

Cancer incidence and metolachlor use in the Agricultural Health Study: An update

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Metolachlor, a widely used herbicide, is classified as a Group C carcinogen by the U.S. Environmental Protection Agency based on increased liver neoplasms in female rats. Epidemiologic studies of the health effects of metolachlor have been limited. The Agricultural Health Study (AHS) is a prospective cohort study including licensed private and commercial pesticide applicators in Iowa and North Carolina enrolled 1993–1997. We evaluated cancer incidence through 2010/2011 (NC/IA) for 49,616 applicators, 53% of whom reported ever using metolachlor. We used Poisson regression to evaluate relations between two metrics of metolachlor use (lifetime days, intensity-weighted lifetime days) and cancer incidence. We saw no association between metolachlor use and incidence of all cancers combined ($n = 5,701$ with a 5-year lag) or most site-specific cancers. For liver cancer, in analyses restricted to exposed workers, elevations observed at higher categories of use were not statistically significant. However, trends for both lifetime and intensity-weighted lifetime days of metolachlor use were positive and statistically significant with an unexposed reference group. A similar pattern was observed for follicular cell lymphoma, but no other lymphoma subtypes. An earlier suggestion of increased lung cancer risk at high levels of metolachlor use in this cohort was not confirmed in this update. This suggestion of an association between metolachlor and liver cancer among pesticide applicators is a novel finding and echoes observation of increased liver neoplasms in some animal studies. However, our findings for both liver cancer and follicular cell lymphoma warrant follow-up to better differentiate effects of metolachlor use from other factors.

Metolachlor is a chloroacetanilide herbicide that was first registered in 1976 and is used primarily on corn, soybeans and sorghum. The US Environmental Protection Agency (USEPA) has classified metolachlor as a “possible human carcinogen” based

on mixed results in rodent studies, with a statistically significant increase in liver neoplasms seen in female rats at high dose levels.¹

Epidemiologic studies of the health effects of metolachlor are quite limited. An ecologic study in Maryland found a nonsignificantly increased risk of childhood bone cancer in areas with detectable levels of metolachlor in groundwater supplies²; interpretation of these results is limited by the presence of multiple contaminants in the groundwater.

To date, the health effects of occupational exposure to metolachlor have been studied only in the Agricultural Health Study (AHS), a prospective study that includes commercial and private pesticide applicators in Iowa and private pesticide applicators in North Carolina. Methodological details of this cohort study have been described elsewhere.^{3,4} Both a nested case-control study of lung cancer⁵ and a prospective follow-up through 2002 of applicators who used metolachlor⁶ observed increased risks for lung cancer with high lifetime days of use. These analyses saw weaker associations with intensity-weighted lifetime days, a metric that accounts for exposure-modifying factors.⁷ Associations between metolachlor use and incidence of a number of other cancers were also examined in the prospective study.⁶ The study found a decreasing risk of prostate cancer with increasing lifetime days of reported use. Rectal cancer was significantly elevated in the highest lifetime-days category

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What's new?

Metolachlor, a widely used herbicide, has been classified as a possible human carcinogen (Group C) by the U.S. Environmental Protection Agency based on an increase in liver neoplasms in female rats. This update of pesticide applicators in the Agricultural Health Study is the first occupational epidemiology assessment to report positive associations between metolachlor use and liver cancer in humans. For both liver cancer and follicular cell lymphoma, lifetime and intensity-weighted lifetime days of metolachlor use showed positive trends that were statistically significant when applicators with no metolachlor use were used as the referent group.

and nonsignificantly elevated using intensity-weighted lifetime days. However, cases were sparse, and the elevations were attenuated when nonexposed applicators were used for comparison.⁶ No statistically significant associations with ever use of metolachlor were seen in AHS studies of multiple risk factors for specific cancers in applicators: melanoma,⁸ pancreatic cancer⁹ and colon or rectal cancer.¹⁰

The current evaluation extended follow-up of the cohort (through 2010 in North Carolina and 2011 in Iowa) to further evaluate the associations between occupational use of metolachlor and cancer incidence. This update also incorporated new exposure information from a follow-up interview. While exposure-response relations for all cancers with at least 20 cases in metolachlor users were examined, results for lung, prostate and rectal cancer were of particular interest in light of previous analyses, and liver cancer was of interest because of the animal data.

Methods**Population**

Pesticide applicators were enrolled at pesticide licensing sessions conducted between 1993 and 1997 in Iowa (private and commercial applicators) and North Carolina (private applicators only). Of the 57,310 licensed restricted-use applicators in this cohort, we excluded 6,259 because they did not provide sufficient information to quantify days of metolachlor use; 1,094 because of a cancer diagnosis other than nonmelanoma skin cancer before enrollment in the AHS; and 341 who had no person-time at risk. Of the 49,616 eligible applicators, 36 were included in the lifetime-days usage analyses but excluded from analyses of intensity-weighted lifetime days because they lacked data for the latter metric.

We ascertained cancer incidence by linking cohort records to cancer registry files through 2010 for North Carolina and 2011 for Iowa. In addition, to determine vital status, we linked cohort members to the death registries of these two states and to the National Death Index.

Exposure assessment for the AHS has been described in depth elsewhere.^{7,11–13} At enrollment, self-administered questionnaires were used to collect data on lifetime pesticide use and application practices, demographic and lifestyle data and personal and family history of cancer. Approximately 5 years later (1999–2005), applicators completed a telephone questionnaire about pesticide use during the most recent year of

application as an indicator of pesticide use over the interval since enrollment.^{14,15} Overall, 36,342 applicators (63% of the full cohort) responded to this second questionnaire. We evaluated two metrics: lifetime days and intensity-weighted lifetime days of metolachlor use. The lifetime days metric was the summation of self-reported use from the enrollment and follow-up questionnaires.¹¹ The weighting factors for the intensity-weighted lifetime days metric were designed to account for use of personal protective equipment, methods of pesticide application, whether the applicator repaired pesticide application equipment and whether the applicator mixed pesticides. These factors were modified since the previous analyses of metolachlor to incorporate refinements based on field measurement data for subgroups of the AHS population, resulting in minor changes to the intensity-weighted lifetime days.⁷ In addition, analyses included a data-driven multiple imputation for days of use for applicators who did not complete the 1999–2005 questionnaire.¹⁵

Statistical analysis

With the availability of 5-year follow-up (AHS phase 2) information, we computed metolachlor use as a time-dependent quantity. We categorized lifetime days and intensity-weighted lifetime days of use with quartiles based on the distribution among exposed cancer cases (excluding nonmelanoma skin cancers). As in the original analysis,⁶ we observed that demographic characteristics for groups with higher metolachlor use were more similar to those using less metolachlor than to unexposed applicators, and therefore again produced two sets of analyses: one restricted to person-time after first metolachlor use and a second without this restriction.

We used Poisson regression to generate rate ratios (RRs) and 95% confidence intervals (CIs) for the relations among metolachlor use and all cancers combined, as well as specific cancer sites, and the MIANALYZE procedure to determine variances for 95% CI calculation when using phase 2 imputed data. We used SAS (version 9.3; SAS Institute, Cary, NC) for all analyses. We accumulated person-time-at-risk for each 2-year calendar increment from the date of enrollment (1993–1997) through the earliest of the study end date (December 31, 2010 in North Carolina and December 31, 2011 in Iowa), date of first cancer diagnosis (other than non-melanoma skin cancer), date of death or the date first moved

out of state. We used the midpoint value of each exposure category treated as a continuous variable to test for linear trend. All tests were two-sided and conducted at $\alpha = 0.05$. To account for disease latency, we selected a 5-year lag for the primary analyses, discounting the five most recent years of exposure, but also conducted unlagged analyses for comparison with earlier AHS analyses.

