carried out in floriculturists in areas where there was extensive production of flowers and ornamental plants with the use of large quantities of pesticides belonging to different chemical classes [53–56]. Higher MN frequencies were also observed in subjects suffering from symptoms of pesticide poisoning or

chronic intoxication [27,31] during the intensive spraying season, or working in specific conditions such as cultivation in the greenhouses [53–56]. Increases in MN frequencies were detected when workers did not use personal protection devices in different application activities, or did not use them properly during non-direct exposure to pesticides, such as during harvesting and packaging fruits [77] or cotton [78], or when processing tobacco leaves [51,52]. This indicates a potential genotoxic risk associated with the exposure to pesticide residues on fruits and foliage further down the handling chain.

In contrast, there were no increases in MN when adequate protection measures or improved methods of pesticide application led to lower genotoxic exposures for the workers. Six [60–65] of twelve studies on agricultural workers involved in mixed activities, pesticide spraying, and different agricultural practices in the production of various crops in different European countries, characterized by a common use of personal protection equipment, reported no significant increases in MN frequencies. Negative results were also shown in a study carried out in Chile where specific safety precautions were followed [44]. The majority of studies (6 out of 7) [33–39] on professional applicators who used a single or few compounds in the framework of specific programs did not show any significant increases in MN frequency associated with the use of the chemicals that were genotoxic at some levels in experimental studies, such as phosphine or malathion. Improved fumigation techniques with phosphine gas in commercial grain stores in Australia, associated with increased CA damage in humans, demonstrated that exposure levels could be at the acceptable standard without producing detectable cytogenetic damage, as was shown by the negative results of a study applying the L-CBMN [33].

A parallel analysis of CA, SCE and DNA damage by comet assay in a number of studies revealed a significant correlation with the MN frequency results.

Exposure to a wide range of toxic chemicals, the absence of data on qualitative or quantitative exposure and on the individual involvement in specific tasks for the large majority of the available studies, makes it difficult to establish a clear association between the genetic damage and specific compounds or classes of compounds. However, an indication of a genotoxic risk can be plausibly derived for specific classes of compounds such as benzimidazoles [56] or single compounds such as glyphosate [40] and diazinon [43], due to consistent positive findings in exposed subjects.

Inter-individual variability in the MN frequency was well characterized in exposed groups of subjects. However, identification of sensitive subgroups through the analysis of specific genetic polymorphisms involved in the metabolism of certain pesticide classes in several studies did not show consistent results and failed to explain the inter-individual variability.

Overall, many of the pesticide studies evaluated had limitations due to the study design, inferior recruitment strategy to enroll exposed and control subjects, low statistical power, and lack of reliable exposure data. The subject selection in many cases did not take into account the different tasks in which the workers were involved and the known confounding factors for the exposed-control matching. The sizes of some of the selected groups of subjects were inadequate to detect a statistically significant difference between the exposed participants and controls. However, the major limitation common to the majority of biomonitoring studies in human populations exposed to pesticides is insufficient or missing data on exposure. The assessment and monitoring is particularly difficult for pesticides because mixtures of compounds belonging to different chemical classes, including active and inert ingredients, are commonly used. Moreover, application varies by seasons of the year, and workers are frequently switching between different sites, tasks, and pesticide formulations.

5. Conclusions

Our systematic review indicates a reliability of the L-CBMN assay to assess genotoxicity in people exposed to various pesticide classes with different mechanisms of action.

The main mechanism proposed to be involved in carcinogenesis and in some of degenerative diseases is mostly through the production of reactive oxygen species and mitotic spindle impairment associated with the exposure to specific classes of pesticides. This process could induce genetic alterations expressed as micronuclei.

The assay is suitable for biomonitoring studies to evaluate the potential genotoxic risk associated with the use of active agrochemical ingredients. However, further improvement of the study design is needed in future studies in order to better characterize the exposure and the confounding factors. One of the main conclusions of our analysis is the relevance of the use of protection equipment and adequate safety measures in reducing the exposure to pesticide and the genotoxic risk, suggesting the promotion of educational programs for safe pesticide use.

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1. Introduction

A "pesticide" is defined as any substance or mixture of substances used to kill, repel or control a "pest", including, fungi, bacteria, insects, snails, worms, rodents and weeds. This general term covers specific groups of agrochemicals such as herbicides, fungicides, insecticides, acaricides, nematicides, molluscicides, rodenticides, growth regulators, repellents, rodenticides and biocides [1]. Pesticides are biologically active compounds designed to selectively affect functional systems or target molecules specific to the "pest". Due to the similarities in the biological macromolecules of all organisms an absolute selectivity is difficult to achieve, therefore the large majority of pesticides is characterized by various degree of toxicity to non-target species, including humans. Pesticides have multiple uses: they are applied to protect crops from insects, weeds and fungal diseases while they are growing, and to prevent food contamination by rats, mice, insects and fungi during storage. Pesticides are also used to control vector-borne diseases and noxious insects and to protect animals from illnesses that can be caused by parasites and fleas. Herbicides are employed to clear roadside weeds, trees and brush and are commonly applied in ponds and lakes to control algae and plants such as water grasses that can interfere with swimming and fishing.

The Pesticide Manual lists 10,400 product names associated with 1630 substances belonging to different chemical classes that are, or have been, used as pesticides [2]. The active ingredients are formulated in a variety of ways, such as liquids, dusts, granules, impregnated pellet-tablets, resin strips and concentrates. The formulations also contain a number of "inert" ingredients, such as solvents and surfactants that play an important role in the effectiveness of a pesticide product. These additives may have toxic effects different from the active ingredients.

Pesticides are used worldwide. The general population can be exposed to low concentrations of agricultural pesticides through contamination of air, water, food supplies [3], and also through household use [4]. High exposures are associated with the production, packaging and application of these compounds in agriculture or for purposes of protection of public health such as malaria prevention. Three main routes are relevant for the exposure to pesticides: inhalation by breathing air containing pesticide aerosol or small particles with adsorbed pesticide, dermal absorption during scattering, mixing and loading of powders and liquids, spraying and crop harvesting, and oral ingestion of contaminated food or water.

Acute pesticide poisoning, associated with specific chemical classes or compounds is a major public health problem, mainly in developing countries. Each year, at least 300 million people worldwide are estimated to suffer from acute pesticide poisoning [5,6].

A number of pesticides have been characterized as possible or probable human carcinogens by IARC based on human and experimental animal data showing links between some pesticides and cancer at multiple sites [7]. Cancers of the lung, prostate, lymphatic and hematopoietic system are most frequently associated with pesticide exposure in epidemiological studies [8,9]. For example, pesticides from different chemical and functional classes were related with an excess risk of non-Hodgkin lymphoma [10]. Moreover, a recent study suggests that occupational maternal pesticide exposure during pregnancy, and paternal exposure prior to conception, may increase the risk of leukemia in the offspring [11].

Epidemiological findings, along with experimental laboratory data, reveal a positive association between pesticide exposure and different neurodegenerative disorders such as Parkinson's disease (PD) [12], Alzheimer's disease (AD) [13], amyotrophic lateral sclerosis (ALS) [14]. Other studies demonstrated that exposure to pesticides may cause other diseases or health effects, such as neurodevelopmental toxicity [15], non-malignant respiratory diseases [16], diabetes [17] and immune toxicity [18]. A number of studies suggest that an interaction between certain gene polymorphisms and exposure to pesticide increases the risk for PD [19], adverse birth outcomes [20], and neurodevelopmental problems [21–23].

Pesticides can exert their toxic effects to humans through various modes of action either related or different from their mechanism of action against the target "pest" [24]. The genotoxic potential of pesticides has been extensively studied as a primary risk factor for long-term health effects such as cancer and degenerative diseases. The majority of pesticides in use have been tested in a wide variety of mutagenicity assays that cover gene mutations, chromosomal alterations, and DNA damage. Experimental data revealed that many agrochemical substances are mutagenic, and induce different genetic endpoints [25]. As a consequence, many pesticides were banned from the market or restricted in use for their carcinogenic and/or genotoxic properties, but due to their bioaccumulation potential, could persist in environment for years.

Current regulations concerning the introduction of pesticides to the market (e.g. Dir. 91/414/EEC; US-EPA regulations) involve the evaluation of the active substances in a comprehensive number of tests in different biological systems *in vitro* and *in vivo* and, do not allow the use of pesticide formulations containing carcinogenic and/or genotoxic components. However, the exclusion of genotoxic potential for single compounds introduced in the market as pesticides may not prevent long-term risk for humans associated with the practical use of agrochemical formulations (including inert ingredients) possibly interacting with each other.

The biomonitoring of genotoxicity in human populations is a useful tool to estimate the exposure and potential risk from an integrated exposure to complex mixtures of chemicals. Biomonitoring studies in different countries have been carried out to elucidate the risk associated to the exposure to specific compounds and pesticide-related occupations, or to specific cultivation practices.

The aim of this review is to retrieve, analyze and summarize published studies that used the lymphocyte cytokinesis-block micronucleus (L-CBMN) in pesticide exposed subjects to:

- Confirm L-CBMN as an informative biomarker associated with exposure to pesticides
- Assess the genotoxicity of pesticides in specific occupational and environmental settings

Table 1
L-CBMN and other genotoxicity assay studies in pesticide exposed subjects: chemical plant workers.

Country Exposure	Number of participants exposed and controls (sex and age)	Exposure period Employment time (years)	Exposure measurement (type and value)	Number of cells evaluated in L-CBMN assay	QS	Results (MN‰ Exposed vs Control)		FR	Reference
Brazil Chemical plant Organochlorines: carbon tetrachloride (group IARC 2B), perchloroethylene (group IARC 2A) HCB (group IARC 2B)	Exposed 41 m (37 y) Controls: 28m (37 y) healthy subjects recruited in the same area without pesticide exposure	Mean 9 y of work in the company	Exposed to complex mixtures (carbon tetrachloride, perchloroethylene HCB) Serum concentrations of HCB: Exposed: 0.4 μg/ml Controls: No detectable	500 BN or quadrinucleated cells	11/27	L-CBMN No correlation with age, smoke, working time and serum concentration of HCB	↑ 9.0‰ vs 2.5‰ ***	3.6	Da Silva et al. [28]
Croatia Chemical plant 2,4-D (group IARC 2B), Atrazine, alaclor, cyanazine, malathion (group IARC 2A)	Exposed 17m + 3f (20) (44.52 ± 9.57/ 44.67 ± 2.51) Controls from the general population 12m + 8f (20) (32.25 ± 7.67/ 29.75 ± 7.90)	22.25 y (4–30)	Not available	500 BN cells	10/27	After 8 months of exposure L-CBMN CA (200 MF) SCE (50 cells) Comet T length After 8 months without exposure L-CBMN CA (200 M) SCE (50 cells) Comet T length	↑ 26.5‰ vs 7.3‰* ↑ 12.05% vs 1.95%*** ↑ 6.62/cell vs 2.96/cell ↑ 46.9 vs 12.0 μm* ↑ 13.6‰ vs 7.3‰ * ↑ 4.71% vs 1.95 %*** ↑ 5.64/cell vs 2.96/ cell*** ↑ 27.5 vs 12.0 μm*	3.63 6.18 2.23 3.9 1.86 2.41 1.9 2.3	Garaj-Vrhovac and Zeljezic [29]
Croatia Chemical plant 2,4-D(group IARC 2B), Atrazine, alaclor, cyanazine, malathion	Exposed 7m + 3f (10) (34.3 ± 9.27/ 44.67 ± 2.51) Controls from the general population 12m + 8f (20) (32.25 ± 7.67/ 29.75 ± 7.90)	22.25 y (4–30)	Not available	500 BN cells	10/27	L-CBMN CA (200 MF) Comet T length	↑ 54.0‰ vs 7.6‰ ** ↑ 13.2 % vs 1.95 %* ↑ 50.13 vs 13.06 μm **	7.1 6.7 3.7	Garaj-Vrhovac and Zeljezic [30]
Pakistan *Pesticide production industry* Organophosphates and pyrethroids	Exposed 29m (34.17 ± 2.96) Controls: healthy subjects recruited in the same area 35m (35.20 ± 3.52)	13.48 ± 3.84 y	Exposure to complex mixtures organophosphates pyrethroids ALT, AST, ALP (↑) Serum cholinesterase: ↓ 8174.59 vs 11715.79	1000 BN cells	15/27	L-CBMN Time-dependence	↑ 12.62‰ vs 6.11‰***	2.1	Bhalli et al. [31]
Croatia Carbofuran production	Exposed 21m + 9f (30) (41.1 ± 1.99) *Controls were* selected from general population 21m + 9f (30) (40.2 ± 1.97)	continuously exposed to active *ingredients for* at least 4 months 15.7 y	No inhibition of serum acetyl-cholinesterase	2000 BN cells Giemsa staining	16/27	L-CBMN NB NBP Comet Tail length Effect of age, alcohol and *smoke and duration of* exposure	↑ 13.4‰ vs 7.4‰ ** ↑ 9.5‰ vs 3.6‰** ↑ 2.3‰ vs 0.3‰* ↔ 14.4 vs 13.9μm	1.8 2.6 7.6	Zeljezic *et al.* [32]

Abbreviations: Males abbreviated as "m", females as "f", years of age indicated as mean or mean ± SD; IARC classification: Group 1: carcinogenic to humans; Group 2A probably carcinogenic to humans; Group 2B possibly carcinogenic to humans; group 3 not classifiable as to its carcinogenicity to humans; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; QS, quality score based on the following parameters: (i) age-matching, (ii) gender-matching, (iii) smoking status-matching, (iv) alcohol intake-matching, (v) nutritional intake- matching, (vi) appropriate measurement of chemical exposure (see Table 1) 27 is the perfect score; BN, binucleated lymphocytes; CA, chromosomal aberrations; M, metaphases; SCE, sister chromatid exchange; NPB, nucleoplasmic bridges; NBUD, nuclear buds; Comet median t – tail length; FR, Frequency Ratio (MN frequency in exposed subjects/MN frequency in Controls); ↑, significant increase; ↓, significant decrease (number following arrow in brackets indicates mean ratio); ↔, no effect; *p < 0.005; **p < 0.001; ***p < 0.0001; NS, not statistically significant.

Table 2
L-CBMN and other genotoxicity assay studies in pesticide exposed subjects. Single compound use by pesticide sprayers.

Country Exposure	Number of participants: exposed and controls (sex and age)	Exposure period Employment time (years) Use of personal protection equipment (PPE)	Exposure measurement (type and value)	Number of cells	QS	Results (MN‰; Exposed vs Control)	FR	Reference
Australia Phosphine fumigation	Exposed 31 fumigators Controls 21 controls working at the same sites	170–200 fumigations per year during Dec–March 1.5–32 y	Environmental monitoring of phosphine at fumigation sites <2.4 ppm/h	1200 BN cells	14/27	L-CBMN ↔ 6.9‰ vs 7.1‰ Age effect		Barbosa and Bonin [33]
USA Medfly eradication program Malathion (group IARC 2A) fumigation	Exposed 29m + 9f (38) (32.4 ± 9.6) Controls: office workers and supervisors 9m + 7f (16) (34.6 ± 5.2)	Exposure during the spraying season (Sept–Nov)	Malathion diacid metabolite: post-shift mean 274 ppb (<5–2200 ppb)	1000 BN cells DAPI staining	12/27	L-CBMN ↔ 17.1‰ vs 18.7‰ No correlation with metabolite in urine		Titenko-Holland et al. [34]
USA Medfly eradication program Malathion fumigation	1992 Exposed 13 (m + f) Controls 4 (m + f) Sept 1993 Exposed 24 (m + f) Controls 10 (m + f) Dec 1993 Exposed 14 (m + f) Controls 6 (m + f)	Exposure during the spraying season (Sept–Nov)	Malathion diacid metabolite	1000 BN cells DAPI staining	11/27	1992 L-CBMN ↔ 20.1‰ vs 14.3‰ Malathion diacid metabolite Not detected ↔ 14.5‰ vs 14.3‰ >100 ppb ↔ 23.0‰ vs 14.3‰ 1993 Sept L-CBMN ↔ 19.4‰ vs 22.1‰ Dec L-CBMN ↔ 17.0‰ vs 18.2‰		Windham et al. [35]
USA Fumigation with methyl bromide (IARC group 3)	Exposed 31m (33.9) Controls: neighbors and friends recruited by exposed workers 27m (32.9)	Employed in fumigation for at least 6 months	Median exposure during 3 weeks preceding the examination: 4 h	1000 BN cells DAPI staining	15/27	L-CBMN ↔ 7.23‰ vs 4.73‰ Oropharyngeal cells MNC ↔ 2.07‰ vs 1.31‰		Calvert et al. [36]
Eastern Kansas (USA) 2,4-D (group IARC 2B) pesticide applicators	Exposed 12 m (27.5 ± 12.5) Controls 9m (24.7 ± 4.3)	4.5 ± 1.9 h of spraying before sampling. Average cumulative time of spraying: 204 h	Urine analysis: Mean 2,4-D concentration: 240 ± 100 ppb	1000 BN cells Giemsa staining	16/27	Before exposure L-CBMN ↔ 11.6‰ vs 11.0‰ After exposure L-CBMN ↔ 9.1‰ vs 11.0‰ RI Before ↔ 1.33 vs 1.29 After ↑ 1.47 vs 1.29**		Figgs et al. [37] Holland et al. [38]
Greece Tobacco field spraying with Metalaxyl and imidacloprid	Exposed 11m (38.2 ± 4.05) Controls: healthy subjects 11m (43.82 ± 3.76)	Duration of spraying: 4–6 h 23.64 ± 4.13	Use of PPE: 50%	1000 BN cells Giemsa staining	11/27	Before exposure L-CBMN ↔ 12.82‰ vs 9.27‰ After exposure L-CBMN ↔ 17.00‰ vs 9.27‰**		Vlastos et al. [39]
Colombia Glyphosate (group IARC 2A) aerial spraying for control illicit crops	Exposed Area 1 Putumayo 29m + 29f (58) (31.4 ± 7.2) Area II Narino 31m + 31f (58) (32.5 ± 7.4) Valle del Cauca 14m + 14f (28) (33.4 ± 8.7) Controls: healthy volunteers from an area where organic coffee is grown without the use of pesticides 30m + 30f (60) 27 ± 5.6	Scheduled air spraying of glyphosate	Application for crop eradication: Glyphosate 3.6 kg/ha mixed with an adjuvant (Cosmoflux)	2000 BN cells Giemsa	18/27	Before spray Putumayo L-CBMN ↑ 3.61‰ vs 1.83‰** Narino L-CBMN ↑ 4.14‰ vs 1.83‰** Valle del Cauca L-CBMN ↑ 5.75‰ vs 1.83‰ Immediately after spray Putumayo L-CBMN ↑ 4.64‰ vs 1.83‰**[1] Narino L-CBMN ↑ 5.98‰ vs 1.83‰**[1] Valle del Cauca L-CBMN ↑ 8.64‰ vs 1.83‰**[1] Four months after spray L-CBMN	1.9 2.4 3.1 2.5 3.3 4.72 3	Bolognesi et al. [40]

Table 2 (Continued)

Country Exposure	Number of participants: exposed and controls (sex and age)	Exposure period Employment time (years)	Exposure measurement (type and value) Use of personal protection equipment (PPE)	Number of cells	QS	Results (MN‰; Exposed vs Control)	FR	Reference
						↑5.61‰ vs 1.83‰**		
						Nariño L-CBMN ↑3.91‰ vs 1.83‰**[l]	2.1	
						Valle del Cauca L-CBMN ↑7.38‰ vs 1.83‰**	4	
						m. Significant increase post vs before spray n. Significant decrease after spray		

Abbreviations: Males abbreviated as "m", females as "f", years of age indicated as mean or mean ± SD; IARC classification: Group 1: carcinogenic to humans; Group 2A probably carcinogenic to humans; Group 2B possibly carcinogenic to humans; group 3 not classifiable as to its carcinogenicity to humans; QS, quality score based on the following parameters: (i) age-matching, (ii) gender-matching, (iii) smoking status-matching, (iv) alcohol intake-matching, (v) nutritional intake- matching, (vi) appropriate measurement of chemical exposure (see Table 1) 27 is the perfect score; BN, binucleated lymphocytes; MNC, micronucleated cells; RI, replicative index; ↑, significant increase; ↓, significant decrease (number following arrow in brackets indicates mean ratio); ↔, no effect; *$p < 0.005$; **$p < 0.001$; ***$p < 0.0001$; NS, not statistically significant; FR Frequency Ratio (MN frequency in exposed subjects/MN frequency in Controls).

- Identify the knowledge gaps and need for future studies.

2. Materials and methods

This review follows the methodology described in the PRISMA statement [26]. Specifically, a literature search was carried out up to December 2015 through *MedLine/PubMed* and *TOXLINE* electronic databases. Key search terms included "micronucleus" and "micronuclei" in combination with "pesticide", "insecticide", "herbicide", "fungicide", "agriculture", and "farmer", supplemented by an internet-based search using Google. A manual search of the reference list of studies and review articles was subsequently performed. Reference sections of retrieved articles were also analyzed to identify any publications which may have been potentially missed in the initial search. The first author (C.B.) did the initial selection based on titles and abstracts. Eligible for the inclusion in the present review were all studies which concerned the application of L-CBMN assay in groups of subjects occupationally or environmentally exposed to pesticides. Only studies where a full English text was available were included.

The articles considered eligible for the review were analyzed with respect to their quality, considering the most relevant parameters, each one scoring from 1 to 3 points. The parameters assessed included: i) number of subjects in control and in exposed groups (1 = <20, 2 = 20–50, 3 = >50); ii) age-,gender-, smoking status-, alcohol intake-, nutritional intake- matching (1 = significantly different or no data collected; 2 = not statistically different; 3 = perfectly matched); iii) the measurement of the exposure (1 = assessed by questionnaire only, 2 = measurement in ambient environment, 3 = measurement in body fluids); iv number of cells scored per subject (1 = <1000, 2 = 1000–2000, 3 = ≥2000). A maximum score was 27 based on the range between 1 and 3 for 9 assessment categories.

The quality scores for individual studies reported in Tables 1–7 summarize the results of analysis of different exposure settings. The selected studies were evaluated considering the demographic characteristics, data on the conditions of occupational exposure, specific tasks and exposure assessment as well as results of the L-CBMN assay and other available biomarkers.

For each study we calculated the ratio between the mean frequency of micronuclei (MN) or of binucleated cells with MN (MNBN) for exposed and control groups (frequency ratio, FR) in order to reduce the bias caused by inter-laboratory variability. The correlation between the MN frequency and data on chemical exposure, if available, was evaluated. The agreement of findings in the MN assay with other DNA damage tests applied in the same groups of subjects was also addressed.

3. Results

The literature search identified 1114 articles, of which 1047 were excluded because they were related to experimental studies or to the use of MN assay in tissues other than lymphocytes. The remaining 67 articles were further evaluated and an additional 12 papers were excluded because the MN assay was in buccal cells, or the publications were review articles or not in English. As a result of this search process, a total of 55 full-text articles, related to 49 studies were included in the present review.

A single study is available on the evaluation of cytogenetic damage in patients diagnosed with pesticide poisoning. In a group of 40 subjects admitted at the Emergency Department of an agricultural region in the Southern Turkey a significant increase of MN and CA, but not SCE was detected at the hospital discharge with respect to the values at the admission [27].

All other studies were analyzed with respect to the conditions of pesticide exposure and the chemical classes of compounds applied in order to group the studies with common characteristics. These categories included chemical plant workers (Table 1), pesticide sprayers (Tables 2 and 3), floriculturists (Table 4), agricultural workers (Table 5), exposure without spraying (Table 6), and non-occupational exposure (Table 7). All these categories are described in detail in the following sections of the review.

3.1. L-CBMN in chemical plant workers

Five studies on workers exposed to mixture of compounds in chemical plants producing pesticides belonging to different chemical classes and classified as possible carcinogens and/or genotoxicants were identified (Table 1 and Fig. 1).

A study of 41 male workers exposed to a complex mixture of organochlorides, mainly perchloroethylene, carbon tetrachloride and hexachlorobenzene (HCB) at the plant in the southeast of Brasil reported higher frequencies of MN in peripheral lymphocytes compared with the control group (N = 28) with a frequency ratio of 3.6 [28]. No correlations with age, smoking, or length of

Table 3
L-CBMN and other genotoxicity assay studies in pesticide exposed subjects: Use of mixture of compounds by pesticide sprayers.

