ORIGINAL ARTICLE

Agricultural pesticide use and risk of glioma in Nebraska, United States

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Background: To evaluate the risk of the adult glioma associated with farming and agricultural pesticide use, the authors conducted a population based case control study in eastern Nebraska.
Methods: Telephone interviews were conducted with men and women diagnosed with gliomas (n = 251) between 1988 and 1993 and controls (n = 498) randomly selected from the same geographical area. Unconditional logistic regression was used to calculate adjusted odds ratios (ORs) for farming and for use of individual and chemical classes of insecticides and herbicides, including pesticides classified as nitrosatable (able to form N-nitroso compounds upon reaction with nitrite). Non-farmers were used as the reference category for all analyses.
Results: Among men, ever living or working on a farm and duration of farming were associated with significantly increased risks of glioma (⩾55 years on a farm OR = 3.9, 95% CI 1.8 to 8.6); however, positive findings were limited to proxy respondents. Among women, there were no positive associations with farming activities among self or proxy respondents. Specific pesticide families and individual pesticides were associated with significantly increased risks among male farmers; however, most of the positive associations were limited to proxy respondents. For two herbicides and three insecticides, use was positively associated with risk among both self and proxy respondents. Based on a small number of exposed cases, ORs were significantly increased for the herbicides metribuzin (OR = 3.4, 95% CI 1.2 to 9.7) and paraquat (OR = 11.1, 95% CI 1.2 to 101), and for the insecticides bufencarb (OR = 18.9, 95% CI 1.9 to 187), chlorpyrifos (OR = 22.6, 95% CI 2.7 to 191), and coumaphos (OR = 5.9, 95% CI 1.1 to 32).
Conclusion: The authors found significant associations between some specific agricultural pesticide exposures and the risk of glioma among male farmers but not among female farmers in Nebraska; however, most of the positive associations were limited to proxy respondents. These findings warrant further evaluation in prospective cohort studies where issues of recall bias are not a concern.

The incidence rate of primary brain tumours was 6.4 per 100 000 in the United States in 2000 and has been gradually increasing.[1] Gliomas represent over 90% of malignant cancers of the brain and central nervous system. Although brain cancer is one of the most lethal cancers, its aetiology is poorly understood. Exposure to ionising radiation has been consistently associated with increased incidence and other suspected risk factors include certain rare genetic disorders, organic solvents, and electromagnetic fields.[2]

A meta-analysis of 33 studies that evaluated brain cancer and farming found a small but significantly increased risk of brain cancer among farmers.[3] Six studies were published after the meta-analysis[4–9] and four[5–8] found non-significant increased risks of glioma among male farmer workers or farm supervisors. Risk was significantly increased among women in one study[5] and significantly inverse in another[8] The increased risk of brain cancer has been hypothesised to be the result of a number of exposures experienced by farmers, including pesticides, fertilisers, solvents, fuels, and zoonotic viruses.[10] Several studies evaluated risk by type of farming operation or pesticide use. Significantly increased risks were found for livestock farming[11] including poultry,[12] dairy,[13 14] and sheep[14] and with use of pesticides[6 15 16] and insecticides[17] in general. However, the associations with animal farming and pesticide use were not entirely consistent across studies and information on specific pesticides used was not obtained.

Italian farmers[17] who used insecticides or fungicides had a significant twofold increased risk of brain cancer. The authors hypothesised that exposure to alkyl ureas present in many copper sulfate products used as fungicides in the study area may account for the increased risk of brain cancer because N-nitroso alkyl ureas and other N-nitroso compounds (NOC) are potent nervous system carcinogens in animals[18–20] Many pesticides contain amine or amide groups which can react with nitrite to form N-nitrosamines and N-nitrosamides, respectively.[21] More than 300 pesticide formulations have been surveyed for the presence of nitrosamines or their ability to react with nitrite[22–24] and NOC contamination has been found in many pesticides commonly used in agriculture.[25]

We conducted a population based case control study in Nebraska to determine if agricultural pesticide exposures were associated with the risk of adult glioma. An additional aim of our study was to evaluate exposure to NOC and NOC precursors and risk of glioma. Here we present results for farming and agricultural pesticide use including use of nitrosatable pesticides.