We adjusted all models for attained age (using restricted cubic splines), cigarette smoking (never/low/high, with the median value of pack-years among smokers used to demarcate low and high categories), alcohol use reported in the year before enrollment (never, and tertiles of number of drinks per month among applicators reporting drinking), family history of cancer at any site in first-degree relatives (yes/no), applicator type (private, commercial) and state of residence (Iowa/North Carolina). For oral cavity cancers, we also assessed the effects of adjusting for ever use of oral tobacco (snuff or chewing tobacco). We adjusted for sex and race (white, nonwhite) for all cancers combined and for race for cancers of the prostate and lung. For the other outcomes of interest, all or almost all cases occurred in white males, so all applicators were retained in the analyses with no adjustment for sex or race (with the exception of exclusions for sex-specific cancers). We identified the five most highly correlated pesticides by categorizing unlagged intensity-weighted lifetime days for each pesticide and calculating the correlation with quartiles of intensity-weighted lifetime days for metolachlor. To adjust for these pesticides in the analyses, we categorized exposure to each as never, low and high, with the median of intensity-weighted lifetime days used to differentiate low from high exposure. A separate category was used to indicate missing data for each correlated pesticide.

Results

Among the 49,616 applicators meeting inclusion criteria, 26,505 (53%) reported any metolachlor use (Table 1). Follow-up for all applicators averaged 14.9 years, double the average follow-up available for the first analysis of cancer incidence and metolachlor use. The applicators were almost all white (97%) and male (97%). For most demographic and exposure characteristics, differences between applicators who did not use metolachlor and those with any use were larger than differences among the groups with different levels of use. Applicators reporting metolachlor usage were more likely to have consumed alcohol in the past year and to have at least a high school education. The largest difference was state of residence, with >70% of applicators in each usage category residing in Iowa, compared to 57% of those who did not use metolachlor. Alcohol consumption and smoking differed by state, with the former higher in Iowa and the latter higher in North Carolina.

Applicators in the highest metolachlor usage quartile differed in some respects from the applicators who did not use metolachlor and those who used less metolachlor; in particular, many more in the highest quartile were commercial

applicators (20.6% of applicators in quartile 4 vs. <8% of applicators in each of the other usage groups and in nonusers of metolachlor). Applicators in the highest usage group were also most likely to have used one or more of the highly correlated pesticides: imazethapyr, alachlor, atrazine, dicamba and/or trifluralin.

Among applicators who ever used metolachlor, with low-exposed applicators as the referent, results for all cancers combined and for most specific cancer sites exhibited few trends or significant elevations in the top quartiles for lifetime days or intensity-weighted lifetime days of use with a 5-year lag (Table 2). However, several cancers did show increased (oral cavity, rectal and testicular cancer) or decreased (leukemia and prostate and stomach cancer) risk with higher levels of metolachlor use. Neither point estimates nor trends were statistically significant. For oral cavity malignancies, adjusting for oral tobacco (snuff or dip) use had little effect on the results.

Although NHL as a grouped outcome showed no evidence of a trend, RRs for the follicular cell lymphoma subtype¹⁶ were elevated in all categories for lifetime days and, in the third and fourth quartiles, for intensity-weighted lifetime days, though trends were not statistically significant. Liver cancer showed nonstatistically significant increased risks in the third and fourth quartiles for both exposure metrics; the tests for trend were not significant ($p = 0.10$). Because liver cancer was the only outcome with fewer than five cases in the lowest metolachlor use category with cutpoints based on all cancers combined, we also performed alternate analyses using categories based on the equal distribution of exposed liver cancer cases. The results were similar (data not shown) to those presented above, although risk estimates increased and the trend approached statistical significance ($p = 0.07$) for intensity-weighted lifetime days.

Analyses with the unexposed as the referent did not change the conclusions for most outcomes (Table 3). Point estimates for lung and pancreatic cancers were lower, with all exposed categories in deficit in these analyses, but neither the category estimates nor trends attained statistical significance. For leukemia, the small suggestion of a negative trend disappeared, and for rectal cancer, point estimates in the top three quartiles decreased. For all outcomes, findings were similar when unlagged data were used (results not shown). Results were also similar for sensitivity analyses excluding applicators who did not respond to the second questionnaire and analyses removing applicator type from the models.

The most notable results of the analyses with the unexposed referent were for follicular cell lymphoma and liver cancer, both of which had statistically significant positive trends. Follicular cell lymphoma showed elevations in all but the first quartile, with the highest lifetime-days quartile attaining statistical significance; trends for both metolachlor use metrics were statistically significant. A test for homogeneity did not show statistically significant differences between NHL subtypes, but cases were sparse for some subtypes,

Table 1. Selected demographic and lifestyle characteristics of applicators by cumulative metolachlor use in the Agricultural Health Study, 1993–2011