Country Exposure	Number of participants: exposed and controls (sex and age)	Exposure period Employment time (years)	Exposure measurement (type and value) Use of personal protection equipment (PPE)	Number of cells evaluated in L-CBMN assay	QS	Results (MN‰; Exposed vs Control)		FR	Reference
Vineyard									
Serbia Vineyard workers	Exposed 27m Non smoking (39 ± 6.1) Controls I. 15m (42.3 ± 7) nonsmoking school teachers from the same area II. 20m Nonsmoking students and employers outside the vine growing area	Spraying period: 3 days-1week 2–9 working hrs/day Average: 4 h/day 6 months/y 12.1 years	Use mixtures of agrochemical formulations (9 listed) dissolved in organic solvents (xylene) Mostly used: Diazinon Cineb	1000 BN cells Giemsa	14/27	Pre-spraying period L-CBMN vs Controls I vs Controls II Unstable CA (100 MF) vs Controls I vs Controls II SCE (50 cells) vs Controls I vs Controls II One-month after spraying L-CBMN vs Controls I vs Controls II Unstable CA (100 M) vs Controls I vs Controls II At the end of spraying season L-CBMN vs Controls I vs Controls II Unstable CA (100M) vs Controls I vs Controls II SCE (50 cells) vs Controls I vs Controls II	↔ 5.41‰ vs 5.90‰ ↔ 5.41‰ vs 5.09‰ ↔ 0.13% vs 0.067% ↔ 0.13% vs 0.050% ↔ 5.41/cell vs 4.96/cell ↔ 5.41/cell vs 4.83/cell ↑ 17.8‰ vs 5.90‰** ↑ 17.8‰ vs 5.09‰ ** ↔ 0.22% vs 0.067% ↔ 0.22% vs 0.050% ↑ 39.9‰ vs 9.63‰** ↑ 39.9‰ vs 5.20‰** ↑ 0.79% vs 0.064% ↑ 0.79% vs 0.05% ↔ 6.2/cell vs 6.0/cell ↔ 6.2/cell vs 6.0/cell	3 3.5 3.2 4.4 4.1 7.6 12.3 15.8	Joksic et al. [42]
Greece Vineyards and olive tree culture	Exposed 11m (46.45 ± 4.21) Controls: healthy volunteers living in the same area 11m (42.18 ± 3.38)	Spraying 6–100 acres 2–15 times/y 26.45 ± 3.38 y	Use of mixture of agrochemical formulations including insecticides, fungicides and herbicides (17 listed) Mostly used: parathion, cypermethrin, paraquat, glyphosate Use of PPE: 50%	1000 BN cells Giemsa	12/27	L-CBMN MN	↔ 8.0‰ vs 6.20‰ ↑ 8.73‰ vs 6.20‰*	1.4	Vlastos et al. [43]
Brazil Vineyard workers	Exposed 108m (41.6 ± 11.6) Controls: office employers living in the same area 65m (37.8 ± 10.6)	Spraying in open field Exposed to pesticides 2–3 times/week About 400 h/y	Complex mixtures of compounds which changed according with the weather Mostly used: carbamates and organophosphates Use of PPE: 90%	2000 BN cells Giemsa	17/27	L-CBMN Comet : damage index PON1 polymorphisms: *Gln/Gln vs Gln/Arg* or *Arg/Arg* (48 vs 48) Combined *PON1* and *OGG1* *PON Gln/Arg* and *OGG1 SEr326Cys* (29 exposed *vs* 21 controls)	↑ 7.34‰ vs 4.33‰ ↑ 20.26 vs 4.42 ↑ 8.44‰ vs 6.23‰* 9.21‰ vs 3.39‰**	1.7 4.6 1.35 2.7	Da Silva et al. [44] Rohor et al. [45]
Tobacco									
Brazil Tobacco farmers	Exposed 111 (65m+47f) (42.4 ± 14.07) Controls: office workers	2 times at 5 months interval: Pesticide application	Exposure to complex mixture of pesticides	2000 BN cells Giemsa	17/27	Pesticide application L-CBMN Comet Damage index	↑ 6.83‰ vs 5.22‰ **	1.3 2.9	Da Silva et al. [46]

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)	2,228 (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	1,087 (9.0)	3,021 (21.2)	8,031 (35.2)
Diazinon	1,908 (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	78.3	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	83	73.0	1.0 (0.6–1.7)	1.6 (0.7–3.8)[e]
Bladder	79	78.0	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)[e]	
	57–2,678	15	0.9 (0.4–1.7)[e]	0.54	337.2–18,241	30	1.4 (0.8–2.5)[e]	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; Potrern 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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International Agency for Research on Cancer

World Health Organization

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IARC Monographs evaluate DDT, lindane, and 2,4-D

Lyon, France, 23 June 2015 - The International Agency for Research on Cancer (IARC), the specialized cancer agency of the World Health Organization, has evaluated the carcinogenicity of the insecticides gamma-hexachlorocyclohexane (lindane) and dichlorodiphenyltrichloroethane (DDT) and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D).

After thoroughly reviewing the latest available scientific literature, a Working Group of 26 experts from 13 countries convened by the IARC Monographs Programme classified the insecticide lindane as *carcinogenic to humans* (Group 1). There was *sufficient evidence* in humans for the carcinogenicity of lindane for non-Hodgkin lymphoma (NHL).

The insecticide DDT was classified as *probably carcinogenic to humans* (Group 2A), based on *sufficient evidence* that DDT causes cancer in experimental animals and *limited evidence* of its carcinogenicity in humans. Epidemiological studies found positive associations between exposure to DDT and NHL, testicular cancer, and liver cancer. There was also strong experimental evidence that DDT can suppress the immune system and disrupt sex hormones. However, overall there was no association between breast cancer and DDT levels measured in samples of blood or fat.

The herbicide 2,4-D was classified as *possibly carcinogenic to humans* (Group 2B), based on *inadequate evidence* in humans and *limited evidence* in experimental animals. There is strong evidence that 2,4-D induces oxidative stress, a mechanism that can operate in humans, and moderate evidence that 2,4-D causes immunosuppression, based on in vivo and in vitro studies. However, epidemiological studies did not find strong or consistent increases in risk of NHL or other cancers in relation to 2,4-D exposure.

A summary of the final evaluations is available online in *The Lancet Oncology*, and the detailed assessments will be published as Volume 113 of the IARC Monographs.

Lindane has been used extensively for insect control, including in agriculture and for treatment of human lice and scabies. High exposures have occurred among agricultural workers and pesticide applicators; however, the use of lindane is now banned or restricted in most countries. Large epidemiological studies of agricultural exposures in the USA and Canada showed a 60% increased risk of NHL in those exposed to lindane.

DDT was introduced for the control of insect-borne diseases during the Second World War and was later applied widely to eradicate malaria and in agriculture. Although most uses of DDT were banned from the 1970s, DDT and its breakdown products are highly persistent and can be found in the environment and in animal and human tissues throughout the world. Exposure to DDT still occurs, mainly through diet. The remaining and essential use of DDT is for disease vector control, mainly for malaria. This use is strictly restricted under the Stockholm Convention.

Since its introduction in 1945, 2,4-D has been widely used to control weeds in agriculture, forestry, and urban and residential settings. Occupational exposures to 2,4-D can occur during manufacturing and application, and the general population can be exposed through food, water, dust, or residential application, and during spraying.

IARC Monographs evaluate DDT, lindane, and 2,4-D

Note to the Editor:

What does the classification mean in terms of risk?
The classification indicates the *strength of the evidence* that a substance or agent causes cancer. The Monographs Programme seeks to identify cancer hazards, meaning the potential for the exposure to cause cancer. However, it does not indicate the *level of risk* associated with exposure. The cancer risk associated with substances or agents assigned the same classification may be very different, depending on factors such as the type and extent of exposure and the strength of the effect of the agent.

What is the difference between risk and hazard?
The IARC Monographs Programme evaluates cancer hazards but not the risks associated with exposure. An agent is considered a *cancer hazard* if it is capable of causing cancer under some circumstances. *Risk* measures the probability that cancer will occur, taking into account the level of exposure to the agent. The distinction between *hazard* and *risk* is important, and the Monographs Programme identifies cancer hazards even when risks are very low at current exposure levels, because new uses or unforeseen exposures could engender risks that are significantly higher.

Read the *IARC Monographs* Q&A

http://www.iarc.fr/en/media-centre/iarcnews/pdf/Monographs-Q&A.pdf

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The International Agency for Research on Cancer (IARC) is part of the World Health Organization. Its mission is to coordinate and conduct research on the causes of human cancer, the mechanisms of carcinogenesis, and to develop scientific strategies for cancer control. The Agency is involved in both epidemiological and laboratory research and disseminates scientific information through publications, meetings, courses, and fellowships. If you wish your name to be removed from our press release e-mailing list, please write to com@iarc.fr.

Increased Cancer Burden Among Pesticide Applicators and Others Due to Pesticide Exposure

Michael C. R. Alavanja, DrPH[1]; Matthew K. Ross, PhD[2]; Matthew R. Bonner, PhD, MPH[3]

A growing number of well-designed epidemiological and molecular studies provide substantial evidence that the pesticides used in agricultural, commercial, and home and garden applications are associated with excess cancer risk. This risk is associated both with those applying the pesticide and, under some conditions, those who are simply bystanders to the application. In this article, the epidemiological, molecular biology, and toxicological evidence emerging from recent literature assessing the link between specific pesticides and several cancers including prostate cancer, non-Hodgkin lymphoma, leukemia, multiple myeloma, and breast cancer are integrated. Although the review is not exhaustive in its scope or depth, the literature does strongly suggest that the public health problem is real. If we are to avoid the introduction of harmful chemicals into the environment in the future, the integrated efforts of molecular biology, pesticide toxicology, and epidemiology are needed to help identify the human carcinogens and thereby improve our understanding of human carcinogenicity and reduce cancer risk. CA Cancer J Clin 2013;63:120-142. ©2013 American Cancer Society.*

Keywords: *pesticides, cancer burden, carcinogen, risk, environmental cancer, public health*

Introduction

A comprehensive and successful strategy for minimizing cancer risk from pesticide use should combine research initiatives aimed at identifying pesticides that are human carcinogens with policies that attempt to reduce these exposures to workers and the general public. In this discussion, pesticides are defined as a diverse group of chemical formulations used to control pests, including insects, molds, and unwanted plants.

Pest problems in public health (ie, vectors of disease), agriculture, and commerce are not static because pests develop resistance to widely used pesticides and are periodically introduced to new geographic areas without effective natural controls. Historically, the evolution of new pests has resulted in the development of new pesticides, followed shortly thereafter by new pesticide problems, such as pest resistance and unintended toxicities. In the United States and other developed countries, regulatory toxicity testing has kept many genotoxic chemicals and animal carcinogens out of the market place.[1] An incomplete understanding of human carcinogenicity, however, seems to have resulted in allowing some human carcinogens on to the worldwide market, resulting in excess cancer risk to those who are highly exposed and those who are particularly vulnerable.[2,3] For example, an International Agency for Research on Cancer (IARC) monograph published in 1991 stated, "occupational exposures in spraying and application of non-arsenical insecticides" as a group are classified as "probable human carcinogens" (category 2A),[2] yet the identification of specific pesticides as human carcinogens has not yet been made. If current regulatory toxicity testing has been inadequate, new data from toxicology and cancer biology will need to be used in conjunction with epidemiology to help improve our regulatory procedures and more reliably identify human carcinogens.

Rather than wait for human carcinogens to be identified, several European countries, including Sweden, Denmark, the Netherlands, and others, have initiated pesticide use reduction policies that have resulted in substantially diminished

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TABLE 1. Most Commonly Used Conventional Pesticide Active Ingredients, Agricultural Market Sector, 2007 Estimates, Ranked by Range in Millions of Pounds of Active Ingredient

ACTIVE INGREDIENT	FUNCTIONAL CLASS	CHEMICAL CLASS	RANK	RANGE
Glyphosate	Herbicide	Phosphinic acid	1	180-185
Atrazine	Herbicide	Triazine	2	73-78
Metam sodium	Fumigant	Dithiocarbamate	3	50-55
S-metolachlor	Herbicide	Acetamide	4	30-35
Acetochlor	Herbicide	Acetamide	5	28-33
1,3-dichloropropene	Fumigant	Organochlorine	6	27-32
2,4-D	Herbicide	Phenoxy acid	7	25-29
Methyl bromide	Fumigant	Methyl halide	8	11-15
Chloropicrin	Fumigant	Organochlorine	9	9-11
Pendimethalin	Herbicide	Dinitroaniline	10	7-9
Ethephon	Plant growth regulator	Ethylene generator	11	7-9
Chlorothalonil	Fungicide	Phthalamide	12	7-9
Metam potassium	Fumigant	Dithiocarbamate	13	7-9
Chlorpyrifos	Insecticide	Organophosphate	14	7-9
Copper hydroxide	Fungicide	Inorganic alkali	15	6-8
Simazine	Herbicide	Triazine	16	5-7
Trifluralin	Herbicide	Dinitroaniline	17	5-7
Propanil	Herbicide	Anilide	18	4-6
Mancozeb	Fungicide	Dithiocarbamate	19	4-6
Aldicarb	Insecticide	Carbamate	20	3-4
Acephate	Insecticide	Organophosphorus	21	2-4
Diuron	Herbicide	Urea	22	2-4
MCPA	Herbicide	Phenoxy acid	23	2-4
Paraquat (dipyridylium)	Herbicide	Bipyridal	24	2-4
Dimethenamid	Herbicide	Acetamide	25	2-4

2,4-D indicates 2,4-dichlorophenoxyacetic acid; MCPA, 2-methyl-4-chlorophenoxyacetic acid.

Source: US Environmental Protection Agency Office of Pesticide Programs. Pesticide Industry Sales and Usage. 2006 and 2007 Market Estimates. Washington, DC: US Environmental Protection Agency; 2007. Available from: epa.gov/pesticides/pestsales/07pestsales/usage2007_2.htm. Accessed November 27, 2012.

pesticide use overall.[4] In the United States, a nationwide use reduction policy has met with resistance politically because of disagreements about the net benefit to health and debate concerning the disproportionate economic impact of these policies on selected groups (eg, farmers, food processors, and pesticide manufacturers) and on food prices.[1] The information available for these policy debates on cost-benefit are not yet equal since identifying the impact of pesticides on cancer risk has been difficult and progress relatively slow, while estimating the immediate economic impact of pesticide use reduction policies on agriculture and commerce is more readily quantifiable. Since pesticides are pervasive in our environment, environmental health policy in the United States has instead focused on reducing human exposure to pesticides by controlling the methods and conditions of use.[1]

The active ingredients of pesticides are a very diverse array of chemical structures. Many pesticide structures are very complex and cannot be categorized simply. A convenient classification is based on the targeted pest (eg, herbicides, insecticides, fungicides, nematicides, and rodenticides). The classes may then be subdivided into smaller subclasses based on chemical structure. Herbicides account for the largest portion of total use, followed by other pesticides, insecticides, and fungicides. The amount of pesticide used in the US in both 2006 and 2007 exceeded 1.1 billion pounds.[5]

TABLE 2. Most Commonly Used Conventional Pesticide Active Ingredient in the Home and Garden Market Sector, 2007 and 2005 Estimates, Ranked by Range in Millions of Pounds of Active Ingredient

ACTIVE INGREDIENT	TYPE	CHEMICAL CLASS	RANK	RANGE
2,4-D	Herbicide	Phenoxy acid	1	8-11
Glyphosate	Herbicide	Phosphinic acid	2	5-8
Carbaryl	Insecticide	Carbamate	3	4-6
MCPP	Herbicide	Phenoxy_acid	4	4-6
Pendimethalin	Herbicide	Dinitroaniline	5	3-5
Pyrethroids	Insecticide	Pyrethroid	6	2-4
Malathion	Insecticide	Organophophorus	7	2-4
Dicamba	Herbicide	Benzoic_acid	8	1-3
Trifluralin	Herbicide	Dinitroaniline	9	1-3
Pelarganoc acid	Herbicide	Fatty acid	10	<1

2,4-D indicates 2,4-Dichlorophenoxyacetic acid; MCPP, methylchlorophenoxypropionic acid.

Does not include moth controls: paradichlorobenzene (30-35 million pounds per year) and naphthalene (2-4 million pounds per year). Also does not include insect repellent N,N-diethyl-meta-toluamide (5-7 millions pound per year).

Source: US Environmental Protection Agency Office of Pesticide Programs. Pesticide Industry Sales and Usage. 2006 and 2007 Market Estimates. Washington, DC: US Environmental Protection Agency; 2007. Available from: epa.gov/pesticides/pestsales/07pestsales/usage2007_2.htm. Accessed November 27, 2012.

The amount of pesticide used in the US accounted for 22% of the total world pesticide amount used, 25% of the world herbicide amount used, 10% of the world insecticide amount used, 14% of the world fungicide amount used, and more than 25% of other pesticide amounts used in both years.[6] The most highly used pesticides in agriculture, home and garden use, and government and commercial use are identified in Tables 1, 2, and 3.[5]

Pesticide Exposures and Control

Among members of the general public who are not applying pesticides, multiple routes of exposure are possible depending on whether the individual is an adult or a child, the location of their residence in relation to pesticide applications, whether a residence was treated with pesticides, the occupations of household members, the volatility of the compound, the persistence of the pesticides

TABLE 3. Most Commonly Used Conventional Pesticide Active Ingredients in the Industry/Commercial/Government Market Sector, 2007, 2005, 2003, and 2001 Estimates, Ranked by Range in Millions of Pounds of Active Ingredient

ACTIVE INGREDIENT	TYPE	CHEMICAL CLASS	RANK	RANGE
2,4-D	Herbicide	Phenoxy acid	1	19-22
Glyphosate	Herbicide	Phosphinic acid	2	13-15
Chlorothalonil	Fungicide	Phthalimide	3	3-5
MSMA	Herbicide	Organoarsenic	4	2-4
Diuron	Herbicide	Urea	5	2-4
Pendimethalin	Herbicide	Dinitroaniline	6	2-4
Triclopyr	Herbicide	Organochlorine	7	2-4
Copper sulfate	Fungicide	Inorganic sulfate	8	2-4
Malathion	Insecticide	Oganophosphorous	9	1-3
Sulfuryl fluoride	Insecticide	Inorganic fluoride	10	1-3

2,4-D indicates 2,4-dichlorophenoxyacetic acid; MSMA, monosodium methyl arsenate.

Includes applications to homes and gardens by professional applicators. Does not include sulfur or petroleum oil. Due to lack of data, the same estimate is used for both 2005 and 2007 in this report.

Source: US Environmental Protection Agency Office of Pesticide Programs. Pesticide Industry Sales and Usage. 2006 and 2007 Market Estimates. Washington, DC: US Environmental Protection Agency; 2007. Available from: epa.gov/pesticides/pestsales/07pestsales/usage2007_2.htm. Accessed November 27, 2012.

TABLE 4. Routes of Pesticide Exposure and Exposure Control Measures

SUBJECT	MAJOR ROUTES OF EXPOSURE	PREVENTIVE OR CORRECTIVE ACTION	REFERENCES
Pesticide applicator	Dermal	1. Use personal protective equipment including chemically resistant gloves. 2. Remove all pesticide-soiled clothing as soon as possible. 3. Wash or shower immediately following application. 4. Follow all pesticide label instructions.	14-18
	Ingestion	1. Do not eat, drink, or smoke during pesticide handling or application.	17
	Inhalation	1. Mix or load pesticides in a well-ventilated area. 2. Wear appropriate respiratory protective equipment according to pesticide label instructions.	14,17
Adult bystander and children's guardians	Dermal	1. Do not enter fields, lawns, or confined spaces where pesticides have been applied for the period specified on label instructions. Do not allow children to do so. 2. Interrupt take-home pathways: a. Encourage family members to remove shoes and other pesticide-soiled clothing outside the home if possible or as soon as possible after entering the home. b. Vacuum rug and/or clean floors if possibly soiled with pesticides. c. Do not store pesticides in living areas or anywhere within the reach of children. Keep all pesticides in a locked cabinet in a well-ventilated utility area or garden shed. 3. Keep children and pets away from areas where pesticides were applied. 4. Encourage family members exposed to pesticides to wash or shower as soon as possible after exposure. 5. Do not have pets enter the living areas of the home when soiled with pesticides until cleaned. 6. Wash clothing soiled with pesticides separately from other laundry.	6-13
	Ingestion	1. Never store pesticides in cabinets with or near food. 2. Always store pesticides in their original containers, complete with labels that list ingredients, directions for use, and first aid in case of accidental exposure. 3. Never transfer pesticides to soft drink bottles or other containers. 4. Rinse fruits and vegetables with water. Scrub with a brush and peel them if possible.	3,5,6,9,10,13
	Inhalation/general	1. Do not stockpile pesticides. Purchase only what you need for immediate application. 2. Follow the pesticide label directions for proper disposal. 3. Report any symptoms possibly related to pesticide exposure to your health care provider. When possible, report the name of the product, the ingredients, and the first aid instructions contained on the product label. 4. If a close neighbor or someone else is applying pesticides outdoors near your home, stay indoors with your children and pets. Keep windows and doors closed.	3,6,14,17
Regulatory agencies, scientific community, and chemical manufacturers	All	1. Identify human carcinogens and remove them from the market place or greatly curtail their use. 2. Identity the persistence and accumulation potential of pesticides and reduce the use of long-lived pesticides wherever possible. 3. Identify good pesticide work practices and educate the public in these practices. 4. Design more effective pesticide containers and application equipment that minimizes pesticide exposure to the applicator and to children who may come into contact with these containers.	3,5,6

in the environment, and several other chemical and physical properties of the pesticides (Table 4).[3,5-18] Pesticide applications to the home by a second party can result in both dermal and respiratory exposure. Other common routes of exposure to the general public include drinking water and dietary sources.[6] To minimize nonoccupational exposures to pesticides, EPA regulations have discouraged the use of the longer-lasting pesticides such as organophosphate (OP) insecticides in the home.[5] A trend toward the use of pyrethroids and other shorter-lived pesticides is resulting in lower OP exposures among the general public.[5]

The National Academy of Sciences[3] suggested that children may experience greater risk from pesticide exposure than adults because of the behavioral, dietary, and physiological characteristics associated with development. Among children, an important source of pesticide exposure results from diet[7]; for example, the consumption of organic produce is associated with a substantially lower concentration of urinary dialkylphosphate levels (which indicate organophosphorus pesticide exposure) than in those eating conventional foods,[7,8] but we do not have substantial evidence suggesting a cancer hazard associated with this exposure.[9] Another important source of pesticide

exposure results from the transfer of pesticides from a person who is occupationally exposed.[10] For example, urinary dialkylphosphate levels have been measured in studies of children and show parental occupation or their household proximity to farmland[7,8,10-12] and self-reported residential use of pesticides by parents[12,13] are important sources of childhood exposure (Table 4).[3,5-18]

Among adults applying liquid pesticides of low volatility, dermal exposures typically account for 90% of pesticide exposures.[14-16] The dermal penetration can vary between 2% and 20% if the pesticide is left on the skin for 8 hours or longer,[15] and therefore the use of proper protective equipment including chemical-resistant gloves and protective suits when handling the pesticide can substantially reduce exposure.[17] When the skin is immediately washed after pesticide use, a substantial additional reduction takes place.[14,18] A larger fraction of the exposure would be by the respiratory route among those applying more volatile pesticides (eg, flying insect spray) and other aerosols, and thus respiratory protection appropriate to the chemical being used is usually recommended (Table 4).[3-18]

To minimize nonoccupational exposures to pesticides, EPA regulations have discouraged the use of the longer-lasting and broad-spectrum pesticides. The lipophilic bioaccumulative organochlorine (OC) insecticides that were widely used in the mid-20th century were subsequently replaced by OPs, carbamates, and pyrethroids because these compounds were more environmentally labile and did not accumulate in the food chain to the same extent as the OCs. Moreover, compounds such as pyrethroids have become extremely attractive for pest control because they exhibit greater selective lethality toward insects compared with mammals.[19] Importantly, when humans are exposed to pyrethroids, OPs, and carbamates, the compounds are generally metabolized and eliminated from the body within 24 to 48 hours as water-soluble metabolites in urine. Physiologically based pharmacokinetic models that predict the internal dose of specific pesticides as a function of time are tools used to assess chemical dosimetry following exposures, although these models are more developed in animal studies than in humans.[20]

Since a total ban on the use of chemical pesticides is unlikely to happen in any country in the foreseeable future, ensuring cancer risk reduction from pesticides will depend on identifying pesticides that are human carcinogens. This review is not exhaustive, but rather it is focused on several cancers (ie, prostate cancer, non-Hodgkin lymphoma [NHL], adult and childhood leukemia, multiple myeloma, and breast cancer) where considerable progress has been made in identifying pesticides likely to be human carcinogens by synthesizing results from epidemiology, toxicology, and cancer biology. Although more than 800 active pesticide ingredients are currently on the market in the United States and other countries, only arsenical insecticides[2] and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (a contaminant of the phenoxy herbicide 2,4,5,-T) have been identified as human carcinogens by the IARC (category 1).[2] However, literature developed subsequent to publication of the IARC monograph suggests that chemicals in every major functional class of pesticides (ie, insecticides, herbicides, fungicides, and fumigants) are associated with human cancer. Table 5 presents a list of pesticides that have been carefully evaluated in well-designed epidemiological and/or toxicological/cancer biology studies for human carcinogenicity of the prostate, NHL, adult leukemia, and multiple myeloma.[21-68] While we discuss a potential link between pesticides and breast cancer and childhood leukemia because of widespread public anxiety about these cancers, no specific pesticide has yet been strongly linked to these cancers and therefore we do not include them in our table. The list is not exhaustive and considers only these 4 cancer sites because the literature is most developed for these sites. Other chemicals are likely to emerge as our understanding of pesticide-induced mechanisms of cancer etiology expands.