MATERIALS AND METHODS

Study population

The current study was part of the Nebraska Health Study II, which was designed to investigate the potential roles of occupational and dietary risk factors in adult glioma, oesophageal adenocarcinoma, and stomach cancer in 66 counties of eastern Nebraska. The study population has been described previously.[26 27] Briefly, all participants were White male or female residents of these 66 counties, aged 21 years or older. Histologically confirmed incident primary adult glioma cases diagnosed between 1 July 1988 and 30 June

Abbreviations: CI, confidence interval; GBM, glioblastoma multiforme; NOC, N-nitroso compounds; OR, odds ratio

1993 were identified from the Nebraska Cancer Registry, or from 11 participating hospitals in Lincoln and Omaha covering more than 94% of adult glioma cases in the study population.

Controls were randomly selected from the control group of a previous population based case control study of non-Hodgkin's lymphoma, Hodgkin's disease, multiple myeloma, and chronic lymphocytic leukaemia conducted in 1986–87 in the same base population.[28] In the previous study the controls were selected by a 3:1 frequency matching by race, sex, vital status, and 5 year age groups to the overall case distribution. Controls under the age of 65 years were selected from the general population by random digit dialing[29] and those 65 years and over were identified from Medicare files. Controls for deceased cases were selected from Nebraska mortality records with the additional matching factor of year of death. Controls for the current study were randomly selected from the previous study controls and were frequency matched by age, sex, and vital status to the combined distribution of the glioma, stomach, and oesophageal cancer cases. Because of inadequate numbers of younger controls for the glioma study, 23 additional controls identified by random digit dialing (n = 3) or from death certificates (n = 20) were interviewed. Self responding controls were oversampled to allow for more study power in subgroup analyses by respondent type.

Interviews

Glioma cases, controls, or their proxies were interviewed by telephone during 1992–94 using a structured questionnaire containing information about demographics, smoking and alcohol consumption, diet, family history of cancer, complete residential and occupational history, medical history, and other factors. Among those who lived or worked on a farm, we asked a detailed history of pesticide use on the farm as well as years of farming activity and the size of the farm where they lived or worked the longest. Subjects were queried about the use of specific pesticides; the pesticide list was developed with the assistance of local agricultural experts and included 20 insecticides and 17 herbicides used on Nebraska crops over the previous 40 years. All of the questions about pesticide use were for the time period before 1985, the time frame of the previous study.

Of 282 eligible cases, interviews were obtained for 251 (89%) (139 men, 112 women). Of the eligible interviewed cases, 87% were astrocytic (56% of cases were glioblastoma multiforme), 8% were oligodendrogliomas, 2% ependymomas, 2% mixed gliomas, and 1% other unspecified gliomas. Due to the severity of the disease, interviews were conducted with proxies for 76% of cases. Most proxy respondents were either spouses (62%) or other first degree relatives (33%).

Of 606 eligible controls, 503 (83%) were interviewed. The response rate for controls in the original study was 87%, giving an overall control response rate of 75% for this study. Five controls were excluded after the interview due to a reported diagnosis of a brain tumour, leaving 498 controls in the analysis. Among these controls, 60% of the interviews were conducted with proxies. Proxy respondents were primarily spouses (45%) or other first degree relatives (46%).

Data analysis

We estimated glioma risk for individual herbicides and insecticides and for chemical family groupings. Additionally, through a review of the literature and the expert judgment of an NOC chemist (W Lijinsky), we grouped the pesticides according to their ability to react with nitrite to form NOCs. We determined the likely carcinogenicity of the NOC derivative through a review of animal studies.[30 31] For nitrosatable pesticides that had not been tested in animals, a judgment was made (by WL) as to their likely carcinogenicity based on the stability of the N-nitroso derivative and the structural similarity to tested compounds. Eleven of the 17 herbicides evaluated were considered nitrosatable (2,4,5-T, 2,4-D as dialkylamine salts, EPTC, glyphosate, trifluralin, atrazine, butylate, cyanazine, dicamba, metolachlor, propachlor), and the derivatives of the first five were judged to be likely animal carcinogens. Five of the 20 insecticides evaluated were considered nitrosatable (bufencarb, carbaryl, carbofuran, famphur, nicotine), and all five nitrosatable insecticides were considered likely animal carcinogens.

We used unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs) with Stata software (version 8.0).[32] All significance tests were two sided ($\alpha = 0.05$). The ORs for farming activity and pesticide use were calculated using non-farmers as a reference group. Subjects who lived or worked on a farm only before age 18 (n = 145) were evaluated separately because their farming experience was of short duration and their pesticide use was generally low. Trend tests were performed by assigning scores to categorical variables using the median value among controls and treating the scored variables as continuous variables in the logistic analyses.

