Published in final edited form as:
Am J Ind Med. 2010 May ; 53(5): 486–496. doi:10.1002/ajim.20789.

Characteristics of non-participation and potential for selection bias in a prospective cohort study

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Abstract

Background—We investigated the potential for selection bias due to non-participation in the follow-up of a large prospective cohort study.

Methods—Licensed pesticide applicators (52,395 private; 4916 commercial) in the Agricultural Health Study provided demographic, health, and pesticide exposure information at enrollment (1993-1997) and in a five-year follow-up telephone interview. Factors associated with non-participation in the follow-up were identified using multiple logistic regression. Potential for selection bias was evaluated by comparing exposure-disease associations between the entire cohort and the follow-up subset.

Results—Sixty-six percent of private and sixty percent of commercial applicators completed the follow-up interview. Private and commercial applicators who did not complete the follow-up reported at enrollment younger age, less education, lower body mass index, poorer health behaviors but fewer health conditions, and lower pesticide use. Estimates of exposure-disease associations calculated with and without non-participants did not indicate strong selection bias.

Conclusions—Differences between non-participants and participants in the follow-up interview were generally small, and we did not find significant evidence of selection bias. However, the extent of bias may depend on the specific exposure and outcome under study.

INTRODUCTION

In order to provide a basis for drawing valid conclusions, study participants should accurately reflect the exposure and outcome prevalence of the population they represent. Because some participants are inevitably lost to follow-up over the course of a study with multiple follow-up time points, it is important to know whether non-participants differ significantly from participants with respect to either disease or exposure status. Differential loss of participants by exposure and disease status simultaneously can lead to selection bias. [Greenland 1977]

The Agricultural Health Study (AHS) is a longitudinal cohort study that has prospectively collected information on a variety of farm-related exposures and health outcomes for over 57 000 licensed pesticide applicators from North Carolina and Iowa. Having completed the first five-year follow-up, we examine the similarities and differences in characteristics at enrollment between participants and non-participants and determine the extent to which differential losses to follow-up could bias exposure-disease associations.

METHODS

The AHS was designed to examine potential health effects of farm-related exposures, in particular exposure to pesticides. All pesticide applicators applying between 1993 and 1997 for a license to use restricted-use pesticides in North Carolina or Iowa were invited to participate. Applicators in Iowa included both private and commercial applicators; in North Carolina, all applicators were private applicators. Those who agreed (82% of private applicators, 42% of commercial applicators) completed an enrollment questionnaire, which included information on demographic characteristics, health history, and lifetime pesticide use practices. Consistent with approved informed consent procedures for questionnaire data at the time, returning an enrollment questionnaire was considered informed consent. Almost one-half (47%) of these applicators provided additional information in a more detailed take-home questionnaire, which they returned by mail. Questionnaires are available on the study website (http://aghealth.nci.nih.gov/questionnaires.html). The study was approved by the Institutional Review Boards of the National Institutes of Health (Bethesda, Maryland) and its contractors.

Between 1999 and 2003, applicators were contacted by phone for the second phase of the study, which used a Computer Assisted Telephone Interview (CATI) to collect updated information on both pesticide exposure and diagnosis of incident disease. Interviews were scheduled to avoid months of peak farm activity. In Iowa, 70% of interviews were completed between November and March, with virtually no interviews completed in April, May, and October. In North Carolina, 75% of interviews were completed between November and April, with relatively few interviews completed in June, August, or September. Of the 50 766 private and 4775 commercial pesticide applicators who enrolled and were still living at the time of the telephone interview, 66% of private and 60% of commercial applicators completed the follow-up CATI after a median follow-up time of 5.4 years. Non-participants were defined as applicators who enrolled in the first phase of the study by completing the enrollment questionnaire but who did not complete the second phase telephone interview. Deaths within the cohort up to the time of follow-up were identified using state mortality files and the National Death Index, and individuals who had died were excluded from all analyses (N = 1629). Proxy interviews with next of kin were not conducted. Incident cancer cases diagnosed before January 01, 2006 were identified by linkage with the state cancer registries in North Carolina and Iowa. Prevalent cases for all health conditions were those reported at enrollment, and incident cases were those reported after enrollment.

Most non-participation occurred because the applicator refused to be interviewed (15%) or could not be reached (14%). Contact information was missing for 1%, another 1% of non-participation was due to chronic illness or language difficulties, and 2% did not participate for other reasons.

Analyses were performed using the P1REL0712.00 and P2REL0612.03 AHS data release files and SAS, version 9.1 (SAS Institute, Inc., Cary, North Carolina). Reported results were restricted to private pesticide applicators; commercial applicators were investigated separately for comparison and differences are noted. Multiple logistic regression was used to calculate the adjusted odds of non-participation; hence an odds ratio greater than one indicates a greater odds of not participating in the follow-up interview.

In order to estimate the effect that potential selection bias may have on estimates of association, we considered three potential exposure-disease associations: chlorpyrifos with prevalent depression, smoking with prevalent chronic lung disease, and smoking with incident cancer. We compared the results for the entire cohort that was originally enrolled

with results for the sub-cohort that participated in the follow-up interview. For depression and chronic lung disease we conducted cross-sectional analyses using enrollment data, and for overall cancer we used incident diagnosis information that was collected prospectively. While the association of smoking with cancer and chronic lung disease is well established, an association between chlorpyrifos and depression has been suggested but not confirmed. [Aldridge, et al. 2005, Beseler, et al. 2008]

RESULTS

Demographics and Health

Among private pesticide applicators, nonparticipation in follow-up was associated with younger age, non-White race, fewer years of education, and North Carolina residency (Table 1). With regard to household characteristics, non-participants were less likely to have children, although the number of children had no significant influence, and to be unmarried. Growing up on a farm was not associated with participation.

Considering health behaviors, non-response was positively associated with smoking and alcohol use and inversely associated with vegetable consumption and vitamin or mineral supplementation. In contrast, non-participants were less likely to be overweight or obese (body mass index over 25). Leisure time physical activity was not significantly associated with participation.

Information on all health conditions was self-reported at enrollment with the exception of incident cancer, which was obtained from cancer registry files (Table 2). For most health conditions, non-participants were less likely to have reported a condition at enrollment than participants, although many differences were not statistically significant. The only health condition for which we had information on incident diagnoses for both participants and non-participants was cancer. Although prevalent cancer cases were more likely to participate in follow-up than applicators with no cancer diagnosis at enrollment (adjusted odds ratio for nonparticipation (OR) 0.74, 95% confidence interval (CI) 0.63 – 0.87), cancer incidence did not differ significantly by follow-up status (OR 0.93, CI 0.85 – 1.01). Results for additional health conditions that were reported on the take-home questionnaire (and thus available for less than half the cohort) are included in Appendix A.

Pesticide Use

Personally mixing or applying pesticides was significantly associated with participation at follow-up (non-response OR 0.52, CI 0.40 .68) (Table 3). Furthermore, applicators in both states who personally applied their own pesticides less than half of the time were less likely to participate (OR 0.85, CI 0.79 – 0.93 in North Carolina; OR 0.86, CI 0.81 – 0.91 in Iowa). In Iowa participation increased with longer lifetime duration of pesticide application but was not associated with the frequency of pesticide application (days applied per year). On the other hand, in North Carolina lifetime years of application was not associated with participation, but a higher frequency of application was associated with non-participation.

While pesticide use was positively associated with participation, the type of pesticide used appeared to have little impact. One exception was fungicide users in North Carolina, who were slightly more likely to be non-participants in the telephone interview (OR 1.13, CI 1.05 – 1.22). Use of chemical-resistant gloves was associated with decreased odds of non-participation in Iowa but not North Carolina. Larger farm size was associated with increased odds of non-participation in both states, although the association was slightly stronger in North Carolina.

AHS investigators previously developed an algorithm that takes into account factors such as frequency of use, application method, and personal protective equipment to estimate intensity of pesticide exposure.[Dosemeci, et al. 2002, Coble, et al. 2005] Increasing exposure intensity score for all pesticide types was associated with a slightly decreased odds of non-participation in Iowa but not in North Carolina (Appendix B). The method used for pesticide application was not associated with non-participation in North Carolina. In Iowa, nearly all application methods were associated with decreased odds of non-participation, suggesting that this was just a surrogate for applying pesticides.

Characteristics associated with follow-up among commercial applicators were similar to the findings for private applicators although some associations were no longer significant due to the smaller sample size (data not shown). The single exception was that commercial applicators who had grown up on a farm were significantly more likely to participate in the follow-up interview than applicators who had not (OR 0.76, CI 0.63 – 0.93).

Bias Estimation

Exposure-outcome relationships for the original enrolled cohort were compared with results from private pesticide applicators who participated in the follow-up interview (Table 4). We examined two cross-sectional relationships: chlorpyrifos exposure with depression and smoking status with chronic lung disease (excluding asthma). Because we were particularly interested in the effect of losses to follow-up on associations with incident disease in addition to prevalent disease, we also included the association between smoking and incident cancer.

From the analyses reported above, we know that depression, chronic lung disease, and smoking are associated with the probability of follow-up although the association was only marginally significant for depression. For chlorpyrifos, non-participation was more likely among exposed applicators in North Carolina (OR 1.11, 1.04-1.19) but less likely in Iowa (OR 0.90, 0.85-0.94). We therefore examined the chlorpyrifos-depression association separately by state. If selection bias were to affect the point estimates, we would expect that losing exposed controls in North Carolina would increase the observed odds ratio and that losing unexposed controls in Iowa would decrease the observed odds ratio.

The odds ratio for the association of smoking with either chronic lung disease or incident cancer did not differ significantly between the entire cohort and those who completed the follow-up. Likewise, estimates did not differ for the chlorpyrifos-depression association in Iowa. However, in North Carolina there was a non-significant increase in the association between chlorpyrifos and depression for the follow-up cohort (OR 1.22, CI 0.98 – 1.51) compared to the enrollment cohort (OR 1.07, CI 0.90 – 1.27).

DISCUSSION

Overall participation of private pesticide applicators in the telephone interview five years after enrollment was 66%. Patterns of response associated with age, education, and marital status were consistent with what has frequently been observed in other studies.[Benfante, et al. 1989, Shahar, et al. 1996, Osler, et al. 2008, Russell, et al. 2001] Participants in the follow-up interview tended to have healthier behaviors with regard to smoking, alcohol intake, vegetable consumption, and vitamin and mineral supplementation than non-participants. In apparent contrast to these healthy behaviors, we found that body mass index was higher among participants and that individuals who reported a health condition at enrollment were more likely to participate in the follow-up.

These findings together support the "worried ill" hypothesis proposed by Veenstra. [Veenstra, et al. 2006] Having been diagnosed with a health condition, these participants were likely instructed to improve their health habits and would therefore be more likely to report having healthier habits than participants without a diagnosis. Furthermore, these participants may have a greater vested interest in the completion of the study than those without any diagnosis. We also know the health conditions reported at enrollment were not severe enough to interfere with participation, and provided that these conditions did not seriously progress in the intervening time, we would expect that participants who reported a condition at enrollment should be capable of participating in the follow-up interview.

One limitation of this analysis is that for health conditions other than overall cancer we were unable to measure the probability of participation among incident cases. However, it was reassuring to find that incident cancer cases were not significantly different from non-cancer cases in their probability of follow-up at interview. Furthermore, we did not observe a significant effect of selection bias when we examined the association of incident cancer with smoking status in the subset of applicators who completed the follow-up interview. It should be noted that while incident cancer was not significantly associated with follow-up, it is still possible for selection bias to occur. [Greenland 1977]

In addition to having limited information on incident disease status, we also could not adequately determine whether the impact of losses due to fatal incident conditions was similar to that from non-fatal health conditions. The loss of deceased participants would be expected to result in an underestimation of disease incidence, and if these participants were more (or less) likely to report pesticide exposure than those with non-fatal health conditions, this could lead to selection bias. Although we were unable to consider specific causes of death because of the small number of deaths between enrollment and follow-up, a comparison of deceased participants with participants who completed the follow-up did not indicate any remarkable differences. Since the percent of participants who died before follow-up was only 3%, we would not expect this exclusion to result in substantial selection bias.

Applicators were more likely not to participate if they had never mixed or applied pesticides or if they personally applied pesticides less than one-half of the time, consistent with the idea that those with more of a connection to the subject of the study would be more likely to participate. Conversely, larger farm size was associated with increased probability of non-participation, suggesting that how busy a participant was had an effect on participation. The relationship of other measures of pesticide use to likelihood of participation differed between the two states. Overall, participation in the telephone interview was greater in Iowa than in North Carolina. Farming activities differ between the two states. North Carolina has a longer growing season and increased crop variety compared to Iowa. Frequency of pesticide use was higher in North Carolina and was associated with increased probability of non-participation, whereas in Iowa there was no significant association. In Iowa applicators reported a significantly greater number of years of pesticide use at enrollment despite being younger on average, and this measure was proportional to the probability of participation. Overall, there did not appear to be a general trend with respect to the level of pesticide exposure and probability of follow-up. Furthermore, the potential for differential non-response or selection bias is likely to vary for specific pesticides.

Farming status at time of interview might also affect participation rates. We did not have information on pesticide license status at time of follow-up and do not know if non-respondents had disproportionately left farming. However, since nearly 20% of those who completed a follow-up interview were no longer farming at follow-up (11.5% in Iowa and

33.5% in North Carolina), leaving farming cannot entirely explain non-response at follow-up.

We investigated the potential for selection bias to affect estimates of exposure-disease associations in the subset of applicators who completed the follow-up by comparing odds ratio estimates between the original cohort and the CATI interview subset. We observed no significant changes in the estimates for any of the three associations. However, the differential loss of participants with no report of depression who were exposed to chlorpyrifos in North Carolina did increase the association from 1.07 to 1.20.

With the exception of cancer, our exploration of selection bias was based on prevalent conditions reported at enrollment. Incident conditions might have a greater impact. The severity of such conditions and the timing with regard to follow-up interview would no doubt influence participation and in turn the possibility of selection bias. For large cohort studies collecting prospective information on self-reported diseases and exposures, it is important to consider the potential for selection bias to occur and to estimate the extent to which it may bias associations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

AHS	Agricultural Health Study
CATI	Computer Assisted Telephone Interview

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Table 1

General population characteristics of private pesticide applicators and participation in follow-up in the Agricultural Health Study 1993 - 2003.

	Non-participants (%) N = 17,307	Participants (%) N = 33,457	Unadjusted OR	95% confidence interval		Adjusted OR*	95% confidence interval	
Age								
<18	0.3	0.1	3.6	2.3	5.7	2.8	1.8	4.4
19-29	12	7	1.9	1.8	2.1	2.0	1.8	2.1
30-39	26	23	1.2	1.2	1.3	1.2	1.2	1.3
40-49	26	28	1.0	Reference		1.0	Reference	
50-59	19	23	0.9	0.8	0.9	0.8	0.8	0.9
60-69	12	15	0.9	0.8	0.9	0.8	0.7	0.8
07-79	4	4	1.2	1.0	1.3	1.0	0.9	1.1
>=80	0.3	0.3	1.2	0.8	1.7	1.0	0.7	1.5
State								
North Carolina	39	35	1.0	Reference		1.0	Reference	
Iowa	61	65	0.9	0.8	0.9	0.9	0.9	0.9
Race, ethnicity								
White, non-Hispanic	96	97	1.0	Reference		1.0	Reference	
Other	4	3	1.3	1.2	1.4	1.1	1.0	1.3
Education								
Some high school	11	9	1.2	1.1	1.2	1.2	1.1	1.3
Completed high school or GED	51	47	1.0	Reference		1.0	Reference	
Some college	23	25	0.9	0.8	0.9	0.8	0.8	0.8
College	15	19	0.7	0.7	0.8	0.7	0.7	0.7
Gender								
Male	98	97	1.0	Reference		1.0	Reference	
Female	2	3	0.8	0.7	0.9	0.8	0.7	0.9
Children								
No	21	15	1.0	Reference		1.0	Reference	
Yes	79	85	0.7	0.7	0.7	0.8	0.8	0.9
Marital status (at enrollment)								
Married or living as	80	86	1.0	Reference		1.0	Reference	

Am J Ind Med. Author manuscript; available in PMC 2011 October 3.

	Non-participants (%) N = 17,307	Participants (%) N = 33,457	Unadjusted OR	95% confidence interval		Adjusted OR[a]	95% confidence interval	
Other	20	14	1.6	1.5	1.7	1.3	1.3	1.4
Grew up on farm[†]								
No	9	8	1.0	Reference		1.0	Reference	
Yes	91	92	0.9	0.8	1.0	0.9	0.8	1.1
Smoking, total years								
Never	53	56	1.0	Reference		1.0	Reference	
up to 5y	10	10	1.1	1.0	1.2	1.1	1.0	1.2
5 - 15y	15	14	1.2	1.1	1.2	1.2	1.1	1.2
15 - 25y	13	11	1.2	1.1	1.2	1.2	1.2	1.3
more than 25y	9	10	0.9	0.9	1.0	1.1	1.0	1.1
Alcohol								
never	32	35	1.0	1.0	1.1	1.0	0.9	1.0
<=5 drinks/mo	26	29	1.0	Reference		1.0	Reference	
5-10 dpm	12	11	1.2	1.1	1.3	1.1	1.1	1.2
10-30 dpm	15	14	1.2	1.2	1.3	1.2	1.1	1.3
>30 dpm	15	11	1.5	1.4	1.6	1.4	1.3	1.4
Vegetable servings								
Less than 1/day	71	67	1.0	Reference		1.0	Reference	
At least 1/day	29	33	0.8	0.8	0.9	0.9	0.8	0.9
Take supplements[†]								
no	70	66	1.0	Reference		1.0	Reference	
yes	30	34	0.8	0.8	0.9	0.9	0.8	0.9
Exercise (summer)[†]								
None	27	27	1.0	Reference		1.0	Reference	
up to 2 hr/wk	35	37	1.0	0.9	1.0	1.0	0.9	1.1
3 hr or more	38	37	1.0	1.0	1.1	1.0	0.9	1.1
Body mass index[†]								
under 18	0.4	0.2	1.6	0.9	2.8	1.4	0.8	2.5
18 to 24.9	26	22	1.0	Reference		1.0	Reference	

	Non-participants (%) N = 17,307	Participants (%) N = 33,457	Unadjusted OR	95% confidence interval		Adjusted OR*	95% confidence interval	
25 and over	74	78	0.8	0.7	0.9	0.8	0.8	0.9

*Odds ratio (OR) of being a non-participant, adjusted for age, state, education, and smoking

†Asked on take-home questionnaire (Non-participants = 5,514; Participants = 16,674)

Table 2

Health conditions reported at enrollment and participation in follow-up among private applicators in the Agricultural Health Study 1993 - 2003.

	Non-participants (%) N = 17,307	Participants (%) N = 33,457	Adjusted OR*	95% confidence interval	
Cancer					
Hodgkin's disease	0.04	0.1	0.39	0.16	0.94
non-Hodgkin's lymphoma	0.09	0.2	0.67	0.36	1.24
Leukemia	0.05	0.09	0.55	0.24	1.28
Melanoma	2.4	3.0	0.91	0.81	1.04
Other skin cancer	3.7	4.8	0.86	0.78	0.95
Other cancer	1.3	1.9	0.78	0.66	0.92
Any Cancer					
Prevalent	1.3	2.0	0.74	0.63	0.87
Incident	5.6	6.6	0.93	0.85	1.01
Cardiovascular					
Heart disease	4.2	5.3	0.88	0.80	0.98
Pulmonary					
Asthma	5.0	5.5	0.92	0.84	1.00
Other chronic lung disease	3.2	3.8	0.85	0.76	0.95
Kidney					
Kidney disease	0.7	1.0	0.73	0.58	0.92
Endocrine					
Diabetes	2.6	2.9	0.98	0.87	1.11
Neurologic/Psychologic					
Parkinson's disease	0.1	0.1	0.98	0.57	1.69
Depression	3.3	3.9	0.92	0.82	1.02
Nervous disorder	1.8	1.8	1.04	0.89	1.20
Infectious					
Tuberculosis	0.2	0.3	0.64	0.40	1.02
Pneumonia	13	15	0.91	0.86	0.97
Any condition	30	34	0.89	0.86	0.93

* Odds ratio (OR) of being a non-participant, adjusted for age, state, education, and smoking

Table 3

Participation (%) and pesticide exposure among private pesticide applicators in the Agricultural Health Study

	NORTH CAROLINA					IOWA				
	Non-Participants (N = 7,119)	Participants (N=33,457)	Adjusted OR[a]	95% confidence interval		Non Participants (N = 10,188)	Participants (N = 21,089)	Adjusted OR[a]	95% confidence interval	
Ever mixed or applied pesticides										
Never mix or apply	2	1	1.00	Reference		1	0.5	1.00	Reference	
Ever	98	99	0.78	0.60	1.02	99	99	0.52	0.40	0.68
Personally apply pesticides										
Less than half the time	23	20	1.00	Reference		22	19	1.00	Reference	
Half the time or more	73	77	0.85	0.79	0.93	75	79	0.86	0.81	0.91
Lifetime years of mixing										
1 year or less	4	3	1.30	1.07	1.59	2	1	1.08	0.88	1.32
2-5 years	16	13	1.00	Reference		12	9	1.00	Reference	
6-10 years	18	17	0.92	0.82	1.03	16	13	0.95	0.86	1.05
11-20 years	30	31	0.94	0.84	1.04	35	35	0.88	0.80	0.96
21-30 years	19	21	1.00	0.89	1.13	24	27	0.79	0.71	0.87
More than 30 years	11	14	0.95	0.83	1.09	10	14	0.65	0.58	0.74
Days of mixing per year										
Less than 5 days	18	22	1.01	0.91	1.13	17	16	1.02	0.95	1.11
5-9 days	15	18	1.00	Reference		27	27	1.00	Reference	
10-19 days	22	24	1.06	0.95	1.18	32	34	0.95	0.89	1.01
20-39 days	24	20	1.30	1.17	1.44	18	18	0.96	0.89	1.04
40-59 days	9	7	1.38	1.20	1.59	3	3	0.98	0.84	1.13
60-150 days	8	5	1.60	1.37	1.86	2	2	0.91	0.74	1.11
More than 150 days	2	1	1.97	1.48	2.62	0.3	0.3	0.88	0.56	1.40
Ever use[†]										
Fungicides	66	65	1.09	1.02	1.18	16	22	0.77	0.72	0.83
Fumigants	43	47	0.90	0.84	0.97	8	12	0.79	0.72	0.86
Herbicides	94	95	0.85	0.74	0.98	97	98	0.70	0.59	0.83
Insecticides	88	90	0.94	0.84	1.05	92	95	0.73	0.66	0.80

	NORTH CAROLINA					IOWA				
	Non-Participants (N = 7,119)	Participants (N=33,457)	Adjusted OR*	95% confidence interval		Non Participants (N = 10,188)	Participants (N = 21,089)	Adjusted OR*	95% confidence interval	
Uses chemically resistant gloves										
No	49	49	1.00	Reference		16	15	1.00	Reference	
Yes	51	51	1.01	0.94	1.07	84	85	0.88	0.82	0.94
Number of acres farmed										
None or didn't work on farm	8	9	1.13	0.99	1.29	1	1	1.49	1.11	1.99
up to 50 acres	30	37	1.00	Reference		2	2	1.00	Reference	
50 to 1,000 acres	49	46	1.27	1.17	1.37	77	80	1.17	0.97	1.41
more than 1,000 acres	13	7	1.82	1.60	2.06	20	17	1.37	1.12	1.66

*Odds ratio (OR) of being a non-participant, adjusted for age, education, and smoking

†All four pesticide groups modeled simultaneously

Table 4

Disease-exposure associations comparing follow-up participants with the entire population of private pesticide applicators in the Agricultural Health Study 1993 - 2003.

NORTH CAROLINA	Depression						
Chlorpyrifos use	Never	%	Ever	%	Adjusted OR*	95% confidence interval	
Entire cohort							
Never	8960	60	329	59	1.00	Reference	
Ever	6062	40	232	41	1.07	0.90	1.27
Phase 2 participants							
Never	5858	61	212	57	1.00	Reference	
Ever	3753	39	161	43	1.22	0.98	1.51

IOWA	Depression						
Chlorpyrifos use	Never	%	Ever	%	Adjusted OR†	95% confidence interval	
Entire cohort							
Never	16147	57	553	50	1.00	Reference	
Ever	12388	43	558	50	1.29	1.15	1.46
Phase 2 participants							
Never	10728	55	396	50	1.00	Reference	
Ever	8635	45	402	50	1.25	1.08	1.44

BOTH STATES	Chronic lung disease (not asthma)						
Chlorpyrifos use	Never	%	Ever	%	Adjusted OR‡	95% confidence interval	
Entire cohort							
Never	24113	55	701	44	1.00	Reference	
Former	12948	30	634	39	1.47	1.31	1.65
Current	6748	15	276	17	1.42	1.23	1.65
Phase 2 participants							
Never	16290	56	506	45	1.00	Reference	
Former	8743	30	460	41	1.47	1.28	1.68
Current	4042	14	167	15	1.33	1.11	1.60

*Odds ratio (OR) of being a non-participant, adjusted for age (two youngest and two oldest categories combined), education, smoking

†Adjusted for age, education, and smoking

‡Adjusted for age, state, and education

Hollingsworth

Heather A. Pigman
dir 202 898 5814
hpigman@hollingsworthllp.com

January 28, 2016

PRIVILEGED AND CONFIDENTIAL

VIA ELECTRONIC MAIL

Dr. Lorelei Mucci
[redacted]

Re: Monsanto Roundup® Litigation

Dear Dr. Mucci:

This letter confirms that Hollingsworth LLP ("HLLP"), on behalf of Monsanto Company ("Monsanto"), has retained you to provide expert consulting services to HLLP, for the purpose of assisting HLLP in representing Monsanto in connection with potential and/or actual litigation against Monsanto involving injuries allegedly caused by Roundup® and/or glyphosate ("the Litigation"). You acknowledge that you have received, and/or likely will receive, confidential information from HLLP and that you likely will generate work product (orally and/or in writing) to assist us in representing Monsanto in the Litigation. You agree that you will maintain all information exchanged between HLLP and you (whether orally or in writing) as strictly confidential and privileged, unless we inform you, at some time in the future, that certain information needs to be disclosed in the Litigation. You also agree to maintain the fact that you have been retained by HLLP as strictly confidential and privileged, unless we inform you, at some time in the future, that your identity as HLLP's expert has been disclosed in the Litigation. Furthermore, you agree to not do any consulting or other work for any other corporation, law firm, or person with respect to any actual or potential legal claims involving Roundup® and/or glyphosate. You will be compensated at your standard hourly rate for time spent working with HLLP on the Litigation, namely $350.00 per hour.

If you agree to these terms, please sign the letter below and send it back to me. We look forward to working with you.

Hollingsworth

Sincerely,

Heather A. Pigman

SEEN AND AGREED:

Dr. Lorelei Mucci

Message

From: SALTMIRAS, DAVID A [AG/1000] [/O=MONSANTO/OU=NA-1000-01/CN=RECIPIENTS/CN=DASALT]
Sent: 5/13/2012 8:34:43 PM
To: FARMER, DONNA R [AG/1000] [/O=MONSANTO/OU=NA-1000-01/CN=RECIPIENTS/CN=180070]; GOLDSTEIN, DANIEL A [AG/1000] [/O=MONSANTO/OU=NA-1000-01/CN=RECIPIENTS/CN=527246]
Subject: RE: Reviewers of Eriksson (2008) and Hardell & Eriksson (1999)

Thanks Donna!

David Saltmiras, Ph.D., D.A.B.T.
Toxicology Manager
Regulatory Product Safety Center
Monsanto
ph [redacted]

From: FARMER, DONNA R [AG/1000]
Sent: Sunday, May 13, 2012 3:13 PM
To: SALTMIRAS, DAVID A [AG/1000]; GOLDSTEIN, DANIEL A [AG/1000]
Subject: RE: Reviewers of Eriksson (2008) and Hardell & Eriksson (1999)

The review of Eriksson was done by Pam Mink.

Hans-Olav and Dimitrios were good friends of John Acquavella. We worked with them a lot when John was here.

http://www.hsph.harvard.edu/faculty/hansolov-adami/

http://www.hsph.harvard.edu/faculty/dimitrios-trichopoulos/

From: SALTMIRAS, DAVID A [AG/1000]
Sent: Saturday, May 12, 2012 9:47 PM

To: GOLDSTEIN, DANIEL A [AG/1000]; FARMER, DONNA R [AG/1000]
Subject: Reviewers of Eriksson (2008) and Hardell & Eriksson (1999)
Importance: High

Donna and Dan,

I am polishing the carcinogenicity lit review for glyphosate Annex I Renewal. Can you please tell me:

- Who wrote up the attached critique/review of Eriksson (2008)?

- Who are Hans-Olav Adami and Dimitrios Trichopoulos and what are their credentials for the attached Hardell & Eriksson (1999) review?

Thanks,

David Saltmiras, Ph.D., D.A.B.T.
Toxicology Manager
Regulatory Product Safety Center
Monsanto
ph [redacted]

Message

From: AH HOOI LIM
Sent: 6/23/1999 4:49:36 PM
To: RICHARD W SCHUMACHER; B K CHIU; YEEW THAI TENG; GUI MEI CHONG; TET JONG CHANG; ENG GUAN LIM; RICHARD A GREUBEL; DONNA R FARMER
CC: LISA M DRAKE; JOHN F ACQUAVELLA
Subject: Re: Popular herbicide Linked To Cancers

Donna,

Thanks for the info.

Teng and Chang, let's meet on May 28, morning to determine our rebuttal and other communication. Teng, we could use Prestige Communication to handle the media part and I am leaving the contact to you.

Rgds...LAH

_____ Reply Separator _____
Subject: Popular herbicide Linked To Cancers
Author: DONNA R FARMER at MONSL125
Date: 6/22/99 3:58 PM

Rich and all:

In response to your e-mail request for information on what we call the "Swedish or Hardell study." John Acquavella and I have been doing the technical work on this issue. Here is what we have been sending around when we receive information requests.

Let us know if you have further questions or need additional help.

Best Regards,

Donna

**********8

Please find below an update on the Hardell situation

Also please note the following 3 attachments:
critique.doc
letter.doc
newscientist.doc

Please don't hesitate to contact us if you need additional information.

Donna and John
****

WHAT WAS THE EPIDEMIOLOGIC PUBLICATION LINKING GLYPHOSATE AND NON-HODGKINS LYMPHOMA (NHL)?

In April 1999, a Swedish epidemiologic study entitled "A Case-Controlled Study of Non-Hodgkin's Lymphoma and Exposure to Pesticides" was published

in the journal Cancer, by Lennart Hardell. This study found statistically significant associations between NHL and reported use of fungicides and herbicides. "Reported use of" glyphosate, along with reported use of several other herbicides, showed a weak, not statistically significant association with NHL. Despite the obvious weakness of the paper (detailed below), the finding was rapidly picked up in the popular press (e.g. New Scientist) and became the basis for allegations against the safety of Roundup herbicides and, indirectly, against Roundup ready crops.

WHO IS LENNART HARDELL?

Hardell has a long history with Monsanto. Our former colleagues in Solutia tell us that "Hardell hates Monsanto." Hardell started the controversy over dioxin and soft tissue sarcoma 20+ years ago based on some flawed epidemiologic research. He also worked to link Agent Orange to cancer based on its presumed dioxin content. Hardell continues to be an expert witness against Solutia in toxic tort cases. Dr. Ralph Cook, the retired Medical Director of Epidemiology at Dow Chemical, told us that Hardell is very arrogant. He has a history of resorting to ad hominem attacks when challenged by industry, so if he feels threatened he may resort to linking us with old allegations made against Monsanto Chemical Company (on dioxin, PCBs and the like). Our Monsanto registration manager in Sweden has indicated that Hardell is personally calling the journalists to inform them of his study.

Hardell likes being in the middle of controversial issues. He recently released a report linking the use of mobile phones and brain tumors.

WHAT IS INDUSTRY DOING?

A Swedish industry group, IVT, has being trying to arrange a meeting with Hardell. June 1, 1999, IVT received a letter from Hardell in which he claimed too be to busy to meet with industry in June, July and August.

WHAT IS THE CONCLUSION OF OUR INTERNAL EXPERT REVIEW OF HARDELL'S PAPER?

Our Monsanto epidemiologist, John Acquavella, has reviewed the NHL paper and prepared the attached critique (critique.doc). The study was found to have several important limitations including reliance on memory of pesticide use from subjects or their next of kin, inability to control for confounding factors, and the very small number of subjects reporting glyphosate use (4 cases). Dr. Acquavella has concluded that the alleged findings are inconsistent with what is known about glyphosate, and that systematic error or chance seem to be the most likely explanations for the glyphosate findings in this study.

IMPACTS TO DATE?

We are not aware of any documented impact on business. What we do know is the story has been picked up in the popular press. It is also being used against us by groups such as Greenpeace, and it has resulted in numerous inquires around the world.

