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Original Contribution

Malathion Exposure and the Incidence of Cancer in the Agricultural Health Study

Matthew R. Bonner[1], Joseph Coble[1], Aaron Blair[1], Laura E. Beane Freeman[1], Jane A. Hoppin[2], Dale P. Sandler[2], and Michael C. R. Alavanja[1]

[1] Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, US Department of Health and Human Services, Bethesda, MD.
[2] Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, US Department of Health and Human Services, Research Triangle Park, NC.

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Malathion is the most common organophosphate insecticide applied in the United States, and while some studies suggest that it may be clastogenic, its carcinogenicity has not been demonstrated in rodents. However, malathion has been associated with non-Hodgkin's lymphoma in several epidemiologic studies. The authors investigated associations between malathion exposure and cancer among 19,717 pesticide applicators enrolled in the Agricultural Health Study between 1993 and 1997. Information on lifetime years and days per year of use and intensity of malathion exposure was obtained with self-administered questionnaires prior to the onset of any cancer. The average follow-up time was 7.5 years (1993–2002). Rate ratios and 95% confidence intervals were calculated using Poisson regression, adjusting for potential confounders. Overall, lifetime days of malathion use (top tertile of exposure, >39 days) was not associated with all cancers combined (rate ratio = 0.97, 95% confidence interval: 0.81, 1.15). The risk of non-Hodgkin's lymphoma was not associated with malathion use, although the number of cases was small. The risk of melanoma with more than 39 lifetime exposure-days was 0.39 (95% confidence interval: 0.14, 1.03). In summary, malathion exposure was not clearly associated with cancer at any of the sites examined. Although the rate ratios for melanoma were reduced, small numbers and lack of experimental evidence suggest that the observed reductions may have arisen by chance.

malathion; neoplasms; pesticides

Abbreviations: AHS, Agricultural Health Study; CI, confidence interval; NHL, non-Hodgkin's lymphoma; RR, rate ratio.

First introduced in 1950, malathion (diethyl(dimethoxythiophosphorylthio)succinate) is the most commonly applied organophosphate insecticide in the United States (1). In agricultural settings, malathion is applied to numerous crops, including wheat and corn (2). It is also used for home and garden applications, mosquito control, and Mediterranean fruit fly eradication and as a topical treatment for head lice (2, 3). The widespread use of this chemical makes it especially important to identify any related health risks.

Organophosphate insecticides, including malathion, irreversibly inhibit acetylcholinesterase, leading to the accumulation of acetylcholine and acute neurotoxicity at high doses. However, how organophosphate insecticides may affect the risk of cancer is currently unknown. Despite the absence of an established carcinogenic mechanism for organophosphate insecticides, several mechanisms have been proposed, including the induction of cellular proliferation (4), oxidative stress (5–7), and immunotoxicity (8).

In the 1980s, the International Agency for Research on Cancer conducted comprehensive evaluations of the literature to assess the potential human carcinogenicity of malathion, concluding that there was ''limited evidence for the

Correspondence to Dr. Michael C. R. Alavanja, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 2160 Executive Blvd., Bethesda, MD 20892-7240 (e-mail: alavanjm@exchange.nih.gov).

mutagenicity of malathion'' (9, 10). Malathion or its metabolite malaoxon was not mutagenic in several *Salmonella* strains (11–13), nor did it induce sex-linked recessive lethal mutations in *Drosophila melanogaster* (14). The findings of both in vitro and in vivo studies of cytogenetic changes in humans and other mammals have been inconsistent (15–33). In contrast, the US Environmental Protection Agency concluded in 2000 that there was ''suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential'' (34).

Malathion exposure was associated with non-Hodgkin's lymphoma (NHL) in two case-control studies (35, 36). However, in a pooled analysis of results from three case-control studies (35, 37, 38), De Roos et al. (39) found no association with NHL (odds ratio = 1.1, 95 percent confidence interval (CI): 0.6, 1.8). More recently, subcutaneous injections of malathion have been shown to increase the risk of mammary tumors in Sprague-Dawley rats (4). The authors hypothesized that malathion, by inhibiting acetylcholinesterase, increases cholinergic stimulation, resulting in mammary epithelial cell proliferation. However, malathion use was not clearly associated with breast cancer either in Hispanic agricultural workers (40) or in a previous report from the Agricultural Health Study (AHS) (41). Additional investigations carried out within the AHS have not demonstrated associations with occupational malathion exposure and cancers of the prostate (42) or lung (43). However, other analyses of specific organophosphate insecticides (i.e., chlorpyrifos (44) and diazinon (45)) have found increased risk of lung cancer in the AHS cohort.

