



children: A pilot study

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ABSTRACT

Rationale: Asthma, frequently associated with rhinitis, is the most common chronic disease in children, and a significant role is played by a range of environmental exposures. Among them, air pollution is of foremost concern. However, little is known about the impact of airborne pesticide exposure on children's respiratory health in rural areas. The objective of this study was to analyze the association between airborne pesticide exposure and asthma and rhinitis in children.

Methods: In a French vineyard rural area, children (3–10 years old) from 4 selected schools were invited to participate in this study over two periods: winter, with no or low air pesticide levels, and summer when fields are frequently treated with pesticides. Two health outcomes were considered: asthma and rhinitis symptoms (ISAAC questionnaire), and peak expiratory flow (PEF). A quantitative score of symptoms was built. Exposure to pesticides was evaluated 1) by measuring 56 pesticides in the ambient outdoor air around schools in the two periods and building a cumulative exposure index, and 2) by measuring ethylenethiourea (ETU) concentrations in urine in a subsample of children ($n = 96$), ETU being a urine biomarker of exposure to dithiocarbamates fungicides. Next, the association between pesticide exposure and respiratory health was studied using a logistic regression model, adjusted for confounders and respiratory status at baseline.

Results: 281 children participated in the study (47% girls, mean age: 7.5 yrs). 25% of the children were living on a farm. 22% had experienced wheezing at least once. 15.8% had asthma diagnosed by a doctor, 12% had current asthma and 35% had allergic rhinitis.

The main pesticides detected in the ambient outdoor air around schools were fungicides (89.3%; mainly folpet and dithiocarbamates) and insecticides (10.6%).

No association was found between the symptom score and pesticides in the outdoor air around schools during summer, when pesticides were applied to vineyards. However, an association was found between ETU urinary concentration ($> 0.974 \mu\text{g/g creatinine}$) and asthma and rhinitis symptoms ($\text{OR} = 3.56$; $\text{IC } 95\% \text{ } 1.04\text{--}12.12$). This result could be explained by extracurricular exposure, which was not considered in our air measurements in the schools. No association was found between peak expiratory flow and exposure to pesticides in the air.

Conclusions: Children living in vineyard rural areas are at a higher risk of airborne dithiocarbamates exposure during the summer period. Despite the limited size of our sample, our results suggest possible links between some pesticide measurements and respiratory and allergic symptoms such as rhinitis.

1. Introduction

Pesticides are chemical agents developed to have cytotoxic effects on, and then to kill, unwanted organisms. They are usually considered according to their targets (fungicides, insecticides, herbicides, acaricides) and they are also classified according to their chemical structure (i.e. dithiocarbamates, pyrethroids) (Li and Jennings, 2017). Agricultural pesticides are commonly used for crop protection around the

world, both in industrialized countries and low-developed countries. In addition, some of them, such as pyrethroids, have domestic uses to control mites, cockroaches and mosquitos in homes, or pests, weeds and diseases in gardens (Saillenfait et al., 2015).

Asthma is one of the most common chronic diseases worldwide with an estimated 300 million affected individuals (Nunes et al., 2017). Rhinitis is often associated with asthma in epidemiological studies (Pols et al., 2015) and is considered as a comorbidity of asthma. Prevalence is

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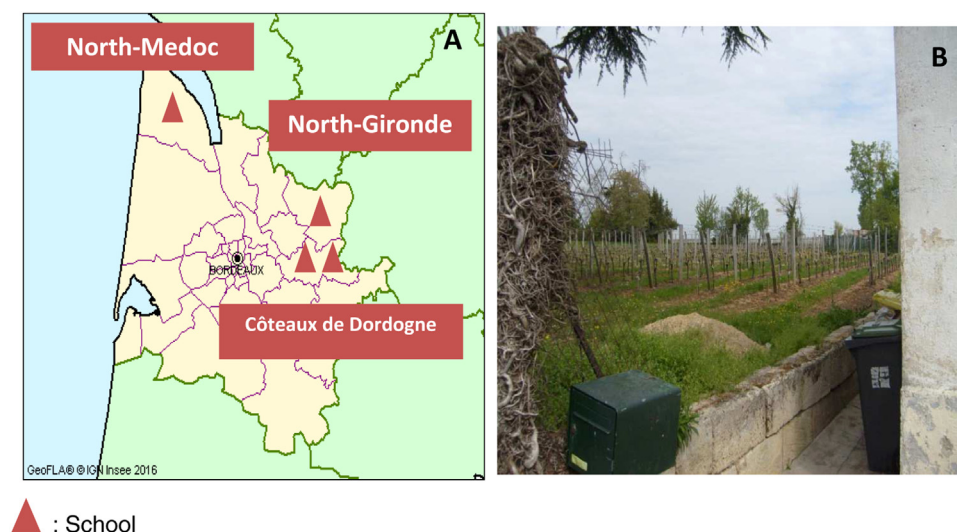


