Cancer Incidence Among Pesticide Applicators Exposed to Atrazine in the Agricultural Health Study

Jennifer A. Rusiecki, Anneclaire De Roos, Won Jin Lee, Mustafa Dosemeci, Jay H. Lubin, Jane A. Hoppin, Aaron Blair, Michael C. R. Alavanja

Background: **Atrazine is the most heavily applied agricultural pesticide for crop production in the United States. Both animal and human studies have suggested that atrazine is possibly carcinogenic, but results have been mixed. We evaluated cancer incidence in atrazine-exposed pesticide applicators among 53 943 participants in the Agricultural Health Study, a prospective cohort study of licensed pesticide applicators in Iowa and North Carolina.** ***Methods:*** **We obtained detailed pesticide exposure information using a self-administered questionnaire completed at the time of enrollment (1993–1997). Cancer incidence was followed through December 31, 2001. We used adjusted Poisson regression to calculate rate ratios (RRs) and 95% confidence intervals (CIs) of multiple types of cancer among atrazine exposed applicators.** P_{trend} **values were calculated using atrazine exposure as a continuous variable, and all statistical tests were two-sided. Two exposure metrics were used: quartiles of lifetime days of exposure and quartiles of intensity-weighted lifetime days of exposure.** ***Results:*** **36 513 (68%) applicators reported ever using atrazine; exposure was not associated with overall cancer incidence. Comparisons of cancer incidence in applicators with the highest atrazine exposure and those with the lowest exposure, assessed by lifetime days (RR_{LD}) and intensity-weighted lifetime days (RR_{IWLD}) of exposure yielded the following results: prostate cancer, RR_{LD} = 0.88, 95% CI = 0.63 to 1.23, P_{trend} = .26, and RR_{IWLD} = 0.89, 95% CI = 0.63 to 1.25, P_{trend} = .35; lung cancer, RR_{LD} = 1.91, 95% CI = 0.93 to 3.94, P_{trend} = .08, and RR_{IWLD} = 1.37, 95% CI = 0.65 to 2.86, P_{trend} = .19; bladder cancer, RR_{LD} = 3.06, 95% CI = 0.86 to 10.81, P_{trend} =.18, and RR_{IWLD} = 0.85, 95% CI = 0.24 to 2.94, P_{trend} = .71; non-Hodgkin lymphoma, RR_{LD} = 1.61, 95% CI = 0.62 to 4.16, P_{trend} = .35, and RR_{IWLD} = 1.75, 95% CI = 0.73 to 4.20, P_{trend} = .14; and multiple myeloma, RR_{LD} = 1.60, 95% CI = 0.37 to 7.01, P_{trend} = .41, and RR_{IWLD} = 2.17, 95% CI = 0.45 to 10.32, P_{trend} = .21.** ***Conclusions:*** **Our analyses did not find any clear associations between atrazine exposure and any cancer analyzed. However, further studies are warranted for tumor types in which there was a suggestion of trend (lung, bladder, non-Hodgkin lymphoma, and multiple myeloma). [J Natl Cancer Inst 2004;96:1375–82]**

Atrazine (2-chloro-4-ethylamino-6-isopropylamino)-s-triazine is a triazine herbicide that is used primarily on corn and soybean crops to control growth of broadleaf and grassy weeds. It is the most heavily used agricultural pesticide in the United States, with an estimated 76.4 million pounds applied annually *(1)*. Human exposure to atrazine occurs occupationally in farming and manufacturing and environmentally through contaminated drinking water or drift. Atrazine is the most commonly detected pesticide in surface water in surveys in the midwestern United States and was the second most frequently detected pesticide in the U.S. Environmental Protection Agency (EPA) National Survey of Pesticides in Drinking Water Wells *(2)*. Use of atrazine has been restricted since 1993, primarily to protect water supplies *(2)*. Only licensed pesticide applicators may purchase atrazine.

Results from animal and human studies on the carcinogenic effects of exposure to atrazine have been mixed. Oral administration of atrazine was associated with increased incidence and earlier onset of mammary tumors in female Sprague–Dawley rats but not in other strains of rats or in other mammals *(3,4)*. Atrazine exposure was also associated with lymphomas and testicular cancer in rats and mice in some studies *(5–7)*. Several epidemiologic studies in humans have evaluated cancer risks associated with atrazine exposure *(8–22)*. Slightly greater than expected numbers of bladder, oral cavity, and lymphohematopoietic cancers were observed in a cohort of triazine herbicide manufacturing workers; however, none of the increases were statistically significant, and the people in the study were exposed to carcinogens other than atrazine *(8)*. This study also found statistically significantly elevated standardized incidence ratios (SIRs) for prostate cancer (SIR = 3.94, 95% confidence interval [CI] = 1.28 to 9.20); however, this increase may have been due to the intensive prostate-specific antigen (PSA) screening of the workers in this cohort *(8)*. A mortality study based on the same population also found an increased standardized mortality ratio for non-Hodgkin lymphoma (standardized mortality ratio = 3.72, 95% CI = 1.01 to 9.52) *(9)*. However, no association was found between atrazine exposure and prostate cancer in a study by Alavanja et al. *(10)* of the Agricultural Health Study cohort, a cohort of pesticide applicators from Iowa and North Carolina enrolled from January 1, 1993, through December 31, 1997.

