International Journal of Epidemiology, 2016, 792–805
doi: 10.1093/ije/dyv195
Advance Access Publication Date: 27 September 2015
Original article

Environmental Exposures and Cancer

Occupational exposure to pesticides and bladder cancer risk

Stella Koutros,[1]* Debra T Silverman,[1] Michael CR Alavanja,[1] Gabriella Andreotti,[1] Catherine C Lerro,[1] Sonya Heltshe,[2] Charles F Lynch,[3] Dale P Sandler,[4] Aaron Blair[1] and Laura E Beane Freeman[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, [2]Seattle Children's Hospital Research Institute, University of Washington School of Medicine, Department of Pediatrics, Seattle, WA, USA, [3]College of Public Health, University of Iowa, Iowa City, IA, USA and [4]Epidemiology Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA

*Corresponding author. Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 9609 Medical Center Dr., Bethesda, MD 20892, USA. E-mail: KoutrosS@mail.nih.gov

Accepted 3 September 2015

Abstract

Background: In the developed world, occupational exposures are a leading cause of bladder cancer. A few studies have suggested a link between pesticide exposures among agricultural populations and bladder cancer.

Methods: We used data from the Agricultural Health Study, a prospective cohort study which includes 57 310 pesticide applicators with detailed information on pesticide use, to evaluate the association between pesticides and bladder cancer. We used Poisson regression to calculate rate ratios (RRs) and 95% confidence intervals (CIs) to estimate the association between each of 65 pesticides and 321 incident bladder cancer cases which accrued over the course of follow-up (1993–2011), adjusting for lifestyle and demographic and non-pesticide farm-related exposures, including those previously linked to bladder cancer. We conducted additional analyses stratified by smoking status (never, former, current).

Results: We observed associations with bladder cancer risk for two imidazolinone herbicides, imazethapyr and imazaquin, which are aromatic amines. Ever use of imazaquin (RR = 1.54, 95% CI: 1.05, 2.26) was associated with increased risk whereas the excess risk among users of imazethapyr was evident among never smokers (RR in highest quartile vs non-exposed = 3.03, 95% CI: 1.46, 6.29, *P*-interaction = 0.005). We also observed increased risks overall and among never smokers for use of several chlorinated pesticides including chlorophenoxy herbicides and organochlorine insecticides.

Conclusions: Several associations between specific pesticides and bladder cancer risk were observed, many of which were stronger among never smokers, suggesting that

possible risk factors for bladder cancer may be more readily detectable in those unexposed to potent risk factors like tobacco smoke.

Key words: Pesticides, bladder cancer, epidemiology

Key Messages

- Occupational exposures are a leading cause of bladder cancer, but occupational pesticide exposure has been little explored as a possible risk factor.
- We observed increased risks for two aromatic amine herbicides, chlorophenoxy herbicides and organochlorine insecticides.
- Several associations were more apparent among never smokers, suggesting that pesticide exposure may be an overlooked exposure in bladder carcinogenesis.
- Our results highlight the difficulty in trying to understand the impact of other exposures on smoking-related cancers.

Introduction

In the developed world, bladder cancer is the fourth and twelfth most common cancer in men and women, respectively.[1] The leading risk factors are cigarette smoking and occupational exposures.[2] Aromatic amines, including 2-naphthylamine, 4-aminobiphenyl, benzidine, ortho-toluidine and others, are established bladder carcinogens that have been described in the occupational setting.[3] Agricultural populations have a lower prevalence of smoking than the general population,[4–6] which may explain why several studies have found either no association or a decreased risk of bladder cancer in this occupational group.[7–13] On the other hand, two studies have shown a link between farming and bladder cancer among non-smokers,[14,15] which suggests a complexity in interpreting the effect of other exposures in the presence of smoking, the primary risk factor for bladder cancer. In addition, some studies have suggested a link between farming, herbicide exposure or specific agricultural settings and risk of bladder cancer.[14–22] Bladder cancer risk might be explained by the urogenous contact hypothesis which proposes that active carcinogens dissolved in urine come into contact with and transform cells of the bladder epithelium.[23] Many pesticides and their metabolites are readily excreted from the body via the urine. Thus, the potential exists for pesticides to adversely affect the bladder. We previously reported an increased risk of bladder cancer[24] in a cohort of farmers occupationally exposed to the aromatic amine herbicide, imazethapyr. Other specific pesticides, however, have been little explored as possible risk factors for bladder cancer. Thus, we used data from the Agricultural Health Study (AHS), a large prospective cohort study of pesticide applicators with detailed pesticide use data, to evaluate the association between several specific pesticides and bladder cancer risk.

Methods

Study population

The AHS is a prospective cohort study that includes 52 394 licensed private pesticide applicators in Iowa and North Carolina and 4916 licensed commercial applicators in Iowa. The cohort has been described in detail.[6,24,25] Briefly, individuals seeking licenses for restricted-use pesticides were recruited from December 1993 through December 1997 (82% of the target population enrolled). The protocol was approved by all relevant institutional review boards. We obtained cancer incidence information by regular linkage to cancer registry files in Iowa and North Carolina. In addition, the cohort is matched to state mortality registries and the National Death Index to identify vital status, and to home address records of the Internal Revenue Service, motor vehicle registration files and pesticide license registries of state agricultural departments to determine residence in Iowa or North Carolina. The current analysis included all incident bladder cancers (invasive and *in situ*) diagnosed from enrolment (1993–97) through 31 December 2010 in North Carolina and 31 December 2011 in Iowa. We censored follow-up at the date of cancer diagnosis, time of death, movement out of state or at the end of the current follow-up time. Because there was only one case of bladder cancer diagnosed among female applicators, we excluded women from the analysis ($n = 1562$), as well as 1071 individuals with prevalent cancer at enrolment and 333 with no follow-up information, leaving

54 344 men for analysis among whom a total of 321 incident bladder cancers were diagnosed.

Exposure assessment

Information on use of individual pesticides was captured in two self-administered questionnaires [http://www.aghealth.nih.gov/collaboration/questionnaires.html] completed during cohort enrolment. All applicators completed the first enrolment questionnaire, which enquired about ever/never use of 50 pesticides, as well as duration (years) and frequency (average days/year) of use for a subset of 22 pesticides. In addition, 44.1% of the applicators returned the second (take-home) enrolment questionnaire, which enquired about duration and frequency of use for the remaining 28 additional pesticides and ever/never use of additional pesticides. A follow-up questionnaire, which ascertained pesticide use since enrolment and last year applied, was administered 5 years after enrolment and completed by 36 342 (63%) of the original participants. For participants who did not complete a follow-up questionnaire (20 968 applicators, 37%), a data-driven multiple imputation procedure was used to impute use of specific pesticides at follow-up. A detailed description of the imputation process and validation is described by Heltshe *et al.*[26] Enrolment and follow-up information were combined to generate cumulative lifetime days of use and intensity-weighted lifetime days of use.

We restricted analyses to those pesticides with 10 or more exposed cases ($n = 65$). Among these, 44 had detailed data to explore associations between cumulative exposure and bladder cancer risk, using two exposure metrics: (i) lifetime days of pesticide use, that is the product of years of use of a specific pesticide and the number of days used per year; and (ii intensity-weighted lifetime days of use, which is the product of lifetime days of use and a measure of exposure intensity. Intensity was derived from an algorithm using questionnaire data on mixing status, application method, equipment repair and use of personal protective equipment.[27] We also used 15-year lagged cumulative exposure, discounting the most recent 15 years of use. Supplementary Table 1 (available as Supplementary data at *IJE* online) provides the complete list of pesticides evaluated and their prevalence of use. Data were obtained from Agricultural Health Study data release versions P1REL201209.00 and P2REL201209.00.

