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Maternal residential pesticide use and risk of childhood leukemia in Costa Rica

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Evidence suggests that early-life exposure to pesticides inside the home may be associated with childhood leukemia, however data from Latin American countries are limited. We examined whether self-reported maternal residential pesticide use and nearby pesticide applications–before and after child's birth–were associated with acute lymphoblastic leukemia (ALL) in the Costa Rican Childhood Leukemia Study (CRCLS), a population-based case-control study (2001-2003). Cases (n = 251 ALL) were diagnosed between 1995 and 2000 (age <15 years at diagnosis) and were identified through the Costa Rican Cancer Registry and National Children's Hospital. Population controls (n = 577) were drawn from the National Birth Registry. We fitted unconditional logistic regression models adjusted for child sex, birth year, and socioeconomic status to estimate the exposure-outcome associations and also stratified by child sex. We observed that self-reported maternal insecticide use inside the home in the year before pregnancy, during pregnancy, and while breastfeeding was associated with increased odds of ALL among boys [adjusted Odds Ratio (aOR) = 1.63 (95% confidence interval [95% CI]: 1.05–2.53), 1.75 (1.13–2.73), and 1.75 (1.12–2.73), respectively. We also found evidence of exposure-response relationships between more frequent maternal insecticide use inside the home and increased odds of ALL among boys and girls combined. Maternal report of pesticide applications on farms or companies near the home during pregnancy and at any time period were also associated with ALL. Our study in Costa Rica highlights the need for education to minimize pesticide exposures inside and around the home, particularly during pregnancy and breastfeeding.

Key words: pesticides, leukemia, childhood cancer, Costa Rica

Abbreviations: AL: acute leukemia; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CI: confidence interval; CLIC: Childhood Leukemia International Consortium; CRCLS: Costa Rican Childhood Leukemia Study; DDT: dichlorodiphenyltrichloroethane; OP: organophosphate; OR: odds ratio

Additional Supporting Information may be found in the online version of this article.

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Introduction

Childhood leukemia accounts for about 30% of all cancers in children under the age of 15 years, and its most common subtype, acute lymphoblastic leukemia (ALL), comprises approximately 75–80% of the cases.[1] Current research, predominantly conducted in the United States and Europe, suggests that exposure to environmental toxicants, such as pesticides, may increase the risk of leukemia in children.[2]

To date, multiple studies have examined the association between early-life residential pesticide exposure and childhood leukemia with mostly consistent results.[3–14] For example, in a pooled analysis of 12 case-control studies from the Childhood Leukemia International Consortium (CLIC), increased odds of ALL were observed among children who had any pesticide exposure in their homes shortly before conception, during pregnancy, or after birth [odds ratio (OR) = 1.39 (95% Confidence Interval (95% CI): 1.25–1.55), 1.43 (95% CI: 1.32–1.54), and 1.36 (95% CI: 1.23–1.61), respectively].[15] Similarly, a study in Brazil, which is the only case-control study to assess the association between residential pesticide exposure and childhood leukemia in a Latin American country to date, found that self-reported maternal contact with pesticides during pregnancy was associated with increased odds of ALL [OR = 2.10 (95% CI:

What's new?

Costa Rica has one of the highest incidence rates of childhood leukemia worldwide. Pesticide use is widespread there, raising questions about whether pesticide exposure is in part responsible for the country's elevated childhood leukemia incidence. Here, acute lymphoblastic leukemia (ALL) in Costa Rican boys was associated with maternal insecticide use in the home as well as with pesticide spraying on nearby farms before and after the child's birth. Among both boys and girls, ALL risk increased in association with frequency of maternal in-home insecticide use. The results offer insight into possible areas of intervention to reduce childhood leukemia risk.

1.14–3.86)] and acute myeloid leukemia (AML) [OR = 5.01 (95% CI: 1.97–12.7)] in infants (aged 0–11 months).[7] In addition, several studies, including the one conducted in Brazil, have reported exposure-response relationships between residential insecticide exposure and risk of childhood leukemia.[3,4,6,7]

Given the findings from previous studies, it is particularly important to understand the association between pesticide exposure and childhood leukemia in Costa Rica, a tropical country with extensive pesticide use.[16,17] Costa Rica is also one of the countries with the highest incidences of childhood leukemia in the world.[18] For example, between 2001 and 2010, the age-standardized incidence rate of childhood leukemia in Central America and the Caribbean was 45.5 cases per million children per year,[19] whereas the incidence rate in Costa Rica was 56.5 cases per million children in 2008.[20] The latter rate is consistent with the incidence rate observed among Hispanic children in the United States from 2006 to 2010 (59.6 cases per million per year),[21] but is higher than rates found among children from other ethnicities in the United States (ranging from 29.9 to 46.9 cases per million).[21]

In order to better understand the association of early-life exposure to environmental toxicants with the risk of leukemia in Costa Rican children, the Costa Rican Childhood Leukemia Study (CRCLS), a nationwide case-control study, was conducted between 2001 and 2003. Previous analyses from our study have shown associations between parental occupational pesticide exposure and an increased risk of childhood leukemia.[22] In the present analyses, we examined whether self-reported maternal pesticide use inside the home and maternal report of nearby pesticide applications before and after the child's birth were associated with ALL in Costa Rican children.