In case–control studies conducted in the midwestern United States, atrazine or triazine use was not associated with Hodgkin disease *(11)*, leukemia *(12)*, multiple myeloma *(13)*, soft tissue sarcoma *(11)*, or colon cancer *(14)*. Atrazine use was weakly or

Affiliations of authors: Occupational and Environmental Epidemiology Branch (JAR, WJL, MD, AB, MCRA) and Biostatistics Branch (JHL), Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Rockville, MD; Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, and Department of Epidemiology, University of Washington, Seattle (ADR); Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, NC (JAH).

Correspondence to: Michael C. R. Alavanja, DrPH, Occupational and Environmental Epidemiology Branch, National Cancer Institute, 6120 Executive Blvd., EPS 8000, Bethesda, MD 20892-7240 (e-mail: alavanjm@mail.nih.gov).

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moderately associated with non-Hodgkin lymphoma (NHL) in case–control studies conducted in Iowa and Minnesota *(15)*, Kansas *(11)*, and Nebraska *(16,17)*, although the association in the Nebraska study was diminished after adjustment for exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) and organophosphate insecticides *(16)*. However, a pooled analysis by De Roos et al. *(18)* of data from these studies found statistically significantly increased odds ratios (ORs) for NHL with atrazine exposure in combination with exposure to one of three other pesticides (diazinon, alachlor, or dicamba). A case–control study of ovarian cancer found an increased risk among women farmers "possibly" and "definitely" exposed to atrazine in their occupation *(19)*. Ecologic studies have shown increased risks of stomach *(20)*, prostate, brain, testicular *(21)*, and breast cancers *(22)* and leukemia and decreased risks of colon *(20)* and breast cancers *(23)* with increasing amounts of triazine herbicides applied or with increasing levels measured in drinking water.

Based on inadequate data for humans and limited data for experimental animals, atrazine was classified as "possibly carcinogenic to humans" (Group 2B) by the International Agency for Research on Cancer in 1999 *(24)*. The EPA has classified atrazine as "not likely to be a human carcinogen" *(25)*. However, the limited data on the effects of atrazine among humans, the provocative findings in animal studies, and the frequency with which this herbicide is used warrant further investigation among exposed populations. We therefore investigated site-specific cancer incidence and risk among pesticide applicators exposed to atrazine in the Agricultural Health Study cohort using a longer follow-up period and a larger number of case patients than the prostate cancer analysis by Alavanja et al. *(10)*.

Subjects and Methods

Cohort Enrollment and Follow-up

The Agricultural Health Study cohort is a prospective study of 57 311 private and commercial applicators licensed to apply restricted-use pesticides who live in Iowa or North Carolina and who were recruited between 1993 and 1997 *(26)*. Cohort members were matched to cancer registry files in Iowa and North Carolina for case identification and to the state death registries and the National Death Index to ascertain vital status. Incident cancers were identified for the time period from the date of enrollment through December 31, 2001, and were coded according to the International Classification of Diseases for Oncology, 2nd edition (ICD-O-2). Cohort members who were alive but no longer residing in Iowa or North Carolina were identified through current address records of the Internal Revenue Service (address information only), Motor Vehicle Registration offices, and pesticide license registries of the state agricultural departments. Person-year accumulation for cancer incidence of individuals who had moved from the state was censored in the year they departed, although they were still followed up for mortality. The mean time of follow-up was 6.5 years. All participants provided verbal informed consent, and the protocol was approved by the institutional review boards of the National Cancer Institute, Batelle, the University of Iowa, and Westat.

Exposure Assessment

A self-administered enrollment questionnaire collected comprehensive exposure data on 22 pesticides and information on ever/never use for 28 more pesticides, use of personal protective equipment, pesticide application methods, pesticide mixing, equipment repair, smoking history, alcohol consumption, cancer history of first-degree relatives, and basic demographics *(27)*. Applicators who completed this questionnaire were also given a self-administered take-home questionnaire, which sought additional information on occupational exposures. The questionnaires may be accessed at http://www.aghealth.org/questionnaires.html.

Data from questionnaires completed at enrollment and measurement data from the pesticide exposure literature were used to calculate estimated intensity of exposure to each pesticide using the following algorithm: intensity level = ([mixing status + application method + equipment repair status] × personal protective equipment use) *(28)*.

The scores assigned to each factor in the intensity-level algorithm were not assigned as nominal or ordinal values but were weighted to reflect intensity of exposure as described in the literature. Mixing status (mix) was a three-level variable based on never mixing, personally mixing less than 50% of the time, and personally mixing more than 50% of the time (mix = 0, 3, and 9, respectively). Application method (applic) was a six-level variable based on never applying, use of aerial-aircraft or distribution of tablets, application in furrow, use of boom on tractor, use of backpack, and use of hand spray (applic = 0, 1, 2, 3, 8, 9, respectively). Equipment repair status (repair) was a two-level variable based on not repairing or repairing (repair = 0, 2, respectively). Personal protective equipment use was an eight-level variable based on type of personal protective equipment used while applying pesticides *(28)*.

