

Residential Pesticide Exposures in Pregnancy and the Risk of Sporadic Retinoblastoma: A Report From the Children's Oncology Group



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- **PURPOSE:** To examine whether parental pesticide exposure contributes to the development of sporadic retinoblastoma.
- **DESIGN:** Case-control study.
- **METHODS:** Data were collected by a large multicenter study of sporadic retinoblastoma in which parents of 99 unilateral and 56 bilateral age-matched case-control pairs were interviewed by telephone. Retrospective exposure information was collected on the type, location, timing, and frequency of residential pesticide use. We used conditional logistic regression analyses to estimate odds ratios for maternal pesticide exposure in the month before or during pregnancy and to assess whether the type of product, and the circumstances under which it was applied, were associated with risk of disease.
- **RESULTS:** Unilateral retinoblastoma was associated with parental insecticide use (odds ratio [OR], 2.8; confidence interval [CI], 1.1–6.7) and the use of professional lawn or landscape services (OR, 2.8; CI, 1.0–8.2). For bilateral disease we observed large point estimates for several exposures but the small number of cases rendered these results uninformative (ie, resulted in wide confidence intervals). Whether parents used the pesticide inside vs outside the home did not appear to modify risk estimates for unilateral retinoblastoma (OR, 2.5; CI, 0.9–7.0 vs OR, 2.5; CI, 1.0–6.5), nor did the type, frequency, timing related to pregnancy, or applicator of pesticide used influence estimates to an appreciable degree for disease.
- **CONCLUSIONS:** Our results suggest that parental pesticide exposure before or during pregnancy may play a role in the development of childhood retinoblastoma. Retrospectively collected exposure data introduces the

possibility of recall bias; therefore, results should be interpreted cautiously until additional studies are conducted. (Am J Ophthalmol 2017;176:166–173. © 2017 Elsevier Inc. All rights reserved.)

RETINOBLASTOMA IS A MALIGNANT TUMOR OF THE retina that occurs because of mutation or loss of both alleles of the *RB1* gene. It is an embryonal tumor most commonly found in young children,¹ with a majority (63%) being diagnosed before age 2 and 95% by age 5. In hereditary retinoblastoma (6%–10% of cases), a germline mutation of the *RB1* gene is inherited from a parent, and most of these cases present bilaterally. In 30% of cases, a *de novo* germline mutation occurs in parental germline cells (most frequently from the father)² or occurs in very early embryonic development (“sporadic heritable retinoblastoma”); these cases also tend to present as bilateral disease. In the remaining cases, 2 somatic mutations occur in the same retinal cell during pregnancy or early life, and these cases present unilaterally (“nonheritable retinoblastoma”).

Though the 10-year survival rate is high in the United States (93%), these rates vary considerably in low to middle-income countries (40–79%).³ Despite the high cure rates, retinoblastoma survivors tend to have a poorer quality of life and have greater school absenteeism.⁴ Further, pediatric cases of heritable retinoblastoma have an elevated risk for developing sarcoma and leukemia by age 14.⁵ In adulthood, these children have an increased risk of chronic medical conditions, including a second malignant neoplasm.⁶

There has been some evidence suggesting a role for occupational or environmental exposures in retinoblastoma development. Retinoblastoma has been related to residential exposure to air pollution,^{7–9} parental occupational exposure to oil mists,¹⁰ and parental employment in radio or television repair,¹¹ shoe or leather work,¹² electrical work,¹² or the metal industry.^{13,14} Although the literature has shown fairly consistent associations between the home use of insecticides or herbicides and other childhood cancer types,^{15,16} thus far findings on retinoblastoma have been equivocal. Parental employment as a pesticide applicator¹⁷ or in horticultural, forestry, or farm work^{18–20} has been associated with a null



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or even weakly decreased risk for “childhood eye cancer” (all types combined) or retinoblastoma in previous studies, with the exception of the Agricultural Health Study that reported a higher risk (standardized incidence ratio, 1.63; confidence interval [CI], 0.41–6.53) with wide confidence intervals.²¹ Only one study¹³ found parental employment-related pesticide exposure to be associated with offspring bilateral retinoblastoma (odds ratio [OR], 2.12; CI, 1.25–3.61). For unilateral disease, increased risk was observed among children whose maternal grandfathers were farmers (OR, 10.0; CI, 1.4–433).¹⁴ With the exception of the latter 2 studies that observed positive associations,^{13,14} none of the others excluded familial cases, nor did they estimate risks separately for nonheritable and heritable disease. Only one previous study investigated the use of home insect or garden sprays, and suggested increases in risk for nonheritable retinoblastoma, but the sample size was very small and the estimates accordingly imprecise (OR, 2.7; CI, 0.6–15.6).²² The purpose of the present study was to conduct an in-depth examination of associations between sporadic retinoblastoma and pesticide exposures.