Feedback from the UK and Swedish regulators indicates that they don't consider the study to be a credible basis for changing their position on glyphosate-containing products.

WHAT HAVE WE DONE TO DEFEND GLYPHOSATE?

Monsanto scientists and a Yale M.D. collaborated to submit a letter to the editor of Cancer critiquing the Hardell paper (see attachment - letter.doc). The letter has been accepted for publication and will appear in the August 15th edition. In addition our former medical director in Europe and two consulting European scientists collaborated to submit a letter to the editor of the New Scientist (see attachment - newscientist.doc, published May 29, 1999).

We have supplied technical support to Monsanto PR, regulatory and business representatives around the world. In addition we have circulated the critique of the paper and the letters to the editors to relevant parties.

We are creating a scientific outreach network of prominent epidemiologists in Europe and the U.S., including Dimitrios Trichopoulos (Harvard/Greece)

and Hans-Olov Adami (Harvard/Sweden), who will assist us in defending glyphosate. We are planning meetings with them and with four prominent epidemiologists in each of the following areas; UK (this meeting is set for August 17th), Scandinavia (targeting Sept.), Italy/Greece (targeting August), Netherlands/France/Germany (targeting Sept.) and the US (targeting October). The purpose of these meetings is to raise awareness of the limitations of Hardell's research and gain support for glyphosate in the epidemiologic community worldwide.

Residential Exposure to Pesticide During Childhood and Childhood Cancers: A Meta-Analysis

Mei Chen, PhD, MS, Chi-Hsuan Chang, MSc, Lin Tao, PhD, Chensheng Lu, PhD, MS

CONTEXT: There is an increasing concern about chronic low-level pesticide exposure during childhood and its influence on childhood cancers.

OBJECTIVE: In this meta-analysis, we aimed to examine associations between residential childhood pesticide exposures and childhood cancers.

DATA SOURCES: We searched all observational studies published in PubMed before February 2014 and reviewed reference sections of articles derived from searches.

STUDY SELECTION: The literature search yielded 277 studies that met inclusion criteria.

DATA EXTRACTION: Sixteen studies were included in the meta-analysis. We calculated effect sizes and 95% confidence intervals (CIs) by using a random effect model with inverse variance weights.

RESULTS: We found that childhood exposure to indoor but not outdoor residential insecticides was associated with a significant increase in risk of childhood leukemia (odds ratio [OR] = 1.47; 95% CI, 1.26–1.72; I^2 = 30%) and childhood lymphomas (OR = 1.43; 95% CI, 1.15–1.78; I^2 = 0%). A significant increase in risk of leukemia was also associated with herbicide exposure (OR = 1.26; 95% CI, 1.10–1.44; I^2 = 0%). Also observed was a positive but not statistically significant association between childhood home pesticide or herbicide exposure and childhood brain tumors.

LIMITATIONS: The small number of studies included in the analysis represents a major limitation of the current analysis.

CONCLUSIONS: Results from this meta-analysis indicated that children exposed to indoor insecticides would have a higher risk of childhood hematopoietic cancers. Additional research is needed to confirm the association between residential indoor pesticide exposures and childhood cancers. Meanwhile, preventive measures should be considered to reduce children's exposure to pesticides at home.

Department of Environmental Health, Harvard T.H. Chan School of Public Health, Boston, Massachusetts

Dr Chen participated in the study conception, design, identification of studies, data collection, study selection, data extraction, data analysis and interpretation, and drafting and revision of the article; Ms Chang participated in data collection, study selection, data analysis, and revision of the article; Dr Tao participated in data collection, study selection, and data analysis; Dr Lu participated in the study conception, design, identification of studies, data collection, study selection, data extraction, analysis, and interpretation, and critical revision of the article; and all authors approved the final manuscript as submitted.

www.pediatrics.org/cgi/doi/10.1542/peds.2015-0006

DOI: 10.1542/peds.2015-0006

Accepted for publication Jul 7, 2015

Address correspondence to Chensheng Lu, PhD, MS, Department of Environmental Health, Harvard T.H. Chan School of Public Health, Landmark Center West 404G, 401 Park Dr, Boston, MA 02215. E-mail: cslu@hsph.harvard.edu

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

Although pesticides are essential for eradication of pests in agriculture and for public health, they are toxic chemicals and can affect children's health in a variety of settings, such as at home, in parks and gardens, and on school grounds. Children greatly increase their chances of pesticide exposure when they play on pesticide-treated surfaces such as a floor or lawn and then put their hands into their mouths. It is known that households with children commonly use and store pesticide products.[1–3] The use of pesticides at child care facilities,[4] on athletic fields,[5] and on school grounds[6] could all present potential exposures and health hazards to children.

Because children's immune systems are still developing, they may provide less protection than adult immune systems. To be specific, their enzymatic and metabolic systems may be less able to detoxify and excrete pesticides than those of adults. Therefore, they are more vulnerable to pesticides. Epidemiologic studies also support the idea that pesticide exposure can have greater impact on children's health than on adults' health.[7,8] Children exposed to pesticides at home or at school have experienced acute toxic effects on their respiratory, gastrointestinal, nervous, and endocrine systems, as well as other serious medical outcomes.[6,9,10] Concern about the health effects of low-level exposure to pesticides in children has been increasing in recent years, generating a substantial number of epidemiologic studies demonstrating associations between pesticide exposures and childhood cancers.[11–16] However, most of these studies focused on parental occupational exposure or agricultural exposure, not exposure in the home. We found a few systematic reviews examining the association between residential pesticide exposure and childhood cancers. But the association was not elucidated in these reviews, because authors included parental occupational exposure data or studies investigating multiple risk factors that increase chance findings through multiple statistical testing.[12–14]

The aim of our study was to perform a systematic review of the currently available epidemiologic evidence to estimate the relationship between residential (or nonoccupational and nonagricultural) childhood pesticide exposure and childhood cancers. We sought to provide scientific evidence for preventive actions and for making legislative decisions.

METHODS

Data Source and Study Selection

We conducted a literature search in PubMed for articles published before February 2014. We used combinations of the following keywords to identify relevant articles: [residential, urban, indoor, house, home, household, domestic or school] AND [pesticide, insecticide, herbicide, fungicide, organochlorine or organophosphorus] AND [children, childhood, youth, teenager, adolescent, toddler, infant, neonate, prenatal or postnatal] AND [cancer, tumor, malignancy, neoplasm, neuroblastoma, lymphoma, leukemia, sarcoma, astrocytoma, glioma, craniopharyngioma, ependymoma, rhabdomyosarcoma or retinoblastoma]. The search was limited to human studies and written in English. All abstracts were screened to determine their suitability for review.

We included original epidemiologic studies reporting on nonoccupational pesticide exposure and children's health. We used the following criteria to exclude articles from the meta-analysis. We excluded those not reporting original results (eg, review articles, ecologic studies, or case reports); toxicological studies; studies conducted in occupational settings, on hazardous waste sites, on farms, or in proximity to agricultural pesticides; studies involving only adults or children with Down syndrome or without reporting children's health outcomes; studies with only pesticides in general (no specific pesticide groups) or studies with a list of chemicals including pesticides; studies without specific windows of exposure; or duplicate studies that included subjects already included in a more complete or more recent study examining a greater number of subjects.

Two authors of this article (M.C. and C.L.) independently retrieved and screened all the titles and abstracts of studies according to the predetermined selection criteria. We also manually screened references in the selected articles for additional relevant studies. The full texts of the studies with potential eligibility were obtained and assessed independently by the 2 authors (M.C. and C.L.) for final inclusion. Any discrepancies were resolved by consensus.

Data Extraction

From each eligible study, 2 authors (M.C. and C.C.) extracted information about the study design, location, study period, study population and control characteristics, exposure assessment method, outcomes, and key findings. The same 2 authors independently extracted and tabulated the most relevant estimators, namely odds ratios (ORs) and 95% confidence intervals (CIs). ORs and CIs are 2 commonly used estimators in most meta-analyses dealing with health risks associated with environmental chemical exposures.[12,13,15,17–21] The results were compared and consensus was obtained before the meta-analysis.

After classification of the studies, the data were subgrouped and calculated by pesticide categories, exposure locations, and type of cancer in the following stratified meta-analyses:

- Pesticide category and exposure locations:
  - Indoor pesticide exposure
    - Indoor insecticide exposure

- Outdoor pesticide exposure
  - Herbicide exposure
  - Outdoor insecticide exposure
- Cancer types: acute leukemia, leukemia, lymphoma, hematopoietic cancers (leukemia and lymphoma), childhood brain tumor, and all childhood cancers (including neuroblastoma, Wilms tumor, and soft tissue sarcoma)

We analyzed data from professional home treatment (ie, the work done by licensed pest control professionals) by performing a meta-analysis on data with professional home treatment together with parental home treatment or by using data for professional home treatments alone (if number of studies was ≥2). We calculated dose effect by performing a separate meta-analysis on data of the highest frequency of pesticide uses.

Data Analysis

We performed the meta-analysis by using the Comprehensive Meta Analysis version 2 (Biostat, Inc, Englewood, NJ) in accordance with Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines.[22] The random effects model was used in this analysis. The random effects summary of ORs and 95% CIs was estimated to provide an indicator of the overall strength of association between childhood pesticide exposure and childhood cancers. These associations are illustrated in the forest plots. In the plots, the CI for each study is represented by a horizontal line and the estimate of summary OR by a box square. The box area is proportional to the weight, which is the inverse of the variance of the effect estimate from each individual study in the meta-analysis. The diamond and broken vertical line for type of cancer represent the subtotal summary estimate, with CI indicated by its width. The null hypothesis is 1 and is represented by the central vertical dashed line from top to bottom of the plot. All statistical tests were 2 sided, and a P value of $<.05$ was considered statistically significant.

Assessment of Heterogeneity

Because the current review includes a limited number of studies, and the conventional statistical approach to evaluating heterogeneity using a χ^2 test (Cochran's Q) has low power when there are few studies,[23] we used the I^2 statistic to quantify the amount of variation in results across studies that is due to heterogeneity. I^2 can be interpreted as a measure of the percentage of the total variation that cannot be explained by chance.[23] An I^2 value of 25%, 50%, or 75% can be taken to mean low, moderate, or high degrees of heterogeneity.[23] A value of 0% indicates no observed heterogeneity, and estimations from either the fixed effects model or random effects model would be the same. The P values for heterogeneity are based on the Q statistic.

Publication Bias

Publication bias was tested with funnel plots and Egger's test.[24] The funnel plot was made by the natural logarithm of the estimate of ORs versus the SE from all included individual studies in a meta-analysis. We tested funnel plot asymmetry, which can result from unpublished small studies without statistically significant effects, by using the linear regression method.[24]

Sensitivity Analysis

To measure the robustness and determine whether some of the factors (or possible biases) have a major effect on the results of this meta-analysis, we conducted several sensitivity analyses by

- Removing the study with highest weight
- Removing the studies reporting extreme ORs (the highest and the lowest)
- Removing hospital-based studies (or performing a meta-analysis including only population-based studies)
- Removing extended exposure windows or ill-defined pesticide categories

RESULTS

Study Identification and Characteristics

Figure 1 describes this study's identification, screening, and selection process. From the initial 277 articles identified from PubMed search, 239 were excluded based on their titles or abstracts, and 17 were excluded based on the full text. We excluded 3 other studies from the analysis. One had a duplicated population, another had a study population located in a region with high agricultural pesticide use, and a third had insufficient data to permit the calculation.[25–27] No additional articles were identified from the references cited in the included articles. A total of 16 articles met the full inclusion criteria and were eventually included in the meta-analysis.[28–43]

The characteristics of the studies used in the meta-analysis are shown in Table 1. All 16 studies are case-controlled studies published between 1993 and 2012. The participation rates for most studies ranged between 65% and 96% for case groups and between 61% and 99% for control groups. The sample sizes ranged from 45[32] to 1184 cases,[38] and the upper age limits of case groups were between 9 and 19 years. Among these studies, 10 focused on hematopoietic malignancies, 5 on childhood brain tumor (CBT), and 2 on Wilms tumor and neuroblastoma. Four other studies reported data on >1 malignancy.[36–38,41]

The current meta-analysis was run separately for the 2 windows of exposure: before and after birth to diagnosis, and after birth to diagnosis. Because the outcomes from either window of exposure were similar (as shown in Supplemental Table 3), the

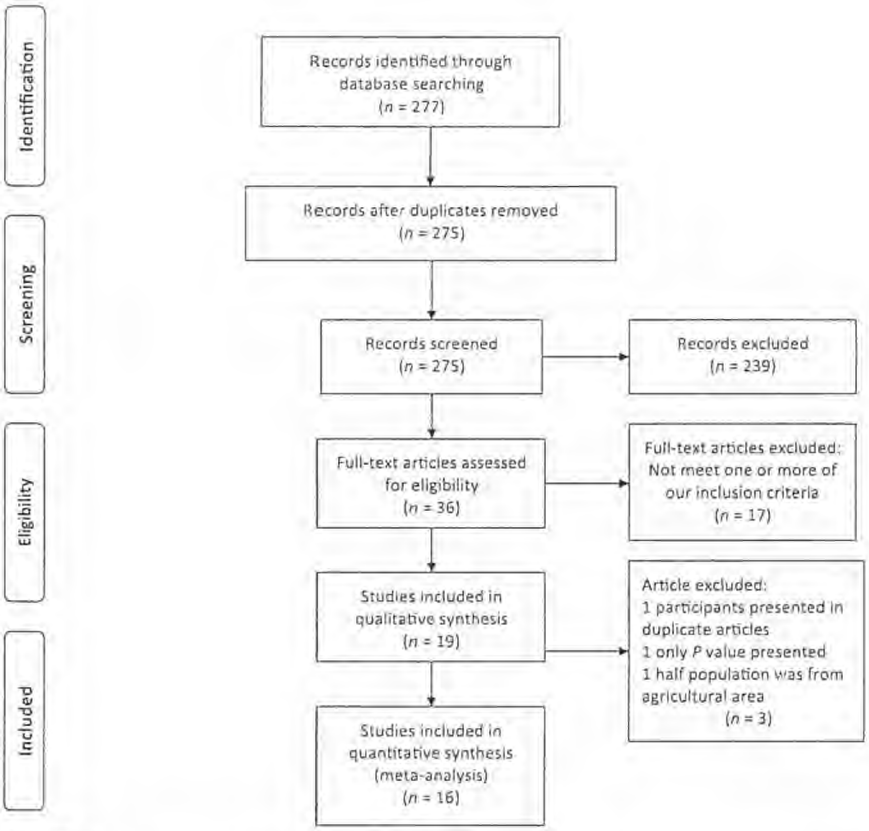

FIGURE 1
PRISMA 2009 Flow Diagram. (Reprinted with permission from Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group. Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med. 2009;6(6):e1000097)

following results and discussion focus on the window from prenatal and after birth until diagnosis.

Publication Bias

We examined the main findings from all studies and included them in an inverse funnel plot of log-transformed odds ratio versus SE. Although we were limited by the small number of studies included, we saw no clear trend of publication bias (or asymmetry) from visual inspection of the plot, with Egger's test *P* values at .92, .10, and .14 for indoor pesticides, herbicides, and outdoor pesticide exposures, respectively.

Study Synthesis

Table 2 summarizes the results of the subgroup meta-analyses and the assessment of heterogeneity. The results of 13 studies on home pesticide exposure, grouped by types of childhood cancer and listed by years of publication, are shown in Fig 2. Exposure to indoor insecticides during childhood was associated with a significant increase in risk of childhood leukemia (OR = 1.47; 95% CI, 1.26–1.72; I^2 = 30%) and childhood lymphomas (OR = 1.43; 95% CI, 1.15–1.78; I^2 = 0%).

Additional subgroup analysis combining studies on acute leukemia (AL) yielded elevated risks for exposure to both home pesticides (OR = 1.55; 95% CI, 1.38–1.75) and indoor insecticides (OR = 1.59; 95% CI, 1.39–1.81) with significantly lower heterogeneities (I^2 of 0%). When we combined studies on leukemia and lymphoma, we observed a statistically significant association between childhood hematopoietic malignancies and home pesticide exposure during childhood (11 out of 12 data were from indoor insecticides). There was low heterogeneity (OR = 1.46; 95% CI, 1.32–1.60; $I^2 \leq 5\%$). A positive but not statistically significant association between home pesticide exposure during childhood and CBT was observed (OR = 1.22; 95% CI, 0.83–1.81; I^2 = 23%) and this association decreased after data were combined with those for professional home treatment (OR = 1.11; 95% CI, 0.87–1.42; I^2 = 5%).

We conducted sensitivity analysis on the results to test whether these results were influenced by 1 or 2 studies (Supplemental Table 3). Sensitivity analysis conducted by removing highest weights, excluding extreme ORs, or deleting hospital and friends controls did not change the associations between home pesticide (or indoor insecticide) exposure and childhood AL, leukemia, lymphoma, and childhood hematopoietic malignancies (shown in Supplemental Table 3), and statistical significance remained. Heterogeneities were significantly lower (most I^2 were 0%) after extreme ORs were removed in the sensitivity analyses. When we replaced the indoor pesticide data of Ma et al[37] with insecticide data in the rerun meta-analysis, the result was very similar. This finding was consistent with the statement by those authors that "there was a considerable overlap between the definition as well as the results between indoor pesticides and insecticides."

Subgroup analysis on dose and multiple-agent effect yielded a statistically significant higher risk for childhood leukemia (OR = 1.92; 95% CI, 1.27–2.89) and hematopoietic malignancies (OR = 2.04; 95% CI, 1.40–2.97). However, when the studies on professional home treatment were grouped together, the seemingly significant increase in risk for childhood leukemia became not statistically significant.

TABLE 1 Overview of the Case-Controlled Studies Included in the Meta-Analysis

Study	Sample Size (case/control)	Age (y)	Study Population, Location, and Period	Exposure Assessment	Cases	Controls
Davis et al (1993), USA	45/85	≤10	Patients in Missouri, diagnosed 1985–1989	Maternal phone interview	CBT	Noncancer friends or other cancer matched with age and gender
Leiss et al (1995), USA	252/222	<15	Patients in Denver, 1976–1983	Parental interview	CBT, Leu, Lym, STS	Noncancer population matched by gender, age, region
Pogoda et al (1997), USA	224/218	≤19	Patients from West Coast, 1984–1991	Maternal phone interview	CBT	Noncancer population matched by gender, age, region
Infante-Rivard et al (1999), Canada	491/491	≤9	Patients from metropolitan Montreal, diagnosed 1980–1993	Parental phone interview	ALL	Noncancer population matched by age, gender, region
Meinet et al (2000), Germany	1184,234, 940/2588	≤15	Patients from West Germany, diagnosed 1992–1994	Mail and parental phone interview	Leu, NHL	Noncancer population matched by gender, age, region
Buckley et al (2000), USA	268/268	≤20	Patients in US, 1986–1990	Maternal phone interview	NHL	Noncancer population matched by age, gender, and race
Daniel et al (2001), USA	390/296	<19	Hospital patients in US and Canada, 1992–1994	Parental phone interview	Neuroblastoma	Noncancer population matched by age, region
Ma et al (2002), USA	162/162	≤14	Hospital patients in northern California, 1995–1999	Maternal in-home personal interview	ALL, Leu	Noncancer population matched by gender, age, mother's race, region
Menegaux et al (2006), France	280/288	<15	Hospital patients in France, diagnosed 1995–1999	Maternal personal interview	AL	Hospital noncancer children matched by age, gender, hospital, race
Rudant et al (2007), France	1060/1681	<15	Patients in France, diagnosed 2003–2004	Maternal phone interview	AL, HL, NHL	Noncancer population matched by age, gender
Urayama et al (2007), USA	294/369	<15	Patients from northern and central California, diagnosed since 1995	In-home interviews with caretaker	ALL	Noncancer children matched by age, gender, Hispanic status, maternal race, region
Cooney et al (2007), USA	523/517	<16	Patients in US and Canada, 1999–2002	Maternal phone interview	Wilms tumor	Noncancer children matched by age and region
Nielsen et al (2010), USA	201/285	≤10	Patients in US west coast, 1984–1991	Maternal in-person interview	CBT	Noncancer children matched by age and gender
Bailey et al (2011), Australia	388/870	<15	Patients in Australia, 2003–2007	Parental questionnaires and phone interviews	ALL	Noncancer population matched by gender, age, region
Ding et al (2012), China	176/180	≤14	Hospital patients in Shanghais China, 2010–2011	Maternal in-person interview and children's urine collections	ALL	Noncancer hospital children matched by gender and age
Greenop et al (2013), Australia	288/917	≤14	Patients in Australia, 2005–2010	Maternal in-person interview	CBT	Noncancer population matched by gender, age, and region

ALL, acute lymphoblastic leukemia; HL, Hodgkin lymphoma; Leu, leukemia; Lym, lymphoma; NHL, non-Hodgkin lymphoma; STS, soft tissue sarcoma.

TABLE 2 Meta-Analysis Using Random Effects Model for the Relationship Between Childhood Cancer and Exposure to Residential Pesticides During Childhood

Subgroup	Study *N*	Summary		Heterogeneity	
		OR	95% CI	*P*	I^2
Indoor pesticides[a,b]					
(A) AL	6	1.59	1.40–1.80	.839	0
Add professional home treatment	7	1.55	1.38–1.75	.794	0
Indoor insecticides	5	1.59	1.39–1.81	.725	0
(B) Leukemia	8	1.48	1.29–1.70	.267	20
Add professional home treatment	9	1.46	1.29–1.65	.327	13
Dose and multiple agents effects[c]	3	1.92	1.27–2.89	.959	0
Professional treatment only	3	2.04*	1.05–3.95	.061	64
Indoor insecticides	7	1.47	1.26–1.72	.197	30
(C) Lymphoma	4	1.43	1.15–1.78	.578	0
Indoor insecticides	4	1.43	1.15–1.78	.578	0
(D) Hematopoietic cancers	12	1.47	1.33–1.62	.457	0
Add professional home treatment	13	1.46	1.32–1.60	.513	0
Indoor insecticides	11	1.46	1.31–1.63	.388	5
Dose and multiple agents effect[c]	4	2.04	1.40–2.97	.894	0
(E) CBTs[a,c,f]	4	1.22	0.83–1.81	.275	23
Add professional home treatment	5	1.11	0.87–1.42	.380	5
(F) All cancers[f,g]	20	1.40	1.28–1.52	.390	5
Outdoor pesticide[a,b]					
(A) Leukemia	6	1.15	0.95–1.38	.190	33
Herbicide	5	1.26	1.10–1.44	.762	0
Yard insecticides[h]	3	1.11	0.60–2.05	.002	84
(B) Lymphoma	4	0.86	0.62–1.19	.131	47
Herbicide	3	1.52*	1.02–2.27	.090	58
Yard insecticides[i]	2	1.12	0.78–1.59	.314	2
(C) Hematopoietic cancers	10	1.04	0.88–1.23	.086	41
Herbicide	8	1.33	1.16–1.52	.350	10
Yard insecticides	5	1.09	0.75–1.58	.007	71
(D) CBTs	3	0.95	0.47–1.89	.012	77
Herbicide	2	1.98	0.94–4.14	.409	0
Yard insecticides[i]	2	1.29	0.86–1.92	.548	0
(E) All cancers[g]	16	1.10	0.93–1.32	.001	62
Herbicide	12	1.35	1.16–1.55	.221	23
Yard insecticides[k]	8	1.14	0.89–1.45	.028	55

*The summary ORs became not statistically significant in the sensitivity analysis when we removed ill-defined herbicide or highest weight or extreme ORs. Study *N*: number of studies included. Hematopoietic cancers include leukemia and lymphoma. All cancers include neuroblastoma and Wilms tumor and soft tissue sarcomas in outdoor pesticides. Study results with case numbers <3 are not included in the summary.

[a] In the study[45] where insecticides against different types of nuisance were reported, data with the highest OR were used.

[b] In the studies where results of different exposure windows in the same study were reported, the windows away from birth were used.

[c] The data of >10 per year were used in the study,[58] and the data of >5 per year were used in the study.[37]

[d] When both cancer-free controls and cancer controls were reported, cancer-free controls were used.

[e] The crude OR and 95% CI were calculated based on the data in the article.[42]

[f] Where >1 home pesticide usage was reported, home pesticides for nuisance pests were used.

[g] In the study[30] where the results were essentially the same during pregnancy and during childhood, the data reported from pregnancy through childhood were treated as during childhood.

[h] Includes studies[35,39,41] and ORs associated with yard pesticides were replaced by yard insecticides in studies.[35,39]

[i] Includes 2 data from the study.[41]

[j] Includes 2 studies.[32,40]

[k] In addition to all yard insecticides in each subgroup, an additional study[30] was included and ORs associated with yard pesticides were replaced by yard insecticides.

Part of the reason could be the small number of studies included.

Combining all studies reporting childhood cancers (including neuroblastoma[31] and Wilms tumor[30]) with childhood home pesticide exposure yielded a meta-rate summary OR of 1.40 (95% CI, 1.28–1.52) with a low degree of heterogeneity (I^2 of 5%). Therefore, the results show that there is a statistically significant risk of childhood cancers associated with exposures to home pesticides, especially indoor insecticides, during childhood.

Outdoor pesticides include outdoor insecticides, herbicides, and fungicides. Table 2 and Fig 3 show the cancer risks from exposure to residential herbicides during childhood. A statistically significant association between childhood leukemia and exposure to herbicides (OR = 1.26; 95% CI, 1.10–1.44, I^2 = 0%) was observed, and the sensitivity analysis confirmed the robustness of this association. The greatest risk estimates were observed in the association between childhood exposure to herbicides and the risk of leukemia. The observed association with increase in risk of childhood lymphoma became not statistically significant during the sensitivity analyses. No association appeared between herbicide exposure and CBT. When studies on all types of childhood cancers were combined, including neuroblastoma[31] and Wilms tumor,[30] a statistically significant association with residential herbicide exposure was observed (OR = 1.35; 95% CI, 1.16–1.55; I^2 = 23%). We did not find any statistically significant association between exposure to outdoor pesticides or outdoor insecticides and any types of childhood cancers (Fig 4). Because only a few studies were available on exposure to residential fungicides and childhood cancers, we did not include exposure to fungicides in the current analysis.

DISCUSSION

In this meta-analysis, we examined 16 epidemiologic studies on the possible association between residential pesticide exposure during childhood and childhood cancers. Overall, the results suggest that cancer risks are related to the type of pesticide and where it was used. Exposure to residential indoor insecticides but not outdoor insecticides during childhood was significantly associated with an

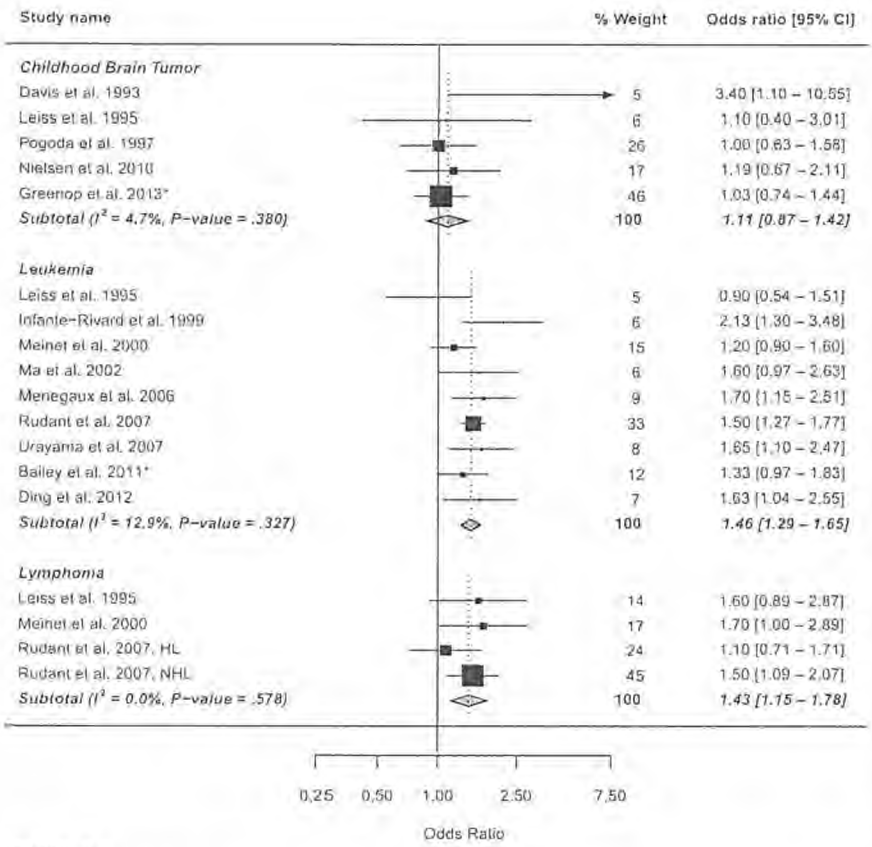

FIGURE 2
Meta-analysis of the association between childhood cancers and exposure to home pesticides during childhood. *Professional home treatments.

increasing risk of childhood cancers including leukemia, AL, and lymphoma but not CBT. Among the 5 studies reporting CBT outcomes in the analyses, 4 studies did not provide specific exposure locations, although the applications were probably indoors. This ambiguity about where pesticides were used could dilute the true effects of residential pesticides and therefore result in the association toward the null. Similarly, the fact that adding professional home treatment in hematopoietic cancers and CBT lowers the summary ORs could also result from the ambiguity of exposure location. The greatest risk estimates were observed in the association between childhood exposure to indoor insecticides and the risk of AL. The risk of childhood hematopoietic malignancies increased with the frequency of use. These observations provide additional support to the positive exposure–response relationship between indoor insecticide use and the increased risk of childhood hematopoietic malignancies.

We did not observe any significant childhood cancer risk associated with exposure to outdoor pesticides. However, when we looked into the different categories of outdoor pesticides, we found that exposure to herbicides was associated with a slightly higher risk of childhood cancers in general, which include leukemia, lymphoma, and CBT, although statistical significance appeared only in association with leukemia. No significant association between outdoor insecticides and childhood cancers was observed. This result emphasizes how important it is to specify the type and location of the pesticide when analyzing pesticide exposure and childhood cancer. Because of the small number of studies included in the current meta-analysis, more studies are needed to confirm these associations.

Results from the current analysis are in agreement with the main findings of 2 previously published studies on residential pesticide exposure and childhood leukemia.[13,14] Both observed significant associations between insecticide exposure and childhood leukemia. Although these results were based on a small number of studies, the consistency of the main findings suggests that there probably is a higher risk of childhood leukemia with indoor insecticide exposure during childhood. We have observed a slightly elevated risk of childhood leukemia associated with exposure to herbicides, with no evidence of heterogeneity. This finding is also consistent with that reported by Van Maele-Fabry et al[14] but not by Turner et al,[13] and both reported a high degree of heterogeneity (I^2 of 61% and 72%, respectively). Neither our study nor the study of Turner et al[13] observed any association between childhood leukemia and exposure to outdoor insecticides during childhood. Like Van Maele-Fabry et al,[14] we also did not observe any association between childhood leukemia and outdoor pesticide exposure.

We also found a positive association between childhood lymphoma and indoor insecticide exposure. Furthermore, the overall childhood cancer risk is elevated with childhood home pesticide exposure. There was a third study reporting that pesticide use at home or in the garden was statistically associated with the elevated risk of lymphoma, leukemia, and CBT.[20] However, Vinson et al[20] did not provide information on specific categories of pesticides or locations of use in their analysis; most of their study results were related to occupational exposure. Therefore, we

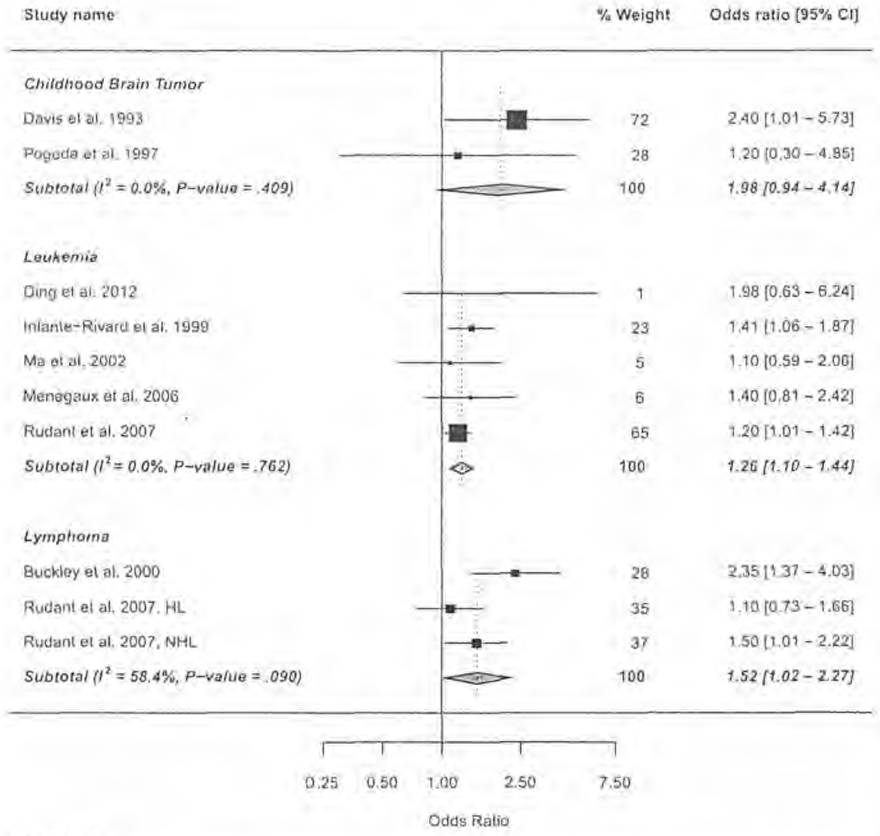

FIGURE 3
Meta-analysis of the association between childhood cancers and exposure to residential herbicides during childhood.

could not directly compare our results with those reported by Vinson et al.[20]

Although most of our findings are consistent with those of the earlier meta-analyses, there are some differences. One main difference is that several studies included in the previous 2 meta-analyses were excluded from the current analysis. These were studies that either were conducted in occupational settings, involved only adults, reported only pesticides in general (not specifying pesticide groups), or included other chemicals with pesticides. Therefore, we eliminate the effects from these studies in the summary ORs.