The inconsistent epidemiologic and experimental data, as well as the ambiguous classification of malathion by the Environmental Protection Agency, emphasize the need for comprehensive epidemiologic investigations with more detailed information on malathion exposure. Data from the AHS provided us with the opportunity to investigate cancer risk among pesticide applicators who reported use of malathion.

MATERIALS AND METHODS

A detailed description of the AHS has been previously published (46). Briefly, the AHS is an ongoing prospective cohort study of 57,311 licensed restricted-use pesticide applicators and 32,347 applicator spouses from Iowa and North Carolina. Licensed pesticide applicators include farmers (private applicators) and commercial applicators employed by pest control companies or businesses that use pesticides on their premises. Recruitment was conducted between December 13, 1993, and December 31, 1997. Vital status was ascertained from the National Death Index and state death registries. Incident cancers were identified through state tumor registries and coded using the *International Classification of Diseases for Oncology*, Second Edition (47). Cases included incident primary cancers diagnosed between December 13, 1993, and December 31, 2002 (AHS data release, version 0412.01). The average follow-up time was 7.5 years. Participants (n = 1,113) who moved out of Iowa or North Carolina were censored at the year in which they moved. The current analysis consisted of 25,291 (44 percent) applicators with complete information on malathion application. Among these applicators, those with prevalent cancer cases (n = 620), those failing to provide information about malathion use (n = 1,137), and those without data on key potential confounders (n = 3,817) were excluded, leaving 19,717 cohort members for this analysis. The protocol was approved by all appropriate institutional review boards, and all participants provided informed consent.

Exposure assessment

The enrollment questionnaire obtained detailed information on the application of 22 different pesticides (http://www.aghealth.org/questionnaires.html). For each pesticide, applicators reported the number of years and the number of days per year that they had personally mixed or applied that particular pesticide. Information on 28 other pesticides, including malathion, was limited to ever use on the enrollment questionnaire. In addition, the enrollment questionnaire gathered information on application methods and the use of personal protective equipment. Data on smoking history, alcohol drinking in the past 12 months, fruit and vegetable consumption, other agricultural activities, and nonfarm occupational exposures were additionally ascertained at enrollment. Participants were also asked to complete a take-home questionnaire that included the same detailed questions about pesticide usage as those on the enrollment questionnaire for malathion and the 27 other pesticides.

Lifetime days of exposure to malathion (lifetime exposure-days) was calculated as the product of the number of years of malathion use and the number of days per year on which malathion was used. In addition, we estimated an exposure intensity score based on an algorithm developed by Dosemeci et al. (48). The exposure intensity score weights aspects of pesticide use that may modify the intensity of exposure, including whether an applicator personally mixed or prepared the pesticides for application, what types of application methods were used, the repair of pesticide application equipment, and the use of personal protective equipment during these activities. The intensity-weighted number of lifetime exposure-days was calculated by multiplying the exposure intensity score by lifetime exposure-days.

Statistical analysis

Rate ratios and 95 percent confidence intervals were calculated with Poisson regression. Nine cancer sites had a sufficient number of cases for statistical analyses (i.e., >5 cases per category of exposure), including lymphatic-hematopoietic cancers combined (i.e., multiple myeloma, leukemia, Hodgkin's lymphoma, and NHL); leukemia; NHL; cancers of the lung, prostate, colon and rectum, kidney, and bladder; and melanoma. Categories for malathion lifetime exposure-days and intensity-weighted lifetime exposure-days were based on tertiles of the exposure distribution for all of the exposed cancer cases combined. The distribution among all cases was used to ensure sufficient numbers of exposed

TABLE 1. Selected characteristics of pesticide applicators according to malathion exposure in the Agricultural Health Study, 1993–1997

