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Passive exposure to agricultural pesticides and risk of childhood leukemia in an Italian community

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ABSTRACT

Background: Exposure to pesticides has been suggested as a risk factor for childhood leukemia, but definitive evidence on this relation and the specific pesticides involved is still not clear.
Objective: We carried out a population-based case-control study in a Northern Italy community to assess the possible relation between passive exposure to agricultural pesticides and risk of acute childhood leukemia.
Methods: We assessed passive pesticide exposure of 111 childhood leukemia cases and 444 matched controls by determining density and type of agricultural land use within a 100-m radius buffer around children's homes. We focused on four common crop types, arable, orchard, vineyard and vegetable, characterized by the use of specific pesticides that are potentially involved in childhood induced leukemia. The use of these pesticides was validated within the present study. We computed the odds ratios (OR) of the disease and their 95% confidence intervals (CI) according to type and density of crops around the children's homes, also taking into account traffic pollution and high-voltage power line magnetic field exposure.
Results: Childhood leukemia risk did not increase in relation with any of the crop types with the exception of arable crops, characterized by the use of 2.4-D, MCPA, glyphosate, dicamba, triazine and cypermethrin. The very few children (n = 11) residing close to arable crops had an OR for childhood leukemia of 2.04 (95% CI 0.50–8.35), and such excess risk was further enhanced among children aged <5 years.
Conclusions: Despite the null association with most crop types and the statistical imprecision of the estimates, the increased leukemia risk among children residing close to arable crops indicates the need to further investigate the involvement in disease etiology of passive exposure to herbicides and pyrethroids, though such exposure is unlikely to play a role in the vast majority of cases.

1. Background

Leukemia is the most common cancer type in children, representing 30% of all childhood cancers (American Cancer Society, 2014). Childhood leukemia (CL) annual rates in Italy are 5/100,000 inhabitants, an incidence comparable to other Western countries (AIRTUM, 2012). The etiology of this disease unfortunately remains largely unknown, though epidemiological studies have identified a number of potential genetic and environmental risk factors. Pesticide exposure is among the latter, but despite several epidemiologic studies carried out during the latest three decades on this possible relation, definitive evidence on their involvement in CL onset is still lacking (Ntzani et al., 2013). Most investigations found an increased risk of disease associated with *in utero* (Menegaux et al.,

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2006) or postnatal (Ma et al., 2002) pesticide exposure through household use or parental occupational exposures (Wigle et al., 2009; Turner et al., 2010; Van Maele-Fabry et al., 2010, 2011; Ntzani et al., 2013; Bailey et al., 2015; Chen et al., 2015). Very few studies have investigated consequent pesticide exposure from over-spray or off-gassing of crops that are situated near resident homes (Lu et al., 2000; Curwin et al., 2005; Rull et al., 2009), despite the tendency of children spending more time outdoors, and increasing their exposure risk, when playing on the floor, ground, lawn and frequently putting hands and objects in their mouths (Freeman et al., 2005).

Under this perspective, crop density (*i.e.* the percentage of land planted with crops) around the residence may be a proxy for environmental exposure to agricultural pesticides (Walker et al., 2007; Carozza et al., 2008; Booth et al., 2015), as also suggested by the correlation between acreage crops nearby the house and pesticide concentrations in home dust (Lu et al., 2000; Fenske et al., 2002; Ward et al., 2006; Harnly et al., 2009; Gunier et al., 2011), or pesticide metabolites in biological samples (Loewenherz et al., 1997; Lu et al., 2000).

Several pesticide categories have been associated with CL risk, including insecticides such as organophosphate, organochlorine and pyrethroid, fumigants, herbicides and fungicides (Reynolds et al., 2002, 2005; Rudant et al., 2007; Rull et al., 2009; Ding et al., 2012). These compounds might favor CL onset through oxidative stress, genotoxicity, endocrine disruption, or cholinesterase inhibition (Perry and Soreq, 2004; Williams et al., 2004; Vidal, 2005; Phillips and Foster, 2008; Androutsopoulos et al., 2013; Hernandez et al., 2013; Mrema et al., 2013; Vakonaki et al., 2013; Choi and Joas, 2016; Hernandez and Menendez, 2016), effects to which children are more susceptible than adults (Au, 2002; Roberts et al., 2012).

Here we report the results of a population-based case-control study in an Italian community investigating the possible etiologic role of residence in proximity to agricultural fields, as an indicator of passive exposure to pesticides, on CL risk.

2. Methods

2.1. Study population

Using an already described study design and population (Malagoli et al., 2010, 2015; Vinceti et al., 2012), through the Italian hospital-based registry of childhood malignancies managed by the Italian Association of Paediatric Haematology and Oncology we identified all cases of CL newly diagnosed in children aged 0–14 residing in the Modena and the Reggio Emilia provinces (total population around 1,200,000) of the Northern Italy Emilia-Romagna region. For the present study, we considered CL cases diagnosed from 1998 to 2011. For each case, we randomly selected four population controls among all residents with the same year of birth, sex, and province and calendar year of residence. Residence address was collected at time of diagnosis for cases and at the corresponding year for matched controls. To verify the residential stability of our study population, we reconstructed the lifetime residential history of a sample of children and we found that the occurrence of address changes was unlikely (only 18% during the entire first 5 years of age), ensuring that our subjects are characterized by low mobility (Malagoli et al., 2010; Vinceti et al., 2012).

Socio-economic status was assessed by paternal income for the index year, collected from the Ministry of Finance.

We modeled air concentration of two pollutants from vehicular traffic, benzene and particulate matter $\leq 10\,\mu\text{m}$ (PM_{10}), at the home of residence as described in a previous study (Vinceti et al., 2012), with the exception of the few subjects residing in sparsely populated mountain municipalities.