Odds ratios were adjusted for age ($\leqslant$49, 50–59, 60–69, $\geqslant$70), sex, and respondent type. Separate analyses by sex and respondent type were also conducted. Women reported very little pesticide use, therefore our analyses of individual pesticides was limited to men. We evaluated as possible confounders variables that were associated with risk of glioma in our study population. These included a history of head injury, marital status, education level, alcohol consumption, medical history of diabetes mellitus, dietary intake of α- and β-carotene, and dietary fibre. None of these factors changed the ORs by more than 10% and the final models were adjusted only for age, sex, and respondent type.

RESULTS

The characteristics of brain cancer cases and controls have been described previously.[26] For both men and women the cases tended to be younger than controls as a result of the frequency matching by age across all case groups which included gastrointestinal cancer cases. Because of the higher matching ratio for self responding cases, there was a higher proportion of self responding controls than cases. A greater proportion of cases completed some education beyond high school and had a family history of brain cancer.

Overall, brain cancer risk was increased among farmers and the association was stronger among men who worked on a farm after age 18 (table 1); however, the increased risk for both groups of farmers was only observed among proxy respondents. Among self respondents, the association with farming was inverse, although it was not statistically significant. We observed a pattern of increasing risk with duration of farming among both self and proxy respondents, although the ORs were much stronger among proxies. Increasing average farm size was not associated with increasing glioma risk. There were no increased risks of glioma among women who lived or worked on a farm before age 18 or as an adult (adult farmers OR = 0.7, 95% CI 0.4 to 1.2), regardless of duration or average farm size (not shown). Among women the ORs for farm work, duration, and farm size were similar for proxy and self respondents (not shown).

Overall, brain cancer risk was increased among adult male farmers reporting that insecticides (OR = 1.8, CI 1.0 to 3.0), herbicides (OR = 1.7, CI 1.0 to 3.0), or nitrosatable pesticides (OR = 1.9, CI 1.1 to 3.4) were used on the farm on which they lived or worked (table 2). Risks of twofold or greater were observed for organophosphate insecticides, dinitroaniline herbicides, and triazine herbicides. The increased risks were

Table 1 Odds ratios (ORs) and 95% confidence intervals (CIs) for brain cancer by farming activity among male farmers

	Overall					Self					Proxy				
		Cases					Cases					Cases			
	Controls	n	OR*	95% CI	p for trend	Controls	n	OR†	95% CI	p for trend	Controls	n	OR†	95% CI	p for trend
Non-farmers	112	49	1.0	Ref‡		40	20	1.0	Ref‡		72	29	1.0	Ref‡	
Farmers															
Only before age 18§	49	27	1.3	0.7–2.4		27	5	0.4	0.1–1.2		22	22	2.5	1.2–5.3	
Adult farmers¶	122	62	1.7	1.0–2.9		35	12	0.7	0.3–1.8		87	50	2.6	1.4–4.9	
Adult farmers**															
Years farmed															
⩽24	29	12	1.2	0.5–2.7		9	2	0.3	0.1–1.8		20	10	2.1	0.8–5.8	
25–54	34	18	1.6	0.8–3.3		11	6	1.0	0.3–3.4		23	12	2.2	0.9–5.5	
⩾55	40	24	3.9	1.8–8.6	0.001	9	3	1.4	0.2–7.9	0.885	31	21	6.2	2.3–16.2	<0.001
Average acres															
<160	38	17	1.7	0.8–3.7		10	3	0.8	0.2–3.4		28	14	2.6	1.0–6.4	
160–320	31	26	3.3	1.6–6.8		4	2	0.9	0.2–5.9		27	24	4.6	2.0–10.6	
>320	35	14	1.3	0.6–2.7	0.061	19	7	0.7	0.2–2.2	0.588	16	7	1.9	0.7–5.8	0.006

*Odds ratio adjusted for age (⩽49, 50–59, 60–69, ⩾70) and respondent type.
†Odds ratio adjusted for age (⩽49, 50–59, 60–69, ⩾70).
‡Reference category: non-farmers.
§Subjects who lived or worked on a farm only before age 18.
¶Subjects who had lived or worked on a farm as an adult (>18 years).
**Numbers may not sum to total adult farmers due to missing information on years farmed and farm size.

limited to proxy respondents; whereas with the exception of dinitroaniline herbicide class, the associations were inverse among self respondents. Among women, there were no significant associations for any of the pesticide classes, regardless of respondent type (data not shown).

