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Agricultural crop density in the municipalities of France and incidence of childhood leukemia: an ecological study

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Abstract:

Background: Pesticide exposure is suspected to play a role in the etiology of childhood leukemia (AL). Various sources of exposure have been explored, but few studies have investigated the risk of childhood AL in relation to residential exposure to agricultural pesticides. Since around 50% of France is agricultural land, with marked pesticide use, France is a suitable location to investigate for an association. We aimed to analyze the association between the agricultural crop density in the municipalities of France and the incidence of childhood AL between 1990 and 2014.

Methods: 11,487 cases of AL diagnosed in children aged 0-14 years were registered by the French National Registry of Childhood Hematological Malignancies over 1990-2014. National agricultural census data for 1990, 2000 and 2010 were used to estimate the densities of the most common crops in France. The incidence of AL was estimated in the 35,512 municipalities, by age and gender, and 3 observation periods, and expressed as the standardized incidence ratio (SIR).

Results: We observed a moderate log-linear association between viticulture density and the incidence of AL, with a 3% increase in SIR for a 10% increase in viticulture density (SIRR = 1.03; 95%CI [1.00-1.06]). The association remained for lymphoblastic AL but not for myeloid AL. The association was stable after stratification by geographic area, age and period, and after adjustment on UV radiation and a French deprivation index. No consistent association was observed for other crop types.

Discussion: This nationwide study shows a moderate increase in incidence of childhood AL in municipalities where viticulture is common. Future individual studies are needed to know whether this observation is confirmed and related to particular use of pesticides.

Keywords: Childhood leukemia, pesticides, agriculture, ecological study, record-based study

Ethics approval

The research undertaken with the RNHE data is covered by agreements on the ethical use of data and the protection of personal data and have been approved by French national authorities (French data protection authority CNIL N° n°998198). This study was part of a wider project called “GEOCAP-AGRI” and the use of the agricultural census data for this project have been approved by the French Committee of the Statistical Confidentiality and the CNIL was notified of data processing (N° 2077682 v0).

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Introduction:

The causes of childhood acute leukemia (AL) have not been fully elucidated. AL is the most common type of childhood cancer in industrialized countries, with an incidence rate of about 5 cases per 100,000 person-years per annum in children aged 0-14 years in France (Lacour et al., 2010). Among children, acute lymphoblastic leukemia (ALL) is more frequent (80% of cases) than acute myeloid leukemia (AML) (15% of cases). Several rare genetic diseases such as Down syndrome, some genetic polymorphisms (Orsi et al., 2012), and high dose ionizing radiation exposure are known risk factors (Hsu et al., 2013; Pearce et al., 2012; Preston et al., 1994). A range of environmental factors are suspected to play a role in the etiology of childhood leukemia, including exposure to pesticides (Bailey et al., 2015).

Pesticides include all the chemicals designed to control insects, weeds, fungi and other pests. Carcinogenic effects of some pesticides have been observed in animal studies but also in epidemiological studies. Most of the latter have focused on occupational exposure and strong associations have been found for adult hematological malignancies (Miligi et al., 2006; Van Maele-Fabry et al., 2007). The general population is also exposed to those compounds, although at lower doses than in the context of occupational exposure. Children constitute a particularly vulnerable population in that they consume more food per kilogram of body weight than adults, have a higher inhalation rate and have a higher body surface area to body weight ratio. They also have less ability to metabolize and eliminate chemicals (Roberts et al., 2012). In addition, early life is a critical time window of heightened susceptibility to environmental aggressions due to the high rate of cell proliferation and the immaturity of organs (Bassil et al., 2007; Ostrea et al., 2009; WHO, 2006). Before birth, embryo and fetus could be exposed through parental occupational and domestic exposures. The main exposure route for children is dietary intake of contaminated food/water and air inhalation. The main sources of airborne particles derived from pesticide application are domestic use and agricultural application on fields near children's homes. Children living close to treated land may be exposed through several pathways: direct pesticide drift, dust tracked into the home after pesticide volatilization, and contact with the clothing and shoes of occupationally exposed family members (Deziel et al., 2017; Gunier et al., 2011).

Several studies have investigated for the potential effects of pesticide exposure on leukemia risk in children. Meta-analyses of home pesticide exposures have shown a consistent positive association between the risk of leukemia and pesticide exposure (Bailey et al., 2015; Van Maele-Fabry et al., 2019). The meta-analysis of the Childhood Leukemia International Consortium (CLIC) (Bailey et al., 2015) included a large pooled analysis of individual data from 12 case-control studies and found evidence of an association between ALL/AML and prenatal exposure to pesticides, and between childhood exposure and ALL. Systematic reviews and meta-analyses investigating parental occupational exposure found evidence of an association with the risk of AL (Van Maele-Fabry et al., 2010; Wigle et al., 2009). More recently, the CLIC conducted a large pooled analysis of 13 case-control studies (Bailey et al., 2014). A positive association between maternal occupational exposure to pesticides during pregnancy and the risk of AML was found. A slight increase in the risk of ALL was observed with paternal exposure around conception. A study from the International Childhood Cancer Cohort Consortium (I4C) pooling five birth cohorts also suggested an increased risk of AML, but not ALL, in children whose father was occupationally exposed to pesticides. Those results were based however on small numbers (Patel et al., 2019). Few studies have investigated the risk of childhood AL in relation to residential exposure to agricultural pesticides. A Spanish record-based case-control study (Gómez-Barroso et al., 2016) showed weak associations between proximity to crops/specific crops and AL. A small Italian case-control study found no association between residential proximity to all or specific crops and

AL(Malagoli et al., 2016). Two Texan ecological studies (Carozza et al., 2009; Walker et al., 2007) did not evidence any associations between AL and crop density/proximity to crops. Two large ecological studies based on numerous US states used county-level agricultural census data to estimate crop density (Booth et al., 2015; Carozza et al., 2008) and found associations between specific crops and the incidence of AL. With regard to specific pesticides, propargite, metam sodium, dicofol, azoles and possibly organophosphates were associated with a higher risk of childhood AL in two Californian studies (Reynolds et al., 2002, 2005b), but in two other similar studies, no clear patterns were found (Reynolds et al., 2005a; Rull et al., 2009).

There were around 27 million hectares of Utilized Agricultural Areas (UAA) in the most recent (2010) French agricultural census ("Ministère de l'agriculture et de l'alimentation - agreste - La statistique, l'évaluation et la prospective agricole - Recensement agricole 2010," n.d.). The area constitutes about 50% of mainland France. The use of agricultural pesticides is thus considerable, and it has been estimated that about 65,000 to 70,000 metric tons of agricultural pesticides, most of them fungicides, are sold each year (INSERM, 2013). Therefore, France is a pertinent location to study the effects of residential exposure to agricultural pesticides on the risk of childhood leukemia. In this study, we investigated the ecological association between the agricultural crop densities in the municipalities of France and the incidence of childhood AL between 1990 and 2014.