Statistical analyses

For each pesticide, we categorized exposure based on the distribution of use among exposed cases. Depending on the prevalence of exposure, we created categories based on the median exposure, tertiles or quartiles. We used Poisson regression to calculate rate ratios (RRs) and 95% confidence intervals (CIs) and used the MIANALYZE procedure in SAS, version 9.3 (SAS Institute, Inc., Cary, NC, USA) to obtain the appropriate variance for the imputed data. Analyses were conducted using ever/never use, the lifetime days, intensity-weighted lifetime days and the 15-year lagged metrics. We evaluated several lifestyle, demographic and non-pesticide farm-related exposures, including those previously linked to bladder cancer (diesel exhaust exposure, welding, painting, grinding metal) as possible confounders of the relationship between pesticides and bladder cancer, and ultimately included the following variables which were independently related to bladder cancer in our population for adjustment of all models: attained age (10-year intervals), race (White, other), cigarette smoking (status, pack-years among former and current smokers) and pipe smoking (ever/never). Smoking status [never, former (smoked at least 100 cigarettes in the past], current) was ascertained at enrolment and subsequently upon cohort follow-up. Duration (years) and intensity (cigarettes/day) of smoking were assessed at enrolment. To fully explore possible confounding due to smoking, we explored adjusting for smoking in two ways: (i) status (never, former, current) and pack-years smoked; and (ii) status and duration (years) of smoking. We also conducted analyses stratified by smoking status (never, former, current). We also explored adjustment for ever use of pesticides most highly associated with a given individual pesticide in multivariate models, as well as mutual adjustment for pesticides that were associated with bladder cancer risk. Likelihood ratio tests were used to assess differences between strata (P-interaction). All tests were two-sided and conducted at the $\alpha = 0.05$ level. Tests for trend used the midpoint value of each exposure category in regression models.

Results

In all, 321 cases of bladder cancer were diagnosed among male applicators through the current follow-up period. Of these, 96% ($n = 307$) were urothelial carcinomas and the majority of these were localized tumours ($n = 272$) (data not shown); 83 cancers were diagnosed among never smokers, 161 among former smokers and 69 among current smokers (Table 1); 13% of cases also reported a history of pipe use (Table 1); and all of these men were former cigarette smokers at enrolment.

Table 2 shows the rate ratios of bladder cancer associated with ever use of specific herbicides, insecticides, fumigants and fungicides. Increased risks of bladder cancer were observed among ever users of the herbicides bentazon

Table 1. Characteristics of incident bladder cancer cases among men in the Agricultural Health Study

Characteristic	Cohort Person-years (total = 802,905.7)	Total Bladder Cancer $n = 321$ n (%)[a]
Age at the end of current follow-up		
<60	402510.437 (50.1)	57 (17.8)
60–69	203258.327 (25.3)	100 (31.2)
70–79	138180.408 (17.2)	114 (35.5)
80+	58956.5777 (7.3)	50 (15.6)
Mean (SD)		69.6 (10.4)
State		
Iowa	534349.517 (66.6)	185 (57.6)
North Carolina	268556.233 (33.4)	136 (42.4)
Applicator Type		
Private/farmer	729393.3 (91.0)	300 (93.5)
Commercial	70440.4 (8.8)	21 (6.5)
Exposed to engine exhaust		
No	268975.2 (33.5)	123 (38.3)
Yes	80786.8 (10.1)	50 (15.6)
Missing	450071.6 (56.1)	148 (46.1)
Paint at least once a year		
No	257887.4 (32.2)	153 (47.7)
Yes	541946.2 (67.5)	168 (52.3)
Missing		
Grind metal in summer and/or winter		
Monthly	93414.5 (11.6)	57 (17.8)
Weekly	145398.4 (18.2)	63 (19.6)
Other	68232.9 (8.5)	36 (11.1)
Missing	490545.0 (61.1)	165 (51.4)
Race		
White	767652.107 (95.6)	317 (98.8)
Black/Other	35253.6427 (4.4)	4 (1.2)
Smoking Status[b]		
Never	416616.101 (51.9)	83 (25.9)
Former	231281.971 (28.8)	161 (50.2)
Current	130657.717 (16.3)	69 (21.5)
Missing	24349.9603 (3.0)	8 (2.5)
Pipe Smoker		
Never	764677.153 (95.2)	278 (86.6)
Ever	38228.5969 (4.8)	43 (13.4)

[a]Percents may not sum to 100 due to rounding.
[b]Assessed at enrolment and follow-up.

(RR = 1.55, 95% CI: 1.10, 2.19), bromoxynil (RR = 1.51, 95% CI: 1.04, 2.20), chloramben (RR = 1.56, 95% CI: 1.10, 2.22), diclofop-methyl (RR = 1.85, 95% CI: 1.01, 3.42) and imazaquin (RR = 1.54, 95% CI: 1.05, 2.26). Additional associations were observed between ever use of 2,4-D (RR = 1.46, 95% CI: 0.98, 2.18) and ever use of sethoxydim (RR = 0.65, 95% CI: 0.43, 1.00), with a positive and an inverse association observed, respectively. The organochlorine insecticides dichlorodiphenyltrichloroethane (DDT) and heptachlor were positively associated with bladder cancer risk (RR = 1.40, 95% CI: 1.10, 1.80 and RR = 1.30, 95% CI: 0.98, 1.74, respectively).

Table 3 shows the associations between cumulative intensity-weighted lifetime days of herbicide use and risk of bladder cancer overall and stratified by smoking status. We observed positive trends for 2,4,5-T [RR in tertile 3 (T3) vs non-exposed = 2.64, 95% CI: 1.23, 5.68, P-trend = 0.02], 2,4-D [RR in quartile 4 (Q4) vs non-exposed = 1.88, 95% CI: 0.94, 3.77, P-trend = 0.02], glyphosate (RR in Q4 vs non-exposed = 1.93, 95% CI: 0.95, 3.91, P-trend = 0.03), and imazethapyr (RR in Q4 vs. non-exposed = 3.03, 95% CI: 1.46, 6.29, P-trend = 0.004) among never smokers. There was evidence of effect modification by smoking on the relationship between cumulative intensity-weighted days of imazethapyr and bladder cancer (P-interaction = 0.005). An inverse trend with 2,4,5-T among former smokers, and a borderline inverse trend with dicamba among current smokers, were also observed.

Table 4 shows the associations between cumulative intensity-weighted lifetime days of insecticide use and risk of bladder cancer overall and stratified by smoking status. Overall, there were no positive trends in risk with increasing levels of insecticide use. Among never smokers, positive gradients in risk were observed with increasing use of two carbamate insecticides, aldicarb [RR high (M2) vs non-exposed = 4.04, 95% CI: 1.20, 13.57, P-trend = 0.03] and carbofuran (RR in T2 vs non-exposed = 1.99, 95% CI: 1.06, 3.75, P-trend = 0.03), two organochlorine insecticides, chlordane (RR T3 vs non-exposed = 2.83. 95% CI: 1.16, 6.90, P-trend = 0.02) and toxaphene (RR high vs non-exposed = 3.75, 95% CI: 1.57, 8.97, P-trend = 0.003), one organophosphate insecticide, fonofos (RR T3 vs non-exposed = 2.01, 95% CI: 1.01, 4.00, P-trend = 0.05) and one pyrethroid insecticide, permethrin use (RR high vs non-exposed = 2.28, 95% CI: 1.08, 4.82, P-trend = 0.04). No trends were observed between bladder cancer and pesticides among former or current smokers. The interaction between exposure and smoking was only evident for carbofuran (P-interaction = 0.04) and chlorpyrifos (P-interaction = 0.01).

There were no associations overall or among any of the smoking strata for use of any fumigants or fungicides evaluated (Supplementary Table 2, available as Supplementary data at *IJE* online) and bladder cancer, with the exception of a positive association among smokers using carbon tetrachloride/carbon disulfide, which was based on only three exposed cases. In addition, Supplementary Table 3 (available as Supplementary data at *IJE* online) provides stratified risks of bladder cancer by smoking status for those pesticides with no cumulative use information. No notable differences in observed associations emerged from analyses of lifetime days or from lagged exposures and these are, therefore, not shown.