Mechanisms of Pesticide Toxicity

Pesticides have diverse chemical structures and exhibit a variety of biological modes of action in both target and nontarget organisms.[69] Following absorption into the body, pesticides are often biotransformed to water-soluble metabolites for the purpose of detoxification and elimination. Rates of biotransformation can be rapid (hours to days), as in the case of OP insecticides, or extremely slow (years to decades), as is noted for OC insecticides, which accounts for the bioaccumulation of these lipophilic compounds in adipose tissue. Multiple mechanisms are likely involved in pesticide-mediated carcinogenesis. Most of the published literature point toward oxidative stress and/or receptor-mediated mechanisms being important determinants, whereas inflammatory and aberrant epigenetic mechanisms caused by pesticide exposure are only in a preliminary stage of development and, consequently, there is not a lot of literature to support these mechanisms at this time. However, epigenetic modifications of tumor suppressor genes and oncogenes that alter their expression in tumors have been shown to be molecular drivers of cancer pathogenesis during the promotion and progression phases. Thus, this section will briefly focus on oxidative stress and receptor-mediated toxicities that are caused by pesticides.

TABLE 5. Epidemiological and Toxicological Evidence of Carcinogenicity for Selected Cancer Sites and Pesticides

CANCER SITE	PESTICIDE	CURRENT US EPA REGULATORY STATUS[a]	IARC CLASSIFICATION (YEAR)[b]	EXPOSURE SOURCE	EPIDEMIOLOGIC EVIDENCE	REFERENCE	TOXICOLOGICAL EVIDENCE	REFERENCE
Prostate	Fonofos (OP)	Not registered	Not evaluated	Occupational	1. Monotonic increase in risk of aggressive PC.	25	No direct evidence for PC.	—
					2. Significant interaction between exposure and genetic variants in 8q24, base excision repair, nucleotide excision repair.	23,24,91	Mutagenic in *S. typhimurium* and *S. cerevisae* genotoxicity assays.	26
	Terbufos (OP)	Registered	Not evaluated	Occupational	1. Monotonic increase in risk of aggressive PC.	25	No direct evidence for PC.	—
					2. Significant interaction between exposure and genetic variants in 8q24, base excision repair.	23,24	Mutagenic in *S. typhimurium* and *S. cerevisae* genotoxicity assays.	26
	Malathion (OP)	Registered	Group 3 (1987)	Occupational	1. Monotonic increase in risk of aggressive PC.	25	No direct evidence for PC.	—
					2. Positively associated with PC.	102		
	Permethrin (pyrethroid)	Registered	Not evaluated	Occupational	1. Significant interaction between exposure and genetic variants in 8q24.	24	No direct evidence for PC.	—
	Aldrin (OC)	Not registered	Group 3 (1987)	Occupational	1. Increased in risk of PC among men with a family history of PC.	25	No direct evidence for PCr;	—
							Hepatocarcinogenesis in mice through a nongenotoxic mode of action.	22
	Chlordecone (OC)	Not registered	Group 2B (1987)	Environmental	1. Increased risk of PC in highest exposure tertile.	29	Androgenic activity in cultured prostate cells.	28
	Lindane (HCH)	Not registered	Group 2B (1987)	Environmental	1. Serum concentrations positively associated with prevalence of PC.	32	Low levels of HCH alter androgen signaling in cultured prostate cells.	31
					2. Positively associated with PC.	102	Lindane induces micronuclei in cultured human prostate cells.	30
	DDT (OC)	Not registered	Group 2B (1991)	Occupational	1. Positively associated with PC.	102	DDE (environmental metabolite of DDT) can bind to androgen receptor in cultured prostate cells.	28
	Dieldrin (OC)	Not registered	Group 3 (1987)	Environmental	1. Serum concentrations positively association with prevalence of PC.	32	No direct evidence for PC.	—
							Induces oxidative stress and hepatocarcinogenic in mice through a nongenotoxic mode of action.	22
							Disrupt normal estrogen and androgen receptor function in cultured cells.	32
	Simazine (triazine)	Registered	Group 3 (1999)	Occupational	1. Positively associated with PC.	102	No direct evidence for PC.	—
	Atrazine (triazine)	Registered	Group 3 (1999)	Occupational	1. Not associated with PC.	105	No direct evidence for PC.	—
	Methyl bromide (methyl halide)	Registered	Group 3 (1999)	Environmental	1. Positively associated with PC.	35	Mutagenic in bacterial assays.	36
							DNA adducts (O^6-methylguanine) detected in rodent forestomach and liver.	36,37
	Oxychlordane (metabolite of chlordane, an OC)	Not registered	Group 2B (2001)	Environmental	1. No association with PC.	38-41	No direct evidence for PC.	—
	HCB (OC)	Not registered	Group 2B (2001)	Environmental	1. No association with PC.	40,113	Low levels of HCB enhance androgen signaling in cultured prostate cells and mouse prostate.	42
					2. Positively associated with PC.	39		
	Mirex (OC)	Not registered	Group 2B (1987)	Environmental	1. No association with PC.	40	No direct evidence for PC.	—
NHL	Lindane (HCH)	Not registered	Group 2B (1987)	Environmental	1. Positively associated with NHL with t(14:18).	43,52	No direct evidence for NHL.	—
					2. Positively associated with NHL.	44		
	Dieldrin (OC)	Not registered	Group 3 (1987)	Environmental	1. Positively associated with NHL with t(14:18).	43,52	No direct evidence for NHL.	—
					2. Positively associated with NHL.	54	Increased CYP1A and 1B expression in female rat liver, kidney, and mammary tissue.	50
					3. No association with NHL.	58,60,126		
	Toxaphene (OC)	Not registered	Group 2B (2001)	Environmental	1. Positively associated with NHL with t(14:18).	43,52	No direct evidence for NHL.	—

TABLE 5 (Continued)

CANCER SITE	PESTICIDE	CURRENT US EPA REGULATORY STATUS[a]	IARC CLASSIFICATION (YEAR)[b]	EXPOSURE SOURCE	EPIDEMIOLOGIC EVIDENCE	REFERENCE	TOXICOLOGICAL EVIDENCE	REFERENCE
	2,4-D (phenoxy herbicide)	Registered	Group 2B (1987)	Occupational	1. Positively associated with NHL. 2. No association with NHL.	121 62,123,124	No direct evidence for NHL. Increased CYP1A and 1B expression in female rat liver, kidney, and mammary tissue.	— 50
	MCPA (phenoxy herbicide)	Registered	Group 2B (1987)	Occupational	1. Positively associated with NHL. 2. Positively associated with NHL among those with asthma or hay fever.	47 61	No direct evidence for NHL.	—
	β-Hexachlorobenzene (a metabolite of HCB; chlorinated hydrocarbon)	Not registered	Group 2B (2001)	Environmental	1. Plasma concentrations positively associated with NHL.	127	No direct evidence for NHL.	—
	HCB (OC)	Not registered	Group 2B (2001)	Environmental	1. No association with NHL. 2. Plasma concentrations positively associated with NHL.	46,48,54,55 58,60,126 127	No direct evidence for NHL.	—
	TCDD (OC)	Not registered	Group 1 (2012)	Occupational	1. Positively associated with NHL mortality.	45	No direct evidence for NHL. Increased CYP1A and 1B expression in female rat liver, kidney, and mammary tissue.	— 50
	DDT (OC)	Not registered	Group 2B (1991)	Environmental	1. Positively associated with NHL. 2. No association with NHL.	48,55,60 126,127 46,54,56 59,131	No direct evidence for NHL.	—
	Chlordane/oxychlordane (OC)	Not registered	Group 2B (2001)	Environmental	1. Positively associated with NHL. 2. No association with NHL.	55,60,126,127 48,54,58	No direct evidence for NHL.	—
	Glyphosate (OP herbicide)	Registered	Not evaluated	Occupational	1. Positively associated with NHL.	47	No direct evidence for NHL.	—
	Atrazine (triazine)	Registered	Group 3 (1999)	Occupational	1. Superadditive effect in combination with alachlor, diazinon, and carbofuran 2. Positively associated with NHL with t(14;18).	128 52	No direct evidence for NHL.	—
	Mirex (OC)	Not registered	Group 2B (1987)	Environmental	1. Positively associated with NHL. 2. No association with NHL.	127 46	No direct evidence for NHL.	—
Adult leukemia	Fonofos (OP)	Not registered	Not evaluated	Occupational	1. Positively associated with leukemia.	148	No direct evidence for leukemia.	—
	Diazinon (OP)	Registered	Not evaluated	Occupational	1. Positively associated with leukemia.	149	No direct evidence for leukemia.	—
	Metribuzin (triazinone herbicide)	Registered	Not evaluated	Occupational	1. Positively associated with leukemia.	150	No direct evidence for leukemia.	—
	Alachlor (aniline herbicide)	Registered	Not evaluated	Occupational	1. Positively associated in the highest-exposure category only.	151	No direct evidence for leukemia.	—
	EPTC (thiocarbamate)	Registered	Not evaluated	Occupational	1. Positively associated in the highest-exposure category only.	65	No direct evidence for leukemia.	—
	Chlordane/heptachlor (OC)	Not registered	Group 2B (2001)	Occupational	1. Positively associated with leukemia.	44	No direct evidence for leukemia.	—
MM	Permethrin (pyrethroid insecticide)	Registered	Group 3 (1991)	Occupational	1. Positively associated with MM.	156	No direct evidence for MM.	—
	Captan (phthalimide fungicide)	Registered	Group 3 (1987)	Occupational	1. Positively associated with MM.	186	No direct evidence for MM.	—
	Carbaryl (carbamate insecticide)	Registered	Group 3 (1987)	Occupational	1. Positively associated with MM.	186	No direct evidence for MM.	—

EPA indicates Environmental Protection Agency; IARC, International Agency for Research on Cancer; OP, organophosphate; PC, prostate cancer; *S. typhimurium*; *Salmonella typhimurium*; *S. cerevisae*, *Saccharomyces cerevisiae*; OC, organochlorine; HCH, hexachlorocyclohexane; DDT, dichloro-diphenyl-trichloroethane; DDE, dichlorodiphenyldichloroethylene; HCB, hexachlorobenzene; NHL, non-Hodgkin lymphoma; CYP1A/1B, cytochrome P4501A/1B; 2,4-D, 2,4-dichlorophenoxyacetic acid; MCPA, 2-methyl-4-chlorophenoxyacetic acid; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; EPTC, S-ethyl-N,N-dipropylthiocarbamate; MM, multiple myeloma.

[a]Regulation status was obtained from the Pesticide Action Network Pesticides Database (pesticideinfo.org [accessed October 20, 2012]).

[b]IARC classifications are as follows: group 1: carcinogenic to humans; group 2A, probably carcinogenic to humans; group 2B, possibly carcinogenic to humans; group 3, not classifiable regarding its carcinogenicity to humans; and group 4: probably not carcinogenic to humans.

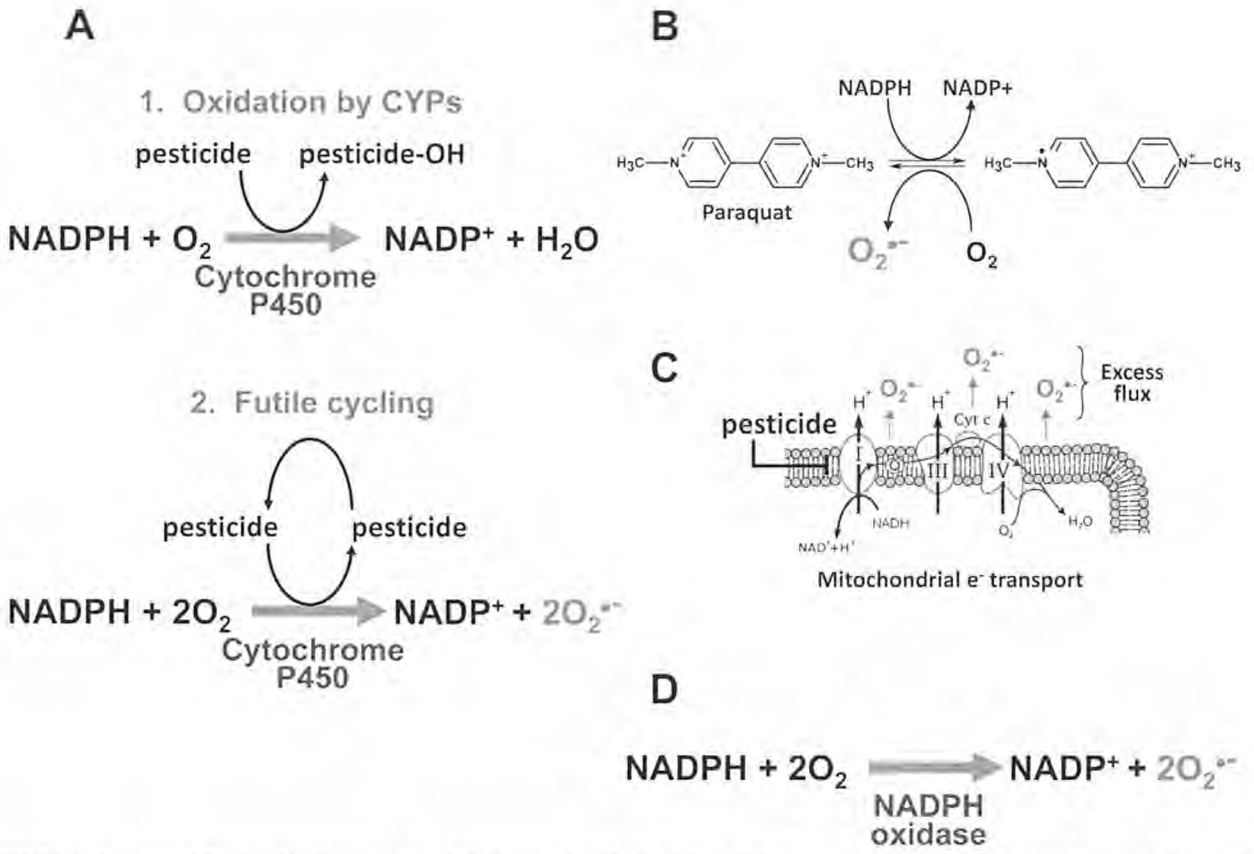

FIGURE 1. Summary of Potential Mechanisms by Which Pesticides Cause Oxidative Stress. (A) Mechanism 1 describes the normal oxidation of a pesticide catalyzed by cytochrome P450 (CYP), leading to a hydroxylated metabolite. Mechanism 2 describes futile oxidative metabolism of a pesticide by CYP450s, leading to reaction uncoupling and superoxide (O_2) production (eg, organochlorines, polychlorinated biphenyls cause futile cycling).[70] (B) Generation of redox-active pesticide metabolites, such as quinones or bipyridinium compounds, which undergo redox cycling leading to superoxide formation (paraquat redox cycling is shown as an example).[71] (C) Impairment of electron transport cascades in mitochondria, leading to excess superoxide flux (eg, rotenone is well known to inhibit complex I).[72] (D) Activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase by pesticides can liberate superoxide.[73] OH indicates hydroxyl radical; H_2O, water.

Oxidative Stress

Exposure to pesticides may cause the net production of reactive oxygen species (ROS) in tissues when antioxidant defense mechanisms are overwhelmed. ROS are often free radicals (ie, oxygen-containing species containing an unpaired electron, such as superoxide [$O_2^{\bullet -}$] and hydroxyl radical [OH]), which renders them highly unstable in a chemical sense. There are generally 4 mechanisms by which pesticides can increase the levels of ROS, such as superoxide (Fig. 1).[70-73] However, regardless of the mechanism by which ROS are produced, a consequence of their overproduction is that they can cause extensive DNA and protein damage in cells. Although the oxidative stress hypothesis of pesticide-induced cancers is attractive, several unanswered questions remain and many details need to be filled in. For example, are tumor suppressor genes or oncogenes specifically targeted by ROS generated by pesticide exposure, thus contributing to disease? Moreover, the identification of specific biomarkers that can distinguish between pesticide exposure, oxidative stress, and disease are needed to establish the links between pesticides and disease endpoints.

Steroid and Xenobiotic Receptors and Pesticides: Endocrine Disruption and Xenobiotic Metabolism

Although most pesticides on the market are not mutagenic in genotoxicity assays such as the Ames mutagenicity test, there is increasing epidemiological evidence of links between pesticide exposure and cancer. Therefore, it is logical to hypothesize alternative mechanisms of action by which pesticides might contribute to cancer beyond canonical DNA damage and mutagenic mechanisms. Endocrine disruptors are chemicals found in the environment (xenochemicals) that block or mimic hormone action, contributing to a wide range of pathologies. They are found in many products, including pesticides. Many xenochemicals can bind to and displace endogenous ligands for the steroid nuclear receptor family, which includes protein receptors that bind to the sex hormones estrogen

TABLE 6. Biomarkers of Exposure, Oxidative Stress, DNA Damage, and Genetic Susceptibility Relevant to Pesticide-Induced Cancers

BIOMARKER	ANALYTE OR ENZYME ACTIVITY ASSAYED	BIOLOGICAL FLUID/SAMPLE USED	REFERENCES
Pesticide exposure	Biomonitoring of pesticides and their metabolites	Urine, serum, plasma	76
	Blood cholinesterase activity and mass spectrometric detection of OP-adducted cholinesterases	Blood	77
Oxidative stress	Malondialdehyde, F2-isoprostanes, thiobarbituric acid-reactive substances	Urine, serum, plasma	78-81
	Catalase and SOD activities	RBCs	78
	8-oxo- or 8-OH-deoxyguanosine	Urine	82
DNA damage	Alkaline comet assay, micronuclei, chromosomal aberrations, sister chromatid exchange	Blood lymphocytes	83-85
	8-oxo- or 8-OH-deoxyguanosine	Urine	86,87
	Apurinic/apyrimidinic endonuclease activity	Blood lymphocytes	83
	"Challenge" assay (DNA repair phenotype)	Blood lymphocytes	88
Genetic susceptibility	Paraoxonase 1 polymorphism	Lipoproteins (HDL)	89,90
	Glutathione transferase, cytochrome P450 polymorphisms	Blood lymphocytes	23,90
	Base excision repair polymorphism	Blood lymphocytes	23
	Nucleotide excision repair polymorphism	Blood lymphocytes	91

OP indicates organophosphate; SOD, superoxide dismutase; RBCs, red blood cells; HDL, high-density lipoprotein.

and androgen, thus aberrantly activating receptor function and leading to changes in gene expression networks.[74,75] Inappropriate activation of androgen and estrogen receptors by pesticides is one hypothesis that might contribute to the excess cancer burden caused by pesticides, particularly the contribution of hydrophobic OCs to prostate and breast cancer risk. Therefore, although pesticides might not be genotoxic per se, their ability to bind steroid and xenobiotic receptors may cause significant alterations in gene expression programs that modulate the carcinogenic activities of common environmental pollutants.

Biomarkers Relevant to Pesticide-Induced Cancers

Biomarkers of exposure, genetic susceptibility, and biological effects such as oxidative stress and DNA damage relevant to pesticide-induced cancers are presented in Table 6.[23,76-91] This list is not exhaustive but highlights both established markers of pesticide exposure (such as cholinesterase activity) and emerging biomarkers (such as the "challenge assay," which assesses the DNA repair capacity of cells). It should be noted that most of these biomarkers are not used in the clinic at present; however, they have usefulness in research studies that aim to determine the etiology of cancers that have been linked to agrochemical exposure.

Cell-Based and Animal Studies to Establish Biomarkers of Pesticide Toxicity

The use of cultured animal and human cells allows high-throughput assays of pesticide toxicity to be assessed at much lower cost compared with whole-animal studies and without the ethical constraints that limit human studies. The purpose of these high-throughput cell-based assays is not to completely replace in vivo studies. Rather, it is a screening process to prioritize the environmental chemicals that will be tested in whole-animal studies. This approach has been embraced by the US EPA and National Institute of Environmental Health Sciences National Toxicology Program to establish the most important environmental chemicals to focus on and to conserve resources.[92] However, effective risk characterization of pesticides will require the integration of in vitro studies, in vivo studies, and epidemiological evidence in order to provide the best protection of public health.

In the US EPA's ToxCast research program, part of the phase 1 study examined 309 chemicals (mostly pesticides) in a high-throughput genotoxicity assay that measured the activity of the p53 transcription factor, which is activated upon DNA damage.[93] As expected, only a small fraction of the tested compounds gave positive hits (10%); a full listing of the chemicals found to be genotoxic can be found at the EPA ToxCast Web site (epa.gov/ncct/toxcast/ accessed November 27, 2012). A caveat to this study is that this high-throughput screen lacked a metabolic activation

system, which might have caused false-negative results to be reported, and positive hits were found at high concentrations of 12.5 μM or higher. With respect to the ability of pesticides to enhance ROS production in cells, high concentrations (approximately 50-100 μM) of organophosphorus pesticides were shown to induce oxidative stress and reduce the activity of antioxidant enzymes in cultured PC-12 cells, which is an in vitro model of dopaminergic neurons.[94] Evidence of DNA damage was also evident in this study. Moreover, these toxic effects could be ameliorated by vitamin E supplementation. However, except for deliberate poisoning episodes, it is highly unlikely that humans would ever be exposed to such high supraphysiological concentrations of pesticide. An earlier study, also using PC-12 cells treated with pesticides (endrin, chlordane, alachlor, fenthion, and chlorpyrifos) but at a much lower concentration (100 nM), demonstrated increased levels of DNA single-strand breaks compared with untreated cells when assessed by the alkaline elution method.[95] Cultured neuroblastoma cells (SH-SY5Y) exposed to fipronil, a phenylpyrazole insecticide, exhibited elevated amounts of ROS and were more likely to undergo apoptosis (cell suicide) compared with untreated cells.[96] Apoptosis was found to correlate with the extent of oxidative stress caused by the fipronil. Thus, these representative descriptive reports do suggest that pesticides can enhance levels of ROS in cultured cells. However, mechanistic information in this area is sparse and much more work is required.

In whole-animal studies, enhanced ROS production and lipid peroxidation in Sprague-Dawley rat liver and brain was found following treatments with the pesticides endrin, chlordane, alachlor, fenthion, or chlorpyrifos.[95,96] In addition, DNA single-strand breaks were also elevated in the livers and brains of pesticide-treated rats. Thus, oxidative stress can be elicited in cultured cells and intact animals by pesticides that have very different chemical structures. There is no chemical similarity between OPs (eg, chlorpyrifos) and OCs (eg, chlordane) and thus it is unlikely that these different classes of pesticides elicit toxicities through a common mode of action. This again highlights the complexity of studying the biological effects of pesticides and trying to find common mechanisms of action. Future studies will need to become more systematic in their approach to selecting pesticides for further mechanistic study. Moreover, animal studies occasionally give conflicting results, even for chemicals thought to exhibit well-defined mechanisms of toxicity. For example, paraquat is well known to induce oxidative stress in human lung, and an in vivo study using rats demonstrated that paraquat could significantly enhance the production of 8-OH-deoxyguanosine, particularly in the brain, lung, and heart.[97] However, in another study, no significant effects on the level of oxidized deoxyguanosine in rat liver, lung, or urine were found following a single intraperitoneal injection of 20 mg/kg of paraquat compared with untreated controls.[98] Therefore, these examples highlight the discordance that often exists between animal and human studies, and the challenge that epidemiologists and toxicologists face when trying to reconcile such conflicting reports.