Magnetic field induction in the proximity of high-voltage power lines ($\geq$132 kV) located in the territories of both provinces was calculated as described in a previous study (Malagoli et al., 2010), so as to identify three 'exposed' corridors surrounding the power lines, with increasing magnetic field intensity ($0.1 \leq B < 0.2$, $0.2 \leq B < 0.4$, $B \geq 0.4\,\mu T$).

2.2. Pesticide exposure assessment

We georeferenced residences of case and control children in a Geographical Information System (GIS) according to the Rome Monte Mario Italy 1 reference system, using Arc-GIS software (version 10.1, ESRI, Redlands, CA 2012). We retrieved the satellite coordinates of the residences from the institutional database of the Modena and Reggio Emilia Provinces and, for addresses not included, through *in loco* measurement with a portable GPS device (GPSmap 60CSx, Garmin Int. Corp., Olathe, KS).

Agricultural use land density (crop density) around the children's residence was used as a proxy for environmental exposure to agricultural pesticides. We assessed the land use type in proximity to each geocoded home according to The Land Use Map 2003 (available at geoportale.regione.emilia.romagna.it/it) for both Modena and Reggio Emilia provinces, containing GIS-based information including multispectral ortophotos with ground resolution of 50 cm, collected by the Emilia-Romagna Region Agency by remote sensing in 2003 (Ward et al., 2000). The Land Use Map 2003 contains a detailed description of land use with the specifications of Corine Land Cover (Bossard and Otahel, 2000). Roads, including names, technical and administrative classification were also obtained from the cartographic archive of Emilia-Romagna Region Agency. With a coupled GIS/Python procedure, we defined a circular buffer with a 100-m radius around each child's home.

For crop-type identification analysis, we used the predefined land use subtype named 'Rural area' present on The Land Use Map 2003. 'Rural area' comprises territories allocated to agriculture with various crop fields, dedicated to different cultivations. We determined crop density within the 100-m buffers for total agricultural land and for the four specific categories of crops available for type broad crop category: arable, orchards, vineyards and vegetables.

2.3. Pesticides

Provinces of Modena and Reggio Emilia are located in the Padana Plain, a flat area in Northern Italy which is very intensively cultivated. Almost 46% of both provinces' surfaces (approximately 640.676 acres) is devoted to agriculture and local farms are estimated to use approximately 1400 t of pesticides every year. Among the pesticides extensively used there with suspected leukaemogenic properties are categories such as phosphorganics, triazoles, phenylalanines, neonicotinoids, phenoxyacetic acids and pyrethroids, and compounds such as dicamba and glyphosate. With the help of the technicians of the public Agricultural Department of the Emilia-Romagna Region, which is in charge of overseeing farmers activities including pesticide use, we estimated and validated the use of pesticide categories or specific compounds for the most common crop types (Table 1). For the purposes of this study, these technicians assessed actual pesticides in a sample of 118 local farms in 2003, by scrutinizing the 'Registers of treatments', which according to the Italian rules each farm must maintain and in which all the details about type, timing and amount of pesticide use has to be recorded.

In the recent years an increasing number of farms located in the Emilia-Romagna Region was certified as being 'pesticide-free', though to date no more than 5% of farms in Modena and Reggio Emilia provinces – around 3% of agricultural land – does not use

Table 1
Specific chemicals and pesticide categories with suspected leukemogenic properties most frequently applied to the different crops in the study area.

Crop	Pesticide (category or compounds)	Intensity of use		
		Insecticides	Herbicides	Fungicides
Arable	2,4-D, MCPA, Glyphosate, Dicamba, Triazines, Pyrethroids	*	***	*
Orchards	Phosphorganics, Triazoles, Phenylalanines, Neonicotinoids	****	*	*****
Vineyards	Phosphorganics, Triazoles, Phenylalanines	**	*	*****
Vegetable	Neonicotinoids, Pyrethroids, Phenylalanines	**	**	****

any pesticides. We traced and georeferenced these farms through the official website of pesticide-free certification agency (http://www.trasparente-check.com/protetta2/index.htm).

2.4. Data analysis

We estimated the relative risk of CL associated with passive residential pesticide exposure by calculating the odds ratio (OR) of disease and the 95% confidence intervals (CI) in a conditional logistic regression model, according to type and percentage of land use (crop density) within a 100-m circular buffer around the children's homes. In addition to the crude analysis, we computed an adjusted OR within a multivariate model taking into account exposure to benzene, PM_{10} and to magnetic fields from high-voltage power lines. Categorical variables were created with zero as the reference group and non-zero values were categorized into two groups based on the median value as cutpoint. To focus on children residing very close to intensely cultivated areas, we also modeled crop density as dichotomous, being <95% or ≥95%, an approach which was feasible only for total agricultural land and for arable crops, since for orchards, vineyards and vegetable crops too few children lived nearby such a high amount of these cultivations. We eventually assessed these associations by stratifying for sex and age, and for the two major leukemia subtypes, acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Data analysis was performed using the clogit command in Stata-14 (StataCorp., College Station, TX. 2015) for conditional maximum-likelihood fitting of stratified logistic model (Rothman et al., 2012).

2.5. Ethical considerations

The Ethics Committee of Modena approved the study protocol, and the study was conducted according to the ethical guidelines and the Italian rules concerning the implementation of epidemiologic and biomedical research.

3. Results

There were 111 newly-diagnosed cases of CL during the study period, including 86 ALL cases (average age at diagnosis 6.1 yrs), 22 AML cases (5.3 yrs) and 3 cases included in the chronic myeloid category. These cases and the 444 controls were comparable with regards to paternal income.

Residences of 270 study subjects (48.6%) included some agricultural land within a 100-m buffer. For these 270 children, the median percentage of overall agricultural land within the 100-m buffer around their residences was 29.2% (10th–90th 2.7–96.2). Arable crops, especially wheat, corn and barley, were the most commonly cultivated crops with 78% of the total agricultural area falling in this category. The other three crop types were rarely found nearby children's residences, with a percentage not exceeding 10% and 7% of the total agricultural area nearby the children for orchards and vineyards, respectively. Since only one child lived in the proximity of vegetable crops, analyses for this crop type were unfeasible. The percentile distribution of study subjects according to crop density around their residence is shown in Table 2.