Methods

Study population

Subject recruitment and procedures for the CRCLS have been described elsewhere.[22] Briefly, all cases of childhood leukemia (ages 0–14 years at diagnosis, $n = 334$) diagnosed between 1995 and 2000 in Costa Rica were identified using information from the Costa Rican Cancer Registry and the National Children's Hospital. Consent to participate in CRCLS was obtained from 90% of eligible cases. Population controls were randomly selected from the National Birth Registry and frequency-matched to cases by birth year. We used information reported by the mother at the time of birth to identify addresses of controls. Because addresses in the National Birth Registry were sometimes restricted to neighborhoods, we also used national electoral databases and local social security clinics to ascertain the exact address of 62% of potential controls. If the control could not be located (either because the exact address was not identified or no one was available at the time of the home visit) or the control refused to participate, we randomly selected a new control of the same age living in the same neighborhood as the originally selected control. Among eligible controls, 91% consented to participate. A total of 300 children with leukemia (252 ALL cases) and 579 healthy controls were enrolled in CRCLS. Once consent was obtained from at least one parent of the child enrolled in the study, we conducted an extensive face-to-face interview with the mother and, when available, the father. The interview included the administration of three questionnaires: one about the mother, one about the father, and one about the child (frequently completed by the mother, and occasionally by the father or a caregiver). Due to the number of participants missing paternal questionnaires (13%) and the etiological differences between ALL and other types of leukemia, we restricted the present analyses to data from maternal questionnaires and included only cases diagnosed with ALL. After excluding two controls with missing maternal exposure data and one case who was missing the date of diagnosis, a total of 251 ALL cases and 577 controls were included in our analyses.

The Ethical Committees of the National Children's Hospital, Ministry of Health of Costa Rica, and Karolinska Institutet in Sweden approved all study materials and procedures. Informed consent was obtained from at least one parent or legal representative for each participant before their data collection began.

Exposure assessment

We collected information on socio-demographic and lifestyle characteristics (e.g., parental age, education, smoking habits, dietary intake, and household income), known or suspected risk factors for childhood leukemia (e.g., X-ray exposure, family history of leukemia, and birth defects), parental medical history, and parental occupational and residential exposure to pesticides and other environmental toxicants. More specifically on residential pesticide exposure, we asked mothers about their home use of pesticides from the following groups: (*i*) insecticides, (*ii*) herbicides, (*iii*) fungicides, and (*iv*) commercial/professional pest control treatments, as well

as exposure to (*v*) pesticides sprayed by the Ministry of Health for vector control, and (*iv*) pesticides sprayed on farms or companies near the home. We also asked mothers about the frequency (i.e., daily, several times per week, weekly, several times per month, monthly, every three months, several times per year, once per year, less than once per year) and periods of their pesticide use inside the home [i.e., in the year prior to pregnancy, during pregnancy, during breastfeeding, <1 year, 1–2 years, 2–4 years, ≥5 years but up until leukemia diagnosis for cases and either the interview date or age of 15 years for controls (whichever occurred first), and always].

Within each period of potential exposure, we estimated in a standardized way the average frequency of exposure in days per year in order to account for the differences in length of the exposure period (e.g., the year before pregnancy had a length of 12 months and pregnancy had a length of ∼9 months) and variation among study participants (e.g., the length of the period after birth varied based on the censoring date). We estimated the frequency of pesticide use across periods of exposure by (*i*) converting each of the individual pesticides that mothers reported using to days per year and then (*ii*) calculating the average frequency of pesticide use (in days per year) for the five exposure periods (i.e., the year before pregnancy, pregnancy, while breastfeeding, after birth, and at any time) for each of the six pesticide groups. For instance, values of 365 were assigned to pesticides used "daily", 208.5 to "several times per week", 52 to "weekly", 32 to "several times per month", 12 to "monthly", 2.5 to "several times per year", 1 to "once per year", and 0 to "less than once per year" or "no use". Values for categories "several times per week", "several times per month", "several times per year", and "less than once per year" were estimated by averaging the values of the categories above and below [e.g., the average of 208.5 days per for "several times per week" was estimated by averaging 365 (value for pesticides used daily) and 52 (value for pesticides used weekly)].