Material and Methods

Geographic units

The municipalities are the smallest administrative subdivisions in France. During the study period, 1990-2014, boundaries changed: some municipalities were merged and others divided. To harmonize the dataset, we regrouped the municipalities where appropriate and obtained 35,512 harmonized units (referred to as "municipalities" hereafter).

Crop densities in the municipalities

Nationwide agricultural censuses are conducted in France by the French Ministry of Agriculture every ten years and collect data based on questionnaires applied to every farm in France. The information relating to a farm is linked to the municipality of the main building on the farm. These data were used to assess the crop densities in the municipalities because of their exhaustiveness, high quality and the detailed list of crops they included. We were able to access the detailed data via a secure platform of the secure data access center (CASD). We extracted UAA by type of crop at the municipality level from the 1988, 2000 and 2010 national agricultural censuses. We calculated the crop densities for a given municipality as the ratio of the crop specific UAA to the total area of the municipality.

We considered the total UAA (sum of permanent crops, arable land and pastures) and the UAA of the most common crops grown in France. Our main analysis focused on two permanent crops: "viticulture" and "arboriculture", the locations of which are relatively stable over time, and 7 groups of non-permanent crops: "corn", "straw cereals", "oilseeds", "potatoes", "fresh vegetables, strawberries, melons", "legumes: beans, peas, protein crops", "industrial crops", which may be rotated annually. In an additional analysis, we addressed 6 other specific non-permanent crops that were aggregated in the main analysis: "durum wheat", "common wheat", "barley", "rapeseed", "sunflower", "beets".

Incidence data

We included all the children registered in the French National Registry of Childhood Hematological Malignancies (RNHE) (Lacour et al., 2010) between 1990 and 2014, who were less than 15 years old, diagnosed with AL, and residing in mainland France at the time of diagnosis. There were 11,487 cases of AL. For each case, the registry provided the date of birth, date of diagnosis and address where the child was living at the time of diagnosis. Since we used three different agricultural censuses (1988, 2000, 2010) to estimate crop densities over time, we subdivided the study period into three sub-periods, in order to obtain incidence data associated with the closest agricultural census: 1990-1994, 1995-2004, 2005-2014.

Population data

The annual estimates of the municipality populations, by year of age and gender, were provided by the French National Institute for Statistics and Economic Studies (INSEE) for the census years: 1990, 1999, 2006-2014. For the inter-census years (1991-1998 and 2000-2005), the municipality populations were estimated using the annual estimates provided by INSEE for the 96 French mainland *départements*, and a linear interpolation of the proportion of children in the municipalities related to their *département* population between 1990 and 1999, and between 1999 and 2006. The national incidence rates of childhood AL, by year of age, gender and period, were used as reference rates to estimate the number of AL cases expected in each municipality under the hypothesis of homogeneous incidence rate over the whole country.

Potential confounder data

In previous studies of the French data (Coste et al., 2017, 2015; Marquant et al., 2016), we evidenced an ecological association between the incidence of childhood ALL and 1) the residential UV radiation exposures of the municipalities, 2) the socio-economic statuses (SES) of the municipalities expressed as a deprivation index based on the 1999 census data. We considered these factors as potential confounders for the associations between ALL incidence and crop density index. Residential UV radiation exposure was defined as a dichotomous variable, with lower exposure for the municipalities with an annual average daily exposure less than or equal to 105.5 J/cm^2. The median income of the households, proportion of blue-collar workers, proportion of unemployed and proportion of baccalaureate (high school diploma) holders were integrated in a principal component analysis. The first component, which explained 68% of the total variation, was used to define the French deprivation index "FDep99" (Rey et al., 2009).

Statistical analysis

All analyses were implemented with SAS v9.4 software. Spearman coefficients were estimated to assess the correlations between the specific crops we selected for the study. The standardized incidence ratio (SIR) was used to characterize the spatial variations of incidence over the study period. Poisson regression models were fitted for all the analyses using the municipalities of residence at diagnosis for each sub-period as the basic statistical units (i.e. 106,536 "municipalities x period"). The corresponding expected numbers of cases were incorporated in the models as the offsets.

For each of the crop types (total UAA, 9 groups of crops, 6 subgroups of crops nested within some of the groups), two models were used to make statistical inferences about the association between municipality crop density and childhood AL incidence. In the first model, the crop density was qualitative and categorized by the following procedure: for the main analysis of total UAA density, the municipalities with a total UAA density less than 5% were grouped into the first category. Above that threshold, four quartiles with equal pediatric populations were defined based on the distribution of total UAA in the municipalities. For the main analyses of a specific crop, the first category was unchanged (total UAA density <5%). The second category consisted of the municipalities with total UAA density greater than or equal to 5% but a density less than 5% for the specific crop "*c*". Above that threshold four quartile categories of equal pediatric population were considered. After obtaining the crop density-based on geographic zoning, which consisted of 5 (total UAA) or 6 (specific crop UAA) groups of municipalities, we fitted the first model:

$$\ln\left(E(O_{kp})\right) = \ln(E_{kp}) + \sum_{h=1}^{5} \beta_h \times X_h$$

In which $E(O_{kp})$ is the expected value of the observed number of cases (O_{kp}) in k^{th} group of municipalities for period p (1990-1994, 1995-2004, 2005-2014); E_{kp} is the corresponding expected number of cases; X_h is a dummy variable with a value of one if h=k, 0 otherwise; and β_h is the corresponding regression coefficient. To test for heterogeneity between categories of agricultural density, we conducted a likelihood ratio test comparing an empty model with the first model.

For the second model, a semi-quantitative discrete crop density variable was built:

$$\ln\left(E(O_{kp})\right) = \ln(E_{kp}) + \alpha_0 + \beta \times Z_{kp}$$

in which Z_{kp} is the pediatric population-weighted average value of the crop density (total UAA or crop-specific) in the k^{th} category for period p. In this model, e^{β} estimated the SIR variation for an increase of 1 unit in Z_{kp} (referred to as SIRR, for standardized incidence rate ratio). In this study, Z_{kp} was calibrated to correspond to a 10% increase in the total area of the municipality. We used a likelihood ratio test comparing both models to test for a departure from the log-linearity hypothesis.

While the null hypothesis to be tested was the absence of any association between AL incidence rate and crop density, the alternative hypothesis of interest was that the association was positive. Therefore, we calculated one-sided p-values for the second model. The Pearson chi-square test based on model residuals was used to check that the models fitted the data well and to detect the existence of any extra-Poisson variability in the observed numbers of cases. The main analyses were conducted for all leukemia, the ALL and AML subgroups, and two age groups (0-6, 7-14 years old).