Although previous meta-analyses took into account exposure locations and pesticide categories when performing stratification analysis, Van Maele-Fabry et al[14] reported indoor and outdoor exposures but gave no information about pesticide category. Stratification analyses based on categories of pesticide exposure were run in the study by Van Maele-Fabry et al,[14] but no analysis was done on the exposure location for each category of pesticide; therefore, the true risk factors could be diluted. There were also no results from sensitivity analyses provided by Van Maele-Fabry et al.[14]

Unlike Van Maele-Fabry et al's[14] report and our observation, Turner et al[13] reported a statistically significant positive association between childhood leukemia and exposure to residential outdoor pesticides but not outdoor insecticides nor herbicides. However, these results were inconsistent with each other because outdoor pesticides were most likely to be outdoor insecticides or herbicides.

In the current meta-analysis, we divided studies into 3 subgroups based on the pesticide use pattern, such as indoor pesticides and insecticides, outdoor pesticides and herbicides, and outdoor pesticides and insecticides. We used a random effects model to estimate the summary ORs for each subgroup. In the home pesticide (mostly indoor insecticides) category, although some subgroup analyses were conducted on only a limited number of studies (<5), the observed heterogeneity was low ($I^2 \leq 13\%$) in these analyses. We also pooled studies to increase the accuracy of estimated summary ORs for hematopoietic malignancy and all cancers, and we observed zero or low levels of heterogeneity. Similarly, there was no observed heterogeneity in the herbicide category, including estimated summary ORs for hematopoietic malignancy and all cancers. These results of zero or low heterogeneity for indoor pesticides and herbicide exposure indicated the consistency of studies included and suggest that combining data is appropriate. However, the heterogeneity for outdoor pesticide or outdoor insecticide exposure was high. Because these studies included in the current meta-analysis differed in study design, study population, and the exposure and timing of exposure, the heterogeneity of the associations should be interpreted with caution.

Overall, our study has shown that childhood cancer risks are related to the type of pesticide use and its application locations during childhood. Childhood exposure to residential indoor insecticides was associated with an increasing risk of childhood cancers but not outdoor insecticides.

Although meta-analysis is a useful tool to assess causal relationships by combining results from different studies, outcomes can be constrained

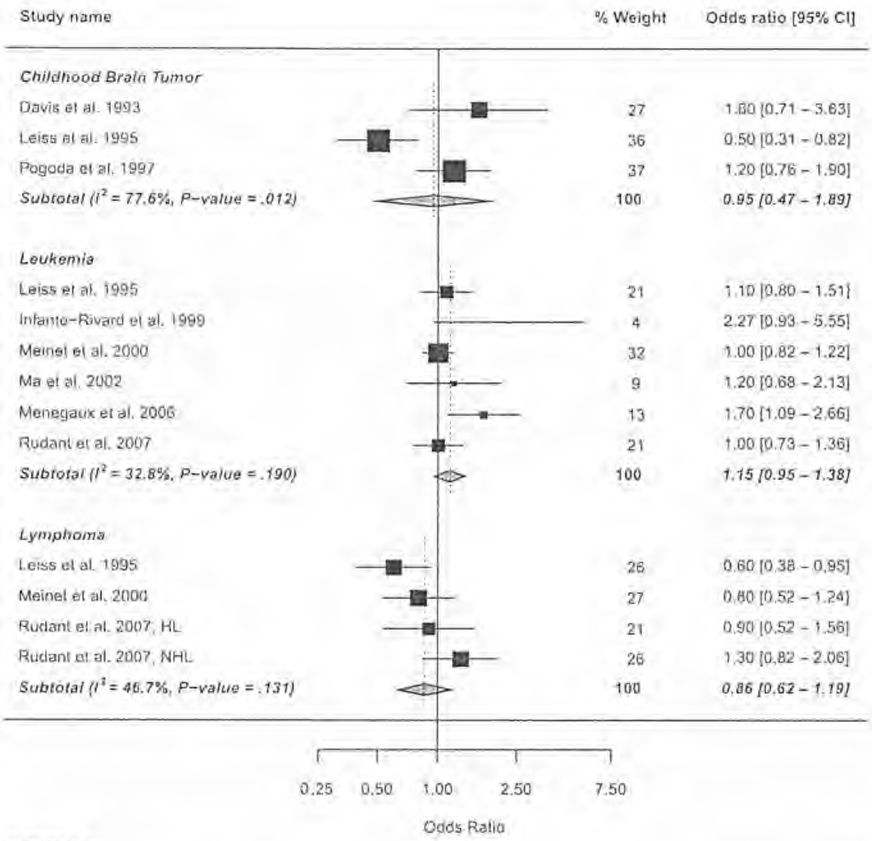

FIGURE 4
Meta-analysis of the association between childhood cancers and exposure to residential outdoor pesticides during childhood.

by the limitations of the original studies. In the current analysis, the small number of studies is a major limitation. Very few studies have assessed pesticide exposures and childhood cancers. In addition, other limitations such as selection bias, recall bias, misclassification, and publication bias might limit the applicability of the findings to the general population. To deal with the potential selection bias associated with hospital or friend controls, we performed a sensitivity analysis by excluding Davis et al[32] and Menegaux et al[39] from each pesticide category to reinforce the associations.

To reduce recall bias and misclassification, the studies we included used several strategies to reduce confounding factors and biases, such as restriction of entry to study of subjects with confounding factors, matching controls to have equal distribution of confounders, using standardized questionnaires, identical interviewing procedures for both cases and controls, and adjustment of the results. Publication bias refers to the fact that studies with less significant findings may be less publishable than those with positive outcomes; therefore, they would be unavailable for meta-analyses. For example, one of the studies from the current analysis stated that "neither residential use of insecticides nor use of pesticides in the garden was found to be significantly more frequent in any group of cases with solid tumors compared with controls, therefore no quantitative data were provided."[38] Although the results from the current meta-analysis do not seem to be significantly influenced by publication bias, this bias cannot be completely excluded. Note that when Van Maele-Fabry et al[14] assessed the impact of exclusion of nonpublished data and studies in languages other than English, they found that rerunning the meta-analysis and including nonpublished and non–English-language studies did not substantially modify the results.

A positive exposure–response relationship between residential indoor insecticide use and occurrence of childhood cancers was observed in the current study. Some studies have also shown that maternal pesticide exposure during pregnancy was associated with childhood cancers.[35,37,39] Although current data do not establish the most critical exposure period for the occurrence of childhood cancers, their development is probably multifactorial and probably includes gene–environment interactions.[11,44–46] Some studies assert a possible association between pesticide exposure with genetic predisposition and defined subtypes of childhood cancers.[26,42,43] Additional studies are needed to examine the potential mechanisms by which childhood exposure to pesticides could lead to the development of childhood cancers.

CONCLUSIONS

The current meta-analysis has revealed positive associations between exposure to home pesticides and childhood cancers, with the strongest association observed between indoor insecticide exposure and acute childhood leukemia. Although epidemiologic research is limited in identifying the association between the adverse health outcomes in young children and pesticide uses in residential areas, the findings from the present meta-analysis and those previously published have consistently demonstrated

associations between pesticide exposure and childhood cancers. While the research community is working toward a better understanding of the causality of pesticides in various childhood diseases, more and more pesticides are being used in farming, in landscape maintenance, and in the home. Therefore, public health policies should be developed to minimize childhood exposure to pesticides in the home. States and local authorities can establish programs, such as integrated pest management, to minimize residential pesticide uses, especially indoor uses.[47,48] In the meantime, parents, school and daycare teachers, and health care providers can learn about common pesticide types and labeling information and can stay aware of the short- and long-term effects of these chemicals.[49,50] Every effort should be made to limit children's exposure to pesticides.

ABBREVIATIONS

AL: acute leukemia
CBT: childhood brain tumor
CI: confidence interval
OR: odds ratio

FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: Supported by the Department of Energy and Environmental Protection, State of Connecticut (contract 042103580).

POTENTIAL CONFLICT OF INTEREST: The authors have indicated they have no potential conflicts of interest to disclose.

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Maternal Smoking and Childhood Leukemia and Lymphoma Risk among 1,440,542 Swedish Children

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Abstract

Possible *in utero* effects of maternal smoking on hemopoietic cancer in the offspring have been addressed previously, although the results are inconclusive. In this investigation, we take advantage of population-based registers in Sweden to examine maternal smoking during pregnancy and childhood risk of leukemia and lymphoma. Prospective data were available from 1,440,542 Swedish children born between 1983 and 1997. Proportional hazard models were used to estimate hazard ratios (HR) and 95% confidence intervals (95% CI) controlling for potential confounders. In the study base, 750 hemopoietic cancers occurred across 11 million person-years. Incidence rates per 100,000 person-years were 4.7 for acute lymphocytic leukemia (ALL), 0.45 for acute myelogenous leukemia, and 0.76 for non-Hodgkin's lymphoma. Maternal smoking was associated with a lower risk of ALL (HR, 0.73; 95% CI, 0.58–0.91). On the other hand, there was a higher risk of acute myelogenous leukemia (HR, 1.41; 95% CI, 0.74–2.67) particularly among heavy (≥10 cigarettes per day) smokers (HR, 2.28; 95% CI, 1.05–4.94). The data also suggested a small excess risk of non-Hodgkin's lymphoma (HR, 1.25; 95% CI, 0.76–2.04). Evidence from this large cohort suggests that maternal smoking affects the risk of childhood leukemia and lymphoma in the offspring. The Swedish registries provide unique opportunities to examine this research question, with a design inherently free of selection and recall biases. The apparent protective effect with ALL needs to be explored further and in no way supports maternal smoking as beneficial, given its adverse association with common pregnancy outcomes. (Cancer Epidemiol Biomarkers Prev 2004;13(9):1528–33)

Introduction

The negative effects of cigarette smoking on cancer risk in adulthood are well documented and include convincing evidence of an increased risk of cancer of the lung and larynx (1), bladder (2), esophagus (3), and oral cavity (4). The possible *in utero* effects of maternal smoking during pregnancy on subsequent cancer risk in the offspring have been addressed more recently through epidemiologic studies, although the results are in large part inconclusive (5, 6). With respect to childhood leukemia and lymphoma, several case-control studies have observed a positive effect of maternal smoking during pregnancy on risk of acute lymphocytic leukemia (ALL; refs. 7, 8), acute myelogenous leukemia (AML; refs. 9, 10), and lymphomas (7, 11). Other studies have found no association between maternal smoking and risk of these cancers (7, 12), whereas others still showed some evidence of a protective effect at least for ALL (13-15) and AML (15, 16).

A well-conducted case-control study is an efficient design to examine *in utero* exposure to cigarette smoking and risk of childhood cancer. However, this study design is vulnerable to potential biases, including selection and recall biases, which could account for the diverging results of prior studies. Given the rarity of childhood leukemia and lymphoma, however, a cohort study, which would avoid these potential limitations, is often difficult to undertake with sufficient statistical power.

In the present investigation, we take advantage of existing population-based registers in Sweden to examine the effect of maternal smoking during pregnancy on childhood risk of leukemia and lymphoma among a cohort of 1,440,542 Swedish children born between 1983 and 1997.

Materials and Methods

Study Population. The study base for the present investigation consists of all live births in Sweden between January 1, 1983 and December 31, 1997 that were registered in the population-based Swedish Medical Birth Registry. The Birth Registry includes >99% of all births in Sweden (17). Follow-up data on this cohort were achieved through linkage of the Birth Registry with the Swedish Cancer Registry and the National Cause of Death Registry. Because each Swedish

Received 11/21/03; revised 4/13/04; accepted 4/15/04.

Grant support: Swedish Cancer Society grant 4242-B00-02XBB and Swedish Foundation for International Cooperation in Research and Higher Education grant, supporting collaboration between Karolinska Institutet and Harvard University.

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Table 1. Characteristics of malignant childhood leukemia and lymphoma (*International Classification of Diseases, Seventh Edition* codes 200.0–207.0) in Sweden among cohort of 1,440,542 children born 1983–1997

	International Classification of Diseases, Seventh Edition code	*n*	Rate per 10^5 person-years	Mean (SD) age at diagnosis	% Male
ALL	204.0	505	4.75	3.7 (2.7)	53.7
AML	205.0	48	0.45	3.5 (3.9)	45.8
Chronic myelogenous leukemia	205.1	13	0.12	5.4 (3.8)	53.9
Other leukemias*	206–207	22	0.21	3.6 (3.7)	45.5
NHL†	200, 204.1	81	0.76	5.7 (3.0)	74.7
Hodgkin's disease	201	20	0.19	7.0 (3.7)	75.0
Reticulosis	202	61	0.57	2.4 (2.9)	57.4

*Includes 6 monocytic leukemias and 16 other and unspecified leukemias.
†Includes two chronic lymphocytic leukemia cases classified as NHL.

resident is assigned a national registration number, which is a unique identifier, it is possible to merge national databases.

Information on incident leukemia and lymphoma cases in the cohort came from the Swedish Cancer Registry, established by the National Board of Health and Welfare in 1958. Swedish law mandates and regulates physicians and pathologists, who confirm the diagnosis of cancer, to report on every newly diagnosed malignant tumor to the Swedish Cancer Registry. Since the early 1980s, all notifications of cancer diagnosis have been sent directly to one of six regional cancer registers, each of which has a strictly defined catchment area. All case reports are verified for completeness at the regional registries and subsequently computerized. Incidence statistics from the six regional registries are pooled in the Swedish Cancer Registry.

Information on all deaths in the cohort was available from the National Cause of Death Registry. The registry includes dates of death from specific causes, which is obtained from death certificates and coded according to the standards of the *International Classification of Diseases, Eighth, Ninth, and Tenth Editions*. Medical certification is carried out by the attending physician or coroner, with use of both clinical records and autopsy reports. This registry, which was established in 1961, maintains date and cause of death for >99% of residents who died after this year.

Among the 1,591,271 Swedish live births between 1983 and 1997, we excluded 3,627 (0.2%) infants who died within the first week of birth and 1,475 (0.1%) with Down syndrome. We excluded from the analysis an additional 97,905 (6.2%) births with missing information on maternal smoking, 47,573 (3.0%) with other missing covariate data, and 149 (0.01%) with erroneous follow-up information. Thus, the sample size of the final cohort for this analysis was 1,440,542 (90.5%) Swedish births during 1983 to 1997.

Data Collection. The Birth Registry includes standardized information from antenatal, obstetric, and neonatal medical records. During the first antenatal visit, normally at 8 to 12 gestational weeks, information from a standardized questionnaire is recorded by a nurse midwife. Information on maternal smoking during the first trimester has been collected routinely since 1983. Women were asked the number of cigarettes that they smoked, which was coded on the questionnaire as 0, 1 to 9, or ≥10 cigarettes per day. Additional covariate data include maternal demographic data, reproductive history, and birth characteristics and outcomes. Through linkage with the Education Registry, years of formal education attained as of December 31, 1998 were obtained from Statistics Sweden. Information on mother's country of birth was provided through linkage to the Immigration Registry and stratified into Nordic (Sweden, Denmark, Norway, Finland, and Iceland) or non-Nordic country of birth.

Lymphoma and Leukemia Cases. The incidence of lymphoma and leukemia (*International Classification of Diseases, Seventh Edition* codes 200–207) in the cohort was based on information provided by the Swedish Cancer Registry. Information available from the Swedish Cancer Registry includes date of diagnosis, malignancy, histologic subtype (WHO/HS/CANC/24.1 Histology Code), basis of diagnosis, and death from cancer. Observation time of the cohort was calculated from date of entry into the cohort (birth date) until the occurrence of a diagnosis of any primary lymphoma or leukemia cancer, or censoring since diagnosis of another cancer, death, or end of the observation period (December 31, 1997).

Statistical Analysis. The relation between maternal smoking and risk of childhood lymphoma or leukemia in the offspring was assessed using information on time to cancer event, which accounts for different amounts of follow-up time in the cohort. First, the incidence rates of cancer in the entire cohort were estimated by dividing the number of cases that occurred during follow-up by the total number of person-years at risk for a given level of exposure. Proportional hazard models using Proc PHREG in SAS version 8.2 were used to estimate the hazard ratio (HR) and 95% confidence interval (95% CI) of hemopoietic cancers, given smoking status, comparing nonsmokers as the reference. To assess whether the dose of cigarettes increased or decreased risk in a linear fashion, we calculated statistical tests for trend. The following covariates were evaluated as potential confounders: maternal age (categorically: ≤19, 20–24, 25–29, 30–34, ≥35 years), maternal education (categorically: ≤9, 10–11, 12, ≥13 years), parental status (cohabitating/not cohabitating), residence at birth (town or rural/large city), maternal birthplace (Nordic/non-Nordic), parity (categorically: 1, 2–3, ≥4), birth year (ordinal), and baby's gender (male/female). Because of concern that

birth weight (ordinally) and gestational age (categorically: <32, 32–36, ≥37 weeks) potentially could be considered on the causal pathway, we controlled for these variables in a secondary analysis.

Because of the early age at onset of ALL, we examined whether the effect of smoking was constant by age at diagnosis. To accomplish this, we stratified models into risk sets of 0 to 1 (completed), 2 to 4, and ≥5 years of follow-up and estimated the effect of maternal smoking in each risk group. Furthermore, we examined whether the effect of maternal smoking on ALL differed among male and female offspring, comparing the estimates formally with a test for interaction.

Results

This cohort of 1,440,542 children born in Sweden between 1983 and 1997 contributed almost 11 million person-years to the study base. ALL was by far the most common occurring of the leukemias and lymphomas, with an incidence rate of 4.75 per 100,000 person-years (Table 1). The characteristics of non-Hodgkin's lymphoma (NHL) and Hodgkin's disease cases were notably different than ALL and AML, with an older mean age and a predominance of male cases.

In Table 2, we present the prevalence of maternal smoking during pregnancy by demographic, reproductive, and birth characteristics. Overall, 24% of women smoked during pregnancy. The proportion of women smoking during pregnancy was higher among younger women, among those with lower levels of education, and among those born in Nordic countries. Maternal smoking was also associated with preterm birth and lower birth weight. Over the course of the study period, there was evidence of notable decreases in smoking prevalence.

Adjusting for potential confounders, maternal smoking was associated with a 30% lower risk of ALL (HR, 0.73; 95% CI, 0.58–0.91; Table 3). The risk reduction was similar for light (1–9 cigarettes per day) and heavy (≥10 cigarettes per day) smokers. On the other hand, there was evidence that maternal smoking was associated with a higher risk of AML. In particular, children whose mothers smoked ≥10 cigarettes per day during early pregnancy had a >2-fold higher risk of AML (HR, 2.28; 95% CI, 1.05–4.94) compared with women who did not smoke. The data also suggested a small excess risk of NHL, although because of the small number of cases, 95% CIs were wide. In the proportional hazard analyses, further adjustment by gestational age and birth weight did not substantial change the HRs, suggesting that these variables are neither confounders nor on the causal pathway.

In Table 4, we present estimates of the effect of maternal smoking on ALL stratified by age at diagnosis and sex. A decreased risk of ALL associated with maternal smoking was evident for each age at diagnosis (Table 4), although the effect was more consistent among those diagnosed at ages 0 to 1 years. Maternal smoking was associated with a significantly protective effect on risk of ALL among males only, but there was no evidence of a statistical interaction between maternal smoking and infant's sex on risk of ALL (P for interaction = 0.32).

Discussion

Evidence from this large cohort of Swedish children suggests that maternal smoking during pregnancy affects the risk of childhood leukemia in the offspring. The data are consistent with a small protective effect of smoking on risk of ALL and with an excess risk of AML. There is also some evidence that maternal smoking increases the risk of NHL, although small numbers of cases in the cohort prevent definitive conclusions. Although there is no statistical evidence of interaction, the effect of maternal smoking on ALL seems more consistent among male compared with female offspring and slightly stronger for infants during the first year of life.

Table 2. Frequency of smoking during pregnancy by maternal and reproductive characteristics among 1,440,542 Swedish births, Sweden, January 1983–December 1997

	N	Smoking during pregnancy (%)
Maternal age (y)		
≤19	37,243	43.6
20–24	311,861	29.8
25–29	538,653	22.2
30–34	379,602	21.1
≥35	173,183	21.7
Maternal education (y)		
≤9	243,553	43.1
10–11	593,128	28.3
12	177,235	15.7
13–14	252,578	12.3
≥15	174,048	8.4
Parental status		
Cohabitating	1,308,277	22.7
Not cohabitating	71,318	48.4
Town/city		
Large city	383,063	23.6
Town/rural	1,057,479	24.2
Maternal birthplace		
Nordic	1,323,945	24.8
Non-Nordic	116,597	15.4
Parity		
1	584,022	24.0
2–3	753,583	23.4
≥4	102,937	29.4
Multiple birth		
Singleton	1,406,909	24.1
Multiple	33,633	23.5
Offspring sex		
Female	700,348	24.0
Male	740,014	24.1
Gestational age (wk)		
≤31	8,143	31.7
32–36	57,624	28.9
≥37	1,373,847	23.8
Birth weight (g)		
<1,500	7,822	32.1
1,500–2,500	51,340	36.1
2,501–3,500	625,964	29.4
3,501–4,500	703,703	19.1
>4,500	46,871	12.7
Birth year		
1983–1986	343,557	30.3
1987–1990	402,512	26.5
1991–1994	432,496	21.6
1995–1997	261,977	16.1

Table 3. Crude and adjusted HRs for the effect of maternal smoking on leukemia and lymphoma, Sweden, January 1983–December 1997

	Cases (*n*)	Rate per 10^5 person-years	Crude HR	Adjusted HR* (95% CI)	Adjusted HR† (95% CI)
Maternal smoking					
ALL					
No	400	5.93	Reference	Reference	Reference
Yes	105	4.01	0.73	0.73 (0.58–0.91)	0.75 (0.60–0.93)
1–9 cigarettes	61	3.80	0.69	0.68 (0.52–0.89)	0.69 (0.52–0.91)
≥10 cigarettes	44	4.35	0.80	0.80 (0.58–1.10)	0.84 (0.61–1.15)
P for trend			0.016	0.012	0.043
AML					
No	33	0.49	Reference	Reference	Reference
Yes	15	0.57	1.28	1.41 (0.74–2.67)	1.28 (0.65–2.49)
1–9 cigarettes	6	0.37	0.83	0.91 (0.38–2.21)	0.75 (0.29–1.96)
≥10 cigarettes	9	0.89	2.00	2.28 (1.05–4.94)	2.20 (1.00–4.83)
P for trend			0.15	0.084	0.13
NHL					
No	56	0.83	Reference	Reference	Reference
Yes	25	0.96	1.17	1.25 (0.76–2.04)	1.22 (0.74–2.02)
1–9 cigarettes	15	0.93	1.14	1.21 (0.68–2.18)	1.15 (0.63–2.11)
≥10 cigarettes	10	0.99	1.21	1.30 (0.65–2.60)	1.33 (0.66–2.68)
P for trend			0.51	0.38	0.39
Reticulosis					
No	44	0.65	Reference	Reference	Reference
Yes	17	0.65	1.11	1.20 (0.67–2.16)	1.12 (0.61–2.05)
1–9 cigarettes	14	0.87	1.48	1.60 (0.86–3.00)	1.47 (0.77–2.79)
≥10 cigarettes	3	0.30	0.51	0.54 (0.17–1.77)	0.54 (0.16–1.77)
P for trend			0.74	0.855	0.739

*Data adjusted for maternal age, maternal education, maternal birthplace, parity, birth year, and baby's gender.
†Data also adjusted for gestational age and birth weight.

In evaluating the results of the study, there are several strengths to consider. The large size and duration of follow-up provide one of the few opportunities to evaluate the research question of maternal smoking on cancer risk using a cohort design. The Swedish Medical Birth and Cancer Registers include 99% of all births and 96% of cancer cases in Sweden (17, 18), respectively. Using these population-based resources almost eliminates the possibility of selection bias and loss to follow-up.

Maternal smoking in this study was assessed at the time women registered for prenatal care, during the first trimester. In this way, the possibility of recall bias is eliminated. However, we do lack exposure information over the course of pregnancy. Because it is unclear what the critical window of exposure is, we may have some misclassification of this time-varying exposure. For example, ~10% of smokers in Sweden cease cigarette smoking after the first antenatal care visit (19). Thus, if the relevant time window were later in pregnancy, we would have classified a small proportion of unexposed person-time as exposed. Moreover, the societal attitudes toward smoking may have led to underreporting of smoking during pregnancy. Because such misclassification of the exposure is nondifferential, the true associations between maternal smoking and leukemia and lymphoma may be greater than reported.

Because of the study design, there are few limitations to consider. The Medical Birth Register lacks information on some reported risk factors, such as exposure to ionizing radiation, parental occupation, and dietary data. These factors may have differed by maternal smoking status, thus leading to potential residual confounding. Of particular concern may be residual confounding by paternal smoking. Some studies suggest that, among

Table 4. Adjusted* HRs for the effect of maternal smoking on ALL stratified by age at diagnosis and sex, Sweden, January 1983–December 1997

	Age at diagnosis			Gender	
	0–1 y HR (95% CI)	2–4 y HR (95% CI)	≥5 y HR (95% CI)	Male HR (95% CI)	Female HR (95% CI)
Maternal smoking					
No	Reference	Reference	Reference	Reference	Reference
Yes	0.56 (0.31–1.01)	0.83 (0.62–1.11)	0.64 (0.42–0.97)	0.63 (0.46–0.86)	0.85 (0.62–1.16)
1–9 cigarettes	0.57 (0.28–1.15)	0.79 (0.55–1.13)	0.55 (0.32–0.95)	0.63 (0.43–0.92)	0.75 (0.50–1.10)
≥10 cigarettes	0.55 (0.22–1.37)	0.89 (0.59–1.35)	0.78 (0.44–1.40)	0.64 (0.40–1.02)	1.02 (0.66–1.57)
P for trend	0.071	0.33	0.10	0.008	0.59

*Data adjusted for maternal age, maternal education, maternal birthplace, parity, birth year, and baby's gender.

nonsmoking mothers, paternal smoking is associated with increased risk of ALL and lymphoma (16, 20). However, in Sweden, paternal smoking is closely associated with maternal smoking (19). Thus, if paternal smoking is associated with increased risk of ALL and NHL also in Sweden, we should have underestimated the protective effect of maternal smoking on ALL and overestimated the effect on NHL.

In this study, mean follow-up time of the cohort is ~8 years, and ~90% of the children were <15 years old at the end of the study. Thus, this study focused on cancers that occurred earlier in the cohort. This observation should be taken into consideration when assessing the generalizability of these findings to malignancies with later age at onset. If the *in utero* effects of smoking play a greater role on later rather than earlier onset cancers (15), then our effect estimates may not be directly applicable to the age groups under study. At least for ALL, our data do not suggest a different effect of smoking by age at diagnosis.

Our results agree with some, but not all, previous studies on the effect of maternal smoking on risk of childhood leukemia and lymphoma. The United Kingdom Cancer Study, which is a nationwide population-based case-control study, evaluated maternal smoking during the second trimester of pregnancy using structured interviews (15). The authors found that maternal smoking was associated with a 24% lower risk of leukemia (P for trend = 0.03). This protective effect was notable for both ALL and AML, however. A large, population-based case-control study undertaken in Germany assessed maternal smoking during the first trimester and found a protective effect for ALL and an increased risk for NHL (14). A meta-analysis based on eight studies, however, found no evidence of an effect of maternal smoking on leukemia (relative risk 1.05; CI, 0.82–1.34; ref. 21).

Few cohort studies examining maternal smoking and risk of childhood hemopoietic cancers have been undertaken. In a study including 54,795 live-born children, there was some evidence of a protective effect of maternal smoking on total leukemia, although the results were not statistically significant (22). In an initial follow-up for the Swedish birth cohort between 1982 and 1987, Pershagen et al. (23) reported no association between maternal smoking and cancers of the lymphatic and hemopoietic system (HR, 1.04; 95% CI, 0.71–1.52). However, in case-control studies nested within the cohort through 1989, there was evidence of a protective effect of ALL (13) and excess risks of AML (10) and NHL (11). Maternal smoking data from the Swedish nested case-control and cohort studies was derived in the same manner as the present study.

Given the inconclusiveness of earlier epidemiologic studies, we can turn to biological plausibility to assess the study findings. First, several components of cigarette smoke, such as benzo[*a*]pyrene and 4-aminobiphenyl, are known to cross the placental membrane and have been detected in the placenta and fetal blood of offspring (24-27). In addition, maternal smoking during pregnancy was positively associated with increased numbers of specific mutations such as deletions in lymphocytes of the offspring (28, 29). Thus, it is biologically plausible that maternal smoking during pregnancy increases the risk of NHL and AML, as observed in our study.

The protective effect of smoking and ALL is more difficult to understand, and little is known about the mechanism by which smoking could exert such an effect. In animal models in which progeny are exposed *in utero* to benzo[*a*]pyrene, a component of tobacco smoke, there is substantial evidence of generalized immune suppression after birth (30-32). In particular, *in utero* exposure to benzo[*a*]pyrene decreases prolymphocytic cells in animals (31) and suppresses B-cell lymphopoiesis and induces pre–B-cell apoptosis in bone marrow cultures (33). Such suppression of immune function could result in a decreased response and lower likelihood of clonal expansion.

Despite the apparent protective effect of smoking on ALL, this study in no way supports that maternal smoking is beneficial. Smoking during pregnancy is linked to several adverse effects, including fetal growth restriction, preterm birth, and perinatal mortality (33-35), outcomes that are significantly more common conditions. This evidence may simply outline a potential mechanism by which ALL could occur.

Clearly, the question of maternal smoking and risk of hemopoietic cancers remains. This study provides supportive evidence of positive associations with AML and NHL and an interesting protective effect with ALL, which needs to be explored further. With additional follow-up time, this unique cohort of Swedish children will help to further elucidate the role of maternal smoking on risk of childhood cancers.

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Cancer Epidemiology, Biomarkers & Prevention

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Maternal Smoking and Childhood Leukemia and Lymphoma Risk among 1,440,542 Swedish Children

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Cancer Epidemiol Biomarkers Prev 2004;13:1528-1533.

ARTICLE

Prospective Study of *Trichomonas vaginalis* Infection and Prostate Cancer Incidence and Mortality: Physicians' Health Study

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Background A recent nested case–control study found that the presence of antibodies against *Trichomonas vaginalis*, a common nonviral sexually transmitted infection, was positively associated with subsequent incidence of prostate cancer. We confirmed these findings in an independent population and related serostatus for antibodies against *T vaginalis* to prostate cancer incidence and mortality.

Methods We conducted a case–control study nested within the Physicians' Health Study that included 673 case subjects with prostate cancer and 673 individually matched control subjects who had available plasma samples. Plasma from blood samples collected at baseline was assayed for antibodies against *T vaginalis* with an enzyme-linked immunosorbent assay. We used conditional logistic regression to estimate the odds ratios (ORs) of incident prostate cancer, extraprostatic prostate cancer, and cancer that would ultimately progress to bony metastases or prostate cancer–specific death.

Results Although not statistically significant, the magnitude of the association between *T vaginalis*–seropositive status and overall prostate cancer risk (OR = 1.23, 95% confidence interval [CI] = 0.94 to 1.61) was similar to that reported previously. Furthermore, a seropositive status was associated with statistically significantly increased risks of extraprostatic prostate cancer (OR = 2.17, 95% CI = 1.08 to 4.37) and of cancer that would ultimately progress to bony metastases or prostate cancer–specific death (OR = 2.69, 95% CI = 1.37 to 5.28).