Fig. 1. Location of the schools in Gironde department (A) and picture of school 2 closed to vineyard (B).

increasing in many countries, especially among children. Asthma is still a major cause of school and work absence (Linneberg et al., 2016). Asthma still causes a large burden, with hospitalization, emergency visits and loss of productivity in the workplace (GINA, 2014). It is well established that asthma is a consequence of an interaction between genetic predisposition and several environmental factors (Martinez and Vercelli, 2013) including allergens (Belgrave et al., 2018), smoking exposure, both active and passive, viral and bacterial infections, air pollution, both indoor and outdoor (Cecchi et al., 2018), and occupational exposure, with the expression of different phenotypes and severities (Wenzel, 2012).

The association between pesticide exposure and asthma was reported first among Canadian (Senthilselvan et al., 1992), French (Baldi et al., 2014), and US farmers (Hopkin et al., 2006a, 2006b, 2008, 2017). However, living near treated fields could also expose the general population to pesticides during or after their application. There is a need for additional data on pesticide exposure in the general population, on biological mechanisms and on genetic susceptibility. This question is now currently being raised by individuals (healthy or asthmatic) who decide to live in rural areas, sometimes to avoid traffic air pollution. Few studies have examined the impact of pesticide exposure on asthma in the general population (Salameh et al., 2003; Salam et al., 2004; Xu et al., 2012; Mamane et al., 2015). A review published by Mamane et al. (Mamane et al., 2015) reported 20 studies dealing with respiratory health and non-occupational pesticide exposure: 14 carried out on children and six on adults. In four out of nine studies on children with biological measurements, mothers' dichlorodiphenyldichloroethylene (DDE) blood levels during pregnancy were associated with asthma and wheezing in young children.

In Southwestern France, the Bordeaux area is characterized by a large area covered by vineyards (about 120,000 ha). Pesticides, and particularly fungicides (Wightwick et al., 2010) (such as Folpet and Dithiocarbamates compounds), are frequently used from May to August to control fungi that could affect grapes before picking. In the villages of these areas, schools are often located in the vicinity of fields, and children in these schools are in close proximity to treated vines and may be exposed to pesticides by inhalation.

The objective of this pilot study was to explore the association between pesticide exposure (measured in the air or estimated from urinary biomarkers) and asthma and rhinitis in schoolchildren, in rural areas, near vineyards.

2. Methods

2.1. Study design

This study was observational and consisted of two phases in the same population of children. Phase I was during winter with no outdoor air pesticide exposure (March–April 2011), and phase II during summer when pesticides are applied to vineyards and consequently outdoor air pesticide exposure is present (May–June 2011). The children were still at school during this period.

2.2. Selection of geographic area

Prior to the selection of schools, the proportion of the area planted with vines in the municipality was calculated on the basis of the utilized agricultural area (UAA), and the exact location of the schools in relation to the vines was identified from satellite images. Among the 50 municipalities with the highest proportion of vines, four primary schools located on the edge of the vineyards were retained for the study.

The selection of schools was performed according to nonrandom sampling in clusters, with four sample unit school. Schools were selected from the fifty schools in the most rural and winegrowing areas of the region according to a ranking using the utilized agricultural area and the proportion of area under vines of municipalities. Next, the schools were chosen when they were close to a large area of vineyards (data collected through a survey conducted on the ground). The location of these four schools is shown in Fig. 1. 665 parents agreed to their children participating in this study (i.e. complete data on health status) (Fig. 2).

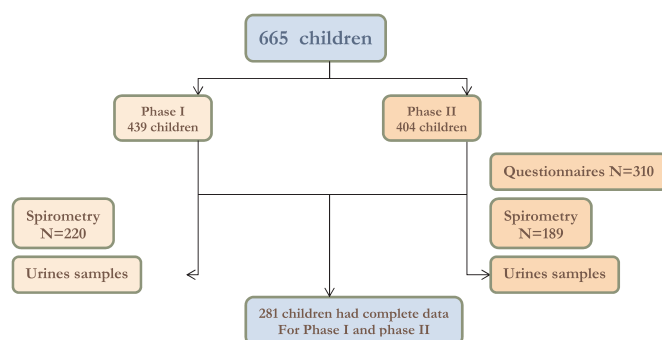


Fig. 2. Description of included population.