Individuals missing data on the time period in which they were potentially exposed a pesticide were excluded from multivariate analyses for that pesticide group (e.g., 1 case and 1 control for insecticides; 13 cases and 8 controls for pesticides sprayed on nearby farms or companies). Missing values for frequency of maternal use of a pesticide during any exposure period were imputed using the participant's average frequency of exposure for that pesticide group from other exposure periods (when available) or the frequency most commonly reported by other participants for that pesticide group at any exposure period (i.e., one time per year for herbicides, professional fumigation, and fumigation for vector control; 2.5 times per year for insecticides, fungicides, and pesticides sprayed on nearby farms or companies).

Statistical analysis

We estimated bivariate associations between the exposures, outcome, and covariates using t-tests for continuous variables and χ^2 tests for categorical variables. We used Spearman correlation coefficients (r_s) to examine whether the average frequency of use of one pesticide group was correlated with (*i*) average frequency of use of other pesticide groups and (*ii*) average frequency of use of that pesticide group at other time periods. We examined exposure-outcome associations using unconditional logistic regression models to estimate ORs and their 95% CIs. We ran separate regression models for each exposure period and three pesticide groups: herbicides, insecticides, and spraying on farms or companies near the home. We did not fit multivariate regression models for the remaining pesticide groups (i.e., fungicides, pesticides used for professional fumigation, and pesticides sprayed for vector control by the Ministry of Health) due to the small number of mothers of cases and controls who reported these exposures. We adjusted our models for birth year (matching variable at enrollment), and child sex and socioeconomic status [two variables identified *a priori* that were associated with at least one of the pesticide groups and the outcome of interest in bivariate analyses ($p < 0.20$)]. Covariates with missing information (i.e., socioeconomic status) were randomly imputed based on observed probability distributions.

Because the average frequency of use for most pesticide groups was low, exposure to each of the groups was dichotomized as none vs. any use. Insecticide use was reported frequently enough to create three categories: low [no use and average use up to 2.5 times per year (which was the median for most exposure periods)], medium [average use between 2.5 and 36 times per year (the latter represented the 75th percentile for most exposure periods)], and high (average use >36 times per year). We examined whether child sex modified the exposure-outcome associations using a product interaction term between child sex and each of the dichotomized exposures and also stratifying by sex. We did not examine sex differences in associations of average frequency of insecticide use (three categories) and childhood leukemia due to our relatively small sample size.

We conducted sensitivity analyses to assess the robustness of our results: (*i*) we fitted our regression models using socioeconomic status variables imputed in two different ways: by substituting the missing observations with medium socioeconomic status (the most frequent category for both cases and controls) and by creating a new category for the missing values; (*ii*) we adjusted our models for parental education (highest combined level) instead of socioeconomic status [because we had imputed a relatively large percentage of socioeconomic status missing values (∼20%)]; (*iii*) we adjusted our regression models for additional potential confounders or strong predictors of ALL [i.e., maternal smoking and alcohol consumption during pregnancy, birth order, birth weight, and breastfeeding] to examine their impact on effect estimates (referred to henceforth as fully-adjusted models); (*iv*) we included all 299 leukemia cases enrolled in the CRCLS (not just ALL cases) in the unconditional logistic regression models; (*v*) we assessed the independent effect of multiple

exposures by adjusting for exposure to insecticides, herbicides, and pesticides sprayed on farms or companies near the home in the same model; and (*vi*) we examined the potential impact of the mother being home during the day when agricultural pesticide spraying would be more common by adjusting for parity (1 vs. >1 pregnancies) in models including child sex, year of birth, and socioeconomic status.

Results

Study population characteristics

ALL was the most common type of leukemia in the CRCLS, accounting for nearly 85% of the cases (AML and other types = 10% and 5% of cases, respectively). ALL cases and controls were similar on most attributes, including maternal age at child's birth, parental education, maternal smoking and alcohol consumption during pregnancy, child sex, and birth order (Table 1). Cases were more likely to have been born between 1991 and 1995 (50%) than controls (42%) and to have a lower socioeconomic status (18%) than controls (14%). The average time period between age of diagnosis (censoring date) and age of interview for the cases was 3.9 years. The average age at the time of interview for controls was 11 years.

Insecticides were the pesticides most frequently reported to have been used by mothers inside their homes (between 58% and 84% of participants reported using them across all five exposure periods), whereas fungicide use, professional fumigation, and fumigation for vector control were relatively rare (<10% exposed in at least one period of exposure; Table 2). Self-reported maternal insecticide and herbicide use was relatively similar among cases and controls for all exposure periods. More mothers of cases than controls reported living near farms or companies that sprayed pesticides in all periods of exposure (39% vs. 31%, $p = 0.01$ at any time period), while more mothers of controls reported living in areas sprayed with pesticides for vector control than mothers of cases after birth (32% vs. 22%, $p < 0.01$) and at any time period (33% vs. 24%, $p < 0.01$; Table 2). Average frequencies of maternal use within a specific pesticide group were highly correlated throughout the different exposure periods ($r_s = 0.61$–0.99); however, average frequencies of use were either weakly or not at all correlated between pesticide groups (see Supporting Information Table 1). For instance, the strongest correlations between pesticide groups were observed for herbicides and spraying on farms or companies near the home ($r_s = 0.24$–0.30).