No consistent and monotonic association was observed between CL and total agricultural land or specific crop density within the 100-m circular buffer around the children's homes, using three categories of exposure, *i.e.* no exposure (referent group), exposure < median of positive value and exposure > the median (Table 3). A non-dose-related positive association emerged only for orchards, due to an elevated OR in the middle-exposure category (2.27, 95% CI 0.64–8.06).

Focusing on children whose residence was deeply surrounded by cultivated areas, i.e. setting the exposure cut-point at ≥95% of crop density (Table 4), we found an increased risk associated not with total agricultural crops but specifically with arable crops (OR = 2.04, 95% CI 0.50–8.35). In sex- and age-stratified analyses, ORs were statistically very unstable, but we observed an increased risk in the age group <5 yrs (OR = 5.76, 95% CI 0.78–42.70) and not in the older children, while ORs were increased in both sexes although more in males. We could not estimate valid risk estimates for the

Table 2
Agricultural land use density and crop types nearby children's residences.

	No. of positives	(%)	Median	(Range in 95%CI)	Percentiles	
					25th	75th
Total agricultural land						
Cases	53	(47.7)	29.8	(22.1–32.1)	9.8	42.4
Controls	217	(48.9)	28.5	(22.3–33.5)	8.4	56.1
Arable crops						
Cases	46	(41.4)	24.4	(13.6–31.0)	8.7	33.0
Controls	201	(45.3)	22.3	(17.2–29.7)	7.2	50.6
Orchards						
Cases	6	(5.4)	11.5	(3.0–39.9)	3.2	22.2
Controls	26	(5.9)	28.1	(13.4–41.7)	7.5	45.0
Vineyards						
Cases	3	(2.7)	3.5	(2.4–57.6)	2.4	57.6
Controls	32	(7.2)	15.6	(4.0–22.1)	1.5	34.5
Vegetables						
Cases	0	–	–	–	–	–
Controls	1	(0.2)	34.6	–	–	–

Table 3
Odds ratios (OR) and 95% confidence intervals (CI) of childhood leukemia associated with crop density within the 100-m circular buffer around children's homes.

	0[a]		<Median		≥Median	
	Cases/ controls	OR	Cases/controls	OR (95%CI)	Cases/controls	OR (95%CI)
Total agricultural land			<28.5%		≥28.5%	
Crude	58/227	Ref.	25/108	0.90 (0.53–1.54)	28/109	1.00 (0.60–1.68)
Adjusted[b]	47/171	Ref.	18/78	0.85 (0.44–1.63)	18/83	0.71 (0.36–1.41)
Arable crops			<22.3%		≥22.3%	
Crude	65/243	Ref.	21/100	0.78 (0.45–1.35)	25/101	0.92 (0.54–1.55)
Adjusted[b]	53/183	Ref.	16/75	0.76 (0.39–1.49)	14/74	0.61 (0.30–1.23)
Orchards			<28.1%		≥28.1%	
Crude	105/418	Ref.	5/13	1.57 (0.53–4.69)	1/13	0.30 (0.04–2.36)
Adjusted[b]	77/311	Ref.	5/10	2.27 (0.64–8.06)	1/11	0.41 (0.05–3.24)
Vineyards				<15.6%		≥15.6%
Crude	108/412	Ref.	2/16	0.42 (0.09–2.01)	1/16	0.24 (0.03–1.78)
Adjusted[b]	80/307	Ref.	2/13	0.57 (0.11–2.83)	1/12	0.33 (0.04–2.57)

[a] Reference category.
[b] Adjusting for exposure to outdoor benzene and PM_{10}, and magnetic fields.

Table 4
Odds ratios (OR) and 95% confidence intervals (CI) of leukemia in children with ≥95% of agricultural use land or arable crops within the 100-m buffer around the home, according to sex and age.

	Total agricultural land				Arable crops			
	<95%[a]		≥95%		<95%[a]		≥95%	
	Cases/controls	OR	Cases/controls	OR (95%CI)	Cases/controls	OR	Cases/controls	OR (95%CI)
All subjects								
Crude	107/420	Ref.	4/24	0.66 (0.22–1.92)	107/437	Ref.	4/7	2.29 (0.67–7.81)
Adjusted[b]	80/313	Ref.	3/19	0.64 (0.18–2.22)	80/326	Ref.	3/6	2.04 (0.50–8.35)
Age <5years								
Crude	54/209	Ref.	2/15	0.53 (0.12–2.33)	54/221	Ref.	2/3	2.67 (0.45–15.96)
Adjusted[b]	36/142	Ref.	2/10	1.18 (0.24–5.82)	36/150	Ref.	2/2	5.76 (0.78–42.70)
Age ≥5 years								
Crude	53/211	Ref.	2/9	0.88 (0.18–4.23)	53/216	Ref.	2/4	2.00 (0.37–10.92)
Adjusted[b]	44/171	Ref.	1/9	0.38 (0.04–3.09)	44/176	Ref.	1/4	0.79 (0.08–7.47)
Males								
Crude	64/251	Ref.	2/13	0.61 (0.13–2.74)	64/261	Ref.	2/3	2.67 (0.45–15.96)
Adjusted[b]	48/189	Ref.	2/11	0.84 (0.18–3.97)	48/197	Ref.	2/3	3.29 (0.53–20.29)
Females								
Crude	43/169	Ref.	2/11	0.72 (0.16–3.32)	43/176	Ref.	2/4	2.00 (0.37–10.92)
Adjusted[b]	32/124	Ref.	1/8	0.55 (0.06–4.82)	32/126	Ref.	1/3	1.71 (0.16–17.89)

[a] Reference category.
[b] Adjusting for exposure to outdoor benzene and PM_{10}, and magnetic fields.

remaining crop types, since too few children lived close to large amounts of these cultivations.