Odds ratios for individual pesticides are presented in table 3 if five or more male cases reported that the pesticide was used. Overall, risk was significantly increased for several herbicides (atrazine, metribuzin, paraquat, pendimethalin, trifluralin) and insecticides (bufencarb, chlorpyrifos, coumaphos, fonofos, terbufos). Among these pesticides with significantly increased risks overall, ORs were increased among both self and proxy respondents for metribuzin, paraquat, bufencarb, chlorpyrifos, and coumaphos. Additionally, non-significant increases of twofold or greater were observed among both self and proxy respondents for the herbicide bentazon and the insecticides benzene hexachloride and disulfoton. We attempted to evaluate trends by number of years and number of days per year these pesticides were used, but there were insufficient numbers of exposed individuals.

Table 4 shows ORs for brain cancer and use of pesticide chemical classes among adult male farmers by histological subtype. Among glioblastoma multiforme (GBM) cases, risk was non-significantly increased twofold for the dinitroaniline, phenoxy, and triazine herbicides. We observed

Table 2 Odds ratios (ORs) and 95% confidence intervals (CIs) for brain cancer for ever-use of pesticides chemical classes among adult male farmers

	Overall				Self				Proxy			
		Cases				Cases				Cases		
	Controls	n	OR*	95% CI	Controls	n	OR†	95% CI	Controls	n	OR†	95% CI
Non-farmers	112	49	1.0	Ref‡	40	20	1.0	Ref‡	72	29	1.0	Ref‡
Insecticides§	77	42	1.8	1.0–3.0	30	10	0.7	0.3–1.7	47	32	3.0	1.5–6.2
Farmers, no use	31	10	1.5	0.6–3.6	3	2	10.1	0.6–181.2	28	8	1.5	0.6–4.2
Carbamate	36	20	1.7	0.8–3.3	17	6	0.7	0.2–2.2	19	14	3.0	1.2–7.5
Organochlorine	47	26	1.9	1.0–3.6	20	5	0.5	0.2–1.8	27	21	3.6	1.6–8.1
Organophosphorus	46	30	2.0	1.1–3.7	23	7	0.6	0.2–1.7	23	23	4.4	1.9–10.1
Herbicides§	70	38	1.7	1.0–3.0	28	9	0.6	0.2–1.7	42	29	2.8	1.4–5.9
Farmers, no use	41	11	1.2	0.5–2.6	6	3	1.5	0.3–8.0	35	8	1.2	0.5–3.3
Acetanilide	34	22	1.8	0.9–3.6	17	7	0.7	0.2–2.1	17	15	3.3	1.3–8.2
Benzoic acid	29	14	1.4	0.6–2.9	15	4	0.5	0.1–2.0	14	10	2.4	0.9–6.5
Carbamate	22	14	1.7	0.8–3.9	9	3	0.7	0.2–3.1	13	11	2.9	1.1–7.8
Dinitroaniline	16	17	2.9	1.3–6.6	8	5	1.1	0.3–3.8	8	12	5.5	1.8–16.4
Phenoxy	56	32	1.8	1.0–3.3	24	7	0.6	0.2–1.6	32	25	3.3	1.5–7.2
Triazine	42	27	2.0	1.0–3.7	20	7	0.7	0.2–2.0	22	20	3.7	1.6–8.7
Nitrosatable pesticides use¶	61	36	1.9	1.1–3.4	27	9	0.7	0.2–1.8	34	27	3.4	1.6–7.3

*Odds ratio adjusted for age (⩽49, 50–59, 60–69, ⩾70) and respondent type.
†Odds ratio adjusted for age (⩽49, 50–59, 60–69, ⩾70).
‡Reference category: non-farmers.
§Individual pesticides were grouped into herbicides (2,4,5-T, 2,4-D, alachlor, atrazine, bentazon, butylate, chloramben, cyanazine, dicamba, EPTC, glyphosate, metolachlor, metribuzin, paraquat, pendimethalin, propachlor, trifluralin) and insecticides (aldrin, benzene hexachloride, bufencarb, carbaryl, carbofuran, chlordane, chlorpyrfios, coumaphos, DDT, diazinon, dieldrin, disulfoton, famphur, fonofos, heptachlor, lindane, malathion, nicotine, phorate, terbufos). We also classified the pesticides into their chemical families: acetanilide herbicides (alachor, metolachlor, propachlor), benzoic acid herbicides (chloramben, dicamba), carbamate herbicides (butylate, EPTC), dinitroaniline herbicides (pendimethalin, trifluralin), phenoxy herbicides (2,4,5-T, 2,4-D), triazine herbicides (atrazine, cyanazine, metribuzin), carbamate insecticides (bufencarb, carbaryl, carbofuran), organochlorine insecticides (aldrin, benzene hexachloride, chlordane, DDT, dieldrin, heptachlor, lindane), organophosphorus insecticides (chlorpyrfios, coumaphos, diazinon, disulfoton, famphur, fonofos, malathion, phorate, terbufos).
¶Nitrosatable pesticides includes 11 herbicides (2,4,5-T, 2,4-D, atrazine, butylate, cyanazine, dicamba, EPTC, glyphosate, metolachlor, propachlor, trifluralin) and five insecticides (bufencarb, carbaryl, carbofuran, famphur, nicotine).