METHODS

FOR OUR CASE-CONTROL STUDY, ETHICS APPROVAL WAS obtained prospectively through the Institutional Review Board (IRB) of the University of Pennsylvania, the Wills Eye Institute, and every participating Children’s Oncology Group (COG) institution prior to subject recruitment. The abovementioned review boards approved the prospective collection of data from subjects through telephone interviews. For the retrospective data analyses of the present study, IRB approval was also obtained from the University of California, Los Angeles prior to receiving the de-identified dataset. All subjects provided verbal and/or written informed consent prior to study participation and all acts complied with Health Insurance Portability and Accountability Act regulations.

The data for this study were collected as part of a large multicenter case-control study of retinoblastoma, which has been described in detail elsewhere.^{23,24} In brief, eligible incident cases were diagnosed with sporadic retinoblastoma on or after July 1, 2006 through June 30, 2011 and were diagnosed and/or treated at a COG institution or at the Wills Eye Institute (Philadelphia, Pennsylvania, USA). Subjects were eligible if they were residing in the United States or Canada, had access to a home or cellular telephone, and had at least 1 biological parent who spoke English or Spanish who was available for participation. Children who were adopted or in foster care were excluded from participation in the present study. In total, 282 cases (186 unilateral and 96 bilateral) were recruited and completed phone interviews.

Case families were asked to nominate 1 or more friends or nonbiological relatives with a child in the same age range as the index child (0–1, 2–3, 4–5, 6–7, 8–9, 10–11, 12–13, or 14–15 years) as potential controls. For bilateral retinoblastoma, paternal exposures are of primary interest; therefore, the “ideal control” was a child in the same age range whose father was not biologically related to the case child’s father. For unilateral retinoblastoma, maternal exposures are of greatest interest; therefore, the “ideal control” was a child in the same age range whose mother was not biologically related to the case child’s mother. The study attempted to contact and recruit control families starting with the child closest in age to the matched case, and moving on to the next on the list if that child was unavailable, until all potential controls had been exhausted. Only maternal questionnaires asked about home pesticide use. Four participants (2 controls, 1 unilateral case, and 1 bilateral case) did not answer questions on home pesticide use and were therefore excluded from the present analyses.

Structured telephone interviews were conducted with parents of cases and controls. In addition to information on demographics and health behaviors during the perinatal period, mothers were asked detailed questions regarding residential pesticide use before conception and during pregnancy, including the use of professional lawn or landscape services; the use of pest control professionals or exterminators for their home; in-home use of insect or rodent killers; use of indoor foggers; home or garden use of herbicides, mold removal products, antifungals, or weed killers; insect repellent; head lice treatment on the children; and, for pets, the use of flea collars or flea or tick shampoos.

When parents indicated that they had applied a product themselves around their home or garden, they were asked to identify the name of the product. Depending on the question asked, between 1% and 43% of parents were able to identify the type of product used, and among those, parents were able to supply the name for approximately 70% of products. A large variety of products were listed by parents; therefore, we were unable to estimate effects by the specific pesticide or active ingredient used.