In a secondary analysis, we systematically adjusted our models with the ALL subtype for the residential UV radiation level and the deprivation level of the municipalities. The temporal and spatial stability of the main analysis results was assessed by examining their consistency across three time periods (1990–1994, 1995–2004, 2005–2014), by excluding the Paris urban unit, where a lower AL incidence rate had been observed in a previous study (Marquant et al., 2016), and then by splitting the rest of the country into 4 large regions (North-West, South-West, North-East, South-East). An additional qualitative model was fitted by splitting the crop density categories into two so as to obtain “semi-quartiles”, in order to better describe the shape of the association observed in the main analysis.

It was difficult to formulate precise credible alternative hypotheses for statistical power calculations because of the lack of *a priori* information on the expected effect size of the associations, and the heterogeneity of the selected crops in terms of frequency, range of spatial variation of their densities, and agricultural practices. However, we calculated minimum detectable SIR ensuring a power of 80 % for the one-sided test at the 5% level under the null hypothesis of no association between crop density and AL. Those calculations were based on the expected number of cases in each leukemia diagnosis group and calculated for the quartile of municipalities with the highest crop density (S1). For AL and ALL, we were able to detect SIR varying from about 1.07, for total agriculture area and cereals area, to about 1.30 for potatoes, arboriculture and fresh vegetables areas. The statistical power was more limited for AML, with minimum detectable SIRs varying from about 1.15 for total agriculture area and cereals area to 1.94 for potatoes and arboriculture areas.

Results

Between 1990 and 2014, the French National Registry of Childhood Hematological Malignancies registered 11,487 cases of childhood AL diagnosed in France, 82,5% of which were ALL cases (n=9,488) and 15.6% AML (n=1,803). ALL was more frequent between 1 and 6 years old, whereas AML had a higher incidence rate among the youngest (less than 1 years old) (Fig 1).

Half of the French pediatric population lived in a municipality with 23.8% or more of its area used for agriculture (S2, Fig 2, S3). Some municipalities had a crop density above 100% because the UAA reported by the farmers was greater than the area of the municipalities where the farms were located. In the agricultural censuses, the UAA reported by a farmer was allocated to the municipality of the main building on the farm, which was not always the municipality where all the agricultural land was situated. There are few non-agricultural municipalities (UAA/area < 5%) in France. They consisted in the most urban areas (Paris region), the Landes forest in the South-West, and the mountainous regions (Vosges, Pyrénées, Alpes, Massif Central, Jura) (Fig 2, S3).

The distributions of the specific crop densities were different from each other. The median of the population-weighted crop density distribution was 3.6% for straw cereals, and 0.1% or less for the others (S2). The highest values of the 99th percentile of the population-weighted distribution were observed for straw cereals (57.9%) and viticulture (35.2%), reflecting a right-skewed distribution. This was the results of the organization of French agriculture, with specialized regions for most of the crops under study, even the most prevalent ones. Straw cereals and corn, for example, are common crops in the North and North-West, respectively (Fig 3, S4), but less common in other regions. Straw cereals density was highly correlated with oilseeds, legumes and industrial crops (Spearman coefficients r > 0.60); potatoes density with fresh vegetables and industrial crops (r ≥ 0.60); and legumes density with industrial cultures (r = 0.57) (S5). Viticulture was not associated with any of the other crops, except moderately with arboriculture (r = 0.22) (S5). Overall, municipal densities of the different types of crops were highly correlated between censuses. However, the reported cultivation of potatoes, legumes and fresh vegetables were less stable over time (S6).

Poisson regression showed no evidence of an association between total agricultural density and the incidence of childhood AL (SIRR = 1.00; 95%CI [0.99-1.01], for a 10% increase in density) or its subgroups (Table 1).

For permanent crops, we observed a positive association between viticulture density and incidence of AL (Table 2). This association was slightly stronger for ALL (SIRR = 1.04; 95%CI [1.01-1.07] for a 10% increase in viticulture density), with increased SIRs in the two categories with the highest viticulture densities (SIR = 1.12; 95%CI [0.97-1.29] and SIR = 1.17; 95%CI [1.01-1.35], respectively, Table 2). No log-linear trend was observed for AML (SIRR = 0.99 (95%CI [0.92-1.07]). For arboriculture, no trend and no pattern were observed for any type of childhood leukemia, but the numbers of cases were more limited, in particular for the AML subgroup (Table 2).

For the cereals group, heterogeneity between the SIRs of the different categories of corn density was detected for all AL and ALL, and the log-linearity of the association was rejected (S7), but the results did not suggest an increase in incidence rate with increasing corn density (Table 3, S7). No association was observed for AML (Table 3, S7). The log-linear regression models showed SIRR between straw cereals density and AL, ALL and AML that were almost equal to 1 (Table 3, S7). Additional analyses on specific straw cereals (common wheat, durum wheat and barley) did not show any evidence of an association with the AL incidence rate (S8).

A positive log-linear association was suggested for oilseeds density and ALL (SIRR = 1.04; 95%CI [0.99-1.09], Table 4), but the categorical model did not show a clear pattern (S7), and there was no evidence of an increased SIR in the category with the highest oilseed densities. Heterogeneity of the SIRs in the categorical model was observed for AML (S7), with a lower SIR in the intermediate oilseed density category, but no evidence of a positive trend (Table 3, S7). Additional analyses of specific oilseeds (rapeseed, sunflower), did not show any association with the AL incidence rate (S8).

For vegetables, no evidence of a log-linear association between potatoes/fresh vegetables, strawberries or melons and any group of AL was found, with no pattern observed in the categorical models (Table 3, S7). For legumes (beans, peas, protein crops), a positive log-linear association was observed for all AL (SIRR = 1.09; 95%CI [0.98-1.22]) for a 10% increase in legume density (Table 3).However the categorical models showed no clear pattern (S7). Analysis of AML showed a positive trend, but with very wide confidence intervals, and based on a limited number of cases (Table 3, S7).

Analysis of industrial crops showed no evidence of an association with any subtype of AL (Table 3, S7). An additional analysis of industrial beets did not detect any heterogeneity of the SIRs or any clear log-linear trend (S8).