2.3. Health data collection

Authorization by the National Commission of Information Technology and Civil Liberties (CNIL) was sought and obtained before conducting this non-interventional survey. The parents of the children were informed by mail of the purposes and modalities of the survey. Their written consent was requested for each core of the study (questionnaire, peak flow measurement, urine samples).

In phase I, parents answered a detailed questionnaire on socio-demographic data, and the respiratory health of the children in terms of asthma and rhinitis, via a ISAAC questionnaire (Weiland et al., 2004; Caillaud et al., 2014), and environmental items (smoking exposure, pets at home, domestic use of pesticides, occupational use of pesticides). In phase II, parents answered a detailed questionnaire on respiratory and rhinitis symptoms during the children's life and over the last two months, as well as environmental items. The fieldworkers delivered the questionnaires to the teachers at school, and each child took them home. Then the completed questionnaires were brought back to school by the children and stored in a specific place by the teachers, to be collected by the fieldworkers.

A symptom score was built by adding together the points below attached to each item of the questionnaire, as presented in Table 1. The symptom score was built to integrate the lack of control of asthma and rhinitis. The score varied from 0 to 18, with 18 indicating uncontrolled asthma and rhinitis. The score was classified into three groups: low score (Score = 0), moderate score (score from 1 to 3) and high score (more than 3).

During the medical examination, we used an electronic device to measure forced expiratory volume at seconds 1 (FEV1) and 6 (FEV6) of a forced vital capacity (FVC) maneuver (% predicted value), to measure bronchial obstruction. Spirometry was monitored by fieldworkers at school, for children aged over 6 years old, at each phase of the study. After training the children through instruction and blowing practice, spirometry was measured before and after the exercise, three times each.

2.4. Pesticide exposure assessment

2.4.1. Measurements of pesticides in outdoor air

Monitoring data during the week of the examination were used as a surrogate for usual child exposure. During the week of health

evaluation, pesticides in the outdoor air were continuously measured by monitoring mobile stations located close to the schools: 17 insecticides, 20 herbicides and 19 fungicides were analyzed in the air samples and measured concentrations were expressed in ng/m³ of air, representing the total cumulative concentration of compounds over a week. The list of these pesticides was drawn up by a French national working group (see Annex A). Exposure to pesticides was evaluated by measuring pesticides in the ambient air of schools in the two periods and building a cumulative exposure index (Phyto index).

$$\text{Phyto index} = \sum_{i=1}^n C_i \times T_i \text{ expressed in ng/m}^3$$

n: number of pesticides detected in ambient air

C_i: concentration of each pesticide in ng/m³

T_i: ratio of acceptable daily intake (ADI) of the more toxic component and ADI of component i.

T_i is the ratio between the toxicity components of the most toxic pesticide (Ethoprophos) under acceptable daily intake of each component. This criterion is the available toxicological parameter. $T_i = \text{ADI (ethoprophos)} / \text{ADI } i$.

2.4.2. Measurements of pesticide biomarkers in urine samples

A large number of pesticides are likely to be used on the vines during a given treatment season. Among these pesticides, fungicides are the most numerous and most frequently used to control grapevine diseases that could compromise the harvest. For biological measurements, two fungicide groups (phthalimides and dithiocarbamates) were chosen because these fungicides are representative of the uses on vines and were detected in outdoor air close to schools in this study during vine treatment. Among the phthalimides, folpet is the most widely used and detected molecule and was therefore retained in this study. The ethylene-bis-dithiocarbamates (EBDCs), a group consisting of several drugs such as maneb, zineb, mancozeb fungicides, could be studied simultaneously considering that they have a common metabolite, ethylenethiourea, in urine samples (19). To take into account diuresis, urine creatinine concentration was also determined and urine ETU concentration was then expressed as ng per g of urine creatinine.

Spot urine samples were collected by the fieldworkers from the children at each phase, in the morning on waking. Specimens were placed in precleaned glass containers with Teflon-lined caps, transported at + 4 °C to the laboratory during the day. After aliquoting, samples were stored at −80 °C until analysis. Creatinine concentrations in urine were determined using a commercially available diagnostic enzyme method. Urine ETU concentrations were obtained by urine sample analysis using a validated method by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). The method was optimized to obtain high sensitivity, which is required for environmental pesticide exposure assessment. The limit of detection (LOD) of the method was 5 ng/L and the limit of quantification (LOQ) was 50 ng/L.