No substantial difference emerged in stratified analysis according to leukemia subtypes, since the increased risks in children residing close to arable crops were comparable for both ALL and AML (Table 5), with similar age-specific trends to that observed in the overall study population although there were very small numbers for these analyses (data not shown).

After taking into account the location of the pesticide-free farms, the overall study results were not affected since no exposed subjects resided close to those farms.

4. Discussion

In this study population, we found an indication of excess CL risk, although statistically very imprecise, among children living in strict proximity to arable crops. On the converse, no excess risk emerged in association with overall agricultural crops or other specific crop types. Our *a priori* hypothesis was that for children living adjacent to cultivated fields, passive exposure to drift of the pesticides used for the specific crop types was not negligible (Lu et al., 2000; Rubino et al., 2012). We selected a 100-m buffer around homes since it represents the distance where substantial pesticide drift occurs according to some studies (Wittich and Siebers, 2002; Siebers et al., 2003; Martin et al., 2008; Wolters et al., 2008; Cornelis et al., 2009; Garron et al., 2009), taking into account that in Italy agricultural fields are sprayed only by ground-based devices and therefore larger drifts are difficult to assume (Tsakirakis et al., 2014). In particular, for arable crops, sprayers' devices are kept in a horizontal position, very close to the plants, so the dispersion of pesticides is very limited. However, since the drift is also affected by other factors such as the weather conditions at the time of application (humidity, wind speed and direction etc.) and children at home may be walking around their neighborhood, we cannot entirely rule out that such exposure might involve larger distances than the one we considered (Wittich and Siebers, 2002; Nsibande et al., 2015). In addition, some studies have shown that agricultural drift pathways may contribute to pesticide exposure in non-occupationally exposed women at larger distance than 100 m, as assessed through pesticide residential dust, even if not all studies are consistent (Deziel et al., 2015). Overall, the assessment of pesticide terrestrial field dissipation is a very complex issue (Doruchowski et al., 2013; Otto et al., 2015) and is still under active investigation (Organisation for Economic Cooperation and Development, 2016).

The most abundant pesticides used in arable crops fall in the herbicide category, though pyrethroids may also be used. Herbicides are widely used in agriculture to control weeds and other unwanted vegetation, with several compounds reported as endocrine disruptors and responsible for reproductive dysfunction (Short and Colborn, 1999). Though the potential carcinogenicity, in partic-

Table 5
Odds ratios (OR) and 95% confidence intervals (CI) of leukemia in children with ≥95% of agricultural use land or arable crops within the 100-m buffer around the home, according to the leukemia subtypes acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).

	ALL				AML			
	<95%[a]		≥95%		<95%[a]		≥95%	
	Cases/controls	OR	Cases/controls	OR (95%CI)	Cases/controls	OR	Cases/controls	OR (95%CI)
Total agricultural land								
Crude	83/325	Ref.	3/19	0.62 (0.18–2.13)	21/84	Ref.	1/4	1.18 (0.10–10.88)
Adjusted[b]	62/241	Ref.	2/15	0.55 (0.12–2.46)	17/68	Ref.	1/4	1.00 (0.09–11.64)
Arable crops								
Crude	83/339	Ref.	3/5	2.40 (0.57–10.04)	21/86	Ref.	1/2	2.00 (0.18–22.06)
Adjusted[b]	62/252	Ref.	2/4	2.14 (0.38–11.93)	17/70	Ref.	1/2	1.84 (0.14–24.24)

[a] Reference category.
[b] Adjusting for exposure to outdoor benzene and PM_{10}, and magnetic fields.

ular leukemogenicity, of these compounds is still unclear (EPA, 2006), some studies have suggested an association between herbicide exposure and CL. Schreinemachers et al. found an excess mortality from CL using wheat acreage as a proxy for chlorophenoxy herbicide exposure (Schreinemachers, 2000). Infante-Rivard et al. found that reported herbicide use in garden, yard or interior plants during pregnancy was related to an increased ALL risk in offspring (Infante-Rivard et al., 1999). Metayer reported an elevated risk of ALL associated with home dust level of herbicides, in particular chlorthal and alachlor, after CL diagnosis in a region with high agricultural pesticide use, though no association with home dust levels at the time of leukemia diagnosis of phenoxyacetic acid herbicides and in particular 2,4-D and MCPA emerged, perhaps because earlier life exposures would be of greater relevance (Metayer et al., 2013).

In the area investigated by our study, arable crops were characterized by the use of a few specific herbicide compounds, in particular two phenoxyacetic acids, *i.e.* 2,4-D and MCPA, glyphosate, dicamba and triazines. Extensive use of phenoxyacetic acid herbicides in adults, particularly in occupationally-exposed subjects, has been associated with increased risk of non-Hodgkin's lymphoma, soft tissue sarcoma, leukemia and other cancers (Morrison et al., 1992; Boers et al., 2012; Coggon et al., 2015), though not all studies were consistent (Hartge et al., 2005). Phenoxyacetic acid pesticides and dicamba may act as synthetic auxins, but the mechanisms of their possible leukemogenic activity are still unclear.

Glyphosate is a broad-spectrum herbicide, whose use significantly increased in recent years and which has been suggested to be a probable human carcinogen, *i.e.* IARC Group 2A (Guyton et al., 2015; Mesnage et al., 2015). However, we are not aware of studies investigating glyphosate effects on CL risk, while data have been provided for non-Hodgkin's lymphoma.

Triazines have been reported as endocrine disrupters, but their role in developing cancer is still controversial (Sathiakumar et al., 2011). A study which explored spatial patterns of childhood cancers and herbicides in groundwater found a possible association between CL and atrazine (Thorpe and Shirmohammadi, 2005), and increased ALL risk has been linked with residential proximity to agricultural applications of several pesticides including triazines (Rull et al., 2009). Herbicides are commonly used for arable crop treatments, and our results give some support to a possible relation of this pesticides category with CL risk.

