2008

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Environmental Research 108 (2008) 400–403

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

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ARTICLE INFO

Article history:
Received 3 April 2008
Received in revised form
10 July 2008
Accepted 23 July 2008
Available online 17 September 2008

Keywords:
Chlorothalonil
Pesticides
Fungicides
Cancer
Agriculture

ABSTRACT

Background: Chlorothalonil is a broad spectrum, non-systemic fungicide widely used to control diseases affecting over 50 fruit, vegetable, and agricultural crops. Despite its extensive use for over 30 years, little is known about the potential human carcinogenicity associated with the routine application of chlorothalonil. Rodent studies have shown evidence of renal tubular carcinomas and adenomas. We explored cancer incidence with chlorothalonil exposure using data from the Agricultural Health Study, a prospective cohort of licensed pesticide applicators in Iowa and North Carolina.

Methods: Licensed private and commercial pesticide applicators were recruited into this study from 1993 to 1997. Detailed information regarding pesticide use was obtained via self-administered questionnaires. Cancer incidence was followed through December 31, 2004. Chlorothalonil exposure was classified by lifetime exposure days and intensity-weighted lifetime exposure days, and then categorized into tertiles. The intensity-weighted lifetime exposure days metric was calculated based on a complex algorithm which includes pesticide application methods among other factors. This may increase or decrease exposure.

Results: Of the 47,625 pesticide applicators included in this analysis, 3657 applicators reported using chlorothalonil with a median of 3.5 application days per year. Chlorothalonil was not associated with overall cancer incidence, nor did we find any association with colon, lung, and prostate cancers—the only cancers for which we had sufficient numbers to explore associations.

Conclusion: We did not find any strong evidence for an association between chlorothalonil and the cancers investigated. Although animal studies have suggested renal cancer may be associated with chlorothalonil, we had insufficient data to evaluate this cancer.

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1. Introduction

Exposure to pesticides is recognized as an important environmental risk factor associated with the development of cancer (Alavanja and Bonner, 2005; Miligi et al., 2006). Despite this knowledge, there are insufficient data on many commercially available pesticides. The Agricultural Health Study (AHS) is a prospective cohort study designed to evaluate both cancer and non-cancer outcomes in pesticide applicators to better understand risk factors for disease. Increased rates for certain cancers among farmers such as leukemia, multiple myeloma, lip, prostate, and stomach have prompted several studies including the AHS (Alavanja et al., 1996; Blair et al., 1992). Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile), CAS number 1897-45-6, is a broad spectrum, non-systemic pesticide that is used primarily as a fungicide and mildewicide (US Environmental Protection Agency (EPA), 1999; Wilkinson and Killeen, 1996). It has been widely used to control diseases affecting more than 50 fruit, vegetable, and agricultural crops and on turf, lawn, and ornamental plants for over 30 years. The most popular uses of chlorothalonil in the US include application to peanuts (about 34% of total chlorothalonil used in the US), potatoes (about 12%), tomatoes (about 7%), and golf courses (about 10%) (US EPA, 1999). Because this fungicide is non-systemic, it is often applied several times a season to the same crop.

Chlorothalonil is resistant to hydrolysis, photolysis, and volatilization. It is also persistent in water when microbial activity is limited. It has been found to be practically or relatively non-toxic

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0013-9351/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.envres.2008.07.018

to avian species, small mammals, and honey bees; however, chlorothalonil is highly toxic in amphibians, crustaceans, and fish (US EPA, 1999). The mechanisms of action for chlorothalonil are unknown, but some sources suggest that chlorothalonil is a multi-site inhibitor affecting various enzymes and other metabolic processes in fungi (Ministry of Agriculture Food and Fisheries of British Columbia, 2004).

The US EPA has classified chlorothalonil as a probable carcinogen (B2), based on sufficient evidence of carcinogenicity from animal studies but no epidemiologic data are available (Orme and Kegley, 2006; US EPA, 1999). The International Agency for Research on Cancers (IARC), on the other hand, has classified chlorothalonil as a possible carcinogen (2B). The most recent monograph (1999) indicates that there are no data available on human carcinogenicity.

Studies in rodents have shown renal tubular adenomas and carcinomas in male rats and mice and in female rats (IARC, 1999). The metabolism of chlorothalonil in rats, by the action of γ-glutamyl transpeptidase and cysteine-conjugate β-lyase resulting in the production of di- and tri-thiols, is thought to be responsible for the toxicity seen in the kidneys. These enzymes may be less active in humans than in rats (IARC, 1999).

The limited data on chlorothalonil in humans, the reported results of animal studies, and the widespread use of this pesticide in agriculture in the United States prompted us to investigate cancer incidence among pesticide applicators exposed to chlorothalonil in the AHS cohort.