Table 3 Odds ratios (ORs) and 95% confidence intervals (CIs) for brain cancer by ever-use of individual pesticides among adult male farmers

	Overall				Self				Proxy			
		Cases				Cases				Cases		
	Controls	n	OR*	95% CI	Controls	n	OR†	95% CI	Controls	n	OR†	95% CI
Non-farmers	112	49	1.0	Ref‡	40	20	1.0	Ref‡	72	29	1.0	Ref‡
Herbicides												
2,4,5-T§¶	17	7	1.3	0.5–3.6	8	2	0.4	0.1–2.3	9	5	2.7	0.7–9.8
2,4-D§¶	56	32	1.8	1.0–3.3	24	7	0.6	0.2–1.6	32	25	3.3	1.5–7.2
Alachlor	28	20	2.0	1.0–4.0	13	7	0.9	0.3–2.6	15	13	3.3	1.3–8.5
Atrazine§	38	25	2.1	1.1–4.0	20	7	0.7	0.2–2.0	18	18	4.2	1.7–10.3
Bentazon	5	6	3.0	0.8–10.9	2	3	3.9	0.5–32.1	3	3	2.4	0.4–13.1
Butylate§	4	5	2.8	0.7–11.5	4	2	1.2	0.2–7.7	0	3	Inf	–
Chloramben	8	5	1.9	0.6–6.6	4	2	0.9	0.1–5.7	4	3	3.1	0.6–17.3
Cyananzine§	12	7	1.6	0.6–4.5	6	2	0.6	0.1–3.2	6	5	3.0	0.8–12.1
Dicamba§	26	11	1.2	0.5–2.7	13	3	0.5	0.1–1.9	13	8	2.0	0.7–5.7
EPTC§¶	21	12	1.6	0.7–3.6	8	2	0.5	0.1–2.6	13	10	2.7	0.97–7.4
Glyphosate§¶	32	17	1.5	0.7–3.1	17	4	0.4	0.1–1.6	15	13	3.1	1.2–8.2
Metolachlor§	14	6	1.2	0.4–3.6	8	2	0.4	0.1–2.3	6	4	2.6	0.6–11.3
Metribuzin	9	9	3.4	1.2–9.7	3	5	3.3	0.6–16.8	6	4	2.9	0.7–12.7
Paraquat	1	5	11.1	1.2–101.2	1	2	4.3	0.3–60.7	0	3	Inf	–
Pendimethalin	5	7	4.0	1.1–14.2	3	1	0.6	0.1–6.6	2	6	11.2	1.9–67.1
Propachlor§	20	11	1.5	0.6–3.4	8	3	0.7	0.2–2.9	12	8	2.3	0.8–6.8
Trifluralin§¶	14	17	3.2	1.4–7.3	7	5	1.2	0.3–4.3	7	12	5.9	1.9–18.2
Insecticides												
Aldrin	19	7	1.7	0.6–4.8	8	2	0.7	0.1–4.0	11	5	3.2	0.9–11.6
Benzene	6	6	2.8	0.8–9.6	2	2	2.5	0.3–22.7	4	4	3.1	0.7–14.8
Hexachloride												
Bufencarb§¶	1	5	18.9	1.9–187.3	0	1	Inf	–	1	4	18.7	1.7–207.6
Carbaryl§¶	28	13	1.4	0.6–3.1	12	2	0.3	0.1–1.5	16	11	3.0	1.1–7.9
Carbofuran§¶	23	12	1.4	0.6–3.2	14	6	0.9	0.3–2.8	9	6	2.1	0.6–6.8
Chlordane	27	14	1.8	0.8–4.1	11	2	0.4	0.1–1.8	16	12	3.8	1.4–10.4
Chlorpyrifos	1	10	22.6	2.7–191.7	0	1	Inf	–	1	9	22.8	2.6–199.6
Coumaphos	2	5	5.9	1.1–32.2	1	2	3.6	0.3–44.2	1	3	8.8	0.8–93.2
DDT	35	18	1.8	0.9–3.6	14	3	0.5	0.1–2.0	21	15	3.2	1.3–8.0
Diazinon	22	11	1.4	0.6–3.3	12	4	0.6	0.2–2.4	10	7	2.5	0.8–8.1
Dieldrin	17	4	0.9	0.3–3.1	7	0	–	–	10	4	2.1	0.5–8.2
Disulfoton	4	5	3.1	0.8–12.9	2	3	2.7	0.4–18.1	2	2	2.8	0.3–24.6
Famphur§¶	12	7	1.8	0.6–5.0	8	4	1.0	0.3–4.2	4	3	2.9	0.5–16.3
Fonofos	17	17	2.8	1.2–6.1	9	5	0.9	0.3–3.3	8	12	5.7	1.9–17.0
Heptachlor	13	8	2.4	0.9–6.8	4	2	1.1	0.2–7.5	9	6	3.6	1.0–12.6
Lindane	20	13	2.2	1.0–5.3	8	4	1.1	0.3–4.3	12	9	3.5	1.2–10.4
Malathion	29	16	2.0	0.9–4.2	12	5	0.8	0.2–2.8	17	11	3.4	1.2–9.3
Nicotine§¶	16	7	1.9	0.7–5.5	4	0	–	–	12	7	3.4	1.1–11.0
Phorate	17	9	1.6	0.6–4.1	9	4	0.9	0.2–3.5	8	5	2.4	0.7–8.8
Terbufos	18	17	2.7	1.2–6.0	13	5	0.7	0.2–2.5	5	12	9.8	2.8–34.0