Pyrethroids are synthetic organic insecticides used in arable crops, but more generally in all crops examined in the present study, and these compounds are considered immune and endocrine disruptors and potential carcinogens (EPA, 2006). Few studies have examined the association between pyrethroids and childhood leukemia. In a Chinese case-control study, Ding et al. observed an increased risk of childhood ALL associated with increased urinary metabolite levels of pyrethroid pesticides (Ding et al., 2012).

We found an increased risk in children aged less than five years, a finding compatible with the hypothesis that younger children may have a greater susceptibility to adverse health effects of pesticides (Tsakiris et al., 2015). In fact, immune systems are still developing in early childhood, and this might explain a higher susceptibility to cancer-promoting substances in the early years of life. In addition, the efficacy of enzymatic and metabolic systems to detoxify and excrete chemicals is lower in the early years of life (Faustman et al., 2000; Sheets, 2000; Alarcon et al., 2005), resulting in higher blood levels of toxic metabolites in foetuses and young children for longer periods than in adults (Garry, 2004; Weiss et al., 2004). It is also possible that passive exposure to agricultural pesticides is higher in the youngest children due to their lower residential mobility compared with older children, with consequently higher exposure through inhalation and also due to a higher tendency at this age group for soil and dust ingestion (Moya and Phillips, 2014; von Lindern et al., 2016).

A strength of our study was the use of crop density as a proxy of pesticide exposure (Ritz and Rull, 2008) with a further validation process directly conducted with farm records which allowed us to confirm the pesticides used for specific crops, focusing on the period during which cases were diagnosed. Other investigative methods such as biologic monitoring or use of carpet dust pesticide levels are also subject to bias since they refer only to a recent evaluation of exposure. Self-reported questionnaire methods could lead to exposure misclassification due to recall bias, and this misclassification may likely be differential. In addition, questionnaire responders may not be aware which crops are planted near their homes and they are unlikely to know which pesticides are used nearby their residences. Finally and most important, our methodological approach allowed the avoidance of selection and recall biases, two usual events in case-control studies resulting from voluntary participation in a survey, and which can severely affect study validity (Filippini et al., 2015).

Our study also has some relevant limitations. We do not have exact information about the effective amount of pesticides used for treatments applied near children's homes and particularly near the exposed ones. Moreover, we could not systematically address for each participant factors affecting fate and drift potential of pesticides in the environment, such as wind speed and direction at the time of application, the mixing of solvents and adjuvants that may affect the persistence and volatility of the active ingredient, and the application method. In addition, use of the residence at the time of diagnosis may be an inaccurate estimate of lifetime residential exposure, though as mentioned above residential mobility in our population is expected to have been very low, particularly at the youngest ages (Malagoli et al., 2010). Finally, and probably more important, we acknowledge that the study was underpowered to yield a satisfactory precision of the risk estimates, and to reliably detect small, subtle effects of the exposures under study. The limited number of exposed cases also indicates that even accepting a role of passive pesticide exposure in the case of vicinity to arable crops, such exposure would be involved in disease etiology only for a small minority of the CL cases, although we could not assess the potential effects of pesticide exposure through diet or through parental occupational exposure. In conclusion, and despite the study limitations with particular reference to statistical power, our results may give some support to an excess CL risk following passive residential exposure to pesticides used for arable crops, known to be herbicides and pyrethroids, while they do no indicate the involvement of other pesticide categories in disease etiology.

Conflict of interest

The authors declare no conflict of interest.

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References

AIRTUM, 2012. Report on Cancer in Children and Adolescents in Italy. http://www.registri-tumori.it/PDF/AIRTUM2012/EP37_1_s1_t001_024_AIRTUM.pdf.

Alarcon, W.A., Calvert, G.M., Blondell, J.M., Mehler, L.N., Sievert, J., Propeck, M., Tibbetts, D.S., Becker, A., Lackovic, M., Soileau, S.B., Das, R., Beckman, J., Male, D.P., Thomsen, C.L., Stanbury, M., 2005. Acute illnesses associated with pesticide exposure at schools. JAMA 294, 455–465.

American Cancer Society, 2014. Cancer Facts & Figs. 2014. http://www.cancer.org/acs/groups/content/@research/documents/webcontent/acspc-042151. pdf.

Androutsopoulos, V.P., Hernandez, A.F., Liesivuori, J., Tsatsakis, A.M., 2013. A mechanistic overview of health associated effects of low levels of organochlorine and organophosphorous pesticides. Toxicology 307, 89–94.

Au, W.W., 2002. Susceptibility of children to environmental toxic substances. Int. J. Hyg. Environ. Health 205, 501–503.

Bailey, H.D., Infante-Rivard, C., Metayer, C., Clavel, J., Lightfoot, T., Kaatsch, P., Roman, E., Magnani, C., Spector, L.G., Th Petridou, E., Milne, E., Dockerty, J.D., Miligi, L., Armstrong, B.K., Rudant, J., Fritschi, L., Simpson, J., Zhang, L., Rondelli, R., Baka, M., Orsi, L., Moschovi, M., Kang, A.Y., Schuz, J., 2015. Home pesticide exposures and risk of childhood leukemia: findings from the childhood leukemia international consortium. Int. J. Cancer 137, 2644–2663.

Boers, D., Portengen, L., Turner, W.E., Bueno-de-Mesquita, H.B., Heederik, D., Vermeulen, R., 2012. Plasma dioxin levels and cause-specific mortality in an occupational cohort of workers exposed to chlorophenoxy herbicides, chlorophenols and contaminants. Occup. Environ. Med. 69, 113–118.

Booth, B.J., Ward, M.H., Turyk, M.E., Stayner, L.T., 2015. Agricultural crop density and risk of childhood cancer in the midwestern United States: an ecologic study. Environ. Health 14, 82.

Bossard, M.F.J., Otahel, J., 2000. CORINE Land Cover Technical Guide. European Enviroment Agency, Copenhagen.