Exposure to Pesticides and Select Cancer Sites

A growing body of epidemiological, molecular biology, and toxicological evidence assessing the link (or lack of a link) between specific pesticides and specific cancers is becoming available in the scientific literature. While space limitations prevent a comprehensive review of all cancers here, the emerging multidiscipline literature is well illustrated in the case of prostate cancer, NHL, leukemia, multiple myeloma, and breast cancer. In should be noted that tumor sites in rodents following treatment with pesticides almost never concord with human epidemiological findings, which is probably due to species differences and different exposure scenarios. An additional challenge is trying to estimate the degree of caution that should be exercised when using a compound if the specific pesticide can induce tumors in nontarget tissues in cancer bioassays. For example, risk assessors would be concerned with their risk estimates if a tested pesticide could cause liver tumors in a rodent, even though it is highly unlikely that the pesticide would cause liver tumors in human epidemiologic data.

Prostate Cancer

Prostate cancer is the most common cancer diagnosed among men in the United States, accounting for an estimated 28.5% of all cancers diagnosed in men in 2012.[99] Approximately 241,740 cases will be diagnosed in 2012, with an estimated 28,170 deaths occurring.[99] Prostate cancer ranks second after lung cancer as the underlying cause of death in men, accounting for an estimated 9.3% of all cancer deaths in men.[99] Prostate cancer risk associated with pesticides has been evaluated in over 100 occupational studies worldwide (mostly among farmers and other pesticide users). Results from meta-analyses based on these studies are consistent with a weak, positive association between farming and prostate cancer.[100] More recent epidemiologic evidence from a number of different studies now, more convincingly, shows that prostate cancer is related to pesticide use specifically.

In one of the largest prospective studies of pesticide exposures published to date, the Agricultural Health Study (AHS), which was conducted in Iowa and North Carolina, a small but significant excess prostate cancer risk was

observed among both farmers (19% excess) and commercial pesticide applicators (28% excess).[21] Among the 1962 incident prostate cancer cases that developed in the AHS cohort from 54,412 pesticide applicators that were cancer free at the start of the observation period,[21] 3 OP insecticides and an OC insecticide were significantly associated with a monotonic increase in the risk of aggressive prostate cancer as the metric of exposure increased. In this study, aggressive prostate cancer was defined as having one or more of the following tumor characteristics: distant stage, poorly differentiated grade, Gleason score of 7 or higher, or fatal prostate cancer (underlying-cause prostate cancer). The OP chemicals identified include fonofos, which is no longer registered for use in the United States, and 2 other OP insecticides currently used widely in the United States and worldwide: malathion and terbufos. However, the biological mechanisms by which these compounds might cause prostate cancer is uncertain. In vitro studies demonstrated that fonofos and terbufos were both genotoxic in *Salmonella typhimurium* and *Saccharomyces cerevisiae*,[26] although no studies have determined whether these 2 OPs can cause DNA damage in mammalian cells. In addition, the recent study by Koutros et al[25] demonstrated that a significantly increased risk of prostate cancer was observed among men with documented exposure to fonofos or aldrin and a family history of prostate cancer, whereas there was no increased risk among men without a family history. These results suggest an important genetic component contributes to the prostate cancer risk associated with selected pesticides.

Aldrin is an OC insecticide that was extensively used worldwide until 1970, when it was banned in the United States and most other countries. Animal studies suggest that OCs such as aldrin and dieldrin can induce hepatocarcinogenesis in mice through a nongenotoxic mode of action in which the slow oxidative metabolism of these compounds, or futile cycling leading to cytochrome P450 decoupling (Fig. 1A), is accompanied by increased levels of ROS, the depletion of hepatic antioxidant defenses (particularly α-tocopherol), and elevated lipid peroxidation.[22] It was also shown that dieldrin, which is structurally related to aldrin, can induce oxidative stress, resulting in the modulation of gene expression that favors the expansion of latent initiated preneoplastic cells in mouse liver.[22] However, the "tumor promoter-like" effects of OCs such as aldrin and dieldrin do not seem to occur in rat, dog, and monkey liver. Thus, because of the inconsistency in the induction of hepatocarcinomas caused by OC exposure in various species, it is unclear whether results from studies in mice can be translated to humans. Moreover, the organ specificity of cancer in the mouse model caused by OCs, such as dieldrin, does not concord with the human epidemiological findings. Furthermore, prostate tumors are not detected in mice following treatment with dieldrin.

In the AHS, significant interactions between terbufos and fonofos exposures and genetic variants on chromosome 8q24,[24] in the base excision repair pathway,[23] and in the nucleotide excision repair pathway[91] and prostate cancer risk were observed. Although more studies are needed to verify these reports, one interpretation of these findings is that DNA damage elicited by terbufos and fonofos is inefficiently repaired by individuals with DNA repair gene variants, which may contribute to disease development. An alternative explanation is that terbufos and fonofos (or their metabolites) do not directly damage DNA; however, these compounds may promote the growth of initiated cells found in genetic backgrounds of inefficient DNA repair.

In other analyses from the AHS project, occupational exposure to petroleum oil herbicides and the presence of single nucleotide polymorphisms (SNPs) in genes that encode xenobiotic metabolizing enzymes caused the risk of prostate cancer to be 3.7 times higher than in individuals who possess the same SNP but did not use petroleum oil herbicides.[101] One xenobiotic metabolizing enzymes identified with a variant allele linked to petroleum oil herbicide exposure and a higher prostate cancer risk was found in the gene that encodes microsomal epoxide hydrolase, which is an important detoxication enzyme of reactive epoxides.[27] Epoxides are chemicals that are formed via cytochrome P450-mediated monooxygenation of carcinogens, such as benzo(a)pyrene found in cigarette smoke and aflatoxin B1, which is produced by the mold *Aspergillus flavus*. Epoxides produced in vivo are often chemically unstable and can covalently modify DNA, thus forming DNA adducts with a propensity to cause mutation. Thus, components of petroleum oil herbicides may be bioactivated to reactive epoxides that can damage DNA, and this risk may be modified by SNPs in microsomal epoxide hydrolase.

In a case-control study of prostate cancer conducted on 709 consecutive cases of histologically confirmed prostate cancer identified between June 2004 and December 2007 in Guadeloupe, a French archipelago in the Caribbean, prostate cancer risk increased with increasing plasma chlordecone concentration (ie, Kepone [Allied Signal Company and LifeSciences Product Company, Hopewell, VA]).[29] Chlordecone is a chlorinated polycyclic ketone insecticide that was used extensively in the French West Indies for more than 30 years, but was banned in the United States in 1975 and worldwide in 2009. Chlordecone is an endocrine disruptor with estrogenic activity.[29] A 1.77-fold excess risk of prostate cancer was observed in individuals in the highest tertile of exposure compared with those not exposed (P for trend = .002).

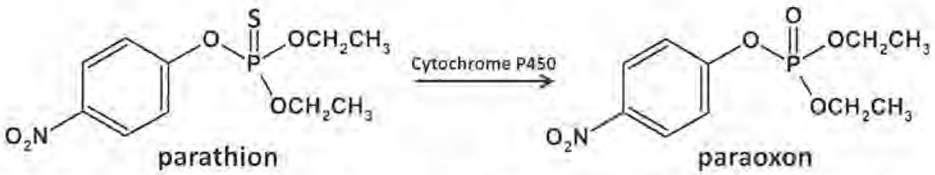

FIGURE 2 Oxidative Desulfuration of the Organophosphate Insecticide Parathion. Parathion is oxidized by cytochrome P450s to the reactive oxon metabolite paraoxon.

Stronger associations were observed among those with a positive family history of prostate cancer. Among subjects with plasma chlordecone concentrations above the limit of detection and 2 at-risk genetic polymorphisms, the risk of prostate cancer was 5.23-fold higher than for those without the exposure or the genetic polymorphism. OC pesticide exposure is often associated with an increased risk of hormone-related cancers, including prostate cancer. After adjustment for other covariates, analysis of National Health and Nutrition Examination Survey (NHANES) data showed that serum concentrations of lindane (P for trend = .02), transnonachlor (P for trend = .002), and dieldrin (P for trend = .04) were significantly associated with the risk of prostate cancer.[32] A popular hypothesis for the toxic mechanism of hydrophobic OCs and other chlorinated pesticides is that they disrupt normal estrogen and androgen receptor functions, thus causing altered gene expression programs to be induced in cells, paving the way for malignant cell development.[42] For example, in vivo and in vitro data from mice and cultured cells suggest that low levels of hexachlorobenzene (HCB) can weakly agonize androgen action and thus enhance androgen signaling, whereas high levels of HCB interfere with androgen signaling.[31] In addition, genotoxic mechanisms may also be in play for OCs. For instance, the OC lindane was found to induce micronuclei in cultured human prostate cells following treatment at very low concentrations (10^{-12}–10^{-10} M) for 24 hours.[30] Thus, both receptor- and genotoxic-mediated toxicities may be at work for OCs and prostate cancer.

Collectively, these studies seem to show that subpopulations with specific genetic characteristics may be particularly vulnerable to the carcinogenic effects of certain OC and OP insecticides. A recent study from Canada also found a significantly increased risk of prostate cancer caused by malathion,[102] and a recent study from the AHS found an excess risk of prostate cancer among occupational users of terbufos.[103] OPs, such as malathion, parathion, and terbufos, can be bioactivated by cytochrome P450–mediated monooxygenation reactions to yield the oxon metabolites (see Figure 2 for example of bioactivation of parathion). Oxons are exquisitely potent compounds that inhibit serine hydrolases via covalent modification of the catalytic serine residue in the enzyme active site.[104] Serine hydrolases participate in a wide variety of physiological and pathophysiological processes, including signal transduction in neural tissue, digestion, immune response, xenobiotic detoxification, and the clotting cascade. Thus, inhibition of these enzymes may lead to a variety of pathological effects. In contrast to most OP compounds, malathion is generally thought to be safe to humans because it contains 2 labile carboxylic acid ester bonds that are easily hydrolyzed by carboxylesterases, thus producing nontoxic products. Nevertheless, human are highly exposed to malathion and this compound can be converted to malaoxon in mammals, which can inhibit serine hydrolases and lead to unwanted toxicities.

A significant association between prostate cancer risk and exposure to dichlorodiphenyltrichlorethane (DDT), a chlorinated insecticide (1.68-fold excess risk for those highly exposed compared with those not exposed); simazine, a triazine herbicide (1.89-fold excess risk for those highly exposed compared with those not exposed); and lindane, a chlorinated insecticide (2.02-fold excess risk for those highly exposed compared with those not exposed) was observed among 1516 prostate cancer cases and 4994 age-matched controls in a population-based case-control study in British Columbia, Canada.[102] Atrazine, a triazine herbicide, was previously suspected of being associated with prostate cancer in a small study of pesticide manufacturing workers,[33] but was not associated with prostate cancer in a much larger evaluation done in the AHS study.[105] Atrazine is one of the most heavily used pesticides in the United States and concerns have been raised about the high levels detected in groundwater. Atrazine is rapidly metabolized to polar metabolites that are readily excreted in the urine of both rodents and humans.[106,107] However, its major quantitative metabolite, dialkylchlorotriazine, was recently shown to covalently modify proteins both in vitro and in vivo,[108] suggesting that dialkylchlorotriazine has the potential to alter protein and cellular function. In addition, there are concerns about the neuroendocrine-disrupting effects of this herbicide.[34]

In contrast to occupational settings, relatively little epidemiology has been conducted to characterize the role that environmental or residential exposures may have in the etiology of prostate cancer. The added complexity in assessing often unknown or poorly quantified environmental exposure to pesticides is a likely explanation.

While the greatest cancer risks from carcinogenic chemicals might be expected to occur among those with long-term occupational exposures, recently, male residents of California's intensely agricultural Central Valley who had ambient exposure to methyl bromide were observed to have a 1.62-fold excess risk of prostate cancer compared with those with no ambient exposure. Similar risks were not observed for simazine, maneb (a dicarbamate fungicide), or paraquat dichloride (a bipyridinium dichloride herbicide).[35] Similar to many methyl halides, methyl bromide was found to be positive in a battery of mutagenicity test systems.[36] Mutation formation is not dependent on the presence of an exogenous enzyme activation system, and thus methyl halides can directly modify DNA because of the relative ease of breaking the carbon-halide bond.[36] Indeed, methyl bromide can directly methylate calf thymus DNA in aqueous solution.[37] Moreover, methyl bromide causes aberrant DNA methylation in rats and mice in vivo,[109,110] and can generate the highly mutagenic O^6-methyl guanine lesion.[37,109] Glutathione conjugation of methyl bromide is the primary mechanism of its detoxification and this reaction is catalyzed by the glutathione S-transferase theta-1 (GSTT1) isoform.[111] The frequency of the GSTT1 null polymorphism in the human population is 20% for whites and 80% for Asians; these individuals do not express a functional GSTT1 enzyme.[112] Future studies that examine the null GSTT1 genotype, methyl bromide exposure, and prostate cancer risk might be worth pursuing because individuals who cannot express GSTT1 would be predicted to have a higher prostate cancer risk. However, it should be noted that methyl bromide is being phased out of use because of its ability to deplete atmospheric ozone.

It is also important to point out that prostate tissue has the ability to both activate and detoxify genotoxins and to repair any consequential DNA damage. The expression of mRNA transcripts for phase 1-activating enzymes such as cytochrome P450 1A2 (*CYP1A2*), *CYP1A1*, and *CYP1B1* has been demonstrated in human prostate.[38] This indicates that carcinogens can be metabolized in situ within the prostate tissue into reactive intermediates that damage macromolecules. Nevertheless, much more mechanistic toxicology studies need to be performed to determine whether occupational exposure to pesticides such as methyl bromide can cause prostate cancer. In light of the increasing epidemiological database linking specific pesticides with prostate cancer, it is reasonable to assume that much more will be learned in the future.

Nonoccupational exposure to OC insecticides was investigated in 4 case-control studies by measuring the concentrations of selected OC insecticides in serum,[41] adipose tissue,[39] or plasma.[40,113] Aronson et al[40] reviewed medical records for male participants aged 50 years to 80 years who visited one of 5 urology clinics in Kingston, Ontario, Canada between 1997 and 1999. Of the 7 OC insecticides assayed (*p,p'*-dichlorodiphenyldichloroethylene [DDE], *p,p'*-DDT, *trans*-nonachlor, oxychlordane, HBC, β-hexachlorocyclohexane, and mirex), none was associated with prostate cancer.[40]

Ritchie and Vial[41] also examined concentrations of OC insecticides in serum from a case-control study of men with prostate cancer in Iowa. Of the 8 analytes reported, only 3 (*p,p'*-DDE [100% cases, 99% controls], *trans*-nonachlor [98% cases, 88% controls], and oxychlordane [91% cases, 82% controls]) had detectable concentrations above 50% for both the cases and controls, but none of these 3 pesticides was clearly associated with prostate cancer. In a case-control study nested in the Japan Public Health Center-based Prospective Study,[113] 201 incident prostate cancer cases were identified through December 31, 2005. Nine analytes were assayed, including *o,p'*-DDT, *p,p'*-DDT, *p,p'*-DDE, trans-nonachlor, cis-nonachlor, oxychlordane, HCB, mirex, and β-HCH. However, none of these analytes was associated with prostate cancer.

In a small case-control study comprised of 58 cases and 23 controls, Hardell et al[39] found positive associations between prostate cancer and HCB (odds ratio [OR], 3.15; 95% confidence interval [95% CI], 1.04-9.54), *p,p'*-DDE (OR, 2.39; 95% CI, 0.81-7.09), trans-chlordane (OR, 3.49; 95% CI, 1.08-11.2), and MC6 (OR, 2.71; 95% CI, 0.87-8.42). With the exception of HCB, none of the ORs achieved statistical significance and all point estimates were imprecise due to the small number of study participants.

In summary, a number of specific pesticides have been linked to prostate cancer risk in occupational settings in an increasing number of studies. In many cases, this risk seems to be enhanced by a family history of prostate cancer. Although the enhanced prostate cancer risk may be a result of common occupational exposures among family members, there is increasing evidence that specific genetic polymorphisms in key genetic pathways may play an important etiologic role. Since the "at-risk genetic polymorphisms" are relatively common in the population, controlling the pesticide exposure rather than genetic testing may be the more desirable public health cancer control measure. Occupational exposures to some, but not all, OP and chlorinated pesticides have been associated with prostate cancer, but other pesticide categories have also been implicated in prostate cancer etiology. Studies of other pesticides with interesting preliminary gene environment analyses are now being completed.

Non-Hodgkin Lymphoma

NHLs are a heterogeneous group of over 20 different B- and T-cell neoplasms affecting the immune system/lymphatic system and arising primarily in the lymph nodes.[114,115] Interest in the etiology of NHL has increased

because incidence rates have nearly doubled in Western countries during the interval from the 1960s through the mid-1990s. The established risk factors for NHL include genetic susceptibility and a previous history of malignant disease[116] and different immunosuppressive states including human immunodeficiency virus; autoimmune diseases such as Sjogren syndrome, systemic lupus erythematosus, rheumatoid arthritis, and psoriasis; and celiac disease.[117] Organ transplant recipients receiving immunosuppressive therapy are at a more than 100-fold excess risk of NHL.[118] However, these conditions cannot account for the increases observed.[118] Exposure to pesticides, particularly phenoxy acid herbicides, has been suggested as a cause of NHL,[119] but the evidence has been inconsistent. In Sweden, Hardell et al observed a 6-fold increased risk of NHL among those who used phenoxy acid herbicides.[120] In Kansas, Hoar et al observed a significant 2-fold increased risk among those who used phenoxy acid herbicides and the risk was highest for those who used 2,4-dichlorophenoxyacetic acid (2,4-D) for 21 days or more during the course of 1 year.[121] In Nebraska, a nonsignificant 50% excess risk of NHL was observed among users of 2,4-D, but the risk did increase to over 3-fold for those who used the herbicide 20 or more days per year.[122] Little evidence of an association between phenoxy acid herbicides and NHL was observed in New Zealand,[123] Washington state,[62] or Minnesota and Iowa.[124] A meta-analysis of 13 case-control studies published between 1993 and 2005 observed an overall significant meta-OR between occupational exposure to pesticides and NHL (OR, 1.35; 95% CI, 1.2-1.5). When observations were limited to those individuals with more than 10 years of exposure, the risk increased (OR, 1.65; 95% CI, 1.08-1.95).[125] While the meta-analysis supports the hypothesis that pesticides are associated with NHL, they lack sufficient detail about pesticide exposure and other information on risk factors for hematopoietic cancers to identify specific causes.[125]

Since the publication of the meta-analysis by Merhi et al,[125] several new population-based studies have been published suggesting that specific pesticides play an important role in NHL etiology. In a case-cohort study using a population-based prospective Danish cohort of 57,053 persons, 256 cohort members were diagnosed with NHL.[126] Eight pesticides and 10 polychlorinated biphenyls congeners were measured in adipose tissue collected at enrollment, prior to cancer onset among the 256 NHL cases and in 256 cancer-free individuals randomly selected from the cohort. A higher risk of NHL was observed among those with higher prediagnostic adipose tissue levels of DDT, cis-nonachlor, and oxychlordane than among those with lower adipose tissue levels.[126] No clear association was found between NHL and polychlorinated biphenyls. A Swedish study by Eriksson et al of 910 cases and 1016 controls observed a significant excess risk of NHL associated with the phenoxy herbicide 2-methyl-4-chlorophenoxyacetic acid. (MCPA) (OR, 2.81; 95% CI, 1.27-6.22) and glyphosate (OR, 2.02; 95% CI, 1.16-3.71). Insecticides overall demonstrated an OR of 1.28 (95% CI, 0.96-1.72) and impregnating agents (ie material used as a water-repellent and antifungal treatment of wood, brick, plaster, and roof tiles) showed an OR of 1.57 (95% CI, 1.07-2.30). 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) have been banned from Sweden and therefore could not be evaluated.[47] Several important observations have been made in a population-based case-control study conducted in 6 Canadian provinces including Quebec, Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia with cases diagnosed between September 1, 1991 and December 31, 1994. An increased risk of 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).[51] In this same case-control study, 6 pesticides/pesticide analytes also showed a significant association with NHL (beta-hexachlorocyclohexane, *p, p'*-dichloro-DDE, HCB, mirex, oxychlordane, and transnonachlor).[127] 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). However, 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 plasma levels of HCB, beta-HCB, or DDE.[46] In yet another case-control study from the 6 Canadian provinces, the risk of NHL increased with the number of different pesticides used.[53] ORs increased even further when the analyses were restricted to "potentially carcinogenic" pesticides; one pesticide had an OR of 1.30 (95% CI, 0.90-1.88), 2 to 4 pesticides had an OR of 1.54 (95% CI, 1.11-2.12), and more than 4 pesticides had an OR of 1.94 (95% CI, 1.17-3.23). These results are somewhat similar to those reported by De Roos et al, who pooled data from 3 NHL case-control studies conducted in the 1980s in 4 American Midwestern states. A superadditive effect was observed in which atrazine amplified the risk of NHL when used in combination with several other pesticides including alachlor, diazinon, and carbofuran.[128] In yet another article from the 6 Canadian provinces study, the joint effect of pesticide exposure and immune suppression was preliminarily evaluated.[61] Study participants with asthma or hay fever had nonsignificantly elevated risks of NHL associated with the use of MCPA (OR, 2.67; 95% CI, 0.90-7.93) compared with participants without any of these conditions (OR, 0.81; 95% CI, 0.39-1.70).

Two epidemiological studies reported that the association of NHL with pesticides was largely limited to NHL cases with the t(14;18) chromosomal translocation.[43,52] In the study by Schroeder et al conducted in Iowa and Minnesota, patients with NHL with the t(14:18) translocation were found to have significantly elevated levels of dieldrin (OR, 3.7; 95% CI, 1.9-7.0), lindane (OR, 2.3; 95% CI, 1.3-3.9), toxaphene (OR, 3.0; 95% CI, 1.5-6.1), and atrazine (OR, 1.7; 95% CI, 1.0-2.8).[52] In the study by Chiu et al conducted in Nebraska, farmers diagnosed with NHL with a t(14:18) translocation were found to have significantly elevated levels of dieldrin (OR, 2.4; 95% CI, 0.8-7.0), toxaphene (OR, 3.2; 95% CI, 0.8-12.5), and lindane (OR, 3.5; 95% CI, 1.4-8.4) compared with nonfarmers. In the prospective AHS, lindane use was associated with a significantly elevated risk of NHL.[44] In a Dutch cohort of workers involved in the manufacturing of chlorophenoxy herbicides, predicted TCDD levels were associated with a significant increase in mortality from NHL (OR, 1.36; 95% CI, 1.06-1.74).[45]

Cytogenetic and molecular studies of individuals exposed to a number of pesticides, such as lindane and 2,4-D, are beginning to reveal a role of pesticides in the induction of chromosomal rearrangements, particularly the t(14;18) translocation that occurs with high frequency in patients with NHL.[57] This translocation appears to be one step in the progression of a normal cell to a cancer cell; however, it is unclear whether pesticides (or other toxicants) cause the t(14;18) translocation or whether they are generated during the course of malignant transformation as a result of the developing genomic instability that arises during disease progression. Polymerase chain reaction-based quantitation of the t(14;18) translocation frequency in peripheral blood lymphocytes, as described by Fuscoe,[129] might be a promising biomarker to use in studies of pesticide-exposed populations. A direct connection between agricultural pesticide use, frequency of the t(14;18) translocation in the blood, and malignant progression to follicular lymphoma has been observed in a prospective cohort study of farmers.[130] This study indicated that the t(14;18) translocation appeared to be an early event in NHL and suggested a molecular connection between agricultural pesticides, the t(14;18) translocation frequency in the blood, and clonal progression, but links to specific pesticides were not possible. However, the mechanistic molecular connection between pesticides and the t(14;18) translocation is still unclear and establishing this link will require much more work. Nevertheless, the higher prevalence of the t(14;18) translocation in pesticide-exposed workers compared with controls is a provocative finding and the replication of this finding in another pesticide-exposed population will be an important follow-up study. Moreover, for the t(14;18) translocation to be used as a biomarker, these findings would ideally be validated in an animal model treated with pesticides. This would provide an even stronger case for studying this biomarker in human populations.

We identified 15 studies that reported on nonoccupational exposure to pesticides and NHL. The vast majority of these studies focused on OC insecticides (11 of 15 studies) and used serum,[48,58,131] plasma,[46,54-56,59] or adipose tissue[53,60,126] concentrations of the OC compounds as the estimate of exposure. Of these 11 studies, 7 measured chlordane/heptachlor or their metabolite (eg, oxychlordane, heptachlor epoxide) concentrations. Four studies[55,60,126,127] observed positive associations between chlordanes and NHL, whereas the 3 other studies did not observe an association.[48,54,58]

In addition to oxychlordane and related compounds (eg, heptachlor), 10 of these studies examined the association between concentrations of DDT or its metabolite, DDE, and NHL. Five studies[48,55,60,126,127] demonstrated either positive or suggestive associations, whereas the other 5 studies[46,54,56,59,131] did not observe an association between DDT or DDE and NHL.