ORs for pesticides with more than five exposed brain cancer cases are shown. Inf, infinity.
*Odds ratio adjusted for age (⩽49, 50–59, 60–69, ⩾70) and respondent type.
†Odds ratio adjusted for age (⩽49, 50–59, 60–69, ⩾70).
‡Reference category: non-farmers.
§Nitrosatable pesticides.
¶Nitrosatable pesticides with experimental evidence of carcinogenicity or were likely to be carcinogenic.

statistically significant positive associations with use of organochlorine insecticides, organophosphorous insecticides, and dinitroaniline herbicides among cases with astrocytoma other than GBM, and non-significantly increased ORs of twofold or greater for carbamate insecticides, and acetanilide, carbamate, and triazine herbicides. Nitrosatable pesticide use was associated with a non-significant twofold increased risk of both GBM and other astrocytomas. As before, the positive associations that we observed were generally limited to proxy respondents (data not shown). The number of cases with other types of glioma was small and we observed no significantly increased risk for any pesticide group. The number of exposed cases was too few to evaluate individual pesticides by histological type.

DISCUSSION

We found an increased risk of brain cancer among men who lived or worked on a farm and risk increased with duration; farming was not a risk factor among women. Among men, the positive association with ever having lived or worked on a farm was limited to proxy respondents. Risk was also significantly increased among men who used specific pesticides and pesticide chemical classes; however, the positive results were mostly limited to proxy respondents. The exceptions were for two herbicides—metribuzin and paraquat—and for three insecticides—bufencarb, chlorpyrifos, and coumaphos—for which we observed significantly increased risks of glioma overall, and positive associations among both self and proxy respondents based on small numbers of exposed cases. When we analysed pesticide chemical classes, including the nitrosatable pesticides, by histological type, we found a pattern of somewhat stronger risks for astrocytomas other than GBM; however, as we observed for cases overall, risk was largely limited to proxy respondents.

Exposure to nitrosatable pesticides or to pesticides contaminated with nitrosamines has been suggested as a possible explanation for the higher rates of brain cancer among farmers because of the long standing hypothesis that NOC exposure is a potential risk factor for this cancer.[18–20 33 34] Bufencarb, which was associated with an increased risk in both self and proxy respondents, is a nitrosatable carbamate

Table 4 Odds ratios (ORs) and 95% confidence intervals (CIs) for brain cancer for ever-use of pesticides classes by histological types among adult male farmers

