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

Mikael Eriksson[1]*, Lennart Hardell[2], Michael Carlberg[2] and Måns Åkerman[3]

[1]*Department of Oncology, University Hospital, Lund, Sweden*
[2]*Department of Oncology, University Hospital, Örebro, Sweden*
[3]*Department of Pathology, University Hospital, Lund, Sweden*

We report a population based case–control study of exposure to pesticides as risk factor for non-Hodgkin lymphoma (NHL). Male and female subjects aged 18–74 years living in Sweden were included during December 1, 1999, to April 30, 2002. Controls were selected from the national population registry. Exposure to different agents was assessed by questionnaire. In total 910 (91%) cases and 1016 (92%) controls participated. Exposure to herbicides gave odds ratio (OR) 1.72, 95% confidence interval (CI) 1.18–2.51. Regarding phenoxyacetic acids highest risk was calculated for MCPA; OR 2.81, 95% CI 1.27–6.22, all these cases had a latency period >10 years. Exposure to glyphosate gave OR 2.02, 95% CI 1.10–3.71 and with >10 years latency period OR 2.26, 95% CI 1.16–4.40. Insecticides overall gave OR 1.28, 95% CI 0.96–1.72 and impregnating agents OR 1.57, 95% CI 1.07–2.30. Results are also presented for different entities of NHL. In conclusion our study confirmed an association between exposure to phenoxyacetic acids and NHL and the association with glyphosate was considerably strengthened.
© 2008 Wiley-Liss, Inc.

Key words: phenoxyacetic acids; MCPA; glyphosate; insecticides; impreganting agents; non-Hodgkin lymphoma

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

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

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

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

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

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

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

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

Material and methods

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

Cases

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

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

Controls

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

Assessment of exposure

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

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

Statistical methods

Unconditional logistic regression analysis (Stata/SE 8.2 for Windows; StataCorp, College Station, TX) was used to calculate odds ratios (OR) and 95% confidence intervals (CI). Adjustment was made for age, sex and year of diagnosis (cases) or enrolment (controls). In the univariate analysis, different pesticides were analyzed separately and the unexposed category consisted of subjects that were unexposed to all included pesticides. When analyzing

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

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

subgroups of NHL all controls were used in the separate analyses. In the dose-response calculations made for agents with at least 20 exposed subjects, median number of days of exposure among controls was used as cut-off. Latency period calculations and multivariate analyses included agents with statistically significant increased OR, or with an OR > 1.50 and at least 10 exposed subjects.

Results

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

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

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

This report presents exposure data regarding different types of pesticides.

Herbicides

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

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

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

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

When different NHL entities were analysed separately, the OR for the subtype small lymphocytic lymphoma/chronic lymphocytic leukaemia (SLL/CLL) was increased for both phenoxy herbicides and, especially, glyphosate, Table III. The entity diffuse large B-cell lymphoma (DLBCL) was significantly associated with exposure to phenoxyacetic acids, but not to other herbicides. On the other hand, the group follicular lymphoma was not clearly associated with phenoxyacetic acids, and only nonsignificantly with

glyphosate. The category "other specified B-cell lymphoma" (*e.g.*, mantle cell lymphoma, marginal zone lymphoma) was significantly associated with exposure to phenoxyacetic acids, and an increased risk was also indicated for glyphosate. T-cell lymphomas seemed to be associated with all types of herbicides, but no statistically significant ORs were found due to relatively few exposed subjects. The least numerous categories ("unspecified NHL") yielded high and statistically significant ORs for phenoxy herbicides and glyphosate.