Characteristic ²	Lifetime days of metolachlor use ¹ , no lag				
	No use (n = 23,111)	Quartile 1 (n = 7,866)	Quartile 2 (n = 5,079)	Quartile 3 (n = 6,757)	Quartile 4 (n = 6,803)
Age (years) number (%)					
<50	4,070 (17.6)	1,824 (23.2)	968 (19.1)	1,254 (18.6)	1,205 (17.7)
50–<60	6,498 (28.1)	2,448 (31.1)	1,664 (32.8)	2,304 (34.1)	2,708 (39.8)
60–<70	6,095 (26.4)	1,951 (24.8)	1,369 (27.0)	1,738 (25.7)	1,747 (25.7)
≥70	6,448 (27.9)	1,643 (20.9)	1,078 (21.2)	1,461 (21.6)	1,143 (16.8)
Sex					
Male	22,060 (95.5)	7,777 (98.9)	5,035 (99.1)	6,710 (99.3)	6,753 (99.3)
Female	1,051 (4.5)	89 (1.1)	44 (0.9)	47 (0.7)	50 (0.7)
Race					
White	22,427 (97.0)	7,683 (97.7)	4,981 (98.1)	6,633 (98.2)	6,687 (98.3)
Non-White	616 (2.7)	167 (2.1)	93 (1.8)	116 (1.7)	98 (1.4)
Missing	68 (0.3)	16 (0.2)	5 (0.1)	8 (0.1)	18 (0.3)
Family history of cancer					
Yes	13,249 (57.3)	4,608 (58.6)	2,851 (56.1)	3,860 (57.1)	3,958 (58.2)
No	9,080 (39.3)	3,038 (38.6)	2,145 (42.2)	2,770 (41.0)	2,728 (40.1)
Missing	782 (3.4)	220 (2.8)	83 (1.6)	127 (1.9)	117 (1.7)
Smoking history (pack-years)					
None	11,810 (51.1)	4,306 (54.7)	2,919 (57.5)	3,752 (55.5)	3,641 (53.5)
Low (<11.25)	7,004 (30.3)	2,154 (27.4)	1,430 (28.2)	1,912 (28.3)	1,906 (28.0)
High (≥11.25)	3,952 (17.1)	1,306 (16.6)	692 (13.6)	1,044 (15.5)	1,221 (17.9)
Missing	345 (1.5)	100 (1.3)	38 (0.7)	49 (0.7)	35 (0.5)
Alcohol consumption over past year (drinks per month)					
Never in past year	8,379 (36.2)	2,262 (28.8)	1,331 (26.2)	1,667 (24.7)	1,565 (23.0)
<1.875	3,362 (14.5)	1,203 (15.3)	798 (15.7)	951 (14.1)	919 (13.5)
≥1.875–<14.5	5,887 (25.5)	2,344 (29.8)	1,648 (32.4)	2,226 (32.9)	2,140 (31.4)
≥14.5	4,926 (21.3)	1,903 (24.2)	1,215 (23.9)	1,823 (27.0)	2,090 (30.7)
Missing	557 (2.4)	154 (2.0)	87 (1.7)	90 (1.3)	89 (1.3)
Education					
<High school	2,338 (10.1)	561 (7.1)	277 (5.5)	345 (5.1)	317 (4.7)
High school graduate/GED	10,704 (46.3)	3,824 (48.6)	2,328 (45.8)	3,158 (46.7)	3,104 (45.6)
>High school	9,525 (41.2)	3,342 (42.5)	2,392 (47.1)	3,148 (46.6)	3,240 (47.6)
Missing	544 (2.4)	139 (1.8)	82 (1.6)	106 (1.6)	142 (2.1)
State of residence					
Iowa	13,202 (57.1)	5,725 (72.8)	4,037 (79.5)	5,422 (80.2)	5,162 (75.9)
North Carolina	9,909 (42.9)	2,141 (27.2)	1,042 (20.5)	1,335 (19.8)	1,641 (24.1)
Applicator type ³					
Private	21,315 (92.2)	7,414 (94.3)	4,792 (94.3)	6,230 (92.2)	5,403 (79.4)
Commercial	1,796 (7.8)	452 (5.7)	287 (5.7)	527 (7.8)	1,400 (20.6)
Five most correlated ⁴ pesticides					
Use of Imazethapyr					
No	17,360 (75.1)	4,247 (54.0)	2,128 (41.9)	2,375 (35.1)	2,085 (30.6)
Yes	5,342 (23.1)	3,389 (43.1)	2,794 (55.0)	4,197 (62.1)	4,506 (66.2)
Missing	409 (1.8)	230 (2.9)	157 (3.1)	185 (2.7)	212 (3.1)

Table 1. Selected demographic and lifestyle characteristics of applicators by cumulative metolachlor use in the Agricultural Health Study, 1993–2011 (Continued)

Characteristic ²	Lifetime days of metolachlor use ¹ , no lag				
	No use (n = 23,111)	Quartile 1 (n = 7,866)	Quartile 2 (n = 5,079)	Quartile 3 (n = 6,757)	Quartile 4 (n = 6,803)
Use of Alachlor					
No	14,800 (64.0)	3,384 (43.0)	1,723 (33.9)	2,197 (32.5)	1,735 (25.5)
Yes	7,793 (33.7)	4,289 (54.5)	3,214 (63.3)	4,371 (64.7)	4,899 (72.0)
Missing	518 (2.2)	193 (2.5)	142 (2.8)	189 (2.8)	169 (2.5)
Use of Atrazine					
No	10,870 (47.0)	2,168 (27.6)	856 (16.9)	854 (12.6)	512 (7.5)
Yes	11,796 (51.0)	5,586 (71.0)	4,177 (82.2)	5,836 (86.4)	6,231 (91.6)
Missing	445 (1.9)	112 (1.4)	46 (1.0)	67 (0.9)	60 (0.9)
Use of Dicamba					
No	14,927 (64.6)	3,695 (47.0)	1,821 (35.9)	2,123 (31.4)	1,835 (27.0)
Yes	7,440 (32.2)	3,905 (49.6)	3,089 (60.8)	4,456 (65.9)	4,752 (69.8)
Missing	744 (3.2)	266 (3.4)	169 (3.3)	178 (2.6)	216 (3.2)
Use of Trifluralin					
No	15,093 (65.3)	3,421 (43.5)	1,553 (30.6)	1,802 (26.7)	1,548 (22.8)
Yes	7,155 (31.0)	4,184 (53.2)	3,373 (66.4)	4,759 (70.4)	5,047 (74.2)
Missing	863 (3.7)	261 (3.3)	153 (3.0)	196 (2.9)	208 (3.0)

¹Based on enrollment and first follow-up information. Metolachlor quartile cutpoints based on distribution of all cancer cases: (i) 0–≤18.75, (ii) >18.75–≤38.75, (iii) >38.75–≤113.5, (iv) >113.5 lifetime days.

²Demographic and lifestyle factors reported at enrollment.

³The term “private applicators” refers primarily to farmers and “commercial applicators” to professional pesticide applicators.

⁴Five most correlated pesticides determined by categorizing unlagged intensity-weighted lifetime days for each pesticide and calculating the correlation with quartiles of intensity-weighted lifetime days for metolachlor.

including follicular cell. For liver cancer, we observed statistically significant increases in the third and fourth quartiles and statistically significant trends for both lifetime days and intensity-weighted lifetime days.

Discussion

This updated examination of metolachlor use and cancer in the AHS cohort found suggestions of positive associations between metolachlor use and incidence of both liver cancer and follicular cell lymphoma, though there were few exposed cases for either outcome ($n = 25$ and 32 , respectively). To our knowledge, this is the first occupational epidemiology study to report positive associations between metolachlor exposure and these two outcomes. The findings for liver cancer are of particular interest, given observations of increased liver neoplasms in some animals exposed to metolachlor.

Studies underlying the USEPA classification of metolachlor as a “possible human carcinogen” found mixed results in rodents, with female rats showing the most strongly positive results for hepatic neoplasms.¹⁷ No increased tumor incidence was observed in Charles River DC-1 mice.¹⁸ Other hepatic effects in rats included induction of liver enzymes,^{19,20} a dose-related increase in hepatocellular hypertrophy (male rats)¹⁷ and cystic cholangioma at high doses (female rats).¹⁷ A study comparing the effects on cells of metolachlor and alachlor, a

structural analog, found metolachlor to be less cytotoxic than alachlor to human hepatoma (HepG2) cells.²¹ Another study found metolachlor to be less cytotoxic than alachlor and acetochlor to rat hepatocytes but of equivalent potency in human hepatocytes. In addition to the hepatic effects,²² increased incidence of nasal cavity tumors was reported in male rats in a high dose group.²³ In our study, there were too few female applicators to evaluate sex differences for specific cancer sites.

The potential mechanism of metolachlor hepatic carcinogenicity is unknown, and results appear to differ for rat and human cells and subcellular components. Metolachlor has been shown to induce hepatic CYP2B1/2 activity in male rats.¹⁹ Researchers have suggested an activation pathway leading to a DNA-reactive dialkylbenzoquinone imine, but a study found that while rat liver microsomes exposed to metolachlor produced one important intermediate [2-chloro-*N*-(2-methyl-6-ethylphenyl)acetamide], human liver microsomes did not.¹⁸ However, other investigators have shown greater potential for cytotoxicity in human than in rat hepatocytes.²² Clarification of the reasons for these differences would facilitate evaluation of the potential for carcinogenicity in humans.