We estimated odds ratios and 95% confidence intervals using conditional logistic regression for the matched-pair design. Selection of variables for adjustment was based on literature review, as well as associations observed in our data.^{22,24–28} In analyses involving unilateral cases we adjusted for mother’s race (white non-Hispanic, Hispanic, other), mother’s employment status (yes/no), whether the father was living at home at the start of pregnancy (yes/no), and the mother’s age (≤ 29 , 30–34, 35+). In analyses of bilateral retinoblastoma, we adjusted for the same variables with the addition of father’s age (≤ 29 , 30–34, 35+). Marital status, mother’s educational attainment, maternal tobacco smoking, interview by proxy, and the child’s sex did not change the estimate more than 10%, and therefore we did not include these variables in the final model. We

TABLE 1. Demographic Characteristics of Participants and Crude Conditional Effect Estimates^a

	Unilateral (N = 99 Pairs)			Bilateral (N = 56 Pairs)		
	Case N (%)	Control N (%)	Crude OR (95% CI)	Case N (%)	Control N (%)	Crude OR (95% CI)
Child's sex						
Male	50 (51)	45 (45)	1.0	29 (52)	25 (45)	1.0
Female	49 (49)	48 (48)	0.9 (0.5–1.6)	27 (48)	30 (54)	0.8 (0.4–1.7)
Mother's race						
White non-Hispanic	68 (69)	74 (75)	1.0	44 (79)	37 (66)	1.0
Hispanic	19 (19)	11 (11)	9.4 (1.2–74.5)	5 (9)	7 (13)	N/A
Other	12 (12)	10 (10)	1.4 (0.4–4.9)	7 (13)	7 (13)	N/A
Mother's age						
≤29	52 (53)	44 (44)	1.0	22 (39)	21 (38)	1.0
30–34	32 (32)	37 (37)	0.7 (0.3–1.5)	18 (32)	21 (38)	0.9 (0.4–2.4)
35+	15 (15)	14 (14)	0.9 (0.3–2.5)	16 (29)	9 (16)	2.5 (0.6–10.7)
Maternal education						
High school or less	18 (18)	17 (17)	1.0 (0.3–3.0)	9 (16)	4 (7)	5.8 (0.7–51.3)
Vocational or some college	15 (15)	17 (17)	0.8 (0.3–2.0)	9 (16)	9 (16)	1.3 (0.4–4.3)
College graduate	48 (48)	40 (40)	1.0	24 (43)	23 (41)	1.0
Post-college	18 (18)	21 (21)	0.8 (0.3–2.0)	14 (25)	15 (27)	0.6 (0.2–2.1)
Number of prior pregnancies						
0	35 (35)	33 (33)	1.0	22 (39)	22 (39)	1.0
1	34 (34)	24 (24)	1.4 (0.7–3.1)	18 (32)	15 (27)	1.3 (0.5–3.4)
2	18 (18)	20 (20)	0.9 (0.4–2.0)	9 (16)	10 (18)	1.1 (0.3–3.6)
3+	11 (11)	16 (16)	0.6 (0.2–1.8)	7 (13)	4 (7)	1.9 (0.4–9.1)
Family income						
<\$25 000	5 (5)	7 (7)	4.3 (0.6–30.6)	9 (16)	4 (7)	N/A
\$25 000 to <\$35 000	7 (7)	6 (6)	3.6 (0.7–19.0)	1 (2)	4 (7)	N/A
\$35 000 to <\$50 000	16 (16)	11 (11)	1.8 (0.5–5.9)	9 (16)	6 (11)	2.0 (0.2–25.6)
\$50 000 to <\$75 000	24 (24)	19 (19)	1.7 (0.7–4.1)	11 (20)	15 (27)	0.3 (0.1–2.1)
\$75 000 to <\$100 000	12 (12)	15 (15)	1.2 (0.4–3.2)	6 (11)	8 (14)	0.3 (0.0–1.9)
≥\$100 000	24 (24)	29 (29)	1.0	15 (27)	11 (20)	1.0
Father's race						
White non-Hispanic	62 (62)	69 (70)	1.0	37 (66)	35 (63)	1.0
Hispanic	16 (16)	14 (14)	1.5 (0.3–6.9)	9 (16)	8 (14)	1.8 (0.2–15.4)
Other	13 (13)	8 (8)	2.3 (0.7–7.6)	9 (16)	8 (14)	1.3 (0.2–9.3)
Father's age						
≤29	29 (29)	34 (34)	1.0	12 (21)	17 (30)	1.0
30–34	33 (33)	29 (29)	1.3 (0.6–3.0)	21 (38)	18 (32)	1.8 (0.6–5.4)
35+	28 (28)	28 (28)	1.3 (0.5–3.6)	21 (38)	17 (30)	2.6 (0.7–9.4)

CI = confidence interval; OR = odds ratio.