The detection of Folpet biomarkers in urines is currently ongoing in our laboratory, and will not be presented in this paper.

2.5. Air pollutant exposure assessment

In parallel with air pesticide measurements, concentrations of PM_{2.5}, NO_x, O₃, SO₂ were measured from Monday to Friday in schoolyards by air pollution monitoring mobile stations during the week in which the children underwent medical examination and provided urine samples.

2.6. Statistical analysis

We tested the association between pesticide exposure at phase II and

Table 1
Detailed points attached to each item of the questionnaire.

Variables	Point
Asthma	
Wheezing	1
Nocturnal wheezing	1
Exercise wheezing	1
Nocturnal dry cough	1
Attack limited speech	3
Rhinitis	
Sneezing and nasal blockage	1
Season of symptoms	
Winter	1
Spring	1
Summer	1
Autumn	1
Daily discomfort	
Not at all	0
A little	1
Moderate	2
Very much	3
Asthma and/or rhinitis	
School absenteeism	1
Medication	
As needed	1
Regular	1

symptom scores (outcome) at phase II, after adjustment for respiratory status if there was no modifying effect. Confounding factors included in the model were as follows: gender, age, mother and father's level of education, personal atopy, smoking exposure, bronchiolitis, pets at home, living on a farm, domestic use of pesticide, occupational exposure to pesticide, index of air quality (pollutants exposure), temperature, and respiratory score at phase I. The temperature was averaged daily and was obtained from the national website Météo France with geolocation of the temperature. Occupational exposure was self-reported by the parents on the questionnaire.

We tested the association between pesticide concentration measured in the air and symptom scores ($n = 281$), and then between pesticide concentration measured in urine using ETU as a biomarker of exposure and symptom scores in a subsample of children ($n = 96$) from schools 2 and 4 (arbitrary selection). To study the effect of pesticide exposure on respiratory symptoms, ordinal logistic regression models with cumulated logit were used; the dependent variable was ordinal (symptoms score). An adequation model was used in accordance with the Hosmer and Lemeshow Test. The analyses were performed with SAS, version 9.4 (SAS Institute Inc., Cary, NC, Etats-Unis).

3. Results

The study population included children who participated in both phases 1 and 2, and who completed the questionnaires. Out of the initial population of 665, $n = 281$ children (41%) were eligible for our analysis (i.e. complete data in health status) and $n = 96$ children gave urine samples in both phases 1 and 2 and completed the questionnaires (Fig. 2).

3.1. Characteristics of the population (Table 2)

Prevalence of at least one instance of wheezing was 22.3%, and prevalence of at least one instance of asthma was 15.8%. 32% had personal atopy such as hay fever or eczema. Two children had a prescription for no exercise because of their asthma. 50% of children were exposed to parental passive smoking. 56% of parents had a pet at home. A high proportion (85.5%) of parents reported regularly using pesticides at home (e.g. against fleas, body lice, insecticides, moths) (Table 2).

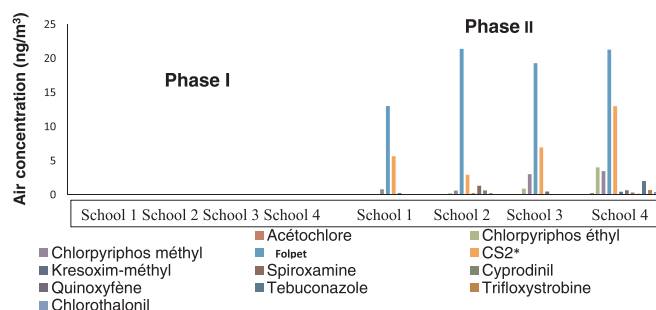
3.2. Concentrations of pesticides in the air in the two phases (Fig. 3)

No pesticide was measured in the air during the winter period (phase I). In contrast, during the pesticide treatment season (phase II), twelve pesticides were detected in the schoolyards, 89.3% of them fungicides and 10.6% insecticides. Among the fungicides, folpet was the most frequent compound detected, followed by the dithiocarbamates compounds (identified through CS_2 measurements in the air) and insecticides (chlorpyrifos) (Fig. 3).

Table 2

Characteristics of the children population ($n = 281$).

Variables	n	%
Age (years; mean $\pm$ sd)	7.47 $\pm$ 2.32	
Gender (girls)	132	46.98
Living in an agricultural exploitation	54	23.38
Professional use of pesticides by the parents	63	24.51
Domestic use of pesticides by the parents	236	85.50
Current wheezing	34	12.32
Current allergic rhinitis	96	34.53
Asthma medication	33	12.04



* CS_2 : carbon disulfur is a marker of dithiocarbamates pesticides

Fig. 3. Concentrations of pesticides in air during the two phases. * CS_2 : carbon disulfur is a marker of dithiocarbamates pesticides.