	Glioblastoma multiforme				Astrocytoma			Other glioma		
		Cases			Cases			Cases		
	Controls	n	OR*	95% CI	n	OR*	95% CI	n	OR*	95% CI
Non-farmers	112	25	1.0	Ref†	15	1.0	Ref†	9	1.0	Ref†
Insecticides	77	23	2.0	1.0–4.1	14	1.7	0.7–4.0	5	1.0	0.3–3.3
Farmers, no use	31	10	2.3	0.9–6.1	0	–	–	0	–	–
Carbamate	36	7	1.3	0.5–3.4	10	2.6	1.0–6.6	3	1.1	0.3–4.7
Organochlorine	47	12	1.7	0.7–3.9	11	2.8	1.1–7.1	3	1.2	0.3–5.6
Organophosphorus	46	13	1.3	0.9–1.9	13	2.6	1.1–6.3	4	1.1	0.3–4.1
Herbicides	70	21	1.9	0.9–3.8	12	1.6	0.7–3.9	5	1.1	0.3–3.7
Farmers, no use	41	8	1.4	0.5–3.6	3	1.2	0.3–5.1	0	–	–
Acetanilide	34	10	1.9	0.8–4.7	9	2.1	0.8–5.5	3	0.9	0.2–3.8
Benzoic acid	29	6	1.2	0.4–9.3	5	1.6	0.5–5.2	3	1.4	0.3–6.3
Carbamate	22	5	1.2	0.4–3.7	6	2.6	0.8–8.1	3	2.2	0.5–9.9
Dinitroaniline	16	5	2.1	0.6–6.7	8	3.9	1.3–11.6	4	3.0	0.7–12.4
Phenoxy	56	17	2.0	0.9–4.3	11	1.8	0.7–4.5	4	1.0	0.3–3.8
Triazine	42	13	2.0	0.9–4.7	10	2.2	0.9–5.6	4	1.3	0.3–4.9
Nitrosatable pesticides use‡	61	18	2.0	0.9–4.2	14	2.2	0.9–5.1	4	0.9	0.3–3.5

*Odds ratio adjusted for age (⩽49, 50–59, 60–69, ⩾70) and respondent type.
†Reference category: non-farmers.
‡Nitrosatable pesticides includes 11 herbicides (2,4,5-T, 2,4-D, atrazine, butylate, cyanazine, dicamba, EPTC, glyphosate, metolachlor, propachlor, trifluralin) and five insecticides (bufencarb, carbaryl, carbofuran, famphur, nicotine).

insecticide for which there is evidence of carcinogenicity of the N-nitroso derivative. However, two other nitrosatable carbamates (carbofuran and carbaryl) did not show consistent elevated risks of glioma among self and proxy respondents.

The assessment of nitrosability of each pesticide was determined by a literature review and an assessment of the chemical structure by an expert chemist (W Lijinsky). However, past NOC contamination of pesticide formulations was variable[25] and likely resulted in misclassification of exposure to nitrosatable pesticides. Also, different pesticides vary in their ability to react with nitrite to form N-nitroso compounds and in their carcinogenicity.[22–24] Animal evidence for N-nitroso compounds as causing central nervous system tumours is strongest for the N-nitroso ureas, which are potent neurocarcinogens.[35] However, none of the nitrosatable pesticides on our questionnaire was a urea compound, because urea pesticides were not commonly used in our study area.

Humans are exposed to preformed N-nitroso compounds from a variety of sources.[21 30 36] Dietary intakes are estimated to contribute the majority of NOC exposure.[36] In this study population, dietary nitrate and nitrite, precursors of NOC, were not significantly associated with risk of brain cancer.[26] It is also important to consider that the routes of exposure are different for nitrosatable pesticides (inhalation and dermal) versus dietary NOC or NOC precursors (ingestion). Relative to other sources of NOC exposure, occupational exposure via agricultural pesticide use may be small and for this reason it may not be significant for the development of brain cancer. However, that does not rule out the possibility of an increased risk among those with higher exposures or among subgroups with susceptible genotypes for activating nitrosamines.[37 38]

The possible aetiological importance of agricultural pesticide exposure in brain cancer risk has been suggested by previous epidemiological studies. Musicco *et al*[17] suggested that exposure to alkyl ureas (components of fungicides used extensively in vineyards) may explain the significant positive association between farming and glioma observed in their case control study in Italy. However, this fungicide was not included in this study because of low usage. A death certificate study in France found significantly increased standardised mortality ratios for vineyard farmers in regions with higher pesticide use compared with farmers in areas with low pesticide use.[39] A record linkage cohort study in Sweden found increased brain cancer incidence among male farmers with pesticide use assessed by a geographic area.[16] Population based case control studies in Sweden[40] and the US[6] found a non-significant increased risk of glioma with occupational pesticide use. A cohort study in China found a significantly increased risk for occupations with pesticide exposure.[15] A cohort of golf course superintendents in the US[41] and licensed pesticide users in Italy[42] had significant excess mortality from brain cancer. However, another study in Norway showed no association between brain cancer incidence or mortality and agricultural pesticide use.[43] One of the largest studies to date found no association between occupational pesticide exposure and glioma risk among men and women.[8] None of these studies evaluated risk in relation to specific pesticides or chemical classes of pesticides.