Table 2. Ever use of pesticides and risk of bladder cancer in the Agricultural Health Study

Pesticide	Exposed Cases	RR[a] (95% CI)
Herbicides		
2,4,5-T[b]	91	1.15 (0.84, 1.59)
2,4,5-TP[b,c]	40	1.07 (0.74, 1.56)
2,4-D	245	**1.46 (0.98, 2.18)**
Acifluorfen, sodium salt[c]	28	1.21 (0.79, 1.85)
Alachlor	158	1.15 (0.86, 1.52)
Atrazine	220	1.22 (0.88, 1.69)
Bentazon[c]	67	**1.55 (1.10, 2.19)**
Bromoxynil[c]	51	**1.51 (1.04, 2.20)**
Butylate	86	0.86 (0.63, 1.19)
Chloramben[b,c]	46	**1.56 (1.10, 2.22)**
Chlorimuron-ethyl	91	0.85 (0.62, 1.17)
Clomazone[c]	24	0.99 (0.64, 1.54)
Cyanazine	101	0.90 (0.67, 1.21)
Dicamba	125	0.84 (0.62, 1.14)
Diclofop-methyl[c]	11	**1.85 (1.01, 3.42)**
EPTC	49	0.98 (0.70, 1.37)
Ethalfluralin[c]	10	0.77 (0.40, 1.45)
Fluazifop-butyl[b,c]	26	1.06 (0.68, 1.64)
Glyphosate	248	1.17 (0.78, 1.77)
Imazaquin[c]	38	**1.54 (1.05, 2.26)**
Imazethapyr	104	1.03 (0.76, 1.40)
Linuron[c]	21	0.97 (0.60, 1.55)
Metolachlor	113	0.86 (0.65, 1.13)
Metribuzin	107	0.75 (0.54, 1.04)
Propachlor[b,c]	27	1.20 (0.78, 1.83)
Paraquat	71	0.86 (0.61, 1.20)
Pendimethalin	113	0.75 (0.55, 1.02)
Petroleum Oil/Petroleum Distillates	130	0.88 (0.65, 1.21)
Sethoxydim[c]	28	**0.65 (0.43, 1.00)**
Simazine[b,c]	16	1.04 (0.61, 1.77)
Thifensulfuron-methyl[c]	14	1.04 (0.59, 1.82)
Trifluralin	139	1.08 (0.80, 1.45)
Insecticides		
Acephate[c]	21	0.91 (0.55, 1.50)
Aldicarb	35	0.88 (0.59, 1.32)
Aldrin[b]	88	1.20 (0.92, 1.57)
Carbaryl	192	1.04 (0.70, 1.54)
Carbofuran	67	0.86 (0.63, 1.16)
Chlordane[b]	97	0.95 (0.74, 1.22)
Chlorpyrifos	108	0.88 (0.67, 1.14)
Coumaphos	19	0.95 (0.59, 1.54)
DDT[b]	136	**1.40 (1.10, 1.80)**
DDVP[b]	25	1.01 (0.65, 1.55)
Diazinon	98	0.74 (0.54, 1.02)
Dieldrin[b,c]	32	1.19 (0.82, 1.72)
Disulfoton[b,c]	15	0.94 (0.54, 1.65)
Ethoprop[c]	11	0.73 (0.39, 1.37)
Fonofos[b]	53	1.09 (0.78, 1.52)
Heptachlor[b]	72	**1.30 (0.98, 1.74)**
Lindane[b]	69	1.08 (0.82, 1.42)
Malathion	223	1.01 (0.65, 1.58)
Methomyl[c]	13	1.17 (0.64, 2.12)
Parathion[b]	62	1.14 (0.81, 1.61)
Permethrin	44	0.75 (0.53, 1.07)
Phorate	96	0.99 (0.72, 1.37)
Terbufos	92	1.05 (0.79, 1.41)
Toxaphene[b]	56	0.96 (0.72, 1.30)
Fumigants		
Aluminum Phosphide	20	1.13 (0.70, 1.83)
Carbon Tetrachloride/Carbon Disulfide[b]	32	1.39 (0.93, 2.09)
Ethylene Dibromide[b,c]	17	0.86 (0.51, 1.46)
Methyl Bromide	48	0.86 (0.60, 1.23)
Fungicides		
Benomyl[b]	42	1.09 (0.74, 1.60)
Captan	32	1.19 (0.81, 1.74)
Chlorothalonil	27	1.09 (0.71, 1.66)
Maneb/Mancozeb	35	0.86 (0.57, 1.29)
Metalaxyl	65	0.66 (0.47, 0.94)

[a]Model adjusted for age, race, state, pack-years of cigarettes and pipe smoking.
[b]No longer registered for use in the USA.
[c]Results available on ever use only.

Discussion

In this analysis, we saw associations between two imidazolinone herbicides, imazethapyr and imazaquin which are aromatic amines, and bladder cancer risk. Ever use of other herbicides, including the general use pesticides bentazon and bromoxynil, the chlorophenoxy herbicide diclofop-methyl and another chlorinated herbicide chloramben, were also associated with bladder cancer. Increased risks of bladder cancer were also observed with regard to use of the chlorinated insecticide DDT; however, no consistent exposure-response relationship was observed in expanded analyses.

Imazethapyr is an imidazolinone herbicide used to control weeds in corn, soybean, dry bean, alfalfa and other crops.[28] Imazaquin is a general-use pesticide used to control grasses and broadleaf weeds.[29] In a previous analysis in the AHS focusing on risk of all cancer in a subcohort of applicators that used imazethapyr, we reported a relationship between imazethapyr and bladder cancer based on 41 exposed cases. In this analysis, which includes 6–7 years of additional follow-up and an additional 100 exposed cases, we did not observe an overall association with imazethapyr. An exposure-response relationship, however, was observed (P-trend = 0.004) among never smokers, with the highest category of exposure experiencing a 3-fold risk.

We also observed that ever use of another imidazolinone herbicide, imazaquin, was associated with bladder cancer risk. Although neither herbicide has demonstrated evidence of carcinogenicity in mice or rats, there is some plausibility for a possible link between exposure to imazethapyr and imazaquin and risk of bladder cancer because these herbicides are aromatic amine compounds, a chemical class which has been linked to bladder cancer, and animal metabolism studies show that these pesticides are readily excreted in the urine predominantly as the parent aromatic compounds.[28,29] The risk associated with imazethapyr exposure, however, was predominantly observed only among a smaller group of never smokers and it was not possible to evaluate quantitative exposure for imazaquin, and thus findings are unclear. Neither imazethapyr nor imazaquin have undergone a complete evaluation for evidence of human carcinogenic potential by the USA Environmental Protection Agency (U.S. EPA) or the International Agency for Research on Cancer (IARC). We are unaware of any other epidemiological study outside the AHS that has evaluated exposure to these pesticides as possible risk factors for cancer.

We also observed an increased risk of bladder cancer associated with ever use of the herbicides bentazon and bromoxynil. Bentazon and bromoxynil are used on a variety of food crops but are also used on lawns, turfs and golf courses. In our data, ever use of bentazon and bromoxynil were moderately correlated ($r = 0.54$). When we mutually adjusted models for these two herbicides, the results for both became non-significant. However, whereas the magnitude of the effect for bromoxynil diminished, the effect of bentazon was similar to that observed overall, and additional analyses stratified by smoking status also showed a strong association between bentazon and bladder cancer among never smokers (RR = 2.14, 95% CI: 1.09, 4.21, Supplementary Table 3, available as Supplementary data at *IJE* online), suggesting the effect is unlikely to be due to smoking and that bentazon might be more important in driving the observed bladder cancer risk than bromoxynil. There are limited experimental data on bentazon as a bladder carcinogen. In a combined chronic toxicity-carcinogenicity study in rats,[30] bentazon was found to result in increases in urine volume along with reduced urinary specific gravity, which may be related to bladder cancer risk.[31] Although there are few other data to support our findings regarding bentazon and bromoxynil, the use of these pesticides in both agricultural and general-use purposes indicates additional evaluation is warranted. Bentazon has been classified as a Group E carcinogen, evidence of non-carcinogenicity to humans, by the U.S. EPA based on animal models[30] and bromoxynil has been classified as a Group C, possible human carcinogen, based on observed liver tumours in animals;[32] neither have been evaluated by IARC.

Several chlorinated pesticides were also shown to influence bladder cancer risk in our analyses. Chloramben is an herbicide used to control weeds on soybean and other crops. No information is available on the carcinogenic effects of chloramben in humans, although a US study reported that oral exposure to chloramben caused liver tumours in mice but not in rats.[33] We also found that ever use of the organochlorine insecticide DDT increased bladder cancer risk, but no trend in risk with increasing use was observed. This may be due, in part, to the lack of detailed information from more than half of those reporting being ever exposed to DDT (only 46% reported days and years of use). Two other organochlorine insecticides, chlordane and toxaphene, showed evidence of increased bladder cancer risk but only among never smokers. Organochlorine insecticides have been linked to several cancer sites,[34] but we are unaware of any studies suggesting a link with bladder cancer.

In subgroup analyses, we also observed some interesting associations between several herbicides and insecticides and bladder cancer among never smokers. Never smoking applicators with the highest use of the chlorophenoxy herbicides 2,4,5-T and 2,4-D had higher risk of bladder cancer, and heavy users of the herbicide glyphosate had increased risk as well. Recently, a cohort of chlorophenoxy herbicide manufacturing workers in The Netherlands was observed to have excess bladder cancer mortality, in particular among workers involved in the manufacture of 2,4,5-T.[35] Because the numbers of observed bladder cancer deaths in this and other manufacturing cohorts was small,[36,37] it is difficult to draw a definitive conclusion. Observational studies in dogs showed that exposure to herbicide-treated lawns, in particular those treated with phenoxy herbicides, was associated with higher bladder cancer risk.[38,39] Interestingly we also observed a positive association between another chlorophenoxy herbicide, diclofop-methyl, and bladder cancer, albeit among few exposed cases ($n = 11$). Diclofop-methyl is classified as likely to be carcinogenic to humans by the U.S. EPA[40] and IARC ranks chlorophenoxy herbicides as possibly carcinogenic to humans (Group 2B). Taken together, these data suggest a possible link between chlorophenoxy herbicide exposure and bladder cancer. Several insecticides showed higher risk of bladder cancer among the never smokers as well, but power was limited to draw conclusions as the numbers of exposed cases were often small, given their lower prevalence of use.