Characteristic	Nonexposed ($n = 7,427$)		Low-exposed* ($n = 5,004$)		High-exposed† ($n = 7,286$)	
	No.	%	No.	%	No.	%
Age (years)						
<40	2,597	35.0	1,487	29.7	1,711	23.5
40–49	1,911	25.7	1,500	30.0	2,181	29.9
50–59	1,509	20.3	1,029	20.6	1,756	24.1
≥60	1,410	19.0	988	19.7	1,638	22.5
Gender						
Male	7,162	96.4	4,893	97.8	7,145	98.1
Female	265	3.6	111	2.2	141	1.9
State						
Iowa	5,278	71.1	3,943	78.8	5,235	71.9
North Carolina	2,149	28.9	1,061	21.2	2,051	28.1
Type of applicator						
Private	6,510	87.7	4,593	91.8	6,567	90.1
Commercial	917	12.3	411	8.2	719	9.9
Cigarette smoking						
Never smoker	4,277	57.6	2,869	57.3	3,857	52.9
Former smoker	2,009	27.1	1,524	30.5	2,420	33.2
Current smoker	1,141	15.4	611	12.2	1,009	13.9
Alcohol drinking‡						
Yes	4,889	65.8	3,535	70.6	5,124	70.3
Education						
High school or less	4,545	61.2	2,514	50.2	3,612	49.6
More than high school	2,882	38.8	2,490	49.8	3,674	50.4
Family history of cancer						
Yes	2,784	37.5	2,202	44.0	3,470	47.6
Alfalfa production						
Yes	1,608	21.7	1,254	25.1	1,671	22.9
Cotton production						
Yes	263	3.5	118	2.4	266	3.7
Other pesticides used (yes)						
Carbaryl	1,739	23.4	2,180	43.6	4,087	56.1
Parathion	132	1.8	316	6.3	912	12.5
Diazinon	709	9.6	1,003	20.0	2,359	32.4
Chlordane	455	6.1	928	18.6	2,075	28.5
Lindane	313	4.2	631	12.6	1,562	21.4
	Mean value					
Person-years of follow-up	7.5 (1.5)§		7.5 (1.5)		7.5 (1.5)	
No. of other pesticides used	10.3 (6.6)		14.7 (6.5)		17.5 (7.3)	
Pack-years of smoking						
Former smoker	14.9 (19.0)		14.7 (18.8)		16.7 (21.0)	
Current smoker	22.3 (20.1)		24.3 (21.4)		25.9 (20.7)	

* First tertile of exposure among exposed cases.
† Second and third tertiles of exposure among exposed cases.
‡ Reported alcohol consumption within the last 12 months.
§ Numbers in parentheses, standard deviation.

TABLE 2. Rate ratios for selected cancers by lifetime exposure-days of exposure to malathion among pesticide applicators in the Agricultural Health Study, 1993–2002

Lifetime exposure-days*	No. of cases	Nonexposed referent group		Low-exposed† referent group	
		RR‡	95% CI‡	RR	95% CI
All cancers					
0	349	1.0	Referent		
>0–9	258	1.05	0.89, 1.23	1.0	Referent
10–39	190	0.97	0.81, 1.16	0.93	0.77, 1.12
>39	203	0.97	0.81, 1.15	0.92	0.77, 1.11
p for trend			0.57		0.51
Lymphatic-hematopoietic cancers					
0	34	1.0	Referent		
>0–9	21	0.87	0.50, 1.50	1.0	Referent
10–39	17	0.90	0.50, 1.61	1.03	0.54, 1.95
>39	24	1.27	0.75, 2.16	1.42	0.79, 2.57
p for trend			0.23		0.20
Leukemia					
0	11	1.0	Referent		
>0–9	7	0.80	0.31, 2.08	1.0	Referent
10–39	5	0.74	0.26, 2.15	0.91	0.29, 2.86
>39	11	1.65	0.71, 3.86	2.07	0.80, 5.40
p for trend			0.11		0.07
Non-Hodgkin's lymphoma§					
0	14	1.0	Referent		
>0–9	7	0.62	0.24, 1.56	1.0	Referent
10–39	7	0.69	0.27, 1.78	1.09	0.38, 3.13
>39	9	0.81	0.33, 2.01	1.07	0.38, 3.04
p for trend			0.96		0.93
Lung cancer					
0	31	1.0	Referent		
>0–9	16	0.75	0.41, 1.38	1.0	Referent
10–39	18	1.08	0.60, 1.94	1.46	0.74, 2.86
>39	22	0.94	0.53, 1.65	1.20	0.62, 2.32
p for trend			0.98		0.88

Table continues

cases in each exposure category. Models were adjusted for factors frequently hypothesized to confound epidemiologic studies of cancer and pesticides, including age at enrollment (<40, 40–49, 50–59, or ≥60 years), gender, education (high school graduate or less vs. more than high school), cigarette smoking (never smoking, <12 pack-years, or ≥12 pack-years), alcohol consumption during the past 12 months (yes/no), history of cancer in a first-degree relative (yes/no), year of enrollment, state of residence (Iowa/North Carolina), and use of the five pesticides most highly correlated with malathion lifetime exposure-days (carbaryl ($r = 0.47$), parathion ($r = 0.44$), diazinon ($r = 0.43$), chlordane ($r = 0.39$), and lindane ($r = 0.39$)). None of the 49 other pesticides was negatively correlated with malathion. For each of these five pesticides, categorical variables were generated with three exposure levels: 1) no exposure, 2) exposure less than or equal to the median, and 3) exposure greater than the median. Most of the observed risk estimates were not influenced by further adjustment for use of these five pesticides. However, because carbaryl and parathion were associated with melanoma and lindane was associated with NHL, results for these two cancers include adjustment for use of these pesticides. All other results are presented adjusted only for the above-listed potential confounders.