2. Materials and methods

2.1. Cohort enrollment and follow-up

The AHS is a prospective cohort study of 57,311 applicators living in Iowa and North Carolina who apply restricted-use pesticides. The participants were recruited between 1993 and 1997 and include private applicators (primarily farmers) and commercial applicators (employees of pest control companies or businesses that use pesticides; from Iowa only). Farmers and commercial pesticide applicators were identified when they sought restricted-use pesticide licenses from their respective states. This cohort represented approximately 82% of eligible applicators from both states during the enrollment period.

The cohort is linked annually with the state population-based cancer registries in Iowa and North Carolina to assess incident cancer cases. Incident cancer cases between enrollment and December 31, 2004 were identified among study participants and reported using the International Classification of Diseases for Oncology, 2nd edition (ICD-O-2). Those cohort subjects who were alive but no longer residing in Iowa or North Carolina were identified as no longer living in the study area through current address records of the Internal Revenue Service, motor vehicle registration offices, and pesticide license registries of the state agricultural departments. Person-years accumulation for cancer incidence of individuals who had moved from the state was censored in the year they departed. Less than 2% of the cohort was lost to follow-up. The mean time of follow-up was 9.2 years. All participants provided verbal informed consent, and the protocol was approved by the institutional review boards of the National Cancer Institute, Batelle, the University of Iowa, and Westat.

2.2. Exposure assessment

Exposure to chlorothalonil was assessed via a self-administered enrollment questionnaire. Comprehensive exposure information, including days of use per year and years of use, was obtained for chlorothalonil, along with 49 other frequently used pesticides. Exposure to chlorothalonil was quantified using information from this questionnaire. The questionnaire may be accessed at http://aghealth.nci.nih.gov/questionnaires.html.

Two chlorothalonil exposure metrics were used in our analyses: lifetime exposure days (LD) and intensity-weighted lifetime exposure days (IWLD). To calculate LD we multiplied the number of application days per year by the number of years of application. The IWLD metric was calculated by multiplying the LD by an intensity score devised by an AHS industrial hygienist (M.D.) and was calculated based on the following algorithm: intensity score = (mixing status+application method+equipment repair) × use of personal protective equipment (PPE) (Dosemeci et al., 2002).

2.3. Statistical analysis

We carried out Poisson regression analyses to evaluate the risk of all cancers combined and specific cancers with chlorothalonil exposure. Cancer sites were selected for analysis if there were 5 or more incident cases of a given cancer for each category of chlorothalonil-exposed subjects and 20 cases of the cancer overall. Four cancer sites met these criteria—all cancers combined, colon, lung, and prostate cancers. Although kidney cancer was the only cancer for which a priori evidence of carcinogenic effects existed, we were unable to evaluate this cancer in this analysis due to the low counts among exposed individuals ($n = 7$).

Prevalent cancer cases identified at or prior to enrollment in this study were excluded from these analyses. Additionally, 2430 applicators were excluded because they did not provide complete information on chlorothalonil use (i.e. missing data on ever/never use, use per year and number of years used), which is required to accurately and consistently calculate the exposure metrics. We included 47,625 applicators (43,968 non-exposed and 3657 exposed) in our analysis.

Chlorothalonil exposure was categorized into tertiles for both LD and IWLD, and comparisons were made to both the lowest exposed tertile and the non-exposed group. Potential confounders identified based on the literature and biological plausibility included: age, smoking history, cancer history of first-degree relative, residence (Iowa or North Carolina), and applicator type (commercial or private). We also considered other pesticides as potential confounders by investigating the correlation coefficients between chlorothalonil (IWLD) and all 49 other pesticides (IWLD) in the AHS. Highly correlated pesticides were identified as those with a correlation coefficient greater than 0.50 and are listed in Table 1. However, when we included these pesticides in our models, they did not significantly change the rate ratios by more than 10%; therefore, we did not include them in our final models. Final models were adjusted for age as a categorical variable (<40, 40–49, 50–59, $\geqslant 60$), smoking history (never, low <12 pack-years, high $\geqslant 12$ pack-years), family history of cancer in first-degree relatives (yes/no), state of residence (Iowa or North Carolina), and applicator type (private, commercial). Further adjustment for race, sex, education level, and alcohol consumption did not change parameter estimates by more than 10% so we did not include them in the final models. Separate analyses were performed using the non-exposed and lowest exposed groups as referent groups. *P*-values for trend (*p*-trend) were calculated using chlorothalonil as a categorical variable, and all statistical tests were two-sided.