An interesting element of this analysis is the observed differences in risk among never smokers for multiple chemicals. Since cigarette smoking is the major risk factor for

Table 3. Cumulative intensity-weighted days for herbicide use and risk of bladder cancer, overall and stratified by smoking status

Pesticide	OVERALL		NEVER		FORMER		CURRENT		
	$n = 321$ cancers		$n = 83$ cancers		$n = 161$ cancers		$n = 69$ cancers		
	Cases	RR[a] (95% CI)	Cases	RR[b] (95% CI)	Cases	RR[c] (95% CI)	Cases	RR[b] (95% CI)	p-interaction
2,4,5-T[d]									
Non-exposed	122	Ref	28	Ref	70	Ref	22	Ref	
T1	14	1.35 (0.77, 2.36)	4	1.73 (0.60, 4.99)	8	1.16 (0.56, 2.43)	1	**	
T2	14	0.99 (0.56, 1.73)	2	0.63 (0.15, 2.66)	9	1.00 (0.50, 2.02)	3	1.54 (0.46, 5.23)	
T3	15	0.83 (0.48, 1.42)	9	2.64 (1.23, 5.68)	3	0.25 (0.08, 0.81)	3	1.12 (0.33, 3.77)	
p-trend		0.45		0.02		0.02		0.82	0.02
2,4-D									
Non-exposed	61	Ref	13	Ref	31	Ref	17	Ref	
Q1	60	1.25 (0.86, 1.82)	13	0.99 (0.44, 2.25)	34	1.26 (0.74, 2.14)	13	1.41 (0.67, 2.94)	
Q2	61	1.01 (0.70, 1.47)	18	1.19 (0.58, 2.44)	30	0.87 (0.51, 1.48)	13	1.16 (0.54, 2.48)	
Q3	61	0.89 (0.61, 1.30)	16	0.90 (0.42, 1.90)	30	0.75 (0.43, 1.31)	15	1.30 (0.63, 2.69)	
Q4	62	1.25 (0.87, 1.81)	23	1.88 (0.94, 3.77)	31	1.12 (0.66, 1.91)	8	0.83 (0.33, 2.04)	
p-trend		0.31		0.02		0.69		0.45	0.65
Alachlor									
Non-exposed	126	Ref	33	Ref	61	Ref	32	Ref	
Q1	37	1.10 (0.75, 1.60)	10	1.10 (0.54, 2.25)	22	1.25 (0.76, 2.07)	5	0.71 (0.26, 1.91)	
Q2	39	0.90 (0.63, 1.30)	12	1.06 (0.54, 2.06)	18	0.83 (0.49, 1.41)	9	0.94 (0.44, 2.03)	
Q3	38	1.23 (0.85, 1.77)	11	1.33 (0.67, 2.63)	21	1.41 (0.85, 2.32)	6	0.82 (0.34, 1.97)	
Q4	39	1.00 (0.70, 1.43)	14	1.43 (0.77, 2.68)	18	0.99 (0.59, 1.68)	7	0.67 (0.29, 1.51)	
p-trend		0.94		0.25		0.99		0.37	0.84
Atrazine									
Non-exposed	89	Ref	23	Ref	52	Ref	14	Ref	
Q1	53	1.30 (0.91, 1.86)	23	1.04 (0.51, 2.11)	29	1.10 (0.68, 1.76)	11	2.39 (1.09, 5.27)	
Q2	55	0.94 (0.65, 1.36)	22	0.63 (0.29, 1.36)	23	0.67 (0.40, 1.12)	21	2.72 (1.32, 5.62)	
Q3	56	0.98 (0.69, 1.39)	26	0.95 (0.5,0 1.83)	28	0.78 (0.48, 1.27)	12	1.67 (0.77, 3.62)	
Q4	55	0.95 (0.67, 1.34)	28	1.03 (0.54, 1.96)	27	0.80 (0.50, 1.29)	10	1.28 (0.56, 2.89)	
p-trend		0.46		0.69		0.43		0.52	0.13
Butylate[d]									
Non-exposed	115	Ref	35	Ref	58	Ref	19	Ref	
Q1	16	1.29 (0.76, 2.19)	3	0.65 (0.20, 2.13)	11	1.81 (0.94, 3.49)	2	1.13 (0.26, 4.92)	
Q2	15	1.44 (0.84, 2.49)	3	0.87 (0.26, 2.84)	10	1.84 (0.93, 3.64)	2	1.39 (0.32, 6.04)	
Q3	16	0.98 (0.58, 1.66)	3	0.57 (0.18, 1.88)	10	1.38 (0.70, 2.73)	3	0.96 (0.28, 3.29)	
p-trend		0.98		0.36		0.32		0.98	0.64
Chlorimuron-ethyl[d]									
Non-exposed	121	Ref	27	Ref	71	Ref	20	Ref	
T1	15	1.07 (0.62, 1.83)	6	1.66 (0.68, 4.07)	6	0.75 (0.32, 1.73)	3	1.30 (0.38, 4.40)	
T2	15	0.88 (0.51, 1.54)	3	0.76 (0.23, 2.52)	7	0.82 (0.37, 1.79)	5	1.31 (0.44, 3.89)	
T3	17	0.79 (0.47, 1.31)	8	1.75 (0.79, 3.88)	6	0.54 (0.23, 1.24)	3	0.62 (0.18, 2.09)	
p-trend		0.33		0.21		0.15		0.43	0.34
Cyanazine									
Non-exposed	175	Ref	48	Ref	87	Ref	40	Ref	
Q1	25	0.71 (0.46, 1.10)	6	0.59 (0.24, 1.46)	17	0.88 (0.51, 1.51)	2	0.33 (0.08, 1.40)	
Q2	25	0.66 (0.42, 1.03)	9	0.90 (0.43, 1.89)	10	0.46 (0.23, 0.94)	6	0.87 (0.36, 2.09)	
Q3	24	1.25 (0.80, 1.95)	5	0.90 (0.35, 2.31)	12	1.22 (0.65, 2.30)	7	1.90 (0.82, 4.40)	
Q4	26	0.81 (0.53, 1.24)	9	1.03 (0.49, 2.15)	14	0.89 (0.49, 1.59)	3	0.42 (0.13, 1.37)	
p-trend		0.59		0.76		0.94		0.31	0.27
Dicamba									
Non-exposed	150	Ref	30	Ref	74	Ref	37	Ref	
Q1	31	0.92 (0.61, 1.38)	9	0.83 (0.38, 1.78)	15	0.85 (0.47, 1.54)	7	1.14 (0.48, 2.74)	
Q2	32	0.70 (0.45, 1.08)	7	0.56 (0.23, 1.34)	20	0.85 (0.49, 1.47)	5	0.54 (0.19, 1.58)	

(continued)