Carozza, S.E., Li, B., Elgethun, K., Whitworth, R., 2008. Risk of childhood cancers associated with residence in agriculturally intense areas in the United States. Environ. Health Perspect. 116, 559–565.

Chen, M., Chang, C.H., Tao, L., Lu, C., 2015. Residential exposure to pesticide during childhood and childhood cancers: a meta-analysis. Pediatrics 136, 719–729.

Choi, J.P.A., Joas, A., 2016. Systematic Literature Review on Parkinson's Disease and Childhood Leukaemia and Mode of Actions for Pesticides. European Food Safety Authority Supporting Publications En-955.

Coggon, D., Ntani, G., Harris, E.C., Jayakody, N., Palmer, K.T., 2015. Soft tissue sarcoma, non-Hodgkin's lymphoma and chronic lymphocytic leukaemia in workers exposed to phenoxy herbicides: extended follow-up of a UK cohort. Occup. Environ. Med. 72, 435–441.

Cornelis, C., Schoeters, G., Kellen, E., Buntinx, F., Zeegers, M., 2009. Development of a GIS-based indicator for environmental pesticide exposure and its application to a Belgian case-control study on bladder cancer. Int. J. Hyg. Environ. Health 212, 172–185.

Curwin, B.D., Hein, M.J., Sanderson, W.T., Nishioka, M.G., Reynolds, S.J., Ward, E.M., Alavanja, M.C., 2005. Pesticide contamination inside farm and nonfarm homes. J. Occup. Environ. Hyg. 2, 357–367.

Deziel, N.C., Friesen, M.C., Hoppin, J.A., Hines, C.J., Thomas, K., Freeman, L.E., 2015. A review of nonoccupational pathways for pesticide exposure in women living in agricultural areas. Environ. Health Perspect. 123, 515–524.

Ding, G., Shi, R., Gao, Y., Zhang, Y., Kamijima, M., Sakai, K., Wang, G., Feng, C., Tian, Y., 2012. Pyrethroid pesticide exposure and risk of childhood acute lymphocytic leukemia in Shanghai. Environ. Sci. Technol. 46, 13480–13487.

Doruchowski, G., Balsari, P., Marucco, P., Herbst, A., Wehmann, H.J., Roettele, M., Gil, E., Codis, S., Pauwelyn, E., 2013. Decision support tools for environmentally safe use of pesticides. Commun. Agric. Appl. Biol. Sci. 78, 37–45.

EPA, U.S, 2006. Permethrin Facts, EPA 738-F-06-012.

Faustman, E.M., Silbernagel, S.M., Fenske, R.A., Burbacher, T.M., Ponce, R.A., 2000. Mechanisms underlying Children's susceptibility to environmental toxicants. Environ. Health Perspect. 108 (Suppl. 1), 13–21.

Fenske, R.A., Lu, C., Barr, D., Needham, L., 2002. Children's exposure to chlorpyrifos and parathion in an agricultural community in central Washington State. Environ. Health Perspect. 110, 549–553.

Filippini, T., Heck, J.E., Malagoli, C., Del Giovane, C., Vinceti, M., 2015. A review and meta-analysis of outdoor air pollution and risk of childhood leukemia. J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 33, 36–66.

Freeman, N.C., Hore, P., Black, K., Jimenez, M., Sheldon, L., Tulve, N., Lioy, P.J., 2005. Contributions of children's activities to pesticide hand loadings following residential pesticide application. J. Expos. Anal. Environ. Epidemiol. 15, 81–88.

Garron, C.A., Davis, K.C., Ernst, W.R., 2009. Near-field air concentrations of pesticides in potato agriculture in Prince Edward Island. Pest Manag. Sci. 65, 688–696.

Garry, V.F., 2004. Pesticides and children. Toxicol. Appl. Pharmacol. 198, 152–163.

Gunier, R.B., Ward, M.H., Airola, M., Bell, E.M., Colt, J., Nishioka, M., Buffler, P.A., Reynolds, P., Rull, R.P., Hertz, A., Metayer, C., Nuckols, J.R., 2011. Determinants of agricultural pesticide concentrations in carpet dust. Environ. Health Perspect. 119, 970–976.

Guyton, K.Z., Loomis, D., Grosse, Y., El Ghissassi, F., Benbrahim-Tallaa, L., Guha, N., Scoccianti, C., Mattock, H., Straif, K., International Agency for Research on Cancer Monograph Working Group, I.L.F, 2015. Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate. Lancet Oncol. 16, 490–491.

Harnly, M.E., Bradman, A., Nishioka, M., McKone, T.E., Smith, D., McLaughlin, R., Kavanagh-Baird, G., Castorina, R., Eskenazi, B., 2009. Pesticides in dust from homes in an agricultural area. Environ. Sci. Technol. 43, 8767–8774.

Hartge, P., Colt, J.S., Severson, R.K., Cerhan, J.R., Cozen, W., Camann, D., Zahm, S.H., Davis, S., 2005. Residential herbicide use and risk of non-Hodgkin lymphoma. Cancer Epidemiol. Biomark. Prev. 14, 934–937.

Hernandez, A.F., Menendez, P., 2016. Linking pesticide exposure with pediatric leukemia: potential underlying mechanisms. Int. J. Mol. Sci. 17.

Hernandez, A.F., Parron, T., Tsatsakis, A.M., Requena, M., Alarcon, R., Lopez-Guarnido, O., 2013. Toxic effects of pesticide mixtures at a molecular level: their relevance to human health. Toxicology 307, 136–145.

Infante-Rivard, C., Labuda, D., Krajinovic, M., Sinnett, D., 1999. Risk of childhood leukemia associated with exposure to pesticides and with gene polymorphisms. Epidemiology 10, 481–487.