Associations between maternal pesticide use inside the home (none vs. any) and childhood ALL

Self-reported maternal insecticide use in the year before pregnancy, during pregnancy, and while breastfeeding was associated with increased odds of ALL among boys [aOR = 1.63 (95% CI: 1.05–2.53), 1.75 (95% CI: 1.13–2.73), and 1.75 (95% CI: 1.12–2.73), respectively, all p-int < 0.20; Table 3]. In contrast, we observed that maternal insecticide use after birth and at any time period were associated with small decreases in odds of ALL among girls [aOR = 0.69 (0.39–1.23) and 0.66 (0.37–1.17), respectively, all p-int > 0.20]. Maternal herbicide use inside the home was not associated with childhood ALL in combined and sex-stratified analyses (Table 3).

Maternal report of pesticides sprayed on farms or companies near the home was also associated with childhood ALL. For example, maternal report of these pesticide applications during pregnancy, while breastfeeding, and during any time period was associated with increased odds of leukemia in combined analyses of boys and girls [aOR = 1.43 (95% CI: 1.00–2.05), 1.41 (95% CI: 0.98–2.03) and 1.52 (95% CI: 1.11–2.09), respectively; Table 3]. In sex-stratified analyses, we observed that these exposure-outcome associations were stronger among boys than among girls, but sex differences were not statistically significant (all p-int > 0.20).

Associations between average frequency of maternal insecticide use inside the home (low, medium, high) and childhood ALL

Children whose mothers reported a high average frequency of insecticide use inside their homes (>36 times/year) in the year before pregnancy, during pregnancy, and while breastfeeding had higher odds of ALL compared to children whose mothers reported a low frequency of insecticide use (<2.5 times/year) during these exposure periods [aOR = 1.56 (95% CI: 1.07–2.27), 1.58 (95% CI: 1.08–2.31), and 1.56 (95% CI: 1.07–2.29), respectively; all p-trend < 0.05; Table 4].

Sensitivity analyses

Effect estimates did not change appreciably after (*i*) using different methods of imputing/handling missing observations for socioeconomic status, (*ii*) adjusting for parental education instead of socioeconomic status, or (*iii*) adjusting regression models for additional potential confounders or strong predictors of the outcome (fully-adjusted models; data not shown). Notably, associations between average frequency of insecticide use (low, medium, high) and childhood leukemia were slightly weaker when we included all leukemia cases ($n = 299$) in the adjusted models, as compared to analyses with ALL cases only (see Supporting Information Table S2). Results did not change appreciably when we stratified by socioeconomic status or year of birth (See Supplementary Information Tables S3 and S4, respectively). Associations between maternal residential pesticide use (none vs. any) and childhood leukemia did not change when we included all leukemia cases (data not shown). In general, effect estimates were slightly lower when we assessed co-pollutant confounding by adjusting for exposure to insecticides, herbicides, and pesticide spraying on farms or companies near home in the same models. Importantly, associations highlighted previously remained in the same direction (data not shown). Because parity was not associated with the outcome ($p = 0.88$) and effect estimates did not change appreciably when the variable was included in the models (See Supplementary Information

Table 1. Socio-demographic characteristics of ALL cases (n = 251) and controls (n = 577) from the Costa Rican Childhood Leukemia Study (CRCLS), 2001–2003