Two reference groups were used for the analyses: 1) pesticide applicators who reported never using malathion and 2) pesticide applicators whose use of malathion was in the lowest tertile of exposure, hereafter called the ''low-exposed'' group. The low-exposed group was used because of concerns that applicators who reported using malathion might differ systematically from those who did not report using malathion with regard to unmeasured factors. Thus,

TABLE 2. Continued

Lifetime exposure-days*	No. of cases	Nonexposed referent group		Low-exposed† referent group	
		RR	95% CI	RR	95% CI
Prostate cancer¶					
0	135	1.0	Referent		
>0–9	116	1.20	0.93, 1.54	1.0	Referent
10–39	76	0.98	0.74, 1.30	0.81	0.61, 1.09
>39	86	1.04	0.79, 1.37	0.88	0.66, 1.16
p for trend			0.86		0.59
Colorectal cancer					
0	40	1.0	Referent		
>0–9	29	1.06	0.65, 1.71	1.0	Referent
10–39	20	0.92	0.54, 1.59	0.88	0.50, 1.56
>39	18	0.84	0.48, 1.48	0.84	0.46, 1.51
p for trend			0.48		0.61
Kidney cancer					
0	8	1.0	Referent		
>0–9	6	0.92	0.31, 2.66	1.0	Referent
10–39	7	1.31	0.47, 3.65	1.43	0.48, 4.28
>39	6	0.98	0.34, 2.89	1.12	0.36, 3.51
p for trend			0.98		0.98
Bladder cancer					
0	15	1.0	Referent		
>0–9	9	0.81	0.35, 1.87	1.0	Referent
10–39	10	1.14	0.51, 2.55	1.40	0.57, 3.44
>39	7	0.71	0.29, 1.77	0.83	0.30, 2.24
p for trend			0.51		0.51
Melanoma#					
0	14	1.0	Referent		
>0–9	15	1.16	0.54, 2.49	1.0	Referent
10–39	9	0.79	0.32, 1.91	0.66	0.28, 1.53
>39	7	0.48	0.17, 1.30	0.39	0.14, 1.03
p for trend			0.09		0.08

* Lifetime exposure-days = years of use × days of use per year.
† First tertile of exposure among exposed cases.
‡ RR, rate ratio; CI, confidence interval.
§ Further adjusted for lindane use.
¶ Person-time was restricted to males for the prostate model. Rate ratios were adjusted for age, gender, smoking, alcohol, education, family history of cancer, year of enrollment, and state of residence.
# Further adjusted for carbaryl and parathion use.

the low-exposed provided a referent group that circumvented the potential for such differences to confound associations. In addition to using lifetime exposure-days and intensity-weighted lifetime exposure-days, we calculated rate ratios based on frequency (days of use per year), duration (years of use), and intensity (intensity score) of malathion exposure. Frequency and duration were assessed in the questionnaire with an ordered categorical scale rather than a continuous scale, thereby limiting our ability to finely categorize these exposure metrics. Therefore, cutpoints for these metrics were derived to ensure that there were at least five cases in each category. In contrast, intensity of exposure was assessed on a continuous scale and was categorized into tertiles based on the distribution among all cancer cases combined.

Analyses were also stratified by state of residence (Iowa or North Carolina) and applicator type (private or commercial). Linear trends were assessed using the *p* value of the coefficient of the exposure in which the median value for each category was treated as a continuous variable while

adjusting for covariates (49). A sensitivity analysis, as described by Greenland (50), was conducted to assess the potential for selection bias among persons completing the take-home questionnaire. Briefly, the proportions or selection probabilities for the exposed cases and nonexposed cases who completed the take-home questionnaire were calculated. Similarly, the proportions of exposed and nonexposed person-time for those who completed the take-home questionnaire were also calculated. These selection probabilities were used to algebraically calculate the selection bias factor. To estimate a rate ratio corrected for the selection bias, we divided the rate ratio by this selection bias factor.

RESULTS

Participants were predominantly male private applicators from Iowa (table 1). Malathion-exposed applicators reported consuming slightly more alcohol at enrollment than did nonexposed applicators. In addition, exposed applicators were more likely to have more than a high school education, were slightly older, and more often reported having a family history of cancer in comparison with nonexposed applicators. Malathion-exposed applicators also smoked more cigarettes, on average, and applied more types of pesticides than the nonexposed applicators.

The data distributions for both malathion lifetime exposure-days and intensity-weighted lifetime exposure-days were highly skewed to the right. The median values were 20 (range, 2.5–5,000) and 11.6 (range, 0.85–43,400), respectively. We used logarithmic transformation to normalize the distributions of these two metrics.