^aAll columns do not add to 100% owing to missing information.

conducted sensitivity analyses examining results when proxy respondents were excluded, as well as when nonideal controls were excluded.

RESULTS

NOT ALL CASES HAD A MATCHED CONTROL; THEREFORE, analyses were conducted on 99 matched pairs of unilateral cases/maternal controls and on 56 bilateral cases/maternal controls who completed the interview. Although efforts were made to primarily recruit age-matched children who

were not biological relatives, in some instances the study accepted controls who either were unmatched or were biological relatives (7.8% of controls). In unilateral analyses, 2% of maternal-case and 5% of maternal-control interviews were conducted with a proxy respondent, typically the other parent. No bilateral interviews were conducted with a proxy respondent.

The demographics of participants are shown in [Table 1](#). The unilateral case group included a larger proportion of Hispanic mothers and the bilateral case group included a greater proportion of mothers with a high school education or less. Both case groups had lower family income than the control group. A total of 256 participants (82%) in our

matched analyses reported ever use of any pesticide in the month before or during pregnancy.

Table 2 shows associations between residential pesticide exposure in the month before or during pregnancy and unilateral retinoblastoma. We found that the use of a product to kill insects or diseases on the lawn was associated with the development of unilateral retinoblastoma (OR, 2.8; CI, 1.1–6.7). Using professional lawn or landscaping services was also associated with increased risk of unilateral disease (OR, 2.8; CI, 1.0–8.2). Home use of weed killer was associated with unilateral retinoblastoma development (OR, 2.3; CI, 0.9–5.4), but the 95% CI was wide and included the null.

Table 3 summarizes associations between maternal residential pesticide exposure in the month before or during pregnancy and bilateral retinoblastoma. High positive point estimates with wide confidence intervals that included the null were estimated for use of professional lawn or landscape services (OR, 3.4; CI, 0.6–18.0). Although for several of the other measures effect estimates were also elevated above 1, our point estimates were similarly imprecise owing to the small number of cases.

Table 4 shows multivariate analyses with more detailed information on the type of product, and the circumstances under which it was applied, using the same types of exposures as shown in the previous tables. In general, the effect estimates for retinoblastoma were similar for the various types, locations, timing, and frequency of pesticide use, with overlapping confidence intervals; that is, risk of unilateral and bilateral retinoblastoma increased regardless of whether families had used sprays or other types of products, whether they used the product inside or outside the home, and whether they used them early or late in pregnancy.

About half of participants reported a product name for which ingredients could be identified, of which many included combinations of pyrethroids or neonicotinoids (e.g. imidacloprid). Other reported products used for weed control included glyphosate, 2,4-D, 2-methyl-4-chlorophenoxyacetic acid (MCPA), and dicamba. Sensitivity analyses excluding proxy respondents and nonideal controls yielded effect estimates similar to those reported here (data not shown).

DISCUSSION

IN OUR CASE-CONTROL STUDY, WHICH STRATIFIED BY laterality of disease, we observed positive associations for some prenatal pesticide use and child sporadic retinoblastoma. For unilateral retinoblastoma, odds ratios were increased more than 2-fold with reported use of professional lawn or landscaping services, and similarly with use of insecticides inside or outside the home. Use of weed and insect control products on the lawn also resulted

in increased odds ratios; however, the confidence intervals included the null. Although there also was some suggestion of increased risks in relation to these exposures for bilateral cases, all point estimates were estimated relatively imprecisely owing to the small number of cases. Our findings for unilateral cases are consistent with the proposed underlying mechanisms of disease, which suggests that unilateral retinoblastoma is likely associated with somatic mutations during fetal development. Given that the present study tests many potential associations, issues of multiple comparison are a concern; however, as most point estimates for pesticide use and retinoblastoma were in the direction of a positive association, we believe our study provides evidence that is suggestive of a true association.

In this study, questions on pesticide use were asked only of the mother, and mothers may not have been aware of all pesticides that were applied by the father in or around the home. If pesticide applications contributed to *de novo* mutations in paternal germline cells, leading to bilateral disease, we were not able to address these exposures. However, the separation of exposures is not easy to make if both parents are living together, and we assume if one reported using the pesticide the other parent is likely exposed as well.