We also observed statistically significant positive trends for follicular lymphoma for both lifetime and intensity-weighted lifetime days. While a previous AHS analysis of

Table 2. Rate ratios¹ for all cancers with 20 or more exposed cases by quartiles of lifetime days and intensity-weighted lifetime days of metolachlor use among Agricultural Health Study applicators (*n* = 26,505) who ever used metolachlor (with person-time in the low-metolachlor use category as referent), 5-year lag

Cancer site	Lifetime days			Intensity-weighted lifetime days		
	<i>N</i> ²	RR (95% CI)	<i>p</i> -Trend	<i>N</i>	RR (95% CI)	<i>p</i> -Trend
All cancers						
Q1 ³	699	1.00 reference		694	1.00 reference	
Q2	626	1.00 (0.90–1.13)		604	0.96 (0.86–1.08)	
Q3	611	1.00 (0.89–1.13)		610	0.97 (0.86–1.10)	
Q4	589	0.97 (0.86–1.11)	0.64	613	0.92 (0.80–1.05)	0.27
Bladder						
Q1	33	1.00		35	1.00	
Q2	21	0.67 (0.38–1.18)		21	0.59 (0.33–1.03)	
Q3	28	0.86 (0.50–1.47)		22	0.60 (0.34–1.06)	
Q4	29	0.84 (0.45–1.57)	0.92	32	0.75 (0.41–1.38)	0.80
Brain						
Q1	7	1.00		10	1.00	
Q2	7	1.29 (0.43–3.86)		5	0.52 (0.16–1.66)	
Q3	10	1.70 (0.58–5.05)		8	0.89 (0.32–2.53)	
Q4	7	1.71 (0.49–6.02)	0.41	8	1.10 (0.35–3.49)	0.57
Colon						
Q1	40	1.00		46	1.00	
Q2	44	1.16 (0.73–1.84)		41	0.97 (0.62–1.52)	
Q3	45	1.20 (0.72–2.04)		43	1.00 (0.61–1.65)	
Q4	45	1.19 (0.72–1.97)	0.60	44	0.96 (0.57–1.63)	0.91
Esophagus						
Q1	7	1.00		9	1.00	
Q2	9	1.26 (0.42–3.76)		4	0.59 (0.16–2.19)	
Q3	7	1.05 (0.33–3.35)		13	1.73 (0.63–4.73)	
Q4	14	1.81 (0.63–5.21)	0.27	12	1.58 (0.50–4.97)	0.24
Kidney						
Q1	20	1.00		19	1.00	
Q2	22	1.14 (0.59–2.18)		21	1.01 (0.51–1.99)	
Q3	24	1.14 (0.57–2.27)		27	1.17 (0.60–2.28)	
Q4	21	0.89 (0.44–1.80)	0.59	20	0.69 (0.32–1.48)	0.24
Liver						
Q1	2	1.00		3	1.00	
Q2	4	1.86 (0.31–11.1)		3	0.85 (0.16–4.52)	
Q3	7	3.13 (0.56–17.4)		8	1.83 (0.42–8.02)	
Q4	10	4.01 (0.68–23.5)	0.10	9	1.71 (0.33–8.83)	0.44
Lung						
Q1	50	1.00		49	1.00	
Q2	41	0.97 (0.63–1.51)		41	0.88 (0.54–1.44)	
Q3	51	1.17 (0.77–1.79)		50	1.07 (0.66–1.72)	
Q4	42	0.90 (0.55–1.48)	0.73	47	0.87 (0.52–1.44)	0.70

Table 2. Rate ratios for all cancers with 20 or more exposed cases by quartiles of lifetime days and intensity-weighted lifetime days of metolachlor use among Agricultural Health Study applicators ($n = 26,505$) who ever used metolachlor (with person-time in the low-metolachlor use category as referent), 5-year lag (Continued)

Cancer site	Lifetime days			Intensity-weighted lifetime days		
	N^2	RR (95% CI)	p -Trend	N	RR (95% CI)	p -Trend
Melanoma						
Q1	29	1.00		38	1.00	
Q2	27	1.10 (0.63–1.91)		17	0.54 (0.30–0.97)	
Q3	29	1.20 (0.68–2.10)		27	0.91 (0.52–1.60)	
Q4	27	1.19 (0.65–2.18)	0.60	30	1.03 (0.55–1.93)	0.43
Oral cavity						
Q1	10	1.00		14	1.00	
Q2	21	2.34 (1.06–5.16)		12	1.06 (0.48–2.36)	
Q3	16	1.88 (0.82–4.31)		19	1.69 (0.79–3.61)	
Q4	14	1.78 (0.72–4.39)	0.63	16	1.66 (0.70–3.96)	0.21
Pancreas						
Q1	14	1.00		12	1.00	
Q2	9	0.82 (0.34–2.00)		13	1.33 (0.57–3.13)	
Q3	9	0.86 (0.35–2.11)		7	0.73 (0.26–2.03)	
Q4	9	0.94 (0.34–2.54)	0.99	9	0.88 (0.30–2.60)	0.57
Prostate						
Q1	306	1.00		292	1.00	
Q2	266	0.94 (0.80–1.12)		276	1.05 (0.88–1.24)	
Q3	255	0.93 (0.77–1.11)		245	0.94 (0.77–1.13)	
Q4	232	0.86 (0.70–1.04)	0.15	245	0.89 (0.72–1.10)	0.15
Rectum						
Q1	13	1.00		11	1.00	
Q2	23	1.87 (0.86–4.07)		25	2.29 (0.95–5.54)	
Q3	24	1.84 (0.87–3.92)		23	2.16 (0.97–4.80)	
Q4	21	1.71 (0.75–3.90)	0.44	23	2.01 (0.80–5.06)	0.45
Stomach						
Q1	13	1.00		12	1.00	
Q2	10	0.84 (0.36–1.98)		9	0.82 (0.33–2.02)	
Q3	10	0.95 (0.40–2.23)		10	0.94 (0.37–2.39)	
Q4	4	0.41 (0.12–1.40)	0.18	7	0.68 (0.23–2.06)	0.57
Testes						
Q1	9	1.00		9	1.00	
Q2	4	0.76 (0.23–2.49)		5	0.98 (0.32–3.06)	
Q3	6	1.24 (0.42–3.62)		7	1.83 (0.61–5.55)	
Q4	5	2.00 (0.58–6.91)	0.20	3	1.60 (0.34–7.56)	0.39
Thyroid						
Q1	8	1.00		9	1.00	
Q2	6	0.95 (0.31–2.92)		7	0.98 (0.34–2.80)	
Q3	5	0.75 (0.22–2.56)		3	0.35 (0.08–1.61)	
Q4	7	1.24 (0.37–4.16)	0.68	7	0.93 (0.26–3.35)	0.91
Lymphohematopoietic malignancies						
Q1	82	1.00		80	1.00	
Q2	69	0.93 (0.67–1.30)		61	0.85 (0.60–1.20)	