Conclusions This large prospective case–control study obtained further support for an association between a seropositive status for antibodies against *T vaginalis* and the risk of prostate cancer, with statistically significant associations identified for the risk of extraprostatic prostate cancer and for clinically relevant, potentially lethal prostate cancer.

J Natl Cancer Inst 2009;101:1–6

A number of inflammation-related factors have been implicated in prostate cancer risk and progression, but the origin of inflammation is unclear (1). Infections are one possible source. *Trichomonas vaginalis* is a common nonviral sexually transmitted infection, with an estimated 174 million annual infections globally (2). Prevalence in American men ranges from approximately 3% among young men in the general population (3) to 65% among military personnel with nongonococcal urethritis (4). Little is known about the prevalence of infection in older men; however, in contrast to other common sexually transmitted infections, the infection has been observed to be more prevalent among men aged 25–39 years than in men aged 18–20 years (3,5). Urethral symptoms associated with *T vaginalis* tend to be less severe than other common sexually transmitted infections, such as those due to *Chlamydia trachomatis* or *Neisseria gonorrhoeae* (6). Furthermore, more recent studies have found that *T vaginalis* is associated with asymptomatic infections in 50%–75% of infected men (5,7). Consequently, many men are unaware that they are infected with the parasite.

Men infected with *T vaginalis* often experience spontaneous resolution, as shown by decreasing rates of infection with time

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See "Funding" and "Notes" following "References."

DOI: 10.1093/jnci/djp306

CONTEXT AND CAVEATS

Prior knowledge

The presence of antibodies against *Trichomonas vaginalis*, a common nonviral sexually transmitted infection, has been positively associated with subsequent incidence of prostate cancer.

Study design

Nested case–control study that included case subjects with prostate cancer and individually matched control subjects who had available plasma samples that were collected at baseline. Plasma was assayed for antibodies against *T vaginalis*. The relationship of incident prostate cancer, extraprostatic prostate cancer, and cancer known to progress to bony metastases or prostate cancer–specific death was investigated.

Contribution

The size of the association between *T vaginalis*–seropositive status and overall prostate cancer risk, although not statistically significant, was similar to that reported previously. A seropositive status was associated with statistically significantly increased risks of extraprostatic prostate cancer, cancer that is known to progress to bony metastases, or prostate cancer–specific death.

Implications

Further investigation is warranted to determine whether local prostatic inflammation could lead to downstream events that influence prostate cancer risk and to confirm the association between *T vaginalis* serostatus and aggressive prostate cancer.

Limitations

The time between *T vaginalis* infection and blood collection was not known. Men with *T vaginalis* infection might visit their physicians more frequently than those without such infection and so increase the possibility of prostate cancer diagnosis. Because other sexually transmitted infections occur concurrently with *T vaginalis* infections, the possibility that *T vaginalis* is acting as a marker for another pathogen cannot be ruled out.

From the Editors

since last sexual contact with an infected partner (8) and natural history studies (9), in which as many as one-third of men cleared the infection within 2 weeks without treatment (7). Nevertheless, a smaller proportion of men experience long-term asymptomatic infection (7,9). *T vaginalis* can ascend the urethra to the prostate and infect the prostate epithelium (10,11), and in that epithelium, it is associated with evidence of acute and chronic inflammation (10). As such, chronic prostatic infection with *T vaginalis* may initiate an inflammatory response that could increase the risk of developing prostate cancer (10) and increase the risk of disease progression.

A recent case–control study (12) nested in the Health Professionals Follow-up Study found that seroprevalence of *T vaginalis* infection was positively associated with subsequent prostate cancer risk, with a suggestion of the greatest risk for more aggressive disease that was defined as high Gleason grade disease. As a follow-up on the positive finding between *T vaginalis* serostatus and prostate cancer risk, we conducted a large nested case–control study within the Physicians' Health Study to further investigate a potential association between *T vaginalis* serostatus and prostate cancer incidence. We also investigated potential associations between *T vaginalis* serostatus and subgroups of prostate cancer defined by tumor stage, tumor grade, age at diagnosis, and cancer that ultimately progressed to bony metastases or prostate cancer–specific death.

Study Subjects and Methods

Study Population

The Physicians' Health Study (13,14) was initiated in 1982 as a randomized, double-blind, placebo-controlled trial of aspirin and β-carotene for the primary prevention of cardiovascular disease and cancer. The study included 22 071 healthy US male physicians aged 40–84 years at baseline. Before being randomly assigned to a treatment group, 14 916 (68%) of the 22 071 men provided a blood sample (15). These participants constitute the study base for the nested case–control study.

We included 673 case subjects who were diagnosed with prostate cancer up to 18 years after blood collection (1982–2000) and who had available plasma samples. We selected 673 control subjects from the population at risk at the time of the case subject's diagnosis (ie, those who had provided blood, had not had a prostatectomy, and had not reported a diagnosis of prostate cancer at the time the case subject was diagnosed with prostate cancer). For statistical efficiency, control subjects were individually matched to case subjects by age (within 1 year), smoking status (never, former, or current), and follow-up time.

Laboratory Assessment

Plasma from prospectively collected blood samples from each case subject and his matched control subject (stored at −80°C) was thawed and assayed for antibodies against *T vaginalis* with an assay that detects IgG antibodies against purified, recombinant α-actinin protein from *T vaginalis*. Enzyme-linked immunosorbent assays were optimized with known negative and positive pooled plasma of uninfected individuals and patients with trichomonosis, respectively, that gave reproducible readings after incubation with microtiter wells containing immobilized α-actinin. In this study, paired plasma samples from case and control subjects were diluted at 1:10 (vol/vol) in phosphate-buffered saline–Tween-20 containing 5% skim milk, and 100 μL of the diluted plasma was added to each well of a 96-well plate (Nunc, Rochester, NY). After incubation for 3 hours at 37°C, the plates were washed three times with phosphate-buffered saline–Tween-20 followed by the addition of 100 μL of secondary goat anti–human IgG (Fc-specific) conjugated to horseradish peroxidase at a 1:1500 dilution in phosphate-buffered saline–Tween-20 containing 5% skim milk to each well. Plates were incubated again for 1 hour at 37°C and then washed three times with phosphate-buffered saline–Tween-20. Color was allowed to develop by adding 100 μL of substrate solution per well (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); phosphate–citrate buffer with 0.03% sodium perborate, Sigma Chemical Co, St. Louis, MO) according to the manufacturer's recommendations, and plates were incubated at room temperature for 10 minutes. Absorbance values at a wavelength of 405 nm were then obtained by examining the supernatants spectrophotometrically with an enzyme-linked immunosorbent assay plate reader (Bio-Tek instruments, Inc, Winooski, VT).

Case–control sample pairs were assayed in adjoining wells, with blinding of laboratory personnel as to the case–control status of the samples. All samples were tested in duplicate and inferences were based on the mean of duplicate values. To create absorbance scores, we used a control plasma panel consisting of pooled plasma from known seronegative patients and four plasma samples with increasing seropositivity. We divided the mean duplicate absorbance value for each seropositive sample in the control panel by the mean duplicate absorbance value of the seronegative control plasma to obtain a minimum positive to negative (P/N) ratio for each absorbance score (0 = 1 to <1.81; 1 = 1.81 to <2.78; 2 = 2.78 to <3.31; 3 = 3.31 to <4.07; or 4 = ≥4.07). The positive to negative ratio was computed for all case subjects with prostate cancer and all control subjects, and the resulting values were then compared with the specified cut points determined from the control panel to assign an absorbance score (ie, 0, 1, 2, 3, or 4). Samples from the control panel were included with each plate to monitor reproducibility; values for these samples always fell within the previously determined range. Samples with absorbance scores of 3 or 4 were considered positive for history of trichomonosis. We also included 29 quality-control duplicate or triplicate samples that were randomly distributed across plates. Concordance in serostatus was achieved for 26 of 29 (90%) of the quality-control samples; 17 of 26 of the concordant replicate samples were seropositive.

Statistical Analysis

We used conditional logistic regression to analyze prostate cancer risk according to serostatus adjusting for matching factors. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by comparing men who were *T vaginalis* seropositive at baseline with men who were *T vaginalis* seronegative. We additionally controlled for randomization to aspirin assignment and body mass index (continuous) and evaluated risk within subgroups of stage and grade at diagnosis. All *P* values were from two-sided statistical tests, with α of .05 considered to be statistically significant.

Analyses were undertaken with the SAS Statistical Analysis version 9.1.3 (SAS Institute, Cary, NC). The research protocol was approved by the institutional review board at Partners Healthcare. Questionnaire data were collected with implied consent, and biomarker data were collected with written authorization.

Results

On average, case subjects were aged 68.7 years (SD = ±7.4 years) at diagnosis. Most case subjects were diagnosed with well-differentiated tumors (54% with a Gleason score of 2–6) at a localized stage (83% with a stage of T1 or T2). Mean time between blood collection and prostate cancer diagnosis was 9.3 years (range = 0.3–17.9 years). The seroprevalence of *T vaginalis* infection was 21% in control subjects and 25% in case subjects (Table 1). *T vaginalis* absorbance scores were not associated with age or baseline prostate-specific antigen (PSA) levels in case subjects or control subjects.

T vaginalis seropositivity was not statistically significantly associated with total prostate cancer risk (OR = 1.23, 95% CI = 0.94 to 1.61) or high-grade disease (OR for Gleason 7–10 scores = 1.10, 95% CI = 0.72 to 1.68). However, serological evidence of *T vaginalis* infection was associated with a statistically significant increase in the risk of diagnosis of advanced-stage prostate cancer (OR = 2.17, 95% CI = 1.08 to 4.37) and in the risk of cancer that would ultimately progress to distant metastases or cancer-specific death (OR = 2.69, 95% CI = 1.37 to 5.28) (Table 1). We also found that the association between *T vaginalis* and prostate cancer was stronger for men who were diagnosed more closely to blood collection (Table 1). Compared with case subjects overall (n = 673), the 94 case subjects who were diagnosed within 5 years of blood collection tended to be somewhat older at diagnosis (eg, those who were aged >65 years = 72 [77%] vs 452 [67%]) and more advanced (eg, those who were at stage T3 or T4, N1, or M1 = 23 [25%] vs 105 [16%]). However, cross-classifying men on these characteristics suggested that the time scale that most influenced effect estimates was the duration between blood collection and diagnosis. Given the observed increased risk for cancer soon after blood collection, we explored the association between *T vaginalis* serostatus and lethal prostate cancer, according to years from blood collection to diagnosis. Among the 39 men diagnosed with lethal cancer within 5 years of blood collection and their matched control subjects, men positive for history of trichomonosis (n = 15) were statistically significantly more likely to develop lethal prostate cancer than seronegative men (OR = 6.4, 95% CI = 1.5 to 27.9).

Discussion

In this large nested case–control study, we provide further evidence to support the previously reported association between a *T vaginalis*–seropositive status and prostate cancer risk (12). The magnitude of the overall association of *T vaginalis*–seropositive status with incidence in our study, although not statistically significant, was similar to that observed in the previous case–control study nested in the Health Professionals Follow-up Study (OR = 1.43, 95% CI = 1.00 to 2.03). The Health Professionals Follow-up Study found a suggestion that infection was primarily associated with more aggressive disease, as shown by the higher Gleason scores at diagnosis, but small numbers prohibited a subgroup analysis among men with advanced disease. In this analysis with more than two decades of follow-up for case subjects with prostate cancer, we found that *T vaginalis*–seropositive status was primarily associated with clinically relevant prostate cancer. That is, compared with a seronegative status, a seropositive status before cancer diagnosis was associated with a statistically significant risk of developing prostate cancer that was diagnosed at an advanced stage. Moreover, *T vaginalis* infection appears to be associated with cancer that will ultimately progress to bony metastases and prostate cancer death, independent of body mass index, smoking status, aspirin randomization group, age at diagnosis, and tumor stage and grade. We found no evidence of a stronger association with higher Gleason grade but the subjectivity of Gleason grading and the shift in scores over time (16–18) could explain this discrepancy, because Gleason scores in the Health Professionals Follow-up Study tended to be assigned more recently and, thus, may be better predictors of lethal disease (18).

Our study had several limitations. Because all men provided blood samples in 1982 and all *T vaginalis* assays of plasma samples were completed in 2008, the performance of the assay should not

Table 1. Association between *Trichomonas vaginalis* antibody serostatus and prostate cancer risk among 673 matched pairs nested in the Physicians' Health Study (1982–2000)*

	T vaginalis serostatus	
	Negative	Positive
Control subjects, No. (%)	529 (78.6)	144 (21.4)
All prostate cancer		
Case subjects, No. (%)	508 (75.5)	165 (24.5)
OR (95% CI)	1.00 (Ref)	1.23 (0.94 to 1.61)
Tumor grade: Gleason 2–6		
Case subjects, No. (%)	238 (76.3)	74 (23.7)
OR (95% CI)	1.00 (Ref)	1.16 (0.77 to 1.74)
Tumor grade: Gleason 7–10		
Case subjects, No. (%)	204 (76.7)	62 (23.3)
OR (95% CI)	1.00 (Ref)	1.10 (0.72 to 1.68)
Tumor stage: localized (T1 or T2)		
Case subjects, No. (%)	406 (76.6)	124 (23.4)
OR (95% CI)	1.00 (Ref)	1.10 (0.81 to 1.49)
Tumor stage: extraprostatic (T3 or T4, N1, and M1)		
Case subjects, No. (%)	70 (66.7)	35 (33.3)
OR (95% CI)	1.00 (Ref)	2.17 (1.08 to 4.37)
Nonlethal cancer		
Case subjects, No. (%)	416 (76.7)	126 (23.3)
OR (95% CI)	1.00 (Ref)	1.01 (0.75 to 1.37)
Lethal cancer or development of bony metastases		
Case subjects, No. (%)	92 (70.2)	39 (29.8)
OR (95% CI)	1.00 (Ref)	2.69 (1.37 to 5.28)
Age at diagnosis: <65 y		
Case subjects, No. (%)	169 (76.5)	52 (23.5)
OR (95% CI)	1.00 (Ref)	1.41 (0.86 to 2.31)
Age at diagnosis: ≥65 y		
Case subjects, No. (%)	339 (75.0)	113 (25.0)
OR (95% CI)	1.00 (Ref)	1.12 (0.81 to 1.56)
Time from blood draw to diagnosis: ≤5 y		
Case subjects, No. (%)	64 (68.1)	30 (31.9)
OR (95% CI)	1.00 (Ref)	2.86 (1.27 to 6.47)
Time from blood draw to diagnosis: >5 y		
Case subjects, No. (%)	444 (76.7)	135 (23.3)
OR (95% CI)	1.00 (Ref)	1.09 (0.81 to 1.46)

* From logistic regression conditioned on age and smoking and additionally adjusted for randomized aspirin assignment and body mass index. CI = confidence interval; OR = odds ratio; Ref = referent.

be differentially influenced by specimen quality according to date of cancer diagnosis. The unknown period of time between infection and blood collection, however, could influence assay sensitivity. Presumably, men who were infected with *T vaginalis* closer to the time of blood collection in 1982 would be more likely to have detectable levels of antibodies. Because case and control subjects were matched on age (range = 40–84 years at blood collection) and timing of infection is more likely to be related to age than calendar time, this misclassification would likely be nondifferential with respect to case–control status and thus lead us to underestimate the true effect estimate.

Two additional biases also warrant attention. First, we found that the association between *T vaginalis* infection and incidence of prostate cancer was stronger among men diagnosed within 5 years of blood collection. Biomarkers most strongly associated with disease occurring early in a study typically raise concerns about reverse causation (ie, because of the influence of early preclinical disease on the measured biomarker). However, in this study and in all studies of prostate cancer, biological heterogeneity and the impact of PSA testing on the type of prostate cancers diagnosed are important considerations. Consequently, the men who were diagnosed with prostate cancer earlier in our follow-up, before the introduction of PSA testing in 1986, are more likely to be clinically relevant. Thus, the association observed among case subjects who were diagnosed early in follow-up is consistent with the strong association between infection and advanced-stage or lethal disease. For reverse causation to account for our study findings, the carcinogenic process would have to lead to higher levels of detectable antibodies. Although no data have been obtained to support or contest the assumption that levels of antibodies against *T vaginalis* increase during cancer development, tumorigenesis is known to alter adaptive immune response (19). Second, our findings could be influenced by detection bias if men with *T vaginalis* infection were more likely to be diagnosed with prostate cancer. To address this possibility, we investigated the relationship of antibody levels to baseline PSA levels but found no association. However, we cannot rule out other urologic symptoms that could bring about diagnosis. Conservatively, serological history of infection with *T vaginalis* may be a marker of clinically relevant disease, as suggested by the association between infection and development of

bony metastases or prostate cancer death. More research is required to establish this association.

Disease heterogeneity could also largely explain the apparent discrepancy between our findings and those of a recent study using data from 616 case subjects and 616 matched control subjects sampled from the Prostate Cancer Prevention Trial, a randomized trial of finasteride in 18 882 men, which found no association between *T vaginalis* seropositivity and the incidence of prostate cancer (20). We found that a *T vaginalis*–seropositive status was principally associated with aggressive, potentially lethal disease. In contrast, most prostate cancers that were analyzed in the Prostate Cancer Prevention Trial were diagnosed at an early stage as a result of annual PSA screening and end-of-study prostate biopsy (21). Evidence is accumulating that the risk factors for lethal and indolent prostate cancer may differ. In an analysis in the Health Professionals Follow-up Study that examined 10 risk factors for total or advanced prostate cancer supported by existing literature (22), only four factors were found to have a statistically significant association with overall incidence: African American race, positive family history, higher tomato sauce intake (inversely), and α-linolenic acid intake. By contrast, recent smoking history, taller height, higher body mass index, positive family history, and high intakes of total energy, calcium, and linolenic acid were all statistically significantly associated with fatal prostate cancer. Consistent with our study, these results suggest that there may be multiple biological pathways that contribute to particular subgroups of prostate cancer.

The proportions of case subjects and control subjects with high seropositivity for antibodies against *T vaginalis* were somewhat higher in this study (24.5% of case subjects and 21.4% of control subjects) than in the Prostate Cancer Prevention Trial (15.2% of case subjects and 15.0% of control subjects) or the Health Professionals Follow-up Study (13% of case subjects and 9% of control subjects) (12). Assays for all three studies were prepared under the direction of the same microbiologist (J. F. Alderete) and used an enzyme-linked immunosorbent assay to detect antibodies against α-actinin protein from *T vaginalis*. In both the Prostate Cancer Prevention Trial and the Physicians' Health Study studies, known seropositive and seronegative control samples were used to determine absorbance score cut points, which were then applied to study case subjects and control subjects. In the Health Professionals Follow-up Study, absorbance score cut points were based on previous serological findings (23,24), because serum samples from positive and negative control subjects were not available. Furthermore, absolute readings of the enzyme-linked immunosorbent assays in all three studies could be influenced by the specific technician conducting the assay and the fact that the laboratory was relocated in December 2007. Thus, differences in assay sensitivity may account for some of the variation in distribution of *T vaginalis* seropositivity across these three studies, especially given that demographic characteristics do not appear to explain the observed variability. All three studies included men from across the United States. Although African American race and lower socioeconomic status are generally associated with higher rates of sexually transmitted infections (25), including *T vaginalis* infections (3), the study with the highest proportion of men with a seropositive status (ie, the Physicians' Health Study) has the smallest proportion of African Americans (<1%) and a relatively high socioeconomic status because all participants are physicians. Further, the mean age at blood collection in all three studies was similar (ie, 66 years in Health Professionals Follow-up Study, 64 years in Prostate Cancer Prevention Trial, and 59 years in Physicians' Health Study).

Because other sexually transmitted infections occur concurrently with *T vaginalis* infections, we cannot rule out the possibility that *T vaginalis* is acting as a marker for another infection. However, two studies (5,6) report that concomitant sexually transmitted infections, including those by *N gonorrhoeae* and *C trachomatis*, occur only in 10%–20% of case subjects, making it unlikely that these particular sexually transmitted infections could account for the observed association. Furthermore, the previous study in the Health Professionals Follow-up Study investigated other common sexually transmitted infections, including those by *N gonorrhoeae*, *C trachomatis*, *Treponema pallidum*, and human papillomavirus, and found no association with prostate cancer, except for an inverse association for human herpesvirus type 8 infection (26,27). Nested case–control studies using data from the Nordic biobank consortium found no association between prostate cancer risk and human papillomavirus types 16, 18, and/or 33 (28), herpes simplex virus-2, or human herpesvirus type 8 (29); however, these studies observed a statistically significant inverse association with serological evidence of *C trachomatis* infection (30). A study nested within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (20) found that seroprevalence of *C trachomatis*, human papillomavirus-16 and -18, herpes simplex virus-2, cytomegalovirus, and human herpesvirus type 8 were not individually associated with prostate cancer risk among white men. Men with one or more sexually transmitted infections, however, had a modest increase in risk of developing prostate cancer (OR = 1.3, 95% CI = 1.0 to 1.6), indicating that the measured infections could perhaps be serving as proxies for another infection such as *T vaginalis*.

Although our study may elucidate one mechanism by which local prostatic inflammation could arise and lead to downstream events that influence prostate cancer development and progression, studies that focus on local response to infection in the prostate are needed to determine whether *T vaginalis* is a causal agent. Nonetheless, in light of the limited understanding of factors that lead to lethal prostate cancer, our finding of an association between *T vaginalis* serostatus and aggressive prostate cancer is noteworthy. If our findings are confirmed, *T vaginalis* could serve as a marker for adverse outcomes in patients for prostate cancer or, more optimistically, as a target for secondary chemoprevention.

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Funding

National Cancer Institute (5R01CA058684-13, 5R01CA042182-20, CA34944, CA40360, CA097193, T32CA009001-32 to J.R.S.), National Heart Lung and Blood Institute (HL-26490, HL-34595), Harvard University Milton Fund (to L.A.M.), Dana-Farber/Harvard Cancer Center Prostate Specialized Program of Research Excellence (to J.R.S.), and Prostate Cancer Foundation (to L.A.M.).

Notes

The authors had full responsibility for the design of the study, the collection of data, the analysis and interpretation of the data, the decision to submit the manuscript for publication, and the writing of the manuscript.

We are grateful to Ms Jennifer Sinnott and Ms Haiyan Zhang for their assistance with computer programming and data management. We thank the participants of the Physicians' Health Study for their longstanding commitment and participation.

Manuscript received February 17, 2009; revised July 21, 2009; accepted August 4, 2009.

Epidemiology of Prostate Cancer Risk and Progression

Prostate Cancer Evidence Academy

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Descriptive Epidemiology

PUBLIC HEALTH BURDEN OF PROSTATE CANCER

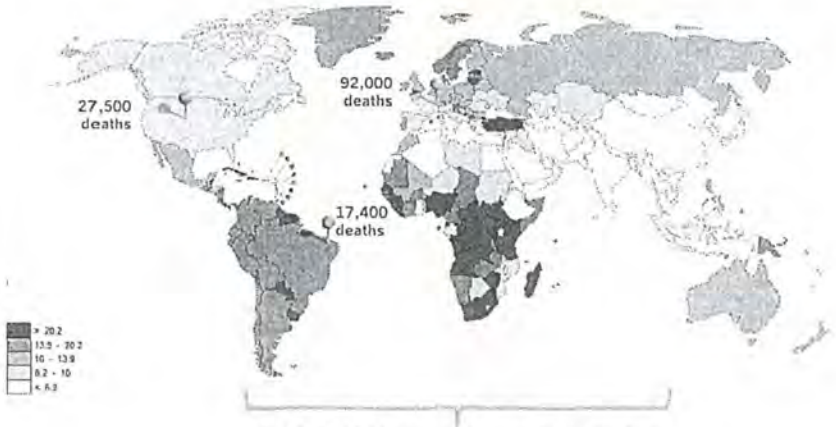
Geographic differences in prostate cancer incidence

→ 1.1 million incident prostate cancers per year

40-fold difference in incidence rates

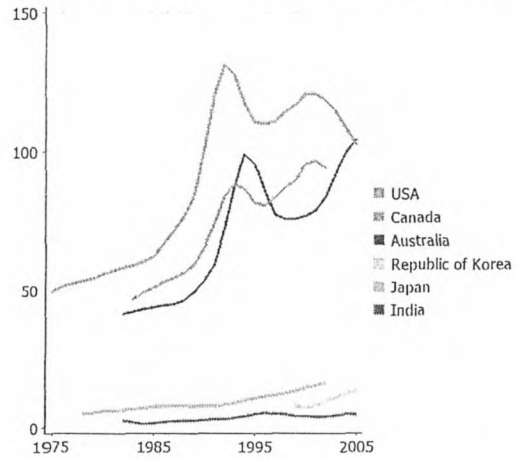
Geographic differences in prostate cancer mortality

→ 370,000 deaths from prostate cancer per year



12-fold difference in mortality rates

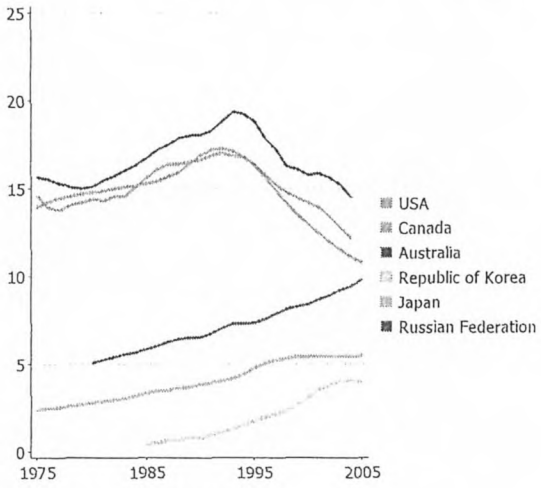
Trends in prostate cancer incidence



Age-standardized rate (W) per 100,000

GLOBOCAN 2008, International Agency for Research on Cancer

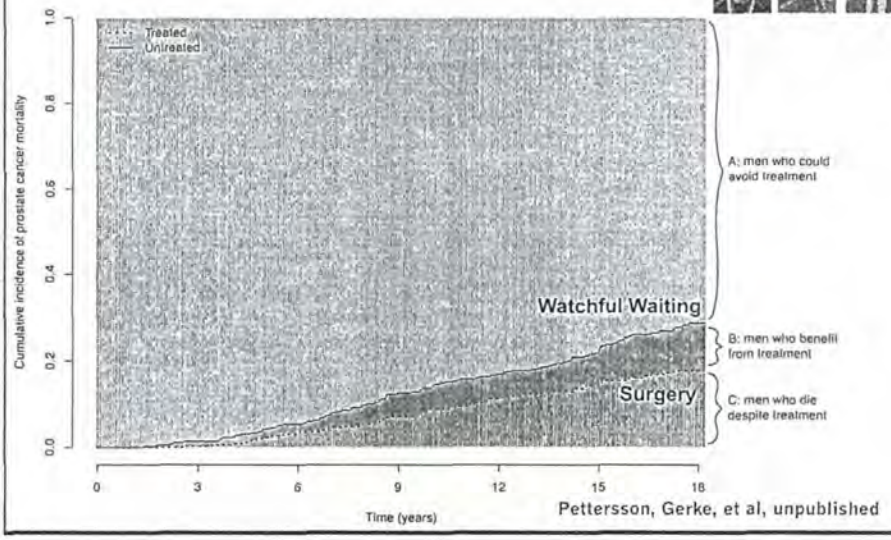
Trends in prostate cancer mortality



Age-standardized rate (W) per 100,000

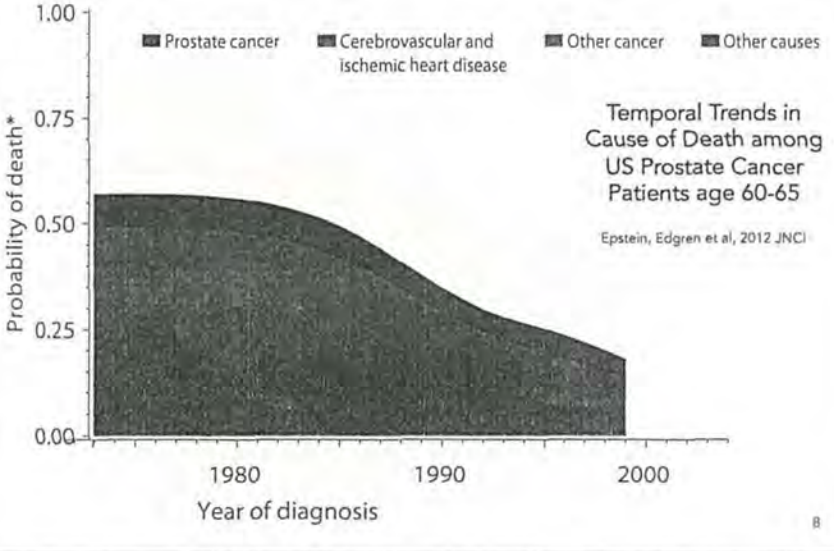
GLOBOCAN 2008, International Agency for Research on Cancer

Variable and long natural history of prostate cancer



Pettersson, Gerke, et al, unpublished

10-year risk of death among prostate cancer patients



RISK FACTORS FOR TOTAL PROSTATE CANCER

Risk factors for total prostate cancer

Established

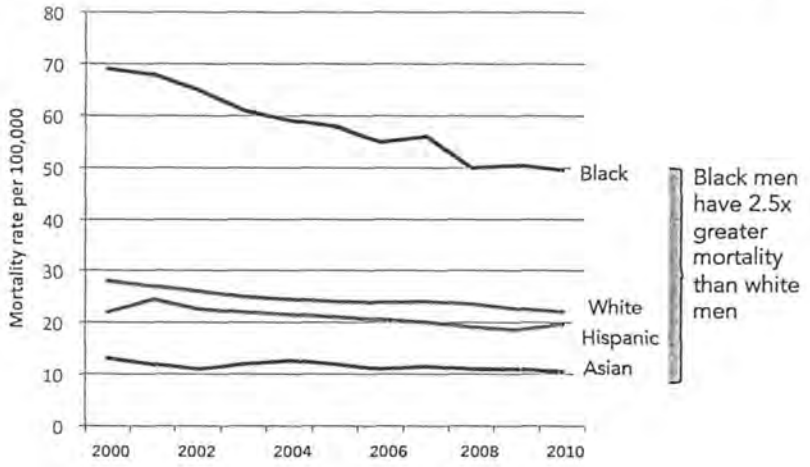
- Older age
- African ancestry
- Family history of prostate cancer
- Genetic risk loci

Probable

- Taller height
- Diabetes (lower risk)

Race/ethnicity

Trends in prostate cancer mortality rates in the US by race/ethnicity



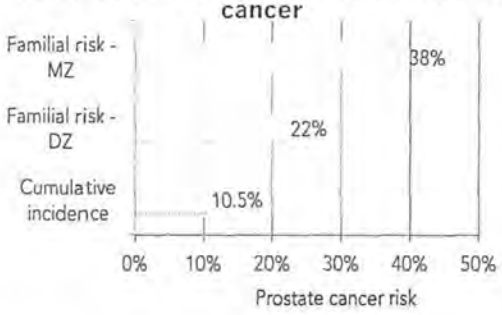
Centers for Disease Control and Prevention

Family history of prostate cancer

The Heritability of Prostate Cancer in the Nordic Twin Study of Cancer

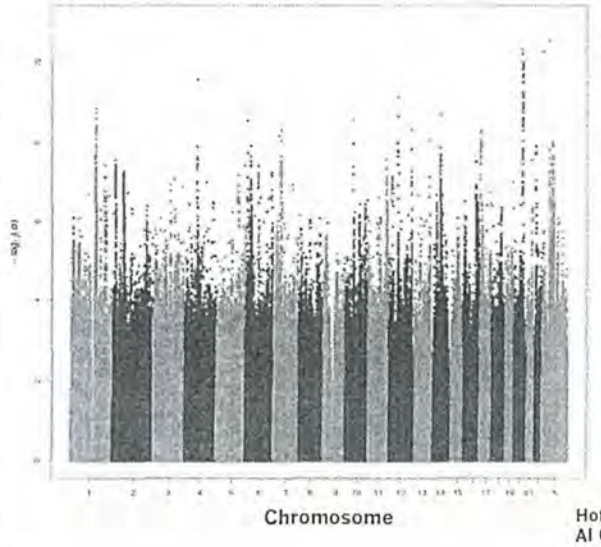
Jacob G. Hjelmborg[1,2], Thomas Scheike[3], Kirsi Holst[3], Axel Skytthe[1,2], Kathryn L. Penney[4,5], Rebecca E. Graff[5], Eero Pukkala[6,7], Kaare Christensen[1,2], Hans-Olov Adami[8,9,10], Niels V. Holm[3,4], Ekrasiah Nuttall[3], Steinunn Thorsteinsdottir[11], Mikael Hemmingsson[11,12,13], Kamila Czene[11], Jennifer R. Harris[14], Jaakko Kaprio[3,6], and Lorelei A. Mucci[4,5,11]

