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Risk of non-Hodgkin's lymphoma and exposure to hexachlorocyclohexane, a nested case-control study

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ABSTRACT

Organochlorines have been linked with non-Hodgkin's lymphoma (NHL) in epidemiological studies. We elucidate the importance of hexachlorocyclohexane (HCH), an organochlorine insecticide, in the aetiology of NHL among individuals with dermal exposure to HCH. This is a case-control study nested in a cohort of sheep owners, collected from records on sheep dipping. The number of dipped sheep was used as surrogate for exposure. No other insecticide was used in sheep dip in Iceland during the study period. Cases ($n = 45$) were identified by record linkage with the national cancer registry (through 1962–2003) and controls ($n = 221$) were selected at random from the cohort. In logistic regression analysis the odds ratio for NHL was 3.86 (95% CI 1.59–8.53), adjusted for age, for individuals who had 100 sheep or more as compared to those who had less than 100 sheep. The results indicate that HCH may be linked to the development of NHL.

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1. Introduction

The organochlorine insecticide hexachlorocyclohexane (HCH) was introduced before 1950; the technical grade HCH was a mixture of different isomers containing around 15% γ -isomer (lindane), which proved later to be the most active insecticide. The International Agency for Research on Cancer evaluated in 1987 the carcinogenicity of HCH. The evidence for carcinogenicity to animals was considered sufficient for technical grade HCH and the α -isomer and limited for the β - and γ -isomers.¹ The evidence for carcinogenicity to humans was considered inadequate.¹

The epidemiological evidence on the relationship between pesticides and cancer was reviewed in 1996 and the authors concluded that organochlorine insecticides were linked with soft tissue sarcoma, non-Hodgkin's lymphoma (NHL), leukae-

mia, and less consistently with lung and breast cancers.² In later studies, exposure to pesticides has been suggested as a possible risk factor for NHL,^{3–6} but few studies provide information on specific pesticides. Both farmers and pesticide applicators have often been exposed to several pesticides and in case-control studies mixed exposure was usually a stronger predictor for NHL than when adjustment had been made for individual pesticides.^{6,7} It has been concluded that lindane does not appear to be strongly linked to the development of NHL, although a weak association has not been excluded.^{6,8}

In previous studies on sheep owners who were obligated to dip their sheep using HCH to combat ectoparasites, the incidence for NHL was near unity when compared to the rates of the general population in Iceland.⁹ Since records on sheep dipping are available, it was considered of interest to determine whether NHL among sheep owners is related to the

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number of dipped sheep, which were looked upon as a surrogate for exposure to HCH.

2. Patients and methods

This is a case-control study nested in a cohort of sheep owners ($n = 7882$, all male), which served as a study base and was collected from paper records on sheep dipping from the Icelandic Veterinary Services, as described in a previous publication.⁹ Record linkage, using the personal identification number of the sheep owners, with the Icelandic Cancer Registry provided information on cases with NHL accumulated up to the end of year 2003, as previously described.⁹ Over 94% of the cancers reported to the Cancer Registry have histologically confirmed diagnoses.¹⁰

Annual dipping of sheep to combat ectoparasites, mainly sheep scab mite, *Psoroptes ovis*, has been compulsory by law in Iceland since 1914.^{11,12} A law adopted in 1959 reduced the requirement to one compulsory dip every other year.¹¹ The obligation to dip the sheep fell on the sheep owner or farmer.^{11–13} The concentrate of HCH (Gamatox®, the drug under this commercial name was used during the study period) was used in sheep dip from 1947 onwards,¹⁴ and was the only insecticide used for this purpose until the entire practice of dipping was replaced around 1980 by annual prophylactic injection of Ivermectin and related medicines to combat ectoparasites. Printed on the reverse side of the records of sheep dipping was advice to sheep owners on how to perform the dipping and how to mix the dip from the concentrate of HCH. Non-infested sheep were to be totally immersed in the dip for 1 min and the head of the animal should be dipped briefly three times. Infested animals should be totally immersed for 3 min, and their heads dipped five times. After dipping a herd, the inside of the sheepshed was to be scrubbed with the remainder of the dip, using a broom. No advice was given concerning personal protection, although dipping was hard and dirty work, known to involve skin contamination, as has also been recognized elsewhere.¹⁵ The farmers in Iceland dipped their sheep inside during the winter, after they had brought in their herds from highland pastures; no contractors were ever engaged in sheep dipping. In the often complicated combat against sheep scab mite in Iceland,¹² the chief veterinary officer expressed concern about the weakness of the arrangements and organisation of the dipping but, according to the legislation, the obligation to dip fell on the sheep owner.^{13,14} The chief veterinary officer considered that the reported dipping had been performed by the sheep owners themselves. That view he shared with retired sheep farmers and according to the latter, farmers could not avoid getting their hands and often also their arms and other parts of their bodies wet while dipping. Frequently they would get splashes on their clothes, their faces, and even in their eyes from the dip.