All statistical analyses were conducted using Stata® statistical software program (release 9.0; Stata Corporation, College Station, Texas). We used the P1REL0502 release of the AHS database.

3. Results

To determine the most appropriate reference group, we evaluated selected characteristics of the chlorothalonil exposed (lowest tertile and highest two tertiles) and non-exposed applicators in the AHS cohort. These results are presented in Table 1. The cohort was comprised primarily of white, male, private applicators with relatively low smoking rates. Exposed and non-exposed groups were similar with regard to age, gender, race, alcohol consumption, and family history of cancer. Although the lowest exposed tertile was more comparable to the highest two tertiles with respect to residence (Iowa or North Carolina) and education level, there was not a clear distinction as to which group would be a more appropriate referent. As such, separate analyses were performed using the non-exposed and lowest exposed groups as the referent.

Table 2 displays adjusted rate ratios (RR) and confidence intervals (CI) for selected cancer sites with respect to chlorothalonil IWLD. As mentioned previously, we also carried out Poisson regression analyses for the LD metric but only present results for IWLD. For all cancers combined ($n = 2457$), there was no statistically significant increased risk of cancer associated with chlorothalonil exposure for comparison to the non-exposed (highest tertile: RR = 1.04, 95% CI = 0.83–1.32) and the lowest exposed (third tertile: RR = 0.95, 95% CI = 0.68–1.32) reference groups. There were statistically significant elevated RRs for lung cancer when the non-exposed group was the reference group in the second tertile of both IWLD (RR = 1.95, 95% CI = 1.18–3.22; *p*-trend = 0.19) and LD (RR = 1.75, 95% CI = 1.06–2.90, *p*-trend = 0.10). However, the association was diminished in

Table 1
Characteristics of applicators by chlorothalonil exposure based on 1993–1997 enrollment data in the agricultural health study

Characteristics	Non-exposed group, no. (%) ($n = 43{,}968$)	Lowest exposed tertile, no. (%) ($n = 1240$)	Highest two tertiles, no. (%) ($n = 2417$)
Age			
<40	15,017 (34.2%)	483 (39.0%)	906 (37.5%)
40–49	12,654 (28.8%)	314 (25.3%)	711 (29.4%)
50–59	8997 (20.5%)	214 (17.3%)	420 (17.4%)
⩾60	7300 (16.6%)	229 (18.5%)	380 (15.7%)
Race[a]			
White	43,199 (98.4%)	1165 (94.0%)	2270 (94.0%)
Other	692 (1.6%)	74 (6.0%)	146 (6.0%)
Sex			
Male	42,838 (97.4%)	1174 (94.7%)	2349 (97.2%)
Female	1,130 (2.6%)	66 (5.3%)	68 (2.8%)
State of Residence			
Iowa	32,373 (73.6%)	357 (28.8%)	338 (14.0%)
North Carolina	11,595 (26.4%)	883 (71.2%)	2079 (86.0%)
Educational level[a]			
High school/GED or less	23,933 (55.5%)	578 (48.2%)	1141 (48.5%)
Beyond high school	19,223 (44.5%)	621 (51.8%)	1213 (51.5%)
Applicator type[b]			
Commercial	4127 (9.4%)	145 (11.7%)	238 (9.8%)
Private	39,841 (90.6%)	1095 (88.3%)	2179 (90.2%)
Smoking history			
Never	24,517 (55.8%)	566 (45.6%)	1144 (55.1%)
Low (<12 pack-yrs)	9944 (22.6%)	337 (27.2%)	564 (22.8%)
High (⩾12 pack-yrs)	9507 (21.6%)	337 (27.2%)	709 (29.3%)
Current alcohol consumption[a]			
Never	13,019 (29.8%)	493 (40.0%)	917 (38.2%)
Ever	30,729 (70.2%)	740 (60.0%)	1484 (61.8%)
Family history of cancer in first-degree relatives			
No	26,359 (60.0%)	764 (61.6%)	1467 (60.7%)
Yes	17,609 (40.0%)	476 (38.4%)	950 (40.0%)
Use of highly correlated pesticides (ever use)			
Dieldrin	682 (3.5%)	30 (5.6%)	21 (2.2%)
Heptachlor	2349 (12.2%)	57 (10.6%)	32 (3.3%)
Ziram	66 (0.3%)	10 (1.9%)	21 (2.2%)

[a] Values do not equal the totals (n) due to missing values.
[b] "Private applicators" refers primarily to individual farmers and "Commercial applicators" refers to professional pesticide applicators.

the highest tertiles for each exposure metric, and tests for trend were not statistically significant. For all other cancers investigated, no elevated risks were found for comparison to either reference group. The results for lifetime days were similar (data not shown).