Because retinoblastoma in children is a rare disease with an estimated mean age-adjusted incidence in the United States of 11.8 per million children aged 0–4 years (95% CI, 10.9–12.8) from 1975 to 2004,²⁹ studies investigating environmental risk factors are limited, and most have thus far not distinguished between unilateral and bilateral cases, nor have they focused only on sporadic cases. The increased risks we see for different types of residential pesticide applications during pregnancy and unilateral cases are corroborated by the only other study of retinoblastoma reporting on the use of home insect or garden sprays.²² This study estimated a similarly increased point estimate for nonheritable unilateral retinoblastoma, although the confidence intervals were wide (OR, 2.7; CI, 0.6–15.6).²² Several other studies examined occupational pesticide exposures. One such study, which assessed paternal occupational exposure based on a job exposure matrix applied to jobs 10 years prior to conception, reported an increased odds ratio for nonfamilial bilateral retinoblastoma of 1.40 (95% CI, 0.85–2.30).¹³ Other occupational studies assessed parental pesticide exposure more crudely, including working as a pesticide applicator¹⁷ or horticultural, forestry, or farm work^{18–20} and child retinoblastoma (or all childhood “eye cancer,” of which retinoblastoma accounts for over 90% of cases), reporting no or even somewhat decreased risks. However, these studies were flawed in that they either obtained all occupational data from birth records (and thus did not have access to information such as specific agents exposed to at work, employment dates, or number of jobs held) or made so many comparisons that any or all of the findings could have occurred by chance. The great majority of these

TABLE 2. Estimate Effects and 95% Confidence Intervals for Parental Pesticide Exposure Inside or Outside the Home in the Month Before or During Pregnancy and Unilateral Retinoblastoma, Conditional Logistic Regression Analyses

	Cases (N = 99)		Controls (N = 99)		Crude	Adjusted ^a
	Exposed N	Unexposed N	Exposed N	Unexposed N	OR	OR (95% CI)
Professional services use						
Lawn or landscape services	21	78	13	86	2.5	2.8 (1.0, 8.2)
Home pest control services	12	87	20	79	0.4	0.6 (0.2, 1.7)
Product to kill insects or diseases						
Inside or outside the home, such as Raid	51	48	42	57	1.8	2.8 (1.1, 6.7)
On the lawn	13	86	9	90	1.5	1.2 (0.4, 3.4)
On plants, vegetables, fruit, flowers, or trees	4	95	7	92	0.6	0.6 (0.2, 2.3)
Use of weed control products on garden/lawn	27	72	19	80	1.8	2.3 (0.9, 5.4)
Use of product to kill mold or fungus	5	94	4	95	1.5	3.2 (0.4, 26.0)
Use of rodent control products	7	92	8	91	0.9	0.8 (0.3, 2.7)
Use of flea/tick products on pets	36	60	33	66	1.1	1.5 (0.7, 3.1)
Use of insect repellent	25	74	28	71	0.7	0.6 (0.3, 1.5)

CI = confidence interval; OR = odds ratio.

^aAdjusted for mother's race, mother's age, mother's work status, and whether the father was living at home at start of pregnancy.

TABLE 3. Estimate Effects and 95% Confidence Intervals for Parental Pesticide Exposure Inside or Outside the Home in the Month Before or During Pregnancy and Bilateral Retinoblastoma, Conditional Logistic Regression Analyses

	Cases (N = 56)		Controls (N = 56)		Crude	Adjusted ^a
	Exposed N	Unexposed N	Exposed N	Unexposed N	OR	OR (95% CI)
Professional services use						
Lawn or landscape services	14	42	7	49	3.0	3.4 (0.6, 18.0)
Home pest control services	13	43	11	45	1.8	1.0 (0.2, 5.7)
Product to kill insects or diseases						
Inside or outside the home, such as Raid	27	29	20	36	1.3	1.2 (0.4, 3.5)
On the lawn	5	51	1	55	4.0	12.5 (0.5, 322.1)
On plants, vegetables, fruit, flowers, or trees	6	50	2	54	4.0	NA
Use of weed control products on garden/lawn	15	41	12	44	1.2	1.0 (0.3, 3.3)
Use of product to kill mold or fungus	1	55	1	55	NA	NA
Use of rodent control products	8	48	4	52	2.5	2.5 (0.2, 26.9)
Use of flea/tick products on pets	21	35	15	41	1.9	1.9 (0.6, 5.8)
Use of insect repellent	13	43	13	43	1.0	1.3 (0.3, 5.6)

CI = confidence interval; NA = not applicable; OR = odds ratio.