Table 3. Continued

Pesticide	OVERALL		NEVER		FORMER		CURRENT		
	n = 321 cancers		*n* = 83 cancers		*n* = 161 cancers		*n* = 69 cancers		
	Cases	RR[a] (95% CI)	Cases	RR[b] (95% CI)	Cases	RR[c] (95% CI)	Cases	RR[b] (95% CI)	p-interaction
Q3	32	0.81 (0.54, 1.22)	9	0.84 (0.39, 1.83)	15	0.70 (0.39, 1.28)	8	1.05 (0.45, 2.42)	
Q4	32	0.77 (0.51, 1.16)	13	1.12 (0.56, 2.27)	17	0.84 (0.48, 1.49)	2	0.23 (0.05, 0.98)	
p-trend		0.31		0.50		0.62		0.05	0.32
EPTC									
Non-exposed	226	Ref	66	Ref	116	Ref	44	Ref	
T1	15	0.72 (0.42, 1.23)	3	0.50 (0.15, 1.60)	8	0.68 (0.33, 1.4)	4	1.29 (0.45, 3.70)	
T2	15	1.33 (0.79, 2.27)	3	0.83 (0.26, 2.67)	5	0.86 (0.35, 2.13)	7	3.75 (1.64, 8.58)	
T3	17	0.96 (0.58, 1.58)	5	1.02 (0.41, 2.55)	11	1.23 (0.65, 2.30)	1	**	
p-trend		0.94		0.93		0.49		0.44	0.09
Glyphosate									
Non-exposed	60	Ref	14	Ref	31	Ref	15	Ref	
Q1	62	1.28 (0.86, 1.89)	19	1.64 (0.75, 3.58)	31	1.22 (0.72, 2.08)	12	1.00 (0.46, 2.13)	
Q2	62	0.96 (0.65, 1.41)	11	0.79 (0.35, 1.77)	36	1.07 (0.64, 1.78)	15	0.88 (0.41, 1.87)	
Q3	62	0.85 (0.58, 1.26)	14	0.85 (0.37, 1.95)	30	0.83 (0.49, 1.39)	16	0.86 (0.40, 1.82)	
Q4	62	1.07 (0.73, 1.56)	23	1.93 (0.95, 3.91)	29	1.00 (0.58, 1.72)	10	0.58 (0.25, 1.34)	
p-trend		0.99		0.03		0.67		0.17	0.19
Imazethapyr									
Non-exposed	167	Ref	41	Ref	87	Ref	39	Ref	
Q1	24	0.82 (0.51, 1.31)	7	1.00 (0.41, 2.27)	12	0.77 (0.40, 1.47)	5	0.79 (0.27, 2.32)	
Q2	26	0.96 (0.61, 1.49)	13	1.88 (0.96, 3.71)	10	0.71 (0.35, 1.42)	3	0.51 (0.15, 1.74)	
Q3	23	0.92 (0.58, 1.46)	3	0.46 (0.14, 1.53)	16	1.27 (0.72, 2.26)	4	0.70 (0.24, 2.05)	
Q4 bottom	14	2.08 (1.18, 3.66)	4	2.12 (0.74, 6.10)	6	1.83 (0.78, 4.28)	4	0.76 (0.26, 2.23)	
Q4 top	13	0.94 (0.52, 1.68)	10	3.03 (1.46, 6.29)	3	0.47 (0.15, 1.53)	0	**	
p-trend		0.63		0.004		0.61		0.20	0.005
Metolachlor									
Non-exposed	168	Ref	40	Ref	86	Ref	42	Ref	
Q1	27	0.88 (0.58, 1.34)	8	0.99 (0.44, 2.20)	17	1.09 (0.63, 1.86)	2	0.28 (0.07, 1.17)	
Q2	27	0.74 (0.49, 1.12)	6	0.69 (0.29, 1.64)	13	0.69 (0.38, 1.28)	8	0.92 (0.43, 1.99)	
Q3	28	0.66 (0.44, 0.99)	14	1.29 (0.69, 2.42)	14	0.65 (0.36, 1.17)	0	**	
Q4	28	0.95 (0.63, 1.44)	10	1.50 (0.74, 3.01)	14	0.97 (0.54, 1.75)	4	0.47 (0.15, 1.46)	
p-trend		0.73		0.18		0.78		0.12	0.01
Metribuzin[d]									
Non-exposed	108	Ref	29	Ref	63	Ref	15	Ref	
Q1	12	1.09 (0.59, 2.01)	3	0.88 (0.26, 2.94)	5	0.72 (0.29, 1.83)	4	3.14 (1.00, 9.86)	
Q2	15	0.85 (0.49, 1.48)	3	0.56 (0.16, 1.89)	7	0.64 (0.29, 1.43)	5	2.37 (0.82, 6.87)	
Q3	10	0.89 (0.46, 1.72)	3	0.86 (0.26, 2.88)	6	0.89 (0.38, 2.09)	1	**	
Q4	17	0.72 (0.43, 1.22)	6	0.89 (0.37, 2.19)	8	0.56 (0.27, 1.20)	2	0.73 (0.16, 3.32)	
p-trend		0.21		0.86		0.17		0.48	0.44
Paraquat[d]									
Non-exposed	130	Ref	33	Ref	70	Ref	24	Ref	
T1	10	0.96 (0.49, 1.89)	3	1.30 (0.39, 4.26)	4	0.63 (0.20, 2.03)	3	1.66 (0.49, 5.67)	
T2	13	1.64 (0.91, 2.96)	5	2.97 (1.10, 8.03)	8	1.96 (0.92, 4.19)	0	**	
T3	12	1.29 (0.69, 2.40)	3	2.20 (0.71, 6.87)	7	1.45 (0.64, 3.28)	2	0.45 (0.06, 3.48)	0.08
p-trend		0.65		0.54		0.45		0.57	
Pendimethalin[d]									
Non-exposed	106	Ref	26	Ref	61	Ref	17	Ref	
T1	19	1.00 (0.60, 1.67)	3	0.59 (0.18, 1.96)	12	1.13 (0.58, 2.20)	3	0.97 (0.28, 3.35)	
T2	22	0.62 (0.39, 0.99)	5	0.67 (0.25, 1.82)	12	0.58 (0.31, 1.09)	5	0.73 (0.25, 2.10)	
T3	23	1.11 (0.67, 1.84)	10	2.08 (0.91, 4.75)	9	0.89 (0.42, 1.86)	4	0.92 (0.30, 2.82)	
p-trend		0.67		0.11		0.80		0.93	0.49

(continued)

Table 3. Continued

Pesticide	OVERALL		NEVER		FORMER		CURRENT		
	$n = 321$ cancers		$n = 83$ cancers		$n = 161$ cancers		$n = 69$ cancers		
	Cases	RR[a] (95% CI)	Cases	RR[b] (95% CI)	Cases	RR[c] (95% CI)	Cases	RR[b] (95% CI)	p-interaction
Petroleum Oil/Petroleum Distillates[d]									
Non-exposed	132	Ref	36	Ref	73	Ref	20	Ref	
T1	10	0.90 (0.46, 1.77)	2	0.68 (0.16, 2.84)	5	0.71 (0.26, 1.95)	3	2.17 (0.64, 7.33)	
T2	10	0.70 (0.37, 1.34)	1	**	6	0.78 (0.34, 1.80)	3	1.34 (0.39, 4.58)	
T3	11	1.10 (0.59, 2.04)	3	1.17 (0.36, 3.80)	6	1.09 (0.47, 2.51)	2	1.40 (0.32, 6.03)	
p-trend		0.78		0.82		0.83		0.70	0.63
Trifluralin									
Non-exposed	133	Ref	36	Ref	71	Ref	26	Ref	
Q1	34	1.23 (0.83, 1.81)	13	1.39 (0.68, 2.82)	14	1.02 (0.57, 1.84)	7	1.48 (0.60, 3.64)	
Q2	33	0.76 (0.50, 1.17)	9	0.76 (0.34, 1.68)	16	0.64 (0.36, 1.15)	8	1.10 (0.49, 2.49)	
Q3	35	0.89 (0.61, 1.30)	7	0.63 (0.28, 1.43)	21	0.95 (0.57, 1.58)	7	1.17 (0.50, 2.76)	
Q4	34	0.86 (0.58, 1.27)	12	1.14 (0.59, 2.23)	15	0.72 (0.41, 1.29)	7	0.92 (0.37, 2.25)	
p-trend		0.39		0.86		0.35		0.75	0.80

[a]Model adjusted for age, race, state, pack-years of cigarettes and pipe smoking.
[b]Model adjusted for age, race, state.
[c]Model adjusted for age, race, state, pipe smoking.
[d]Detailed information for these chemicals was collected on the take-home questionnaire at enrolment.

bladder cancer, it is perhaps not surprising that smoking may obscure the effect of another exposure, particularly if that effect is weaker than the smoking effect. Recently, a study of agricultural workers in Egypt found that the associations between farming and bladder cancer were more evident among those who never smoked, and there are other historical examples of positive risks for bladder cancer in association with several factors among never smokers.[14,41–43] A common challenge in these studies, as in ours, is the low precision of estimated associations and lack of statistical interaction, given that the number of never smokers who develop bladder cancer is small. Thus, much larger studies will be needed to fully evaluate a relationship between pesticides, smoking and risk of bladder cancer. Along the same lines, studies have also suggested an interaction with smoking for some exposures, where risk can either be potentiated[42] or diminished[44] across smoking strata. These data and ours suggest that evaluating possible bladder cancer risk factors such as pesticides across strata of smoking may provide valuable insights into bladder cancer risk; however, large studies will be needed to be able to detect risks among specific subgroups and true interactions.