	Cases n (%)	Controls n (%)	p^1
Child characteristics			
Age at diagnosis (years)			
<1	10 (4.0)	–	
1–4	115 (45.8)	–	
5–9	83 (33.1)	–	
10–15	43 (17.1)	–	
Year of birth			
1979–1985	22 (8.8)	83 (14.4)	0.06
1986–1990	62 (24.7)	158 (27.4)	
1991–1995	125 (49.8)	240 (41.6)	
1996–2000	42 (16.7)	96 (16.6)	
Sex			
Boy	137 (54.6)	283 (49.0)	0.14
Girl	114 (45.4)	294 (51.0)	
Birth order[2]			
1st	73 (29.1)	177 (30.7)	0.86
2nd	67 (26.7)	145 (25.1)	
3rd or more	111 (44.2)	255 (44.2)	
Birth weight (grams)[2,3]			
<2,500	18 (7.2)	36 (6.2)	0.30
≥2,500	233 (92.8)	541 (93.8)	
Breastfeeding[2]			
<6 months	113 (45.0)	265 (46.0)	0.60
≥6 months	138 (55.0)	312 (54.0)	
Parental/household characteristics			
Maternal age at delivery (years)[2,3]			
<25	125 (49.8)	256 (44.4)	0.43
25–29	63 (25.1)	150 (26.0)	
30–34	37 (14.7)	104 (18.0)	
≥35	26 (10.4)	67 (11.6)	
Parity[2]			
1 pregnancy	13 (5.20)	35 (6.07)	0.88
>1 pregnancy	237 (94.80)	542 (93.93)	
Parental education[2]			
≤6th grade	95 (37.8)	239 (41.4)	0.58
>6–11th grade	67 (26.7)	139 (24.1)	
High school completed	89 (35.5)	199 (34.5)	
Socioeconomic status[2,4]			
Low	44 (17.5)	80 (13.9)	0.08
Medium	175 (69.7)	444 (76.9)	
High	32 (12.8)	53 (9.2)	
Maternal smoking during pregnancy[2]			
No	241 (96.0)	554 (96.0)	0.99
Yes	10 (4.0)	23 (4.0)	
Maternal alcohol consumption during pregnancy[2]			
No	240 (96.0)	547 (95.0)	0.62
Yes	11 (4.0)	30 (5.0)	

[1]p Value for χ^2 test.
[2]Missing data before simple random imputation: 2 cases (0.8%) for birth order, 48 cases (19.1%) and 82 controls (14.2%) for birth weight, 46 cases (18.3%) and 89 controls (15.4%) for breastfeeding, 1 Case (0.4%) and 4 controls (0.7%) for maternal age at delivery, 1 Case (0.4%) for parity, 55 cases (21.9%) and 107 controls (18.5%) for SES, 4 cases (1.6%) and 6 controls (1.0%) for maternal smoking during pregnancy, and 5 cases (2.0%) and 4 controls (0.7%) for maternal alcohol consumption during pregnancy.
[3]Modeled as continuous variables in multivariate analyses.
[4]Socioeconomic status assessed by interviewers based on house materials, road material, type of neighborhood, and electronics in house.

Table S5), we decided to maintain our original covariate selection process and did not include parity in the final models.

Discussion

In this case-control study of Costa Rican children, we found that maternal report of insecticide use inside the home before birth and during breastfeeding was associated with increased odds of childhood ALL among boys; this increased risk was more evident among children whose mothers reported using insecticides more frequently. Maternal report of pesticide spraying on farms or companies near the home before and after the child's birth was also associated with increased odds of childhood ALL among boys and girls combined. A slight reduction in odds of childhood ALL was found among girls who were exposed to insecticides and herbicides used inside their homes and to pesticides that had been sprayed on farms or businesses near their homes at different periods of exposure.

Results from our study are largely consistent with previous studies indicating that home insecticide use during pregnancy may be associated with an increased risk of childhood leukemia.[3,4,6,7,15] For instance, in a CLIC pooled analysis of 12 case-control studies, household use of insecticides or miticides within 3 months of conception, during pregnancy, and after birth was associated with increased odds of ALL [pooled OR = 1.34 (95% CI: 1.19–1.51), 1.28 (95% CI: 1.18–1.38), and 1.23 (95% CI: 1.12–1.34), respectively].[15]

In our study, we also observed that children whose mothers reported using insecticides more frequently while pregnant or while breastfeeding had higher odds of ALL than children whose mothers reported using these pesticides less frequently. This is consistent with previous studies showing exposure-response relationships between the frequency of insecticide exposure and risk of childhood leukemia.[3,5,7,11] It

Table 2. Residential pesticide use for ALL cases (n = 251) and controls (n = 577) by period of exposure, Costa Rican Leukemia Study (CRCLS), 2001–2003