Recently, the Agricultural Health Study, a large cohort study of pesticide applicators in Iowa and North Carolina, reported a statistically significant increased risk of brain cancer associated with exposure to chlorpyrifos,[44] which is supportive of our positive finding. Chlorpyrifos has neurotoxicological effects in the brains of rats[45] and this association warrants further study. Except for alachlor,[46] which was not associated with brain cancer risk, use of other pesticides by Agricultural Health Study members has not been evaluated in relation to brain cancer risk due to the small number of cases accrued to date.

Besides pesticides, exposure to animals has been evaluated in several studies. Increased brain cancer risk has been observed among farmers raising specific livestock including cattle, poultry, and sheep.[6 11–14 47] However, a large international case control study that evaluated this hypothesis[4] found no overall association between contact with farm animals and glioma risk. Most of the studies did not evaluate specific exposures related to animal farming which include zoonotic viruses, dust, and pesticides. A recent case control study in the US[6] found increased risk of glioma associated with spending time in animal confinement buildings, as well as for personally applying pesticides to crops and livestock.

Our case control study of glioma is one of the few studies with comprehensive and detailed information on pesticide use among farmers. The major limitation of our study was the large proportion of proxy respondents. Most of the associations we observed were limited to proxy respondents.

Main messages

- We found positive associations between agricultural pesticide use and the risk of brain cancer, primarily among proxy respondents.
- For metribuzin, paraquat, bufencarb, chlorpyrifos, and coumaphos, we found significant positive associations overall and positive associations among both self and proxy respondents.

Policy implications

- These findings warrant further evaluation in prospective cohort studies where issues of recall bias are not a concern.

Possible explanations include chance, misclassification of exposure by proxy respondents, or a larger risk among deceased farmers who may have had a more aggressive disease. Additionally, deceased controls may be unrepresentative of the general population which may bias associations.

Chance is a possible explanation, but the larger risk among proxy respondents in each pesticide family and for most histological types argues against this. Use of proxy respondents may introduce non-differential misclassification if proxies are less knowledgeable and tend to underreport use.[48–50] In our study, proxies of cases and controls were more likely to provide ''don't know'' responses and were less likely to report use of specific pesticides than subjects themselves. However, this would lead to ORs biased towards the null among proxy respondents, which was the opposite of what we observed.

A spurious positive association with farming would be observed among proxy respondents, if farmers are under-represented among deceased controls compared with the general population.[51] Although the association with duration of farming was much stronger among proxies, we found a pattern of increased risk among both self and proxy respondents. Moreover, an analysis of stomach and oesophagus cancer cases and pesticide use in this population, which relied on the same controls,[52] found null or modest inverse associations among self and proxy respondents for specific pesticides and pesticide families, arguing against an unrepresentative control group.

Differential misclassification because of better recall by proxy respondents of cases than controls could explain the positive associations among proxies. We found no evidence of differential misclassification for other information including dietary intakes;[26] however, recall of pesticide use could be different. We have some evidence that proxies did not overreport pesticide use, because our questionnaire included a ''fake'' pesticide (called Scope) embedded with actual pesticides used in Nebraska and for which we asked details of use. Proxy respondents for both cases and controls reported no or little use of the ''fake'' pesticide (cases, 0%; controls, 1.5%), whereas self responding cases reported more use than self responding controls (cases, 8.3%; controls, 1.9%). Therefore, although it is unlikely that overreporting of pesticide use by proxies explains the observed increases in the risk among proxy respondents, we cannot rule out more accurate recall by proxies for cases and underreporting by proxies for controls.

Few women reported pesticide use on farms where they lived or work, so most of our analyses were limited to men. Lower exposures to pesticides and other farming exposures may explain why farming was not associated with an increased risk of glioma among women.

In summary, we found positive associations between agricultural pesticide use and the risk of brain cancer, primarily among proxy respondents. Although we have no clear explanation for the differences we observed between proxy and self respondents, we are concerned that many of the positive associations may be due to differential misclassification. For two herbicides, metribuzin and paraquat, and for three insecticides, bufencarb, chlorpyrifos, and coumaphos, we found significant positive associations overall and positive associations among both self and proxy respondents. However, our analyses of individual pesticides among self respondents were limited by small numbers, making it difficult to draw clear conclusions. Recent results from the Agricultural Health Study[44] support our finding of a positive association between chlorpyrifos exposure and brain cancer risk. The positive associations we observed for chlorpyrifos and the other pesticides warrant further evaluation, particularly in prospective cohort studies where issues of recall bias are not a concern.

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