Table 2. Rate ratios for all cancers with 20 or more exposed cases by quartiles of lifetime days and intensity-weighted lifetime days of metolachlor use among Agricultural Health Study applicators ($n = 26,505$) who ever used metolachlor (with person-time in the low-metolachlor use category as referent), 5-year lag (Continued)

Cancer site	Lifetime days			Intensity-weighted lifetime days		
	N^2	RR (95% CI)	p -Trend	N	RR (95% CI)	p -Trend
Q3	55	0.79 (0.55–1.13)		63	0.87 (0.60–1.26)	
Q4	61	0.93 (0.64–1.36)	0.73	62	0.86 (0.57–1.29)	0.64
Leukemia						
Q1	13	1.00		14	1.00	
Q2	12	0.99 (0.43–2.26)		8	0.51 (0.20–1.31)	
Q3	5	0.46 (0.16–1.33)		9	0.65 (0.26–1.61)	
Q4	10	0.83 (0.32–2.11)	0.61	10	0.63 (0.24–1.67)	0.62
Non-Hodgkin lymphoma (NHL) ⁴						
Q1	66	1.00		64	1.00	
Q2	51	0.87 (0.60–1.27)		50	0.90 (0.62–1.33)	
Q3	49	0.88 (0.59–1.30)		48	0.88 (0.59–1.33)	
Q4	50	0.97 (0.64–1.48)	0.94	52	0.95 (0.60–1.49)	0.95
Chronic/small/prolymphocytic/mantle B-cell NHL						
Q1	19	1.00		17	1.00	
Q2	16	0.95 (0.47–1.92)		16	1.20 (0.58–2.47)	
Q3	19	1.12 (0.55–2.29)		20	1.36 (0.64–2.88)	
Q4	13	0.98 (0.44–2.16)	0.98	14	1.11 (0.46–2.69)	0.93
Diffuse large B-cell lymphoma						
Q1	13	1.00		15	1.00	
Q2	12	0.90 (0.39–2.07)		8	0.61 (0.25–1.49)	
Q3	8	0.72 (0.28–1.89)		8	0.50 (0.18–1.35)	
Q4	9	0.76 (0.28–2.05)	0.57	12	0.83 (0.30–2.26)	0.96
Follicular cell lymphoma						
Q1	5	1.00		7	1.00	
Q2	10	2.48 (0.84–7.32)		6	1.08 (0.36–3.24)	
Q3	7	1.84 (0.53–6.34)		10	2.04 (0.71–5.88)	
Q4	9	3.24 (0.96–11.0)	0.14	8	2.08 (0.61–7.10)	0.21
Multiple myeloma						
Q1	15	1.00		11	1.00	
Q2	6	0.42 (0.15–1.14)		9	0.77 (0.30–1.99)	
Q3	8	0.54 (0.22–1.35)		8	0.79 (0.30–2.12)	
Q4	10	0.74 (0.29–1.88)	0.93	11	1.04 (0.37–2.93)	0.76

¹Adjusted for age, smoking, alcohol, applicator status (private or commercial), family history of cancer (any site), state of residence and the pesticides most highly correlated with metolachlor (imazethapyr, alachlor, atrazine, dicamba, trifluralin). All cancers combined also adjusted for sex and race. Lung and prostate cancers also adjusted for race.

²Median number of cases over five imputations.

³For lifetime-days analyses with a 5-year lag, Q1 >0–≤15 days, Q2 >15–≤38.75 days, Q3 >38.75–≤108.5 days, Q4 >108.5 days. For intensity-weighted lifetime-days analyses, Q1 >0–≤490, Q2 >490–≤1,403, Q3 >1,403–≤4,103, Q4 >4,103 units.

⁴Subtypes for non-Hodgkin lymphoma as defined by Morton *et al.*¹⁶

metolachlor did not find associations between metolachlor use and either NHL or lymphohematopoietic cancers in general,⁶ an AHS examination of cancer incidence related to alachlor use reported a positive, statistically significant trend for lymphohematopoietic cancers.²⁴ In human lymphocytes, metolachlor has not been found to induce sister chromatid

exchanges.²⁵ Whether metolachlor produces a clastogenic response in human lymphocytes is unclear, with both positive²⁰ and negative²⁶ reports.

The findings from this update of the cohort are similar to those in the earlier report on cancer incidence and metolachlor use⁶ for rectal cancer, with nonsignificant elevations in all

Table 3. Rate ratios¹ for cancers with at least 20 exposed cases by quartiles of lifetime exposure days and intensity-weighted lifetime exposure days to metolachlor among Agricultural Health Study cohort applicators (with unexposed person-time as the referent), 5-year lag

Cancer site	Lifetime days			Intensity-weighted lifetime days		
	N ²	RR (95% CI)	p-Trend	N	RR (95% CI)	p-Trend
All cancers						
Unexposed	3,248	1.00 (referent)		3,248	1.00 (referent)	
Q1 ³	619	0.95 (0.86–1.04)		619	0.98 (0.89–1.08)	
Q2	626	0.96 (0.88–1.06)		604	0.95 (0.86–1.05)	
Q3	611	0.97 (0.88–1.06)		610	0.96 (0.87–1.07)	
Q4	589	0.94 (0.85–1.04)	0.30	613	0.92 (0.83–1.02)	0.14
Bladder						
Unexposed	168	1.00		168	1.00	
Q1	32	0.99 (0.65–1.51)		33	1.11 (0.74–1.68)	
Q2	21	0.64 (0.39–1.06)		21	0.64 (0.39–1.05)	
Q3	28	0.88 (0.56–1.40)		22	0.70 (0.43–1.15)	
Q4	29	0.92 (0.58–1.48)	0.74	32	0.98 (0.63–1.54)	0.74
Brain						
Unexposed	38	1.00		38	1.00	
Q1	7	0.81 (0.32–2.04)		10	1.19 (0.56–2.57)	
Q2	7	0.91 (0.38–2.17)		5	0.57 (0.20–1.67)	
Q3	10	1.20 (0.53–2.71)		8	1.01 (0.42–2.46)	
Q4	7	1.21 (0.47–3.08)	0.56	8	1.31 (0.52–3.29)	0.59
Colon						
Unexposed	241	1.00		241	1.00	
Q1	38	0.80 (0.55–1.16)		44	0.94 (0.65–1.36)	
Q2	44	0.89 (0.61–1.30)		41	0.85 (0.59–1.23)	
Q3	45	0.90 (0.62–1.31)		43	0.86 (0.60–1.24)	
Q4	45	0.88 (0.60–1.28)	0.60	44	0.81 (0.55–1.19)	0.28
Esophagus						
Unexposed	40	1.00		40	1.00	
Q1	7	0.78 (0.32–1.89)		8	0.84 (0.35–2.00)	
Q2	9	0.95 (0.41–2.23)		4	0.47 (0.16–1.35)	
Q3	7	0.82 (0.34–1.95)		13	1.35 (0.67–2.72)	
Q4	14	1.33 (0.61–2.88)	0.45	12	1.16 (0.52–2.61)	0.47
Kidney						
Unexposed	112	1.00		112	1.00	
Q1	18	0.84 (0.49–1.45)		17	0.90 (0.52–1.57)	
Q2	21	0.97 (0.58–1.60)		21	0.92 (0.55–1.55)	
Q3	24	1.03 (0.63–1.70)		27	1.16 (0.72–1.87)	
Q4	21	0.86 (0.50–1.50)	0.74	20	0.74 (0.42–1.30)	0.44
Liver						
Unexposed	17	1.00		15	1.00	
Q1	2	0.97 (0.17–5.50)		3	1.65 (0.37–7.23)	
Q2	4	1.79 (0.54–5.93)		3	1.33 (0.35–4.99)	
Q3	7	3.06 (1.05–8.90)		8	3.14 (1.11–8.88)	
Q4	10	3.99 (1.43–11.1)	<0.01	9	3.18 (1.10–9.22)	0.03