While a number of other OC insecticides were measured in these studies, coverage of specific insecticides was less frequent. For instance, only one study[127] assayed for mirex, finding a positive association (OR, 1.44; 95% CI, 1.08-1.92). Conversely, HCB was assessed in 8 of these studies,[46,48,54,55,58,60,126,127] of which only one observed an association. β-Hexachlorocyclohexane concentrations were positively associated with NHL in only 2[52,123] of the 6 studies that measured it in either plasma, serum, or adipose tissue. Dieldrin levels were assayed in 4 studies,[54,58,60,126] with only one[54] finding evidence of a positive association with NHL.

In summary, NHL is not one disease but many related diseases with seemingly different etiologies. Few studies of pesticides have been large enough to evaluate the potential link between NHL subtypes and specific pesticide exposures. Nonetheless, new evidence linking NHL with specific chlorinated pesticide use and 2 studies linking the number of different pesticides used with NHL give further support to earlier findings suggesting that specific pesticides are etiologically linked to NHL. Preliminary evidence suggests asthma, allergies, or asthma and allergies and hay fever combined with the use of specific pesticides (eg, MCPA) may enhance the risk of NHL. Although it is possible that t(14;18) translocations are an initiating event in a causative cascade leading to an NHL subtype, follicular lymphoma, much more work needs to be done to establish this. Nevertheless, it has been shown that NHL subtypes with t(14;18) translocations are associated with the chlorinated insecticides dieldrin, lindane, and toxaphene and the triazine herbicide atrazine. Lindane also has been

observed to be directly associated with NHL in a large prospective study performed in the United States. In yet another large case-control study in Sweden, the authors linked the use of glyphosate and MCPA to NHL. Although the epidemiological evidence for certain pesticides and NHL is growing, little is known about the biological/toxicological mechanisms by which these compounds may be contributing to this disease (Table 5).

Leukemia

Childhood Leukemia

Acute lymphocytic leukemia comprises about 80% of all childhood leukemia cases, while acute myeloid leukemia comprises most of the remaining 20%.[49] Male children have a higher incidence of leukemia overall compared with female children. It is estimated that less than 10% of childhood leukemia cases have an identified etiology. Established associations include ionizing radiation, Down syndrome, and other genetic syndromes.[132] In the United States and Europe, there is concern that overall rates of childhood cancer have been increasing since 1970.[133] Early life exposures to pesticides are suspected to be responsible for some of these childhood leukemias. A number of recent systematic reviews of the etiological literature[134-137] reached a somewhat similar conclusion (ie, the current literature is limited). Chief among these limitations are that exposure measures relying on substitutes for information about parental pesticide use itself such as in farm-related activities or crops produced has proven to be inadequate; case-control studies tended to suffer from at least some case-recall bias; cohort studies have been too small to generate a sufficient number of exposed cases, thereby mitigating firm etiological conclusions; many available studies (both case-control and cohort) were too small to reliably evaluate leukemia subtypes and all were too small to identify specific pesticides that might be linked to childhood leukemia; and controlling for potentially confounding factors is difficult when so little is known about the etiology of childhood leukemia generally. Nonetheless, a number of important observations have been made in meta-analyses associated with these reviews (ie, an excess risk of overall leukemia is observed with maternal pesticide exposure from home and garden use[135] or maternal occupational exposure but not with paternal occupational pesticide exposure).[136,137] Meta-analyses of childhood leukemia were elevated for prenatal maternal occupational exposure to both insecticides and herbicides.[136] While elevated risks of childhood leukemia were also observed in meta-analyses of children living in homes where professional pesticide applications were done before pregnancy, during pregnancy, and during the first 3 years of the child's life,[134] Vinson et al observed the maternal-associated leukemia risks to be particularly high for exposures that took place prior to birth.[135] While data are limited, it seems both acute lymphocytic leukemia and acute myeloid leukemia in children may be linked to pesticide exposure.[136] Excess childhood leukemia risks did not appear to be related to the proximity of a home to a farm,[137] nor to carpet-tested levels of chlordane, DDT, DDE, methoxychlor, or pentachlorophenol.[138]

Experimental studies in animal models support the biological plausibility of a link between maternal pesticide exposure and leukemia because the exposure of pregnant females to carcinogens can produce cancer in offspring.[139] Transplacental exposure to select fungicides produced lymphomas in mice.[140] Furthermore, the role of epigenetics in germ cell genomic reprogramming has gained increased attention since it was shown that exposure of gestating female rats during the period of gonadal development to either vinclozolin (a fungicide) or methoxychlor (an insecticide) induced elevated incidences of male infertility and altered sperm quality in offspring up to 4 generations.[141,142] Moreover, prostate lesions, altered gene expression patterns, and cancer were detected in some adult progeny.[142] These provocative findings have caused renewed interest in developmental and reproductive toxicities, such as childhood leukemias, caused by environmental chemicals. At this point, work in this area is in a nascent stage of development and much more needs to be done.

Linking specific pesticides to childhood leukemia would most likely lead to the cancelation of registration of that pesticide in the United States and many other nations. Since such a specific link has not yet been made, prudent public health policy would dictate limiting maternal exposure to pesticides prenatally and during early child- hood and limiting direct childhood exposure whenever possible.

Adult Leukemia

Adult-onset leukemias are a heterogeneous category of hematopoietic malignancies, including chronic and acute subtypes that have different etiologies. Causal associations with leukemia have been demonstrated for 3 agents: benzene,[63] formaldehyde,[64] and ionizing radiation.[143] Other suspected occupational causes include pesticides, infectious agents, electromagnetic fields, and solvents and aromatic hydrocarbons.[144]

A meta-analysis of 14 cohort studies of workers in plants manufacturing pesticides showed a meta-rate ratio of 1.43 (95% CI, 1.05-1.94) for leukemia.[145] A recent meta-analysis of 13 cases and controls examining the association between occupational exposures and hematopoietic cancers observed an OR of 1.35 (95% CI, 0.9-2.0).[125] Epidemiological evidence was insufficient to permit the identification of a specific pesticide in either of these meta-analyses.

OPs have been associated with leukemia and other immunologically related cancers in the epidemiological literature.[65,146-151] The leukemogenic effects of OPs may be related to immune function perturbation. In the AHS, leukemia risk was elevated for the high category of intensity-weight exposure-days for the OP insecticide fonofos (relative risk [RR], 2.67; 95% CI, 1.06-6.70 [*P* value for trend = .04])[148] and diazinon was associated with leukemia (RR, 3.36; 95% CI, 1.08-10.49 [*P* value for trend = 0.026]).[149] A positive association with leukemia was also observed for several herbicides including metribuzin, a selective triazinone herbicide (RR, 2.42; 95% CI, 0.82-7.19 [*P* value for trend = .08]),[150] and the use of the herbicides alachlor[151] and S-ethyl-N,N-dipropylthiocarbamate (EPTC),[65] although the risk associated with both of these herbicides was limited to the highest exposure group and thus further follow-up will be necessary.

The IARC has judged that the weight of evidence suggests that the OC insecticides chlordane, heptachlor, DDT, and toxaphene are possible human carcinogens, whereas other OCs are not classifiable as to their carcinogenicity.[66] In the AHS, chemical-specific associations with leukemia were observed for chlordane/heptachlor (RR, 2.1 [95% CI, 1.1-3.9]), which are structurally related compounds that occur together in technical-grade products of each chemical.[44]

In a prospective study of peripheral blood obtained up to 77 months before a diagnosis of chronic lymphocytic leukemia (CLL) was made, prediagnostic B-cell clones were present in 44 of 45 patients with CLL.[67] Use of B-cell clones as prediagnostic markers of CLL may be a valuable tool in evaluating the link between specific pesticides and CLL.

While the evidence linking pesticide exposure to leukemia is abundant, the evidence linking a specific pesticide to a specific leukemia subtype, which could be used to more stringently regulate use of the pesticide or cancel its registration, is largely nonexistent. Recent epidemiological evidence linking specific pesticides to leukemia has established hypotheses that need to be evaluated in other studies (eg, the associations between leukemia overall and diazinon [an OP insecticide currently in widespread use] and several OC insecticides no longer in use in the United States or other developed countries are of particular interest).[65,66,146-151] Linking leukemia to specific pesticides that are used at high levels occupationally should help to identify the chemical agents responsible for childhood cancers as well. The use of preclinical biomarkers (eg, monoclonal B-cell lymphocytosis) to study the etiology of CLL may be a powerful approach for this leukemia subtype.[67] In addition, it has been shown that arylhydrocarbon receptor activation and cyclooxygenase-2 overexpression in lymphoma cell lines lead to resistance to apoptosis,[152] which might be relevant for the development of lymphomas in vivo caused by pesticide exposures.

Multiple Myeloma

Multiple myeloma is a malignancy of the blood, characterized by a clonal expansion of plasma cells and the production of a monoclonal immunoprotein that can be found in the blood or urine. Clonal expansion of plasma cells is accompanied by osteolytic bone destruction, renal failure, anemia, and hypercalcemia.[153] Following a diagnosis of multiple myeloma, the median length of survival is approximately 3 years. Approximately 21,700 new cases are diagnosed annually.[99] Incidence among blacks is twice that among whites but the survival among blacks is significantly better compared with whites.[154] The underlying cause of multiple myeloma is unknown.[153]

A systematic review of case-control studies of the role of occupational exposure to pesticides in the development of multiple myeloma showed a pooled OR for working farmers of 1.39 (95% CI, 1.18-1.65) and an OR for pesticide exposure of 1.47 (95% CI, 1.11-1.94). For working on a farm for more than 10 years, the OR was 1.87 (95% CI, 1.15-3.16).[125] None of these studies, however, was able to identify a specific exposure that was associated with multiple myeloma. In the AHS, an excess risk of multiple myeloma was observed in the cohort.[155] In a follow-up study, a 1.42-fold (95% CI, 1.00-fold to 1.81-fold) risk of multiple myeloma was observed among cohort members in North Carolina compared with the rest of the state, but a similar excess risk was not observed in Iowa.[21] The cause of this excess could not yet be explained, but a separate analysis of the AHS cohort observed a statistically significant risk of multiple myeloma among pesticide applicators in the highest exposure group for the insecticide permethrin (RR, 5.72; 95% CI, 2.76-11.87 [*P* value for trend = .01]) compared with never-users.[156] A cautious interpretation of these results is warranted because the analysis was driven by only 10 exposed cases in the highest exposure group. Positive associations between the fungicide captan (OR, 2.35; 95% CI, 1.11-3.27) and the insecticide carbaryl (OR, 1.89; 95% CI, 0.98-3.67) and multiple myeloma were observed in a recent Canadian population-based case-control study conducted among men in 6 Canadian provinces (ie, Quebec, Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia).[186] The study consisted of 342 multiple myeloma cases and 1506 controls.

Recent data have shown that multiple myeloma is consistently preceded by monoclonal gammopathy of undetermined significance (MGUS).[157] MGUS is a premalignant plasma cell proliferative disorder without

symptoms or evidence of end-organ damage, but cases do have a lifelong 1% annual risk of progression to multiple myeloma.

In the AHS cohort, the age-adjusted prevalence of MGUS was 1.9-fold (95% CI, 1.3-fold to 2.7-fold) higher among male pesticide applicators compared with men from Olmsted County, Minnesota.[158] In the AHS cohort, a 5.6-fold (95% CI, 1.9-fold to 16.6-fold), 3.9-fold (95% CI, 1.5-fold to 10.0- fold), and 2.4-fold (95% CI, 1.1-fold to 5.3-fold) increased risk of MGUS was observed among users of the chlorinated insecticide dieldrin, the fumigant mixture carbon tetrachloride/carbon disulfide, and the fungicide chlorothalonil, respectively. A previous AHS examination determined that a relationship between exposure and disease is not likely confounded by farming or nonfarming activities.[68]

In summary, although the evidence linking pesticide exposure to multiple myeloma has increased in recent years, few studies have been able to assess the link between specific pesticides and multiple myeloma or its precursor MGUS. It is therefore not surprising that we do not yet observe consistent associations. Clearly, additional epidemiological evidence is needed to test the hypothesis that specific pesticides are positively associated with multiple myeloma before firm conclusions can be reached. The use of preclinical biomarkers of multiple myeloma (ie, MGUS) may be a powerful approach to evaluate these etiological hypotheses.

Nonoccupational OC Insecticide Exposure and Breast Cancer

Breast cancer is the most common cancer among women in the United States, accounting for an estimated 226,870 cases in 2012 and 39,510 deaths.[99] Male breast cancer is relatively rare, with an estimated 2190 cases in 2012 and 410 deaths.[99] Epidemiologic studies of occupational pesticide exposure and breast cancer risk are quite limited. Conversely, the open literature is replete with epidemiologic studies that have investigated nonoccupational exposure to OC compounds, including OC insecticides. Given this paucity in occupational studies, we will focus only on the nonoccupational studies of OC insecticides and breast cancer.

In 1993, Wolff et al[159] published a report observing that the risk of breast cancer was higher among women with high serum concentrations of DDE, the major metabolite of DDT, compared with women with low levels. Since then, a substantial number of epidemiologic studies have been conducted and published investigating this hypothesis.

In 2002, Calle et al[160] published a review article evaluating the then-current literature and concluded that: "At present, there is substantial epidemiologic evidence regarding the possible association between organochlorines (as measured in blood and adipose tissue) and the risk of breast cancer. The evidence does not support an association."[160]

Lopez-Cervantes et al[161] arrived at a similar conclusion using meta-analysis to review the epidemiologic evidence for tissue DDE concentrations and breast cancer. In our current review, we update the literature since 2002. We identified 11 published studies[32,162–171] that reported on associations between measured serum, plasma, or adipose tissue concentrations of OC insecticides and breast cancer, which were not included in either the review by Calle et al or Lopez-Cervantes et al.[160,161] Two studies[162,167] were excluded from our review because risk estimates (eg, ORs) were not reported. A third study[32] was excluded because the case definition included prevalent breast cancer. Of the remaining 8 studies, the results were mixed. While 4 studies[163,165,169,170] did not observe an association between OC concentrations, the other 4 studies[164,166,168,171] did observe positive associations.

However, an important caveat to this conclusion remains largely unexplored: the importance that age at exposure may have in breast cancer development. Lopez-Cervantes et al point out that there is a paucity of evidence regarding exposure at critical time periods.[161] Exposures that occur during early life and adolescence are hypothesized to have etiologic importance for breast cancer.[172,173] During mammary gland development, breast epithelium may be particularly susceptible to environmental carcinogens.[174,175] For instance, exposure to ionizing radiation at an early age confers an increased risk of developing breast cancer as compared with exposure that occurs at later ages.[176,177] Regarding early-life exposure to OC insecticides and breast cancer risk, Cohn et al[168] conducted a nested case-control study among a cohort of female members of the Kaiser Permanente Health Plan in Oakland, California and used stored blood samples that were collected between 1959 and 1967 to assay for serum *p,p'*-DDT. They found that that increasing serum *p,p'*-DDT concentrations were positively associated with breast cancer risk, but only among those women exposed prior to 14 years of age.[168] Caution is warranted in interpreting the results for this one study. While the unique circumstances surrounding the study permitted the investigation of early-life exposure to DDT and future breast cancer risk during a time when DDT was actively being used in the United States, replication will be difficult, as the authors note. Overall, these additional studies do not provide compelling evidence to revise the overall conclusion of the previous reviews that the evidence does not support an association between OC insecticides and breast cancer risk.

While the number of epidemiologic studies that have investigated OC compounds is substantial, few epidemiologic studies have been conducted to investigate

non-OC pesticides and breast cancer risk. We identified just 8 published studies that reported on nonoccupational and non-OC insecticide exposure and breast cancer.[178-185] Of these 8 reports, 4 were case-control studies[181-184] that lacked pesticide-specific exposure information and the fourth was an ecologic study in design.[185] The 3 remaining studies[178-180] assessed exposure to a number of specific pesticides, but overall, these studies are too few to provide a meaningfully review.

Conclusions

Assessing the magnitude of the cancer risk from pesticide exposures in the workplace can be difficult because exposures are usually intermittent, pesticide metabolites have a short half-life, and biomarkers of exposure are often nonspecific to the exposure. Assessing cancer risk from pesticide exposures in the general environment is even more challenging. Nonetheless, the available scientific evidence does strongly suggest that pesticides do cause cancer in both those who use the pesticides directly and those who are exposed because of applications others make. The problem may well be more extreme in developing counties where regulatory controls are weaker or nonexistent.

The mechanisms by which pesticides cause cancer are probably numerous, but are incompletely understood. Cancer risk does not seem to be limited to one functional class of pesticides (eg, herbicide, insecticide, or fungicide) or to one chemical class (eg, OCs, OPs, or triazines). Direct genotoxicity is an important mechanism but many nongenotoxic mechanisms seem to be operating as well. Genetic susceptibility to the carcinogenic effects of some pesticides also appears to be an important aspect of the disease mechanism. The genetic susceptibilities that have been identified to date are common to large segments of the population and therefore do not lend themselves to controlling risk through the identification of susceptible individuals. Controlling exposures is the key to limiting cancer risk. Well-designed epidemiological studies with molecular components will help to identify human carcinogens currently on the market, while an increased understanding of the underlying mechanisms of carcinogenesis will help prevent the introduction of new carcinogens to the marketplace.

Until a more complete understanding of pesticide carcinogenesis is achieved, balancing the potential, albeit uncertain, carcinogenic risk with the health benefits derived from the use of pesticides that can mitigate disease-carrying pests or increase fruit and vegetable production will remain a public health and clinical quandary. In the meantime, health care providers should emphasize the importance of minimizing personal exposures to all pesticides to control cancer risk. ■

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Non-Hodgkin Lymphoma Risk and Insecticide, Fungicide and Fumigant Use in the Agricultural Health Study

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Abstract

Farming and pesticide use have previously been linked to non-Hodgkin lymphoma (NHL), chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). We evaluated agricultural use of specific insecticides, fungicides, and fumigants and risk of NHL and NHL-subtypes (including CLL and MM) in a U.S.-based prospective cohort of farmers and commercial pesticide applicators. A total of 523 cases occurred among 54,306 pesticide applicators from enrollment (1993–97) through December 31, 2011 in Iowa, and December 31, 2010 in North Carolina. Information on pesticide use, other agricultural exposures and other factors was obtained from questionnaires at enrollment and at follow-up approximately five years later (1999–2005). Information from questionnaires, monitoring, and the literature were used to create lifetime-days and intensity-weighted lifetime days of pesticide use, taking into account exposure-modifying factors. Poisson and polytomous models were used to calculate relative risks (RR) and 95% confidence intervals (CI) to evaluate associations between 26 pesticides and NHL and five NHL-subtypes, while adjusting for potential confounding factors. For total NHL, statistically significant positive exposure-response trends were seen with lindane and DDT. Terbufos was associated with total NHL in ever/never comparisons only. In subtype analyses, terbufos and DDT were associated with small cell lymphoma/chronic lymphocytic leukemia/marginal cell lymphoma, lindane and diazinon with follicular lymphoma, and permethrin with MM. However, tests of homogeneity did not show significant differences in exposure-response among NHL-subtypes for any pesticide. Because 26 pesticides were evaluated for their association with NHL and its subtypes, some chance finding could have occurred. Our results showed pesticides from different chemical and functional classes were associated with an excess risk of NHL and NHL subtypes, but not all members of any single class of pesticides were associated with an elevated risk of NHL or NHL subtypes. These findings are among the first to suggest links between DDT, lindane, permethrin, diazinon and terbufos with NHL subtypes.

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Introduction

Since the 1970s, epidemiologic studies of non-Hodgkin lymphoma (NHL) and multiple myeloma (MM) have shown increased risk among farmers and associations with the type of farming practiced [1–6]. While farmers are exposed to many agents that may be carcinogenic [7]; there has been a particular focus on pesticides. Studies from around the world have suggested increased risk of NHL or MM [8,9] and other NHL subtypes [10] in relation to the use of specific pesticides in different functional classes (i.e., insecticides, fungicides, fumigants and herbicides). A meta-analysis of 13 case-control studies published between 1993–2005 observed an overall significant meta-odds ratio (OR) between occupational exposure to pesticides and NHL (OR = 1.35; 95% CI: 1.2–1.5) [11]. This risk was greater among individuals with more than 10 years of exposure (OR = 1.65; 95% CI: 1.08–1.95) [11], but the meta-analysis lacked details about the use of specific pesticides and other risk factors [11]. Although the International Agency for Research on Cancer (IARC) has classified "Occupational exposures in spraying and application of non-arsenical insecticides" as "probably carcinogenic to humans", the human

evidence for the 17 individual pesticides evaluated in this monograph was determined to be inadequate for nine and there were no epidemiological studies for eight pesticides [12]. Since then, more studies have focused on cancer risk from specific pesticides, although the information is still relatively limited for many cancer-pesticide combinations [8,9].

To help fill the current information gap we evaluated the relationships between the use of specific insecticides, fungicides and fumigants and NHL in the Agricultural Health Study (AHS), a prospective cohort of licensed private (i.e., mostly farmer) and commercial pesticide applicators. Because the etiology of NHL and its B and T cell subtypes may differ by cell type[13], we also evaluated risk by subtype while controlling for potential confounding factors suggested from the literature [13], and the AHS data.

Novelty and Impact

These findings on occupationally exposed pesticide applicators with high quality exposure information are among the first to suggest links between DDT, lindane, permethrin, diazinon and terbufos and specific NHL subtypes in a prospective cohort study.

Materials and Methods

Study Population

The AHS is a prospective cohort study of 52,394 licensed private pesticide applicators (mostly farmers) in Iowa and North Carolina and 4,916 licensed commercial applicators in Iowa (individuals paid to apply pesticides to farms, homes, lawns, etc.), and 32,346 spouses of private applicators. Only applicators are included in this analysis. The cohort has been previously described in detail [14,15] and study questionnaires are available on the AHS website (www.aghealth.nih.gov). Briefly, individuals seeking license to apply restricted use pesticides were enrolled in the study from December 1993 through December 1997 (82% of the target population enrolled). At enrollment, subjects did not sign a written informed consent form. However, the cover letter of the questionnaire booklet informed subjects of the voluntary nature of participation, the ability to not answer any question, and it provided an assurance of confidentiality (including a Privacy Act Notification statement). The letter also included a written summary of the purpose of research, time involved, benefits of research, and a contact for questions about the research. The cover letter to the take-home questionnaire included all of the above and also informed the participant that they had the right to withdraw at any time. Finally, subjects were specifically informed that their contact information (including Social Security Number) would be used to search health and vital records in the future. The participants provided consent by completing and returning the questionnaire booklet. These documents and procedures were approved in 1993 by all relevant institutional review boards (i.e., National Cancer Institute Special Studies Institutional Review Board, Westat Institutional Review Board, and the University of Iowa Institutional Review Board-01).

Excluded from this analysis were study participants who had a history of any cancer at the time of enrollment (n = 1094), individuals who sought pesticide registration in Iowa or North Carolina but did not live in these states at the time of registration (n = 341) and were thus outside the catchment area of these cancer registries and individuals that were missing information on potential confounders (i.e., race or total herbicides application days [n = 1,569]). This resulted in an analysis sample of 54,306. We obtained cancer incidence information by regular linkage to the population-based cancer registry files in Iowa and North Carolina. In addition, we linked cohort members to state mortality registries of Iowa and North Carolina and the nation-wide National Death Index to determine vital status, and to the nation-wide address records of the Internal Revenue Service, state-wide motor vehicle registration files, and pesticide license registries of state agricultural departments to determine residence in Iowa or North Carolina. The current analysis included all incident primary NHL, as well as CLL and MM (which are now classified as NHL) [13] ($n = 523$) diagnosed from enrollment (1993–1997) through December 31, 2010 in North Carolina and from enrollment (1993–1997) through December 31, 2011 in Iowa, the last date of complete cancer incidence reports in each state. We ended follow-up and person-year accumulation at the date of diagnosis of any cancer, death, movement out of state, or December 31, 2010 in North Carolina and December 31, 2011 in Iowa, whichever was earlier.