Among persons completing the take-home questionnaire, having ever applied malathion was not associated with all cancers combined (rate ratio (RR) = 0.99, 95 percent CI: 0.88, 1.13) in comparison with the never users; results were similar among all applicators enrolled in the AHS (RR = 0.99, 95 percent CI: 0.89, 1.09). Lifetime exposure-days of malathion use was not associated with all cancers combined when either the nonexposed (p for trend = 0.57) or the low-exposed (p for trend = 0.51) were used as the referent group (table 2). None of the individual cancer sites had statistically significant rate ratios, although the rate ratios were less than 1.0 for the relation of colorectal cancer, melanoma, and bladder cancer with lifetime exposure-days of malathion use. In addition, the risk of leukemia was elevated with more than 39 lifetime days of exposure when either the nonexposed or the low-exposed were used as the referent group, although the numbers of leukemia cases were low in the intermediate exposure categories.

The rate ratios for colorectal cancer were inversely associated with lifetime exposure-days regardless of the reference group used (nonexposed or low-exposed), with a 16 percent lower risk being observed among persons in the highest tertile of exposure (>39 lifetime exposure-days). Further adjustment for body mass index, fruit and vegetable intake at enrollment, and the number of hours per week during the summer and winter that cohort members reported engaging in strenuous exercise in their leisure time did not appreciably influence the rate ratios (data not shown).

Malathion use was inversely associated with melanoma risk among persons in the highest tertile of exposure (>39 lifetime exposure-days) for both referent groups. The rate ratios for melanoma in the highest tertile were 0.48 (95 percent CI: 0.17, 1.30) and 0.39 (95 percent CI: 0.14, 1.03) with use of the nonexposed and low-exposed referent groups, respectively. However, the p values for trend were not significant, and the exposure-response gradient was not monotonic when the nonexposed were used as the referent group. Further adjustment for hours spent in the sun, eye color, hair color, and skin reactivity to sun exposure did not alter the risk estimates (data not shown).

The rate ratios for bladder cancer were less than 1.0 in the highest exposure category when either the nonexposed or the low-exposed were used as the referent group. The inverse association was not monotonic, however. The rate ratio in the middle tertile was near unity (RR = 1.14, 95 percent CI: 0.51, 2.55) when the nonexposed were used as the referent group and was elevated (RR = 1.40, 95 percent CI: 0.57, 3.44) when the low-exposed were used as the referent group.

Rate ratios for colorectal cancer, melanoma, and bladder cancer were examined separately according to the frequency, intensity, and duration of malathion exposure (table 3). Greater than 5 days of malathion use per year was associated with a nonsignificant decrease for melanoma (RR = 0.66, 95 percent CI: 0.27, 1.62) and bladder cancer (RR = 0.65, 95 percent CI: 0.26, 1.62), but no association was observed for colorectal cancer. The p values for trend were not significant for any of these sites. The rate ratios were also less than 1.0 in the highest tertile of malathion exposure intensity for both colorectal cancer (RR = 0.87, 95 percent CI: 0.51, 1.47) and melanoma (RR = 0.61, 95 percent CI: 0.25, 1.49), though the p values for trend were not significant. The rate ratios were also less than 1.0 for bladder cancer; however, the exposure-response gradient was not monotonic. The number of years of malathion use was not associated with cancer at any of these sites.

Rate ratios for cancers of the lung and for melanoma were 1 or less in the highest tertile of intensity-weighted lifetime exposure-days, but not for any of the other cancer sites (table 4), although a slight excess risk was observed for leukemia. When the nonexposed were used as the referent group, the pattern of melanoma rate ratios for intensity-weighted lifetime exposure-days was similar to the pattern for lifetime exposure-days. The risk of melanoma was slightly increased among persons in the first tertile of exposure (RR = 1.44, 95 percent CI: 0.67, 3.10) but declined in the two subsequent tertiles. The rate ratios for bladder cancer were *not* decreased with exposure, regardless of the referent group used. The rate ratio for colorectal cancer was less than 1.0 in the middle tertile, but the exposure-response gradient was not monotonic and the rate ratio shifted closer to unity in the top tertile.