We constructed two lifetime atrazine exposure metrics for this analysis, each categorized into quartiles, based on the quartile levels among all cancer cases: 1) lifetime days of exposure, based on the product of the midpoints of the questionnaire categories of number of years an applicator personally applied or mixed atrazine and number of days in an average year an applicator personally mixed or applied atrazine (i.e., years of use × number of days used per year, resulting in the following quartiles: ≤19.9, 20.0–56.0, 56.1–178.5, ≥178.5) and 2) intensity-weighted lifetime days of exposure, which was the product of lifetime days of exposure and intensity level (i.e., years of use × number of days used per year × intensity level, resulting in the following quartiles: ≤101.9, 102.0–326.7, 326.8–911.4, ≥911.4).

Statistical Analysis

Prevalent cancer case patients identified at or prior to the time of enrollment (n = 1074) and applicators who did not provide information on atrazine use (n = 2294) were excluded from this analysis, leaving 53 943 applicators. Analyses of first primary incident cancer case patients enabled us to obtain exposure data from each case patient prior to the onset of cancer.

To examine internal exposure–response relationships among participants who reported having ever used atrazine, Poisson regression analyses were carried out for individual cancer sites to estimate rate ratios (RRs) and 95% confidence intervals (CIs) associated with quartiles of lifetime days of exposure (RR_{LD}) or intensity-weighted lifetime days of exposure (RR_{IWLD}), using the lowest quartile as the referent. We investigated only cancer sites for which there were at least 20 case patients with atrazine

exposure. *P* values for trend were calculated using atrazine exposure as a continuous variable, and all statistical tests were two-sided. Rate ratios were adjusted for age at enrollment (as a continuous variable), sex, educational level (high school/GED or lower, beyond high school), alcohol consumption (ever/never), family history of cancer in first-degree relatives (yes/no), state of residence (Iowa/North Carolina), and cigarette smoking history (never/low/high: the median value of pack-years [11.25] among smokers was used to classify low and high categories of smokers). In addition, we carried out the same Poisson analyses described above and included second primary incident cancers as case patients (i.e., both first and second primary cancer case patients were included) to increase the numbers of case patients. Variation ranged from one additional case patient with esophageal cancer and leukemia to 28 additional case patients with prostate cancer.

To ensure the use of the most appropriate reference group—either applicators never exposed to atrazine or applicators exposed to atrazine in the lowest exposure quartile—we carried out a comparison of baseline characteristics between different types of pesticide applicators: 1) applicators never exposed to atrazine, 2) applicators with atrazine exposure in the lowest quartile of lifetime days of exposure, and 3) applicators with atrazine exposure in the highest three quartiles of lifetime days of exposure. We postulated that applicators with baseline characteristics similar to those of the applicators in the highest exposure group would be most appropriate as a reference group for the Poisson regression analyses. Too much difference with respect to these baseline characteristics might introduce residual confounding from a variety of unidentified sources.

Potential confounding from exposure to other pesticides was controlled by adjusting exposure to 10 other pesticides (dicamba, cyanazine, alachlor, trifluralin, 2,4-D, chlorimuronethyl, metribuzine, butylate, phorate, and heptachlor). These pesticides were identified as the 10 most strongly correlated with atrazine out of 50 pesticides measured in the Agricultural Health Study, based on either strength of the correlation coefficient for intensity-weighted lifetime days of exposure (highest: $r = .78$; lowest: $r = .58$) or strength of association for ever/never comparison between atrazine and each of the 28 pesticides in the Agricultural Health Study for which there is ever/never data only. None of the pesticides we evaluated was negatively correlated with atrazine. In the final models, exposure levels of dicamba, cyanazine, alachlor, trifluralin, and 2,4-D were categorized as never, low, and high. The low and high group of each pesticide was classified by the median intensity-weighted exposure-days of each pesticide. For the pesticides chlorimuronethyl, metribuzine, butylate, phorate, and heptachlor, we had information only on ever/never use, so these five were categorized as such.

RESULTS

Selected characteristics of the atrazine exposed (lowest quartile and combined highest three quartiles) and nonexposed applicators in the Agricultural Health Study cohort are presented in Table 1. Among 53 943 subjects with complete exposure information, 36 513 (68%) reported ever having used atrazine, and they contributed a total of 237 045 person-years to the analysis. The cohort, both exposed and nonexposed, comprised primarily white, male, private applicators with relatively low smoking rates; in both the exposed and nonexposed groups, about half the subjects reported that they had never smoked. Exposed and nonexposed subjects were similar with respect to age, smoking history, alcohol consumption, educational level, and family history of cancer in a first-degree relative. The group consisting of the lowest exposed quartile is observed to be more similar to the group comprising the highest three quartiles than is the nonexposed group on a number of important variables. These include applicator status (i.e., private/commercial), state of residence, involvement in corn production, and use of the 10 pesticides most highly correlated with atrazine. Because of these similarities, we determined that the most appropriate reference group for the exposure–response analyses was applicators in the lowest quartile of atrazine exposure. However, to ensure that we did not overlook any potential associations and to verify our findings, we also carried out exposure–response analyses using the nonexposed applicators as the reference group (data not shown).