Our study had both strengths and limitations. Detailed self-reported pesticide use information, at two points in time, was used to evaluate cancer risk. Information on pesticide use provided by farmers in the AHS has been found to be accurate and reliable,[45,46] allowing for this exploration of the relationship between specific pesticide exposures and bladder cancer risk. Nonetheless, there is potential for exposure misclassification though it is probably non-differential and would bias relative risks toward the null, diminishing any real exposure-response gradients.[47] Smoking status information was collected at enrolment for use in analyses but also reconciled with data from two follow-up questionnaires that allowed us to carefully characterize this important bladder cancer risk factor. In addition, we performed several sensitivity analyses related to smoking, including exploring adjustment for status and intensity and status and duration, which provided comparable results. We also had information on the ever use of other tobacco products reported at enrolment. Using detailed questionnaire data, we were also able to control for several other suggested bladder cancer risk factors, including exposure to diesel exhaust[48] and grinding metal,[49] none of which changed the estimates between pesticide exposures and bladder cancer risk. In addition we were able to take into consideration the use of pesticides that were correlated with the pesticide of interest and, except for where stated (bentazon and bromoxynil), we found only weak correlation among pesticides, whcih did not influence the calculated risk estimates. Although we evaluated a large number of pesticides ($n = 65$), we observed more positive associations than would have been expected by chance alone (6 observed less than $P = 0.05$ and 3 additional borderline positive associations, wheras 3.25 (or 5%) would have been expected by chance, Table 2). Still, we cannot rule out the possibility that some of our findings

Table 4. Cumulative intensity-weighted days for insecticide use and risk of bladder cancer, overall and stratified by smoking status

Pesticide	OVERALL		NEVER		FORMER		CURRENT		
	n = 321 cancers		*n* = 83 cancers		*n* = 161 cancers		*n* = 69 cancers		
	Cases	RR[a] (95% CI)	Cases	RR[b] (95% CI)	Cases	RR[c] (95% CI)	Cases	RR[b] (95% CI)	p-interaction
Aldicarb[d,h]									
Non-exposed	153	Ref	39	Ref	85	Ref	26	Ref	
M1	8	1.18 (0.56, 2.48)	2	1.75 (0.39, 7.94)	3	0.73 (0.22, 2.39)	2	1.42 (0.30, 6.65)	
M2	8	1.25 (0.56, 2.79)	4	4.04 (1.20, 13.57)	2	0.71 (0.17, 2.98)	2	0.81 (0.09, 6.88)	
p-trend		0.58		0.03		0.61		0.84	0.23
Aldrin[e,h]									
Non-exposed	113	Ref	30	Ref	59	Ref	21	Ref	
T1	15	0.88 (0.50, 1.53)	6	1.38 (0.55, 3.48)	9	0.94 (0.46, 1.94)	0	**	
T2	18	1.61 (0.96, 2.68)	1	**	11	1.75 (0.90, 3.40)	6	2.98 (1.15, 7.71)	
T3	17	1.51 (0.89, 2.55)	6	2.30 (0.92, 5.75)	9	1.44 (0.71, 2.96)	2	1.01 (0.23, 4.40)	
p-trend		0.08		0.12		0.21		0.57	0.05
Carbaryl[d,h]									
Non-exposed	73	Ref	23	Ref	34	Ref	14	Ref	
Q1	25	1.10 (0.68, 1.78)	6	0.82 (0.31, 2.17)	15	1.25 (0.66, 2.38)	4	1.25 (0.41, 3.82)	
Q2	28	1.93 (1.21, 3.09)	5	1.06 (0.36, 3.12)	16	2.35 (1.25, 4.41)	7	2.77 (1.10, 7.00)	
Q3	26	1.49 (0.92, 2.41)	6	1.50 (0.57, 3.91)	13	1.38 (0.68, 2.81)	6	1.94 (0.69, 5.42)	
Q4	27	0.91 (0.55, 1.50)	6	0.90 (0.32, 2.53)	18	1.19 (0.60, 2.34)	2	0.34 (0.07, 1.61)	
p-trend		0.29		0.84		0.90		0.08	0.45
Carbofuran[d]									
Non-exposed	206	Ref	50	Ref	110	Ref	46	Ref	
T1	21	0.52 (0.33, 0.82)	4	0.39 (0.14, 1.09)	13	0.55 (0.31, 0.97)	4	0.62 (0.22, 1.73)	
T2	23	0.98 (0.64, 1.51)	12	1.99 (1.06, 3.75)	8	0.65 (0.32, 1.33)	3	0.60 (0.19, 1.92)	
T3	22	0.90 (0.58, 1.40)	11	1.81 (0.94, 3.50)	7	0.55 (0.26, 1.19)	4	0.73 (0.26, 2.05)	
p-trend		0.77		0.03		0.12		0.51	0.04
Chlordane[e,h]									
Non-exposed	120	Ref	33	Ref	60	Ref	24	Ref	
T1	14	1.21 (0.69, 2.12)	1	0.35 (0.05, 2.56)	12	1.75 (0.94, 3.26)	1	**	
T2	15	0.78 (0.45, 1.34)	3	0.62 (0.19, 2.03)	10	0.93 (0.47, 1.82)	2	0.66 (0.16, 2.83)	
T3	15	1.46 (0.85, 2.52)	6	2.83 (1.16, 6.90)	8	1.34 (0.64, 2.84)	1	**	
p-trend		0.24		0.02		0.55		**	0.27
Chlorpyrifos[f]									
Non-exposed	200	Ref	45	Ref	117	Ref	38	Ref	
Q1	22	0.67 (0.43, 1.05)	8	1.02 (0.47, 2.21)	7	0.34 (0.16, 0.73)	7	1.34 (0.60, 3.00)	
Q2	23	0.84 (0.54, 1.31)	6	0.86 (0.37, 2.01)	7	0.43 (0.18, 0.99)	10	2.08 (1.03, 4.17)	
Q3	23	0.99 (0.64, 1.54)	11	1.86 (0.96, 3.61)	10	0.74 (0.37, 1.46)	2	0.55 (0.13, 2.31)	
Q4	23	0.69 (0.45, 1.06)	10	1.23 (0.62, 2.44)	9	0.50 (0.25, 0.98)	4	0.54 (0.19, 1.53)	
p-trend		0.14		0.42		0.06		0.19	0.01
Coumaphos[f]									
Non-exposed	245	Ref	74	Ref	121	Ref	50	Ref	
M1	8	0.49 (0.24, 0.99)	2	0.36 (0.09, 1.49)	4	0.46 (0.17, 1.25)	2	0.78 (0.19, 3.20)	
M2	11	1.79 (0.98, 3.27)	0	**	9	2.91 (1.48, 5.73)	2	1.66 (0.40, 6.86)	
p-trend		0.09		**		0.003		0.50	0.07
Diazinon[f,h]									
Non-exposed	133	Ref	39	Ref	70	Ref	22	Ref	
T1	11	0.76 (0.41, 1.40)	1	**	8	0.99 (0.47, 2.06)	2	0.97 (0.23, 4.11)	
T2	10	0.52 (0.26, 1.04)	1	**	6	0.40 (0.14, 1.15)	3	1.56 (0.47, 5.21)	
T3	13	1.03 (0.56, 1.90)	2	0.78 (0.18, 3.35)	7	1.06 (0.47, 2.37)	3	1.07 (0.24, 4.66)	
p-trend		0.96		**		0.95		0.86	0.34

(continued)