Insecticides

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

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

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

Fungicides and rodenticides

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

Impregnating agents

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

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

TABLE II – EXPOSURE TO VARIOUS HERBICIDES

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

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

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

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

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

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

TABLE IV – EXPOSURE TO VARIOUS OTHER PESTICIDES

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

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

Multivariate analysis

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

Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Acknowledgements

References

1. Jaffe ES, Harris NL, Stein H, Vardiman JW. World Health Organization classification of tumours. Pathology and genetics. Tumours of haematopoetic and lymphoid tissues. Lyon: IARC Press, 2001.
2. Hardell L, Eriksson M. Is the decline of the increasing incidence of non-Hodgkin lymphoma in Sweden and other countries a result of cancer preventive measures? Environ Health Perspect 2003;111: 1704–6.
3. Hardell L, Axelson O. Environmental and occupational aspects on the etiology of non-Hodgkin's lymphoma. Oncol Res 1998;10:1–5.
4. Pluda JM, Venzon DJ, Tosato G, Lietzau J, Wyvill K, Nelson DL, Jaffe ES, Karp JE, Broder S, Yarchoan R. Parameters affecting the development of non-Hodgkin's lymphoma in patients with severe human immunodeficiency virus infection receiving antiretroviral therapy. J Clin Oncol 1993;11:1099–107.
5. Patton DF, Wilkowski CW, Hanson CA, Shapiro R, Gajl-Peczalska KJ, Filipovich AH, McClain KL. Epstein-Barr virus–determined clonality in posttransplant lymphoproliferative disease. Transplantation 1990;49:1080–4.
6. Lehtinen T, Lumio J, Dillner J, Hakama M, Knekt P, Lehtinen M, Teppo L, Leinikki P. Increased risk of malignant lymphoma indicated by elevated Epstein-Barr virus antibodies—a prospective study. Cancer Causes Control 1993;4:187–93.
7. Potter M. Pathogenetic mechanisms in B-cell non-Hodgkin's lymphomas in humans. Cancer Res 1992;52:5522S–5528S.
8. Hardell L, Eriksson M, Lenner P, Lundgren E. Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study. Br J Cancer 1981;43:169–76.
9. Hardell L. Malignant lymphoma of histiocytic type and exposure to phenoxyacetic acids or chlorophenols. Lancet 1979;1:55–6.
10. International Agency for Research on Cancer. Polychlorinated dibenzo-para-dioxins. IARC Monogr Eval Carcinog Risks Hum 1997;69:333–343.
11. Vos JG, Moore JA, Zinkl JG. Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on the immune system of laboratory animals. Environ Health Perspect 1973;5:149–62.
12. Exon JH, Talcott PA, Koller LD. Effect of lead, polychlorinated biphenyls, and cyclophosphamide on rat natural killer cells, interleukin 2, and antibody synthesis. Fundam Appl Toxicol 1985;5:158–64.
13. Lu YC, Wu YC. Clinical findings and immunological abnormalities in Yu-Cheng patients. Environ Health Perspect 1985;59:17–29.
14. Kerkvliet NI, Brauner JA. Mechanisms of 1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin (HpCDD)-induced humoral immune suppression: evidence of primary defect in T-cell regulation. Toxicol Appl Pharmacol 1987;87:18–31.
15. Faustini A, Settimi L, Pacifici R, Fano V, Zuccaro P, Forastiere F. Immunological changes among farmers exposed to phenoxy herbicides: preliminary observations. Occup Environ Med 1996;53: 583–5.
16. Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R, Fraumeni JF, Jr. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. JAMA 1986;256:1141–7.
17. Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP, Blair A. A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. Epidemiology 1990;1:349–56.
18. Hardell L, Eriksson M. A case-control study of non-Hodgkin lymphoma and exposure to pesticides. Cancer 1999;85:1353–60.
19. Hardell L, Eriksson M, Degerman A. Exposure to phenoxyacetic acids, chlorophenols, or organic solvents in relation to histopathology, stage, and anatomical localization of non-Hodgkin's lymphoma. Cancer Res 1994;54:2386–9.
20. Hardell L, Eriksson M, Axelson O, Flesch-Janys D. Epidemiological studies on cancer and exposure to dioxins and related compounds. In: Schecter A, Gasiewicz T, eds. Dioxins and health. Hoboken, NJ: John Wiley & Sons, 2003. p 729–64.
21. Miligi L, Costantini AS, Veraldi A, Benvenuti A, Vineis P. Cancer and pesticides: an overview and some results of the Italian multicenter case-control study on hematolymphopoietic malignancies. Ann N Y Acad Sci 2006;1076:366–77.
22. Waddell BL, Zahm SH, Baris D, Weisenburger DD, Holmes F, Burmeister LF, Cantor KP, Blair A. Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). Cancer Causes Control 2001;12:509–17.
23. Zheng T, Zahm SH, Cantor KP, Weisenburger DD, Zhang Y, Blair A. Agricultural exposure to carbamate pesticides and risk of non-Hodgkin lymphoma. J Occup Environ Med 2001;43:641–9.
24. Purdue MP, Hoppin JA, Blair A, Dosemeci M, Alavanja MC. Occupational exposure to organochlorine insecticides and cancer incidence in the agricultural health study. Int J Cancer 2007;120:642–9.
25. Colt JS, Davis S, Severson RK, Lynch CF, Cozen W, Camann D, Engels EA, Blair A, Hartge P. Residential insecticide use and risk of non-Hodgkin's lymphoma. Cancer Epidemiol Biomarkers Prev 2006;15:251–7.
26. Rusiecki JA, De Roos A, Lee WJ, Dosemeci M, Lubin JH, Hoppin JA, Blair A, Alavanja MC. Cancer incidence among pesticide applicators exposed to atrazine in the Agricultural Health Study. J Natl Cancer Inst 2004;96:1375–82.
27. Fritschi L, Benke G, Hughes AM, Kricker A, Turner J, Vajdic CM, Grulich A, Milliken S, Kaldor J, Armstrong BK. Occupational exposure to pesticides and risk of non-Hodgkin's lymphoma. Am J Epidemiol 2005;162:849–57.