The Poisson regression rate ratios of selected cancers for which there were at least 20 atrazine-exposed case patients are presented in Table 2. For all cancers combined, there was no statistically significantly increased risk with increasing quartiles of lifetime days of exposure to atrazine or intensity-weighted lifetime days of exposure. Prostate cancer was the most frequent cancer in the cohort (n = 554); we did not detect any increased risk for prostate cancer with increasing atrazine exposure, whether assessed using lifetime days of exposure (highest quartile: RR = 0.88, 95% CI = 0.63 to 1.23; $P_{trend} = .26$) or intensity-weighted lifetime days of exposure (highest quartile: RR = 0.89, 95% CI = 0.63 to 1.25; $P_{trend} = .35$), even in subjects exposed for more than 178.5 days. We detected a statistically nonsignificant increased risk for lung cancer with increasing quartiles of lifetime days of exposure (highest quartile: $RR_{LD} = 1.91$, 95% CI = 0.93 to 3.94; $P_{trend} = .08$). The risk of lung cancer with intensity-weighted lifetime days of exposure, however, was less consistent across quartiles and diminished somewhat compared with that of lifetime days of exposure in the highest exposure quartile ($RR_{IWLD} = 1.37$, 95% CI = 0.65 to 2.86). Further analyses among never smokers, former smokers, and current smokers showed that the rate ratios of lung cancer were increased only in former smokers. However, we did not detect a statistically significant interaction between atrazine exposure and smoking history with respect to lung cancer. For bladder cancer, we also found no association between risk and exposure. A statistically nonsignificantly increased risk was observed with lifetime days of exposure (highest quartile: $RR_{LD} = 3.06$, 95% CI = 0.86 to 10.81; $P_{trend} = .18$) but not with intensity-weighted lifetime days of exposure (highest quartile: $RR_{IWLD} = 0.85$, 95% CI = 0.24 to 2.94). Elevated risks were suggested for NHL for both the analysis using lifetime days of exposure and the analysis using intensity-weighted lifetime days of exposure (highest quartile: $RR_{LD} = 1.61$, 95% CI = 0.62 to 4.16, $P_{trend} = .35$; highest quartile: $RR_{IWLD} = 1.75$, 95% CI = 0.73 to 4.20, $P_{trend} = .14$) and multiple myeloma (highest quartile: $RR_{LD} = 1.60$, 95% CI = 0.37 to 7.01, $P_{trend} = .41$; highest quartile: $RR_{IWLD} = 2.17$, 95% CI = 0.45 to 10.32, $P_{trend} = .21$). However, the numbers of applicators with NHL (n = 68) and multiple myeloma (n = 23) were small, RR estimates were not statistically significant, and there were no indications of a linear dose-response trend. We found no evidence of increased risks for cancers of the oral cavity, colon, rectum, pancreas, or kidney or for melanoma or leukemia.

Table 1. Selected characteristics of applicators by atrazine exposure in the Agricultural Health Study based on 1993–1997 enrollment data

Characteristics	Nonexposed group, No. (%) (n = 17 430)	Lowest exposed quartile, No. (%) (n = 9566)*	Highest three quartiles combined, No. (%) (n = 26 947)†
Age, y			
<40	6741 (38.7)	3012 (31.5)	7906 (29.3)
40–49	4064 (23.3)	2761 (28.9)	8191 (30.4)
50–59	3121 (17.9)	1933 (20.2)	6038 (22.4)
≥60	3504 (20.1)	1859 (19.4)	4811 (17.9)
Sex			
Male	16 272 (93.4)	9439 (98.7)	26 759 (99.3)
Female	1158 (6.6)	127 (1.3)	188 (0.7)
State of residence			
Iowa	8684 (49.8)	6787 (71.0)	19 875 (73.8)
North Carolina	8746 (50.2)	2779 (29.0)	7072 (26.2)
Applicator type‡			
Private	15 010 (86.1)	9208 (96.3)	24 952 (92.6)
Commercial	2420 (13.9)	358 (3.7)	1995 (7.4)
Smoking history			
Never	8671 (49.7)	5244 (54.8)	14 523 (53.9)
Low (<11.25 pack-years)	3941 (22.6)	2050 (21.4)	5815 (21.6)
High (≥11.25 pack-years)	4135 (23.7)	2005 (21.0)	5847 (21.7)
Missing	683 (4.0)	267 (2.8)	762 (2.8)
Alcohol consumption			
No	6267 (36.0)	2847 (29.8)	7415 (27.5)
Yes	10 113 (58.0)	6318 (66.0)	18 740 (69.5)
Missing	1050 (6.0)	401 (4.2)	792 (3.0)
Educational level			
High school/GED or lower	9722 (55.8)	5348 (55.9)	14 934 (55.4)
Beyond high school	7254 (41.6)	4037 (42.2)	11 505 (42.7)
Missing	454 (2.6)	181 (1.9)	508 (1.9)
Family history of cancer in first-degree relatives			
No	9812 (56.3)	5140 (53.7)	14 369 (53.3)
Yes	5521 (31.7)	3554 (37.2)	10 491 (38.9)
Missing	2097 (12.0)	872 (9.1)	2087 (7.8)
Corn production			
No	9764 (56.0)	2293 (24.0)	4879 (18.1)
Yes	7666 (44.0)	7273 (76.0)	22 068 (81.9)
Ever exposure to 10 pesticides most highly correlated with atrazine			
Dicamba	3713 (23.6)§	4940 (56.5)‖	16 246 (63.9)¶
Cyanazine	1636 (10.4)§	3686 (42)‖	15 258 (59.6)¶
Alachlor	3452 (22.1)§	4815 (54.7)‖	17 872 (69.5)¶
Trifluralin	4055 (26.1)§	5030 (57.0)‖	16 989 (66.2)¶
2,4-Dichlorophenoxyacetic acid	8954 (52.1)§	7586 (80.0)‖	23 160 (86.5)¶
Chlorimuronethyl	2538 (16.7)§	3010 (34.6)‖	13 144 (52.1)¶
Metribuzin	2540 (16.7)§	3901 (44.8)‖	15 528 (61.5)¶
Butylate	1328 (8.8)§	2295 (26.5)‖	11 896 (47.3)¶
Phorate	2091 (13.8)§	2714 (31.2)‖	10 783 (42.9)¶
Heptachlor	847 (5.6)§	1113 (12.9)‖	5201 (20.9)¶