The distribution of the French deprivation index in the pediatric population by crop density category showed a moderate positive trend with all the specific crop densities of the municipalities except for viticulture, oilseeds, fresh vegetables and legumes (S9). Indeed, for most crop types, the proportion of children living in the most deprived municipalities was higher (about 25-30%) in the highest crop density categories than in the other categories. Additionally, the residential exposure to UV radiation in the pediatric population was not homogeneous by crop density category. Strong positive trends between residential exposure to UV radiation $> 105.5\ J/cm^2$ and viticulture and arboriculture densities were observed. Strong negative trends for potatoes, legumes and industrial crops densities were observed (S9). Additional analyses of the association between crop densities and ALL incidence with systematic adjustment for both residential UV radiation and the French deprivation index were possible for all cultures except potatoes, legumes and industrial crops. For the latter three crops, which were rarely grown in the municipalities with higher UV radiation levels, we considered only municipalities with UV radiation ≤ 105.5 J/cm^2 and adjustment on the deprivation index was conducted (Table 4). After adjustment, we found no change in the associations observed in the main analysis except for viticulture, for which there was a slight decrease in the log-linear slope (SIRR = 1.02; 95%CI [0.99-1.06] for a 10% increase in density); and legumes, for which there was a slight increase in the slope (SIRR = 1.12; 95%CI [0.99-1.27] for a 10% increase in density). Nevertheless, for legumes, the SIRs in the qualitative model adjusted for the French deprivation index did not differ from those in the main analysis (data not shown). Residential exposure to UV radiation was strongly correlated with viticulture density (r = 0.44), and a supplementary analysis with stratification on the two categories of UV radiation (UV > 105.5 J/cm^2 versus UV ≤ 105.5 J/cm^2) showed a weaker association with ALL in the highest category of UV radiation exposure (SIRR = 1.02; 95%CI [0.98-1.06] for a 10% increase in density) compared to the results of the main analysis. The slope in the lowest category of exposure was similar to that of the main analysis (SIRR=1.04; 95%CI [0.97-1.10] for a 10% increase in density) (S10). However, no significant interaction was detected (p = 0.60).

The associations between the incidences of AL and ALL and viticulture density were unchanged when the Paris region was excluded from the analysis (Table 5). When the country was subdivided into four large regions excluding Paris (Table 5), the log-linear model showed somewhat positive trends for AL and ALL by viticulture density in the North-East, North-West and South-East. There was no trend for the South-West, and no significant interaction was observed (p = 0.44 for AL and p = 0.61 for ALL). Stratification by two age groups (Table 5) did not modify the associations between viticulture density and AL and ALL; the log-linear trend was slightly stronger for ALL for children aged 7 to 14 years, compared to the younger children (Table 5). The association remained stable for both the AL and ALL groups when stratified by three periods (Table 5). Given that viticulture was not strongly correlated with any other specific crop, we did not adjust on other crop densities. We subdivided the viticulture density quartiles into semi-quartiles, and observed no change in the log-linear trend, but a stronger SIR in the highest exposure category (municipalities with a viticulture density greater than 38%) for AL (SIR = 1.24; 95%CI [1.03-1.48]) and ALL (SIR = 1.25; 95%CI [1.02-1.52]) (S11).

Discussion

We found a moderate positive log-linear trend for the viticulture densities of the French municipalities and the incidence rate of childhood AL over the period 1990-2014, with a 3% increase in SIR for a 10% increase in viticulture density (SIRR = 1.03; 95%CI [1.00-1.06]). In the categorical model, the highest category of exposure had an SIR of 1.16; 95%CI [1.02-1.32]. In that category, the number of cases observed over 25 consecutive years (O = 226) was greater, by 31.5, than the corresponding expected number of cases (E = 194.5) based on national age-gender specific incidence rates. However, no evidence of any other positive associations between AL incidence and total agricultural density or any of the specific crop densities considered was observed. When considering the main subgroups of AL and two age groups, the association with viticulture remained for ALL, but not for AML, and was stable for the two age groups. A secondary analysis of ALL subgroups with adjustment on two potential confounders, residential UV radiation and SES of the municipality, showed a small decrease in the association of ALL with viticulture density, because of a strong positive correlation between UV radiation and viticulture density, and an increase in the association with legumes. Stratification on periods and geographic regions of the association between viticulture density and incidence of AL and ALL showed no heterogeneity of the log-linear slope between the strata. Analysis of the semi-quartiles showed a stronger association in the highest category of viticulture density.

This is the first study of the association between municipal agricultural densities and the incidence of childhood leukemia in France. Few studies have investigated the role of environmental exposure to agricultural pesticides and the risk of childhood leukemia, because of the difficulty of combining precise exposures and a large sample size in order to detect small effects. The previous studies were mainly conducted in the United States (Booth et al., 2015; Carozza et al., 2008; Reynolds et al., 2005b, 2005a, 2002; Rull et al., 2009; Thompson et al., 2008; Walker et al., 2007) and based on different designs and exposure assessment methods. Most of the studies analyzed the association between AL and either total agricultural land density (or proximity) or specific crop densities (or proximities). The results with total agriculture land density (or proximity) are inconsistent (Booth et al., 2015; Carozza et al., 2008; Gómez-Barroso et al., 2016; Walker et al., 2007). We did not find any evidence of an association between total UAA density and the incidence of AL. The area considered consisted in arable land, permanent cropland and permanent pastures. The quantities and types of pesticides spread on those land categories are very heterogeneous and, in addition, permanent pastures may be a proxy for contact with farm animals, an exposure that was inversely associated with childhood leukemia in some recent studies (Orsi et al., 2018). Moreover, total agricultural land density may not be a pertinent indicator for the assessment of exposure to agricultural pesticides because of the wide range of agricultural practices covered.

The analyses of specific crops showed a moderate log-linear association between AL incidence rate and viticulture density. Two large US ecological studies of numerous states, based on agricultural census data at county level, found positive associations between AL and specific crops such as oats (Booth et al., 2015; Carozza et al., 2008), corn (Carozza et al., 2008), soybean (Carozza et al., 2008), sugar beets (Booth et al., 2015), and dry beans (Booth et al., 2015). Viticulture was not considered in either of the studies. A Spanish population-based case-control study based on land use maps showed a moderate positive trend for leukemia and proximity of irrigated and heterogeneous cropland, but no association with vineyards or olive groves (Gómez-Barroso et al., 2016). However, the study was based on a smaller number of subjects than in our study, and the controls and cases had different exposure time windows (time of diagnosis for the cases, time of birth for the controls). An Italian case-control study conducted in two agricultural regions and based on land use maps and a very limited number of exposed subjects found a positive but non-significant association with arable land (Malagoli et al., 2016). California is the only state for which comprehensive data on pesticides spread on croplands since the 1990s are available. Epidemiological studies based on the Californian Pesticides Use Reporting database and investigating the role of environmental exposure to agricultural pesticides in the risk of childhood leukemia found moderate associations with some specific compounds such as metam sodium, propargite and dicofol but there were no clear patterns (Reynolds et al., 2005b, 2005a, 2002; Rull et al., 2009). Although now prohibited, those three compounds were used in France during our study period, but their use was not restricted to viticulture only. However, it is very difficult to compare our results with those of the Californian or US-based studies because the crops grown, their proportions, and agricultural practices are different from those in France (S12). In addition, the majority of the pesticides used in France are fungicides (INSERM, 2013), and they are mainly used for vineyards, while the majority used in the USA are herbicides (US EPA, 2015).