Table 3. Rate ratios for cancers with at least 20 exposed cases by quartiles of lifetime exposure days and intensity-weighted lifetime exposure days to metolachlor among Agricultural Health Study cohort applicators (with unexposed person-time as the referent), 5-year lag (Continued)

Cancer site	Lifetime days			Intensity-weighted lifetime days		
	N ²	RR (95% CI)	p-Trend	N	RR (95% CI)	p-Trend
Lung						
Unexposed	330	1.00		330	1.00	
Q1	45	0.81 (0.58–1.14)		43	0.86 (0.60–1.24)	
Q2	42	0.77 (0.54–1.10)		41	0.75 (0.51–1.10)	
Q3	51	0.91 (0.65–1.28)		50	0.89 (0.63–1.26)	
Q4	42	0.70 (0.47–1.02)	0.12	47	0.72 (0.49–1.05)	0.13
Melanoma						
Unexposed	134	1.00		134	1.00	
Q1	24	0.86 (0.54–1.38)		33	1.15 (0.75–1.76)	
Q2	27	1.00 (0.64–1.57)		17	0.63 (0.36–1.09)	
Q3	28	1.09 (0.69–1.71)		27	1.06 (0.66–1.69)	
Q4	27	1.07 (0.66–1.74)	0.64	31	1.20 (0.74–1.94)	0.44
Oral cavity						
Unexposed	69	1.00		69	1.00	
Q1	9	0.63 (0.30–1.34)		13	0.87 (0.45–1.67)	
Q2	21	1.41 (0.82–2.44)		12	0.85 (0.44–1.64)	
Q3	16	1.14 (0.63–2.07)		19	1.36 (0.76–2.41)	
Q4	14	1.08 (0.56–2.10)	0.64	16	1.29 (0.68–2.45)	0.28
Pancreas						
Unexposed	73	1.00		73	1.00	
Q1	11	0.72 (0.36–1.44)		9	0.59 (0.27–1.28)	
Q2	9	0.64 (0.31–1.34)		13	0.95 (0.50–1.79)	
Q3	9	0.61 (0.29–1.30)		7	0.49 (0.22–1.12)	
Q4	9	0.64 (0.29–1.42)	0.25	9	0.60 (0.27–1.32)	0.15
Prostate						
Unexposed	1,242	1.00		1,242	1.00	
Q1	276	1.04 (0.91–1.20)		261	1.00 (0.86–1.16)	
Q2	266	1.00 (0.86–1.15)		272	1.07 (0.93–1.23)	
Q3	255	0.98 (0.84–1.15)		245	0.96 (0.82–1.13)	
Q4	232	0.92 (0.78–1.08)	0.25	245	0.92 (0.78–1.08)	0.26
Rectum						
Unexposed	104	1.00		104	1.00	
Q1	12	0.66 (0.35–1.26)		10	0.55 (0.28–1.11)	
Q2	23	1.29 (0.79–2.12)		25	1.38 (0.83–2.28)	
Q3	24	1.32 (0.80–2.18)		22	1.36 (0.81–2.29)	
Q4	21	1.32 (0.76–2.28)	0.18	23	1.37 (0.79–2.36)	0.14
Stomach						
Unexposed	47	1.00		47	1.00	
Q1	13	1.68 (0.87–3.26)		12	1.64 (0.82–3.29)	
Q2	10	1.18 (0.55–2.52)		9	1.14 (0.52–2.51)	
Q3	10	1.25 (0.59–2.64)		10	1.18 (0.54–2.56)	
Q4	4	0.52 (0.17–1.58)	0.25	7	0.82 (0.34–2.02)	0.61

Table 3. Rate ratios for cancers with at least 20 exposed cases by quartiles of lifetime exposure days and intensity-weighted lifetime exposure days to metolachlor among Agricultural Health Study cohort applicators (with unexposed person-time as the referent), 5-year lag (Continued)

Cancer site	Lifetime days			Intensity-weighted lifetime days		
	N ²	RR (95% CI)	p-Trend	N	RR (95% CI)	p-Trend
Testes						
Unexposed	23	1.00		23	1.00	
	8	1.47 (0.62–3.46)		8	1.20 (0.48–2.96)	
	4	0.90 (0.29–2.77)		5	0.97 (0.34–2.73)	
Q3	6	1.28 (0.48–3.38)		7	1.44 (0.56–3.75)	
Q4	5	1.47 (0.47–4.62)	0.56	3	0.80 (0.20–3.14)	0.91
Thyroid						
Unexposed	39	1.00		39	1.00	
Q1	7	0.81 (0.33–2.00)		8	0.93 (0.38–2.24)	
Q2	6	0.79 (0.31–2.01)		7	0.96 (0.40–2.28)	
	5	0.65 (0.23–1.84)		3	0.37 (0.10–1.43)	
Q4	7	1.05 (0.41–2.71)	0.98	7	1.00 (0.38–2.61)	0.76
Lymphohematopoietic malignancies						
Unexposed	307	1.00		307	1.00	
Q1	70	1.11 (0.84–1.48)		67	1.11 (0.83–1.48)	
Q2	69	1.12 (0.84–1.49)		61	1.01 (0.74–1.38)	
Q3	55	0.95 (0.69–1.30)		63	1.06 (0.78–1.45)	
	61	1.13 (0.82–1.56)	0.66	62	1.06 (0.77–1.47)	0.74
Leukemia						
Unexposed	52	1.00		52	1.00 referent	
Q1	12	1.26 (0.61–2.57)		12	1.43 (0.72–2.87)	
	12	1.38 (0.68–2.79)		8	0.82 (0.35–1.95)	
Q3	5	0.68 (0.26–1.80)		9	1.20 (0.55–2.60)	
Q4	10	1.40 (0.64–3.08)	0.67	10	1.34 (0.60–2.98)	0.52
Non-Hodgkin lymphoma (NHL) ⁴						
Unexposed	247	1.00		247	1.00	
Q1	55	1.09 (0.79–1.50)		53	1.05 (0.76–1.45)	
Q2	52	1.03 (0.74–1.44)		51	1.04 (0.74–1.45)	
Q3	50	1.04 (0.74–1.45)		51	1.02 (0.72–1.44)	
Q4	50	1.13 (0.80–1.61)	0.55	53	1.09 (0.76–1.56)	0.68
Chronic/small/prolymphocytic/mantle B-cell NHL						
Unexposed	72	1.00		72	1.00	
Q1	17	1.10 (0.60–2.03)		14	0.85 (0.44–1.64)	
Q2	16	1.12 (0.63–2.00)		16	1.17 (0.65–2.11)	
Q3	19	1.36 (0.77–2.39)		20	1.38 (0.78–2.42)	
	13	1.32 (0.69–2.52)	0.69	14	1.20 (0.62–2.35)	0.41
Diffuse large B-cell lymphoma						
Unexposed	64	1.00		64	1.00	
Q1	8	0.67 (0.30–1.48)		10	0.85 (0.41–1.78)	
Q2	12	0.88 (0.44–1.76)		8	0.68 (0.31–1.48)	
Q3	8	0.69 (0.31–1.54)		8	0.54 (0.22–1.29)	
Q4	9	0.64 (0.28–1.47)	0.30	12	0.83 (0.38–1.80)	0.52