Records of sheep dipping were kept by community-appointed inspectors, who were responsible to the district veterinarians, who in their turn were responsible to the Icelandic Veterinary Services. The paper records which were available covered the period from 1962–1980. They were written on a special form and signed by the inspectors as verification. The inspectors attested to the correct mixing of the dip, the general conditions under which the dipping was performed,

and that all sheep were dipped.^{12,13} The records contained information on the time of the dip, the name, address, community, gender of the sheep owner and the number of his/her sheep. The records were eventually all computerised. During the period 1962 to 1980 the records from certain districts were missing for some years and sometimes records from certain communities were missing, thus the collection of records was not complete. Some sheep owners were mentioned almost every year, mostly with the same addresses, while others appeared only once in the files. Altogether 7882 males could be identified from the records of sheep dipping; the male population in Iceland was 115,000 in the year 1980. In this 19-year period sheep owners from every community in Iceland were nevertheless included several times, which means that there were sheep owners with addresses in the capital Reykjavik and the capital area, as well as individuals from a whole range of communities from small towns to villages to the smallest rural communities. In the urban area the owners tend to have fewer sheep, however, there were also small owners in the rural areas. Altogether there were 26,499 records through the period from 1962 to 1980. The mean number of sheep per owner was 150 and the median value was 127 and the range of sheep per owner was 3–683.

Cases and controls were identified from the cohort of sheep owners, as mentioned previously. Altogether 45 histologically confirmed cases of NHL according to the International Classification of Diseases 7th Revision, code 200 or 202, diagnosed between the years 1966 and 2003, were found in the Cancer Registry, all of which were included in the study. Controls, 221 individuals, were selected at random from the non-cases of the cohort who did not have cancer. The rationale to select controls from non-cancer cohort members was to eliminate possible bias in the case exposure to HCH and lindane was associated with other types of cancer among these sheep owners. The number of dipped sheep per owner was taken as a surrogate for exposure to HCH. At the beginning the drug contained technical grade HCH, which is a mixture of isomers. The mixture of isomers in the drug was replaced with γ -HCH (lindane) in the mid-1970s, although the exact year is not known. It was decided to use the highest number of sheep ever occurring on the records for each individual. A comparison of cases and controls was done on the material stratified on age, based on Mantel-Haenszel test.¹⁶ If the expected values were less than five, the Fisher exact test was used to calculate 95% confidence intervals (CI),¹⁷ this was completed in Epi-Info 6. This calculation was conducted using a cut-off of 100 between a high and a low number of sheep. This number was chosen because according to retired farmers the smaller owners were less likely to have dipped their sheep themselves, rather that their sheep were dipped by larger sheep owners in their neighbourhood. A multivariate case-control analysis was performed using a logistic regression analysis.¹⁸ The adjusted odds ratio and exact computation of 95% CI were calculated using the SPIDA software package.¹⁹ Case-control status was the dependent variable. Age was treated as a continuous variable expressed in years. The number of owned sheep was treated as a continuous variable.

In a separate analysis the ownership of sheep was treated as a dichotomous variable, classified as 100 sheep or more, or less than 100 sheep. In yet another analysis the number of

sheep owned was divided into three categories: less than 100 sheep, 100 to 199 sheep, and 200 sheep or more and treated as an ordinal variable.

The National Bioethics Committee and the Data Protection Commission approved this study.