One of the challenges of epidemiological studies of the health effects related to exposure to agricultural pesticides is to accurately assess the exposure. Because there is no database for the use of pesticides on cropland in France, we used the agricultural crop density as a proxy for agricultural pesticide use. Agricultural census data are advantageous in that they are exhaustive for the entire country, available for the various study time points and provide a detailed list of crops on the municipal scale. This enabled our results to be broadly linked with national data on agricultural practices. In 2000, viticulture accounted for less than 4% of the total UAA and 20% of the pesticides, mainly fungicides, sold (INRA, 2011). However, even though the agricultural census affords data on the types of crop grown in the census year, crop rotations are not taken into account. Annual rotations on the same land may be a reason why several crop density indicators were correlated. In consequence, we probably had less power to detect an association with rotated crops than with permanent crops. In addition, even if the three censuses allowed us to consider temporal variations, local changes occurring between the censuses could not be accounted for.

Another limitation of the proxy is that farmers report their UAA for the municipality where the farm is located and not for the municipalities where the crops are located. This may introduce non-differential misclassification of the crop density in the municipalities. The misclassification may either over- or underestimate the observed effects (Jurek et al., 2005). Nevertheless, when the farmland is not situated in the municipality of the farm, it is likely to be located in a neighboring municipality as there is a clear regional structure to the type of crops grown. The agricultural crop density of the municipality is therefore likely to be an accurate reflection of the crop density in the area where the children lived.

This is one of the largest studies on this issue, with 25 years of observations and 11,847 AL cases, based on a national registry of childhood cancers with a high degree of completeness, and exhaustive agricultural census data for the various time points. However, the analysis addresses a rare disease and exposure. The analyses of leukemia subgroups were limited by the number of subjects for AML.

We conducted several statistical tests in this study, but we did not take them into formal account. Given the descriptive nature of this investigation, we focused not only on statistical significance but also on the quantitative values of the SIR and SIRR observed to describe our results. From this point of view, the associations observed between viticulture and AL and ALL were consistent in the log-linear and categorical models, and the results were quite stable after several sensitivity analyses.

We did not incorporate an autocorrelated structure in our models, although the crop densities of the municipalities were highly autocorrelated, because our outcome was not spatially autocorrelated in all of the studies conducted in France (Goujon et al., 2018).

Previous French studies have found associations between ALL and residential UV radiation exposure (Coste et al., 2017, 2015) and SES of the residence area (Marquant et al., 2016). We therefore took those two factors into account in a secondary analysis. Adjusting on SES did not materially modify the association of ALL with viticulture or lack of association of ALL with any of the other crops, except for legumes. As expected, ambient UV radiation was spatially positively or negatively strongly associated with specific crops throughout France. There was a strong association between ambient UV radiation and vineyard density; the adjusted log-linear association between ALL and vineyards density was slightly less than in the main analysis. After stratification, we still found a positive association of the same magnitude as in the crude analysis in the stratum with the lowest exposure to UV radiation, even though the confidence interval was somewhat wider. However, adjusting on ambient UV radiation exposure did not modify the observed lack of association between ALL and the other crops.

We were unable to adjust on other potential environmental confounders such as traffic-related air pollution, which has been found to be associated with the risk of childhood leukemia in numerous studies and may be inversely associated with crop density. In addition, other sources of pesticide exposure, such as domestic or parental exposure, were not considered and may modify the association. We cannot either exclude that the association observed could reflect at least in part an association between AL and parental occupational exposure, as our ecological exposure metric do not allow to disentangle environmental, parental and parent mediated exposures.

Conclusion

This is the first French study to analyze the association between the incidence of childhood leukemia and crop density, a proxy of environmental exposure to agricultural pesticides. The study was based on the high-quality data of the French National Registry of Childhood Hematological Malignancies and the exhaustive agricultural census data at three time points on the municipality scale. We did not find any clear association with specific crops, except a moderate positive association between viticulture density and AL incidence rate. In France, vineyards undergo intense pesticide treatments, mainly with fungicides. However, fungicides or intense treatments are used in other agricultural sectors such as arboriculture. In addition, the results may reflect an effect of factors other than pesticide treatment that may have a spatial distribution similar to that of vineyards. This study was part of a wider project that will include a complementary study based on a record-based case-control study with precise geocoding of children's addresses and a combination of different land use data. The study will have an observation period somewhat shorter than that of the present study but with much more precise localization of the croplands. It will also be possible to adjust for other environmental risk factors. Further studies at the individual level and with better exposure assessment are needed to clarify the role of agricultural pesticides in the etiology of childhood leukemia. Improving exposure assessment implies deploying an effective surveillance system for the pesticides spread on fields in order to identify the specific compounds utilized.

Authors contributions

AC, SG, DH, JC designed the study. AC extracted and prepared the agricultural census data from the CASD platform and performed the statistical analyses. SG extracted and prepared the RNHE and INSEE data and contributed to the statistical analysis. LF was involved in data extraction of the RNHE. SG, DH, JC supervised the analyses. AC, SG, JC, DH interpreted the results. AC drafted the initial report. All coauthors revised the report and approved the final version. AC, SG, DH, JC are responsible for the overall content as the guarantors of this paper.

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Table 1 Association between the incidence rate of childhood leukemia and agricultural density[1] in the French municipalities, 1990-2014

				Acute leukemia					Acute lymphoblastic leukemia					Acute myeloid leukemia				
	Average density[2]	**Number of units[3]**	**PY/year**	**O**	**E**	**SIR**	**CI**	**P[4]**	**O**	**E**	**SIR**	**CI**	**P[4]**	**O**	**E**	**SIR**	**CI**	**P[4]**
Total utilized agricultural area (UAA)																		
[0-5[	0.9	6,081	3,241,048	3,315	3,336.2	0.99	0.96-1.03		2,708	2,752.4	0.98	0.95-1.02		556	526.3	1.06	0.97-1.15	
[5-20.3[	12.1	10,871	2,027,237	2,021	2,054.2	0.98	0.94-1.03		1,654	1,695.6	0.98	0.93-1.02		323	323.5	1.00	0.90-1.11	
[20.3-39.6[	29.9	18,178	2,026,946	2,097	2,034.7	1.03	0.99-1.08		1,757	1,680.6	1.05	1.00-1.10		306	319.4	0.96	0.86-1.07	
[39.6-63[	50.6	27,470	2,027,319	2,051	2,028.9	1.01	0.97-1.06		1,719	1,677.5	1.02	0.98-1.07		304	317.1	0.96	0.86-1.07	
≥63	84.3	43,936	2,027,190	2,003	2,033.0	0.99	0.94-1.03		1,650	1,681.8	0.98	0.93-1.03		314	316.8	0.99	0.89-1.11	
Total		106,536	11,349,740	11,487	11,487.0				9,488	9,488.0				1,803	1,803.0			
Test of heterogeneity								0.52					0.15					0.59
Test of departure from log-linearity								0.35					0.08					0.62
SIRR for Δx=10%[5]						1.00	0.99-1.01	0.50			1.00	0.99-1.01	0.35			0.99	0.98-1.01	0.85