Tumor Characteristics

Information on tumor characteristics was obtained from state cancer registries. We followed the definition of NHL and six subtypes of NHL used by the Surveillance Epidemiology and End Results (SEER) coding scheme [16] which was based on the Pathology Working Group of the International Lymphoma Epidemiology Consortium (ICD-O-3 InterLymph modification) classification (Table S1 in File S1, [17], i.e., 1. Small B-cell lymphocytic lymphomas (SLL)/chronic B-cell lymphocytic lymphomas (CLL)/mantle-cell lymphomas (MCL); 2. Diffuse large B-cell lymphomas; 3. Follicular lymphomas; 4. 'Other B-cell lymphomas' consisting of a diverse set of B-cell lymphomas; 5. Multiple myeloma; and 6. T-cell NHL and undefined cell type). There were too few T-cell NHL cases available for analysis [n = 19] so this cell type was not included in the subtype analysis). The ICD-O-3 original definition (used in many earlier studies of pesticides and cancer) of NHL [18] was also evaluated in relation to pesticide exposure to allow a clearer comparison of our results with previous studies.

Exposure Assessment

Initial information on lifetime use of 50 specific pesticides (Table S2 in File S1), including 22 insecticides, 6 fungicides and 4 fumigants was obtained from two self-administered questionnaires [14,15] completed during cohort enrollment (Phase 1). All 57,310 applicators completed the first enrollment questionnaire, which inquired about ever/never use of 50 pesticides, as well as duration (years) and frequency (average days/year) of use for a subset of 22 pesticides including 9 insecticides, 2 fungicides and 1 fumigant. In addition, 25,291 (44%) of the applicators returned the second (take-home) questionnaire, which inquired about duration and frequency of use for the remaining 28 pesticides, including 13 insecticides, 4 fungicides and 3 fumigants.

A follow-up questionnaire, which ascertained pesticide use since enrollment, was administered approximately 5 years after enrollment (1999–2005, Phase 2) and completed by 36,342 (63%) of the original participants. The full text of the questionnaires is available at www.aghealth.nih.gov. For participants who did not complete the Phase 2 questionnaire (20,968 applicators, 37%), a data-driven multiple imputation procedure which used logistic regression and stratified sampling [19] was employed to impute use of specific pesticides in Phase 2. Information on pesticide use from Phase 1, Phase 2 and imputation for Phase 2 was used to construct three cumulative exposure metrics: (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); (ii) intensity-weighted lifetime days of use (i.e., the product of lifetime days of use and a measure of exposure

intensity) and (iii) ever/never use data for each pesticide. Intensity was derived from an exposure-algorithm, which was based on exposure measurements from the literature and individual information on pesticide use and practices (e.g., whether or not they mixed pesticides, application method, whether or not they repaired equipment and use of personal protective equipment) obtained from questionnaires completed by study participants [20].

Statistical Analyses

We divided follow-up time into 2-year intervals to accumulate person-time and update time-varying factors, such as attained age and pesticide use. We fit Poisson models to estimate rate ratios (RRs) and 95% confidence intervals (95% CI) to evaluate the effects of pesticide use on rates of overall NHL and the five NHL subtypes.

We evaluated pesticides with 15 or more exposed cases of total NHL, thereby excluding aluminum phosphide, carbon tetrachloride/carbon disulfide, ethylene dibromide, trichlorfon, and ziram leaving 26 insecticides, fungicides and fumigants for analysis (permethrin for animal use and crop use were combined into one category, all insecticides, fungicides and fumigants are listed in Table S2 in File S1). For each pesticide, we evaluated ever vs. never exposure, as well as tertiles of exposure which were created based on the distribution of all NHL exposed cases and compared to those unexposed. In the NHL subtype analysis and in circumstances where multiple pesticides were included in the model we categorized exposure for each pesticide into unexposed (i.e., never users) and two exposed groups (i.e., low and high) separated at the median exposure level. The number of exposed cases included in the ever/never analysis and in the trend analysis can differ because of the lack of information necessary to construct quantitative exposure metrics for some individuals.

Several lifestyle and demographic factors associated with NHL in the AHS cohort or previously suggested as possible confounders in the NHL literature[13] were evaluated as potential confounders in this analysis. These included: age at enrollment, gender, race, state, license type, education, autoimmune diseases, family history of lymphoma in first-degree relatives, body mass index, height, cigarette smoking history, alcohol consumption per week and several occupational exposures[1–13] including number of livestock, cattle, poultry, whether they raised poultry, hogs or sheep, whether they provided veterinary services to their animals, number of acres planted, welding, diesel engine use, number of years lived on the farm, total days of any pesticide use, and total days of herbicide use. However, since most of these variables did not change the risk estimates for specific pesticides, we present results adjusted for age, race, state and total days of herbicide use, which impacted risk estimates by more than 10% for some subtypes. We also performed analyses adjusting for specific insecticides, fungicides and fumigants shown to be associated with NHL or a specific NHL subtype in the current analysis. Tests for trend used the median value of each exposure category. All tests were two-sided and conducted at $\alpha = 0.05$ level. Analysis by NHL subtype was limited to insecticides, fungicides, and fumigants with 6 or more exposed cases.

We also fit polytomous logit models, where the dependent variable was a five-level variable (i.e., five NHL subtypes) and a baseline level (i.e., no NHL) to estimate exposure-response odds ratios (ORs) and 95% confidence intervals (CIs) for each subtypes of NHL. We then used polytomous logit models to estimate exposure-response trend while adjusting for age, state, race and total days of herbicide use, as in the Poisson models, and tested homogeneity among the 5 NHL subtypes.

Poisson models were fit using the GENMOD procedure and polytomous logit models were fit using the LOGISTIC procedure of the SAS 9.2 statistical software package (SAS Institute, Cary, NC). Summary estimates of NHL and NHL subtype risks for both Poisson models and polytomous logit models incorporated imputed data and were calculated along with standard error estimates, confidence intervals, and p-values, using multiple imputation methods implemented in the MIANALYZE procedure of SAS 9.2.

We also evaluated the impact of the additional pesticide exposure information imputed for Phase 2 on risk estimates. We compared risk estimates for those who completed both the phase 1 enrollment and take-home questionnaires and the phase 2 questionnaires (n = 17,545) with risk estimates obtained from the combined completed questionnaire data plus the imputed phase 2 data (n = 54,306). We also explored the effect of lagging exposure data 5 years because recent exposures may not have had time to have an impact on cancer development. For comparison to previous studies, we also assessed the exposure-response association for NHL using the original ICD-O-3 definition of NHL [18] and the new definition [16] in Table S3 in File S1. Unless otherwise specified, reported results show un-lagged exposure information from both Phase 1 and Phase 2 including Phase 2 imputed data for lifetime exposure-days and intensity-weighted lifetime days of use and NHL defined by the InterLymph modification of ICD-O-3 [17]. Data were obtained from AHS data release versions P1REL201005.00 (for Phase 1) and P2REL201007.00 (for Phase 2).

Results

The 54,306 applicators in this analysis contributed 803,140 person-years of follow-up from enrollment through December 31, 2010 in North Carolina and December 31, 2011 in Iowa (Table 1). During this period, there were 523 incident cases of NHL, including 148 SLL/CLL/MCL, 117 diffuse large B-cell lymphomas, 67 follicular lymphomas, 53 'other B-cell lymphomas' (consisting of a diverse set of B-cell lymphomas) and 97 cases of MM. Another 41 cases consisting of T-cell lymphomas (n = 19) and non-Hodgkin lymphoma of unknown lineage (n = 22) were excluded from cell type-specific analyses because of small numbers of cases with identified cell types. Between enrollment and the end of follow-up, 6,195 individuals were diagnosed with an incident cancer other than NHL, 4,619 died without a record of cancer in the registry data, and 1,248 cohort members left the state and could not be followed-up for cancer. Person-years of follow-up accumulated for all of these study participants after enrollment until they were censored for the incident cancer, death or moving out of the state (data not shown). The risk of NHL increased significantly and monotonically with age in the AHS cohort in this analysis (p = 0.001) and age-adjusted risks were significant for state and NHL overall and race for multiple myeloma (data not shown). Total days of herbicide use had a small but significant effect on the risk of some NHL subtypes, but not on NHL overall. No other demographic or occupational factors showed evidence of confounding so they were not included in the final models.

In Table 2 we present ever/never results for 26 insecticides, fungicides and fumigants by total NHL and by NHL subtype adjusted for age, race, state and herbicide use (total life-time days). Terbufos was the only pesticide associated with an increased risk of total NHL in the ever/never use analysis (RR = 1.2 [1.0–1.5]), although the trend for increasing use and risk of total NHL was not significant (p trend = 0.43) (Table 3). In contrast, there were a few chemicals that were not associated with ever/never use, but

Table 1. Baseline characteristics of AHS study participants in the NHL incidence analysis[1,2].

Variables	All NHL cases (%)	Cohort Person-years.
Age at Enrollment		
<45	84 (16.1)	426,288
45–49	51 (9.8)	101,018
50–54	75 (14.3)	84,998
55–59	90 17.2)	74,440
60–64	78 (14.9)	56,978
65–69	79 (15.1)	35,071
≥70	66 (12.6)	24,347
Race		
White	509 (97.3)	787,799
Black	14 (2.7)	15,341
State		
IA	332 (63.5)	537,252
NC	191 (36.5)	265,888
Lifetime Total Herbicide Exposure Days		
0–146 days	170 (32.5)	251,401
147–543 days	169 (32.3)	273,107
544–2453 days	184 (35.2)	278,632

[1]During the period from enrollment (1993–1997) to December 31, 2010 in NC and December 31, 2011 in Iowa.
[2]Individuals with missing ever/never exposure information or missing confounding variable information were not included in the table.
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did show evidence of an exposure-response association. Lindane was the only pesticide that showed a statistically significant increasing trend in risk for NHL with both exposure metrics, for lifetime-days of lindane use the RR were = 1.0 (ref), 1.2 (0.7–1.9), 1.0 (0.6–1.7), 2.5 (1.4–4.4); p trend = 0.004 and intensity-weighted lifetime-days of use the: RR were: = 1.0 (ref), 1.3 (0.8–2.2), 1.1 (0.7–1.8), 1.8 (1.0–3.2); p trend = 0.04. DDT showed a significant trend for NHL risk with life-time days of use RR = 1.0 (ref), 1.3 (0.9–1.8), 1.1 (0.7–1.7), 1.7 (1.1–2.6); p trend = 0.02, while the intensity weighted lifetime days of use of DDT was of borderline significance: RR = 1.0 (ref), 1.2 (0.8–1.8), 1.1 (0.8–1.7), 1.6 (1.0–2.3); p trend = 0.06. The number of lifetime days of use of lindane and DDT was weakly correlated (coefficient of determination = 0.04), and the pattern of NHL risk showed little change when both were included in the model. The results for lindane adjusted for DDT were, RR = 1.0 (ref), 1.2 (0.7–2.0), 1.0 (0.5–1.8), 1.6 (0.9–3.3); p trend = 0.07 and the results for DDT adjusted for lindane were, RR = 1.0 (ref), 1.3 (0.9–2.0), 0.9 (0.6–1.6), 1.6 (0.9–2.6); p trend = 0.08).

We also evaluated pesticides by NHL sub-type. In the ever/never analyses (Table 2), permethrin was significantly associated with multiple myeloma, RR = 2.2 (1.4–3.5) and also demonstrated an exposure-response trend (RR = 1.0 (ref), 1.4 (0.8–2.7), 3.1 (1.5–6.2); p trend = 0.002) (Table 4). Similarly, there was an elevated risk of SLL/CLL/MCL with terbufos in ever/never analyses RR = 1.4 (0.97–2.0) and an exposure response trend (RR = 1.0 (ref), 1.3 (0.8–2.0), 1.6 (1.0–2.5); p trend = 0.05). For follicular lymphoma, lindane showed an elevated but non-significant association for ever use, RR = 1.7 (0.96–3.2) and a significant exposure-response association (RR = 1.0 (ref), 4.9 (1.9–12.6), 3.6 (1.4–9.5); p trend = 0.04). There were also two chemicals with evidence of exposure-response that were not associated with specific subtypes in the ever/never analyses: DDT (Dichlorodiphenyltrichloroethane) with SLL/CLL/MCL (RR = 1.0 (ref), 1.0 (0.5–1.8), 2.6 (1.3–4.8; p trend = 0.04); and diazinon with follicular lymphoma (RR = 1.0 (ref), 2.2 (0.9–5.4), 3.8 (1.2–11.4); p trend = 0.02) (Table 4).

The pattern of increased CLL/SLL/MCL risk with increased use of DDT and terbufos remained after both insecticides were placed in our model concurrently. CLL/SLL/MCL risk increased with DDT use (RR = 1.0 (ref), 0.9 (0.5–4.7); 2.4 (1.1–4.7); p trend = 0.04), and a pattern of increased CLL/SLL/MCL risk was also observed with terbufos use (RR = 1.0 (ref), 1.1 (0.6–2.1), 1.7 (0.9–3.3) p trend = 0.07), although the trend was not significant for terbufos. Similarly, the pattern of increased follicular lymphoma risk with lindane use and diazinon use remained after both insecticides were placed in our model concurrently. Follicular lymphoma risk increased with diazinon use (RR = 1.0 (ref), 4.1 (1.5–11.1); 2.5 (0.9–7.2); p trend = 0.09), and a similarly, pattern of increased follicular lymphoma risk was observed with lindane use (RR = 1.0 (ref), 1.6 (0.6–4.1), 2.6 (0.8–8.3) p trend = 0.09), although neither remained statistically significant (Table 4).

Three chemicals showed elevated risks in ever/never analyses for certain subtypes, with no apparent pattern in exposure-response analyses: metalaxyl and chlordane with SLL/CLL/MCL, RR = 1.6 (1.0–2.5) and RR = 1.4 (0.97–2.0) respectively, and methyl bromide with diffuse large B-cell lymphoma RR = 1.9 (1.1–3.3). Although there was evidence of association by subtype, and polytomous logit models indicated homogeneity across subtypes for lindane (p = 0.54), DDT (p = 0.44) and any other pesticide evaluated in this study (e.g., permethrin (p = 0.10), diazinon (p = 0.09), terbufos (p = 0.63), (last column in Table 4).

There was no evidence of confounding of the total NHL associations with either lindane or DDT. We also calculated RR for those who completed both the phase 1 enrollment and take-home questionnaires and the phase 2 questionnaire (n = 17,545) and found no meaningful difference in the RR that also included imputed exposures, although there was an increase in precision of

Table 2. Pesticides exposure (ever/never) and adjusted Relative Risk of total NHL and NHL Subtype[1].

Insecticide	Total NHL Cases[2]		SLL/CLL/MCL Cases[2]		Diffuse Large B-Cell Cases[2]		Follicular B-Cell Cases[2]		Other B-cell Cases[2]		Multiple Myeloma Cases[2]	
Pesticide (chemical-functional class)	Ever/Never Exposed	RR[3,4] (95% CI)	Ever/Never Exposed	RR[3,4] (95% CI)	Ever/Never Exposed	RR[3,4] (95% CI)	Ever/Never Exposed	RR[3,4] (95% CI)	Ever/Never Exposed	RR[3,4] (95% CI)	Ever/Never Exposed	RR[3,4] (95% CI)
Aldicarb	47/435	1	14/124	1.1	8/98	0.7	6/54	0.9	7/41	1.6	10/82	1.2
(carbamate-insecticide)		(0.7–1.4)		(0.6–1.8)		(0.4–1.5)		(0.3–2.2)		(0.7–3.5)		(0.6–2.2)
Carbofuran	147/317	1.1	48/86	1.2	26/78	0.8	18/39	1	13/31	0.8	31/56	1.3
(carbamate-insecticide)		(0.9–1.3)		(0.8–1.8)		(0.5–1.3)		(0.5–1.7)		(0.4–1.6)		(0.8–2.1)
Carbaryl	272/225	1	75/66	1	58/53	0.8	37/24	0.8	24/28	0.9	58/34	0.9
(carbamate-insecticide)		(0.8–1.2)		(0.7–1.5)		(0.5–1.3)		(0.5–1.3)		(0.5–1.6)		(0.6–1.4)
Chlorpyrifos	210/300	1	62/84	1	44/70	0.9	32/33	1.3	21/31	0.8	36/58	1
(organophosphate-insecticide)		(0.8–1.2)		(0.7–1.4)		(0.6–1.4)		(0.8–2.2)		(0.5–1.5)		(0.6–1.5)
Coumaphos	46/411	1.1	15/120	1.2	10/93	1	8/48	1.6	5/40	xxx	7/78	1
(organophos-phate-insecticide)		(0.8–1.5)		(0.7–2.1)		(0.5–2.1)		(0.8–3.5)				(0.1–2.1)
DDVP	55/407	1.1	13/124	0.8	10/93	1	8/48	1.3	6/39	1	12/73	1.7
(dimethyl phosphate-insecticide)		(0.8–1.5)		(0.5–1.5)		(0.5–1.9)		(0.6–2.7)		(0.4–2.4)		(0.9–3.2)
Diazinon	144/342	1	46/93	1.3	30/78	0.9	22/38	1.3	12/37	0.8	27/64	1
(organophosphorous-insecticide)		(0.8–1.3)		(0.9–1.9)		(0.6–1.4)		(0.7–2.3)		(0.4–1.6)		(0.6–1.6)
Fonofos	115/349	1.1	35/100	1.1	25/81	1.2	13/45	0.9	15/30	1.3	19/66	1.3
(organophosphorous-insecticide)		(0.9–1.4)		(0.7–1.6)		(0.7–1.9)		(0.5–1.7)		(0.7–2.5)		(0.8–2.3)
Malathion	332/163	0.9	99/43	1	72/37	0.9	46/14	1.3	30/21	0.6	61/32	0.9
(organophosphorous-insecticide)		(0.8–1.1)		(0.7–1.4)		(0.6–1.4)		(0.7–2.4)		(0.3–1.0)		(0.6–1.5)
Parathion (ethyl or methyl)	69/411	1.1	20/117	1	14/91	1	10/48	1.1	7/44	1.1	14/77	1
(organophosphorous insecticide		(0.8–1.4)		(0.7–1.4)		(0.6–1.4)		(0.8–1.5)		(0.7–1.5)		(0.8–1.5)
Permethrin (animal and crop applications)	112/363	1.1	32/106	1	18/81	0.7	18/81	1.1	9/14	0.8	20/72	**2.2**
(pyrethroid insecticide)		(0.8–1.3)		(0.6–1.5)		(0.4–1.2)		(0.6–2.0)		(0.4–1.6)		**(1.4–3.5)**
Phorate	160/325	1	53/87	1.1	31/76	0.9	20/40	0.9	19/31	0.9	26/64	1
(organophosphorous-insecticide)		(0.8–1.2)		(0.8–1.6)		(0.5–1.3)		(0.5–1.6)		(0.5–1.6)		(0.6–1.7)
Terbufos	201/267	**1.2**	64/72	1.4	42/63	1.1	31/26	1.2	26/19	1.8	32/59	1.2
(organophosphorous-insecticide)		**(1.0–1.5)**		(0.97–2.0)		(0.7–1.7)		(0.7–2.1)		(0.94–3.2)		(0.7–1.9)
Chlorinated Insecticides												
Aldrin	116/364	0.9	53/99	0.9	15/91	0.8	13/45	0.8	12/37	0.6	29/62	1.5
(chlorinated insecticide)		(0.7–1.1)		(0.6–1.4)		(0.4–1.6)		(0.4–1.6)		(0.3–1.3)		(0.9–2.5)
Chlordane	136/344	1	49/90	1.4	20/86	0.6	18/41	1.2	13/36	1	31/60	1.2
(chlorinated insecticide)		(0.8–1.3)		(0.99–2.1)		(0.4–1.0)		(0.7–2.1)		(0.7–2.0)		(0.8–1.9)
DDT	182/300	1	59/79	1.2	34/73	0.8	18/41	0.9	20/31	1.1	40/50	1.1

Table 2. Cont.

Insecticide												
	Total NHL Cases[2]		SLL/CLL/MCL Cases[2]		Diffuse Large B-Cell Cases[2]		Follicular B-Cell Cases[2]		Other B-cell Cases[2]		Multiple Myeloma Cases[2]	
Pesticide (chemical-functional class)	Ever/Never Exposed	RR[3,4] (95% CI)	Ever/Never Exposed	RR[3,4] (95% CI)	Ever/Never Exposed	RR[3,4] (95% CI)	Ever/Never Exposed	RR[3,4] (95% CI)	Ever/Never Exposed	RR[3,4] (95% CI)	Ever/Never Exposed	RR[3,4] (95% CI)
(chlorinated insecticide)		(0.8–1.3)		(0.8–1.8)		(0.5–1.3)		(0.5–1.6)		(0.6–2.1)		(0.7–1.8)
Dieldrin	35/442	0.9	5/130	xxx	4/101	xxx	4/54	xxx	7/42	1	10/81	0.9
(chlorinated insecticide)		(0.6–1.2)								(0.7–2.0)		(0.5–1.4)
Heptachlor	90/384	1	33/104	1.1	10/95	1.1	9/48	1.1	13/36	0.9	17/72	1.1
(chlorinated insecticide)		(0.7–1.2)		(0.7–3.0)		(0.3–3.1)		(0.5–3.2)		(0.5–2.7))		(0.6–2.0)
Lindane	85/396	1	27/113	1.2	12/95	0.6	16/41	1.7	9/40	0.7	13/73	1.1
(chlorinated insecticide)		(0.8–1.2)		(0.6–1.5)		(0.3–1.1)		(0.96–3.2)		(0.4–1.2)		(0.5–2.0)
Toxaphene	79/397	1	21/116	0.9	14/90	0.8	9/47	1	10/40	1.1	19/73	1.1
(chlorinated insecticide)		(0.7–1.2)		(0.5–1.5)		(0.4–1.4)		(0.6–2.0)		(0.6–2.0)		(0.6–1.9)
Fungicides												
Benomyl	54/428	1.1	18/123	1.2	12/95	1.1	4/51	xxx	4/51	xxx	11/80	1.1
(carbamate fungicide)		(0.8–1.5)		(0.7–2.0)		(0.6–1.9)						(0.6–2.0)
Captan	60/406	1.1	18/118	1.1	12/91	0.9	5/51	xxx	6/39	1.1	12/76	1.2
(phthalimide fungicide)		(0.8–1.4)		(0.6–1.8)		(0.5–1.8)				(0.5–2.7)		(0.6–2.2)
Chloro-thalonil	35/474	0.8	9/135	0.9	6/107	0.5	5/60	xxx	2/50	xxx	11/84	1.2
(poly-chlorinated aromatic thalonitrile fungicide)		(0.5–1.2)		(0.4–1.9)		(0.2–1.3)						(0.6–2.3)
Maneb/	44/437	0.9	13/127	1.1	12/95	1.1	4/60	xxx	5/49	xxx	10/79	0.8
Mancozeb		(0.7–1.3)		(0.6–2.1)		(0.6–2.1)						(0.4–1.7)
(dithiocarbamate fungicide)												
Metalaxyl	108/381	1	34/106	**1.6**	27/82	1.1	10/48	0.7	10/40	0.9	21/71	0.8
(acylalanine fungicide)		(0.8–1.3)		**(1.0–2.5)**		(0.6–1.8)		(0.4–1.4)		(0.4–1.7)		(0.4–1.3)
Fumigant												
Methyl bromide	85/425	1.1	18/126	0.9	28/86	**1.9**	7/58	0.6	8/44	2.2	19/76	1
(methyl halide fumigant)		(0.9–1.5)		(0.5–1.7)		**(1.1–3.3)**		(0.2–1.4)		(0.9–5.7)		(0.6–1.8)

[1]During the period from enrollment (1993–1997) to December 31, 2010 in NC and December 31, 2011 in Iowa.
[2]Numbers of cases by NHL subtype do not sum to total number of NHL cases (n = 523) due to missing data.
[3]Adjusted RR: age (<45, 45–49, 50–54, 55–59, 60–64, 65–69, ≥70), State (NC vs. IA), Race (White vs. Black), AHS herbicides (tertiles of total herbicide use-days). Statistically significant RR and 95% confidence limits are bolded.
[4]RR was not calculated if the number of exposed cases in a pesticide-NHL subtype cell was <6 and the missing RR was marked with an XXX. Statistically significant RRs and 95% confidence limits are bolded.
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Table 3. Pesticide exposure (lifetime-days & intensity weighted life-time days) and adjusted risks of total NHL incidence[1].