*First quartile of lifetime days of exposure (years of use × days of use per year).

†Second, third, and fourth quartiles of lifetime days of exposure (years of use × days of use per year).

‡"Private applicators" refers primarily to individual farmers and "commercial" refers to professional pesticide applicators.

§Ever exposed to indicated chemical but not to atrazine (thus, numbers in columns do not sum to 100%).

‖Ever exposed to indicated chemical and in lowest quartile of atrazine exposure (thus, numbers in columns do not sum to 100%).

¶Ever exposed to indicated chemical and in the highest three quartiles of atrazine exposure (thus, numbers in columns do not sum to 100%).

Cancer risk patterns were similar when we used never exposed applicators as the reference group, making comparisons for each of the four quartiles of atrazine exposure (data not shown). For prostate cancer, there was no increased risk, whether we used lifetime days of exposure (Q1, RR_{LD} = 0.98; Q2, RR_{LD} = 0.87; Q3, RR_{LD} = 0.74; Q4, RR_{LD} = 0.83; P_{trend} = .09) or intensity-weighted lifetime days of exposure (Q1, RR_{IWLD} = 0.91; Q2, RR_{IWLD} = 0.94; Q3, RR_{IWLD} = 0.78; Q4, RR_{IWLD} = 0.79; P_{trend} = .11). For both lung and bladder cancers, there were statistically nonsignificantly elevated rate ratios only for the highest quartile of lifetime days of exposure; again, for intensity-weighted lifetime days of exposure, the effect was diminished in the highest quartile. For NHL, there was a steadily increasing, statistically nonsignificant linear trend for quartiles of both exposure metrics (highest quartile: RR_{LD} = 2.16, 95% CI = 0.84 to 5.59, P_{trend} = .06; highest quartile: RR_{IWLD} = 2.78, 95% CI = 1.16 to 6.68, P_{trend} = .02). For multiple myeloma, there was a similar pattern for both metrics (highest quartile: RR_{LD} = 4.75, 95% CI = 0.68 to 33.08, P_{trend} = .14; highest quartile: RR_{IWLD} = 4.71, 95% CI = 0.72 to 30.69, P_{trend} = .07). For all other cancers investigated, no associations were found when applicators never exposed to atrazine were used as the comparison group.

Table 2. Rate ratios (RRs) and 95% confidence intervals (CIs) from Poisson regressions for selected cancers* by lifetime days of exposure and intensity-weighted days of exposure to atrazine† among Agricultural Health Study cohort applicators