O: Observed number of AL cases; E: expected number of AL cases under the hypothesis of a homogeneous incidence rate throughout mainland France; SIR: Standardized Incidence Ratio, standardized on age and sex ratio by period; CI: 95% Confidence Interval;

[1] The agricultural density (all crops) in a municipality is defined as the ratio of the total area used for agriculture over the total area of the municipality (based on national agricultural census data);

[2] Average agriculture density weighted by the pediatric population at risk living in the municipalities of the category;

[3] Number of statistical units, defined as "municipalities × period" in each category;

[4] p-value of the tests (chi-square test of heterogeneity between categories of agricultural density, departure from log-linearity test, one-sided test of the regression coefficient in the semi-quantitative model, H_0: slope = 0 *vs* H_1: slope >0);

[5] SIRR (for Δ x = 10%) = Relative Standardized Incidence Ratio with 95% confidence limits: Multiplicative variation of the SIR for a 10% increase in the crop density derived from a log-linear Poisson regression model , where the outcome is the observed number of cases by municipality x period; the offset is the expected number of cases; and the independent covariate is the crop density.

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Table 2 Association between the incidence rate of childhood leukemia and permanent crop density[1] in the French municipalities, 1990-2014

				Acute leukemia					Acute lymphoblastic leukemia					Acute myeloid leukemia				
	Average density[2]	Number of units[3]	PY/year	O	E	SIR	CI	P[4]	O	E	SIR	CI	P[4]	O	E	SIR	CI	P[4]
Viticulture																		
% UAA [0-5[	0.1	6,081	3,241,048	3,315	3,336.2	0.99	0.96-1.03		2,708	2,752.4	0.98	0.95-1.02		556	526.3	1.06	0.97-1.15	
% UAA≥5 & % vit. <5	0.2	92,487	7,333,552	7,365	7,373.6	1.00	0.98-1.02		6,096	6,093.5	1.00	0.98-1.03		1,136	1,154.9	0.98	0.93-1.04	
[5-9.9[	7.2	2,087	193,902	198	193.5	1.02	0.89-1.18		168	159.9	1.05	0.90-1.22		27	30.3	0.89	0.61-1.30	
[9.9-15.9[	12.5	1,565	193,855	179	195.1	0.92	0.79-1.06		149	161.4	0.92	0.79-1.08		26	30.4	0.86	0.58-1.26	
[15.9-25[	19.7	1,577	193,241	204	194.0	1.05	0.92-1.21		179	160.1	1.12	0.97-1.29		24	30.7	0.78	0.52-1.17	
>=25	43.1	2,739	194,142	226	194.5	1.16	1.02-1.32		188	160.8	1.17	1.01-1.35		34	30.4	1.12	0.80-1.56	
Total		106,536	11,349,740	11,487	11,487.0				9,488	9,488.0				1,803	1,803.0			
Test of heterogeneity								0.22					0.13					0.42
Test of departure from log-linearity								0.58					0.52					0.30
SIRR for Δx=10%[5]						1.03	1.00-1.06	0.02			1.04	1.01-1.07	0.01			0.99	0.92-1.07	0.59
Arboriculture																		
% UAA [0-5[	0.1	6,081	3,241,048	3,315	3,336.2	0.99	0.96-1.03		2,708	2,752.4	0.98	0.95-1.02		556	526.3	1.06	0.97-1.15	
% UAA≥5 & % arb. <5	0.3	98,337	7,827,851	7,906	7,869.1	1.00	0.98-1.03		6,555	6,503.1	1.01	0.98-1.03		1,207	1,232.3	0.98	0.93-1.04	
[5-6.4[	5.7	586	71,194	66	71.2	0.93	0.73-1.18		55	58.7	0.94	0.72-1.22		10	11.3	0.88	0.48-1.64	
[6.4-9.3[	7.6	619	69,322	60	69.8	0.86	0.67-1.11		50	57.7	0.87	0.66-1.14		10	10.9	0.92	0.49-1.70	
[9.3-13.2[	11.2	397	70,126	70	70.4	0.99	0.79-1.26		57	58.0	0.98	0.76-1.27		13	11.2	1.16	0.68-2.00	
>=13.2	23.4	516	70,199	70	70.3	1.00	0.79-1.26		63	58.0	1.09	0.85-1.39		7	11.1	0.63	0.30-1.33	
Total		106,536	11,349,740	11,487	11,487.0				9,488	9,488.0				1,803	1,803.0			
Test of heterogeneity								0.83					0.72					0.49
Test of departure from log-linearity								0.75					0.58					0.52
SIRR for Δx=10%[5]						0.98	0.90-1.07	0.68			1.01	0.92-1.11	0.43			0.88	0.69-1.12	0.85

O: Observed number of cases; E: expected number of cases under the hypothesis of a homogeneous incidence rate by period, for each age x sex category throughout mainland France; SIR: Standardized Incidence Ratio = O/E; CI: 95% Confidence Interval; UAA: total utilized agricultural area

[1] The crop density in a municipality is defined as the ratio of the area used for the crop over the total area of this municipality (based on national agricultural census data);

[2] Average crop density weighted by the pediatric population at risk living in the municipalities of the category;

[3] Number of statistical units, defined as "municipalities x period" in each category;

[4] p-value of the tests (chi-square test of heterogeneity between categories of agricultural density, departure from log-linearity test, one-sided test of the regression coefficient in the semi-quantitative model (H_0: slope = 0 *vs* H_1: slope >0);

[5] SIRR (for Δ x = 10%) = Relative Standardized Incidence Ratio with 95% confidence limits: Multiplicative variation of the SIR for a 10% increase in the crop density derived from of a log-linear Poisson regression model adjusted to the observations (the outcome is the observed number of cases by municipality x period; the offset is the expected number of cases; and the independent covariate is the crop density).