3.3. Urine biomarker of pesticide exposure: urine ETU concentrations in the two phases (Table 3)

In a subsample of children ($n = 96$), two urine samples per child were available for the determination of urine ETU concentrations by LC-MS/MS analysis. Comparison of this subsample to the whole study population did not find any significant differences regarding respiratory health. However, the children included in this subsample were more likely to live on a farm ($p < 0.0001$), and their parents reported using more pesticides for occupational purposes ($p < 0.0003$). The parents had a higher level of education and were less frequently smokers than those of children with no complete urine sample in the two phases of the study.

Between phase I and phase II, there was a significant increase in mean and median values of urine ETU concentration (Table 3). It is noteworthy that the maximum value of ETU concentration was higher in phase II than phase I (12.4 and 4.4 $\mu\text{g/g}$ of creatinine, respectively) (Table 3). During phase I, urine ETU concentration was significantly higher in children from school 2 than in those from school 4 ($p = 0.01$). During phase II, urine ETU concentration was higher in children from school 4, but the difference was not significant.

As there is no reference value in the French general population, we take into account the American value of the 95th percentile of distribution in the NHANES cohort. This value of ETU concentration of 0.974 $\mu\text{g/g}$ of creatinine was obtained in 2007–2008 with 382 children. This value was used in our study as the threshold value to classify children with the environmental ETU concentration or above.

Taking into account the threshold value of 0.974 $\mu\text{g/g}$ of creatinine for urine ETU concentration, 23.7% ($n = 23/96$) of children had an ETU urine concentration above this value during the first phase of the study, despite no dithiocarbamates fungicides in the outdoor air around schools. In contrast, in the second phase, during the vineyard treatment period, the proportion of children having an ETU urine concentration above this value increased significantly, up to 38.5% ($n = 37/96$) ($p = 0.012$).

Moreover, among asthmatic children, those who have high ETU urine concentrations ($> 0.974 \mu\text{g/g}$) reported taking significantly more asthma medication than children with low ETU urine concentrations (24.2% vs 8.6%; $p = 0.04$).

3.4. Association between pesticide levels in the air in phase II and symptom scores (Table 4)

A significant association was found between asthma scores and domestic pesticide use by the parents ($p = 0.04$) in a univariate analysis. After adjustment for confounding factors, there was no significant association between any pesticide and the asthma and rhinitis scores respectively (Table 4).

Table 3
Urine ETU concentration ($\mu\text{g/g}$ creatinine).

Variables	N ^a	Mean	SD	Min	Quartile1	Median	Quartile3	Max	p
Phase I									
[ETU] school 2+4	96	0.73	0.81	0.01	0.19	0.43	0.93	4.45	0.0108
[ETU] school 2	30	1.03	0.81	0.11	0.35	0.83	1.45	2.89	
[ETU] school 4	66	0.56	0.80	0.01	0.08	0.24	0.66	4.45	
Phase II									
[ETU] school 2+4	96	1.45	2.27	0.01	0.31	0.66	1.63	12.42	0.44
[ETU] school 2	30	1.22	2.05	0.06	0.35	0.62	1.53	11.37	
[ETU] school 4	66	1.54	2.67	0.01	0.28	0.64	1.63	12.42	

P value: comparison Urine ETU concentration between school 2 and school 4.

Table 4
Multivariate analysis between pesticides exposure in air (using phyto index) and symptoms score.

Scores	N ^a	OR	CI 95%	p
Total score	177	^c 0.54	0.07–4.03	0.5441
Asthma score	177	^b 3.93	0.40–38.44	0.2398
Rhinitis score	177	^d 0.27	0.03–2.35	0.2362
^c Score 0 versus score 1–2		0.21	0.06–5.16	
^e Score 0–1 versus 2		0.55	0.02–1.87	

Obtained by comparing children in school 4 (the most vulnerable) to other children (reference); the values of the scores null references.

^a Complete data.

^b Adjusted to the age, sex, level of education of parents, passive smoking, personal atopy, history of bronchitis, the presence of animal, considered score in phase I, using professional pesticides, habitat on a farm.

^c adjusted on the variables listed above and on the domestic use of pesticides.

^d Rhinitis score: assumption of the sides proportional no respected (score test $p < 0, 0237$).