Cancer site	Exposure days (quartiles)‡	Exposure to atrazine					
		N§	RR_{LD} (95% CI)‖	P_{trend}¶	N§	RR_{IWLD} (95% CI)#	P_{trend}¶
All cancers		1361			1355		
	1–20	357	1.00 (referent)		340	1.00 (referent)	
	21–56	348	1.05 (0.87 to 1.27)		338	0.97 (0.80 to 1.18)	
	57–178.5	358	0.90 (0.74 to 1.11)		338	0.96 (0.79 to 1.18)	
	>178.5	298	1.01 (0.82 to 1.26)	.68	339	0.88 (0.71 to 1.11)	.28
Oral cavity		38			38		
	1–20	9	1.00 (referent)		12	1.00 (referent)	
	21–56	19	1.93 (0.71 to 5.25)		10	0.74 (0.25 to 2.16)	
	57–178.5	5	0.69 (0.20 to 2.38)		10	0.86 (0.30 to 2.47)	
	>178.5	5	0.50 (0.11 to 2.24)	.18	6	0.36 (0.10 to 1.38)	.20
Esophagus		20			20		
	1–20	4	1.00 (referent)		3	1.00 (referent)	
	21–56	8	1.69 (0.39 to 7.28)		8	5.57 (0.63 to 49.05)	
	57–178.5	7	0.77 (0.14 to 4.23)		6	3.03 (0.29 to 31.62)	
	>178.5	1	0.29 (0.03 to 3.35)	.27	3	2.70 (0.23 to 31.21)	.77
Colon		110			108		
	1–20	28	1.00 (referent)		30	1.00 (referent)	
	21–56	22	0.79 (0.40 to 1.57)		24	0.65 (0.33 to 1.28)	
	57–178.5	35	1.12 (0.59 to 2.10)		21	0.61 (0.30 to 1.25)	
	>178.5	25	0.88 (0.41 to 1.89)	.98	33	0.86 (0.43 to 1.73)	.64
Rectum		52			52		
	1–20	11	1.00 (referent)		12	1.00 (referent)	
	21–56	8	1.11 (0.41 to 3.00)		8	0.79 (0.29 to 2.16)	
	57–178.5	17	0.97 (0.35 to 2.69)		14	0.88 (0.32 to 2.42)	
	>178.5	16	1.38 (0.47 to 4.02)	.65	18	0.84 (0.29 to 2.44)	.79
Pancreas		21			21		
	1–20	4	1.00 (referent)		4	1.00 (referent)	
	21–56	9	1.26 (0.27 to 5.84)		9	0.96 (0.23 to 4.00)	
	57–178.5	5	1.14 (0.24 to 5.49)		5	0.57 (0.11 to 2.90)	
	>178.5	3	1.13 (0.19 to 6.61)	.97	3	0.56 (0.10 to 3.10)	.42
Lung		118			117		
	1–20	27	1.00 (referent)		27	1.00 (referent)	
	21–56	25	0.87 (0.40 to 1.87)		19	0.69 (0.30 to 1.57)	
	57–178.5	37	1.13 (0.56 to 2.29)		37	1.56 (0.78 to 3.14)	
	>178.5	29	1.91 (0.93 to 3.94)	.08	34	1.37 (0.65 to 2.86)	.19
Melanoma		52			52		
	1–20	12	1.00 (referent)		14	1.00 (referent)	
	21–56	12	1.06 (0.44 to 2.56)		10	0.57 (0.22 to 1.46)	
	5–178.5	13	1.18 (0.50 to 2.79)		17	1.31 (0.59 to 2.92)	
	>178.5	15	1.05 (0.39 to 2.84)	.84	11	0.41 (0.14 to 1.20)	.36
Prostate		554			552		
	1–20	160	1.00 (referent)		143	1.00 (referent)	
	21–56	135	0.89 (0.66 to 1.21)		143	1.03 (0.76 to 1.41)	
	57–178.5	143	0.75 (0.56 to 1.03)		132	0.86 (0.62 to 1.20)	
	>178.5	116	0.88 (0.63 to 1.23)	.26	134	0.89 (0.63 to 1.25)	.35
Bladder		47			47		
	1–20	10	1.00 (referent)		10	1.00 (referent)	
	21–56	12	2.25 (0.67 to 7.62)		14	1.21 (0.39 to 3.74)	
	57–178.5	9	1.04 (0.27 to 4.05)		12	1.01 (0.31 to 3.29)	
	>178.5	16	3.06 (0.86 to 10.81)	.18	11	0.85 (0.24 to 2.94)	.71
Kidney		40			40		
	1–20	12	1.00 (referent)		13	1.00 (referent)	
	21–56	8	0.78 (0.27 to 2.33)		8	0.10 (0.01 to 0.83)	
	57–178.5	11	0.33 (0.08 to 1.32)		8	0.50 (0.15 to 1.63)	
	>178.5	9	0.58 (0.15 to 2.25)	.22	11	0.43 (0.12 to 1.54)	.27
Non-Hodgkin lymphoma		68			68		
	1–20	17	1.00 (referent)		17	1.00 (referent)	
	21–56	19	1.56 (0.66 to 3.69)		16	0.88 (0.35 to 2.27)	
	57–178.5	17	1.59 (0.67 to 3.79)		15	1.36 (0.56 to 3.28)	
	>178.5	15	1.61 (0.62 to 4.16)	.35	20	1.75 (0.73 to 4.20)	.14
Multiple myeloma		23			23		
	1–20	7	1.00 (referent)		6	1.00 (referent)	
	21–56	4	0.57 (0.10 to 3.13)		2	0.71 (0.12 to 4.30)	
	57–178.5	5	1.19 (0.31 to 4.65)		7	1.85 (0.42 to 8.24)	
	>178.5	7	1.60 (0.37 to 7.01)	.41	8	2.17 (0.45 to 10.32)	.21

(Tables continues)

Table 2 (continued).