Cumulative and familial risk of prostate cancer



Heritability of prostate cancer = 58%

Inherited genetic risk loci



- >107 validated inherited risk SNPs
- Explain <1/3 of heritability
- Unique genetic risk loci associated with ethnicity
- Most risk SNPs associated similarly with aggressive and indolent prostate cancer

Hoffman et al, Cancer Discov 2015
Al Olaama et al, Nat Genet 2014
Shui et al, Eur Urology 2014

Association between diabetes and prostate cancer risk

Results from the Multiethnic Cohort

	Total prostate cancer
Whites	**Diabetes associated with 35% lower risk**
Blacks	**Diabetes associated with 11% lower risk**
Asian	**Diabetes associated with 19% lower risk**
Latinos	**Diabetes associated with 22% lower risk**

Waters et al, Am J Epidemiol 2009

Genetic variants associated with diabetes and prostate cancer

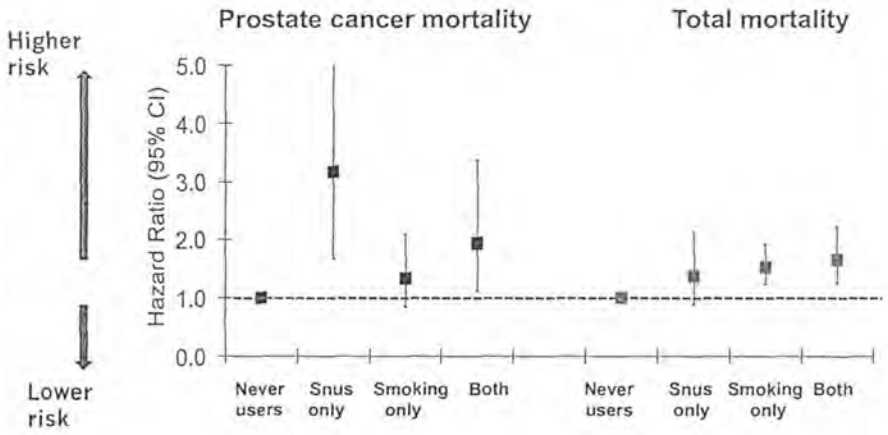
36 genetic risk loci associated with type II diabetes

- 10 associated with prostate cancer risk: NOTCH2, ADCY5, JAZF1, CDKN2A/B, TCF7L2, KCNQ1, MTNR1B, FTO, **HNF1B**
- **HNF1B and prostate cancer risk, Odds ratio = 0.76, p = 0.00008**
- Analysis suggests effect of the genetic loci is through pathways unrelated to diabetes

Michaela et al, AJE 2012

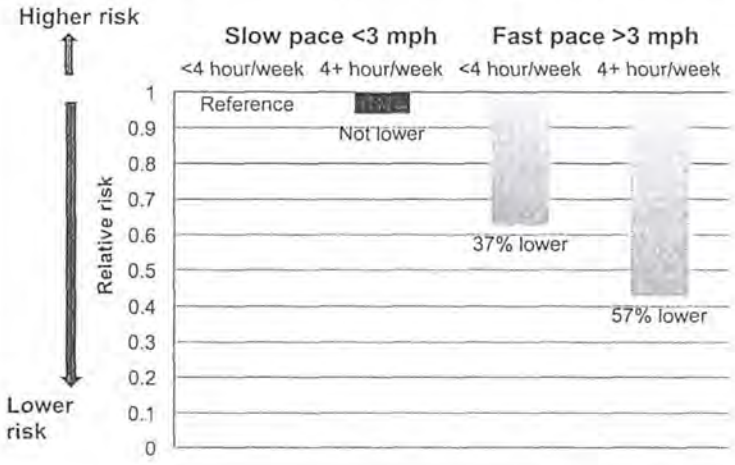
RISK FACTORS FOR PROSTATE CANCER PROGRESSION

Smoking and snus use among 9,500 Swedish men with prostate cancer



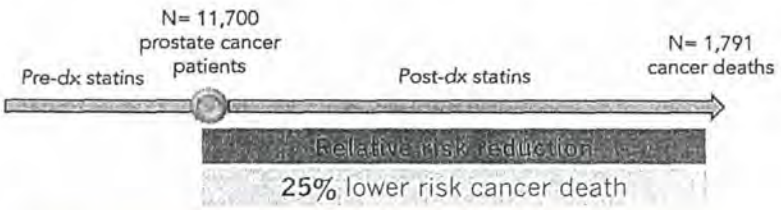
Wilson K., unpublished

Physical activity and prostate cancer recurrence among prostate cancer patients



Richman E, 2011 Cancer Res

Statins and prostate cancer: pre- vs. post- diagnosis



Relative risk reduction
25% lower risk cancer death

Pre-dx use?	Relative risk reduction
NO	18% lower risk cancer death
YES	45% lower risk cancer death

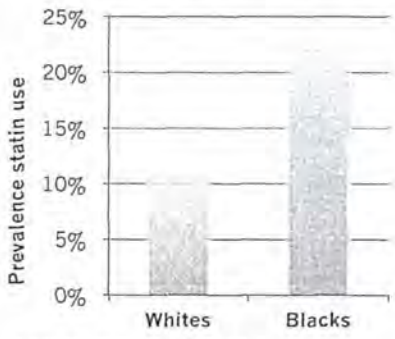
35% lower risk for hydrophilic statins: pravastatin and rosuvastatin
23% lower risk for lipophilic statins: atorvastatin, simvastatin, fluvastatin, and cerivastatin

Yu et al, JCO 2014

Statins, race/ethnicity and prostate cancer

In Southern Community Cohort Study

Prevalence of statin use



Statins and high grade cancer

	Relative risk reduction
Whites	45% lower risk
Blacks	36% lower risk

Kantor, et al., 2014 SER Meeting

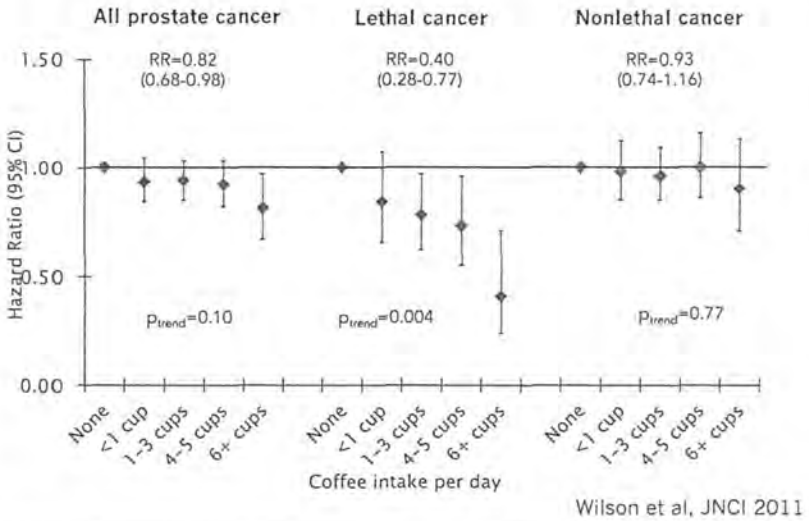
Coffee and prostate cancer risk

Coffee

↓ Insulin ↓ Inflammation ↑ Antioxidants

↓ Prostate cancer progression

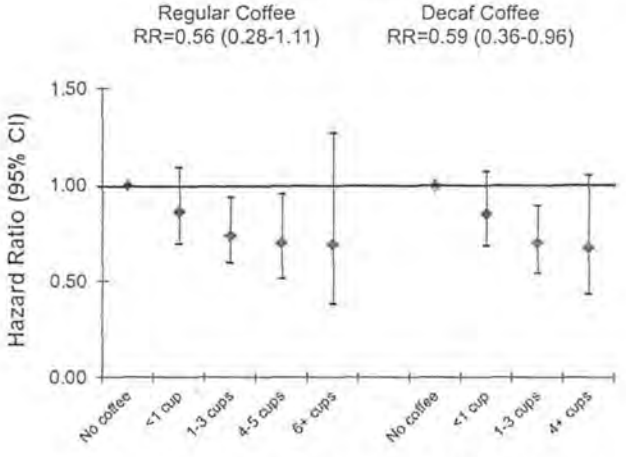
Coffee and prostate cancer risk



Wilson et al, JNCI 2011

Regular vs. Decaf

Lethal cancer



Wilson et al, JNCI 2011

Lycopene and alpha-tocopherol

Circulating carotenoids and prostate cancer risk
Pooled analysis of N=11,239 cases and 18,541 controls

	Lycopene OR (95% CI)	Alpha-tocopherol OR (95% CI)
Total prostate cancer	0.97 (0.87-1.08)	0.86 (0.79-0.93)
Stage of disease		
T1/T2 (localized)	1.04 (0.92-1.18)	0.94 (0.85-1.05)
T3/T4/N1/M1 (advanced)	0.73 (0.54-0.99)	0.71 (0.57-0.88)
Smoking		
Never	1.00 (0.84-1.19)	0.99 (0.82-1.18)
Current	0.76 (0.59-0.96)	0.82 (0.73-0.93)

Key et al, AJCN 2015

Summary of risk factors for advanced/ lethal prostate cancer

Lifestyle factor	Direction of association	Strength of evidence
Cigarette smoking	↑↑	Strong
Obesity	↑↑	Strong
Physical activity	↓↓	Strong
Statins	↓↓	Probable
Lipid levels	↑	Possible
Lycopene/cooked tomatoes	↓↓	Probable
Alpha-tocopherol	↑↑	Probable
Coffee	↓	Possible
Phosphorus/ Calcium	↑	Possible
Vitamin D	↓	Possible

FUTURE DIRECTIONS

Future directions

- Identifying risk factors for molecular subtypes of prostate cancer
  - E.g. TMPRSS2:ERG, PTEN loss
- Identifying unique risk factors for prostate cancer by race/ethnicity
- Evidence for novel risk factors
  - Vasectomy, ejaculation frequency, sexually transmitted infections, circadian rhythm

Date of last revision: September 21, 2015

TITLE

An evaluation of glyphosate use and the risk of non-Hodgkin lymphoma major histological sub-types in the North American Pooled Project (NAPP)

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TARGET JOURNAL

Occupational and Environmental Medicine

ARTICLE TYPE

Original article

KEY TERMS

Glyphosate; Lymphoma, Non-Hodgkin; Pesticides; Case-Control Studies

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WORD COUNT

Abstract: limit 250 (count 249)

Text: limit 4500 (count 6222)

Date of last revision: September 21, 2015

TABLE COUNT

In manuscript: limit 5 (count 4)

In supplement: no limit (count 2)

FIGURE COUNT

In manuscript: no limit (count 1)

In supplement: no limit (count 0)

REFERENCE COUNT

Limit 40 (count 33)

WHAT THIS PAPER ADDS

- Exposure to glyphosate, a broad-spectrum and frequently used herbicide, may be associated with non-Hodgkin lymphoma (NHL). Little is known about how risks may differ by glyphosate exposure levels and NHL sub-types.
- To address this research gap, this analysis integrated detailed, self-reported glyphosate use information with assessments of NHL risk overall and by major histological sub-type using pooled data from 1690 NHL cases and 5131 controls from the U.S. Midwest and Canada.
- Subjects who ever used glyphosate had elevated odds ratios for NHL overall and for all subtypes except follicular lymphoma. Significant or nearly significant risks of NHL overall were observed for >2 days per year (OR=2.42, 95% CI: 1.48, 3.96) and >7 lifetime days (OR=1.55, 95% CI: 0.99, 2.44) of glyphosate use, with some differences in risk by sub-type.
- Glyphosate use may be associated with elevated NHL risk. Although the pattern of risks was not clear across exposure categories, these findings from a large dataset offer more precision than results from previous studies.

Date of last revision: September 21, 2015

ABSTRACT (249)

Objectives: Glyphosate is the most frequently used herbicide worldwide. Some epidemiological studies have found positive associations between glyphosate exposure and non-Hodgkin lymphoma (NHL). This study aimed to evaluate NHL risk overall and by major histological sub-type using detailed glyphosate use metrics.

Methods: The NAPP, composed of pooled case-control studies from the U.S. and Canada, includes NHL cases (N=1690) and controls (N=5131) who provided information on pesticide use. Cases (follicular lymphoma [FL], diffuse large B-cell lymphoma [DLBCL], small lymphocytic lymphoma [SLL], other) from cancer registries and hospitals were frequency-matched to population-based controls. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) by ever/never, duration, frequency, and lifetime days of glyphosate use. Models were adjusted for age, sex, location, proxy respondent, family history of lymphohematopoietic cancer, and personal protective equipment.

Results: Cases who ever used glyphosate (N=133) had a significantly elevated risk of NHL overall (OR=1.43, 95% CI: 1.11, 1.83). Subjects who used glyphosate for >3.5 years had increased SLL risk (OR=1.98, 95% CI: 0.89, 4.39) and those who handled glyphosate for >2 days/year had significantly elevated odds of NHL overall (OR=2.42, 95% CI: 1.48, 3.96) and DLBCL (OR=2.83, 95% CI: 1.48, 5.41). There were suggestive increases (p-trend ≤0.02) in risk of NHL overall, FL, and SLL with more days/year of glyphosate use.

Conclusions: Glyphosate use may be associated with increased NHL risk. Although risk differences by histological sub-type were not consistent across glyphosate use metrics, the NAPP's large sample size yielded more precise results than possible in previous studies.

Date of last revision: September 21, 2015

INTRODUCTION

Glyphosate [N-(phosphonomethyl)glycine] is a broad-spectrum herbicide that is one of the most frequently applied pesticides in the world. First developed commercially for agricultural use in the early 1970s, glyphosate quickly became a popular chemical; as of 2012, it was used in more than 750 products with an annual global production volume exceeding 600,000 tonnes (1). In the U.S., the highest levels of agricultural use occur in the mid-west on crops such as corn, soybeans, and wheat (2). These crops are also examples of the many different types of plants that have been genetically engineered to be resistant to glyphosate.

Commented [AB1]: Check to make sure all these crops have genetically modified seed on the market. I do not think that is the case for wheat yet. I think rice was to be available this year

Glyphosate has been examined as a potential risk factor for lymphatic and hematopoietic cancers including non-Hodgkin lymphoma (NHL). In Canada, NHL ranks as the fifth most incident cancer in males following neoplasms of the prostate, colorectum, lung, and bladder (3). In the American mid-west NHL accounts for an unusually large number of cancers in agricultural areas where populations tend to have lower cancer rates overall (4). The causes of NHL are largely unknown (Hartge P, Wang SS, Bracci PM, Devesa SS, Holly EA. Non-Hodgkin Lymphoma. In Cancer epidemiology and Prevention, 3rd Edition. Shottenfeld D, Fraumeni JF, Jr. (Eds.). Oxford University Press, NY, Ny, 2006), pp. 898-918.). ~~Male~~ NHL has been associated with farming (Blair et al., 1992)~~gender, advanced age, and immune suppression are the best-known risk factors. Agricultural exposures are hypothesized to be involved in the development of NHL~~ and this has prompted studies focused on pesticides.

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In the 1980s and 1990s Four~~four~~ population-based case-control studies were conducted in the U.S. mid-west and six Canadian provinces to examine putative associations between agricultural exposures and pesticides and the risk of NHL. Individual study results showed positive associations between self-reported glyphosate use and NHL risk, although there was variation in the magnitude and statistical significance of risks between studies. In an analysis of the Canadian study the odds ratio [OR] for NHL was 1.26 (95% confidence interval [CI]: 0.87, 1.80) for the use of glyphosate with adjustment for age and province (N=51 exposed cases) (5). The OR was slightly higher from~~A similar risk estimate was found in a separate analysis of men who reportedly ever handled glyphosate in Iowa and Minnesota (6) and higher odds were calculated in~~ a pooled analysis that included 36 exposed male cases from Iowa, Minnesota, Kansas, and Nebraska (logistic regression OR=2.1, 95% CI: 1.1, 4.0 adjusted for age, study site, and other pesticides) (7).

Other studies involving glyphosate exposure and NHL risk have been conducted and many were included in a systematic literature review and meta-analysis of epidemiological studies of pesticide exposure and NHL risk (8). This meta-analysis found~~demonstrated~~ that glyphosate exposure was significantly associated with ~~elevated risks of~~ NHL ~~overall~~ (meta risk ratio [mRR]=1.5, 95% CI: 1.1-2.0, 6 papers). The OR for ~~and~~ B cell lymphoma, ~~(mRR=2.0, 95% CI: 1.1-3.6, 2 papers),~~ a commonly diagnosed NHL sub-type in the regions from which included studies were drawn, was (mRR=2.0, 95% CI: 1.1-3.6, 2 papers). ~~However, meta-analyses were based on a small number of included papers and each study contained low numbers of exposed subjects. Only one included study (9) reported risks by NHL sub-type and only three (5, 9, 10) reported risks by glyphosate exposure level.~~

Date of last revision: September 21, 2015

A comprehensive evaluation of glyphosate carcinogenicity was recently undertaken by the International Agency for Research on Cancer (IARC) (11). This review of mechanistic, animal, and epidemiological evidence classifiedled to the evaluation of glyphosate as a "probable" (group 2A) carcinogen for NHL based on limited evidence in humans and sufficient evidence in experimental animals. The assessment of limited evidence from epidemiological studies was based on case-control studiesprimarily focused on evidence from case-control studies of occupational glyphosate exposure in the U.S., Canada, and Sweden that reported increased risks of NHL that persisted after adjustment for other pesticides. No association between NHL and use of glyphosate was seend in the Agricultural Health Study (AHS), a large prospective study of farmers and commercial pesticide applicators in the U.S.(11). In bioassays, gGlyphosate was was associated with renal tubule carcinoma, pancreatic islet-cell adenoma, and skin tumors (11). able to cause different cancers in mice, postulated to occur through initiation and promotion. Mechanistic and other data supported the "probable" carcinogen conclusion by providing strong evidence for genotoxicity and oxidative stress, both of which are mechanisms of action that can take place in humans (11).

There are several research gaps that need to be addressed in order to better understand the role and impact of glyphosate exposure on the development ofcancer risk, specifically NHL. Individual studies often have limited power for glyphosate exposure, lack evaluation of NHL by sub-type, and do not adjust risk estimates for other pesticides and other exposures (8, 11). MAdditionally, most studies do not have quantitative exposure data needed to perform more sensitive epidemiological analyses and few have addressed potential effect modifiers to identify if glyphosate exposure has a different impact on NHL risk under certain circumstances. Schinasi and Leon (8) have suggested pooling studies as an attempt to overcome some of these limitations. ; AGRICOH, a consortium of agricultural cohorts, is a global effort of this kind (12). Other existing studies can be similarly leveraged for enhancing our knowledge and understanding about glyphosate exposure and NHL risk.

The North American Pooled Project (NAPP) is a pooled resource of population-based case-control studies previously conducted in the U.S. and Canada. The primary objective of this effortstudy was to provide larger numbers for more detailed analyses of possible relationships between NHL and pesticide use. In this paper we evaluate the association between glyphosate use and the risk of NHL among men and women in the NAPP. in the North American Pooled Project (NAPP), a pooled resource of population-based case-control studies previously conducted in the U.S. and Canada. NHL risk was assessed overall and by histological sub-type using detailed self-reported glyphosate use information and adjustment for other pesticides and possible risk factors. The secondary aim of this study was to examine the effects of personal protective equipment (PPE) on the association between glyphosate use and NHL risk overall.

METHODS

Study population

The NAPP is a large and newly established resource of pooling ofed data from four previously conducted case-control studies of men and women who were diagnosed with soft tissue sarcoma and lymphatic

and hematopoietic cancers, including NHL, in the U.S. and Canada. NHL cases were recruited from cancer registries and hospitals during the 1980s in four states (Iowa, Minnesota, Kansas, and Nebraska) and between 1991 and 1994 in six provinces (Quebec, Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia). Cases were 19 years of age or older in all jurisdictions (I think the 19 age cut is correct, just check each study to make sure). Controls were selected from the general population in each state or province. Selection procedures varied by study but included ~~by~~ random digit dialing, voters' lists, health insurance records, Medicare listings for those older than 65 years, and from state mortality files for deceased cases. Controls were matched to NHL cases in each state/province on the basis of age (±2 or 5 years). In some states, cases and controls were matched on the additional variables of sex (Nebraska), race (Nebraska), and vital status and year of death for deceased cases (Iowa, Minnesota, Nebraska, Kansas). All states and provinces included men; women were only included in Nebraska. Deceased cases and controls were eligible for inclusion in the U.S. ~~case-control~~ studies. The Canadian study only considered alive cases and controls. The present analysis used data from both men and women and from alive and deceased NHL cases (N=1690) and controls (N=5131).

Data collection

Participants, or surrogates, provided detailed information about demographic characteristics, pesticide use, agricultural exposures, and exposure to other known or suspected NHL risk factors including lifestyle, medical and occupational history. Interviewer-administered questionnaires were conducted by telephone (Kansas and Nebraska) or in person (Iowa and Minnesota) with cases and controls or their surrogates if subjects were deceased or too ill to respond themselves. In Canada, all cases and controls were mailed a questionnaire to complete themselves (or by their surrogates). Participants who indicated that they had used pesticides were subsequently interviewed over the telephone for details about their pesticide exposure. The Canadian questionnaire was modified from the telephone interview questionnaires that were used in Kansas and Nebraska. The questionnaires from all case-control studies were very similar since they shared a common research objective, involved overlapping groups of principal investigators, and were developed during the same time period. This made the data highly amenable to pooling ~~at present~~. The complete methodologies of each case-control study have been described by Cantor et al., 1992 (Iowa and Minnesota) (6), Hoar et al., 1986 (Kansas) (13), Zahm et al., 1990 (Nebraska) (14), and McDuffie et al., 2001 (Canada) (5).

The NAPP contains extensive information about pesticide use and agricultural exposures reported by cases and controls. In general, pesticide classifications are available from ~~data were collected beginning with the~~ broadest categories (e.g. occupations with potential pesticide exposure), to ~~followed by~~ major chemical classes (e.g. herbicides), to chemical groups (e.g. phenoxy herbicides), and finally individual compounds (e.g. 2,4-D). For each individual compound reported, information was collected for dichotomous use (ever/never), duration of use (number of years), and frequency of personal handling (number of days/year). Duration data were not collected in Kansas and frequency information was not collected in Iowa, Minnesota, and Kansas ~~and Kansas~~. In Kansas participants were asked to open-endedly recall the details of their pesticide use whereas in all other jurisdictions subjects were prompted by a list of chemicals and their trade names. Participants were also asked to report if they had used any

type of PPE in general (Nebraska and Canada) and with herbicides (Iowa, Minnesota, and Kansas) and specific individual pesticides (Iowa and Minnesota).

Assessment of glyphosate use

Self-reported glyphosate use was examined using several different metrics: dichotomous, duration, frequency, and lifetime days (derived by multiplying number of years used with number of days/year handled). Ordinal categories were created for duration, frequency, and lifetime days analyses based on the median of glyphosate used/handled in controls. Since information about duration of glyphosate use was not collected in Kansas, cases and controls from Kansas were omitted from duration analyses. Similarly, cases and controls from Iowa, Minnesota, and Kansas were excluded from frequency and lifetime days analyses owing to the lack of frequency data collected in these states. Participants who had missing or unknown glyphosate use information, but who were from jurisdictions where glyphosate use information was collected, were coded as "never used" in dichotomous analyses. ~~;~~ fFor duration and frequency analyses, missing values were assigned based on the median duration or frequency by state/province, age, and NHL sub-type (simple imputation, rounded to the nearest whole number). Subjects who reported that they used glyphosate were coded as "ever used" or used/handled for the number of years and days/year that they had reported. Continuous analyses were also conducted in order to determine possible trends and changes in risk for every 5 years, 5 days/year, and 10 lifetime days of glyphosate use.

NHL classification

NHL cases in these s tudies were diagnosed ~~at different time periods~~ during the 1980s and 1990s. NHL cases were classified in Iowa, Minnesota, and Nebraska according to the Working Formulation (15, 16); in Kansas and Quebec by the International Classification of Diseases for Oncology First Edition (ICD-O-1) (1976) (17); and in Ontario, Manitoba, Saskatchewan, Alberta, and British Columbia by ICD-O-2 (1990) (18). The original histology codes used in each study were revisited to classify NHL cases using a single or similar scheme for the NAPP. We used ICD-O-1 to code NHL overall and sub-types in the NAPP since histological sub-types were classified in all jurisdictions according to ICD-O-1. These sub-types were follicular lymphoma (FL), diffuse large B cell lymphoma (DLBCL), small lymphocytic lymphoma (SLL), and other. The "other" sub-type included all cases whose histologies were unknown or not FL, DLBCL, or SLL. Pathology reviews were conducted on 84% of Canadian cases (5), 87% of Kansas cases (13), and for all interviewed cases in Iowa and Minnesota (6) and Nebraska (14) in order to validate NHL diagnoses.

Power and sample size

A power and sample size analysis was conducted using the U.S. National Cancer Institute's (NCI) Power Version 3.0 program (19, 20) by inputting the following parameters: number of controls = 5131; number of cases = 1690; control:case ratio = 3; type I error (two-sided) = 0.05; type II error = 0.2; probability of NHL at baseline = 0.04 (21).

Of all 5131 controls available in the NAPP, 244 (4.76%) reported that they ever used glyphosate. A 5% prevalence of pesticide exposure in controls corresponds to ~~a~~perfect power of (1.00) to detect ORs of

2.00 or higher and a, but lower power of(0.46) to detect an OR of 1.25. Given that approximately 5% of controls reported ever being exposed to glyphosate, at a power level of 0.80, a total of 1103 NHL cases would be required to detect an OR of 1.50 (Appendix 1). The numbers of NHL cases and controls in the NAPP appear to be suitable tofor detecting low to moderate relative risks associated with glyphosate exposure in this population.

Statistical analyses

Descriptive statistics were used to characterize the study population and identify potentially confounding variables. Based on previously published literature, a priori possible confounders included age, sex, state/province, use of a proxy respondent (5, 6, 22), lymphatic or hematopoietic cancer in a first-degree relative (23), and diagnosis with select medical conditions related to immune suppression (any allergies, food allergies, drug allergies, asthma, hay fever, mononucleosis, arthritis, or tuberculosis; ever received chemotherapy or radiation) (24-26). History of living or working on a farm or ranch was also evaluated as a potential confounder.

It was possible that the use of other pesticides in the NAPP may confound the relationship between glyphosate use and NHL risk. A two-pronged approach was used to identify potentially confounding by other pesticides. First, a correlation matrix of pooled data was produced to determine the presence and extent of correlation between glyphosate and each individual herbicide, insecticide, and fungicide reportedly used by NAPP subjects. Second, previously published articles based on the individual case-control studies comprising the NAPP were searched to identify any positive or significant relationships between individual pesticides and NHL risk, as would be required for confounding to occur. Pesticides that were most strongly correlated with glyphosate (defined in this study as Spearman coefficients ≥0.35 and Cohen's Kappa value ≥0.30) and that were significantly or strongly associated with NHL in previous studies were evaluated as confounders. These were the herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) (5, 6) and dicamba (5, 7), as well as the insecticide malathion (5, 7).

The use of PPE with glyphosate could theoretically modify NHL risk by reducing subjects' exposure to glyphosate. Although such information was sought in some studies, data were on a sizableThere was a large proportion of the study subjectsmissing data for the more specific variables of PPE used for herbicides and glyphosate and. Therefore, effect modification analyses could only be conducted using involving any lifetime PPE use were conducted using data reported by cases and controls fromin Nebraska and Canada. Any lifetime PPE usage was also included as a confounding variable in models where it was not evaluated as a possible effect modifier.

Unconditional multiple logistic regression was performed using the LOGISTIC procedure ofon the SAS 9.2 statistical software package (SAS Institute, Cary, North Carolina) to calculate pooled ORs and 95% CIs for associations between glyphosate exposure (dichotomous, duration, frequency, lifetime days, and as a continuous variable) and the risk of NHL overall and by histological sub-type (FL, DLBCL, SLL, and other). Primary logistic regression models (OR[a]) contained the following variables as confounders: age, sex, state/province, lymphatic or hematopoietic cancer in a first-degree relative, use of a proxy respondent, and use of any PPE. Secondary logistic regression models (OR[b]) contained the covariates in the primary

model plus reported use of the pesticides 2,4-D, dicamba, and malathion. Medical conditions and history of living or working on a farm or ranch were found not to~~did not appear to play a role in~~ confounding the relationship between glyphosate use and NHL risk and were not included in the models. Use-response trends for duration, frequency, and lifetime days analyses were deemed to be statistically significant if the two-sided p-value for the ordinal glyphosate use category was ≤0.05. The reference group for all analyses was subjects who never used glyphosate. There was a small proportion of subjects (N=175, 2.57% of all participants) with missing age values; these were imputed based on state/province- and case/control-specific means rounded to the nearest whole number.

Sensitivity tests were conducted by excluding proxy respondents from the main analyses. Proxy respondents were excluded from the analyses of PPE as a potential effect modifier in order to minimize the possibility of bias. For the effect modification analyses, glyphosate use was classified dichotomously and by duration, frequency, and lifetime days and overall NHL risks were calculated using logistic regression models adjusted for age, sex, state/province, lymphatic or hematopoietic cancer in a first-degree relative, and use of 2,4-D, dicamba, and malathion.

Ethics approval

Approval to conduct this analysis was obtained from the University of Toronto Health Sciences Research Ethics Board (#25166) and an ethics exemption was obtained from the U.S. NCI Office of Human Subjects Research (#11351). Individual studies had obtained human subjects approval prior to collection of the data and aAll participants provided informed consent before taking part in the studies included in the NAPP analyses.

RESULTS

Characteristics of NHL cases and controls

A total of 1690 NHL cases and 5131 controls were available in the NAPP for analysis. All participants were included in analyses that encompassed proxy respondents. For assessments involving the duration of glyphosate use, 1520 cases and 4183 controls were available; in frequency and lifetime days analyses, 898 cases and 2938 controls were included. The numbers of cases and controls available for the sensitivity analyses excluding proxy respondents were smaller~~lower~~ (Figure 1).

The most frequently diagnosed histological sub-type was DLBCL (38.28%), followed by FL (27.69%), other (23.91%), and SLL (10.12%) (Table 1). Nebraska yielded the highest proportion of cases (22.78%) and controls (27.91%) compared to other states and provinces. The average ages of cases and controls were 62.72 and 61.66 years, respectively. The majority of subjects were male. A similar proportion of proxy respondents were used by cases and controls. Cases were more than twice as likely to report that a first-degree relative was diagnosed with lymphatic or hematopoietic cancer compared to controls (OR=2.13, 95% CI: 1.69, 2.67). Medical history variables were evaluated as potential confounders but they did not have an appreciable impact on adjusted ORs in the main analyses (OR[a] and OR[b]) and were thus excluded from logistic regression models.

Missing glyphosate use data

There were 7 cases with missing values for the number of years of glyphosate used and 13 cases with missing values for the number of days/year of glyphosate handled in the jurisdictions where duration and frequency of glyphosate use data were collected. The median values for the number of years of glyphosate use in cases all subjects with missing values ranged from 0-2 based on jurisdiction, NHL sub-type, and age. The median value for days/year for subjects with missing information was 0 (zero).

Glyphosate use and NHL risks overall and by major histological sub-type

Overall, 113/1690 cases (6.69%) and 244/5131 (4.76%) controls reported that they had used glyphosate at any point in their lifetime. There was a significant association between glyphosate use and the risk of NHL overall (OR^a=1.43, 95% CI: 1.11, 1.83) (Table 2). Risks were elevated for most NHL sub-types but the magnitude of risk differed by sub-type. The greatest risk was observed in SLL cases (OR^a=1.77, 95% CI: 0.98, 3.22) and the lowest risk was found for FL (OR^a=1.00, 95% CI: 0.65, 1.54). Similar and significant excesses were observed for DLBCL (OR^a=1.60, 95% CI: 1.12, 2.29) and other (OR^a=1.66, 95% CI: 1.04, 2.63) sub-types. Associations were attenuated and no longer statistically significant when the model represented by OR^a was further adjusted for ever use of 2,4-D, dicamba, and malathion (OR^b). The odds of SLL did not change even after adjusting risk estimates for these three pesticides.

When glyphosate use was examined by duration (Table 2), there was a general inverse trend in risks except for cases of SLL, where the odds increased with longer duration of glyphosate use (OR^a=1.98, 95% CI: 0.89, 4.39 for >3.5 years versus OR^a=1.49, 95% CI: 0.63, 3.58 for >0 and ≤3.5 years) and this trend was of borderline statistical significance (p-trend for OR^a=0.08). Additional adjustment for the chemicals 2,4-D, dicamba, and malathion generally resulted in attenuated risk estimates (OR^b) compared to models unadjusted for these pesticides (OR^a) except for SLL, for which the addition of these agents in logistic regression models had no substantial effect on risk (e.g. for >3.5 years of glyphosate use, OR^b=1.94, 95% CI: 0.79, 4.80).