Loewenherz, C., Fenske, R.A., Simcox, N.J., Bellamy, G., Kalman, D., 1997. Biological monitoring of organophosphorus pesticide exposure among children of agricultural workers in central Washington State. Environ. Health Perspect. 105, 1344–1353.

Lu, C., Fenske, R.A., Simcox, N.J., Kalman, D., 2000. Pesticide exposure of children in an agricultural community: evidence of household proximity to farmland and take home exposure pathways. Environ. Res. 84, 290–302.

Ma, X., Buffler, P.A., Gunier, R.B., Dahl, G., Smith, M.T., Reinier, K., Reynolds, P., 2002. Critical windows of exposure to household pesticides and risk of childhood leukemia. Environ. Health Perspect. 110, 955–960.

Malagoli, C., Fabbi, S., Teggi, S., Calzari, M., Poli, M., Ballotti, E., Notari, B., Bruni, M., Palazzi, G., Paolucci, P., Vinceti, M., 2010. Risk of hematological malignancies associated with magnetic fields exposure from power lines: a case-control study in two municipalities of northern Italy. Environ. Health 9, 16.

Malagoli, C., Malavolti, M., Costanzini, S., Fabbri, S., Tezzi, S., Palazzi, G., Arcolin, E., Vinceti, M., 2015. Increased incidence of childhood leukemia in urban areas: a population-based case-control study. Epidemiol. Prev. 39, 102–107.

Martin, S., Westphal, D., Erdtmann-Vourliotis, M., Dechet, F., Schulze-Rosario, C., Stauber, F., Wicke, H., Chester, G., 2008. Guidance for exposure and risk evaluation for bystanders and residents exposed to plant protection products during and after application. J. Verbr. Lebensm. 3, 272–281.

Menegaux, F., Baruchel, A., Bertrand, Y., Lescoeur, B., Leverger, G., Nelken, B., Sommelet, D., Hemon, D., Clavel, J., 2006. Household exposure to pesticides and risk of childhood acute leukaemia. Occup. Environ. Med. 63, 131–134.

Mesnage, R., Defarge, N., Spiroux de Vendomois, J., Seralini, G.E., 2015. Potential toxic effects of glyphosate and its commercial formulations below regulatory limits. Food Chem. Toxicol. 84, 133–153.

Metayer, C., Colt, J.S., Buffler, P.A., Reed, H.D., Selvin, S., Crouse, V., Ward, M.H., 2013. Exposure to herbicides in house dust and risk of childhood acute lymphoblastic leukemia. J. Expo. Sci. Environ. Epidemiol. 23, 363–370.

Morrison, H.I., Wilkins, K., Semenciw, R., Mao, Y., Wigle, D., 1992. Herbicides and cancer. J. Natl. Cancer Inst. 84, 1866–1874.

Moya, J., Phillips, L., 2014. A review of soil and dust ingestion studies for children. J. Expo. Sci. Environ. Epidemiol. 24, 545–554.

Mrema, E.J., Rubino, F.M., Brambilla, G., Moretto, A., Tsatsakis, A.M., Colosio, C., 2013. Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology 307, 74–88.

Nsibande, S.A., Dabrowski, J.M., van der Walt, E., Venter, A., Forbes, P.B., 2015. Validation of the AGDISP model for predicting airborne atrazine spray drift: a South African ground application case study. Chemosphere 138, 454–461.

Ntzani, E.E., Chondrogiorgi, M., Ntritsos, G., Evangelou, E., Tzoulaki, I., 2013. Literature Review on Epidemiological Studies Linking Exposure to Pesticides and Health Effects. European Food Safety Authority Supporting Publications EN-497.

Organisation for Economic Cooperation and Development, 2016. ENV/JM/MONO(2016)6. Guidance Document for Conducting Pesticide Terrestrial Field Dissipation Studies, Series on Testing & Assessment No. 232; Series on Pesticides No. 82. IOMC.

Otto, S., Loddo, D., Baldoin, C., Zanin, G., 2015. Spray drift reduction techniques for vineyards in fragmented landscapes. J. Environ. Manage. 162, 290–298.

Perry, C., Soreq, H., 2004. Organophosphate risk of leukemogenesis. Leuk. Res. 28, 905–906.

Phillips, K.P., Foster, W.G., 2008. Key developments in endocrine disrupter research and human health. J. Toxicol. Environ. Health B Crit. Rev. 11, 322–344.

Reynolds, P., Von Behren, J., Gunier, R.B., Goldberg, D.E., Hertz, A., Harnly, M.E., 2002. Childhood cancer and agricultural pesticide use: an ecologic study in California. Environ. Health Perspect. 110, 319–324.

Reynolds, P., Von Behren, J., Gunier, R., Goldberg, D.E., Hertz, A., 2005. Agricultural pesticides and lymphoproliferative childhood cancer in California. Scand. J. Work. Environ. Health 31 (Suppl. 1), 46–54, discussion 45–47.

Ritz, B., Rull, R.P., 2008. Assessment of environmental exposures from agricultural pesticides in childhood leukaemia studies: challenges and opportunities. Radiat. Prot. Dosim. 132, 148–155.

Roberts, J.R., Karr, C.J., Council On Environmental Health, 2012. Pesticide exposure in children. Pediatrics 130, e1765–1788.

Rothman, K.J., Lash, T.L., Greenland, S., 2012. Modern Epidemiology. Lippincott, Williams, & Wilkins, Philadelphia.

Rubino, F.M., Mandic-Rajcevic, S., Ariano, E., Alegakis, A., Bogni, M., Brambilla, G., De Paschale, G., Firmi, A., Minoia, C., Micoli, G., Savi, S., Sottani, C., Somaruga, C., Turci, R., Vellere, F., Tsatsakis, A., Colosio, C., 2012. Farmers' exposure to herbicides in North Italy: assessment under real-life conditions in small-size rice and corn farms. Toxicol. Lett. 210, 189–197.