^aAdjusted for mother's race, mother's age, mother's work status, father's age, and whether the father was living at home at start of pregnancy.

studies used data linkage techniques to obtain cancer diagnoses based on participant address at 1 time period such as birth, which would exclude subjects who migrated, potentially misclassifying the results.^{17,18,20,21} The Agricultural Health Study cohort identified 50 incident cases of various child cancers but only 2 retinoblastomas; thus the reported 60% increased risk for parental pesticide exposures had wide CI for this cancer and has to be interpreted cautiously.²¹ Notably, the above studies mixed familial bilateral and unilateral cases and

thus the findings are not directly comparable to ours. Our own study of air toxins based on ambient monitoring data suggested increases in unilateral retinoblastoma related to several volatile chemicals such as xylenes and toluene,⁹ which supports the notion that environmental toxins act as risk factors during pregnancy and contribute to the etiology of sporadic retinoblastoma in children.

Studies examining associations between pesticide use and childhood cancer so far have mostly focused on leukemia. Two recent systematic reviews/meta-analyses

TABLE 4. Effect Estimates and 95% Confidence Intervals for Combined Parental Pesticide Exposures in the Month Before or During Pregnancy and Sporadic Retinoblastoma, Conditional Logistic Regression

	Unilateral (N = 99 Pairs)				Bilateral (N = 56 Pairs)			
	Cases	Controls	Crude OR	Adjusted OR (95% CI) ^a	Cases (N = 56)	Controls (N = 56)	Crude OR	Adjusted OR (95% CI) ^a
Type of pesticide used								
None used	31	32	Ref	Ref	13	21	Ref	Ref
Spray	49	41	1.6	2.4 (0.9, 6.0)	26	19	2.1	2.8 (0.7, 12.3)
Other ^b	18	20	1.1	1.4 (0.6, 3.7)	17	12	2.8	4.1 (0.9, 20.1)
Where was the pesticide was used								
None used	35	38	Ref	Ref	19	27	Ref	Ref
Inside home	33	31	1.5	2.5 (0.9, 7.0)	23	11	2.3	1.9 (0.6, 6.1)
Outside home	30	24	1.7	2.5 (1.0, 6.5)	14	14	1.3	1.9 (0.5, 6.8)
When was the pesticide was used								
None used	20	22	Ref	Ref	10	15	Ref	Ref
In the month before pregnancy or first trimester	55	47	1.4	1.7 (0.6, 4.6)	31	25	2.0	1.9 (0.4, 8.4)
In second or third trimester	23	24	1.3	1.4 (0.4, 4.4)	15	12	1.7	3.1 (0.7, 14.0)
Who applied the pesticides								
None used	19	20	Ref	Ref	10	15	Ref	Ref
Mother	44	44	1.1	1.7 (0.6, 4.7)	24	19	2.0	2.5 (0.6, 10.7)
Father or other	35	29	1.4	2.4 (0.8, 7.1)	22	18	1.7	2.4 (0.5, 10.4)
Frequency of pesticide use								
None used	20	21	Ref	Ref	10	15	Ref	Ref
Most days or 1–3 times a week	15	19	1.0	1.5 (0.5, 5.2)	12	7	1.0	1.5 (0.5, 5.2)
1–3 times a month, less than 1 time a month, or once or twice only	63	53	1.3	1.8 (0.7, 4.8)	34	30	1.3	1.8 (0.7, 4.8)

CI = confidence interval; OR = odds ratio; Ref = reference group.

^aAdjusted for mother's race, mother's age, mother's work status, and whether the father was living at home at start of pregnancy.