In contrast to duration of glyphosate use, a more consistent pattern of NHL risk emerged in association with frequency of glyphosate personally handled (Table 2). Subjects who handled glyphosate for >2 days/year had NHL risks that were approximately two times the odds observed in participants who handled glyphosate for >0 and ≤2 days/year. This finding was consistent for NHL overall and all sub-types. Elevated risks in the highest category (>2 days/year) were significant for NHL overall (OR^a=2.42, 95% CI: 1.48, 3.96) and DLBCL (OR^a=2.83, 95% CI: 1.48, 5.41) compared to subjects who did not handle glyphosate at all. Significant trends in risk were also found for NHL overall (p-trend for OR^a=0.02) and DLBCL (p-trend for OR^a=0.04). For NHL overall and DLBCL, ORs associated with handling glyphosate for >2 days/year were attenuated but remained statistically significant even after adjusting for the use of 2,4-D, dicamba, and malathion. The pattern of increased risks with more frequent glyphosate handling was still apparent for NHL overall and all sub-types although trends were no longer statistically significant upon adjusting for these three pesticides.

The analysis of lifetime days, derived from the product of number of years used and days/year handled, generally showed risk increases for NHL overall and most sub-types (except "other") in association with

a greater number of lifetime days of glyphosate use (Table 2). These trends were significant for NHL overall (p-trend for OR[a]=0.02), FL (p-trend for OR[a]=0.02), and SLL (p-trend for OR[a]=0.01). There were elevated risks of NHL among participants who had used glyphosate for >7 lifetime days; this was most pronounced for SLL (OR[a]=2.13, 95% CI: 0.76, 5.96). Adjusting for 2,4-D, dicamba, and malathion attenuated risks compared to odds that were unadjusted for these chemicals; however, the general pattern of increased risks remained intact and in some cases (i.e. SLL), was still statistically significant (p-trend for OR[b]=0.03).

Sensitivity analysis

Proxy respondents were used for deceased cases and controls and for alive cases who were too ill to respond to the case-control study questionnaires themselves. The use of proxy respondents might have introduced misclassification of glyphosate use. To account for this possibility, glyphosate use data provided by proxy respondents were excluded from the main analysis presented in Table 2. This generally resulted in reduced ORs compared to risks that included data provided by both self- and proxy respondents, with little effect on the width of confidence intervals and the same general patterns of risks for dichotomous, duration, frequency, and lifetime days analyses (Table 3). For instance, there were significant trends for lifetime days of glyphosate use and the risks of NHL overall (p-trend for OR[a]=0.04), FL (p-trend for OR[a]=0.03), and SLL (p-trend for OR[a]=0.01) (Table 3) that paralleled the trends found in the analysis of data provided by both self- and proxy respondents (Table 2).

However, there were some exceptions to this overall observation. Odds ratios for SLL mostly strengthened with the exclusion of proxy respondents in models both unadjusted for 2,4-D, dicamba, and malathion and models adjusted for these chemicals. For instance, among subjects who ever used glyphosate the risk of SLL excluding data from proxy respondents was 1.89 (OR[a], 95% CI: 1.03, 3.49) which was slightly greater than the risk of SLL based on data provided by self- and proxy respondents (OR[a]=1.77, 95% CI: 0.98, 3.22). Trends of increasing risk of SLL in association with longer duration, greater frequency and lifetime days of glyphosate use were also marginally stronger when data from proxy respondents were excluded.

Effect of PPE

Potential effect modification by PPE usage was evaluated based on data pooled from Canadian and Nebraskan participants. The association between ever glyphosate use and NHL risk overall was generally higher among subjects who reportedly used any type of PPE in their lifetime (OR=0.83, 95% CI: 0.40, 1.73) compared to subjects who never used any type of PPE (OR=0.65, 95% CI: 0.31, 1.35) (Table 4). This pattern of elevated NHL risks in subjects who ever used PPE compared to subjects who never used PPE persisted when glyphosate use was also evaluated by duration, frequency, and lifetime days. Similar to the results in Tables 2 and 3, there were inverse associations between the duration of glyphosate use and NHL risk and positive (increasing) associations between frequency and lifetime days of glyphosate use and NHL risk, regardless of PPE use status. There were many subjects with unknown or missing PPE use information and they were separately modeled in order to reduce the possibility of analyzing

misclassified PPE use data. Risks were high and unstable in this latter group due to the small number of subjects in each glyphosate usage category.

DISCUSSION

The objective of this study was to evaluate potential associations between glyphosate use and NHL risk in the NAPP, a large pooled dataset with detailed information about glyphosate use reported by 1690 NHL cases and 5131 controls. Glyphosate use was associated with elevated NHL risk, a finding that was consistent with previous analyses. Odds somewhat differed by histological sub-type, although there wasn't a consistent pattern across glyphosate use metrics. The novelty of this analysis and increased precision of risk estimates compared to smaller individual studies were major strengths. Yet, the limitations of this study illustrate the need for more research that can better characterize the relationship between glyphosate exposure and the development of NHL.

This report confirms previous analyses indicating increased risks of NHL in association with glyphosate exposure. The odds of NHL for glyphosate use was 1.43 (OR[a], 95% CI: 1.11, 1.83), a value that was situated approximately in between the risks observed in earlier analyses of the Canadian study (OR=1.26, 95% CI: 0.87, 1.80, adjusted for age and province, N=51 exposed cases) (5) and the three pooled U.S. studies (logistic regression OR=2.1, 95% CI: 1.1, 4.0, adjusted for age, study site, and other pesticides, N=36 exposed cases) (7). Further adjusting OR[a] for the pesticides 2,4-D, dicamba, and malathion resulted in an attenuated risk of NHL overall in the NAPP (OR[b]=1.13, 95% CI: 0.84, 1.51). De Roos et al. (2003) (7) used a more conservative approach, a hierarchical regression model, for assessing NHL risk in the three U.S. pooled case-control studies and found that this reduced the odds of NHL overall (OR=1.6, 95% CI: 0.9, 2.8, adjusted for age, study site, and other pesticides). A statistically significant excess of NHL was found in association with more than 2 days per year of use (OR=2.12, 95% CI: 1.20, 3.73) (5) in the Canadian study, a finding that was in agreement with our analogous pooled risk estimate for NHL (OR[a]=2.42, 95% CI: 1.48, 3.96).

Our results are also aligned with findings from epidemiological studies of other populations that found an elevated risk of NHL for glyphosate exposure and with a greater number of days/year of glyphosate use (9), as well as a meta-analysis of glyphosate use and NHL risk (8). From an epidemiological perspective, our results were supportive of the IARC evaluation of glyphosate as a probable (group 2A) carcinogen for NHL (11).

The large sample size of the NAPP was conducive to analyzing NHL risks with different metrics of glyphosate use. Evaluations of dichotomous glyphosate use showed nearly universal increases in risks of NHL overall and by sub-type, but results were more varied upon further examination by duration, frequency, and lifetime days. The odds of NHL, overall and by sub-type, were higher among subjects who reportedly used glyphosate more often in a year or who had greater cumulative use in their lifetime compared to unexposed subjects. Subjects who used glyphosate reported mostly initiating its use in the year 1980. Glyphosate was used by cases and controls for an average of 5 years and handled for an average of 5 days/year. The short duration of use made it challenging to calculate risks associated with longer-term usage, although the mean frequency of handling was typical of how often farmers

reportedly apply glyphosate to agricultural crops (27). For the days/year and lifetime days analyses some trends and risks were statistically significant while others were not, likely due to the lack of sufficient numbers of exposed cases for some sub-types.

There were some differences in risks by sub-type but these were not consistent between the different glyphosate use metrics and were unlikely to be statistically significant. For example, the significant trends observed for lifetime days of glyphosate use and the risks of NHL overall, FL, and SLL were not present for the frequency analysis, where significant trends were only found for NHL overall and DLBCL. In the duration analysis an upward trend was observed for SLL but not for any of the other sub-types or for NHL overall. Despite these uneven results the risks of FL were consistently lower than other sub-types in association with any of the glyphosate use metrics. There was a relatively large number of FL cases in this analysis compared to the numbers available for other sub-types, lessening the likelihood that findings for FL were primarily due to chance. FL is a type of B-cell lymphoma that is the second most common type of NHL, accounting for 22% of all NHLs (28). The observation of lowered FL risks for glyphosate use in this study was a lead for further evaluation. Additionally, the classification of NHL has changed since the case-control studies in the NAPP were conducted. Multiple myeloma is now considered a sub-type of NHL but was not evaluated in this analysis.

A fairly consistent decrease in NHL risk was found when ORs were further adjusted for the pesticides 2,4-D, dicamba, and malathion. This observation suggested that elevated risks of NHL may be attributed, in part, to pesticides other than glyphosate. Formulations of glyphosate reported by NAPP subjects may have contained other active ingredients. In addition or alternatively, glyphosate may have been used in combination with other pesticide active ingredients at the time of application or in the same growing season or year. It is relatively unknown how combinations of pesticides might interact, and we were not able to evaluate this in our analysis. There is a need to further investigate other individual compounds with respect to NHL risk, such as the herbicide 2,4-D, which IARC recently assessed as possibly carcinogenic to humans based on inadequate evidence in humans and limited evidence in animals for NHL (29).

Glyphosate and covariate data provided by self-respondents generally resulted in attenuated risks compared to odds derived from information provided by both self- and proxy respondents. The proportion of proxy respondents used for cases and controls was similar (about one third). Excluding proxies appreciably reduced the numbers of subjects in the sensitivity analysis which might have partly explained differences in risks. There was also the possibility of exposure misclassification by proxy respondents due to inaccurate recall of glyphosate use, which was likely non-differential (27, 30). Non-differential pesticide exposure misclassification was also an issue amongst self-respondents (31). There was less agreement between self-respondents and surrogates for detailed glyphosate use metrics (years and days/year) compared to the dichotomous variable (32). Nevertheless, significant trends of increasing risks in association with greater lifetime days of glyphosate use persisted for NHL overall, FL, and SLL, even when the analysis was limited to self-respondents.

The evaluation of PPE as an effect modifier of the relationship between glyphosate use and overall NHL risk raised some interesting observations. We expected that the use of any PPE such as masks, gloves,

clothing and/or other equipment may confer a protective effect on the development of NHL from glyphosate use by reducing the probability and degree of dermal, respiratory, and oral contact with glyphosate. However, in this study PPE was found to have no effect on the association between glyphosate use and NHL risk overall. This analysis was limited because PPE usage was not specific to glyphosate use or the type or timing of PPE worn. It was also based on pooled data from Canada and Nebraska only and there was a large proportion of missing data. This hypothesis warrants further investigation in larger studies with more information about PPE used with glyphosate in particular.

The exact causes of lymphatic and hematopoietic cancers are not yet known. A suppressed immune system is the most well established risk factor for NHL. It has been hypothesized that pesticides may play a role in modifying immune function (24-26), but there is little evidence to support this hypothesis for glyphosate specifically (11, 25). An alternative or additional explanation is that pesticides may influence the risk of lymphatic and hematopoietic cancers through pathways involving oxidative stress and receptor-mediated mechanisms. The pathway that glyphosate affects in plants is not present in mammals, but there is strong evidence from mechanistic studies that glyphosate causes genotoxicity and the production of reactive oxygen species (11).

The limitations of this study were primarily related to statistical power for some analyses and the possibility of biases and unmeasured confounding. We endeavoured to use data from all subjects for this analysis as reflected by the inclusion of both men and women and alive and deceased subjects. In Canada alone, 50 NHL cases and 133 controls reported ever using glyphosate; pooling resulted in an additional 63 NHL cases and 111 controls who ever used glyphosate in Iowa, Minnesota, Kansas, and Nebraska. Nevertheless, there were small numbers for some categories of duration, frequency, and lifetime days by NHL sub-type due to the absence of duration data collected in Kansas and frequency and lifetime days information from Iowa, Minnesota, and Kansas. Risk estimates based on small numbers may be unstable and could represent chance findings.

To evaluate possible recall bias of self-reported pesticide use, in the study in Kansas, pesticide suppliers were asked to provide information on crops and pesticide purchases for a sample of 130 subjects with farming experience (13, 27). In the Iowa and Nebraska studies, case recall bias was assessed by comparing information on pesticides used that was volunteered versus information that required probing by the interviewer (14, 27, 33). In the Iowa and Minnesota study, interviews were conducted with both farmers and their wives for a sample of subjects (32). There was a moderate level of correspondence between pesticide use information reported by farmers and their pesticide suppliers in Kansas (13, 27). In Iowa and Nebraska, the number of insecticides and herbicides voluntarily identified was similar and suggested the absence of case-response bias, but probing increased the number of positive responses for individual agents (14, 27, 33). In Iowa and Minnesota, surrogate responders were generally a poorer source of information compared to farmers as they had reported a smaller number of pesticides ever used and a greater proportion of "I don't know" answers (32). No similar analysis of recall bias has been conducted in the Canadian case-control study, but the similarity of study designs between the U.S. and Canada make it likely that recall bias is not a major concern in the Canadian study and NAPP as a whole.

Adjusting for several pesticides (2,4-D, dicamba, and malathion) was a useful way to attempt to disentangle the effect of glyphosate from other pesticides on NHL risk. These agents have been shown to be independently associated with NHL in individual case-control studies (5-7). However, they are somewhat correlated with glyphosate exposure in the NAPP and thus their inclusion as confounders may have introduced some degree of collinearity. Unmeasured confounding by other pesticides, agricultural exposures, or unknown factors cannot be ruled out.

While these results are not independent from previous studies, the evaluations by histological sub-type and for detailed glyphosate use metrics are a new and important contribution to the epidemiological literature. NHL is a constellation of heterogeneous cancers that each has its own causes, risk factors, and etiologies. Pesticides, including individual agents such as glyphosate, may exert different effects on these sub-types, and the large size of the NAPP made it possible to parse this out.

The large sample size also resulted in more precise results than possible in previous smaller studies that only had sufficient power to assess risks for dichotomous glyphosate exposure. We were able to model different glyphosate use categories and identify potential trends in NHL risk by sub-type with increasing duration, frequency, and lifetime days of glyphosate use. This made it possible to characterize possible dose-response relationships between glyphosate exposure and lymphoma risk. The effect modification analysis by PPE further allowed an examination of factors that might modify glyphosate exposure (and risk). Both agricultural and non-agricultural uses of glyphosate were reported by cases and controls in this population-based, pooled case-control study, making this evaluation externally valid.

The results of this analysis may be considered in future scientific and regulatory reviews of glyphosate in North America and globally. Stakeholders may also use these results as part of future approaches that communicate the health risks of pesticides using information directly ascertained from the North American population. This will help to inform efforts aimed at mitigating occupational and environmental exposure to pesticides. It will also provide high-quality risk estimates that can be used in future estimations of the burden of cancer from pesticide exposure.

ACKNOLWEDGEMENTS

The authors thank Mr. Joe Barker at IMS, Inc., for his computer programming services to pool the case-control datasets. The authors also thank all of the principal investigators of the individual case-control studies for allowing the data to be pooled.

COMPETING INTERESTS

The authors declare no competing interests.

FUNDING

This analysis was conducted with the support of a Prevention Research Grant from the Canadian Cancer Society Research Institute (#703055). There was no involvement in conducting the NAPP, preparing this article, or deciding to submit this paper for publication.

Date of last revision: September 21, 2015

AUTHORS' CONTRIBUTION

MP designed and conducted this analysis and wrote this manuscript. SAH, JJS, and LBF collectively form the NAPP Executive Committee and approved the proposal for this analysis and provided scientific input during the analytic and manuscript preparation phases. AB, SHZ, DDW, and KPC led the original case-control studies in the U.S. JJS, JAM, and JAD were among the principal investigators of the CCSPH in Canada. All co-authors reviewed and approved this manuscript for submission.

DATA SHARING

Unpublished NAPP data is available upon formal request to the NAPP Executive Committee (SAH, JJS, LBF).

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IARC Monographs on the Evaluation of Carcinogenic Risks to Humans
VOLUME 105: DIESEL AND GASOLINE ENGINE EXHAUSTS AND SOME NITROARENES
Lyon, France: 5-12 June 2012

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[1] Because of a 2001 U.S. District Court ruling involving the NCI/NIOSH Diesel Study, involved scientists are barred from publicly releasing data underlying the articles from the diesel study. Roel Vermeulen will participate in the meeting respecting this position.
[2] Aaron Cohen is the principal scientist of the Health Effects Institute (HEI) which conducts research worldwide on the health effects of air pollution. The Institute's core funding comes in equal part from the U.S. Environmental Protection Agency and the makers of motor vehicles for sale in the United States.
[3] David B. Kittelson has received significant research funding from Caterpillar on the influence of biofuels on particulate emissions (ended in 2009); and from BP for methods of measuring ash in engine exhausts (current).
[4] Martie van Tongeren has received significant research funding from Statoil, CONCAWE and CEFIC.

Representatives of national and international health agencies

Matthias Möhner, Federal Institute for Occupational Safety and Health, Germany
Matteo Redaelli, French Agency for Food, Environment and Occupational Health Safety (ANSES), France
Cheryl Siegel Scott, U.S. Environmental Protection Agency, USA

Observers

Nicole Falette, for the Léon Bérard Centre, France
John F. Gamble, for the IARC Review Stakeholder Group[5], USA[6]
Daniel S. Greenbaum, for the Health Effects Institute, USA[7]
Thomas W. Hesterberg, for the IARC Review Stakeholder Group[5], USA[8]
Timothy L. Lash, for the Association of American Railroads (AAR), USA[9]
Markus Mattenklott, German Social Accident Insurance (DGUV), Germany (8-12 June)
Roger O. McClellan, for the IARC Review Stakeholder Group[5], USA[10]
Peter Morfeld, for the European Research Group on Environment and Health in the Transport Sector (EUGT e.V), Germany[11]
Dirk Pallapies, German Social Accident Insurance (DGUV), Germany[12] (5-7 June)
John Carson Wall, for the IARC Review Stakeholder Group[5], USA[13]

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[5] The IARC Review Stakeholder Group represents the AAM (Alliance of Automobile Manufacturers), ACEA (European Automobile Manufacturers Association), AECC (Association for Emissions Control by Catalyst), API (American Petroleum Institute), CONCAWE (Conservation of Clean Air Water and Environment, the oil companies European association for environment, health, and safety in refining and distribution), EMA (Truck and Engine Manufacturers of America), IPIECA (International Petroleum Industry Environmental Conservation Association), MECA (Manufacturers of Emission Controls Association), and OICA (International Organization of Motor Vehicle Manufacturers).

[6] John Gamble has received significant research funding from CONCAWE.

[7] Dan Greenbaum is the President of the Health Effects Institute (HEI) which conducts research worldwide on the health effects of air pollution. The Institute's core funding comes in equal part from the U.S. Environmental Protection Agency and the makers of motor vehicles for sale in the United States.

[8] Thomas Hesterberg is a full-time employee of Navistar, Inc., a manufacturer of diesel trucks and engines. He provided expert opinion to California Air Resources Board in 2010 regarding emissions from diesel engines.

[9] Timothy L. Lash served as a consultant to the diesel industry through Cambridge Environmental Inc.

[10] Roger McClellan serves as a consultant for the Engine Manufacturers Association, Navistar International, Cummins Engine Co., Shell Exploration and Production Co., Union Pacific, and the American Petroleum Institute.

[11] Peter Morfeld is a member of the Scientific Advisory Group of European Research Group on Environment and Health in the Transport Sector (EUGT); in addition, he has received significant research funding from EUGT.

[12] Dirk Pallapies holds small amounts of stock of Daimler-Benz AG and was employed until 2008 by BASF, a chemical company with business in trap technology, catalysts and additives for diesel and gasoline engines.

[13] John C. Wall is Vice President – Chief Technical Officer of Cummins Inc., a manufacturer of diesel engines. He also holds stock and patents of Cummins Inc.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans
VOLUME 105: DIESEL AND GASOLINE ENGINE EXHAUSTS AND SOME NITROARENES
Lyon, France: 5-12 June 2012

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Jelle Vlaanderen, Section of Environment and Radiation

NOTE REGARDING CONFLICTS OF INTERESTS: Each participant submitted WHO's Declaration of Interests, which covers employment and consulting activities, individual and institutional research support, and other financial interests. Participants identified as Invited Specialists did not serve as meeting chair or subgroup chair, draft text that pertains to the description or interpretation of cancer data, or participate in the evaluations. The Declarations were updated and reviewed again at the opening of the meeting.

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Posted on 12 April 2012, updated on 6 June

[14] Dana Loomis consulted in a lawsuit involving exposure to diesel exhaust (ceased in 2011).
[15] Suzanne Moore holds significant stock of BHP Billiton Limited, a global natural resources company with business in oil and gas exploration, production, development and marketing.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans
VOLUME 116: COFFEE, MATE AND VERY HOT BEVERAGES
Lyon, France: 24-31 May 2016

Working Group Members and Invited Specialists serve in their individual capacities as scientists and not as representatives of their government or any organization with which they are affiliated. Affiliations are provided for identification purposes only.

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Farhad Islami, American Cancer Society, Inc., USA (unable to attend)
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Invited Specialists

None

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans
VOLUME 112: SOME ORGANOPHOSPHATE INSECTICIDES AND HERBICIDES:
DIAZINON, GLYPHOSATE, MALATHION, PARATHION, AND TETRACHLORVINPHOS
Lyon, France: 3-10 March 2015

LIST OF PARTICIPANTS

Working Group Members and Invited Specialists served in their individual capacities as scientists and not as representatives of their government or any organization with which they are affiliated. Affiliations are provided for identification purposes only.

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Invited Specialists

Christopher J. Portier, Agency for Toxic Substances and Disease Registry, USA [retired][2]

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[1] Peter P Egeghy received "in kind" support and reimbursement of travel expenses of on average less than US $2.000 per year during the last 4 years from participation in meetings sponsored by the American Chemistry Council, an industry trade association for American chemical companies, and the Health and Environmental Sciences Institue (HESI), a nonprofit scientific research organization based in Washington and funded by corporate sponsors.

[2] Christopher J Portier receives a part-time salary from the Environmental Defense Fund, a United States–based nonprofit environmental advocacy group.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans
VOLUME 112: SOME ORGANOPHOSPHATE INSECTICIDES AND HERBICIDES:
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Lyon, France: 3-10 March 2015

Representatives of national and international health agencies

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Catherine Eiden, U.S. Environmental Protection Agency, USA (Unable to attend)
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Béatrice Fervers, for the Léon Bérard Centre, France
Elodie Giroux, University Jean-Moulin Lyon 3, France
Thomas Sorahan, for Monsanto Company, USA[4]
Christian Strupp, for the European Crop Protection Association, Belgium[5]
Patrice Sutton, for the University of California, San Francisco, Program on Reproductive Health and the Environment, USA[6]

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Maria Leon Roux, Section of the Environment and Radiation

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[3] Mette Kristine Boye Kristensen is employed by Cheminova A/S, Denmark, a global company developing, producing and marketing crop protection products.

[4] Tom Sorahan is a member of the European Glyphosphate Toxicology Advisory Panel, and received reimbursement of travel cost from Monsanto to attend EuroTox 2012.

[5] Christian Strupp is employed by ADAMA Agricultural Solutions Ltd, Israel, a producer of Diazinone and Glyphosphate.

[6] Patrice Sutton's attendance of this Monographs meeting is supported by the Clarence E. Heller Charitable Foundation, a philanthropic charity with a mission to protect and improve the quality of life through support of programs in the environment, human health, education and the arts.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans
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Posted on 26 January 2015, updated 19 October 2016

HARVARD T.H. CHAN
SCHOOL OF PUBLIC HEALTH

The Nutrition Source

Research Roundup

Noteworthy nutrition studies highlighted by members of The Chan School's Department of Nutrition

Glyphosate, the primary active ingredient in the herbicide "Roundup," is a broad-spectrum, non-selective, systemic herbicide, which effectively kills all plant types. Glyphosate-based herbicide was introduced to the US in 1974 and now has become the world's most common herbicide.

1) Guyton KZ, Loomis D, Grosse Y, et al. (2015) Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. *The Lancet Oncology* 16(5): 490-1.

In March, 2015, 17 experts from 11 countries assessed the carcinogenicity of five pesticides including glyphosate at the International Agency for Research on Cancer. A summary of the final evaluations was published in *The Lancet Oncology*.

- In this report, glyphosate was classified as "probably carcinogenic to humans" (Group 2A) for non-Hodgkin lymphoma, indicating there was limited evidence of carcinogenicity in humans and sufficient evidence of carcinogenicity in animals. Specifically, increased risk of non-Hodgkin lymphoma was consistent across case-control studies of occupational exposure in the USA, Canada, and Sweden. However, no evidence of increased risk of non-Hodgkin lymphoma was observed in the large Agricultural Health Study cohort (AHS).
- The evidence of other cancer sites (skin tumors, renal tubule carcinoma, haemangiosarcoma, and pancreatic islet-cell adenoma) was limited to animal studies.
- Evidence suggested the potential mechanisms for cancer were primarily through two pathways: First, the chemicals damaged DNA, which caused mutations or alterations in their gene codes. Second, glyphosate could induce oxidative stress. Oxidative stress

happens when highly reactive chemicals overwhelm the capacity of cells to deactivate them. Often, free radicals will be produced during this process, and they can interact with molecules in the body and damage various cell components. If the cells cannot effectively counteract this production, cells can become necrotic and die.

2) Mesnage R, Arno M, Costanzo M, et al. (2015) Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure. *Environmental Health* 14(1): 70.

An experimental study published in *Environmental Health* showed that chronic exposure to an ultra-low dose of glyphosate resulted in liver and kidney damage in rats.

- In this study, researchers administered 2-year minute doses (0.1ppb) of Roundup via drinking water, which was representative of what could be found in contaminated tap water.
- First, the authors observed the signs of pathological and biochemical changes in the liver and kidneys of the exposed rats.
- Then, they analyzed the changes in gene expression of these organs. Compared to the control group, more than 4000 gene transcript clusters in the liver and kidneys showed alterations in the exposed rats.
- The findings demonstrated that chronic exposure to glyphosate at an environmental level resulted in liver and kidney damage in an animal toxicity model, which may potentially have health implications for both animal and human populations.

3) Balbuena MS, Tison L, Hahn ML, et al. (2015) Effects of sublethal doses of glyphosate on honeybee navigation. *The Journal of Experimental Biology* 218(Pt 17): 2799-805.

An experimental study published in *The Journal of Experimental Biology* showed that exposure to sublethal doses of glyphosate affect the homeward flight path of honeybees in an open field.

- The authors performed an experiment in which forager honeybees were fed with a sugar solution containing traces of glyphosate in three sublethal concentrations (2.5, 5, and 10 mg/l) and released from a new site.

- The honeybees treated with a higher glyphosate concentration (10mg/l) spent more time performing homeward flights than control bees or bees treated with lower concentrations.
- The results suggest that exposure to glyphosate in a level commonly found in agricultural settings impaired the honeybees' navigation, with potential long-term negative consequences for the foraging success of honeybees.

Due to widespread use of glyphosate, the residues are found in American's urine, breast milk, and drinking water. The IARC has concluded that glyphosate is probably carcinogenic for non-Hodgkin lymphoma, and the risk of other cancer sites is inconclusive. In addition to health concerns, weed resistance to glyphosate has been increasing, which will adversely affect farm production. Due to the developing weed resistance, the Environmental Protection Agency is planning to place new restrictions on glyphosate. However, the details of the regulations have not yet been released at this time.

This month's Research Roundup was compiled by Yu-Han Chiu, a third year doctoral student who has been researching dietary factors in relation to semen quality and other reproductive outcomes. Dr. Chiu has been working with her advisor Dr. Jorge Chavarro and her colleagues on developing a dietary pesticide burden score to estimate an individual's pesticide exposure from food intake. Using this method, they recently presented important new data on pesticide exposure via fruit and vegetable intake in relation to semen quality in the journal Human Reproduction.

Emily H Phares October 16, 2015 Research Roundup

PREVIOUS

Coffee Talk: How It Stacks Up Against Water

NEXT

How risky is it to eat red meat?

HARVARD T.H. CHAN
SCHOOL OF PUBLIC HEALTH

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Research

Philippe Grandjean was born in Denmark in 1950. He graduated with his MD from the University of Copenhagen at age 23, and six years later he defended his doctoral thesis on the 'Widening perspectives of lead toxicity'. He became Professor of Environmental Medicine at the University of Southern Denmark in 1982. A Fulbright Senior Scholarship award brought him to Mt.Sinai Hospital in New York, and he later served as Adjunct Professor of Neurology and Environmental Health at Boston University. In 2003, he became Adjunct Professor of Environmental Health at Harvard University. In 2004, he received an unusual recognition – the Mercury Madness Award for excellence in science in the public interest, from eight US environmental organizations. He has also received the Science Communication Award from the University of Southern Denmark, and in 2015, he received the Bernardino Ramazzini Award for "his long career conducting and promoting environmental health research, especially his groundbreaking work on the effects of methylmercury and other environmental toxins affecting children and for his tireless advocacy of the need to protect future generations from the devastating effects of neuro- and developmental toxins." In 2016, Grandjean received the John F. Goldsmith Award from the International Society for Environmental Epidemiology for his sustained and outstanding contributions to the knowledge and practice of environmental epidemiology.

He lives in Copenhagen, Denmark and in Cambridge, MA, and travels widely to study environmental problems and to examine children whose lives have been affected by pollution, more specifically, the delayed effects of developmental exposure to environmental chemicals.

His most recent projects examine brain development and immune functions in regard to exposures to environmental pollutants, such as perfluorinated compounds and mercury. The results have inspired downward revisions of methylmercury exposure limits internationally and, most recent, the UN's Minamata Convention. Other recent studies have targeted age-related functional deficits and degenerative diseases, such as Parkinson's disease, cardiovascular disease, and type 2 diabetes in regard to life-time exposure to methylmercury, arsenic, persistent lipophilic contaminants, and perfluorinated compounds. Other efforts relate to biomarker development and validation, endocrine disruption caused by organochlorine substances. adverse effects of fluoride exposure, and the neurotoxicity of lead. Dr. Grandjean has also published on research ethics, genetic susceptibility, the setting of exposure limits, and the impact of the precautionary principle on prevention and research.

Recent News

Consensus document: Consensus on early origins (2015)

Web Site: Chemical Brain Drain

Video: Chemical Brain Drain

Open Access publishing: Champion

New Book: Only One Chance

Publications

(Selected articles from 2012-2016)

Grandjean P. Paracelsus Revisited: The dose concept in a complex world. Basic Clin Pharmacol Toxicol 2016; 119: 126-32.

Grandjean P. Learning from Bernardino Ramazzini, a tribute to the Magister from Carpi and to the Fellows of the Collegium Ramazzini. Eur J Oncol 2016: 21: 51-60.

Yorifuji T, Kato T, Ohta H, Bellinger DC, Matsuoka K, Grandjean P. Neurological and neuropsychological functions in adults with a history of developmental arsenic poisoning from contaminated milk powder. Neurotoxicol Teratol 2016; 53: 75-80.

Debes F, Weihe P, Grandjean P. Cognitive deficits at age 22 years associated with prenatal exposure to methylmercury. Cortex 2016; 74: 358-69.

Grandjean P, Clapp R. Perfluorinated alkyl substances: emergence of insights into health risks. New Solutions 2015; 25: 147-63.

Jensen TK, Andersen LB, Kyhl HB, Nielsen F, Christensen HT, Grandjean P. Association between perfluorinated compounds and miscarriage in a case-control study of Danish pregnant women. PLoS One 2015; 10: e0123496.

Mogensen UB, Grandjean P, Nielsen F, Weihe P, Budtz-Jørgensen E. Breastfeeding as an exposure pathway for perfluorinated alkylates. Environ Sci Technol 2015; 49: 10466-73.

Bellanger M, Demeneix B, Grandjean P, Zoeller RT, Trasande L. Neurobehavioral deficits, diseases and associated costs of exposure to endocrine disrupting chemicals in the European Union. J Clin Endocrinol Metab 2015; 100: 1256-66.

Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. Lancet Neurol 2014; 13: 330-8.

Audouze K, Brunak S, Grandjean P. Computational approach to chemical etiologies of diabetes. Sci Comm 2013; 3: 2712.

Julvez J, Davey-Smith G, Golding J, Ring S, St. Pourcain B, Gonzalez JR, Grandjean P. Prenatal methylmercury exposure and genetic predisposition to cognitive deficit at age 8 years. Epidemiology 2013; 24: 643-50.

Grandjean P, Ozonoff D. Transparency and translation of science in a modern world. Environ Health 2013; 12: 70.

Balbus JM, Barouki R, Birnbaum LS, Etzel RA, Gluckman PD, Grandjean P, Hancock C, Hanson MA, Heindel JJ, Hoffman K, Jensen GK, Keeling A, Neira M, Rabadán-Diehl C, Ralston J, Tang KC. Early-life prevention of non-communicable diseases (Comment). Lancet 2013; 381: 3-4.