^e Presentation of the results following the no proportionality of sides.

3.5. Multivariate analysis between ETU exposure and symptom scores (Table 5)

The analysis included 66 children with both complete data in the questionnaire and two urine samples (Table 5).

Urine ETU concentration in phase II was not associated with an increased risk of asthma symptoms, or rhinitis symptoms separately. In contrast, urine concentration of ETU in phase II was significantly associated with an increased risk of asthma and rhinitis (OR: 3.6 [1.04–12.1] after adjustment for age, gender, parents' education, smoking exposure, personal atopy, past bronchiolitis, pet at home, symptom score in phase I, home pesticide use, occupational pesticide use, living on a farm, and pollutants (air quality index).

Finally, no significant association was found between peak expiratory flow in phase II and exposure to pesticides in the air, or urine ETU concentration respectively. In addition, no significant association was found between a decrease in PEF and or/FEV1 between the two phases and exposure to pesticides in the air, or urine ETU concentration respectively. Exposure to pesticide in the air was not associated with

Table 5
Multivariate analysis between urine ETU concentration and symptoms score.

Scores	N ^a	OR	CI 95%	p
Total score	66	^c 3.56	1.04–12.12	0.0425
Asthma score	66	^c 2.01	0.54–7.52	0.2936
Rhinitis score	66	^b 2.83	0.75–10.75	0.1262

^a Complete data.

^b Adjusted to the age, sex, level of education of parents, passive smoking, personal atopy, history of bronchitis, the presence of domestic animals, score in phase I.

^c adjusted on the variables listed above and habitat on a farm.

lung function (OR: 0.73[0.20–2.72]; $p = 0.73$). In the same way, no association was found between urine ETU and lung function (OR: 1.52[0.24–9.55]; $p = 0.89$).

4. Discussion

In this pilot study, we described the short-term impact of pesticide exposure on respiratory and allergic health in children in schools located near treated fields in vineyards in South-West France. Pesticide exposure was assessed both by measurements in the air using a mobile station for one week, and by measurements in urines of a subsample of children using biomarkers of dithiocarbamates, which were fungicides measured in the air during the vine treatment phase (phase II).

We found that even during winter, when fields near schools are not sprayed, a proportion on children had detectable urine levels of ETU, a specific biomarker of dithiocarbamates exposure.

During the spraying period, we did not find any significant association between fungicides and insecticides measured in the air and asthma symptoms, rhinitis symptoms or PEF. In contrast, in a subsample of children with complete data (clinical and biomarker), after adjustment, urine ETU concentration was found to be associated with an increased risk of asthma and rhinitis symptoms.

The NHANES study showed an association between insecticides in the kitchen or dining in the room and wheezing and coughing among children (Xu et al., 2012). In Lebanon, a cross-sectional study of children aged 5–16 years old showed an increase in wheezing among children living near treated fields compared to non-exposed children (Salameh et al., 2003). In general, the assessment of exposure is based on self-reports. In the Pima birth cohort, the authors aimed to assess the proximity of participants' homes to treated fields as a proxy for environmental exposure to agricultural pesticides (Bukalasa et al., 2017).

More recently, a study carried out in Costa Rica on 437 residents living near banana plantations treated by mancozeb (a dithiocarbamate fungicide) showed that ETU concentration in urine was 45% higher in women living 48 m from the plantation than women living more than 565 m away (van Wendel de Joode et al., 2014). Another cross-sectional study in Germany on a sample of schoolchildren showed a significant association between urinary concentration of DDE (insecticides) and asthma (Karmaus et al., 2001). Biomarkers for insecticides were developed in NHANES (Perla et al., 2015).

The mechanism that could explain the impact between pesticides and respiratory health is still being debated. A randomized cross-over study included 25 asthmatic patients exposed to low levels of insecticide aerosols and showed a fall in lung function (FEV1) and an increase in hyper-responsiveness to methacholine 90 min after exposure (Salome et al., 2000). Experimental studies showed that granulometry of particles of folpet fungicide from commercial forms, suspended in water and sprayed on vines, had a size under 5 μm that could easily be inhalable by humans and had cytotoxic and oxidative stress effects on human bronchial epithelial cells (Canal-Raffin et al., 2008). Available data suggest that ethylenebisdithiocarbamates (EBDCs) may have

immunomodulatory effects (Karmaus et al., 2005). A significant increase in CD19 + cells, in both percentage and absolute number, and a significant decrease in the percentage of CD25 + cells were found in post-exposure samples compared to controls (Corsini et al., 2005). The estimated half life for eliminating ETU through the kidneys was close to 100 h (4 days), which could suggest acute exposure to fungicide (Kurtio et al., 1990).