In analyses stratified by state of residence, the melanoma rate ratios were reduced in the higher exposure categories for residents of both states, although confidence intervals were wide and included the null value because of small numbers (data not shown). There were too few commercial

TABLE 3. Rate ratios for colorectal cancer, melanoma, and bladder cancer, by frequency, duration, and intensity of malathion exposure, Agricultural Health Study, 1993–2002

Malathion exposure	Colorectal cancer			Melanoma*			Bladder cancer		
	No. of cases	RR†	95% CI†	No. of cases	RR	95% CI	No. of cases	RR	95% CI
Frequency (days of use per year)									
0	40	1.0	Referent	14	1.0	Referent	15	1.0	Referent
<5	45	0.96	0.62, 1.47	21	0.94	0.46, 1.94	19	1.00	0.51, 1.99
≥5	22	0.94	0.55, 1.59	10	0.66	0.27, 1.62	7	0.65	0.26, 1.62
p for trend‡		0.84			0.32			0.32	
Intensity									
0	40	1.0	Referent	14	1.0	Referent	15	1.0	Referent
Tertile 1	26	1.13	0.68, 1.85	14	1.27	0.57, 2.81	8	0.88	0.37, 2.09
Tertile 2	18	0.83	0.47, 1.46	8	0.76	0.30, 1.90	8	0.95	0.40, 2.26
Tertile 3	22	0.87	0.51, 1.47	9	0.61	0.25, 1.49	10	0.85	0.38, 1.91
p for trend‡		0.40			0.13			0.75	
Duration (years of use)									
0	40	1.0	Referent	14	1.0	Referent	15	1.0	Referent
≤10	43	0.83	0.54, 1.29	22	0.84	0.41, 1.72	19	0.90	0.45, 1.77
>10	24	1.27	0.76, 2.12	9	0.86	0.34, 2.18	7	0.83	0.34, 2.06
p for trend‡		0.25			0.85			0.71	

* Further adjusted for carbaryl and parathion use.
† RR, rate ratio; CI, confidence interval.
‡ Adjusted for age, gender, smoking, alcohol, education, family history of cancer, year of enrollment, and state of residence.

applicators with colorectal cancer or melanoma to assess consistency by applicator type (i.e., private vs. commercial).

DISCUSSION

In this analysis of data from the AHS, occupational exposure to malathion did not appear to be associated with increased risk for any of the cancers examined. Although risk of leukemia was elevated for applicators with more than 39 lifetime exposure-days as compared with those with less than 9 days, further division at the median of the third tertile showed that leukemia risk did not increase monotonically with exposure. Moreover, the rate ratios were attenuated with the intensity-weighted lifetime exposure-days metric. The exposure-response gradients for leukemia were markedly different depending on the reference group (nonexposed or low-exposed). In the analysis using the reference group comprising nonexposed applicators, a J-shaped exposure-response curve was noticeable with both lifetime exposure-days and intensity-weighted lifetime exposure-days. In contrast, when the reference group comprised low-exposed applicators, rate ratios were elevated only in the highest tertile of exposure. The small number of malathion-exposed leukemia cases ($n = 23$), especially in the intermediate exposure categories, and the apparent attenuation of the point estimates with the use of the intensity-weighted exposure metric together limit the interpretation that malathion is associated with leukemia risk.

Suggestive inverse associations were observed for melanoma. The inverse association with malathion use was relatively consistent across exposure metrics and referent groups, although the inverse association was statistically significant only for intensity-weighted lifetime exposure-days, using the low-exposed referent group. None of the *p* values for trend were significant in these analyses. An examination of the exposure metric components—frequency, intensity, and duration—showed attenuated nonsignificant inverse associations.

The evaluation of bladder cancer risk showed relatively inconsistent inverse associations across exposure metrics and referent groups, with considerable variability surrounding all of these risk estimates. In addition, there was little evidence of exposure-response gradients with any of these metrics for bladder cancer.

The healthy worker effect may be one possible explanation for the inverse associations observed for melanoma, colorectal cancer, and bladder cancer. The healthy worker effect is a combination of three processes: 1) a healthy hire effect, 2) a healthy survivor effect, and 3) a time-since-hire decline in health (51). The use of internal comparisons in this occupational cohort study should have mitigated any potential bias due to a healthy hire effect. The healthy survivor and time-since-hire effects, in contrast, are not remedied with internal comparisons (52). However, if a healthy worker survivor effect or time-since-hire effect were operating, years of use should have been inversely associated with risk, and none of these sites—melanoma, colorectal

TABLE 4. Rate ratios for selected cancers, by intensity-weighted lifetime exposure-days of exposure to malathion among pesticide applicators in the Agricultural Health Study, 1993–2002