3. Results

Table 1 shows the characteristics of controls and cases. The crude odds ratio for NHL associated with whether the sheep owner had 100 sheep or more as compared to those who had less than 100 sheep was 3.06 (95% CI 1.32–7.94). The odds ratio for the three age strata, the Mantel-Haenszel estimate, and 95% CI for cases and controls according to the age strata and ownership of sheep are shown in Table 2.

Table 1 – Characteristics of cases and controls

	Controls n = 221 (%)	Cases n = 45 (%)
Age		
Born before 1910	57 (25.8)	12 (26.7)
Born 1910 to 1919	40 (18.1)	14 (31.1)
Born 1920 and later	124 (56.1)	19 (42.2)
Number of owned sheep		
Less than 100	88 (39.8)	8 (17.8)
100 to 199	71 (32.1)	22 (48.9)
200 or more	62 (28.1)	15 (33.3)
A life year 2003	125 (56.6)	15 (33.3)
Urban/rural community		
Urban, more than 2000 inhabitants	18 (8.1)	3 (6.7)
Rural, less than 2000 inhabitants	203 (91.9)	42 (93.3)

Table 2 – Number of cases of non-Hodgkin's lymphoma and controls, odds ratio, and 95% confidence intervals (CI) according to three age strata and whether they have owned less than 100 sheep or more

	Controls (n = 221)	Cases (n = 45)	Odds ratio	95% CI ^a
Born before 1910				
Number of sheep				
<100	33	4		
≥100	24	8	2.75	0.64 to 13.77
Born 1910 to 1919				
Number of sheep				
<100	16	2		
≥100	24	12	4.00	0.72 to 40.63
Born 1920 and later				
Number of sheep				
<100	39	2		
≥100	85	17	3.90	0.85 to 36.20
Crude estimate			3.06	1.32 to 7.94
Mantel-Haenszel estimate			3.46	1.42 to 8.97

a Fisher exact results.

Table 3 – Adjusted odds ratio and 95% confidence intervals (CI) from logistic regression of non-Hodgkin's lymphoma risk among cases and controls according to age and number of sheep

	Controls (n = 221)	Cases (n = 45)	Odds ratio ^a	95% CI
Age (years)			0.98	0.96 to 1.00
Number of sheep				
3 to 99	88	8	1	Reference
100 to 199	71	22	3.83	1.58 to 9.31
200 to 683	62	15	3.44	1.31 to 9.04

a Odds ratios have been calculated in a unique multivariate analysis, taking into account simultaneously all the variables.

The odds ratio for NHL associated with number of owned sheep, treated as a continuous variable, adjusted for age, was 1.002 (95% CI 0.999–1.004). Table 3 shows that the odds ratios for NHL was 3.84 (95% CI 1.58–9.31) for those individuals who had 100 to 199 sheep and 3.44 (95% CI 1.31–9.04) for those individuals who had more than 200 sheep, as compared to those who had less than 100 sheep, adjusted for age. Age entered as a continuous variable was significantly associated with the risk of NHL. The odds ratio for NHL was 3.86 (95% CI 1.59–8.53) for individuals who had 100 sheep or more as compared to those who had less than 100 sheep, adjusted for age.

4. Discussion

The calculations show a significant association between number of sheep, measured as 100 or more or 200 or more, and the risk of NHL adjusted for age. The ownership of sheep serves as a surrogate for exposure to HCH. Although the number of cases in the study is small, the odds ratio was high and the 95% confidence intervals did not include unity. This observed association accords with some previous studies on farmers exposed to lindane.^{3,6} However, one of these studies found a higher risk from lindane used on crops than from that used on animals.⁶ These large population-based case-control studies, one cross-Canada,³ the other used data pooled from studies in Iowa, Minnesota, Kansas and Nebraska⁶ have observed association of NHL and self-reported exposure to lindane. These associations were decreased after adjustment, in multivariate analyses, for potential confounding from other insecticides/pesticides, i.e. 2,4-dichlorophenoxyacetic acid and diazinon.^{3,6} In these studies there were few exposed men whose exposure was limited to lindane or the category organochlorine. A nested case-control study, in Maryland, United States, evaluated the risk of NHL and prediagnostic serum measurement of organochlorine.⁸ There was not observed association between NHL and serum levels of β -HCH, but γ -HCH, lindane, was detected in only a few serum samples as little bioaccumulation of lindane occurs.⁸ In this study the serum samples were collected after the use of technical grade HCH was abandoned and lindane used instead.