Table 4. Continued

Pesticide	OVERALL		NEVER		FORMER		CURRENT		
	$n = 321$ cancers		$n = 83$ cancers		$n = 161$ cancers		$n = 69$ cancers		
	Cases	RR[a] (95% CI)	Cases	RR[b] (95% CI)	Cases	RR[c] (95% CI)	Cases	RR[b] (95% CI)	p-interaction
DDT[e,h]									
Non-exposed	102	Ref	31	Ref	48	Ref	21	Ref	
Q1	15	0.96 (0.55, 1.66)	4	0.98 (0.34, 2.86)	11	1.19 (0.61, 2.32)	0	**	
Q2	16	1.43 (0.84, 2.44)	1	**	13	1.97 (1.05, 3.67)	2	1.25 (0.29, 5.41)	
Q3	15	0.76 (0.43, 1.32)	4	0.80 (0.27, 2.34)	6	0.56 (0.24, 1.33)	4	1.24 (0.41, 3.72)	
Q4	16	1.11 (0.64, 1.90)	4	1.29 (0.44, 3.79)	11	1.40 (0.71, 2.73)	1	**	
p-trend		0.78		0.59		0.48		0.34	0.18
DDVP[f]									
Non-exposed	253	Ref	69	Ref	129	Ref	55	Ref	
M1	12	0.85 (0.47, 1.54)	3	0.65 (0.20, 2.08)	8	1.04 (0.51, 2.15)	1	**	
M2	12	0.93 (0.52, 1.67)	4	1.05 (0.38, 2.89)	7	0.97 (0.45, 2.09)	1	**	
p-trend		0.82		0.92		0.94		**	0.77
Fonofos[f]									
Non-exposed	220	Ref	57	Ref	116	Ref	47	Ref	
T1	15	0.72 (0.42, 1.22)	5	0.88 (0.35, 2.23)	7	0.57 (0.26, 1.24)	3	0.93 (0.29, 3.05)	
T2	17	0.92 (0.56, 1.53)	5	1.01 (0.40, 2.57)	9	0.86 (0.43, 1.71)	3	0.92 (0.28, 2.99)	
T3	18	0.92 (0.57, 1.50)	10	2.01 (1.01, 4.00)	7	0.64 (0.30, 1.39)	1	**	
p-trend		0.78		0.05		0.28		0.20	0.37
Heptachlor[e,h]									
Non-exposed	139	Ref	34	Ref	76	Ref	26	Ref	
M1	14	0.82 (0.46, 1.44)	4	0.91 (0.31, 2.66)	7	0.65 (0.30, 1.44)	3	1.49 (0.44, 5.11)	
M2	14	1.10 (0.63, 1.93)	6	1.91 (0.78, 4.70)	8	1.06 (0.51, 2.23)	0	**	
p-trend		0.75		0.15		0.89		**	0.21
Lindane[e]									
Non-exposed	139	Ref	36	Ref	77	Ref	23	Ref	
M1	12	0.77 (0.43, 1.37)	4	0.82 (0.29, 2.32)	5	0.56 (0.22, 1.39)	3	1.49 (0.44, 5.03)	
M2	12	1.43 (0.78, 2.62)	4	2.00 (0.71, 5.63)	6	1.21 (0.53, 2.81)	2	1.62 (0.38, 6.97)	
p-trend		0.27		0.20		0.72		0.45	0.54
Malathion[f,h]									
Non-exposed	49	Ref	17	Ref	24	Ref	7	Ref	
Q1	28	1.00 (0.62, 1.59)	4	0.35 (0.11, 1.11)	17	1.16 (0.62, 2.17)	6	1.88 (0.62, 5.67)	
Q2	27	1.15 (0.71, 1.86)	9	1.09 (0.49, 2.43)	13	1.03 (0.52, 2.04)	5	1.80 (0.57, 5.72)	
Q3	29	1.14 (0.71, 1.83)	9	1.05 (0.45, 2.44)	15	1.13 (0.59, 2.15)	4	1.26 (0.33, 4.90)	
Q4	29	0.95 (0.60, 1.52)	6	0.66 (0.26, 1.71)	19	1.11 (0.60, 2.04)	4	1.17 (0.34, 4.01)	
p-trend		0.73		0.63		0.85		0.82	0.44
Parathion[f,h]									
Non-exposed	148	Ref	41	Ref	77	Ref	27	Ref	
M1	7	1.05 (0.49, 2.26)	2	1.09 (0.26, 4.60)	5	1.28 (0.51, 3.19)	0	**	
M2	8	1.13 (0.55, 2.36)	1	**	5	1.39 (0.54, 3.54)	2	1.54 (0.35, 6.84)	
p-trend		0.74		**		0.90		**	0.62
Permethrin[g]									
Non-exposed	239	Ref	64	Ref	123	Ref	52	Ref	
T1	13	0.92 (0.52, 1.61)	4	0.96 (0.36, 2.65)	7	0.90 (0.42, 1.93)	2	0.79 (0.19, 3.26)	
T2	13	0.45 (0.25, 0.81)	4	0.46 (0.17, 1.28)	5	0.33 (0.13, 0.81)	4	0.75 (0.25, 2.25)	
T3	15	1.11 (0.65, 1.87)	8	2.28 (1.08, 4.82)	5	0.72 (0.30, 1.77)	2	0.62 (0.15, 2.58)	
p-trend		0.93		0.04		0.31		0.49	0.44
Phorate[f,h]									
Non-exposed	115	Ref	30	Ref	62	Ref	21	Ref	
T1	16	0.74 (0.43, 1.27)	4	0.61 (0.21, 1.76)	8	0.66 (0.31, 1.42)	4	1.24 (0.41, 3.73)	
T2	16	0.99 (0.58, 1.69)	3	0.64 (0.19, 2.13)	10	1.13 (0.57, 2.26)	2	0.89 (0.21, 3.87)	

(continued)

Table 4. Continued

Pesticide	OVERALL		NEVER		FORMER		CURRENT		
	$n = 321$ cancers		$n = 83$ cancers		$n = 161$ cancers		$n = 69$ cancers		
	Cases	RR[a] (95% CI)	Cases	RR[b] (95% CI)	Cases	RR[c] (95% CI)	Cases	RR[b] (95% CI)	p-interaction
T3	17	0.98 (0.58, 1.64)	7	1.42 (0.62, 3.28)	8	0.89 (0.42, 1.88)	2	0.71 (0.17, 3.07)	
p-trend		0.96		0.36		0.90		0.62	0.76
Terbufos[f]									
Non-exposed	182	Ref	47	Ref	96	Ref	39	Ref	
T1	29	0.83 (0.56, 1.24)	7	0.76 (0.34, 1.71)	14	0.68 (0.38, 1.20)	8	1.48 (0.68, 3.20)	
T2	30	0.93 (0.63, 1.38)	16	1.77 (0.99, 3.15)	10	0.59 (0.31, 1.14)	4	0.69 (0.24, 1.94)	
T3	30	0.82 (0.55, 1.21)	8	0.80 (0.38, 1.71)	18	0.92 (0.55, 1.55)	4	0.57 (0.20, 1.59)	
p-trend		0.35		0.74		0.81		0.22	0.11
Toxaphene[e,h]									
Non-exposed	135	Ref	30	Ref	77	Ref	25	Ref	
M1	13	1.13 (0.64, 2.01)	6	2.34 (0.97, 5.68)	5	0.74 (0.30, 1.84)	2	1.14 (0.27, 4.86)	
M2	16	1.40 (0.82, 2.39)	7	3.75 (1.57, 8.97)	8	1.10 (0.52, 2.33)	1	**	
p-trend		0.24		0.003		0.80		**	0.09

[a]Model adjusted for age, race, state, pack-years of cigarettes and pipe smoking.
[b]Model adjusted for age, race, state.
[c]Model adjusted for age, race, state, pipe smoking.
[d]Carbamate insecticide.
[e]Organochlorine insecticide.
[f]Organophosphate insecticide.
[g]Pyrethroid insecticide.
[h]Detailed information for these chemicals was collected on the take-home questionnaire at enrolment.

might be due to chance, in particular in some of the stratified analyses where the number of exposed cases is small. Thus, future follow-up in the AHS to further evaluate the relationship between pesticides and bladder cancer, and to evaluate whether smoking modifies this relationship, are anticipated.

In conclusion, we observed increased risk of bladder cancer with two aromatic amine herbicides, the imidazolinone herbicides imazethapyr and imazaquin. The relationship between bladder cancer and imazethapyr, as well as for several other agricultural and general use herbicides, was more apparent among never smokers and highlights the complexity of trying to understand the impact of other exposures on smoking-related cancers. Associations with bladder cancer incidence and use of several chlorinated pesticides, including chlorophenoxy herbicides and organochlorine insecticides, were observed for the first time. Because farmers generally have lower rates of bladder cancer compared with the general population, few studies have explored whether pesticides, which readily pass through the bladder, might be risk factors for bladder cancer. Collectively, our data suggest that pesticide exposure may be an overlooked exposure in bladder carcinogenesis. Future studies with detailed pesticide information on specific active ingredients and those that explore risks across smoking status are needed.

Supplementary Data

Supplementary data are available at *IJE* online.

Funding

This work was supported by the Intramural Research Program of the National Institutes of Health, NCI, Division of Cancer Epidemiology and Genetics (Z01CP010119), NIEHS (Z01ES0490300), the Iowa Cancer Registry (HHSN261201300020I) and Iowa's Holden Comprehensive Cancer Center (P30CA086862) as well as the NIEHS-funded Environmental Health Sciences Research Center at the University of Iowa (P30ES005605).

Acknowledgement

We thank the participants of the Agricultural Health Study.

Conflict of interest: None declared.