Intensity-weighted lifetime exposure-days*	No. of cases	Nonexposed referent group		Low-exposed† referent group	
		RR‡	95% CI‡	RR	95% CI
All cancers					
0	349	1.0	Referent		
>0–58	218	1.10	0.92, 1.30	1.0	Referent
59–245	218	0.93	0.78, 1.10	0.85	0.70, 1.02
>245	207	0.98	0.82, 1.16	0.89	0.73, 1.08
p for trend			0.60		0.59
Lymphatic-hematopoietic cancers					
0	34	1.0	Referent		
>0–58	16	0.81	0.45, 1.48	1.0	Referent
59–245	22	0.98	0.57, 1.69	1.20	0.63, 2.28
>245	24	1.25	0.73, 2.12	1.49	0.78, 2.84
p for trend			0.25		0.23
Leukemia					
0	11	1.0	Referent		
>0–58	6	0.84	0.31, 2.29	1.0	Referent
59–245	7	0.88	0.34, 2.30	1.05	0.35, 3.13
>245	10	1.45	0.61, 3.48	1.77	0.64, 4.95
p for trend			0.25		0.20
Non-Hodgkin's lymphoma§					
0	14	1.0	Referent		
>0–58	5	0.53	0.19, 1.51	1.0	Referent
59–245	9	0.74	0.31, 1.79	1.30	0.43, 3.90
>245	9	0.83	0.34, 2.04	1.29	0.41, 4.00
p for trend			0.92		0.80
Lung cancer					
0	31	1.0	Referent		
>0–58	14	0.89	0.47, 1.68	1.0	Referent
59–245	21	1.00	0.57, 1.75	1.11	0.56, 2.20
>245	18	0.78	0.43, 1.41	0.83	0.41, 1.70
p for trend			0.42		0.43

Table continues

cancer, or bladder cancer—were inversely associated with years of malathion use.

While two previous case-control studies (35, 36) observed an increased risk of NHL with malathion use, we did not. Recall bias in these case-control studies may be one possible explanation for the discordance with our results. In addition, differences between exposure assessment and the methods used to categorize exposure between these case-control studies and the AHS make it difficult to directly compare results. For instance, Cantor et al. (35) presented results based on ever use of malathion and did not quantify malathion exposure. However, when we examined malathion use dichotomously (i.e., ever vs. never), malathion remained unassociated with NHL (adjusted RR = 0.82, 95 percent CI: 0.43, 1.58). In the other case-control study, McDuffie et al. (36) reported malathion exposure as days per year (unexposed, ≤ 2, and >2), whereas the AHS assessed exposure days per year categorically, with less than 5 days being the lowest category of use from which applicators who reported using malathion could select. While we did not find a strong indication for an association between malathion and NHL, when the low-exposed were used as the referent group, we did observe slight excesses in risk with both lifetime exposure-days and intensity-weighted lifetime exposure-days, although the number of NHL cases was relatively small ($n = 23$).

There is little information regarding the plausibility that malathion could reduce the incidence of melanoma.

TABLE 4. Continued

Intensity-weighted lifetime exposure-days*	No. of cases	Nonexposed referent group		Low-exposed† referent group	
		RR	95% CI	RR	95% CI
Prostate cancer¶					
0	135	1.0	Referent		
>0–58	94	1.20	0.92, 1.56	1.0	Referent
59–245	92	0.98	0.75, 1.28	0.83	0.62, 1.10
>245	88	1.06	0.80, 1.39	0.90	0.67, 1.21
p for trend			0.98		0.81
Colorectal cancer					
0	40	1.0	Referent		
>0–58	28	1.21	0.75, 1.98	1.0	Referent
59–245	15	0.58	0.32, 1.06	0.49	0.26, 0.93
>245	23	1.08	0.64, 1.82	0.96	0.55, 1.69
p for trend			0.81		0.48
Kidney cancer					
0	8	1.0	Referent		
>0–58	4	0.78	0.23, 2.60	1.0	Referent
59–245	10	1.53	0.60, 3.92	2.00	0.62, 6.39
>245	5	0.82	0.26, 2.54	1.12	0.30, 4.27
p for trend			0.68		0.66
Bladder cancer					
0	15	1.0	Referent		
>0–58	8	0.91	0.38, 2.15	1.0	Referent
59–245	9	0.85	0.37, 1.95	0.93	0.36, 2.41
>245	9	0.91	0.39, 2.11	0.95	0.36, 2.52
p for trend			0.91		0.97
Melanoma#					
0	14	1.0	Referent		
>0–58	15	1.44	0.67, 3.10	1.0	Referent
59–245	9	0.66	0.27, 1.60	0.46	0.20, 1.05
>245	7	0.47	0.17, 1.28	0.31	0.12, 0.83
p for trend			0.06		0.06

* Intensity-weighted lifetime exposure-days = intensity score × lifetime exposure-days.
† First tertile of exposure among exposed cases.
‡ RR, rate ratio; CI, confidence interval.
§ Further adjusted for lindane use.
¶ Person-time was restricted to males for the prostate cancer model. Rate ratios were adjusted for age, gender, smoking, alcohol, education, family history of cancer, year of enrollment, and state of residence.
# Further adjusted for carbaryl and parathion use.