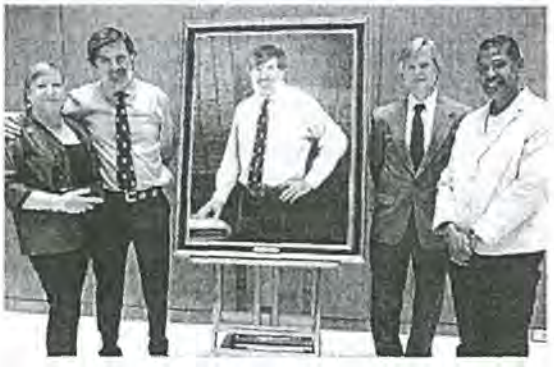
Budtz-Jørgensen E, Bellinger D, Lanphear B, Grandjean P, International Pooled Lead Study Investigators. An international pooled analysis for obtaining a Benchmark dose for environmental lead exposure in children. Risk Anal 2013; 33: 450-61.

Grandjean P, Andersen EW, Budtz-Jørgensen E, Nielsen F, Mølbak K, Weihe P, Heilmann C. Decreased serum vaccine antibody concentrations in children exposed to perfluorinated compounds. JAMA 2012; 307: 391-7.

Choi AL, Sun G, Zhang Y, Grandjean P. Developmental fluoride neurotoxicity: A systematic review and meta-analysis. Environ Health Perspect 2012; 120: 1362-8.

News from the School

Helping Harvey survivors



David Hunter honored

Social responsibility

Brand marketing gone bad

UNITED STATES DISTRICT COURT

NORTHERN DISTRICT OF CALIFORNIA

IN RE: ROUNDUP PRODUCTS LIABILITY LITIGATION	MDL No. 2741
	Case No. 16-md-02741-VC
THIS DOCUMENT RELATES TO ALL CASES	

CONFIDENTIAL - SUBJECT TO PROTECTIVE ORDER

VIDEOTAPED DEPOSITION OF AARON EARL BLAIR, Ph.D.

WASHINGTON, D.C.

MONDAY, MARCH 20, 2017

8:59 A.M.

Reported by: Leslie A. Todd

most updated AHS data should be considered by IARC, correct?

A Yes.

Q You would agree that it would be --

A Well, wait, wait. If it's been published.

Q And you would agree with Dr. Alavanja that it would be irresponsible for the AHS -- Agricultural Health Study investigators not to publish the updated findings on pesticides and NHL in time to influence IARC's decision, correct?

A No. I don't agree with that. And the reason is because the timetable about when you have to have it published is arbitrary. And doing analyses and writing papers is not wedded to a timetable. And what is irresponsible is to rush something out that's not fully analyzed or thought out.

Q Let me ask you --

A That's irresponsible.

Q I'm sorry. Let me ask you then about the e-mails you were talking about previously with respect to the North American Pooled Project, and we can go back to those if you want. But as I remember, Dr. Pahwa was discussing the possibility of doing

you could to try to get the data published in time for the IARC monograph meeting, correct?

A Yeah.

Q But then after we -- after you determined and found out what the data showed with respect to glyphosate and these cancers, the data wasn't published, correct?

A The paper wasn't finished, and you have to finish things in the analysis and the writing before you can publish it.

Q Okay. So let's go back then to what the IARC analysis was and what the working group did.

So the IARC working group then in its analysis of the epidemiology was relying upon -- was not relying upon the most up-to-date AHS data, correct?

A It was relying upon the most up-to-date published data, and that's always the standard at IARC.

Q I understand. But just so the record is clear, IARC was not relying upon the most updated analysis that you were aware of from the AHS data with respect to glyphosate and non-Hodgkin lymphoma, correct?

A Now you present it as if the analyses

were completed. Analyses were done, manuscripts were in description, but the work wasn't finished, which means it's incomplete, and that you don't want to be reporting on. And we didn't.

Q So -- understood.

And because of the fact that you had not completed the manuscript that was in at least manuscript form in March of 2013 in time for it to be a publication by March 2015, IARC didn't have that information?

A That's correct.

Q Now, going back to this issue of publication bias, did the Agricultural Health Study decide not to include data regarding glyphosate and non-Hodgkin lymphoma in its updated publication because the data did not show a positive association?

A No. It decided to do pesticides first because we proceeded -- insecticides first, we sort of proceeded down that line early on and didn't think we had time to switch and do the other when IARC become clear that that's what they were going to look at.

Q Now, you and other AHS investigators are certainly aware, and we looked at some of this discussion previously, that questions have arisen

Q That's what the panel unanimously thought, right?

MR. LASKER: Objection to form.

THE WITNESS: Yes.

BY MR. MILLER:

Q Okay. Has anything you've been shown by Monsanto's lawyers in the 3 hours and 40 minutes that he questioned you changed the opinions that you had at the IARC meeting about glyphosate and non-Hodgkin lymphoma?

MR. LASKER: Objection to form, beyond the scope.

BY MR. MILLER:

Q You can answer.

A No.

MR. MILLER: I didn't even use an hour. Thank you for your time.

MR. LASKER: I have like three questions, but I will ask them from here. We don't have to go off.

MR. MILLER: Sure. Sure. If the doctor is okay with it, I'm okay with it.

THE WITNESS: That's fine.

RECROSS-EXAMINATION

BY MR. LASKER:

Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate

Published Online March 20, 2015 http://dx.doi.org/10.1016/S1470-2045(15)70134-8

For more on the IARC Monographs see http://monographs.iarc.fr

Upcoming meetings
June 2–9, 2015, Volume 113: Some organochlorine insecticides and some chlorphenoxy herbicides
Oct 6–13, 2015, Volume 114: Red meat and processed meat

Monograph Working Group Members
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In March, 2015, 17 experts from 11 countries met at the International Agency for Research on Cancer (IARC; Lyon, France) to assess the carcinogenicity of the organophosphate pesticides tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate (table). These assessments will be published as volume 112 of the IARC Monographs.[1]

The insecticides tetrachlorvinphos and parathion were classified as "possibly carcinogenic to humans" (Group 2B). The evidence from human studies was scarce and considered inadequate. Tetrachlorvinphos induced hepatocellular tumours (benign or malignant) in mice, renal tubule tumours (benign or malignant) in male mice,[2] and spleen haemangioma in male rats. Tetrachlorvinphos is a reactive oxon with affinity for esterases. In experimental animals, tetrachlorvinphos is systemically distributed, metabolised, and eliminated in urine. Although bacterial mutagenesis tests were negative, tetrachlorvinphos induced genotoxicity in some assays (chromosomal damage in rats and in vitro) and increased cell proliferation (hyperplasia in rodents). Tetrachlorvinphos is banned in the European Union. In the USA, it continues to be used on animals, including in pet flea collars.

For parathion, associations with cancers in several tissues were observed in occupational studies, but the evidence in humans remains sparse. In mice, parathion increased bronchioloalveolar adenoma and/or carcinoma in males, and lymphoma in females. In rats, parathion induced adrenal cortical adenoma or carcinoma (combined),[3] malignant pancreatic tumours, and thyroid follicular cell adenoma in males, and mammary gland adenocarcinoma (after subcutaneous injection in females).[4] Parathion is rapidly absorbed and distributed. Parathion metabolism to the bioactive metabolite, paraoxon, is similar across species. Although bacterial mutagenesis tests were negative, parathion induced DNA and chromosomal damage in human cells in vitro. Parathion markedly increased rat mammary gland terminal end bud density.[5] Parathion use has been severely restricted since the 1980s.

The insecticides malathion and diazinon were classified as "probably carcinogenic to humans" (Group 2A). Malathion is used in agriculture, public health, and residential insect control. It continues to be produced in substantial volumes throughout the world. There is limited evidence in humans for the carcinogenicity of malathion. Case-control analyses of occupational exposures reported positive associations with non-Hodgkin lymphoma in the USA,[6] Canada,[6] and Sweden,[7] although no increased risk of non-Hodgkin lymphoma was observed in the large Agricultural Health Study cohort (AHS). Occupational use was associated with an increased risk of prostate cancer in a Canadian case-control study[8] and in the AHS, which reported a significant trend for aggressive cancers after adjustment for other pesticides.[9] In mice, malathion increased hepatocellular adenoma or carcinoma (combined).[10] In rats, it increased thyroid carcinoma in males, hepatocellular adenoma or carcinoma (combined) in females, and mammary gland adenocarcinoma after subcutaneous injection in females.[4] Malathion is rapidly absorbed and distributed. Metabolism to the bioactive metabolite, malaoxon, is similar across species. Malaoxon strongly inhibits esterases; atropine reduced carcinogenesis-related effects in one study.[4] Malathion induced DNA and chromosomal damage in humans, corroborated by studies in animals and in vitro. Bacterial mutagenesis tests were negative. Compelling evidence supported disruption of hormone pathways. Hormonal effects probably mediate rodent thyroid and mammary gland proliferation.

Diazinon has been applied in agriculture and for control of home and garden insects. There was limited evidence for diazinon carcinogenicity

	Activity (current status)	Evidence in humans (cancer sites)	Evidence in animals	Mechanistic evidence	Classification*
Tetrachlorvinphos	Insecticide (restricted in the EU and for most uses in the USA)	Inadequate	Sufficient	-	2B
Parathion	Insecticide (restricted in the USA and EU)	Inadequate	Sufficient	-	2B
Malathion	Insecticide (currently used; high production volume chemical)	Limited (non-Hodgkin lymphoma, prostate)	Sufficient	Genotoxicity, oxidative stress, inflammation, receptor-mediated effects, and cell proliferation or death	2A†
Diazinon	Insecticide (restricted in the USA and EU)	Limited (non-Hodgkin lymphoma, leukaemia, lung)	Limited	Genotoxicity and oxidative stress	2A†
Glyphosate	Herbicide (currently used; highest global production volume herbicide)	Limited (non-Hodgkin lymphoma)	Sufficient	Genotoxicity and oxidative stress	2A†

EU=European Union. *See the International Agency for Research on Cancer (IARC) preamble for explanation of classification system (amended January, 2006). †The 2A classification of diazinon was based on limited evidence of carcinogenicity in humans and experimental animals, and strong mechanistic evidence; for malathion and glyphosate, the mechanistic evidence provided independent support of the 2A classification based on evidence of carcinogenicity in humans and experimental animals

Table: IARC classification of some organophosphate pesticides

in humans. Positive associations for non-Hodgkin lymphoma, with indications of exposure–response trends, were reported by two large multicentre case-control studies of occupational exposures.[3,6] The AHS reported positive associations with specific subtypes, which persisted after adjustment for other pesticides, but no overall increased risk of non-Hodgkin lymphoma.[11] Support for an increased risk of leukaemia in the AHS was strengthened by a monotonic increase in risk with cumulative diazinon exposure after adjustment for other pesticides. Multiple updates from the AHS consistently showed an increased risk of lung cancer with an exposure–response association that was not explained by confounding by other pesticides, smoking, or other established lung cancer risk factors.[12] Nonetheless, this finding was not replicated in other populations. In rodents, diazinon increased hepatocellular carcinoma in mice and leukaemia or lymphoma (combined) in rats, but only in males receiving the low dose in each study. Diazinon induced DNA or chromosomal damage in rodents and in human and mammalian cells in vitro. Some additional support for human relevance was provided by a positive study of a small number of volunteers exposed to a diazinon formulation.[13]

Glyphosate is a broad-spectrum herbicide, currently with the highest production volumes of all herbicides. It is used in more than 750 different products for agriculture, forestry, urban, and home applications. Its use has increased sharply with the development of genetically modified glyphosate-resistant crop varieties. Glyphosate has been detected in air during spraying, in water, and in food. There was limited evidence in humans for the carcinogenicity of glyphosate. Case-control studies of occupational exposure in the USA,[14] Canada,[6] and Sweden[7] reported increased risks for non-Hodgkin lymphoma that persisted after adjustment for other pesticides. The AHS cohort did not show a significantly increased risk of non-Hodgkin lymphoma. In male CD-1 mice, glyphosate induced a positive trend in the incidence of a rare tumour, renal tubule carcinoma. A second study reported a positive trend for haemangiosarcoma in male mice.[15] Glyphosate increased pancreatic islet-cell adenoma in male rats in two studies. A glyphosate formulation promoted skin tumours in an initiation-promotion study in mice.

Glyphosate has been detected in the blood and urine of agricultural workers, indicating absorption. Soil microbes degrade glyphosate to aminomethylphosphoric acid (AMPA). Blood AMPA detection after poisonings suggests intestinal microbial metabolism in humans. Glyphosate and glyphosate formulations induced DNA and chromosomal damage in mammals, and in human and animal cells in vitro. One study reported increases in blood markers of chromosomal damage (micronuclei) in residents of several communities after spraying of glyphosate formulations.[16] Bacterial mutagenesis tests were negative. Glyphosate, glyphosate formulations, and AMPA induced oxidative stress in rodents and in vitro. The Working Group classified glyphosate as "probably carcinogenic to humans" (Group 2A).

We declare no competing interests.

Kathryn Z Guyton, Dana Loomis, Yann Grosse, Fatiha El Ghissassi, Lamia Benbrahim-Tallaa, Neela Guha, Chiara Scoccianti, Heidi Mattock, Kurt Straif, on behalf of the International Agency for Research on Cancer Monograph Working Group, IARC, Lyon, France

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For the Preamble to the IARC Monographs see http://monographs.iarc.fr/ENG/Preamble/index.php

For declarations of interests see http://monographs.iarc.fr/ENG/Meetings/vol112-participants.pdf

A Section 508–conformant HTML version of this article is available at http://dx.doi.org/10.1289/ehp.1409149.

Commentary

IARC Monographs: 40 Years of Evaluating Carcinogenic Hazards to Humans

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Background: Recently, the International Agency for Research on Cancer (IARC) Programme for the Evaluation of Carcinogenic Risks to Humans has been criticized for several of its evaluations, and also for the approach used to perform these evaluations. Some critics have claimed that failures of IARC Working Groups to recognize study weaknesses and biases of Working Group members have led to inappropriate classification of a number of agents as carcinogenic to humans.

Objectives: The authors of this Commentary are scientists from various disciplines relevant to the identification and hazard evaluation of human carcinogens. We examined criticisms of the IARC classification process to determine the validity of these concerns. Here, we present the results of that examination, review the history of IARC evaluations, and describe how the IARC evaluations are performed.

Discussion: We concluded that these recent criticisms are unconvincing. The procedures employed by IARC to assemble Working Groups of scientists from the various disciplines and the techniques followed to review the literature and perform hazard assessment of various agents provide a balanced evaluation and an appropriate indication of the weight of the evidence. Some disagreement by individual scientists to some evaluations is not evidence of process failure. The review process has been modified over time and will undoubtedly be altered in the future to improve the process. Any process can in theory be improved, and we would support continued review and improvement of the IARC processes. This does not mean, however, that the current procedures are flawed.

Conclusions: The IARC Monographs have made, and continue to make, major contributions to the scientific underpinning for societal actions to improve the public's health.

Citation: Pearce N, Blair A, Vineis P, Ahrens W, Andersen A, Anto JM, Armstrong BK, Baccarelli AA, Beland FA, Berrington A, Bertazzi PA, Birnbaum LS, Brownson RC, Bucher JR, Cantor KP, Cardis E, Cherrie JW, Christiani DC, Cocco P, Coggon D, Comba P, Demers PA, Dement JM, Douwes J, Eisen EA, Engel LS, Fenske RA, Fleming LE, Fletcher T, Fontham E, Forastiere F, Frentzel-Beyme R, Fritschi L, Gerin M, Goldberg M, Grandjean P, Grimsrud TK, Gustavsson P, Haines A, Hartge P, Hansen J, Hauptmann M, Heederik D, Henneberger PK, Hemon D, Hertz-Picciotto I, Hoppin JA, Huff J, Jarvholm B, Kang D, Karagas MR, Kjaerheim K, Kjuus H, Kogevinas M, Kriebel D, Kristensen P, Kromhout H, Laden F, Lebailly P, LeMasters G, Lubin JH, Lynch CF, Lynge E, 't Mannetje A, McMichael AJ, McLaughlin JR, Marrett L, Martuzzi M, Merchant JA, Merler E, Merletti F, Miller A, Mirer FE, Monson R, Nordby KC, Olshan AF, Parent ME, Perera FP, Perry MJ, Pesatori AC, Pirastu R, Porta M, Pukkala E, Rice C, Richardson DB, Ritter L, Ritz B, Ronckers CM, Rushton L, Rusiecki JA, Rusyn I, Samet JM, Sandler DP, de Sanjose S, Schernhammer E, Seniori Costantini A, Seixas N, Shy C, Siemiatycki J, Silverman DT, Simonato L, Smith AH, Smith MT, Spinelli JJ, Spitz MR, Stallones L, Stayner LT, Steenland K, Stenzel M, Stewart BW, Stewart PA, Symanski E, Terracini B, Tolbert PE, Vainio H, Vena J, Vermeulen R, Victora CG, Ward EM, Weinberg CR, Weisenburger D, Wesseling C, Weiderpass E, Zahm SH. 2015. IARC Monographs: 40 years of evaluating carcinogenic hazards to humans. Environ Health Perspect 123:507–514; http://dx.doi.org/10.1289/ehp.1409149

Introduction

Important advances in human health have come from the recognition of health hazards and the development of policy actions to address them (Brownson et al. 2009; Espina et al. 2013; Samet 2000). Government and nongovernmental organizations use expert panels to review the scientific literature and to assess its relevance to public health policies. Scientific experts are charged with reviewing the quality and quantity of the scientific evidence and providing scientific interpretations of the evidence that underpin a range of health policy decisions.

The *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* of the International Agency for Research on Cancer (IARC) are a prominent example of such an expert review process. The goal of the Monograph Programme is to assess carcinogenic hazards from occupational, environmental, and lifestyle exposures and agents, thus providing an essential step in the societal decision-making process to identify and then control carcinogenic hazards. For these evaluations, IARC assembles groups of scientists with a range of relevant scientific expertise (called "Working Groups") to review and assess the quality and strength of evidence from informative publications and perform a hazard evaluation to assess the likelihood that the agents of concern pose a cancer hazard to humans (Tomatis 1976). IARC has used this approach for four decades, since the first Monograph in 1972 (IARC 1972). Although widely accepted internationally, there have been criticisms of the classification of particular agents in the past, and more recent criticisms have been directed at the general approach adopted by IARC for such evaluations (Boffetta et al. 2009; Epidemiology Monitor 2012; Ioannidis 2005; Kabat 2012; McLaughlin et al. 2010, 2011).

The Monographs are widely used and referenced by governments, organizations, and the public around the world; therefore, it is critical that Working Group conclusions be clear and transparent. In addition to the actual evaluation, a major contribution of the Monographs is the assembly of relevant literature and its dissemination to the public. We recognize that no system of evaluation is perfect. It is important to foster continuing improvement of the methods used by IARC and other bodies that review scientific evidence. The IARC process itself has been modified from time to time (e.g., addition of specific evaluation of mechanistic data and greater use of formal meta-analyses and data-pooling approaches). Indeed, as recently as April 2014, the IARC Monographs program has been a subject of a review by the Advisory Group to recommend priorities for IARC Monographs during 2015–2019 (Straif et al. 2014). The Advisory Group has made a number of recommendations on further improvements in the Monographs process specifically related to conflict of interest, transparency, and the use of the systematic review procedures in data gathering and evaluation. Thus, possible changes to the process are periodically considered by IARC

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This work was conducted with no direct funding, but was supported in part by the Intramural Research Program of the NIH (NCI and NIEHS).

The views expressed are those of the authors and do not necessarily reflect the official views of the Uniformed Services University of the Health Sciences, the Department of Defense, or the U.S. Food and Drug Administration.

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Received: 14 September 2014; Accepted: 20 February 2015; Advance Publication: 24 February 2015; Final Publication: 1 June 2015.

governing groups (Scientific Council and Governing Council) and Advisory Groups.

Here, we focus on current IARC processes and practices because these have been the focus of recent criticisms. The authors of this Commentary are scientists from a wide range of disciplines who are involved in designing and conducting studies that provide data used in hazard evaluations, such as those performed by IARC. Many (but not all) of us have served on IARC Monograph Working Groups, but none are current IARC staff. We first discuss the history of IARC, and describe how the IARC evaluations are performed in order to foster evidence-based policy. We then describe why unbiased evaluations, based on the evidence and free of conflicts of interest, are necessary for public health decision making. Finally, we discuss the recent criticisms of the IARC approach.

The IARC Monographs

History of the IARC Monographs. Shortly after IARC's establishment, its parent entity, the World Health Organization (WHO), asked IARC to prepare a list of agents known to cause cancer in humans. IARC recognized the need for a systematic process to determine which agents should be listed. Such a process was launched in 1972 by Lorenzo Tomatis, then Chief of the Division of Carcinogenicity of IARC (Tomatis 1976). IARC is funded by the governments of 24 countries that have decided to become members, in addition to competitive grants from funding agencies. The IARC Monograph Programme is mainly funded by the U.S. National Cancer Institute through a renewable grant subject to peer review of the program. Other sources of external funding have included the European Commission Directorate-General of Employment, Social Affairs and Equal Opportunities; the U.S. National Institute of Environmental Health Sciences; and the U.S. Environmental Protection Agency.

The IARC process antedates current systematic review methods, but anticipated some of them, for example, with regard to transparent literature identification. In the IARC process, agents are assessed for carcinogenic hazard and assigned to one of five categories, ranging from carcinogenic to humans to probably not carcinogenic to humans (Appendix 1). The classification categories are described in the preamble to the Monographs (IARC 2006). Carcinogenic hazard identification refers to an assessment of whether an agent causes cancer. Hazard identification does not predict the magnitude of cancer risks under specific conditions; this can be determined only with appropriate exposure–response information (National Research Council 2009).

The IARC Monograph process. The process for the preparation of an IARC Monograph is clearly described in the Preamble, which is published as part of each Monograph (e.g., IARC 2014a). It starts with the nomination of candidate agents. Nominations come from national regulatory agencies, scientists, and stakeholders, including public health professionals, experts in environmental or occupational hygiene, industry representatives, and private citizens. It is important to note that anyone (including private citizens) can participate in the nomination process. The Monograph Programme convenes meetings of special Advisory Groups (composed of external scientists that possess a broad range of relevant professional skills) to review agents nominated for evaluation and to suggest IARC priorities for such reviews (Ward et al. 2010). Announcements of a review are made on the IARC website (http://monographs.iarc.fr/ENG/Meetings/). For example, in 2013 IARC sought nominations for agents to be evaluated in 2015–2019 (IARC 2014b). An Advisory Group reviewed the nominated agents and exposures, added several new ones, and discussed the priorities for each.

The IARC staff makes the final selection of agents for review by taking into account the prevalence and intensity of exposure (of both occupational groups and the general population) and availability of sufficient literature for an evaluation of carcinogenicity, as well as advice from the Advisory Groups. The large majority of evaluations concern specific compounds, but there are also monographs on various occupations or industries, for example, aluminum production, insecticide applicators, firefighters, manufacture of leather goods, leather tanning and processing, welding, painters, petroleum refining, and pulp and paper manufacturing. Some individual exposures that occur in these settings have also been evaluated.

The next step is the selection of members of the Working Group (WG). IARC staff review the literature to identify Working Group candidates and specialists in relevant areas of expertise; they also seek names of possible candidates from the scientific community and advisory groups. The list of potential members, including disclosure of relevant conflicts of interest, is posted on the IARC website (http://monographs.iarc.fr/ENG/Meetings/) before the WG is convened, and anyone can send comments. Members are typically scientists who have conducted research relevant to the agent under review, but not necessarily on the specific agent. Selection procedures are evaluated yearly by the Scientific and the Governing Councils. The IARC Section of Monographs also has an external Advisory Board, made up of independent scientists, that periodically peer reviews its activities. In addition to Working Group members, invited specialists, representatives of health agencies, stakeholder observers, and the IARC Secretariat also attend meetings.

The responsibility of the Working Group is to review the literature before the Monograph meeting, discuss the literature at the meeting, and then classify whether an agent is carcinogenic, probably carcinogenic, possibly carcinogenic, not classifiable, or probably not carcinogenic to humans (see Appendix 1). Working Group members are also responsible for writing the IARC Monograph, which must both review the literature and explain why the Working Group came to their specific conclusions.

The procedures used to evaluate the scientific evidence are described in the Preamble to the Monographs (IARC 2006). It is important to stress that only Working Group members conduct the actual evaluation (Wild and Cogliano 2011; Wild and Straif 2011). IARC staff facilitate the evaluation process and ensure that the procedures described in the Preamble are followed; however, they do not determine the outcomes.

IARC assessments of carcinogenicity are based on, and necessarily limited to, scientific evidence available at the time of the review. The evidence comes from epidemiologic studies, animal bioassays, pharmacokinetic/mechanistic experiments, and surveys of human exposure. The aim is to include all relevant papers on cancer in humans and experimental animals that have been published, or accepted for publication, in peer-reviewed scientific journals and also any publicly available government or agency documents that provide data on the circumstances and extent of human exposure. To that end, the search of the literature takes a comprehensive approach. Papers that are found not to provide useful evidence can be excluded later in the process. IARC staff first use previous IARC Monographs (if available), database searches using relevant text strings, and contact with investigators in the field to identify potentially relevant material. Thus, the initial assembly of the literature is performed by individuals who are not engaged in the actual evaluation. Working Group members are then assigned various writing tasks and are instructed to perform their own literature searches to identify any further papers that might have been missed. In addition, all of the papers assembled by IARC are made available to the full Working Group before they meet, and any member can recommend other papers not previously identified that they think should be considered. Finally, papers can be recommended by stakeholder representatives before or during the Working Group meeting.

At the meeting of the Working Group, the assembled documents are reviewed and summarized by discipline-related subgroups.

However, any member of the Working Group has access to all of the assembled literature. The summaries are distributed to all subgroups, and information from all disciplines is discussed in plenary sessions prior to assigning the agents to a specific carcinogenicity category.

Because new findings continually emerge in the literature, agents are reconsidered when IARC and IARC Advisory Groups judge that there is sufficient additional information that might alter a previous evaluation. Thus, conclusions regarding human carcinogenicity of particular substances may change as new evidence becomes available. For some agents, this reevaluation has resulted in progression toward greater certainty regarding their human carcinogenicity, whereas for others the progress has been moved toward less certainty. Such movements are expected in an open, transparent, and evidence-based process. A comprehensive update of all Group 1 carcinogens was recently accomplished in Volume 100 A through F (http://monographs.iarc.fr/ENG/Monographs/PDFs/index.php).

Usually, several agents are evaluated in a single meeting lasting more than 1 week. After discussing the evidence fully, the Working Group members follow the published IARC procedures for combining information from epidemiologic studies and bioassays to arrive at a preliminary classification (IARC 2014a). Mechanistic data are then considered in order to determine whether they warrant a change from the preliminary classification. The Working Group then votes on the final determination. Many votes are unanimous, but on occasion some reviewers may favor a higher or lower ranking than the majority. When there is dissent, alternative interpretations and their underlying reasoning are sometimes reported in the rationale for the evaluation if the dissenters feel their point of view is not sufficiently addressed in the monograph.

Consideration of the totality of the evidence. IARC Working Groups make every effort to provide full and transparent documentation of what evidence was assembled, how it was evaluated, and which papers were most important for the hazard evaluation. Consequently, the monographs are often quite lengthy, containing many evidence tables [see, for example, the recent monograph on trichloroethylene (IARC 2014c)]. Evaluations involve consideration of all of the known relevant evidence from epidemiologic, animal, pharmacokinetic/mechanistic, and exposure studies to assess cancer hazard in humans. Information on human exposure is not formally graded as part of the overall assessment of carcinogenic hazard; however, these data make a critical contribution to the process by characterizing the timing, duration, and levels of exposure in the population, and in evaluating the quality of the exposure assessment in epidemiologic studies.

Doubts and criticisms have sometimes been expressed about the relative weights attributed to evidence from individual disciplines to the assessment of cancer hazards to humans; however, each discipline provides important evidence toward the overall evaluation of causality according to the Bradford Hill considerations (Hill 1965). Because the totality of the evidence is considered, deficiencies in one discipline are often offset by strengths in another. For example, epidemiologic studies may focus on population-relevant exposures, whereas findings from animal experiments usually involve higher exposures but are less susceptible to confounding.

Long-term animal bioassays and mechanistic studies provide critical information on the capacity of an agent to produce cancer in mammalian systems, including humans, and to contribute to decisions that would lead to better protection of human health. Bioassays are the backbone of regulatory science because they provide the opportunity to rigorously evaluate potential hazards before there is widespread human exposure. Bioassays and mechanistic studies are sometimes criticized for employing exposure routes and doses that in most instances humans would not experience, although experimental dose categories sometimes approach exposure levels found in occupational situations. There is evidence that carcinogenicity in human and animal studies is often concordant, although data may differ as to the affected cancer site (Haseman 2000; Maronpot et al. 2004; Tomatis 2002). A major effort to evaluate the concordance between animal and human results is currently under way; two Working Groups were convened at IARC in 2012, and a systematic evaluation of the correspondence between human and animal data was undertaken (a report is not yet publicly available).

Criticisms of the IARC Process

IARC Monographs are widely used to identify potential carcinogenic hazards to humans and serve as reference documents summarizing the literature on many different agents. In recent years, however, individuals have criticized both the classification of individual agents as well as the general evaluative approach (Boffetta et al. 2009; Epidemiology Monitor 2012; Kabat 2012; McLaughlin et al. 2010, 2011). We discuss four of these criticisms below.

Criticisms of epidemiology. Some of the criticisms of the IARC process have occurred in the context of more general criticisms of epidemiology as a science (Kabat 2008); these were discussed in detail by Blair et al. (2009). Potential methodological weaknesses for observational epidemiologic studies are well recognized and can be found in any epidemiologic textbook (Checkoway et al. 2004; Rothman et al. 2008). Most studies are subject to one or more methodological limitations, but this does not necessarily invalidate their findings (Blair et al. 2009). In fact, the value of epidemiologic studies has been shown by the identification of a number of well-established human carcinogens, including tobacco, asbestos, benzene, hexavalent chromium, and some viruses, in multiple studies. Some critics also argue that small or nonexistent health risks are unjustifiably highlighted and hyped by researchers who have a vested interest in continued research funding and the need to publish to benefit their careers (Boffetta et al. 2008; Kabat 2008; McLaughlin et al. 2010, 2011; Taubes 1995). However, such overstated results are unlikely to exert much of an influence in a Monograph because IARC evaluations are based on the totality of the evidence. The problem would have to occur in multiple studies, and the Working Group would have to be unable to identify it or be unwilling to weigh such studies appropriately. Incorrect positive conclusions regarding carcinogenicity may also occur in reviews of multiple studies because of publication bias, which may selectively populate the literature only with "positive" findings. However, once a topic is recognized as scientifically important, reports on relevant studies will be published regardless of the findings, so publication bias is mainly a concern for newly arising issues. To evaluate the potential for publication bias, Working Groups consider whether stronger negative studies (both in terms of design and sample size) have emerged after publication of an initial cluster of smaller and/or weaker positive studies. Funnel plots help in the assessment of bias relating to sample size and publication bias (Borenstein et al. 2009). In contrast, there are no established statistical techniques to clearly characterize strength of design.

One of the distinctive features of epidemiology is that criticism and self-criticism are firmly embedded in the discipline. A great deal of work has been done on developing methods for critical appraisal (Elwood 2007) and for assessing the likely strength and direction of possible biases (Rothman et al. 2008). Epidemiologists and other members on Working Groups routinely use various approaches to assess possible bias in study design and analysis when weighing the strengths of different studies.

The issue of false positives. Epidemiology specifically has been criticized for a tendency to produce false-positive results (i.e., individual study associations not borne out by the weight of the evidence) or to preferentially report positive findings over negative

or inconclusive findings (i.e., publication bias) (Bofferta et al. 2008, 2009; Ioannidis 2005; Kabat 2012; McLaughlin and Tarone 2013). This criticism has been most often applied to potential false positives from individual studies, but it has been inferred that this problem may also apply to overall hazard evaluations, which use findings from multiple studies. We will consider each of these issues in turn.

False-positive findings may occur by chance, particularly when many combinations of exposures and health outcomes have been examined in a single study without strong prior expectations of association; this happens often, for example, in genome-wide association studies where thousands of gene–disease associations are evaluated. Chance, of course, operates in all disciplines and in both observational and experimental studies. However, there are well-known statistical techniques to reduce the probability of declaring chance findings as "positive" (Rothman et al. 2008). Independent replication, however, is the most convincing way of checking for "chance" findings; hazard evaluations, such as those conducted by IARC Working Groups, rely heavily on reproducibility in independent studies and also interpret data following Bradford Hill principles (Hill 1965).