Our study also has some limitations. The first limitation is the size of the sample, due to a low rate of participation in the study between the two phases.

Regarding pesticides in the air, exposure was evaluated by weekly concentration, and not by daily concentration. Additionally, exposure to pesticide in schoolyards was not representative of individual exposure for children, and we could not apply a time-series analysis. The duration of measurement in the air may also have been too short to evaluate the impact of exposure with an under-estimation of pesticide exposure. A measurement campaign throughout the spraying period would be better in order to include exposure variations and cumulative exposure.

We used ETU as a urine biomarker of air exposure to dithiocarbamates. However, the detection of ETU in urine samples could also be the result of oral-route exposure due to diet (fruits, vegetables) and water. Another important exposure route that might cause symptoms in children is the inhalation of pesticide contaminated soil dust, because they usually spend more time playing outdoors than other population groups (Jennings and Li, 2014; Li, 2018). In addition, ETU is one biomarker of a group of fungicides, but did not allow us to conclude on the health impact of other pesticides found in the air. The threshold used for this biomarker came from the NHANES study; however, we cannot rule out a measure bias that could influence the results. The detection limit of the ETU method in NHANES was 240 ng/g of creatinine, and in our study the detection limit was 5 ng/L. A classification bias cannot be ruled out, with an underestimation of the association observed in our study.

We decided to use a symptom score for asthma and rhinitis from the ISAAC questionnaire, in order to take into account both asthma control and severity of rhinitis. However, our score has not been previously validated. In addition, asthmatic and allergic children were taking their medication as usual.

In the pediatric population, proximal obstruction is generally extremely rare. In our sample, the mean FEV1 was high, at around 96%, and only 1.4% of children had FEV1 lower than the predicted 80%. We think that measuring distal obstruction or FENO for bronchial inflammation or a 6-min running test could be more appropriate in future studies than static lung function measurement. In addition, there is no good correlation between asthma symptoms and lung function, particularly in children.

Airflow limitation may be absent at the time of evaluation in some patients and variable airflow limitation could be documented in a second step (GINA, 2014).

Lastly, we took into account pollution measurements, but not pollen exposure, as there is only one pollen monitoring station in Bordeaux, and it is quite far from the rural area where the schools were located.

Despite these limitations, the originality of our pilot study lies in its use of two approaches to pesticide exposure: air pesticide concentrations using an aggregate index (phyto-index), and urine pesticide concentration using ETU as a biomarker of exposure to dithiocarbamates pesticides. We adjusted our multivariate analysis for known confounding factors.

In conclusion, children living in vineyard rural areas seem to be at higher risk of airborne dithiocarbamate exposure during the summer period. Despite the limited size of our sample, our results suggest an association between a known pesticide metabolite and respiratory symptoms.

In the future, pesticide monitoring in the air should be done routinely near treated fields, in order to assess more precisely child exposures. Biomonitoring of the general population exposed to airborne pesticides should be discussed for public health policies related to respiratory and allergic health.

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Conflict of interest statement

Dr. Raheison reports grants from Bordeaux University Foundation during the conduct of the study; personal fees from Astra Zeneca, personal fees from Chiesi, personal fees from ALK, personal fees from Boehringer Ingelheim, personal fees from Glaxo SmithKline, personal fees from MundiPharma, and personal fees from Novartis, outside the submitted work.

Other authors

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In accordance with European regulations, French observational studies from data obtained without any additional therapy or monitoring procedure do not need the approval of an IRB/IEC when surveys are performed. Authorization by the National Commission of Information Technology and Civil Liberties (CNIL) was sought and obtained before conducting this non-interventional survey. Informed written consent was obtained from the parents.

Annex A. List of pesticides measured in the air during the survey (phase I and II)

Insecticides	Herbicides	Fongicides
Bifenthrine	2,4-D 2-butoxyéthyl ester	Captane
Chlorpyrifos éthyl	2,4-MCPA 2-éthylhexyl ester	Chlorothalonil
Chlorpyrifos méthyl	Acétochlore	Cyprodinil
Cyperméthrine	Aclonifen	Diméthomorphe
Deltaméthrine	Alachlore	Diphénylamine
Diazinon	Atrazine	Dithiocarbamates*
Dichlorvos	Dichlorprop 2-éthylhexyl ester	Epoxiconazole
Dicofol	Diclobénil	Fenhexamide
Endosulfan alpha	Diméthénamide	Fenpropiidine

Endosulfan beta	Fluorochloridone	Fenpropimorphe
Ethoprophos	Mécoprop 2-butoxyéthyl ester	Folpel
Fénoxycarbe	Métazachlore	Kresoxim-méthyl
Fipronil	Métolachlore	Procymidone
Lindane	Oxadiazon	Pyriméthanil
Parathion éthyl	Pendiméthaline	Quinoxifène
Parathion méthyl	Propachlore	Spiroxamine
Perméthrine	Prosulfofocarbe	Tebuconazole
	T é butame	Tolyfluanide
	Terbutylazine	Trifloxystrobine
	Trifluraline	Vinchlozoline

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2018.11.002.