^bOther types of pesticides include liquids, granules, foggers, powders, baits, and traps.

involving 13 and 15 studies, respectively, estimated positive associations between childhood leukemia and prenatal residential pesticide exposures in the pooled analyses.^{15,30} Studies on pesticides and other child cancers are much rarer. The embryonal central nervous system (CNS) tumors, and the sympathetic nervous system tumors such as neuroblastoma, share with retinoblastomas a common origin in the germinal neuroectodermal layer.^{1,31} A relatively large recent study from 10 pediatric oncology centers in Australia found associations between childhood CNS cancer and preconception and early pregnancy exposure to home treatment with pesticides, and paternal occupational pesticide exposure.³² However, a meta-analysis of neuroblastoma and paternal occupational exposure based on 7 case-control and 2 cohort studies did not report positive associations.³³ Overall, there is a growing body of evidence that prenatal or preconceptional pesticide exposure contributes to some childhood cancers, including those of developmental origin.

Although we do not have a record of actual types of pesticides used by professional landscapers, an exposure type related to increased risk in our study, it is likely that such services typically use herbicides applied widely around

the home. In 2007, the most commonly applied pesticides in US homes and gardens were 2,4-D, glyphosate, carbaryl, mecoprop (MCPP), and pendimethalin.³⁴ Also related to increased risk in our study was the use of a "Product to kill insects or diseases inside or outside the home, such as Raid." Home-use products frequently contain mixtures of 2 or more active substances. Although we were unable to examine specific types or classes of pesticides, most subjects who used home pesticides were likely exposed to more than 1 toxic agent during sensitive developmental periods, and further research is needed to identify whether and how the mixture of these substances may be involved in the development of retinoblastoma.

Previous studies have described retinoblastoma incidence with 1 postconception hit for bilateral cases and 2 such hits for unilateral cases.³⁵ Children who inherit 1 defective copy of the *RBI* gene have an increased susceptibility to develop retinoblastoma through inactivation of the second allele (approximately 90%).³⁶ It is suggested that the *RBI* gene inactivation occurs during DNA replication in proliferating retinal progenitor cells and retinal progenitor cell proliferation occurs only in the fetal retina.^{37,38} Although genetic alterations to both copies of the *RBI* gene have been

shown to be necessary to induce retinoblastoma, it is still unclear which type and location of mutational events may be required for tumorigenesis.³⁹ Thus, this etiology is consistent with elevated risks we estimated related to prenatal pesticide exposure for unilateral cases acting as a “second hit.” Overall our findings for use of pesticides and unilateral vs bilateral retinoblastoma in children are consistent with the underlying postulated etiology.

Friend controls are likely to have been more similar to cases on many factors that relate to socioeconomic status, including race and income. A previous study by our group observed a greater number of concordant case-control sets than would be randomly expected for demographic characteristics such as race/ethnicity, education, income, and paternal age.⁴⁰ However, this relationship was not observed when reviewing potential exposures of interest, suggesting that using friend controls may not have resulted in over-matching for several exposures, although it likely provides cases and controls that are more closely matched on possible covariates, reducing confounding. To further mitigate the effects of confounding bias attributable to maternal race, we adjusted for this in all of our models.

Although for this very rare childhood cancer ours was a large study, the numbers in the different categories are small, which is a limitation of the study and may lead to unstable estimates. An additional limitation of our study is the possibility of recall errors. In retrospective studies in which mothers are asked about environmental exposures during or prior to pregnancy, it is possible that case mothers

over-report exposures or that control mothers under-report exposures.⁴¹ Yet the reported frequency of pesticide use was similar to those reported in population-based studies,³⁴ particularly those asking about prenatal or early childhood application frequency.⁴² The study design did not allow for the collection of biological samples or water tests to examine the presence of chemical metabolites in mothers or children. Ideally, we would like to gather prospective data on pesticide use and other potential risk factors, along with toxicologic samples, at multiple time points within a cohort study; however, for rare diseases such as retinoblastoma in children, this design is not feasible.

In conclusion, the observed increase in unilateral retinoblastoma associated with prenatal pesticide use around the home during pregnancy and the suggestion of several pesticide exposures contributing to bilateral cases adds to the body of evidence that suggests that pesticide use during critical prenatal or preconceptional time periods increases risks for these rare childhood cancers. There are few case-control studies on retinoblastoma to date worldwide, and our results require replication in other populations. More detailed information on types of pesticides in the home and from other sources such as diets would be desirable in future studies. Although retinoblastoma is a very rare disease, for affected individuals and families the implications are devastating; thus it is important to identify preventable risk factors. Our findings indicate that there is a need to raise awareness of the implications of home use of pesticides during pregnancy.

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