However, Huang (53) found that malathion treatment (50 μg/ml or 100 μg/ml) inhibited the growth of human hematopoietic cells, although the inhibition at the lower dose (50 μg/ml) was transient and at the higher dose (100 μg/ml), growth inhibition was attributed to extensive cytotoxicity. In addition, low doses of malathion (10 nM) have induced apoptosis in mouse fibroblast cells (54).

In contrast, other experimental evidence suggests that malathion or its derivatives may be carcinogenic. Using single-gel electrophoresis, Blasiak et al. (17) showed that malaoxon and isomalathion, but not malathion, induced DNA damage. Both malaoxon and isomalathion are impurities found in commercial-grade malathion (55). Additionally, normal human mammary epithelial cells treated with malathion showed differential gene expression in several genes that may be related to carcinogenesis (56). Gwinn et al. (56) found increased expression of three genes (*AKR1C1*, *AKR1C2*, *EBBP*) that are involved in xenobiotic

and steroid metabolism and may play a role in the metabolism of carcinogens. Three other genes (*CPF*, *RFC3*, and *TYMS*) that are involved in DNA replication were also found to have decreased expression, and their downregulation may contribute to carcinogenesis as well. In addition, Cabello et al. (4) hypothesized that malathion, by inhibiting acetylcholinesterase, may lead to cholinergically stimulated cell proliferation of mammary epithelial cells and contribute to the induction of carcinogenesis. While we did not have a sufficient number of cases to examine occupational malathion exposure and breast cancer in applicators, a previous report on breast cancer among applicator spouses from this cohort did not find clear evidence of an association (41).

The AHS is a large prospective cohort study with comprehensive exposure assessment that should minimize, though not entirely eliminate, the potential for exposure misclassification. The reliability of information obtained through the AHS questionnaire has been shown to be similar to that of other factors for which information is routinely obtained by questionnaire for epidemiologic studies (57). The percentage of exact agreement for ever use of malathion was 81. For the other pesticides, exact agreement ranged between 70 percent and 90 percent.

The potential for the use of other pesticides and farm-related exposures (e.g., petrochemicals, biologic dusts, viruses, zoonoses, and solvents) to confound epidemiologic findings on pesticide use has been widely purported (58–60), although specific agents have generally not been clearly identified. We systematically assessed lifetime exposure to 49 other frequently used pesticides and controlled for the pesticides most correlated with malathion use in our analyses. Confounding from farm-related exposures is likely to have been minimal in the AHS. Coble et al. (61) examined the prevalence of exposure to solvents, metals, grain dust, and other hazards in the AHS and found little potential for these farm-related exposures to confound the risk estimates obtained in the AHS. We also directly examined potential confounding by occupational exposure to solvents (excluding gasoline) and gasoline used to as a solvent and found no evidence that these factors confounded the risk estimates for the various cancer sites examined. In addition, neither body mass index nor fruit and vegetable intake at enrollment confounded the risk estimates. While we controlled for these factors, residual confounding due to measurement error could not be ruled out.

The analysis of malathion was restricted to 25,291 (44 percent) pesticide applicators who completed the take-home questionnaire. Because applicators self-selected themselves to complete the take-home questionnaire, selection bias may potentially have affected the risk estimates. However, Tarone et al. (62) previously demonstrated few differences between applicators who completed the take-home questionnaire and those who did not, suggesting that selection bias is unlikely. Moreover, we compared the incidence rates for the reported cancer sites between participants who completed the take-home questionnaire and those who did not, and none of the rate ratios were associated with completing the take-home questionnaire, indicating that the cancer risks for these groups were similar.

We also conducted a sensitivity analysis (50) to further explore the potential for selection bias in our analyses. We directly calculated the selection probabilities for malathion-exposed cases, unexposed cases, exposed person-time, and unexposed person-time, using the enrollment questionnaire to determine ever versus never use of malathion. The selection bias factor for malathion exposure was 0.95, indicating that there was slight underestimation of the rate ratios due to a small selection bias. The rate ratio for cancer comparing persons who reported ever using malathion with those who did not was 0.99 (95 percent CI: 0.88, 1.13). After correction for the selection bias, the rate ratio was 1.04 (corrected RR = 0.99/0.95). This bias was quite small, and we think it would not have materially influenced our inferences about the risk estimates.

In summary, we examined occupational malathion use for potential associations with nine cancer sites in the AHS and found no conclusive evidence that occupational exposure to malathion is associated with increased risk of these cancers. Although the reduced risk for melanoma is interesting, the lack of experimental evidence suggests that this may be a chance finding. Continued follow-up of the AHS cohort should provide increased precision, allowing for more definitive conclusions, especially for the less common cancers.

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