	Cases n (%)	Controls n (%)	p^1
Insecticides			
Year before pregnancy	160 (63.7)	338 (58.6)	0.30
Pregnancy	160 (63.7)	335 (58.1)	0.24
Breastfeeding	166 (66.1)	341 (59.1)	0.12
After birth[3]	203 (80.9)	481 (83.4)	0.60
Any time period	206 (82.1)	486 (84.3)	0.44
Herbicides[2]			
Year before pregnancy	44 (17.5)	73 (12.7)	0.12
Pregnancy	42 (16.7)	72 (12.5)	0.18
Breastfeeding	41 (16.3)	75 (13.0)	0.30
After birth[3]	61 (24.3)	145 (25.1)	0.68
Any time period	68 (27.1)	147 (25.5)	0.63
Fungicides			
Year before pregnancy	2 (0.8)	7 (1.2)	0.28
Pregnancy	2 (0.8)	7 (1.2)	0.28
Breastfeeding	2 (0.8)	8 (1.4)	0.25
After birth[3]	7 (2.8)	29 (5.0)	0.11
Any time period	7 (2.8)	29 (5.0)	0.15
Professional fumigation[2]			
Year before pregnancy	8 (3.2)	5 (0.9)	0.01
Pregnancy	6 (2.4)	3 (0.5)	0.02
Breastfeeding	7 (2.8)	4 (0.7)	0.02
After birth[3]	22 (8.8)	35 (6.1)	0.16
Any time period	24 (9.6)	36 (6.2)	0.09
Fumigation for vector control[2]			
Year before pregnancy	17 (6.8)	40 (6.9)	0.32
Pregnancy	18 (7.2)	32 (5.5)	0.21
Breastfeeding	17 (6.8)	35 (6.1)	0.29
After birth[3]	54 (21.5)	186 (32.2)	<0.01
Any time period	59 (23.5)	192 (33.3)	<0.01
Spraying on farm or company near the home[2]			
Year before pregnancy	60 (23.9)	118 (20.7)	0.27
Pregnancy	62 (24.7)	113 (19.8)	0.10
Breastfeeding	61 (24.3)	113 (19.8)	0.10
After birth[3]	79 (31.5)	160 (28.0)	0.27
Any time period	98 (39.0)	174 (30.5)	0.01

[1] p Value for χ^2 test.
[2] Missing data for frequency of use before imputation: 4 cases (1.6%) and 5 controls (0.9%) for herbicides, 3 controls (0.5%) for professional fumigation, 1 Case (0.4%) for fumigation for vector controls, and 6 cases (2.4%) and 12 controls (2.1%) for spraying on farm or company near the home.
[3] Breastfeeding is included in the period after birth.

is important to consider that, while the use of insecticides inside and around the home has helped reduce mortality and morbidity due to common vector-borne diseases such as dengue and malaria in Costa Rica and other tropical countries,[23,24] results from the current study highlight the importance of developing alternative strategies to mitigate the risk of these diseases, such as integrated pest management.

Our findings that exposure to pesticides sprayed on farms or companies near the home may be associated with higher odds of ALL are consistent with other studies that have examined associations between residential proximity to agricultural pesticide applications and childhood leukemia and other childhood cancers.[7,25–28] Notably, previous studies have used exposure assessment methods such as geographic information systems to map pesticide use near the homes,[25] analysis of pesticide use registry data,[26] measurement of pesticide residues in home dust samples,[27] or assessment of parental report of living in an agricultural area with pesticide use.[7] Associations between exposure to agricultural pesticides sprayed near the home and childhood leukemia could potentially be explained by the fact that, compared to children living in non-agricultural areas, children living near farms may be disproportionately exposed to pesticides used in agriculture[26,29] through pathways including pesticide drift and the take-home exposure pathway.[30–33]

In the present study, we did not observe increased odds of leukemia among children whose mothers reported using herbicides inside the home at any time period; however, some other studies have found modest associations for this pesticide group.[6,15] These studies also dichotomized herbicide exposures (i.e., none vs. any use), however, it is possible that there are inconsistencies across the studies due to differences in the types of herbicides used in different countries and the frequencies of herbicide use in the different exposure periods.

One of the most significant findings from our study is that use of some pesticide groups during breastfeeding may increase the risk of childhood ALL among boys. These findings are consistent with the only other study that has examined associations between residential pesticide exposure during breastfeeding and childhood leukemia.[7] However, our results do not show that breastmilk itself contains pesticide residues and an alternative interpretation is that the period of a child's life when breastmilk is consumed may be a time of particular susceptibility to pesticides and other environmental toxins. Breastmilk is an important nutrition source for infants[34] and breastfeeding has been associated with numerous health benefits,[35] including a reduced risk of childhood acute leukemia among breastfed infants,[36] and should never be discouraged. Practices and policies aimed at minimizing the use of pesticides while breastfeeding and during early childhood are warranted to protect children's health.

Understanding the role of residential pesticide exposure in childhood leukemia and other cancers can be difficult because children may be exposed to a mixture of pesticides with different toxicities and modes of application, and also through varying sources and routes of exposure.[37] While challenges exist in collecting sufficient toxicological and epidemiological evidence to evaluate the carcinogenicity of

Table 3. Adjusted[1] associations [aOR (95% CI)] of maternal report of pesticide exposure (none vs. any) with childhood acute lymphocytic leukemia (ALL) by exposure period for all children and stratified by child sex, Costa Rican Childhood Leukemia Study (CRCLS), 2001–2003 (n = 251 cases and 577 controls)