Insecticides						
Pesticide (chemical-functional class) [days of lifetime exposure for each category]	**NHL Cases[2]**	**Non-Cases[2]**	**RR[3,4] (95% CI) by Total Days of Exposure**	**NHL Cases[2,]**	**Non-Cases**	**RR[3,4] (95% CI) Intensity-weighted days of exposure**
Aldicarb (carbamate-insecticide)						
None	238	21557	1.0 (ref)	238	21557	1.0 (ref)
Low [≤8.75]	7	633	1.1 (0.5–2.3)	6	383	1.3 (0.6–3.3))
Medium [>8.75–25.5]	5	522	0.9 (0.3–2.5)	6	853	0.9 (0.4–1.9)
High [>25.5–224.75]	5	1266	0.5 (0.2–1.3)	5	1183	0.5 (0.2–1.3)
			P trend = 0.23			P trend = 0.22
Carbofuran (carbamate-insecticide)						
None	317	36296	1.0 (ref)	317	36296	1.0 (ref)
Low [≤8.75]	63	4775	1.2 (0.9–1.6)	46	3695	1.2 (0.9–1.6)
Medium [>8.75–38.75]	32	3648	0.8 (0.6–1.2)	46	4590	1.0 (0.7–1.3)
High [>38.75–767.25]	44	4370	0.97 (0.7–1.4)	45	4477	1.0 (0.7–1.4)
			P trend = 0.69			P trend = 0.74
Carbaryl (carbamate-insecticide)						
None	128	12864	1.0 (ref)	128	12864	1.0 (ref)
Low [≤8.75]	54	4128	1.1 (0.7–1.6)	46	3962	1.0 (0.7–1.5)
Medium [8.75–56]	43	5096	0.9 (0.6–1.2)	45	4433	0.9 (0.7–1.5)
High [>56–737.5]	39	3281	1.0 (0.7–1.6)	44	4029	1.0 (0.6–1.5)
			P trend = 0.87			P trend = 0.94
Chlorpyrifos (organophosphate-insecticide)						
None	300	30393	1.0 (ref)	300	30393	1.0 (ref)
Low [≤8.75]	71	6493	1.1 (0.9–1.5)	61	6383	1.1 (0.8–1.4)
Medium [>8.75–44]	65	6892	1.1 (0.8–1.4)	60	7549	0.9 (0.7–1.2)
High [>44–767.25]	67	9380	0.8 (0.6–1.1)	60	7044	1.0 (0.7–1.3)
			P trend = 0.11			P trend = 0.85
Coumaphos (organophosphate-insecticide)						
None	411	44846	1.0 (ref)	411	44846	1.0 (ref)
Low [<8.75]	16	1510	1.0 (0.6–1.7)	15	1132	1.3 (0.8–2.1)
Medium [>8.75–38.75]	14	1076	1.2 (0.7–2.1)	14	1452	1.0 (0.6–1.6)
High [>38.75–1627.5]	13	1175	1.2 (0.7–2.0)	14	1170	1.2 (0.7–2.1)
			P for trend = 0.50			P trend = 0.48
DDVP (dimethyl phosphate-insecticide)						
None	407	44551	1.0 (ref)	407	44551	1.0 (ref)
Low [≤8.75]	19	1342	1.4 (0.9–2.1)	18	1281	1.4 (0.9–2.3)
Medium [>8.75–87.5]	17	1519	1.2 (0.7–1.9)	18	1633	1.1 (0.7–1.8)
High [>87.5–2677.5]	17	1893	0.9 (0.6–1.5)	17	1824	1.0 (0.6–1.6)
			P trend = 0.78			P trend = 0.83
Diazinon (organophosphorous-insecticide)						
None	187	17943	1.0 (ref)	187	17943	1.0 (ref)
Low [≤8.75]	28	2506	1.1 (0.7–1.6)	23	2047	1.1 (0.7–1.8)
Medium [>8.75–25]	19	1515	1.0 (0.6–1.8)	24	2246	0.9 (0.5–1.5)
High [>25–457.25]	23	1990	1.2 (0.7–1.9)	22	1708	1.3 (0.8–2.1)
			P trend = 0.52			P trend = 0.33

Table 3. Cont.

Insecticides						
Pesticide (chemical-functional class) [days of lifetime exposure for each category]	**NHL Cases[2]**	**Non-Cases[2]**	**RR[3,4] (95% CI) by Total Days of Exposure**	**NHL Cases[2,]**	**Non-Cases**	**RR[3,4] (95% CI) Intensity-weighted days of exposure**
Fonofos (organophosphorous-insecticide)						
None	349	39570	1.0 (ref)	349	39570	1.0 (ref)
Low [≤20]	47	3812	1.3 (0.96–1.8)	37	2906	1.4 (0.97–1.9)
Medium [>20–50.75]	28	2819	1.1 (0.7–1.6)	38	3487	1.1 (0.8–1.6)
High [>50.75–369.75]	37	3385	1.1 (0.7–1.5)	36	3606	1.0 (0.7–1.4)
			P trend = 0.83			P trend = 0.87
Malathion (organophosphorous-insecticide)						
None	90	8368	1.0 (ref)	90	8368	1.0 (ref)
Low [≤8.75]	75	7284	0.97 (0.7–1.3)	60	5535	1.0 (0.7–1.4)
Medium [>8.75–38.75]	47	5779	0.7 (0.5–1.1)	59	6899	0.8 (0.6–1.1)
High [>38.75–737.5]	57	5037	0.9 (0.6–1.3)	59	5588	0.9 (0.6–1.2)
			P trend = 0.63			P trend = 0.46
Parathion (ethyl or methyl) (organophosphorous insecticide)						
None	228	21457	1.0 (ref)	228	21457	1.0 (ref)
Low [≤8.75]	9	693	1.0 (0.5–2.0)	7	612	0.9 (0.4–2.0)
Medium [>8.75–24.5]	6	351	1.4 (0.6–3.2)	8	462	1.4 (0.7–2.9)
High [>.24.5–1237.5]	6	652	0.8 (0.3–1.8)	6	621	0.8 (0.4–1.9)
			P trend = 0.64			P trend = 0.74
Permethrin (animal and crop applications)						
(pyrethroid insecticide)						
None	371	37496	1.0 (ref)	371	37496	1.0 (ref)
Low [≤8.75]	38	4315	1.1 (0.8–1.5)	33	4263	0.9 (0.6–1.3)
Medium [>8.75–50.75]	31	4611	0.8 (0.5–1.2)	33	4200	1.0 (0.7–1.4)
High [>50.75–1262.25]	33	4121	1.2 (0.8–1.7)	32	4553	1.0 (0.7–1.5)
			P trend = 0.54			P trend = 0.99
Phorate (organophosphorous-insecticide)						
None	171	16834	1.0 (ref)	171	16834	1.0 (ref)
Low [≤8.75]	27	2521	0.8 (0.5–1.2)	26	2320	0.9 (0.6–1.4)
Medium [8.75–24.5]	33	1819	1.4 (0.96–2.1)	27	1951	1.1 (0.7–1.7)
High [>24.5–224.75]	18	2246	0.6 (0.4–1.1)	25	2409	0.8 (0.5–1.3)
			P trend = 0.25			P trend = 0.44
Terbufos (organophosphorous-insecticide)						
None	267	31076	1.0 (ref)	267	31076	1.0 (ref)
Low [≤24.5]	82	8410	1.2 (0.9–1.5)	64	6895	1.1 (0.9–1.5)
Medium [>24.5–56]	54	3925	1.6 (1.2–2.1)	64	4642	1.6 (1.2–2.2)
High [>56–1627.5]	57	6080	1.1 (0.8–1.5)	63	6842	1.1 (0.8–1.5)
			P trend = 0.43			P trend = 0.44
Chlorinated Insecticides						
Aldrin (chlorinated insecticide)						
None	193	19743	1.0 (ref)	193	19743	1.0 (ref)
Low [≤8.75]	27	1613	0.9 (0.6–1.4)	20	1212	0.9 (0.6–1.4)
Medium [>8.75–24.5]	16	1002	0.8 (0.5–1.3)	20	1279	0.8 (0.5–1.3)

Table 3. Cont.

Pesticide (chemical-functional class) [days of lifetime exposure for each category]	NHL Cases[2]	Non-Cases[2]	RR[3,4] (95% CI) by Total Days of Exposure	NHL Cases[2]	Non-Cases	RR[3,4] (95% CI) Intensity-weighted days of exposure
Insecticides						
High [>24.5–457.25]	17	903	0.9 (0.5–1.5)	19	1026	0.9 (0.6–1.5)
			P trend = 0.58			P trend = 0.74
Chlordane (chlorinated insecticide)						
None	179	19115	1.0 (ref)	179	19115	1.0 (ref)
Low [≤8.75]	47	2687	1.3 (0.97–1.9)	23	1303	1.4 (0.9–2.2)
Medium[5]	0	0	xxx	24	1747	1.0 (0.6–1.5)
High [>8.75–1600]	23	1450	1.1 (0.7–1.7)	22	1085	1.4 (0.9–2.2)
			P trend = 0.43			P trend = 0.16
DDT (chlorinated insecticide)						
None	152	18543	1.0 (ref)	152	18543	1.0 (ref)
Low [≤8.75]	43	2121	1.3 (0.9–1.8)	33	1601	1.2 (0.8–1.8)
Medium [>8.75–56]	28	1598	1.1 (0.7–1.7)	32	1760	1.1 (0.8–1.7)
High [>56–1627.5]	27	953	1.7 (1.1–2.6)	32	1305	1.6 (1.0–2.3)
			P trend = 0.02			P trend = 0.06
Dieldrin (chlorinated insecticide)						
None	235	22510	1.0 (ref)	235	22510	1.0 (ref)
Low [≤8.75]	7	472	0.7 (0.3–1.5)	6	363	0.8 (0.4–1.8)
Medium [>8.75–24.5]	8	154	2.3 (1.1–4.7)	5	106	2.2 (0.9–5.3)
High [>24.5–224.75]	2	140	0.7 (0.2–2.9)	5	298	0.8 (0.3–2.0)
			P trend = 0.47			P trend = 0.84
Heptachlor (chlorinated insecticide)						
None	205	20844	1.0 (ref)	205	20844	1.0 (ref)
Low [≤8.75]	21	1261	1.0 (0.6–1.6)	15	1110	0.8 (0.5–1.4)
Medium [>8.75–24.5]	18	679	1.5 (0.9–2.4)	16	425	2.0 (1.2–3.4)
High [>24.5–457.25]	7	600	0.7 (0.3–1.4)	14	1001	0.8 (0.5–1.4)
			P trend = 0.82			P trend = 0.88
Lindane (chlorinated insecticide)						
None	205	20375	1.0 (ref)	205	20375	1.0 (ref)
Low [≤8.75]	18	1285	1.2 (0.7–1.9)	15	976	1.3 (0.8–2.2)
Medium [>8.75–56]	13	1103	1.0 (0.6–1.7)	16	1205	1.1 (0.7–1.8)
High [>56–457.25]	14	467	2.5 (1.4–4.4)	14	673	1.8 (1.0–3.2)
			P trend = 0.004			**P trend = 0.04**
Toxaphene (chlorinated insecticide)						
None	214	20911	1.0 (ref)	214	20911	1.0 (ref)
Low [≤8.75]	14	1198	0.8 (0.5–1.4)	11	630	1.3 (0.7–2.3)
Medium [>8.75–24.5]	13	564	1.5 (0.9–2.7)	12	931	0.9 (0.5–1.6)
High [>24.5–457.25]	6	686	0.6 (0.3–1.4)	10	886	0.8 (0.4–1.5)
			P trend = 0.50			P trend = 0.38
Fungicides						
Benomyl (carbamate fungicide)						
None	219	21425	1.0 (ref)	219	21425	1.0 (ref)
Low [≤12.25]	14	896	1.7 (0.9–2.9)	9	432	2.2 (1.1–4.3)
Medium [>12.25–24.5]	4	214	2.4 (0.9–6.6)	10	732	1.7 (0.9–3.2)

Table 3. Cont.

Insecticides						
Pesticide (chemical-functional class) [days of lifetime exposure for each category]	**NHL Cases[2]**	**Non-Cases[2]**	**RR[3,4] (95% CI) by Total Days of Exposure**	**NHL Cases[2,]**	**Non-Cases**	**RR[3,4] (95% CI) Intensity-weighted days of exposure**
High [>24.5–457.25]	8	834	1.0 (0.5–2.1)	7	779	0.9 (0.4–2.0)
			P trend = 0.93			P trend = 0.75
Captan (phthalimide fungicide)						
None	407	43433	1.0 (ref)	407	43433	1.0 (ref)
Low [≤0.25]	15	2334	0.8 (0.5–1.4)	15	2108	0.9 (0.6–1.5)
Medium [>0.25–12.25]	16	1004	1.5 (0.8–2.6)	15	1171	1.2 (0.7–2.2)
High [>12.25–875]	14	1823	0.8 (0.5–1.5)	14	1805	0.8 (0.5–1.5)
			P trend = 0.69			P trend = 0.52
Chlorothalonil (polychlorinated aromatic thalonitrile fungicide)						
None	474	48442	1.0 (ref)	474	48442	1.0 (ref)
Low [≤12.25]	13	1509	0.9 (0.5–1.6)	10	1800	0.6 (0.3–1.2)
Medium [>12.25–64]	9	1492	0.8 (0.4–1.6)	11	1501	0.9 (0.5–1.7)
High [>64–395.25]	9	1678	0.6 (0.3–1.3)	9	1362	0.8 (0.4–1.6)
			P trend = 0.16			PP trend = 0.52
Maneb/Mancozeb (dithiocarbamate fungicide)						
None	228	21512	1.0 (ref)	228	21512	1.0 (ref)
Low [≤7]	8	400	1.9 (0.9–3.9)	8	486	1.6 (0.8–3.3)
Medium [>7–103.25]	9	990	0.9 (0.4–1.7)	9	680	1.3 (0.6–2.6)
High [>103.25–737.5]	7	454	1.4 (0.6–2.9)	7	677	0.9 (0.4–1.9)
			P trend = 0.49			P trend = 0.78
Metalaxyl (acylalanine fungicide)						
None	209	18833	1.0 (ref)	209	18833	1.0 (ref)
Low [≤6]	16	1439	1.0 (0.6–1.8)	15	1079	1.3 (0.8–2.2)
Medium [>6–28]	15	2182	0.7 (0.4–1.3)	15	2203	0.8 (0.4–1.3)
High [>28–224.75]	13	1566	1.1 (0.6–2.1)	14	1893	0.9 (0.5–1.6)
			P trend = 0.76			P trend = 0.63
Fumigant						
Methyl bromide (methyl halide fumigant)						
None	425	45265	1.0 (ref)	425	45265	1.0 (ref)
Low [≤8]	37	2060	2.0 (1.4–2.9)	26	1680	1.8 (1.2–2.7)
Medium [>8–28]	24	3011	0.9 (0.6–1.4)	25	2501	1.1 (0.7–1.8)
High [>28–387.5]	17	2768	0.6 (0.4–1.0)	25	3571	0.8 (0.5–1.2)
			P trend = 0.04			P trend = 0.10

[1]During the period from enrollment (1993–1997) to December 31, 2010 in NC and December 31, 2011 in Iowa.
[2]Numbers of cases in columns do not sum to total number of NHL cases (n = 523) due to missing data. In the enrollment questionnaire, lifetime-days & intensity weighted life-time days of pesticide use was obtained for the insecticides: carbofuran, chlorpyrifos, coumaphos, DDVP, fonofos, permethrin and terbufos; the fungicides: captan, chlothalonil and the fumigant: methyl bromide. In the take home questionnaire lifetime-days & intensity weighted life-time days of pesticide use were obtained for the insecticides: aldicarb, carbaryl, diazinon, malathion, parathion, and phorate, the chlorinated insecticides: aldrin, chlordane, DDT, dieldrin, heptachlor, lindane, and toxaphene, the fungicides: benomyl, maneb/mancozeb and metalaxyl, therefore, numbers of NHL cases can vary among pesticides listed in the table.
[3]Adjusted RR: age (<45, 45–49, 50–54, 55–59, 60–64, 65–69, ≥70), State (NC vs. IA), Race (White vs. Black), AHS herbicides (tertiles of total herbicide use-days). Statistically significant P trends are bolded.
[4]Permethrin for animal use and crop use were combined into one category.
[5]The distribution of life-time days of chlordane exposure was clumped into two exposed groups those who with, ≤8.75 life-time days of exposure and those with >8.75 life-time days of exposure.
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Table 4. Pesticide exposure (Lifetime-Days of Exposure) and adjusted risks for NHL Subtypes.

Insecticides											
	SLL, CLL, MCL		Diffuse Large B-cell		Follicular B-cell		Other B-cell types		Multiple Myeloma		
	RR[3,4] (95% CI)	N[2]	RR[3,4] (95% CI)	N[2]	RR[3,4] (95% CI)	N[2]	RR[3,4] (95% CI)	N[2]	RR[3,4] (95% CI)	N[2]	NHL subtype
											Homo-geneity
											Test
											(p-value)
Carbaryl											
None	1.0 (ref)	42	1.0 (ref)	29	1.0 (ref)	11	1.0 (ref)	14	1.0 (ref)	22	
Low	1.1 (0.6–2.2)	19	0.8 (0.4–1.6)	17	1.6 (0.6–3.9)	10	1.8 (0.7–4.3)	10	0.7 (0.3–1.4)	14	
High	0.6 (0.3–1.3)	15	1.3 (0.6–2.8)	15	2.8 (1.0–7.4)	10	0.4 (0.1–1.5)	3	1.1 (0.7–1.8)	13	
	p trend = 0.16		p trend = 0.33		p trend = 0.06		p trend = 0.63		p trend = 0.98		0.19
Carbofuran											
None	1.0 (ref)	87	1.0 (ref)	78	1.0 (ref)	39	1.0 (ref)	33	1.0 (ref)	56	
Low	1.1 (0.7–1.8)	28	0.9 (0.5–1.7)	13	1.3 (0.7–2.4)	15	0.8 (0.4–1.8)	8	1.9 ((1.1–3.3)	16	
High	1.5 (0.9–2.5)	19	0.8 (0.5–1.3)	13	0.4 (0.1–1.4)	3	0.7 (0.2–2.0)	4	0.9 (0.4–1.6)	12	
	p trend = 0.16		p trend = 0.37		p trend = 0.31		p trend = 0.46		p trend = 0.57		0.52
Chlorpyrifos											
None	1.0 (ref)	84	1.0 (ref)	70	1.0 (ref)	33	1.0 (ref)	31	1 (ref)	58	
Low	1.2 (0.8–1.8)	31	0.9 (0.6–1.5)	22	1.6 (0.9–2.9)	20	1.2 (0.6–2.2)	14	1.0 (0.6–1.8)	17	
High	0.9 (0.6–1.3)	30	1.1 (0.6–1.7)	22	1.0 (0.5–2.1)	11	0.5 (0.2–1.3)	7	0.7 (0.4–1.3)	14	
	p trend = 0.45		p trend = 0.80		p trend = 0.94		p trend = 0.13		p trend = 0.27		0.90
Coumaphos											
None	1.0 (ref)	120	1.0 (ref)	92	1.0 (ref)	48	1.0 (ref)	40	1.0 (ref)	78	
Low	1.1 (0.5–2.2)	8	0.7 (0.3–1.9)	4	2.1 (0.7–5.8)	4	xxx-	4	0.7 (0.2–2.2)	3	
High	1.5 (0.6–3.4)	6	1.6 (0.6–4.5)	4	1.4 (0.5–4.0)	4	xxx-	1	1.2 (0.4–4.0)	3	
	p trend = 0.35		p trend = 0.42		p trend = 0.47		p trend = xxx		p trend = 0.84		0.63
Diazinon											
None	1.0 (ref)	53	1.0 (ref)	40	1.0 (ref)	15	1.0 (ref)	20	1.0 (ref)	41	
Low	1.4 (0.7–2.7)	14	1.5 (0.7–3.2)	9	2.2 (0.9–5.4)	8	xxx	3	0.4 (0.1–1.2)	4	
High	1.9 (0.98–3.6)	12	1.1 (0.5–2.4)	8	3.8 (1.2–11.4)	7	xxx	2	0.5 (0.2–1.7)	3	
	p trend = 0.06		p trend = 0.72		**p trend = 0.02**		p trend = xxx		p trend = 0.35		0.09
DDVP											
None	1.0 (ref)	124	1.0 (ref)	93	1.0 (ref)	48	1.0 (ref)	39	1.0 (ref)	73	
Low	0.8 (0.4–1.9)	6	1.1 (0.4–2.7)	5	1.5 (0.6–3.9)	5	1.1 (0.4–3.7)	3	2.7 (1.2–5.8)	7	
High	0.7 (0.3–1.7)	6	0.9 (0.4–2.3)	5	1.0 (0.3–3.4)	3	0.9 (0.3–3.1)	3	1.0 (0.3–2.7)	4	
	p trend = 0.49		p trend = 0.87		p trend = 0.90		p trend = 0.91		p trend = 0.81		0.96
Fonofos											
None	1.0 (ref)	100	1.0 (ref)	81	1.0 (ref)	45	1.0 (ref)	30	1.0 (ref)	66	
Low	1.2 (0.7–2.0)	20	1.2 (0.7–2.2)	13	1.5 (0.8–3.0)	11	1.4 (0.6–3.1)	8	1.2 (0.6–2.5)	9	
High	1.0 (0.6–1.8)	15	1.2 (0.6–2.3)	11	0.3 (0.1–1.2)	2	1.1 (0.4–2.7)	6	1.4 (0.7–3.0)	9	
	p trend = 0.96		p trend = 0.65		p trend = 0.19		p trend = 0.84		p trend = 0.33		0.35
Malathion											
None	1.0 (ref)	27	1.0 (ref)	20	1.0 (ref)	6	1.0 (ref)	11	1.0 (ref)	17	
Low	0.7 (0.4–1.3)	29	0.96 (0.5–1.8)	23	1.0 (0.4–2.9)	12	1.0 (0.5–2.4)	11	1.0 (0.5–2.1)	18	
High	1.0 (0.6–1.8)	22	1.0 (0.5–2.0)	20	1.6 (0.6–4.4)	11	0.3 (0.1–0.8)	6	1.0 (0.5–2.0)	17	
Ever/Never	1.0 (0.7–1.4)		0.9 (0.6–1.4)		1.3 (0.7–2.4)		0.6 (0.3–1.0)		0.9 (0.6–1.5)		
	p trend = 0.65		p trend = 0.88		p trend = 0.25		p trend = 0.17		p trend = 0.86		0.33
Permethrin											

Table 4. Cont.