28. van Balen E, Font R, Cavalle N, Font L, Garcia-Villanueva M, Benavente Y, Brennan P, de Sanjose S. Exposure to non-arsenic pesticides is associated with lymphoma among farmers in Spain. Occup Environ Med 2006;63:663–8.

29. Steinrucken HC, Amrhein N. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase. Biochem Biophys Res Commun 1980;94:1207–12.

30. US EPA. U.S. Environmental Protection Agency Registration Eligibility Decision (RED) Glyphosate. EPA-R-93-014. Washington DC: US Environmental Protection Agency, 1993.

31. World Health Organization. International programme on chemical safety. Glyphosate. Environmental health criteria 159. Geneva: WHO, 1994.

32. De Roos AJ, Blair A, Rusiecki JA, Hoppin JA, Svec M, Dosemeci M, Sandler DP, Alavanja MC. Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. Environ Health Perspect 2005;113:49–54.

33. Bolognesi C, Bonatti S, Degan P, Gallerani E, Peluso M, Rabboni R, Roggieri P, Abbondandolo A. Genotoxic activity of glyphosate and its technical formulation Roundup. J Agric Food Chem 1997;45:1957–62.

34. Lioi MB, Scarfi MR, Santoro A, Barbieri R, Zeni O, Di Berardino D, Ursini MV. Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro. Mutat Res 1998; 403:13–20.

35. Lioi MB, Scarfi MR, Santoro A, Barbieri R, Zeni O, Salvemini F, Di Berardino D, Ursini MV. Cytogenetic damage and induction of pro-

oxidant state in human lymphocytes exposed in vitro to glyphosate, vinclozolin, atrazine, and DPX-E9636. Environ Mol Mutagen 1998;32:39–46.

36. Hardell L, Eriksson M, Nordstrom M. Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies. Leuk Lymphoma 2002; 43:1043–9.

37. McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA, Robson D, Skinnider LF, Choi NW. Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. Cancer Epidemiol Biomarkers Prev 2001;10: 1155–63.

38. De Roos AJ, Zahm SH, Cantor KP, Weisenburger DD, Holmes FF, Burmeister LF, Blair A. Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. Occup Environ Med 2003;60:E11.

39. Tatham L, Tolbert P, Kjeldsberg C. Occupational risk factors for subgroups of non-Hodgkin's lymphoma. Epidemiology 1997;8:551–8.

40. Rothman N, Cantor KP, Blair A, Bush D, Brock JW, Helzlsouer K, Zahm SH, Needham LL, Pearson GR, Hoover RN, Comstock GW, Strickland PT. A nested case-control study of non-Hodgkin lymphoma and serum organochlorine residues. Lancet 1997;350:240–4.

41. Persson B, Dahlander AM, Fredriksson M, Brage HN, Ohlson CG, Axelson O. Malignant lymphomas and occupational exposures. Br J Ind Med 1989;46:516–20.