Cancer site	Exposure days (quartiles)‡	Exposure to atrazine					
		N§	RR_{LD} (95% CI)‖	P_{trend}¶	N§	RR_{IWLD} (95% CI)#	P_{trend}¶
Leukemia		41			40		
	1–20	9	1.00 (referent)		7	1.00 (referent)	
	21–56	12	1.04 (0.39 to 2.74)		16	1.64 (0.63 to 4.25)	
	57–178.5	10	0.61 (0.21 to 1.78)		6	0.41 (0.11 to 1.49)	
	>178.5	10	0.57 (0.17 to 1.91)	.22	11	0.56 (0.17 to 1.86)	.11

∗Cancers for which there were at least 20 exposed case patients or an *a priori* hypothesis about an association with atrazine. Rate ratio adjusted for age, sex, alcohol consumption, residence on a farm, smoking status, educational level, family history of cancer, state of residence, and use of 10 most highly correlated pesticides with atrazine.

†Total number exposed to atrazine = 36 513.

‡Quartiles for lifetime days of exposure. Units for intensity-weighted lifetime days of exposure are not displayed in this table because they do not have an intrinsic value.

§Number of cancer-specific case patients exposed to atrazine (total and for each quartile of exposure).

‖RR_{LD} = rate ratio of lifetime days of exposure (i.e., years of use × number of days of use per year).

¶*P* values were two-sided.

#RR_{IWLD} = rate ratio of intensity-weighted lifetime days of exposure (i.e., years of use × number of days of use per year × intensity index).

We carried out the same Poisson analyses described above and included second primary incident cancers as case patients (i.e., both first and second primary cancer case patients included; data not shown) to increase the numbers of case patients. Variation ranged from one additional case patient with esophageal cancer and leukemia to 28 additional case patients with prostate cancer. The results did not differ substantially from those presented in Table 2.

Discussion

We found no associations between cancer incidence and atrazine exposure, whether atrazine was analyzed as a cumulative measure (lifetime days of exposure) or as an intensity-weighted cumulative measure (intensity-weighted lifetime days of exposure). Although rate ratios for NHL, multiple myeloma, lung cancer, and bladder cancer increased with both lifetime days and intensity-weighted lifetime days of atrazine exposure, confidence intervals were wide, and tests for trend were not statistically significant. Similar results were seen whether we used applicators in the lowest exposed quartile or applicators never exposed to atrazine as the reference group.

A recent study of cancer incidence among triazine herbicide manufacturing workers in a plant in Louisiana found a statistically significant excess of prostate cancer for actively working company employees (excluding contract or inactive company employees), compared with the general population in that region (SIR = 394, 95% CI = 128 to 902) *(8)*. However, the high observed incidence of prostate cancer in the Louisiana plant workers may have been due to the frequent PSA testing of these employees, 98% of whom had at least one PSA test before the age of 45. Of the 11 cases, nine were diagnosed at an early clinical stage. In our study, there was considerable power to investigate risk of prostate cancer ($1\text{-}\beta = 0.89$ to detect a rate ratio of 1.3 in the highest quartile, assuming a trend over all quartiles) with atrazine exposure, and we found no increased risk, even for those who had applied atrazine for more than 178.5 days (the highest quartile of exposure) or had the highest intensity-weighted lifetime days of exposure.

Our data suggest no clear association between NHL and multiple myeloma incidence and atrazine exposure. However, we did see some evidence of such an association, and further follow-up is needed to determine whether such an association exists. The only other prospective study on cancer and atrazine is from a cohort of triazine herbicide manufacturing workers, in which there were increased standardized incidence ratios for all lymphatic and hematopoietic cancers (n = 7, 4.4 expected), NHL (n = 3, 2.3 expected), and multiple myeloma (n = 2, 0.4 expected) among a group of men with "definite" or "probable" exposure *(8)*. A mortality study based on the same population detected increased standardized mortality ratios for NHL (n = 4, 1.1 expected); however, the data did not have statistical power to show trends in rates by years worked and years since hire *(9)*. Increased risk of NHL in men was associated with atrazine use after adjustment for other commonly used pesticides in a pooled analysis of the NCI-sponsored case–control studies conducted in Nebraska, Kansas, and Iowa/Minnesota *(18)*. This study also found some evidence of a possible interaction between exposure to atrazine and other pesticides and the risk of NHL. The number of NHL cases in the Agricultural Health Study cohort is too small to provide the statistical power to attempt such an analysis at the present time. In an analysis of NHL by presence or absence of the t(14;18) chromosomal translocation, a statistically significant increased risk was associated with atrazine exposure for patients with the translocation, but not among those lacking it *(29)*, suggesting that further refinement of case definition in future studies may be worthwhile. A previous case–control study observed a weak association between atrazine exposure and multiple myeloma incidence (OR = 1.3, 95% CI not reported) *(13)*, whereas another study found no association (OR = 0.8, 95% CI = 0.4 to 1.6) between multiple myeloma and mixing, handling, or applying atrazine *(30)*.