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Online supplement

Table A: Univariable analysis between symptoms score and exposure to pesticides (ETU urine concentration)

Variables	Scores*		
	Total	Asthma	Allergic rhinitis
	<i>p</i>	<i>p</i>	<i>p</i>
Âge	0,7531	0,9868	0,9224
gender	0,9221	0,8677	0,8812
Socio-economic status	0,1560	0,0834	0,2375
Personal atopy	0,0304	0,0665	0,0177
Bronchitis	0,4019	0,2879	0,5726
pets	0,5201	0,6171	0,8938
passive smoking exposure	0,0534	0,0263	0,3079
Scholl	0,7283	0,8603	0,8318
Symptoms score at phase I	0,0021	0,0062	0,0124
Living on a farm ^a	0,1105	0,0604	0,3888
Professional pesticides ^a	0,7867	0,7055	0,6717
domestic pesticides ^a	0,2476	0,1509	0,3378
School (ref : school 2)	0,7924	0,9111	0,7932
ETU, phase I	0,2354	0,7893	0,1108
random intercept, variance (value <i>p</i> ; -2LL)*			
School	0,00 (~1,0000 ; -8,72.10 ⁻¹¹)	0,00 (~1,0000 ; -4,26. 10 ⁻¹⁴)	0,00 (~1,0000 ; -5,08.10 ⁻¹²)
Home	0,00 (~1,0000 ; 8,73.10 ⁻¹¹)	0,00 (~1,0000 ; 2,84.10 ⁻¹⁴)	0,00 (~1,0000 ; 4,84.10 ⁻¹²)

* Value of null score are reference value ; ^a variables non included in multivariate analysis in case of $p > 0.25$; ** Values obtained by PROC GLIMMIX (SAS[®] to take into account random intercept, likelihood ratio method , : -2LL).

Table B: Description of subsample of children with urine sample (n=96) according to limit detection value

Variables	Reference* (N=60)			Exposed* (N=36)			P ^b
	N ^a	n	%	N ^a	n	%	
Socio-demographic data							
Gender	60			36			0.1263
Girls		33	55.00		14	38.89	
Boys		27	45.00		22	61.11	
Age ^b (years)			8.23±1.90			7.56±2.21	^d 0.1981
Parents education level	59			36			0.2494
Low		25	42.37		11	30.56	
High		34	57.63		25	69.44	
Home exposure							
Smoking exposure	59			36			0.9163
No		36	61.02		21	58.33	
paternal smoking		8	13.56		6	16.67	
maternal smoking		15	25.42		9	25.00	
pets	75			45			0.0783
yes		32	42.67		12	26.67	
No		43	57.33		33	73.33	
Domestic pesticides							
between Phase I and Phase II	58			34			0.7895
yes		9	15.52		6	17.65	
No		49	84.48		28	82.35	

Professional pesticides	57			36			0.5732
yes		19	33.33		10	27.78	
No		38	66.67		26	72.22	
Living on a farm	53			34			0.1299
yes		15	28.30		15	44.12	
No		38	71.70		19	55.88	
Urine ETU. phase I	60			36			0.1948
≤0.974 µg/g of creatinine (reference)		17	28.33		6	16.67	
>0.974 µg/g of creatinine		43	71.67		30	83.33	
health status phase I							
Current asthma **	59			35			0.9870
yes		5	8.47		3	8.57	
No		54	91.53		32	91.43	
asthma medication	58			33			0.0406
yes		5	8.62		8	24.24	
no		53	91.38		25	75.76	
Atopy	56			32			0.1697
yes		18	32.14		15	46.88	
no		38	67.86		17	53.13	
Bronchitis/bronchiolitis	60			35			0.5797
yes		36	60.00		23	65.71	
No		24	40.00		12	34.29	

Figure A. Location of mobile monitoring station in the schools



Figure B. Close-proximity of vineyards and schools