Table 3. Rate ratios for cancers with at least 20 exposed cases by quartiles of lifetime exposure days and intensity-weighted lifetime exposure days to metolachlor among Agricultural Health Study cohort applicators (with unexposed person-time as the referent), 5-year lag (Continued)

Cancer site	Lifetime days			Intensity-weighted lifetime days		
	N ²	RR (95% CI)	p-Trend	N	RR (95% CI)	p-Trend
Follicular cell lymphoma						
Unexposed	24	1.00		24	1.00	
Q1	4	0.93 (0.31–2.79)		6	1.37 (0.52–3.57)	
Q2	10	2.43 (1.07–5.52)		6	1.45 (0.56–3.78)	
Q3	7	1.76 (0.64–4.81)		10	2.67 (1.10–6.49)	
Q4	9	2.89 (1.13–7.38)	0.03	8	2.57 (0.95–6.95)	0.04
Multiple myeloma						
Unexposed	52	1.00		52		
Q1	15	1.46 (0.77–2.78)		11	1.13 (0.54–2.34)	
Q2	6	0.63 (0.25–1.60)		9	0.87 (0.39–1.92)	
Q3	8	0.86 (0.39–1.92)		8	0.94 (0.42–2.12)	
Q4	10	1.20 (0.55–2.60)	0.87	11	1.27 (0.59–2.72)	0.60

¹Adjusted for age, smoking, alcohol, applicator status (private or commercial), family history of cancer (any site), state of residence and the pesticides most highly correlated with metolachlor (alachlor, atrazine, dicamba, imazethapyr, trifluralin). All cancers combined also adjusted for sex and race. Lung and prostate cancers also adjusted for race.

²Median number of cases over five imputations.

³For lifetime-days analyses with a 5-year lag, unexposed = 0 days, Q1 >0–≤15 days, Q2 >15–≤38.75 days, Q3 >38.75–≤108.5 days, Q4 >108.5 days. For intensity-weighted lifetime-days analyses, unexposed = 0 days, Q1 >0–≤490, Q2 >490–≤1,403, Q3 >1,403–≤4,103, Q4 >4,103 units.

⁴Subtypes for non-Hodgkin lymphoma as defined by Morton *et al.*¹⁶

categories above the referent but no significant trend. Prostate cancer continues to have decreasing risks with increasing lifetime days of exposure, although the results in the update are not statistically significant.

The excess lung cancer at higher lifetime days of metolachlor exposure previously seen in the cohort^{5,6} is absent in this update, with no observed exposure response and no notable or statistically significant increase in any exposure quartile. When we replicated the original analysis using the original exposure information collected at enrollment, but incorporating the 6 years of additional follow-up, the relations with both lifetime days and intensity-weighted lifetime days were less strong than in the previous analysis.⁶ Quite a number of lung cancer cases have accrued in the interim; while the original analysis had 46 metolachlor-using cases with no lag imposed, the current analysis has almost 200 such cases with a 5-year lag. When we then added the exposure information from the follow-up questionnaire, the exposure response was dampened further, although this effect of the additional exposure data was more modest than the effect of extending follow-up. Sensitivity analyses, which (i) removed the imputed values and (ii) limited analysis to participants who completed the follow-up questionnaire, did not produce large changes in the results. Pesticides most correlated with metolachlor use differed in the two studies, but an additional sensitivity analysis using the original pesticide confounders also failed to explain the difference between the two analyses. Collectively, these results suggest that the addition of person-time and new cases with different metolachlor use

patterns account for the majority of the attenuation of the previously observed relation between metolachlor and lung cancer. In the update, only 15% of applicators reported using metolachlor in the most recent farming year, in contrast with 48% reporting ever-use of metolachlor on the enrollment questionnaire; 20% of the cohort were no longer farming as of phase 2 follow-up. In addition, the volume of metolachlor used in the United States has declined since the mid-1990s,^{27,28} and increased use of personal protective equipment, other changes in application methods and changes in metolachlor formulation during the follow-up period may have decreased exposures. The apparent attenuation of lung cancer risk may indicate that diminishing use has reduced risk, that the previous results were due to chance or that latency may be important. In our study, with unexposed applicators in the reference group, the relation is in fact negative, though not statistically significant.

Study strengths include the size of the cohort, which comprises 26,505 metolachlor users, as well as 23,111 nonusers of this herbicide. The questionnaires used were quite comprehensive, including information on use of other pesticides, use of personal protective equipment and methods of application. The imputation method used was developed based on applicator characteristics and, for metolachlor, data withheld from development of the imputation algorithm were quite consistent with results generated by the algorithm.¹⁵ Recall bias was minimized by initial collection of pesticide use and lifestyle information at study entry, and assessments of the reliability of pesticide use report data in the study have been positive.^{12,13}

Our inability to distinguish between two metolachlor isomers, *S*-metolachlor and *R*-metolachlor, is a limitation of the study. *S*-metolachlor is the more herbicidally active isomer. The original metolachlor marketed was a 50–50 mixture of the *R*- and *S*-isomers. In 1997, the manufacturer registered a new product, *S*-metolachlor, which contained 88% *S*-isomer, 12% *R*-isomer. *S*-metolachlor can be applied at a lower rate than the old product because it has more of the active isomer. The old formulation was taken off the market in September 1999, but was brought back onto the market as a generic in 2003.²⁹ Chronic toxicity testing on *Daphnia magna* found *R*-metolachlor to be significantly more toxic with respect to longevity and reproductive outcomes than *S*-metolachlor³⁰; smaller amounts of product with higher concentrations of *S*-metolachlor can be applied because of its greater efficacy, leading to the potential for reduced applicator exposures.

Additional follow-up time would allow for sensitivity analyses using a multiplier assuming reduced intensity calculation for days accrued from 1999 forward. However, one study reported that while *S*-metolachlor did not increase the frequency of micronuclei, a commercial formulation of *S*-metolachlor did; the authors suggest that additional xenobiotics in the commercial formulation may be responsible for this effect.³¹ If “other or inert” ingredients (which may vary over time and by commercial formulation and are often not identified in commercial products), rather than metolachlor itself,

are responsible for apparent associations, then accounting for the change in the relative proportions of the isomers may do little to improve assessment of the relations between metolachlor and these malignancies.

The finding of a positive exposure–response relation between liver cancer and metolachlor use comprises the first report suggesting that the increased liver neoplasms observed in rats may have a correlate in humans. The trends for liver cancer results, as well as for follicular cell lymphoma, were positive regardless of the reference group but attained statistical significance in analyses using unexposed person-time as the referent. Additional follow-up would facilitate assessment of whether the differences in the results reflect greater statistical power with a larger reference category or other exposure-related factors that we were unable to control for in our analyses. Further follow-up would also permit better assessment of the role of latency in these associations, as well as evaluation of the role of metolachlor exposure in other health outcomes, particularly those for which cases are sparse or for which a longer lag period may be more biologically plausible.