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Table 3 Association between the incidence rate of childhood leukemia and non-permanent crop density[1] in the French municipalities, 1990-2014

			Acute leukemia (n=11,487)			Acute lymphoblastic leukemia (n=9,488)			Acute myeloid leukemia (n=1,803)		
Crop density	**Average density[2]**	**% population in a municipality with crop density ≥ Q3[3]**	**SIRR[4]**	**CI**	**P[5]**	**SIRR[4]**	**CI**	**P[5]**	**SIRR[4]**	**CI**	**P[5]**
Corn	12.3	4.6		-			-		0.98	0.90-1.08	0.65
Straw cereals	19.3	11.3	1.00	0.98-1.01	0.47	1.00	0.98-1.02	0.48	1.00	0.96-1.03	0.43
Oilseeds	11.2	3.5	1.03	0.98-1.07	0.13	1.04	0.99-1.09	0.07		-	
Potatoes	10.3	0.5	0.98	0.87-1.10	0.65	0.94	0.83-1.07	0.82	1.12	0.86-1.47	0.20
Fresh vegetables	10.5	0.7	1.01	0.92-1.11	0.43	0.99	0.88-1.10	0.60	1.06	0.84-1.34	0.30
Legumes	8.5	0.8	1.09	0.98-1.22	0.06	1.07	0.95-1.21	0.13	1.17	0.90-1.53	0.12
Industrial crops	11.2	1.6	1.00	0.94-1.06	0.49	1.00	0.93-1.07	0.48	1.01	0.87-1.18	0.44

CI: 95% Confidence Interval

[1] The crop density in a municipality is defined as the ratio of the area used for the crop over the total area of this municipality (based on national agricultural census data);

[2] Average of the specific crop densities in the municipalities of France with a density ≥ 5%, weighted by the pediatric population at risk living in the municipalities.

[3] % pediatric population living in a municipality with a specific crop density greater than or equal to the limit value of the third quartile of the crop density distribution in the qualitative model

[4] SIRR = Relative Standardized Incidence Ratio with 95% confidence limits: multiplicative variation of the SIR for a 10% increase in the crop density derived from of a log-linear Poisson regression model where the outcome is the observed number of cases by municipality x period; the offset is the expected number of cases; and the independent covariate is the crop density. The SIRR is shown when log-linearity is not rejected by the test of departure from log-linearity.

[5] p-value of the one-sided test of the regression coefficient in the semi-quantitative model (H_0: slope = 0 *vs* H_1: slope >0).

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Table 4 Association between crop densities and childhood acute lymphoblastic leukemia incidence rate in the French municipalities, jointly adjusted for UV radiation and deprivation index (FDep99), 1990-2014 (9,422 cases of ALL, because of some missing values for Fdep99)

Semi-quantitative log-linear model adjusted on potential confounders[1]	**SIRR[2]**	**CI**	**P[3]**
Total agricultural density (Δx = 10% increase in total UAA density)	1.00	0.997-1.01	0.16
UV>105.5 J/cm² versus UV≤105.5 J/cm²	1.08	1.04-1.13	0.0003
FDep99 most deprived quintile versus Less deprived	0.92	0.88-0.97	0.003
Viticulture density (Δx = 10% increase in viticulture density)	1.02	0.99-1.06	0.07
UV radiation >105.5 J/cm² versus UV radiation ≤105.5 J/cm²	1.07	1.03-1.12	0.002
FDep99 most deprived quintile versus Less deprived	0.93	0.88-0.97	0.003
Arboriculture density (Δx = 10% increase in arboriculture density)	0.98	0.89-1.08	0.66
UV radiation >105.5 J/cm² versus UV radiation ≤105.5 J/cm²	1.09	1.04-1.14	0.0003
FDep99 most deprived quintile versus Less deprived	0.93	0.88-0.98	0.004
Straw cereals density (Δx = 10% increase in cereal density)	1.01	0.99-1.03	0.10
UV radiation >105.5 J/cm² versus UV radiation ≤105.5 J/cm²	1.09	1.04-1.14	0.0002
FDep99 most deprived quintile versus Less deprived	0.92	0.88-0.97	0.003
Oilseeds density (Δx = 10% increase in oilseed density)	1.04	0.99-1.09	0.05
UV radiation >105.5 J/cm² versus UV radiation ≤105.5 J/cm²	1.08	1.04-1.13	0.0003
FDep99 most deprived quintile versus Less deprived	0.92	0.88-0.97	0.003
Potatoes density[4] (Δx = 10% increase in potatoes density)	0.96	0.84-1.10	0.73
FDep99 most deprived quintile versus Less deprived	0.93	0.88-0.99	0.02
Fresh vegetables density (Δx = 10% increase in fresh vegetables density)	0.99	0.89-1.10	0.58
UV radiation >105.5 J/cm² versus UV radiation ≤105.5 J/cm²	1.08	1.04-1.13	0.0003
FDep99 most deprived quintile versus Less deprived	0.93	0.88-0.98	0.004
Legumes density[4] (Δx = 10% increase in legumes density)	1.12	0.99-1.27	0.04
FDep99 most deprived quintile versus Less deprived	0.93	0.87-0.98	0.01
Industrial crops density[4] (Δx = 10% increase in industrial crops density)	1.03	0.96-1.11	0.19
FDep99 most deprived quintile versus Less deprived	0.93	0.87-0.98	0.01

CI: 95% Confidence Interval

[1]Results for corn not shown because log-linearity was rejected in the main analysis in table 4

[2]SIRR = Relative Standardized Incidence Ratio with 95% confidence limits

[3] p-value of the tests: for crop density, one-sided test of the adjusted regression coefficient in the semi-quantitative model, with H_0: slope = 0 vs H_1 slope >0; two-sided test for the adjusted regression coefficients for UV radiation and the deprivation index, included as binary covariates in the model

[4] Restriction of the sample to the municipalities where UV radiation ≤ 105.5 J/cm², because the crops were rarely grown in the municipalities where UV radiation > 105.5 J/cm²

Table 5 Sensitivity analyses on the association between viticulture density and acute leukemia

Variable	Strata	Average density[1]	% population in a municipality ≥ 25% viticulture Density[2]	Acute leukemia					Acute lymphoblastic leukemia				
				O	E	SIRR[3]	CI	P[4]	O	E	SIRR[3]	CI	P[4]
Geographic area	Paris excluded	20.6	2.1	9572	9572	1.03	1.00-1.06	0.04	7943	7943	1.03	1.00-1.06	0.02
	North-West of France	15.7	0.5	2293	2293	1.08	0.97-1.19	0.08	1918	1918	1.08	0.97-1.21	0.09
	North-East of France	20.9	0.6	2811	2811	1.03	0.96-1.12	0.19	2302	2302	1.02	0.93-1.11	0.34
	South-East of France	22.3	5.9	2752	2752	1.01	0.98-1.04	0.27	2752	2752	1.02	0.98-1.06	0.15
	South-West of France	19.9	3.5	1432	1432	1.00	0.94-1.07	0.50	1208	1208	1.01	0.95-1.09	0.35
Age	0-6 years old	20.6	1.7	7411	7411	1.03	0.99-1.06	0.07	6257	6257	1.03	0.99-1.07	0.09
	7-14 years old	20.6	1.7	4076	4076	1.03	0.99-1.08	0.07	3231	3231	1.06	1.00-1.11	0.02
Period	1990-1994	21.1	2.0	2225	2225	1.01	0.95-1.08	0.37	1831	1831	1.03	0.97-1.10	0.16
	1995-2004	20.9	1.7	4508	4508	1.05	1.01-1.10	0.01	3686	3686	1.05	1.01-1.10	0.02
	2005-2014	20.1	1.6	4754	4754	1.02	0.97-1.07	0.24	3971	3971	1.02	0.97-1.07	0.20

O: Observed number of cases; E: expected number of cases; CI: 95% Confidence Interval

[1] Average of the viticulture density in each stratum, weighted by the pediatric population at risk living in the municipalities of the category

[2] % pediatric population living in a municipality with 25% or more viticulture density (25% = limit value of the third quartile of the viticulture density distribution in the qualitative model)

[3] SIRR (for $\Delta x = 10\%$) = Relative Standardized Incidence Ratio with 95% confidence limits: Multiplicative variation of the SIR for a 10% increase in the crop density derived from a log-linear Poisson regression model, where the outcome is the observed number of cases by municipality x period; the offset is the expected number of cases; and the independent covariate is the crop density.