References

1. Ferlay J, Soerjomataram I, Ervik M *et al*. *Cancer Incidence and Mortality Worldwide*. IARC CancerBase No. 11. Lyon, France: International Agency for Research on Cancer, 2013.
2. Silverman DT, Devesa SS, Moore LE, Rothman N. Bladder cancer. In: Schottenfeld D, Fraumeni JF Jr (eds). *Cancer Epidemiology and Prevention*. 3rd edn. New York, NY: Oxford University Press, 2006.

3. Baan R, Straif K, Grosse Y *et al*. Carcinogenicity of some aromatic amines, organic dyes, and related exposures. *Lancet Oncol* 2008;**9:**322–2 3.
4. Acquavella J, Olsen G, Cole P *et al*. Cancer among farmers: a meta-analysis. *Ann Epidemiol* 1998;**8:**64–74.
5. Blair A, Zahm SH, Pearce NE, Heineman EF, Fraumeni JF Jr. Clues to cancer etiology from studies of farmers. *Scand J Work Environ Health* 1992;**18:**209–15.
6. Koutros S, Alavanja MC, Lubin JH *et al*. An update of cancer incidence in the Agricultural Health Study. *J Occup Environ Med* 2010;**52:**1098–105.
7. Ronco G, Costa G, Lynge E. Cancer risk among Danish and Italian farmers. *Br J Ind Med* 1992;**49:**220–25.
8. Mills PK, Kwong S. Cancer incidence in the United Farmworkers of America (UFW), 1987-1997. *Am J Ind Med* 2001;**40:**596–603.
9. Laakkonen A, Pukkala E. Cancer incidence among Finnish farmers, 1995-2005. *Scand J Work Environ Health* 2008;**34:**73–79.
10. Settimi L, Comba P, Bosia S *et al*. Cancer risk among male farmers: a multi-site case-control study. *Int J Occup Med Environ Health* 2001;**14:**339–47.
11. Franceschi S, Barbone F, Bidoli E *et al*. Cancer risk in farmers: results from a multi-site case-control study in north-eastern Italy. *Int J Cancer* 1993;**53:**740–45.
12. Brownson RC, Reif JS, Chang JC, Davis JR. Cancer risks among Missouri farmers. *Cancer* 1989;**64:**2381–86.
13. Reif J, Pearce N, Fraser J. Cancer risks in New Zealand farmers. *Int J Epidemiol* 1989;**18:**768–74.
14. Kabat GC, Dieck GS, Wynder EL. Bladder cancer in nonsmokers. *Cancer* 1986;**57:**362–67.
15. Amr S, Dawson R, Saleh DA *et al*. Agricultural workers and urinary bladder cancer risk in Egypt. *Arch Environ Occup Health* 2014;**69:**3–10.
16. La Vecchia C, Negri E, D'Avanzo B, Franceschi S. Occupation and the risk of bladder cancer. *Int J Epidemiol* 1990;**19:**264–68.
17. Silverman DT, Levin LI, Hoover RN, Hartge P. Occupational risks of bladder cancer in the USA: I White men. *J Natl Cancer Inst* 1989;**81:**1472–80.
18. Settimi L, Comba P, Carrieri P *et al*. Cancer risk among female agricultural workers: a multi-center case-control study. *Am J Ind Med* 1999;**36:**135–41.
19. Viel JF, Challier B. Bladder cancer among French farmers: does exposure to pesticides in vineyards play a part? *Occup Environ Med* 1995;**52:**587–92.
20. Forastiere F, Quercia A, Miceli M *et al*. Cancer among farmers in central Italy. *Scand J Work Environ Health* 1993;**19:**382–89.
21. Kristensen P, Andersen A, Irgens LM, Laake P, Bye AS. Incidence and risk factors of cancer among men and women in Norwegian agriculture. *Scand J Work Environ Health* 1996; **22:**14–26.
22. Cassidy A, Wang W, Wu X, Lin J. Risk of urinary bladder cancer: a case-control analysis of industry and occupation. *BMC Cancer* 2009;**9:**443.
23. Parkash O, Kiesswetter H. The role of urine in the etiology of cancer of the urinary bladder. *Urol Int* 1976;**31:**343–48.
24. Koutros S, Lynch CF, Ma X *et al*. Heterocyclic aromatic amine pesticide use and human cancer risk: results from the U.S. Agricultural Health Study. *Int J Cancer* 2009;**124:**1206–12.
25. Alavanja MC, Sandler DP, McMaster SB *et al*. The Agricultural Health Study. *Environ Health Perspect* 1996;**104:**362–69.
26. 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.
27. 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.
28. USA Environmental Protection Agency. *Health Effects Division (HED) Risk Assessment for Imazethapyr*. Report No.: EPA-HQ-OPP-2002-0189-0003. Washington, DC: EPA, 2002.
29. USA Environmental Protection Agency. *Imazaquin and Its Salts: HED Chapter of the Tolerance Reassessment Eligibility Decision (TRED)*. Report No.: EPA-HQ-OPP-2005-0478-0005. Washington, DC: EPA, 2005.
30. U.S.Environmental Protection Agency. *Reregistration Eligibility Decision (RED): Bentazon*. Report No.: EPA 738-R-94-029. Washington, DC: EPA, 1994.
31. Koutros S, Baris D, Fischer A *et al*. Differential urinary specific gravity as a molecular phenotype of the bladder cancer genetic association in the urea transporter gene, SLC14A1. *Int J Cancer* 2013;**133:**3008–13.
32. U.S.Environmental Protection Agency. *Reregistration Eligibility Decision (RED): Bromoxynil*. Report No.: EPA738-R-98-013. Washington, DC: EPA, 1998.
33. National Toxicology Program. Bioassay of chloramben for possible carcinogenicity. *Natl Cancer Inst Carcinog Tech Rep Ser* 1977;**25:**1–90.
34. International Agency for Research on Cancer. *Occupational Exposures in Insecticide Application, and Some Pesticides*. Lyon, France: IARC, 1991.
35. Boers D, Portengen L, Bueno-de-Mesquita HB, Heederik D, Vermeulen R. Cause-specific mortality of Dutch chlorophenoxy herbicide manufacturing workers. *Occup Environ Med* 2010;**67:**24–31.
36. Kogevinas M, Becher H, Benn T *et al*. Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. An expanded and updated international cohort study. *Am J Epidemiol* 1997;**145:**1061–75.
37. Becher H, Flesch-Janys D, Kauppinen T *et al*. Cancer mortality in German male workers exposed to phenoxy herbicides and dioxins. *Cancer Causes Control* 1996;**7:**312–21.
38. Knapp DW, Peer WA, Conteh A *et al*. Detection of herbicides in the urine of pet dogs following home lawn chemical application. *Sci Total Environ* 2013;**456–7:**34–41.
39. Glickman LT, Raghavan M, Knapp DW, Bonney PL, Dawson MH. Herbicide exposure and the risk of transitional cell carcinoma of the urinary bladder in Scottish Terriers. *J Am Vet Med Assoc* 2004;**224:**1290–97.
40. U.S.Environmental Protection Agency. *Evaluation of the Carcinogenic Potential of Diclofop-Methyl (Second Review)*. Report No.: HED DOC. NO. 014172. Washingon, DC: EPA, 2000.
41. Escolar PA, Gonzalez CA, Lopez-Abente G *et al*. Bladder cancer and coffee consumption in smokers and non-smokers in Spain. *Int J Epidemiol* 1993;**22:**38–44.

42. Hoover RN, Strasser PH. Artificial sweeteners and human bladder cancer. Preliminary results. *Lancet* 1980;**1:**837–40.
43. Mills PK, Beeson WL, Phillips RL, Fraser GE. Bladder cancer in a low risk population: results from the Adventist Health Study. *Am J Epidemiol* 1991;**133:**230–39.
44. Silverman DT, Samanic CM, Lubin JH *et al*. The Diesel Exhaust in Miners study: a nested case-control study of lung cancer and diesel exhaust. *J Natl Cancer Inst* 2012;**104:**855–68.
45. 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:**94–99.
46. Hoppin JA, Yucel F, Dosemeci M, Sandler DP. 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.
47. Blair A, Thomas K, Coble J *et al*. Impact of pesticide exposure misclassification on estimates of relative risks in the Agricultural Health Study. *Occup Environ Med* 2011;**68:**537–41.
48. Boffetta P, Silverman DT. A meta-analysis of bladder cancer and diesel exhaust exposure. *Epidemiology* 2001;**12:**125–30.
49. Colt JS, Friesen MC, Stewart PA *et al*. A case-control study of occupational exposure to metalworking fluids and bladder cancer risk among men. *Occup Environ Med* 2014;**71:**667–74.