Rudant, J., Menegaux, F., Leverger, G., Baruchel, A., Nelken, B., Bertrand, Y., Patte, C., Pacquement, H., Verite, C., Robert, A., Michel, G., Margueritte, G., Gandemer, V., Hemon, D., Clavel, J., 2007. Household exposure to pesticides and risk of childhood hematopoietic malignancies: the ESCALE study (SFCE). Environ. Health Perspect. 115, 1787–1793.

Rull, R.P., Gunier, R., Von Behren, J., Hertz, A., Crouse, V., Buffler, P.A., Reynolds, P., 2009. Residential proximity to agricultural pesticide applications and childhood acute lymphoblastic leukemia. Environ. Res. 109, 891–899.

Sathiakumar, N., MacLennan, P.A., Mandel, J., Delzell, E., 2011. A review of epidemiologic studies of triazine herbicides and cancer. Crit. Rev. Toxicol. 41 (Suppl. 1), 1–34.

Schreinemachers, D.M., 2000. Cancer mortality in four northern wheat-producing states. Environ. Health Perspect. 108, 873–881.

Sheets, L.P., 2000. A consideration of age-dependent differences in susceptibility to organophosphorus and pyrethroid insecticides. Neurotoxicology 21, 57–63.

Short, P., Colborn, T., 1999. Pesticide use in the U.S. and policy implications: a focus on herbicides. Toxicol. Ind. Health 15, 240–275.

Siebers, J., Binner, R., Wittich, K.P., 2003. Investigation on downwind short-range transport of pesticides after application in agricultural crops. Chemosphere 51, 397–407.

Thorpe, N., Shirmohammadi, A., 2005. Herbicides and nitrates in groundwater of Maryland and childhood cancers: a geographic information systems approach. J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 23, 261–278.

Tsakirakis, A.N., Kasiotis, K.M., Charistou, A.N., Arapaki, N., Tsatsakis, A., Tsakalof, A., Machera, K., 2014. Dermal & inhalation exposure of operators during fungicide application in vineyards. Evaluation of coverall performance. Sci. Total Environ. 470–471, 282–289.

Tsakiris, I.N., Goumenou, M., Tzatzarakis, M.N., Alegakis, A.K., Tsitsimpikou, C., Ozcagli, E., Vynias, D., Tsatsakis, A.M., 2015. Risk assessment for children exposed to DDT residues in various milk types from the Greek market. Food Chem. Toxicol. 75, 156–165.

Turner, M.C., Wigle, D.T., Krewski, D., 2010. Residential pesticides and childhood leukemia: a systematic review and meta-analysis. Environ. Health Perspect. 118, 33–41.

Vakonaki, E., Androutsopoulos, V.P., Liesivuori, J., Tsatsakis, A.M., Spandidos, D.A., 2013. Pesticides and oncogenic modulation. Toxicology 307, 42–45.

Van Maele-Fabry, G., Lantin, A.C., Hoet, P., Lison, D., 2010. Childhood leukaemia and parental occupational exposure to pesticides: a systematic review and meta-analysis. Cancer Causes Control 21, 787–809.

Van Maele-Fabry, G., Lantin, A.C., Hoet, P., Lison, D., 2011. Residential exposure to pesticides and childhood leukaemia: a systematic review and meta-analysis. Environ. Int. 37, 280–291.

Vidal, C.J., 2005. Expression of cholinesterases in brain and non-brain tumours. Chem. Biol. Interact. 157–158, 227–232.

Vinceti, M., Rothman, K.J., Crespi, C.M., Sterni, A., Cherubini, A., Guerra, L., Maffeis, G., Ferretti, E., Fabbi, S., Teggi, S., Consonni, D., De Girolamo, G., Meggiato, A., Palazzi, G., Paolucci, P., Malagoli, C., 2012. Leukemia risk in children exposed to benzene and PM10 from vehicular traffic: a case-control study in an Italian population. Eur. J. Epidemiol. 27, 781–790.

Walker, K.M., Carozza, S., Cooper, S., Elgethun, K., 2007. Childhood cancer in Texas counties with moderate to intense agricultural activity. J. Agric. Saf. Health 13, 9–24.

Ward, M.H., Nuckols, J.R., Weigel, S.J., Maxwell, S.K., Cantor, K.P., Miller, R.S., 2000. Identifying populations potentially exposed to agricultural pesticides using remote sensing and a geographic information system. Environ. Health Perspect. 108, 5–12.

Ward, M.H., Lubin, J., Giglierano, J., Colt, J.S., Wolter, C., Bekiroglu, N., Camann, D., Hartge, P., Nuckols, J.R., 2006. Proximity to crops and residential exposure to agricultural herbicides in iowa. Environ. Health Perspect. 114, 893–897.

Weiss, B., Amler, S., Amler, R.W., 2004. Pesticides. Pediatrics 113, 1030–1036.

Wigle, D.T., Turner, M.C., Krewski, D., 2009. A systematic review and meta-analysis of childhood leukemia and parental occupational pesticide exposure. Environ. Health Perspect. 117, 1505–1513.

Williams, R.D., Boros, L.G., Kolanko, C.J., Jackman, S.M., Eggers, T.R., 2004. Chromosomal aberrations in human lymphocytes exposed to the anticholinesterase pesticide isofenphos with mechanisms of leukemogenesis. Leuk. Res. 28, 947–958.

Wittich, K.P., Siebers, J., 2002. Aerial short-range dispersion of volatilized pesticides from an area source. Int. J. Biometeorol. 46, 126–135.

Wolters, A., Linnemann, V., van de Zande, J.C., Vereecken, H., 2008. Field experiment on spray drift: deposition and airborne drift during application to a winter wheat crop. Sci. Total Environ. 405, 269–277.

von Lindern, I., Spalinger, S., Stifelman, M.L., Stanek, L.W., Bartrem, C., 2016. Estimating children's soil/dust ingestion rates through retrospective analyses of blood lead biomonitoring from the bunker hill superfund site in idaho. Environ. Health Perspect. 124, 1462–1470.