False negatives are more difficult to address, and perhaps they occur more frequently than false positives because of low statistical power, nondifferential misclassification of exposure and/or outcome, and incomplete follow-up, which tends to reduce the observed difference in risk between the exposed and nonexposed populations (Ahlbom et al. 1990; Blair et al. 2009; Grandjean 2005; Rothman et al. 2008). A new positive association stimulates research, whereas studies finding no associations tend to stifle further work.

There are difficulties in conducting epidemiologic studies of agents that are relatively "weak" carcinogens, or for stronger carcinogens where exposure is very low because bias and confounding can obscure weak positive associations (MacMahon et al. 1981). In general, weak carcinogens and low levels of exposure result in a smaller "signal-to-noise" ratio making the real signal more difficult to detect. Although the identification of small relative risks to humans poses special challenges to scientific research, the refinement of study designs, improvements in methods of exposure assessment, and the use of biomarkers have helped to address the problems (e.g., newer studies on the effects of air pollution, the growth in opportunities to examine gene–environment interactions) (Gallo et al. 2011). In some situations, there is less of a problem. For example, in occupational studies, exposures and relative risks may be higher while differences in lifestyle factors between different groups of workers are smaller (Checkoway et al. 2004); thus, any confounding by nonoccupational factors is likely to be weak, even from potent causes of cancer such as cigarette smoking (Siemiatycki et al. 1988). Of course, the interpretation of such studies is enhanced when there is supporting evidence from bioassays and/or mechanistic studies.

False-positive and false-negative findings in individual studies may arise by chance or bias, including bias due to confounding (Rothman et al. 2008). However, the evaluation of multiple independent epidemiologic studies from various geographic locations, involving a variety of study designs, as well as evidence from experimental studies, reduces the possibility that false-positive findings from any individual study influences the overall evaluation process. Some studies may have greater influence than others because of methodological strengths and/or large sample size. The use of information from a variety of study designs reduces the likelihood of false-positive evaluations because it is unlikely that the same biases will occur in multiple studies based on different populations under different study designs. Moreover, apparently conflicting results from epidemiologic studies do not necessarily indicate that some are false positive or false negative. This might, for example, reflect differences in levels of exposure or susceptibility to the effects of exposure (effect modification). Finally, judgment by the Working Group is not based exclusively on epidemiologic studies but usually also on results from laboratory and mechanistic studies that provide further evidence and biological coherence. For the Monographs that evaluate carcinogenic hazards associated with specific occupations or industries, the exposures of interest usually involve a complex mixture of chemicals. For these evaluations, most information comes from epidemiologic studies, although exposures to individual agents occurring at these workplaces may have been evaluated in experimental studies.

Discontent with IARC Monograph processes. The IARC Monograph evaluation process has been criticized and it has been alleged that "a number of scientists with direct experience of IARC have felt compelled to dissociate themselves from the agency's approach to evaluating carcinogenic hazards" (Kabat 2012). This is a serious charge. However, the author of this claim provided no evidence to support the charge that a "number of scientists" have dissociated themselves from the process, nor has there been any indication of how many scientists have taken this step, or for what reason. In science, we expect sweeping statements such as this to be appropriately documented. We have not been able to identify any credible support for this contention.

There is an IARC Governing Council and a Scientific Council to provide oversight and guidance to the agency. The Governing Council represents the participating states and sets general IARC policy. It appoints the IARC Director and members of the Scientific Council. The latter are independent scientists who are selected to provide scientific expertise and not as representatives of the member states. They serve for 4 years and serve without pay. The voting members of Monograph Working Groups are not employed by IARC, and they perform this task without financial compensation. There have been 111 volumes, including six separate documents under Volume 100, and three Supplements. Over the years, as the number of publications for each agent to be evaluated increased, the size of Working Groups has increased. Early in the process they were sometimes as small as 10, but now they sometimes include as many as 30 scientists. We estimate that over the entire Monograph series, approximately 1,500 scientists have served as Working Group members, and of course many scientists have also served on the Advisory Groups, Scientific Council, and Governing Council. Thus, if even a small percentage of these scientists were disenchanted with the IARC process, it would result in a considerable number of such individuals and should be easy to document. To be taken seriously, the "dissociation" criticism needs to be supported by documented information describing the number of scientists who have taken this action.

Criticisms of specific evaluations. Some criticisms of the IARC process relate to specific agents, where it is asserted that the hazard evaluations of category 2B, 2A, or 1 are not supported by the scientific literature. In the 111 volumes of the Monographs produced over the four decades since 1971, 970 agents have been considered, 114 (12%) have been classified as carcinogenic to humans (Group 1), 69 (7%) as probably carcinogenic (Group 2A), 283 (29%) as possibly carcinogenic (Group 2B), 504 (52%) as not classifiable regarding their carcinogenicity (Group 3), and 1 (< 1%) as probably not carcinogenic to humans (Group 4). Thus, even for this highly select group of agents (i.e., those selected for evaluation because there was some concern that they might be carcinogenic), more than one-half were "not classifiable" or "probably not carcinogenic," and a further 29% were placed into the category of possibly carcinogenic to humans. This distribution, based on nearly 1,000 evaluations in which fewer than one in five agents were classified as carcinogenic or probably carcinogenic to humans, does not support a conclusion that the process is heavily biased

toward classifying agents as carcinogenic (Boffetta et al. 2009; Kabat 2012).

The monographs for formaldehyde, coffee, DDT, and radiofrequency electromagnetic radiation have been cited as examples of problematic evaluations by some (Kabat 2012) [among these, only formaldehyde was classified as known to be carcinogenic to humans (Group 1) by an IARC Working Group]. These are important agents. However, to accept the charge that IARC evaluations are fundamentally biased, one has to assume that the scientists who were members of the Working Groups were incapable of appropriately evaluating weaknesses in the data, or that they distorted the evaluative process because of personal biases. In our experience, neither of these assertions is correct. Dissent among scientists is not unusual in any area of science. It is a strength of the scientific process. The IARC process capitalizes on this by bringing scientists from different disciplines together in one room to evaluate the literature and to reach a reasoned conclusion. Differences of opinion occur among Working Group members. These differences, however, typically involve disputes related to assignment to adjacent classification categories. It is instructive that there are no instances in which a carcinogen classified at the Group 1 level by one Working Group has been reversed by another. The recent review of all Group 1 agents for Volume 100 provided ample opportunity to reverse such previous classifications, but none occurred. Every scientist could probably name a substance that has been reviewed by IARC that they might personally place in a different category from that assigned by the Working Group, but this is one opinion against the collective wisdom and process of the Working Group.

Criticisms of the composition of the working groups. The composition of the Working Groups has also been criticized (Erren 2011; McLaughlin et al. 2010, 2011); it has been argued that members of the Working Groups who have conducted research on the agents under evaluation have a vested interest in advancing their own research results in the deliberations. This criticism has been addressed directly by Wild and colleagues (Wild and Cogliano 2011; Wild and Straif 2011) from IARC, and we know of no evidence to support this contention. Even if some scientists on the Working Group have performed research on some of the agents being considered, they make up a minority of the Working Group because several agents are usually evaluated in a single meeting, so the number of Working Group members who have conducted research on any one agent is typically small. Our experience has been that having some scientists who are knowledgeable about the studies of the agent under evaluation (and can therefore answer technical queries) and others from different, but related, fields provides a knowledgeable and balanced mix of scientific backgrounds for a thoughtful evaluation of the literature.

Working Group members do not receive any fee for their work, but they are paid travel expenses, and there is some prestige associated with service on an IARC Monograph. However, most scientists asked to serve on IARC Working Groups have already achieved some measure of scientific stature, and there is no reason why this should bias their evaluation in one direction or the other. In addition,

Appendix 1: Classification Categories for the Overall Evaluation for the IARC Monographs (IARC 2006)

Group 1: The agent is *carcinogenic to humans.*
This category is used when there is sufficient evidence of carcinogenicity in humans. Exceptionally, an agent may be placed in this category when evidence of carcinogenicity in humans is less than sufficient but there is sufficient evidence of carcinogenicity in experimental animals and strong evidence in exposed humans that the agent acts through a relevant mechanism of carcinogenicity.

Group 2.
This category includes agents for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost *sufficient*, as well as those for which, at the other extreme, there are no human data but for which there is evidence of carcinogenicity in experimental animals. Agents are assigned to either Group 2A (*probably carcinogenic to humans*) or Group 2B (*possibly carcinogenic to humans*) on the basis of epidemiological and experimental evidence of carcinogenicity and mechanistic and other relevant data. The terms *probably carcinogenic* and *possibly carcinogenic* have no quantitative significance and are used simply as descriptors of different levels of evidence of human carcinogenicity, with *probably carcinogenic* signifying a higher level of evidence than *possibly carcinogenic*.

Group 2A: The agent is *probably carcinogenic to humans.*
This category is used when there is *limited evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals. In some cases, an agent may be classified in this category when there is *inadequate evidence of carcinogenicity* in humans and *sufficient evidence of carcinogenicity* in experimental animals and strong evidence that the carcinogenesis is mediated by a mechanism that also operates in humans. Exceptionally, an agent may be classified in this category solely on the basis of *limited evidence of carcinogenicity* in humans. An agent may be assigned to this category if it clearly belongs, based on mechanistic considerations, to a class of agents for which one or more members have been classified in Group 1 or Group 2A.

Group 2B: The agent is *possibly carcinogenic to humans.*
This category is used for agents for which there is *limited evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals. It may also be used when there is *inadequate evidence of carcinogenicity* in humans but there is *sufficient evidence of carcinogenicity* in experimental animals. In some instances, an agent for which there is *inadequate evidence of carcinogenicity* in humans and less than *sufficient evidence of carcinogenicity* in experimental animals together with supporting evidence from mechanistic and other relevant data may be placed in this group. An agent may be classified in this category solely on the basis of strong evidence from mechanistic and other relevant data.

Group 3: The agent is *not classifiable as to its carcinogenicity to humans.*
This category is used most commonly for agents for which the evidence of carcinogenicity is *inadequate* in humans and *inadequate* or *limited* in experimental animals.
Exceptionally, agents for which the evidence of carcinogenicity is *inadequate* in humans but *sufficient* in experimental animals may be placed in this category when there is strong evidence that the mechanism of carcinogenicity in experimental animals does not operate in humans.

Agents that do not fall into any other group are also placed in this category.

An evaluation in Group 3 is not a determination of noncarcinogenicity or overall safety. It often means that further research is needed, especially when exposures are widespread or the cancer data are consistent with differing interpretations.

Group 4: The agent is *probably not carcinogenic to humans.*
This category is used for agents for which there is *evidence suggesting lack of carcinogenicity* in humans and in experimental animals. In some instances, agents for which there is *inadequate evidence of carcinogenicity* in humans but *evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of mechanistic and other relevant data, may be classified in this group.

IARC strictly requires that any conflict of interests be divulged, and does not allow those with conflicts of interest to serve on Working Groups, although nonvoting observers who may have conflicts of interest are able to attend the Working Group meetings.

Conclusions

For more than four decades the IARC Monograph Programme has provided evaluations of cancer hazards to humans from many different exposures and agents. These are often the first evaluations of new and emerging threats to public health and, consequently, are subject to intense scrutiny. Although these evaluations are widely respected and used by many organizations, institutions, companies, and government agencies to improve the public's health, IARC has recently been subject to criticism over conclusions on specific agents, the process that leads to such conclusions, and membership of the Working Groups. Debate and criticism facilitate self-correction and a check on the validity in science. We are concerned, however, that the criticisms expressed by a vocal minority regarding the evaluations of a few agents may promote the denigration of a process that has served the public and public health well for many decades for reasons that are not supported by data.

There has been very broad involvement of the scientific community in the IARC Monograph Programme through participation in the Working Groups and service on the IARC Governing and Scientific Councils and ad hoc Advisory Board for the Monograph Programme. The long list of scientists who are coauthors of this paper attests to the strong support that IARC has in the scientific community. Many exposures that IARC has evaluated have also been independently evaluated by other institutions, such as the U.S. National Toxicology Program (https://ntp.niehs.nih.gov/); U.S. Environmental Protection Agency (http://www.epa.gov/); National Academy of Sciences (http://www.nasonline.org/); the American Conference of Governmental Industrial Hygienists (ACGIH) Threshold Limit Values and Biological Exposure Indices (http://www.acgih.org/); the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (http://www.av.se/arkiv/neg/); Institute of Occupational Medicine (http://www.iom-world.org/); World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR) Expert Reports; European Chemicals Agency (https://echa.europa.eu); Swedish Criteria Group for Occupational Standards (2013); California Office of Environmental Hazard Assessment (Proposition 65; http://oehha.ca.gov/prop65/background/p65plain.html); Health Canada Bureau of Chemical Safety (http://www.hc-sc.gc.ca/ahc-asc/branch-dirgen/hpfb-dgpsa/fd-da/hcs-bsc/index-eng.php); Scientific Committee on Occupational Exposure Limits (SCOEL), European Commission, Employment, Social Affairs and Inclusion (http://ec.europa.eu/social/main.jsp?catId=148&langId=en&intPageId=684); European Food Safety Authority (EFSA 2013); and European Chemicals Agency (ECHA; http://echa.europa.eu/). Assessments from these groups typically come to conclusions similar to those from IARC. This further indicates broad agreement within the scientific community regarding evidence on carcinogenicity in the scientific literature and expands the number of scientists who do not have a "vested interest" but who have generally agreed with those conclusions.

Disagreement with the conclusions in an IARC Monograph for an individual agent is not evidence for a failed or biased approach. Some disagreement about the carcinogenic hazard of important agents seems inherent to the scientific enterprise and is unavoidable at early stages of the hazard evaluation, where IARC usually operates. Because the evaluations are not—and should not be—static, it is difficult to see how such assessments could be addressed any differently. Substances now universally recognized as human carcinogens (e.g., tobacco, asbestos) at one time went through a quite lengthy period of contentious debate (Michaels 2006, 2008). Any process can in theory be improved with fair and constructive criticism; appropriate reviews may take place from time to time, and we would support continued review and improvement of the IARC processes. However, as a group of international scientists, we have looked carefully at the recent charges of flaws and bias in the hazard evaluations by IARC Working Groups, and we have concluded that the recent criticisms are unfair and unconstructive.

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Global Cervical Cancer: HPV Vaccination and Diagnostics

The risk of dying from cervical cancer is unequally borne by women in developing countries

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In response to new etiologic evidence, improved technology, and promising HPV vaccine efforts, cervical cancer epidemiologic and preventive efforts are being reshaped throughout the world. The Harvard School of Public Health (Center for Health Decision Science), the International Agency for Research on Cancer (IARC), PATH, and the World Health Organization (WHO) are pursuing a coordinated strategy to make new diagnostics and HPV vaccines accessible, affordable, and sustainable in developing countries. The objective of this project is to promote evidence-based decision making in a global effort to prevent deaths from cervical cancer, and to catalyze global cancer prevention efforts by synthesizing the best available data and identifying effective, cost-effective, and affordable strategies to prevent cancer-causing HPV infection using new vaccines, and to detect infection at a treatable stage using new diagnostics. Specific goals include:

(1) To develop regional and country-specific models representing different epidemiologic settings using empiric data from multiple study sites on cancer incidence, type-specific HPV prevalence and distribution across the disease spectrum, and key cofactors.

(2) To conduct comprehensive policy analyses to estimate the avertable burden of disease and cost-effectiveness of various HPV vaccination strategies, and identify potential synergies between vaccination and screening, and the most influential factors on the sustainability and affordability of different policy alternatives.

(3) To develop a Core Modeling Center that will analytically support partner activities (e.g., PATH operational research in four countries), assist with or conduct cost-effectiveness analyses for different stakeholders in the HPV vaccine initiative (e.g., analyses to support GAVI investment case), and inform country decision making with analyses that reflect local costs and regional priorities.

Our partners include:

(1) The International Agency for Research on Cancer (IARC), which coordinates and conducts epidemiological and laboratory research on the causes of cancer. In this partnership, IARC collates published data on HPV type distribution in cervical cancer around the globe and co-ordinates new studies in regions where such data are missing, with special reference to populations where HIV is common. IARC also conducts surveys to determine the age-specific and genotype-specific prevalence of HPV in populations where very little or no knowledge is available.

(2) PATH, an international nonprofit organization that improves the health of people around the world through sustainable and culturally-relevant health related solutions. PATH is organizing HPV vaccination operational research projects in four countries (India, Peru, Uganda, and Vietnam) to generate experience addressing the sociocultural, logistic, policy, and clinical needs related to HPV vaccine introduction. In addition, PATH is negotiating partnerships with HPV vaccine manufacturers to accelerate access to HPV vaccine in developing countries. PATH is working with the partners to develop an investment case for public-sector HPV vaccine financing by potential funders (the GAVI Alliance, bilateral donors, and countries), and will disseminate research

project results and other educational and advocacy messages to global, regional, and national audiences.

(3) The World Health Organization's Initiative for Vaccine Research (WHO-IVR), charged with reinforcing linkages between vaccine research and development and immunization. WHO-IVR focuses on harmonizing and standardizing laboratory procedures and creating a global HPV Laboratory Network to facilitate vaccine licensure and monitoring in developing countries. Additionally, WHO-IVR generates an enabling environment for HPV vaccine introduction by creating an international multidisciplinary policy platform and setting a global agenda for future HPV vaccine introduction in consultation with regions and countries.

(4) Catalan Institute of Oncology (ICO)'s Epidemiology and Cancer Registration Unit, in Barcelona, Spain, which has been involved in the design and development of research initiatives around the world related to the causes and prevention of cancer. ICO analyzes data to assess the prevalence and natural history of HPV infections, the etiology of cervical cancer, and the attributable risk due to cofactors. In partnership with WHO, ICO has created an Information Centre on HPV and Cervical Cancer to facilitate global, regional, and country-specific decisions on current and novel options for cervical cancer prevention.

HARVARD T.H. CHAN | SCHOOL OF PUBLIC HEALTH
Center for Health and the Global Environment

About Us: Scientific Advisor

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What does working with the Center mean to you?

My interest in the Center began when it was co-founded by my dear friend, the late Dr. Paul Epstein, and throughout its twenty-year history. I look forward to providing advice and assistance in its next phase of work, including the health impacts of poor indoor air quality.

Biography

An epidemiologist with more than forty years experience in public health practice, teaching, and consulting, Richard (Dick) Clapp is a both an Emeritus Professor of Environmental Health at Boston University School of Public Health and an Adjunct Professor at the University of Massachusetts Lowell. His research interests have focused on analyzing data related to environmental and occupational causes

of cancer and other diseases. He served as Director of the Massachusetts Cancer Registry from 1980-1989 and is a former Co-Chair of Greater Boston Physicians for Social Responsibility.

Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA)

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The International Agency for Research on Cancer (IARC) Monographs Programme identifies chemicals, drugs, mixtures, occupational exposures, lifestyles and personal habits, and physical and biological agents that cause cancer in humans and has evaluated about 1000 agents since 1971. Monographs are written by ad hoc Working Groups (WGs) of international scientific experts over a period of about 12 months ending in an eight-day meeting. The WG evaluates all of the publicly available scientific information on each substance and, through a transparent and rigorous process,[1] decides on the degree to which the scientific evidence supports that substance's potential to cause or not cause cancer in humans.

For Monograph 112,[2] 17 expert scientists evaluated the carcinogenic hazard for four insecticides and the herbicide glyphosate.[3] The WG concluded that the data for glyphosate meet the criteria for classification as a *probable human carcinogen*.

The European Food Safety Authority (EFSA) is the primary agency of the European Union for risk assessments regarding food safety. In October 2015, EFSA reported[4] on their evaluation of the Renewal Assessment Report[5] (RAR) for glyphosate that was prepared by the Rapporteur Member State, the German Federal Institute for Risk Assessment (BfR). EFSA concluded that 'glyphosate is unlikely to pose a carcinogenic hazard to humans and the evidence does not support classification with regard to its carcinogenic potential'. Addendum 1 (the BfR Addendum) of the RAR[5] discusses the scientific rationale for differing from the IARC WG conclusion.

Serious flaws in the scientific evaluation in the RAR incorrectly characterise the potential for a carcinogenic hazard from exposure to glyphosate. Since the RAR is the basis for the European Food Safety Agency (EFSA) conclusion,[4] it is critical that these shortcomings are corrected.

THE HUMAN EVIDENCE

EFSA concluded 'that there is very limited evidence for an association between glyphosate-based formulations and non-Hodgkin lymphoma (NHL), overall inconclusive for a causal or clear associative relationship between glyphosate and cancer in human studies'. The BfR Addendum (p. ii) to the EFSA report explains that 'no consistent positive association was observed' and 'the most powerful study showed no effect'. The IARC WG concluded there is *limited evidence of carcinogenicity in humans* which means "A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence."[1]

The finding of *limited evidence* by the IARC WG was for NHL, based on high-quality case–control studies, which are particularly valuable for determining the carcinogenicity of an agent because their design facilitates exposure assessment and reduces the potential for certain biases. The Agricultural Health Study[6] (AHS) was the only cohort study available providing information on the carcinogenicity

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of glyphosate. The study had a null finding for NHL (RR 1.1, 0.7–1.9) with no apparent exposure–response relationship in the results. Despite potential advantages of cohort versus case–control studies, the AHS had only 92 NHL cases in the unadjusted analysis as compared to 650 cases in a pooled case–control analysis from the USA.[7] In addition, the median follow-up time in the AHS was 6.7 years, which is unlikely to be long enough to account for cancer latency.[8]

The RAR classified all of the case–control studies as 'not reliable,' because, for example, information on glyphosate exposure, smoking status and/or previous diseases had not been assessed. In most cases, this is contrary to what is actually described in the publications. Well-designed case–control studies are recognised as strong evidence and routinely relied on for hazard evaluations.[9 10] The IARC WG carefully and thoroughly evaluated all available epidemiology data, considering the strengths and weaknesses of each study. This is key to determining that the positive associations seen in the case–control studies are a reliable indication of an association and not simply due to chance or methodological flaws. To provide a reasonable interpretation of the findings, an evaluation needs to properly weight studies according to quality rather than simply count the number of positives and negatives. The two meta-analyses cited in the IARC Monograph[11] are excellent examples of objective evaluations and show a consistent positive association between glyphosate and NHL.

The final conclusion[5] (Addendum 1, p.21) that "there was no unequivocal evidence for a clear and strong association of NHL with glyphosate" is misleading. IARC, like many other groups, uses three levels of evidence for human cancer data.[1] *Sufficient evidence* means 'that a causal relationship has been established' between glyphosate and NHL. BfR's conclusion is equivalent to deciding that there is not *sufficient evidence*. Legitimate public health concerns arise when 'causality is credible', that is, when there is *limited evidence of carcinogenicity*.

EVIDENCE FROM ANIMAL CARCINOGENICITY STUDIES

EFSA concluded 'No evidence of carcinogenicity was confirmed by the majority of the experts (with the exception of one minority view) in either rats or mice due to a lack of statistical significance in pairwise comparison tests, lack of consistency in multiple animal studies and slightly increased incidences only at dose levels at or above the limit dose/maximum tolerated dose (MTD), lack of preneoplastic lesions and/or being within historical control range'. The IARC WG review found a significant positive trend for renal tumours in male CD-1 mice,[12] a rare tumour, although no comparisons of any individual exposure group to the control group were statistically significant. The WG also identified a significant positive trend for hemangiosarcoma in male CD-1 mice,[13] again with no individual exposure group significantly different from controls. Finally, the WG also saw a significant increase in the incidence of pancreatic islet cell adenomas in two studies in male Sprague-Dawley rats.[14–16] In one of these rat studies, thyroid gland adenomas in females and liver adenomas in males were also increased. By the IARC review criteria,[1] this constitutes *sufficient evidence* in animals.

The IARC WG reached this conclusion using data that were publicly available in sufficient detail for independent scientific evaluation (a requirement of the IARC Preamble[1]). On the basis of the BfR Addendum, it seems there were three additional mouse studies and two additional rat studies that were unpublished and available to EFSA. Two of the additional studies were reported to have a significant trend for renal tumours, one in CD-1 mice (Sugimoto. *18-Month Oral Oncogenicity Study in Mice*. Unpublished, designated ASB2012–11493 in RAR. 1997), and one in Swiss-Webster mice (Unknown. *A chronic feeding study of glyphosate (roundup technical) in mice*. Unpublished, designated ABS2012–11491 in RAR. 2001). One of these studies (Sugimoto. Unpublished, 1997) also reported a significant trend for hemangiosarcoma. The RAR also reported two studies in CD-1 mice showing significant trends for malignant lymphoma (Sugimoto. Unpublished, 1997; Unknown. *Glyphosate Technical: Dietary Carcinogenicity Study in the Mouse*. Unpublished, designated ABS2012–11492 in RAR. 2009).

The RAR dismissed the observed trends in tumour incidence because there are no individual treatment groups that are significantly different from controls and because the maximum observed response is reportedly within the range of the historical control data (Table 5.3–1, p.90). Care must be taken in using historical control data to evaluate animal carcinogenicity data. In virtually all guidelines,[1 17 18] scientific reports[19] and publications[20–23] on this issue, the recommended first choice is the use of concurrent controls and trend tests, even in the EC regulations cited in the RAR[18] (see p.375). Trend tests are more powerful than pairwise comparisons, particularly for rare tumours where data are sparse. Historical control data should be from studies in the same time frame, for the same animal strain, preferably from the same laboratory or the same supplier and preferably reviewed by the same pathologist.[17 18] While the EFSA final peer review[4] mentions the use of historical control data from the original laboratory, no specifics are provided and the only referenced historical control data[24] are in the BfR addendum.[5] One of the mouse studies[12] was clearly done before this historical control database was developed, one study (Sugimoto. Unpublished, 1997) used Crj:CD-1 mice rather than Crl:CD-1 mice, and one study[13] did not specify the substrain and was reported in 1993 (probably started prior to 1988). Hence, only a single study (Unknown. Unpublished, 2009) used the same mouse strain as the cited historical controls, but was reported more than 10 years after the historical control data set was developed.

The RAR dismissed the slightly increased tumour incidences in the studies considered because they occurred "only at dose levels at or above the limit dose/maximum tolerated dose (MTD)", and because there was a lack of preneoplastic lesions. Exceeding the MTD is demonstrated by an increase in mortality or other serious toxicological findings at the highest dose, not by a slight reduction in body weight. No serious toxicological findings were reported at the highest doses for the mouse studies in the RAR. While some would argue that these high doses could cause cellular disruption (eg, regenerative hyperplasia) leading to cancer, no evidence of this was reported in any study. Finally, a lack of preneoplastic lesions for a significant neoplastic finding is insufficient reason to discard the finding.

MECHANISTIC INFORMATION

The BfR Addendum dismisses the IARC WG finding that 'there is strong evidence that glyphosate causes genotoxicity' by suggesting that unpublished evidence not seen by the IARC WG was overwhelmingly negative and that, since the reviewed studies were not done under guideline principles, they should get less weight. To maintain transparency, IARC reviews only publicly available data. The use of confidential data submitted to the BfR makes it impossible for any scientist not associated with BfR to review this conclusion. Further weakening their interpretation,

the BfR did not include evidence of chromosomal damage from exposed humans or human cells that were highlighted in Tables 4.1 and 4.2 of the IARC Monograph [3]

The BfR confirms (p.79) that the studies evaluated by the IARC WG on oxidative stress were predominantly positive but does not agree that this is strong support for an oxidative stress mechanism. They minimise the significance of these findings predominantly because of a lack of positive controls in some studies and because many of the studies used glyphosate formulations and not pure glyphosate. In contrast, the WG concluded that (p.77) 'Strong evidence exists that glyphosate, AMPA and glyphosate-based formulations can induce oxidative stress'. From a scientific perspective, these types of mechanistic studies play a key role in distinguishing between the effects of mixtures, pure substances and metabolites.

Finally, we strongly disagree that data from studies published in the peer-reviewed literature should automatically receive less weight than guideline studies. Compliance with guidelines and Good Laboratory Practice does not guarantee validity and relevance of the study design, statistical rigour and attention to sources of bias.[25 26] The majority of research after the initial marketing approval, including epidemiology studies, will be conducted in research laboratories using various models to address specific issues related to toxicity, often with no testing guidelines available. Peer-reviewed and published findings have great value in understanding mechanisms of carcinogenicity and should be given appropriate weight in an evaluation based on study quality, not just on compliance with guideline rules.

GENERAL COMMENTS

Science moves forward on careful evaluations of data and a rigorous review of findings, interpretations and conclusions. An important aspect of this process is transparency and the ability to question or debate the findings of others. This ensures the validity of the results and provides a strong basis for decisions. Many of the elements of transparency do not exist for the RAR.[5] For example, citations for almost all references, even those from the open scientific literature, have been redacted. The ability to objectively evaluate the findings of a scientific report requires a complete list of cited supporting evidence. As another example, there are no authors or contributors listed for either document, a requirement for publication in virtually all scientific journals where financial support, conflicts of interest and affiliations of authors are fully disclosed. This is in direct contrast to the IARC WG evaluation listing all authors, all publications and public disclosure of pertinent conflicts of interest prior to the WG meeting.[27]

Several guidelines have been devised for conducting careful evaluation and analysis of carcinogenicity data, most after consultation with scientists from around the world. Two of the most widely used guidelines in Europe are the OECD guidance on the conduct and design of chronic toxicity and carcinogenicity studies[17] and the European Chemicals Agency Guidance on Commission Regulation (EU) No 286/2011;[18] both are cited in the RAR. The methods used for historical controls and trend analysis are inconsistent with these guidelines.

Owing to the potential public health impact of glyphosate, which is an extensively used pesticide, it is essential that all scientific evidence relating to its possible carcinogenicity is publicly accessible and reviewed transparently in accordance with established scientific criteria.

SUMMARY

The IARC WG concluded that glyphosate is a 'probable human carcinogen', putting it into IARC category 2A due to *sufficient evidence* of carcinogenicity in animals, *limited evidence* of carcinogenicity in humans and *strong* evidence for two carcinogenic mechanisms.

- The IARC WG found an association between NHL and glyphosate based on the available human evidence.
- The IARC WG found significant carcinogenic effects in laboratory animals for rare kidney tumours and hemangiosarcoma in two mouse studies and benign tumours in two rat studies.
- The IARC WG concluded that there was strong evidence of genotoxicity and oxidative stress for glyphosate, entirely from publicly available research, including findings of DNA damage in the peripheral blood of exposed humans.

The RAR concluded[5] (Vol. 1, p.160) that 'classification and labelling for carcinogenesis is not warranted' and 'glyphosate is devoid of genotoxic potential'.

- EFSA[4] classified the human evidence as 'very limited' and then dismissed any association of glyphosate with cancer without clear explanation or justification.
- Ignoring established guidelines cited in their report, EFSA dismissed evidence of renal tumours in three mouse studies, hemangiosarcoma in two mouse studies and malignant lymphoma in two mouse studies. Thus, EFSA incorrectly discarded all findings of glyphosate-induced cancer in animals as chance occurrences.
- EFSA ignored important laboratory and human mechanistic evidence of genotoxicity.
- EFSA confirmed that glyphosate induces oxidative stress but then, having dismissed all other findings of possible carcinogenicity, dismissed this finding on the grounds that oxidative stress alone is not sufficient for carcinogen labelling.

The most appropriate and scientifically based evaluation of the cancers reported in humans and laboratory animals as well as supportive mechanistic data is that glyphosate is a *probable human carcinogen*. On the basis of this conclusion and in the absence of evidence to the contrary, it is reasonable to conclude that glyphosate formulations should also be considered likely human carcinogens. The CLP Criteria[18] (Table 3.6.1, p.371) allow for a similar classification of Category 1B when there are 'studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals'.

In the RAR, almost no weight is given to studies from the published literature and there is an over-reliance on non-publicly available industry-provided studies using a limited set of assays that define the minimum data necessary for the marketing of a pesticide. The IARC WG evaluation of *probably carcinogenic to humans* accurately reflects the results of published scientific literature on glyphosate and, on the face of it, unpublished studies to which EFSA refers.

Most of the authors of this commentary previously expressed their concerns to EFSA and others regarding their review of glyphosate[28] to which EFSA has published a reply.[29] This commentary responds to the EFSA reply.

The views expressed in this editorial are the opinion of the authors and do not imply an endorsement or support for these opinions by any organisations to which they are affiliated.

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Competing interests CJP, MTS and DDW are providing advice to a US law firm involved in glyphosate litigation. CJP also works part-time for the Environmental Defense Fund on issues not related to pesticides.

Provenance and peer review Commissioned; externally peer reviewed.

To cite Portier CJ, Armstrong BK, Baguley BC, *et al*. *J Epidemiol Community Health* 2016;70:741–745.

Published Online First 3 March 2016

J Epidemiol Community Health 2016;70:741–745. doi:10.1136/jech-2015-207005

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Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA)

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J Epidemiol Community Health 2016 70: 741-745 originally published online March 3, 2016
doi: 10.1136/jech-2015-207005