Slight suggestions of increased risk were found for lung and bladder cancer in the highest quartile of lifetime days of exposure to atrazine. However, the rate ratios in the intensity-weighted lifetime days of exposure analyses were weak for lung cancer and essentially null for bladder cancer. We also found similar patterns using the never exposed applicators as a reference group. Because the respiratory system may be an important route of exposure for lung cancer, use of the intensity algorithm, which weighs dermal exposure more heavily, may have increased measurement error. We further investigated the relationship between lung cancer and atrazine by stratifying the popu-

lation into never smokers, former smokers, and current smokers. That the rate ratios were highest among former smokers and null among current smokers suggests that our findings of a slight increase in risk may not be attributable solely to smoking. To our knowledge, there are no *a priori* hypotheses for an association between atrazine exposure and lung cancer. However, atrazine was found in lung tissue at autopsy of a suicide victim poisoned by ingestion of an herbicide mix containing atrazine *(31).* The lung was one of the organs that showed the highest concentrations of atrazine. The inconsistencies between the two analyses for bladder cancer leave us doubtful. We will continue to follow up both cancers with respect to atrazine exposure.

The toxicologic activity of atrazine in humans is unclear. Toxicity studies have examined various endpoints from atrazine exposure, including carcinogenicity, genotoxicity, endocrine disruption, and immunotoxicity. The majority of animal studies indicate that atrazine has low genotoxicity, but there has been no study of genotoxicity in humans. In male and female rats, atrazine disrupts hypothalamic stimulation of pituitary function, resulting in attenuation of luteinizing hormone levels *(24).* This mechanism results in increased rates of mammary tumors in some strains of female rats. In male rats, atrazine causes decreased production of testosterone by Ledig cells *(32)* and reduced seminal vesicle and prostate weights *(32).* However, the potential for endocrine disruption in humans from atrazine exposure and its implications for carcinogenesis are not known. Several studies have observed immunotoxicity of atrazine in animals *in vivo* and in human and animal cells *in vitro;* however, the evidence to date has not established the immune system as a target for atrazine toxicity. Two studies in rodents showed that atrazine exposure decreased levels of circulating lymphocytes, although several other immune parameters were unchanged *(33,34).* Several recent studies have observed impaired immune function associated with administration of atrazine to cells *in vitro,* including impaired cytokine production (interferon γ, interleukin 5, and tumor necrosis factor-α) by human peripheral blood mononuclear cells *(35)* and decreased ability of human natural killer cells to lyse tumor cells *(36).* Immunotoxicity may be particularly relevant for lymphohematopoietic cancers.

The Agricultural Health Study has several important strengths. It is the largest study to date of pesticide applicators exposed to atrazine. Exposure information was gathered prior to cancer diagnosis, thereby minimizing recall bias. In general, farmers provide reliable information and considerable detail regarding their pesticide application history *(37–40).* The Agricultural Health Study cohort consists of licensed pesticide applicators who are responsible for thoroughly understanding pesticide regulations and for purchasing and applying chemicals on their farms *(41).* Recall of pesticide use by the Agricultural Health Study cohort has been shown to be consistent with the dates these pesticides came on the market *(41).* To our knowledge, this is the first human study of atrazine to use a semiquantitative method to assess exposure; comprehensive questionnaire data were used to quantify atrazine exposure levels, providing greater discrimination between high and low exposures than previous studies that broadly defined exposure as "ever used" atrazine. In addition, detailed information on the use of many common pesticides and lifestyle characteristics allowed us to adjust for potential confounding factors.

Certain limitations of our data reduce the number and kinds of inferences we can make regarding atrazine and its association with specific cancers. Although the Agricultural Health Study cohort is large and many participants reported atrazine use, the small number of selected cancers occurring during the 6.5-year average follow-up period prevented estimation of precise effects. In addition, most atrazine applicators were male (99%), precluding our ability to assess the association between atrazine exposure and female cancers, including ovarian and breast cancers, which have been associated with exposure to triazine herbicides *(19,22).* Our analysis provides limited information on the timing of pesticide use in relation to disease. Additionally, with only 6.5 years of follow-up, our ability to make conclusions concerning latency and secular changes in personal protective equipment is limited. We will be able to better address these issues with a longer follow-up period and more exposure data from subsequent phases of the study. Finally, there are hypotheses concerning gestation and early childhood as periods sensitive to endocrine disruptors *(42),* and because our study focused on adult exposures, we cannot address the risk associated with exposures in early life. Although our study used more detailed exposure estimates than did earlier studies, estimates for lifetime days of exposure and intensity-weighted lifetime days of exposure, as well as measures of confounding, include error that could bias our results toward the null. For example, there is some variation we could not account for with respect to the categorical attainment of days exposed in each year. Another source of variation is the number of hours worked in a day of pesticide application. Later phases of the Agricultural Health Study will address these exposure variables and will provide a more precise estimate of exposure.

Despite the limitations noted above, our prospective study of cancer incidence among atrazine exposed pesticide applicators provided an opportunity afforded in few other studies to evaluate cancer risks associated with exposure to atrazine, while adjusting for other common pesticide exposures and lifestyle factors. No increased risk of prostate cancer was observed among 554 atrazine exposed cases with increasing exposure to atrazine, even among those with more than 178.5 days of lifetime use. Statistical power was limited for some cancers, but certain intriguing suggestions of association were observed for non-Hodgkin lymphoma, multiple myeloma, lung cancer, and bladder cancer, which we intend to monitor and further investigate as more cases develop in this cohort.

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NOTE

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