[4] p-value of the tests (chi-square test of heterogeneity between categories of agricultural density, departure from log-linearity test, one-sided test of the regression coefficient in the semi-quantitative model (H_0: slope = 0 *vs* H_1: slope >0)

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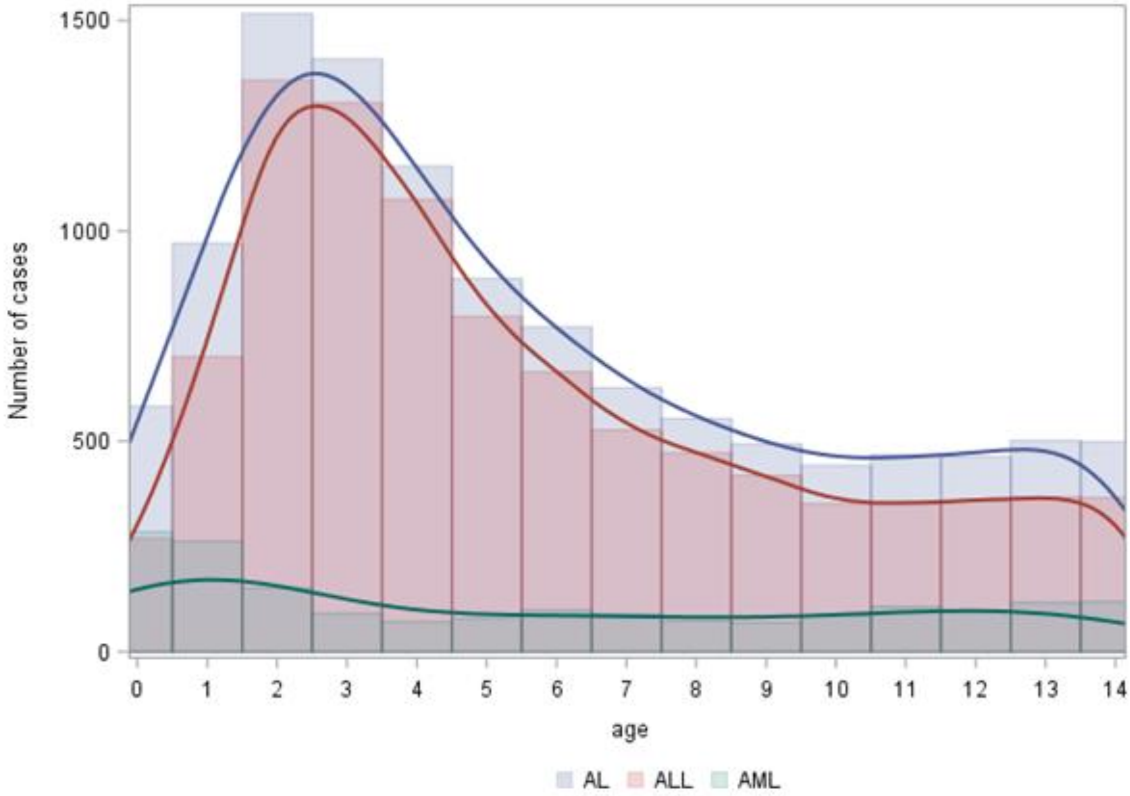
Figure 1 Distribution of childhood acute leukemia (AL) cases by age and main types, mainland France, 1990-2014



AL: acute leukemia; ALL: acute lymphoblastic leukemia; AML acute myeloid leukemia

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Figure 2 Density of agriculture in the municipalities of France – Agricultural census 2000

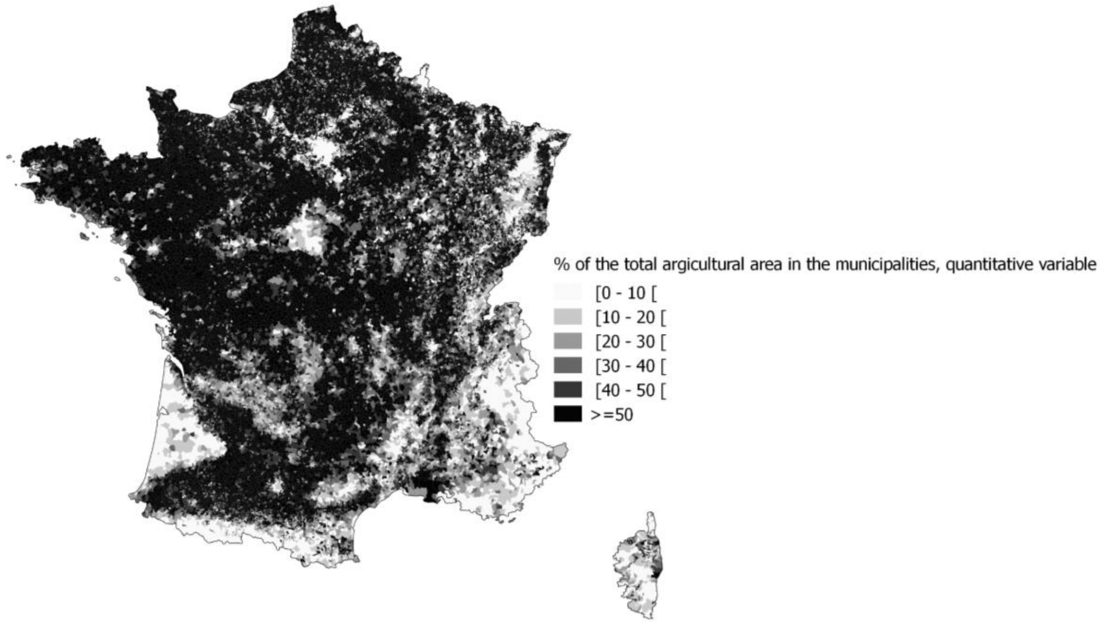

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Figure 3 Density of the specific crops studied in the municipalities of France – Agricultural census 2000