	Year before pregnancy		Pregnancy		Breastfeeding		After birth[2]		Any time period	
	Exposed cases/ controls (n)	OR (95% CI)	Exposed cases/ controls (n)	OR (95% CI)	Exposed cases/ controls (n)	OR (95% CI)	Exposed cases/ controls (n)	OR (95% CI)	Exposed cases/ controls (n)	OR (95% CI)
Insecticides										
All	160/338	1.28 (0.94–1.75)	160/335	1.31 (0.96 1.80)	166/341	1.36 (0.99–1.86)	203/481	0.84 (0.57–1.24)	206/486	0.84 (0.57–1.26)
Boys	91/159	1.63 (1.05–2.53)	92/157	1.75 (1.13 2.73)	96/163	1.75 (1.12–2.73)	112/231	0.97 (0.56–1.67)	114/233	1.03 (0.59–1.80)
Girls	69/179	1.01 (0.64–1.59)	68/178	0.99 (0.63 1.55)	70/178	1.06 (0.67–1.67)	91/250	0.69 (0.39–1.23)	92/253	0.66 (0.37–1.17)
		p-int = 0.17		p-int = 0.09		p-int = 0.13		p-int = 0.48		p-int = 0.44
Herbicides										
All	44/73	1.44 (0.95–2.17)	42/72	1.39 (0.91–2.12)	41/75	1.28 (0.84–1.96)	61/145	0.97 (0.68–1.38)	68/147	1.09 (0.78–1.54)
Boys	22/32	1.43 (0.78–2.63)	20/31	1.32 (0.71–2.46)	19/33	1.17 (0.63–2.19)	33/66	1.08 (0.66–1.77)	36/66	1.21 (0.74–1.96)
Girls	22/41	1.52 (0.85–2.73)	22/41	1.51 (0.85–2.71)	22/42	1.47 (0.82–2.62)	28/79	0.91 (0.55–1.52)	32/81	1.04 (0.64–1.70)
		p-int = 0.84		p-int = 0.99		p-int = 0.85		p-int = 0.51		p-int = 0.61
Spraying on farm/company near the home										
All	60/118	1.29 (0.90–1.85)	62/113	1.43 (1.00–2.05)	61/113	1.41 (0.98–2.03)	79/160	1.32 (0.95–1.84)	98/174	1.52 (1.11–2.09)
Boys	39/63	1.54 (0.95–2.50)	40/61	1.67 (1.03–2.71)	39/60	1.64 (1.01–2.67)	49/85	1.47 (0.93–2.31)	61/92	1.74 (1.13–2.69)
Girls	21/55	0.99 (0.57–1.75)	22/52	1.15 (0.66–2.01)	22/53	1.13 (0.64–1.98)	30/75	1.11 (0.67–1.84)	37/82	1.28 (0.79–2.05)
		p-int = 0.24		p-int = 0.32		p-int = 0.33		p-int = 0.43		p-int = 0.21

[1]Adjusted for child sex (only in models for boys and girls combined), year of birth, and socioeconomic status.
[2]Breastfeeding is included in the period after birth.

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Table 4. Adjusted[1] associations [aOR (95% CI)] of maternal report of frequency of residential insecticide use[2] with acute lymphocytic leukemia (ALL) by exposure period, Costa Rican Childhood Leukemia Study (CRCLS), 2001–2003 (n = 251 cases and 577 controls)

Frequency of use and exposure period	Exposed cases/ controls (n)	OR (95% CI)	p^3
Year before pregnancy			
Low	132/340	Reference	0.03
Medium	56/131	1.12 (0.77–1.63)	
High	62/105	1.56 (1.07–2.27)	
Pregnancy			
Low	134/341	Reference	0.03
Medium	55/134	1.05 (0.72–1.53)	
High	61/101	1.58 (1.08–2.31)	
Breastfeeding			
Low	129/337	Reference	0.02
Medium	60/136	1.16 (0.80–1.68)	
High	61/103	1.56 (1.07–2.29)	
After birth[4]			
Low	101/237	Reference	0.75
Medium	72/168	1.02 (0.71–1.47)	
High	77/171	1.06 (0.74–1.52)	
Any time period			
Low	98/235	Reference	0.66
Medium	74/167	1.08 (0.75–1.56)	
High	78/174	1.08 (0.75–1.55)	

[1]Adjusted for child sex, year of birth, and socioeconomic status.
[2]Low <2.5 times/year; medium 2.5–36 times/year; high >36 times/year.
[3]p Value from test of trend.
[4]Breastfeeding is included in after birth period.

exposure to specific pesticides and combinations of pesticides, multiple organophosphates (OPs) and pyrethroids commonly used in agriculture and inside the homes (e.g., glyphosate,[38] malathion,[38] parathion,[38] permethrin,[39] tetramethrin[39]) have been classified as possible or probable human carcinogens. There is also evidence that pesticides, including dichlorodiphenyltrichloroethane (DDT), pyrethroids, and chlorinated pesticides, may play a role in the development of childhood ALL through the deregulation of the immune system.[40,41] While we only examined broad pesticide classes in our exposure-outcome analyses, it is important to highlight that most of the mothers who reported using insecticides inside their homes described applying them in the form of sprays (which contain predominantly pyrethroids and/or OPs) and coils [which contain pyrethroids and other carcinogenic agents such as polycyclic aromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs)].[42,43] Future studies may consider investigating the effects of specific pesticides and active ingredients on childhood leukemia in order to minimize knowledge gaps regarding the potential carcinogenic action of different pesticide groups.