Because chlorothalonil is a fungicide, the average number of days of application per year are few (median of 3.5 days; range: 0.5–25 days), thus the lifetime days metric is weighted more by years of use than frequency of use (days per year). To assess whether frequency of use was a better exposure metric, we ran an additional analysis based on the days of use (average days applied/year). The results for this analysis were similar for LD and for those presented in Table 2 for IWLD.

Additionally, we repeated our analyses for the selected cancer sites, restricting the data to applicators in North Carolina only (data not shown) because chlorothalonil use was uncommon in Iowa. The results were similar to those presented in Table 2.

4. Discussion

In this study, we evaluated the association between cancer incidence and chlorothalonil exposure using a number of exposure metrics (LD, IWLD, and average days/year). None of these metrics provided evidence for an association between chlorothalonil and the four cancer sites analyzed. Although RRs for lung cancer were elevated in the second tertile of IWLD when the reference group was the non-exposed applicators, the RRs for the third tertiles were not elevated, and tests for trend were not statistically significant.

This study has several major strengths. The large, prospective design of the AHS separates this from other pesticide-related studies. Exposure information was collected prior to cancer diagnosis which minimized recall bias, and the comprehensive questionnaire data enabled us to categorize chlorothalonil exposure into tertiles, providing greater discrimination between high and low exposures. In general, farmers provide accurate and reliable information regarding their pesticide use (Blair et al., 2002; Hoppin et al., 2002). Additionally, detailed information on the use of many common pesticides and lifestyle characteristics allowed us to adjust for potential confounding factors. Case identification through cancer registries provides a consistency in the outcome measures.

Limitations of this study include the relatively small number of incident cases of cancer in the chlorothalonil exposed population ($n = 4375$), thus precluding our ability to evaluate less common and potentially relevant cancer sites (e.g. kidney cancer). As mentioned previously, the most recent IARC monograph for

Table 2
Rate ratios[a] for selected cancers through December 2004 by intensity-weighted lifetime exposure days (IWLD)[b] of exposure to chlorothalonil[c] among agricultural health study cohort applicators

Cancer Site	IWLD Exposure	Cases (*n*)	Non-exposed referent		Lowest exposed referent	
			RR	95% CI	RR	95% CI
			p-trend[d]		*p*-trend[d]	
All cancer	No exposure	2457	1.00 (referent)			
	<70	75	1.13	0.90–1.43	1.00 (referent)	
	70–368	77	1.11	0.88–1.39	1.00	0.72–1.38
	>368	78	1.04	0.83–1.32	0.95	0.68–1.32
			p-trend = 0.36		*p*-trend = 0.75	
Colon	No exposure	187	1.00 (referent)			
	<70	7	1.41	0.65–3.02	1.00 (referent)	
	70–368	5	0.97	0.39–2.40	0.77	0.24–2.48
	>368	8	1.46	0.70–3.03	1.16	0.39–3.41
			p-trend = 0.31		*p*-trend = 0.77	
Lung	No exposure	226	1.00 (referent)			
	<70	11	1.37	0.74–2.52	1.00 (referent)	
	70–368	17	1.95	1.18–3.22	1.41	0.66–3.02
	>368	10	0.96	0.51–1.83	0.72	0.30–1.72
			p-trend = 0.19		*p*-trend = 0.46	
Prostate	No exposure	1038	1.00 (referent)			
	<70	32	1.21	0.85–1.74	1.00 (referent)	
	70–368	26	0.97	0.65–1.44	0.81	0.48–1.37
	>368	23	0.79	0.52–1.21	0.65	0.37–1.12
			p-trend = 0.47		*p*-trend = 0.12	

[a] RR adjusted for age, smoking history, family history of cancer in first-degree relative, state of residence, and applicator type.
[b] IWLD = years of use × number of days of use each year × intensity score.
[c] Total number exposed to chlorothalonil included in this regression analysis = 2557.
[d] *p*-Values are two sided.

chlorothalonil indicated a lack of human carcinogenic data (IARC, 1999). To our knowledge, this is the first study to use a prospective, population-based sample to evaluate cancer risk and chlorothalonil exposure. Although the number of exposed cases was somewhat limited by the relatively short follow-up time, the prospective design and on-going data collection will allow further analyses as more cases occur.

Acknowledgments

This work was supported by the intramural research program of the National Institutes of Health, National Institute of Environmental Health Sciences and National Cancer Institute.

IRB approvals: All participants provided verbal informed consent prior to participation in this study, and the protocol was approved by the institutional review boards of the National Cancer Institute, Batelle, the University of Iowa, and Westat. These approvals will be provided upon request.

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