The drug for the dip, i.e. HCH, was the only insecticide used in sheep dip in Iceland since the year 1947, so there is no confounding from the use of other insecticides in dipping. The concentrate of HCH was in the beginning of the period 1962–1980

technical grade HCH, but after about mid-1970 the drug was changed to contain 98–99% γ -HCH, i.e. the other isomers were almost eliminated from the drug. The number of sheep each individual owned is a surrogate for the exposure to HCH. The owners were obligated by law to dip their sheep¹¹ and discussion on dipping from this period confirms the general rule that the owners dipped their sheep.^{12–14} This is consistent with information obtained by interviews with some retired sheep farmers. The commercial name of the drug to be used in the dip was written on the reverse side of the paper records on sheep dipping. The design of the present study is a case-control study nested in a cohort, and the information on ownership of the sheep, i.e. the surrogate for exposure, was collected prior to the diagnosis of the cases by the community appointed inspectors and with the good intentions of eliminating sheep scab mite. The role of the inspectors was to see that the dip was correctly blended from concentrate of HCH and that all sheep were dipped.

The controls were selected randomly from persons in the cohort of sheep owners who had not been diagnosed with cancer according to the record linkage to the Cancer Registry.⁹ The reason for selecting the controls from the non-cancer individuals rather than the non-NHL individuals of the cohort is that in previous studies lindane was associated with cancers of other sites, that is brain cancer,²⁰ leukaemia²¹ and prostate cancer.²² A study on exposure to insecticide used on animals was also associated with soft-tissue sarcoma.²³ The SIRs for these cancers in the cohort study were near unity⁹ and that was also the case for stomach cancer and multiple myeloma. If exposure to HCH in this cohort is associated with cancers other than NHL including cases with cancers among the controls could possibly have masked the effect of the exposure.

Age is adjusted for in the stratified and the multivariate analyses. Other important possible confounding factors for NHL are not adjusted for in the study, and their distribution between cases and controls is unknown. This concerns genetic factors, acquired immunodeficiency, lifestyle factors and other occupation-related factors, discussed by other researchers.^{4,24,25} Having a home address in the capital area or larger towns, together with a low number of sheep owned, indicates that the persons in question were not sheep farming for their living but obviously had another main job. It is most likely that the small sheep owners kept their sheep for domestic needs. The proportion of urban/rural habitation of controls and cases are fairly equally distributed (Table 1), thus it is unlikely that holding another main job than farming is introducing a bias. The present study focuses on sheep owners who were engaged in dipping their sheep and thus exposed to HCH and lindane; however, no information is available on their possible other farm activity, i.e. whether they had other types of livestock as well, such as cattle, or how long they had been farming. Poultry and pig raising was infrequent before 1980 and grain farming was almost non-existent.²⁶ One can also speculate about confounding by other factors related to sheep farming such as sheep viruses.

NHL is known to be associated with compromised immune system.²⁴ More subtle immune alteration such as asthma have been considered a potential risk factor for NHL and one study have found indications that the risk of NHL among

asthmatics with pesticide exposure may be higher than among non-asthmatics with pesticide exposure.²⁷ HCH have been found to alter the immune system in exposed humans,²⁸ making it biologically plausible that HCH exposure may be etiologically linked with an increased risk for NHL. However, the carcinogenicity of technical grade HCH and lindane are less clear in humans than in laboratory animals.¹

The use of the comprehensive population registers in Iceland, especially the use of the Cancer Registry strengthens our study. Misclassification of case and control status is very unlikely because the recording of cases in the Cancer Registry, which is nationwide, is based on histological examination and is independent of occupation and residence. The primary information on ownership of dipped sheep was collected prior to follow-up and diagnosis of the NHL cases, which eliminates the possibility of recall bias concerning the exposure. The small size of the study did not allow us to separate out the possible effects of induction period and calendar time.

In conclusion, the number of dipped sheep, used as surrogate for exposure to dermal contact with HCH and lindane, was associated with a more than threefold risk of NHL, adjusted for age, indicating that HCH may be linked with the development of NHL among these sheep owners.

Conflict of interest statement

None declared.

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