	SLL, CLL, MCL		Diffuse Large B-cell		Follicular B-cell		Other B-cell types		Multiple Myeloma		
	RR[3,4] (95% CI)	N[2]	RR[3,4] (95% CI)	N[2]	RR[3,4] (95% CI)	N[2]	RR[3,4] (95% CI)	N[2]	RR[3,4] (95% CI)	N[2]	NHL subtype Homo-geneity Test (p-value)
Insecticides											
None	1.0 (ref)	108	1.0 (ref)	89	1.0 (ref)	41	1.0 (ref)	38	1.0 (ref)	64	
Low	1.1 (0.6–2.0)	15	0.6 (0.3–1.2)	8	1.3 (0.6–2.7)	8	0.9 (0.3–2.7)	5	1.4 (0.8–2.7)	13	
High	0.8 (0.5–1.5)	15	1.0 (0.5–2.1)	8	1.0 (0.5–2.4)	8	0.5 (0.2–1.7)	4	3.1 (1.5–6.2)	12	
	p trend = 0.53		p trend = 0.99		p trend = 0.88		p trend = 0.28		**p trend = 0.002**		0.10
Phorate											
None	1.0 (ref)	48	1.0 (ref)	37	1.0 (ref)	20	1.0 (ref)	16	1.0 (ref)	36	
Low	1.0 (0.6–1.9)	14	1.4 (0.7–2.7)	15	1.1 (0.4–3.0)	5	0.9 (0.3–2.2)	6	0.7 (0.3–1.8)	6	
High	0.8 (0.4–1.6)	11	0.7 (0.3–2.1)	4	0.8 (0.3–2.2)	5	1.1 (0.4–3.5)	4	0.8 (0.3–2.4)	4	
	p trend = 0.51		p trend = 0.80		p trend = 0.67		p trend = 0.91		p trend = 0.73		0.77
Terbufos											
None	1.0 (ref)	72	1.0 (ref)	63	1.0 (ref)	31	1.0 (ref)	19	1.0 (ref)	59	
Low	1.3 (0.8–2.0)	32	1.2 (0.8–1.9)	29	1.6 (0.9–3.1)	15	1.8 (0.9–3.6)	17	1.1 (0.6–1.9)	12	
High	1.6 (1.0–2.5)	31	1.0 (0.5–2.0)	12	0.8 (0.4–1.7)	10	1.6 (0.7–3.9)	8	1.3 (0.7–2.7)	5	
	p trend = 0.05		p trend = 0.90		p trend = 0.48		p trend = 0.29		p trend = 0.42		0.63
Chlorinated Insecticides											
Aldrin											
None	1.0 (ref)	53	1.0 (ref)	46	1.0 (ref)	22	1.0 (ref)	20	1.0 (ref)	34	
Low	1.0 (0.5–2.0)	11	xxx	2	1.2 (0.4–3.8)	4	0.4 (0.1–1.5)	3	2.1 (0.9–4.7)	8	
High	1.0 (0.5–2.0)	10	xxx	3	0.8 (0.3–2.5)	4	1.1 (0.3–3.9)	3	1.2 (0.5–3.2)	6	
	p trend = 0.70		p trend = xxx		p trend = 0.21		p trend = 0.67		p trend = 0.40		0.98
Chlordane											
None	1.0 (ref)	48	1.0 (ref)	42	1.0 (ref)	20	1.0 (ref)	21	1.0 (ref)	32	
Low	1.8 (1.0–3.1)	16	1.0 (0.5–2.2)	8	1.7 (0.7–4.3)	6	xxx	2	1.7 (0.9–3.3)	13	
High	1.5 (0.7–3.3)	8	1.4 (0.6–3.3)	7	1.3 (0.4–4.6)	3	xxx	2	0.7 (0.2–2.2)	3	
	p trend = 0.34		p trend = 0.69		p trend = 0.70		p trend = xxx		p trend = 0.57		0.85
DDT											
None	1.0 (ref)	42	1.0 (ref)	34	1.0 (ref)	17	1.0 (ref)	16	1.0 (ref)	28	
Low	1.0 (0.5–1.8)	16	1.6 (0.4–3.1)	2	3.3 (1.4–8.1)	9	0.4 (0.3–2.5))	5	1.2 (0.6–2.6)	10	
High	2.6 (1.3–4.8)	15	1.4 (0.6–3.5)	3	1.1 (0.3–3.6)	4	2.1 (0.7–6.5)	5	0.8 (0.4–1.8)	9	
	p trend = 0.04		P trend = 0.17		p trend = 0.80		p trend = 0.64		p trend = 0.37		0.44
Heptachlor											
None	1.0 (ref)	58	1.0 (ref)	47	1.0 (ref)	24	1.0 (ref)	21	1.0 (ref)	40	
Low	1.1 (0.5–2.3)	9	xxx	3	xxx	2	xxx	3	1.3 (0.4–3.8)	4	
High	1.4 (0.7–3.0)	9	xxx	1	xxx	1	xxx	2	1.2 (0.4–3.6)	4	
	p trend = 0.16		p trend = xxx		p trend = xxx		p trend = xxx		p trend = 0.91		0.68
Lindane											
None	1.0 (ref)	57	1.0 (ref)	49	1.0 (ref)	16	1.0 (ref)	21	1.0 (ref)	43	
Low	1.2 (0.6–2.5)	10	0.6 (0.2–1.7)	4	4.9 (1.9–12.6)	6	xxx	2	xxx	3	
High	2.6 (1.2–5.6)	9	2.0 (0.6–6.5)	3	3.6 (1.4–9.5)	6	xxx	1	xxx	2	
	p trend = 0.13		p trend = 0.96		**p trend = 0.04**		p trend = xxx		p trend = xxx		0.54
Toxaphene											
None	1.0 (ref)	68	1.0 (ref)	47	1 (ref)	23	1.0 (ref)	22	1.0 (ref)	40	

Table 4. Cont.

Insecticides	SLL, CLL, MCL		Diffuse Large B-cell		Follicular B-cell		Other B-cell types		Multiple Myeloma		
	RR[3,4] (95% CI)	N[2]	RR[3,4] (95% CI)	N[2]	RR[3,4] (95% CI)	N[2]	RR[3,4] (95% CI)	N[2]	RR[3,4] (95% CI)	N[2]	NHL subtype Homo-geneity Test (p-value)
Low	0.9 (0.4–2.3)	5	1.3 (0.5–3.3)	5	xxx	2	xxx	3	0.7 (0.2–2.0)	4	
High	0.4 (0.1–1.6)	2	0.9 (0.3–3.0)	3	xxx	2	xxx	2	0.7 (0.2–2.9)	2	
	p trend = 0.08		p trend = 0.77		p trend = xxx		p trend = xxx		p trend = 0.64		0.34
Fungicides											
Captan											
None	1.0 (ref)	118	1.0 (ref)	91	1.0 (ref)	52	1.0 (ref)	39	1.0 (ref)	76	
Low	0.9 (0.4–1.9)	7	1.1 (0.5–2.4)	7	xxx	2	xxx	3	1.4 (0.5–3.4)	5	
High	1.1 (0.5–2.6)	7	0.7 (0.1–3.1)	4	xxx	1	xxx	2	1.2 (0.5–2.9)	5	
	p trend = 0.78		p trend = 0.58		p trend = xxx		p trend = xxx		p trend = 0.75		0.92
Chlorothalonil											
None	1.0 (ref)	135	1.0 (ref)	107	1.0 (ref)	60	1.0 (ref)	50	1.0 (ref)	84	
Low	0.9 (0.4–2.3)	5	1.1 (0.4–3.1)	4	xxx	3	−xxx	1	1.1 (0.4–2.8)	5	
High	1.1 (0.4–3.3)	4	0.3 (0.1–1.2)	2	xxx	2	−xxx	1	0.7 (0.6–2.3)	3	
	p trend = 0.83		p trend = 0.09		p trend = xxx		p trend = xxx		p trend = 0.56		0.76
Metalaxyl											
None	1.0 (ref)	60	1.0 (ref)	45	1.0 (ref)	25	1.0 (ref)	23	1.0 (ref)	39	
Low	2.8 (1.4–5.8)	9	1.1 (0.4–2.6)	7	xxx	3	−xxx	2	0.4 (0.1–1.1)	4	
High	1.1 (0.4–2.8)	6	1.0 (0.4–2.7)	5	xxx	2	−xxx	1	1.1 (0.4–3.2)	4	
	p trend = 0.99		p trend = 0.97		p trend = xxx		p trend = xxx		p trend = 0.87		0.92
Maneb/ Mancozeb											
None	1.0 (ref)	69	1.0 (ref)	49	1.0 (ref)	25	1.0 (ref)	26	1.0 (ref)	41	
Low	2.1 (0.7–6.0)	4	4.0 (1.4–11.6)	4	xxx	2	−xxx	0	1.0 (0.4–2.5)	5	
High	1.2 (0.3–4.0)	3	0.9 (0.3–3.1)	3	−xxx	1	−xxx	0	2.2 (0.5–9.5)	2	
	p trend = 0.84		p trend = 0.74		p trend = xxx		p trend = xxx		p trend = 0.28		0.82
Fumigant											
Methyl Bromide											
None	1.0 (ref)	126	1.0 (ref)	86	1.0 (ref)	58	1.0 (ref)	44	1.0 (ref)	76	
Low	1.1 (0.5–2.2)	9	4.0 (2.2–7.4)	15	1.4 (0.5–4.2)	4	3.6 (1.3–9.8)	5	1.0 (0.5–2.1)	8	
High	0.8 (0.4–1.8)	8	1.0 (0.5–2.1)	11	0.3 (0.1–1.1)	3	1.3 (0.3–5.0)	3	0.8 (0.4–1.8)	8	
	p trend = 0.58		p trend = 0.67		p trend = 0.08		p trend = 0.56		p trend = 0.63		0.59

[1]During the period from enrollment (1993–1997) to December 31, 2010 in NC and December 31, 2011 in Iowa.
[2]Numbers of cases in columns do not sum to total number of NHL cases (n = 523) due to missing data. Ever/never use of all 26 pesticides (table 3) do not always match with exposure-response data in table 4 because of missing data to calculate lifetime-days of use.
[3]Adjusted for age (<45, 45–49, 50–54, 55–59, 60–64, 65–69, ≥70), State (NC vs. IA), Race (White vs. Black), AHS herbicides (in tertiles of total herbicide use-days). Significant RR and 95% confidence limits are bolded.
[4]RR was not calculated if the number of exposed cases for any NHL subtype was <6 and these cells are marked XXX. Four pesticides included in Table 2 (i.e., aldicarb, benomyl, dieldrin and parathion) were not included in Table 4 because no NHL subtype included≥6 cases of a specific cell types with lifetime-days of exposure.
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risk estimates (i.e., narrower confidence intervals) when we included phase 2 imputed data (n = 54,306) (data not shown). Lagging exposures by five years did not meaningfully change the association between lindane or DDT and total NHL (data not shown). The significant exposure-response trends linking use of a particular pesticide to NHL and certain NHL subtypes did not always correspond to a significant excess risk among those who ever used the same pesticide. For chemicals for which the detailed information was only asked about in the take-home questionnaire, we evaluated potential differences between the ever/never analyses based on the enrolment questionnaire and data from the same sub-set of participants who completed the exposure-

response in the take-home questionnaire and found no meaningful differences in the results. We also evaluated the impact of using an updated definition of NHL; when using the original ICD-O-3 definition of NHL[19], lifetime-days of lindane use remained significantly associated with NHL risk (RR = 1.0 (ref), 1.3 (0.7–2.6), 1.2 (0.6–2.8), 2.7 (1.3–5.4), p trend = 0.006). The trend between total NHL and lifetime-days of DDT, however, was less clear and not statistically significant (RR = 1.0 (ref) 1.3 (0.9–1.8), 1.1 (0.5–2.1), 1.4 (0.8–2.6), p trend = 0.32) [Table S3 in File S1]. Carbaryl and diazinon showed non-significant trends with the older definition of NHL, but not with the newer definition used here.

Discussion

A significant exposure–response trend for total NHL was observed with increasing lifetime-days of use for two organochlorine insecticides, lindane and DDT, although RRs from ever/never comparisons were not elevated. On the other hand, terbufos use showed a significant excess risk with total NHL in ever vs. never exposed analysis, but displayed no clear exposure-response trend. Several pesticides showed significant exposure-response trends with specific NHL subtypes however, when polytomous models were used to test the difference in parametric estimates of trend among the five NHL subtypes, there was no evidence of heterogeneity in the sub-types for specific chemicals. The subtype relationships that looked particularly interesting were DDT and terbufos with the SLL/CLL/MCL subtype, lindane and diazinon with the follicular subtype, and permethrin with MM. These pesticide-NHL links should be evaluated in future studies.

Lindane (gamma-hexachlorocyclohexane) is a chlorinated hydrocarbon insecticide. Production of lindane was terminated in the United States in 1976, but imported lindane was used to treat scabies and lice infestation and for agricultural seed treatment [21] until its registration was cancelled in 2009 [22], the same year production was banned worldwide [23]. In our study, 3,410 people reporting ever using lindane (6%) prior to enrollment, 433 reported use at the phase 2 questionnaire (1%), indicating that use had dropped substantially. Oral administration of lindane has increased the incidence of liver tumors in mice and less clearly, thyroid tumors in rats [24]. Lindane produces free radicals and oxidative stress (reactive oxygen species [ROS]) [25] and has been linked with chromosomal aberrations in human peripheral lymphocytes in vitro [26].

Lindane has been linked with NHL in previous epidemiologic studies. A significant association between lindane use and NHL was observed in a pooled analysis of three population-based case-control studies conducted in the Midwestern US, with stronger relative risks observed for greater duration and intensity of use [27]. NHL was also associated with lindane use in a Canadian case-control study [28]. Lindane was significantly associated with NHL risk in an earlier report from the AHS [29]. We are not aware of any previous study that assessed the association between a NHL subtype and lindane use. The exposure-response pattern with total NHL and the follicular lymphoma subtype indicates a need for further evaluation of lindane and NHL.

DDT is an organochlorine insecticide that was used with great success to control malaria and typhus during and after World War II [29] and was widely used for crop and livestock pest control in the United States from the mid-1940s to the 1960s [30]. Its registration for crop use was cancelled in the US in 1972 [30] and banned worldwide for agricultural use in 2009, but continues to be used for disease vector control in some parts of the world [23]. In our study, 12,471 participants (23%) reported ever using DDT prior to enrollment; 12%, 8.7% and 2.3% responding to the take-home questionnaire reported their first use occurred prior to the 1960s, during the 1960s, and during the 1970s, respectively. The National Toxicology Program classifies DDT as "reasonably anticipated to be a human carcinogen" [31] and IARC classifies DDT as a "possible human carcinogen (2B)" [12], both classifications were based on experimental studies in which excess liver tumors were observed in two rodent species. Epidemiology data on the carcinogenic risk of DDT is inconsistent. NHL was not associated with use of DDT in a pooled analysis of three case-control studies in the U.S. where information on exposure was obtained from farmers by questionnaire [32]. There also was no association between the use of DDT and NHL in our study when we used an earlier definition of NHL [18], suggesting some of the inconsistency may be due to disease definition. In the large Epilymph study, no meaningful links between DDT and the risk of NHL, or diffuse large B cell lymphoma were observed, and only limited support was found for a link to CLL [33], although a case-control study of farmers in Italy suggested increased risk of NHL and CLL with DDT exposure [34]. NHL was not associated with serum levels of DDT in a prospective cohort study from the U.S. [35], but NHL was associated with the DDT-metabolite p, p'-DDE, as well as chlordane and heptachlor-related compounds (oxychlordane, heptachlor epoxide) and dieldrin, in a study with exposure measured in human adipose tissue samples [36]. In a Danish cohort, a higher risk of NHL was associated with higher prediagnostic adipose levels of DDT, cis-nonachlor, and oxychlordane [37]. In a Canadian study, analytes from six insecticides/insecticide metabolites (beta-hexachlorocyclohexane, p, p'-dichloro-DDE, hexachlorobenzene (HCB), mirex, oxychlordane and transnonachlor) were linked with a significant increased risk with NHL [38]. However, in an analysis of plasma samples from a case-control study in France, Germany and Spain, the risk of NHL did not increase with plasma levels of hexachlorobenzene, beta-hexachlorobenzene or DDE [39]. In this analysis, NHL was significantly associated with reported use of DDT, but not with the other organochlorine insecticides studied (i.e., aldrin, chlordane, dieldrin, heptachlor, toxaphene). Our findings add further support for an association between DDT and total NHL and our results on SLL/CLL/MCL are novel and should be further explored.

Permethrin is a broad-spectrum synthetic pyrethroid pesticide widely used in agriculture and in home and garden use as an insecticide and acaricide, as an insect repellant, and as a treatment to eradicate parasites such as head lice or mites responsible for scabies [40]. This synthetic pyrethroid was first registered for use in the United States in 1979 [40]. The U.S. Environmental Protection Agency classified permethrin as "likely to be carcinogenic to humans" largely based on the observed increase incidence of benign lung tumors in female mice, liver tumors in rats and liver tumors in male and female mice [41]. Permethrin was not associated with NHL overall in our study, nor in pooled case-control studies of NHL from the U.S (the NHL definition in use at the time of the study did not include MM) [42]. In our analysis, however, the risk of MM increased significantly with lifetime-days of exposure to permethrin, as had been noted in an earlier analysis of AHS data [43]. We are unaware of other studies that have found this association.

Terbufos is an organophosphate insecticide and nematicide first registered in 1974 [44]. The EPA classifies terbufos as Group E, i.e., "Evidence of Non-Carcinogenicity for Humans" [44]. We found some evidence for an association between terbufos use and NHL, particularly for the SLL/CLL/MCL subtype. NHL was not associated with terbufos in the pooled case-control studies from the

U.S. [42] but there was a non-significant association between terbufos and small cell lymphocytic lymphoma [10].

Diazinon is an organophosphate insecticide registered for a variety of uses on plants and animals in agriculture [45]. It was commonly used in household insecticide products until the EPA phased out all residential product registrations for diazinon in December 2004 [45,46]. In an earlier evaluation of diazinon in the AHS, a significant exposure-response association was observed for leukemia risk with lifetime exposure-days [47]. While there was no link between diazinon and NHL overall in this analysis, there was a statistically significant exposure-response association between diazinon and the follicular lymphoma subtype and an association with the SLL/CLL/MCL subtype that was not statistically significant. Diazinon was previously associated with NHL in pooled case-control studies from the U.S. and particularly with SLL [10].

Several other insecticides, fungicides and fumigants cited in recent reviews of the pesticide-cancer literature suggested etiological associations with total NHL [8,9], these include: oxychlordane, trans-nonachlor, and cis-nonachlor which are metabolites of chlordane; and dieldrin and toxaphene among NHL cases with t(14,18) translocations. We did not find a significant association between chlordane and total NHL nor with any NHL subtype, but we did not have information about chlordane metabolites to make a more direct comparison. Similarly we did not observe a significant association between dieldrin nor toxaphene and total NHL nor with any NHL subtypes. Mirex (1,3-cyclopentadiene), an insecticide, and hexachlorobenzene, a fungicide, were also associated with NHL risk [8,9] but we did not examine these compounds in the AHS.

This study has a number of strengths. It is a large population of farmers and commercial pesticide applicators who can provide reliable information regarding their pesticide use history [48]. Information on pesticide use and application practices was obtained prior to onset of cancer. An algorithm that incorporated several exposure determinants which predicted urinary pesticide levels was used to develop an intensity-weighted exposure metric in our study [20]. Exposure was ascertained prior to diagnosis of disease, which should eliminate the possibility of case-response bias [14]. Because of the detailed information available on pesticide use, we were able to assess the impact for the use of multiple pesticides. For example, we evaluated total pesticide use-days, and specific pesticides found to be associated with NHL or its subtypes in the AHS. We found no meaningful change in the associations with DDT, lindane, permethrin, diazinon and terbufos from such adjustments. Information on many potential NHL risk factors was available and could be controlled in the analysis.

Most epidemiological investigations of NHL prior to 2007 [17] did not include CLL and MM as part of the definition. These two subtypes made up 37% (193/523) of the NHL cases in this analysis. This is a strength of our study in that the definition of NHL used here is based on the most recent classification system [16,17] and will be relevant for comparisons with future studies. On the other hand, the inclusion of MM and CLL in the recent definition of NHL makes comparisons of our findings with earlier literature challenging, because the NHL subtypes may have different etiologies. For example, DDT was not significantly associated with NHL using the older definition, but was significantly associated with the NHL using the most recent definition of NHL because of its association with the SLL/CLL/MCL subtype (Table S1 in File S1). On the other hand, carbaryl and diazinon were associated with the old definition of NHL (although non-significantly) but not with the new definition. Lindane, however, was associated with both definitions of NHL. Lindane was significantly associated with the follicular lymphoma subtype and this subtype was included in the older and newer definition of NHL. No other pesticides were significantly associated with NHL under the old definition (Table S3 in File S1).

Although this is a large prospective study, limitations should be acknowledged. A small number of cases exposed to some specific pesticides could lead to false positive or negative findings. We also had reduced statistical power to evaluate some pesticides for total days of use and intensity-weighted days of use because some participants did not complete the phase one take-home questionnaire and the tests of homogeneity between specific pesticides and specific NHL subtypes were underpowered. Some chance associations could occur because of multiple testing, i.e., a number of pesticides, several NHL subtypes, and more than one exposure metric. Despite the generally high quality of the information on pesticide use provided by AHS participants [48,50], misclassification of pesticide exposures can occur and can have a sizeable impact on estimates of relative risk, which in a prospective cohort design would tend to produce false negative results [49].

Conclusion

Our results showed pesticides from different chemical and functional classes were associated with an excess risk of NHL and NHL subtypes, but not all members of any single class of pesticides were associated with an elevated risk of NHL or NHL subtypes, nor were all chemicals of a class included on our questionnaire. Significant pesticide associations were between total NHL and reported use of lindane and DDT. Links between DDT and terbufos and SLL/CLL/MCL, lindane and diazinon and follicular lymphoma, and permethrin and MM, although based on relatively small numbers of exposed cases, deserve further evaluation. The epidemiologic literature on NHL and these pesticides is inconsistent and although the findings from this large, prospective cohort add important information, additional studies that focus on NHL and its subtypes and specific pesticides are needed. The findings from this large, prospective cohort add important new information regarding the involvement of pesticides in the development of NHL. It provides additional information regarding specific pesticides and NHL overall and some new leads regarding possible links with NHL subtypes that deserve evaluation in future studies.

Supporting Information

File S1 This file contains Table S1, Table S2, and Table S3. Table S1, Frequency of NHL in Agricultural Health Study applicators using New (Interlymph hierarchical classification of lymphoid neoplasms) and Older Definitions (ICD-O-3). Table S2, Pesticides included in the Agricultural Health Study questionnaires by Chemical/Functional Class. Table S3, Pesticide exposure (lifetime-days) and adjusted risks of total NHL incidence (Older definition [ICD-O-3]).
(DOC)

Acknowledgments

Disclaimer: The findings and conclusions in this report are those of the author(s) and do not necessarily represent the views of the National Institute for Occupational Safety and Health. The United States Environmental Protection Agency through its Office of Research and Development partially funded and collaborated in the research described here under Contracts 68-D99-011 and 68-D99-012, and through Interagency Agreement DW-75-93912801-0. It has been subjected to Agency review and approved for publication.

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Author Contributions

Conceived and designed the experiments: MCA DPS AB. Performed the experiments: MCA CFL KT CJH. Analyzed the data: MCA JNH CFL CJH KHB JB DWB KT DPS JAH SK GA JHL AB LEB. Contributed reagents/materials/analysis tools: MCA JB DWB CFL. Wrote the paper: MCA LEBF JNH CFL CJH KT AB DWB JHL. Designed the software: JB DWB.

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DRAFT-
Lymphoma risk and pesticide use in the Agricultural Health Study

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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.

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ABSTRACT

Background: Farming and eExposure to pesticides haves 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 applicatorsregistered 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 historyhealth 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.

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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). 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 notthey 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; Cocco et al., 2004), organophosphates (Waddell et al., 2001, Hohenadel et al., 2011)dicamba (McDuffie et al., 2001), nitro-derivatives (Miligi et al., 2005); 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

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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 (Smiard 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

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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.

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Commented [a17]: Yes, we removed all prevalent cancers and included only primary NHL cases.-clarification made in sentence.-no other change necessary.

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

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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.

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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 five5 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/non-use of pesticides in phase 2~~.

Commented [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).

Commented [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 pesticide ~~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.

Commented [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 exposure 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

Commented [a26]: Correction suggested by Cindy.

Commented [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 adjustment 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

Commented [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~~ 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

Commented [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.?

Commented [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.

Commented [AB31]: The Methods says they were significant risk factors.

Commented [a32]: Previous table 2 deleted and discussion of potential confounding variables shortened as suggested by Laura.

Commented [t33]: It's not clear why you are showing these 22 pesticides

Commented [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.

Commented [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~~ confounding 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.

Commented [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.

Commented [a37]: Point estimates deleted to reduce word count as recommended.

Commented [a38]: Need to define the pesticides included in each group appendix 2-done

Commented [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. ,

Commented [lbf40]: I don't think you mention this in the results.

Commented [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).

Commented [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).

Commented [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.

Commented [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

Commented [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.

Commented [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.

Commented [a47]: These will be adjusted for total number of exposure days to chemicals in this class.-Done

DISCUSSION

Commented [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

Commented [a49]: See changes made throughout to address these points.

Commented [lbf50]: *This paper just came out and used the most recent definitions of NHL. Actually supportive of these AHS findings.*
*Occup Environ Med*2013;**70**:91-98 doi:10.1136/oemed-2012-100845

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

AB – I think we need to start with the big picture comparisons first. I suggest the order for the discussion should be: (1) Everi/ever comparisons for NHL overall;, (2) Then move to trends for NHL overall; (3) Then move to subtypes. (4) Next have a discussion of how the comparison

NHL definition might affect comparison of our results with those from the literature. (5) Comparison of these results with literature pesticide vs. 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 subtype. 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.)

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

Commented [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.

Commented [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.

Commented [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.

Commented [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.

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 triazine 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.

Commented [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.

Commented [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

Commented [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.

Commented [lbf58]: Expand to discuss what these actually show—similar to ours? Not similar to ours?

Commented [a59]: Modified sentence in response to comment.

Commented [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 be 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)

Commented [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/omissions 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.

Commented [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 make 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 RR's). Previously, NHL subtypes with t (14;18) translocations were associated with the chlorinated insecticides dieldrin, lindane, and toxaphene

Commented [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 q14;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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Commented [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?

Commented [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)

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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).								
Insecticides, fungicide and fumigant								
	CLL, SLL, 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**
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

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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

Commented [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

Commented [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)

Commented [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.

Commented [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)

Commented [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

Commented [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)

Commented [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.

Commented [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.

Commented [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)

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		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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