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References

- EPA Reregistration Eligibility Decision (RED) Metolachlor. U.S. Environmental Protection Agency Office of Pesticide Programs (OPP), Prevention, Pesticides, and Toxic Substances, Washington, DC, 1995. Available at: <http://www.epa.gov/opp00001/reregistration/REDs/0001.pdf>. Accessed July 14, 2014.
- Thorpe N, Shirmohammadi A. Herbicides and nitrates in groundwater of Maryland and childhood cancers: a geographic information systems approach. *J Environ Sci Health C* 2005; 23:261–78.
- Alavanja MC, Sandler DP, Lynch CF, et al. Cancer incidence in the Agricultural Health Study. *Scand J Work Environ Health* 2005;31 (Suppl 1):39–45, discussion 5–7.
- Alavanja MC, Sandler DP, McMaster SB, et al. The Agricultural Health Study. *Environ Health Perspect* 1996;104:362–9.
- Alavanja MC, Dosemeci M, Samanic C, et al. Pesticides and lung cancer risk in the Agricultural Health Study cohort. *Am J Epidemiol* 2004;160:876–85.
- Rusiecki JA, Hou L, Lee WJ, et al. Cancer incidence among pesticide applicators exposed to metolachlor in the Agricultural Health Study. *Int J Cancer* 2006;118:3118–23.
- Coble J, Thomas KW, Hines CJ, et al. An updated algorithm for estimation of pesticide exposure intensity in the Agricultural Health Study. *Int J Environ Res Public Health* 2011;8: 4608–22.
- Dennis LK, Lynch CF, Sandler DP, et al. Pesticide use and cutaneous melanoma in pesticide applicators in the agricultural health study. *Environ Health Perspect* 2010;118: 812–17.
- Andreotti G, Hou L, Beane Freeman LE, et al. Body mass index, agricultural pesticide use, and cancer incidence in the Agricultural Health Study cohort. *Cancer Causes Control* 2010;21:1759–75.
- Lee WJ, Sandler DP, Blair A, et al. Pesticide use and colorectal cancer risk in the Agricultural Health Study. *Int J Cancer* 2007;121:339–46.
- Dosemeci M, Alavanja MC, Rowland AS, et al. A quantitative approach for estimating exposure to pesticides in the Agricultural Health Study. *Ann Occup Hyg* 2002;46:245–60.
- Hoppin JA, Yucel F, Dosemeci M, et al. Accuracy of self-reported pesticide use duration information from licensed pesticide applicators in the Agricultural Health Study. *J Expo Anal Environ Epidemiol* 2002;12:313–18.
- Blair A, Tarone R, Sandler D, et al. Reliability of reporting on life-style and agricultural factors by a sample of participants in the Agricultural Health Study from Iowa. *Epidemiology* 2002;13: 94–9.
- Hoppin JA, Long S, Umbach DM, et al. Lifetime organophosphorous insecticide use among private pesticide applicators in the Agricultural Health Study. *J Expo Sci Environ Epidemiol* 2012;22:584–92.
- Heltshe SL, Lubin JH, Koutros S, et al. Using multiple imputation to assign pesticide use for non-responders in the follow-up questionnaire in the Agricultural Health Study. *J Expo Sci Environ Epidemiol* 2012;22:409–16.
- Morton LM, Turner JJ, Cerhan JR, et al. Proposed classification of lymphoid neoplasms for epidemiologic research from the Pathology Working Group of the International Lymphoma Epidemiology Consortium (InterLymph). *Blood* 2007;110:695–708.
- US EPA Toxicology Branch Peer Review Committee, Memorandum on Metolachlor, May 30. Available at: http://www.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-108801_23-Aug-85_166.pdf. Accessed June 23, 2014.
- Coleman S, Linderman R, Hodgson E, et al. Comparative metabolism of chloroacetamide herbicides and selected metabolites in human and rat liver microsomes. *Environ Health Perspect* 2000;108:1151–7.
- Dalton SR, Miller RT, Meyer SA. The herbicide metolachlor induces liver cytochrome P450s 2B1/2 and 3A1/2, but not thyroxine-uridine dinucleotide phosphate glucuronosyltransferase and associated thyroid gland activity. *Int J Toxicol* 2003;22:287–95.
- Dearfield KL, McCarroll NE, Protzel A, et al. A survey of EPA/OPP and open literature on selected pesticide chemicals. II. Mutagenicity and carcinogenicity of selected chloroacetanilides and related compounds. *Mutat Res* 1999;443:183–221.
- Miranda SR, Meyer SA. Cytotoxicity of chloroacetanilide herbicide alachlor in HepG2 cells independent of CYP3A4 and CYP3A7. *Food Chem Toxicol* 2007;45:871–7.
- Kale VM, Miranda SR, Wilbanks MS, et al. Comparative cytotoxicity of alachlor, acetochlor, and metolachlor herbicides in isolated rat and cryopreserved human hepatocytes. *J Biochem Mol Toxicol* 2008;22:41–50.
- US EPA. Second Peer Review of Metolachlor, 1991. Available at: <http://www.epa.gov/pesticides/>

- chem_search/cleared_reviews/csr_PC-108801_17-Apr-91_189.pdf. Accessed June 23, 2014.
24. Lee WJ, Hoppin JA, Blair A, et al. Cancer incidence among pesticide applicators exposed to alachlor in the Agricultural Health Study. *Am J Epidemiol* 2004;159:373–80.
 25. Hill AB, Jefferies PR, Quistad GB, et al. Dialkylquinoneimine metabolites of chloroacetanilide herbicides induce sister chromatid exchanges in cultured human lymphocytes. *Mutat Res* 1997;395:159–71.
 26. Grisolia CK, Ferrari I. In vitro and in vivo studies demonstrate non-mutagenicity of the herbicide metolachlor. *Braz J Genet* 1997;20:411–414.
 27. US EPA Pesticides Industry Sales and Usage: 2006 and 2007 Market Estimates. Available at: http://www.epa.gov/opp00001/pestsales/07pestsales/table_of_contents2007.htm. Accessed August 12, 2014.
 28. US EPA Pesticide Industry Sales and Usage: 1994 and 1995 Market Estimates. In: Agency USEP, ed., 1997. Available at: http://www.epa.gov/opp00001/pestsales/95pestsales/table_of_contents1995.htm. Accessed August 12, 2014.
 29. Prostko E, Grey T. Impact of dual magnum on peanut yields in Georgia. Department of Crop and Soil Sciences, University of Georgia College of Agricultural and Environmental Sciences, 2013. Available at: <http://www.gaweed.com/slides/prostko-apres-2013/prostko-apres-2013.pdf>. Accessed June 23, 2014.
 30. Liu H, Ye W, Zhan X, et al. A comparative study of rac- and S-metolachlor toxicity to *Daphnia magna*. *Ecotoxicol Environ Safe* 2006;63:451–5.
 31. Nikoloff N, Escobar L, Soloneski S, et al. Comparative study of cytotoxic and genotoxic effects induced by herbicide S-metolachlor and its commercial formulation Twin Pack Gold(R) in human hepatoma (HepG2) cells. *Food Chem Toxicol* 2013;62:777–81.