While child sex is an established risk factor for childhood leukemia,[44,45] this is the first study to our knowledge to examine sex differences in the association between residential pesticide exposure and childhood leukemia. Evidence suggests that childhood leukemia is a multi-step process, with initiation taking place *in utero* and progression of acute disease taking place after birth,[2] and our results highlight the need for more research investigating the mechanisms of *in utero* leukemia initiation in boys and girls. Further research may benefit from investigating gene-environment interactions and mechanisms of pesticide toxicity to elucidate sex differences in the etiology of childhood leukemia and also in incidence and survival rates.[46]

Our study has several limitations. First, it can be difficult to identify periods of etiologic importance, as maternal reports of use of a pesticide group were highly correlated across exposure periods (e.g., if insecticides were used in one time period, they were likely to be used in other time periods as well). Second, given that the exposure periods of interest occurred multiple years before the interviews, non-differential exposure misclassification may have occurred. Nevertheless, non-differential misclassification would have biased our effect estimates towards the null. Third, we cannot rule out recall bias, particularly for exposure to pesticide applications on farms or businesses near the home,[47] as our analyses relied on maternally-reported pesticide use. However, we have no evidence to believe that mothers' report would have varied by timing or amount of exposure or by child sex. Fourth, exposure assessment is a challenge in many case-control studies and the majority of investigations examining associations between residential pesticide exposure and childhood leukemia have relied on parental report of pesticide use. Our results are largely consistent with two hospital-based case-control studies in Shanghai that assessed maternal self-reported residential pesticide use as well as the analysis of spot urine samples for nonspecific dialkyl phosphate (DAP) metabolites of OP pesticides[5] and nonspecific metabolites of pyrethroid pesticides.[48] However, these metabolites are not specific to residential pesticide use and largely reflect recent dietary exposures, rather than exposure during any previous etiologic period.[49] Lastly, it is possible that the associations we observed could be partially attributable to residual confounding due to parental occupational pesticide exposure. Costa Rica has some of the highest rates of agricultural pesticide use in the world[16,17] and it is extremely difficult to disentangle the impacts of exposure to various pesticides via different pathways and sources of exposure, particularly for children living in agricultural areas.[33] Future studies should employ more comprehensive exposure assessment approaches to better account for the various sources and pathways contributing to children's pesticide exposures, including para-occupational exposures, residential applications, and diet.

Despite its limitations, our study contributes to a growing body of literature associating prenatal exposure to various environmental toxicants with childhood leukemia. This is the first study to examine the associations between home pesticide use and childhood leukemia in any country in Central America, including Costa Rica, a tropical country with extensive pesticide use and high incidence rates of childhood leukemia. It is also the first study to explore sex differences for this exposure-outcome association and the second study to investigate the association of residential pesticide exposure during breastfeeding with childhood leukemia.[7]

Conclusion

Our study sought to determine whether maternal pesticide use inside the home was associated with childhood leukemia in Costa Rica, and if factors such as period or frequency of exposure to different pesticide groups were associated with increased odds of leukemia in this population. Overall, our results are consistent with previous research suggesting that residential exposure to pesticides increases the risk of childhood leukemia. More specifically, we found that (*i*) maternal insecticide use inside the home and pesticide spraying in farms or companies near the home during pregnancy or during breastfeeding were associated with an increase in the risk of leukemia among boys; and (*ii*) there was a positive exposure-response relationship between frequency of insecticide use and risk of leukemia among boys and girls combined. Our study highlights the need for educational programs to inform parents about alternative methods for pest control inside and around the home, and restricting pesticide use during important periods of susceptibility, such as during pregnancy and while breastfeeding. While the use of pesticides in tropical countries like Costa Rica has been successful in reducing morbidity and mortality from vector-borne diseases, it is critical to promote safe use practices and non-chemical pest control methods to reduce the risk of leukemia and other diseases.

Authorship

CH participated in the data analyses and preparation and editing of the manuscript. RG, CM, and MB collaborated in the supervision of data analyses and editing of the manuscript. CW designed the study and participated in fieldwork supervision and editing of the manuscript. AMM collaborated in the supervision of data analyses and preparation and editing of the manuscript.

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